SPECIFICATION

TITLE: ANTIBODY-DRUG CONJUGATE

[0001] Priority

[0002] This application claims the priority of Chinese Patent Application No. CN202410732731.9, filed with the China National Intellectual Property Administration on June 6, 2024, and titled with "DEUTERATED CAMPTOTHECIN DERIVATIVE AND LIGAND DRUG CONJUGATE", which is hereby incorporated by reference in its entirety.

FIELD

[0003] The present disclosure relates to the field of biomedicine, in particular to an antibody and an antigen binding fragment thereof, a camptothecin compound, and a linker and an antibody drug conjugate thereof.

BACKGROUND

[0004] Antibody drug conjugates (ADCs) represent a novel class of biological drugs formed by coupling a monoclonal antibody with a potent cytotoxic payload through a biologically active linker. Their mechanism of action is that the antibody drug conjugate utilizes the monoclonal antibody to achieve specific binding to the target antigen and then is internalized into the target cancer cells, after which the cytotoxic payload exerts cytotoxicity to kill the cancer cells (Fu Z, Li S, Han S, et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy[J]. Signal Transduction and Targeted Therapy, (2022)7:93.). Antibody drug conjugates can specifically target cell surface markers that are differentially expressed between tumor and normal cells, enabling selective delivery of potent cytotoxic agents to target cancer cells. Therefore, ADCs are called "biological missiles" in the field of anti-tumor therapy. Since the first ADC Mylotarg (gemtuzumab ozogamicin) was launched in 2000, there are only 16 ADC drugs (see Table 1 for basic information) approved for marketing worldwide as of January 2025. In clinical application, significant unmet therapeutic needs remain due to the requirement for target-positive patient and

the problem of drug resistance during later stages of ADC therapy. Moreover, ADC development is greatly difficult due to high technical barriers.

Table 1 Basic information on globally approved ADC drugs

Number	Product name	Drug name	R&D institutions	Toxin	Target
1	Mylotarg	Gemtuzumab ozogamicin	Pfizer	Calicheamicin	CD33
2	Adcetris	Brentuximab vedotin	Seagen/Takeda Pharmaceuticals	MMAE	CD30
3	Kadcyla	Trastuzumab emtansine	Roche/ ImmunoGen	DM1	HER2
4	Besponsa	Inotuzumab ozogamicin	Pfizer/Celltech	Calicheamicin	CD22
5	Lumoxiti	Moxetumomab pasudotox	AstraZeneca	PE38	CD22
6	Polivy	Polatuzumab vedotin	Roche	MMAE	CD79B
7	Padcev	Enfortumab vedotin	Seagen/Astellas Pharma	MMAE	HER2
8	Enhertu	Trastuzumab deruxtecan	Daiichi Sankyo/AstraZeneca	DXD	NECTIN-4
9	Trodelvy	Sacituzumab govitecan	Gilead Sciences	SN38	TOROP2
10	Blenrep	Belantamab mafodotin	GSK	MMAF	BCMA
11	Zynlonta	Loncastuximab	ADC Therapeutics	PBD	CD19

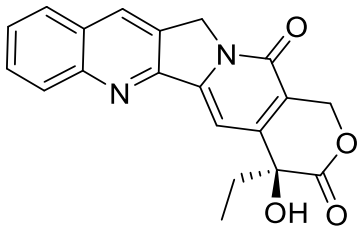
		tesirine			
12	Aidixi	Disitamab vedotin	RemeGen Co., Ltd.	MMAE	HER2
13	Tivdak	Tisotumab vedotin	Genmab/ Seagen	MMAE	TF
14	Elahere	Mirvetuximab soravtansine	Immunogen/ Huadong Medicine Co., Ltd.	DM4	FRA
15	Akalux	Cetuximab Sarotalocan Sodium	Rakuten Medical	IRDye700DX	EGFR
16	Jiatailai	Sacituzumab tirumotecan	Kelun Biotech	KL610023	Trop2

[0005] CDCP1 (CUB domain-containing protein 1) is a transmembrane glycoprotein which is widely expressed in a variety tumor cells, including colorectal cancer, lung cancer, breast cancer, etc. Studies have shown that CDCP1 plays an important role in tumor initiation, invasion and metastasis, and thus has become one of the potential targets for cancer therapy (Khan, Tashbib et al. “The CDCP1 Signaling Hub: A Target for Cancer Detection and Therapeutic Intervention.” Cancer research vol. 81,9 (2021): 2259-2269. doi:10.1158/0008-5472.CAN-20-2978. Siegel, R. L., et al. (2021). "Cancer Statistics, 2021." CA: A Cancer Journal for Clinicians, 71(1), 7-33. DOI: 10.3322/caac.21654.) As the role of CDCP1 in tumorigenesis is increasingly recognized, the development of CDCP1-based antibody drug conjugates is emerging as a major focus in the field of tumor treatment. The antibody drug conjugates based on novel anti-CDCP1 antibodies can enable a precision targeted therapy, so that anti-cancer drugs can specifically act on CDCP1-positive tumor cells to maximize the therapeutic effect. At present, although there are antibody drug conjugates targeting CDCP1 in the preclinical research stage, none have yet entered the clinical trial stage. The development of anti-CDCP1 antibody drug conjugates still faces many challenges in specificity of antibody, designing payload, optimizing conjugation mode, stability and release mechanism of drug in vivo, and the like. Current research focuses on overcoming these technical

difficulties and improving the clinical efficacy of anti-CDCP1 antibody drug conjugates.

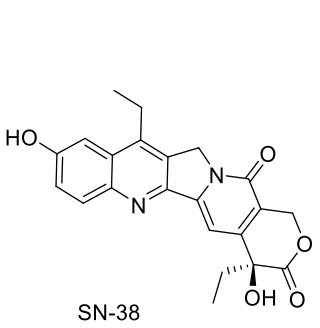
[0006] From the perspective of designing drug payload, the types of toxins that can be used to construct effective ADCs are currently very limited. The 16 marketed ADCs involve only 10 toxins, including calicheamicin, PE38 (a fragment of *Pseudomonas aeruginosa* exotoxin), pyrrolobenzodiazepine (PBD), auristatins (MMAE, MMAF), maytansinoids (such as DM1, DM4), and camptothecin derivatives (such as DXD, SN38, KL610023).

[0007] Wherein, camptothecin derivatives, a general term, refer to a class of compounds obtained by modifying one or more sites of the camptothecin (CPT). Camptothecin (CPT) is a topoisomerase I inhibitor and can be isolated from the bark of *Camptotheca acuminata* (a traditional Chinese medicine used to treat cancer). Its specific molecular structure was first disclosed by Wall et al. in 1966 (Wall M .Plant antitumor agents I. The isolation and structure of camptothecin-A novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata[J].J Am Chem Soc, 1966, 88.), as shown below.

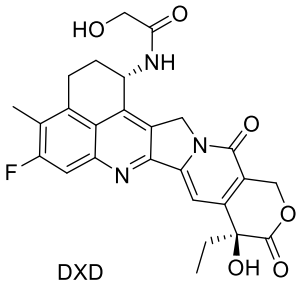
Molecular Structure	Name
	Camptothecin

[0008] Camptothecin (CPT) can bind to topoisomerase and DNA through hydrogen bonds, preventing DNA recombination and damaging DNA to cause cell apoptosis. However, camptothecin has many limitations in its application due to its poor water solubility, low targeting ability, high toxicity to normal tissues, and instability in plasma. (Dancey J, Eisenhauer E A .Current perspectives on camptothecins in cancer treatment[J].Br J Cancer, 1996; Proulx M E, Desormeaux A, Marquis J F, et al. Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin[J]. Antimicrobial Agent and Chemotherapy, 2019.). It has been more than 50 years since the structure of camptothecin was first disclosed. A multitude of camptothecin compounds have been disclosed in the later period, and some have been utilized as toxins in the research of constructing antibody drug conjugates. Nevertheless, to date, only a select

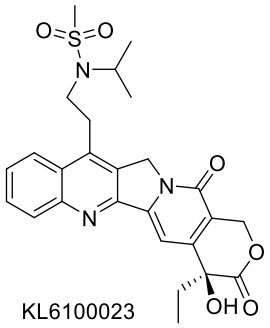
few antibody drug conjugates that contain camptothecin compounds have demonstrated significant druggability. As illustrated in Table 1, only three of the 16 antibody drug conjugates currently on the market utilize camptothecin compounds, namely DXD in trastuzumab deruxtecan, SN38 in sacituzumab govitecan, and KL6100023 in sacituzumab tirumotecan (the structures are shown below). Although the antibody drug conjugates developed based on these three payloads have achieved remarkable results, there is still a need for improvement in the development of novel camptothecin payloads. This is due to the fact that each payload has different killing effects and safety profile in different types of tumor diseases with high variability, and also involves combinations with different linkers, which will bring about differences in therapeutic effects.



SN-38



DXD



KL6100023

[0009] From the perspective of drug composition and technical characteristics, ADC has undergone four iterations in the development process: first-generation ADC drugs, second-generation ADC drugs, third-generation ADC drugs and fourth-generation ADC drugs. (1) The first-generation ADC drugs (Mylotarg, Besponsa) are primarily conventional chemotherapy drugs connected to mouse antibodies through non-cleavable linkers. The efficacy of these ADC drugs is not superior to that of free cytotoxic drugs, and these ADC drugs often cause issues such as immunogenicity, uncontrolled release of toxic payloads, antibody aggregation, metabolic instability, narrow safety windows, and high druggability challenges. (2) The second-generation ADC drugs (Adcetris, Kadcyla) have optimized monoclonal antibodies, cytotoxic payloads, and linkers. They employ IgG1 isotype monoclonal antibodies, which are more suitable for bioconjugation with small molecule payloads and have higher cancer cell targeting capabilities than IgG4. Another breakthrough is the use of more effective cytotoxic drugs with enhanced water solubility and conjugation level. Moreover, in the second-generation ADCs, linkers are optimized to achieve superior plasma stability and drug uniformity. The improvement of the three components, namely the antibody, linker, and toxin, has led to enhanced clinical efficacy and safety in the

second-generation ADCs. However, there are still limitations, including an insufficient therapeutic window due to off-target toxicity, aggregation or rapid clearance of ADCs with a high DAR value. (3) The third-generation ADC drugs (Polivy, Padcev) employ site-specific coupling technology to produce homogeneous ADCs with well-characterized DAR values (2 or 4) and expected cytotoxicity. Such ADCs exhibit low off-target toxicity, low immunogenicity, and high pharmacokinetic efficiency, but still have problems such as insufficient payload release and high drug resistance. (4) The fourth-generation ADC drugs (Enhertu, Trodelvy) adopt a strategy of high DAR value (4-8), medium and low activity cytotoxic payload. The payload has a high bystander effect and demonstrates good efficacy on tumors with both low and high expression of tumor surface antigens. Both two fourth-generation ADC drugs currently on the market, Enhertu and Trodelvy, use camptothecin compounds as cytotoxic payloads. The linker-toxin of Enhertu will be shed in the systemic circulation, causing cytotoxicity, and the payload used by Tropelvy has weak activity and poor stability in the systemic circulation.

[0010] Deuterium is a stable non-radioactive isotope of hydrogen in nature. It was first discovered and named in 1932. Deuterium has very similar physical and chemical properties to hydrogen. Due to its greater atomic mass than hydrogen, compared to the C-H bond, C-D bond has lower vibrational frequency, lower ground state energy, higher activation energy for dissociation, and slower cleavage speed, making the C-D bond more stable (6-9 times) than the C-H bond. Replacing the hydrogen in the drug molecule with deuterium may block the metabolic site and reduce the generation of toxic metabolites. In addition, deuterated drugs can remain stable under a variety of metabolic enzymes, slow down the system clearance rate and thus prolong the half-life of drugs in the body. Therefore, deuterated drug treatment can achieve the goal of reducing the toxic and side effects of the drug without affecting the pharmacological activity, by reducing single dose amount. In 2017, US FDA approved the first deuterated drug in history—Austedo from Teva, which was approved for marketing in China on May 2020. Austedo is a deuterated tetrabenazine. Its prototype drug, tetrabenazine, has been the mainstream drug for the treatment of Huntington's disease, but has defects such as short half-life and low patient dependence. Deuterated tetrabenazine replaces the hydrogen atoms in the two methoxy groups on the tetrabenazine benzene ring with deuterium atoms, which significantly reduces the speed of drug metabolism and prolongs the half-life of the drug, thereby reducing the dosage of the drug. It also inhibits the withdrawal

reaction caused by the decrease of drug blood concentration. Although deuteration has many advantages in drug development, the unpredictability of deuteration effects still remains, including large differences in the effects caused by deuteration at different sites. In terms of synthesis, deuteration poses challenges in controlling both the site and extent of deuterium enrichment. There are currently no deuterated antibody drug conjugates on the market.

[0011] In conclusion, after decades of development, there are still limited drugs and targets approved. Up to now, there are only 16 ADCs approved for 13 targets. Considering the complexity and diversity of cancer, the existing drugs cannot meet the needs of all patients. Although the therapeutic efficacy of antibody drug conjugates containing camptothecin compounds has been significantly improved, they still exhibit pharmacodynamic difference, safety and drug resistance issues. It is therefore of crucial importance to further develop and screen for high-affinity antibodies for tumor-specific highly expressed targets and appropriate payloads and linkers, determining the effectiveness and safety of ADCs.

[0012] Therefore, in view of the above problems and challenges, there remains a need in the field to improve the payloads, antibodies and linkers in order to develop antibody drug conjugates with further improved safety and efficacy or with differential therapeutic effects.

SUMMARY

[0013] The present disclosure provides an antibody or antigen binding fragment thereof targeting CDCP1, a novel linker, a payload and use thereof in an antibody drug conjugate.

[0014] The present disclosure further provides an antibody drug conjugate comprising the antibody, a nucleotide, a polynucleotide combination, an expression vector and a host cell for encoding or expressing the antibody targeting CDCP1, a pharmaceutical composition comprising the antibody targeting CDCP1 or the antibody drug conjugate and use thereof in the manufacture of a medicament for treating or preventing a cancer.

[0015] In an aspect, provided is an antibody drug conjugate targeting CDCP1 comprising a targeting moiety targeting CDCP1, wherein the targeting moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

[0016] (1) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 19, CDR-H2 set forth in SEQ ID NO: 20, and CDR-H3 set forth in SEQ ID NO: 21,

[0017] and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 22, CDR-L2 set forth in SEQ ID NO: 23, and CDR-L3 set forth in SEQ ID NO: 24;

[0018] or

[0019] (2) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 29, CDR-H2 set forth in SEQ ID NO: 30, and CDR-H3 set forth in SEQ ID NO: 31,

[0020] and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 32, CDR-L2 set forth in SEQ ID NO: 33, and CDR-L3 set forth in SEQ ID NO: 34; and

[0021] the CDRs are defined according to IMGT numbering system.

[0022] In some embodiments, for the antibody drug conjugate,

[0023] (1) the heavy chain variable region (VH) comprises a sequence set forth in SEQ ID NO: 25 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 25, and the light chain variable region (VL) comprises a sequence set forth in SEQ ID NO: 26 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 26; or

[0024] (2) the heavy chain variable region (VH) comprises a sequence set forth in SEQ ID NO: 35 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 35, and the light chain variable region (VL) comprises a sequence set forth in SEQ ID NO: 36 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 36.

[0025] In some embodiments, the targeting moiety of the antibody drug conjugate is selected from the group consisting of an antibody and an antigen binding fragment thereof.

[0026] In some embodiments, for the antibody drug conjugate, the antibody or the antigen binding fragment thereof is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a multispecific antibody, Fab fragment, F(ab') fragment, $F(ab')_2$ fragment, Fv fragment, dAb, Fd and single-chain variable fragment (scFv).

[0027] In some embodiments, the targeting moiety of the antibody drug conjugate further comprises a constant region of an immunoglobulin, further preferably, the immunoglobulin is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4.

[0028] In some embodiments, the targeting moiety comprises a heavy chain and a light chain, wherein,

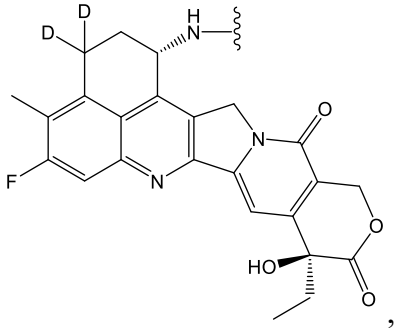
[0029] (1) the heavy chain comprises a sequence set forth in SEQ ID NO: 27 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 27, and the light chain comprises a sequence set forth in SEQ ID NO: 28 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 28; or

[0030] (2) the heavy chain comprises a sequence set forth in SEQ ID NO: 37 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 37, and the light chain comprises a sequence set forth in SEQ ID NO: 38 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 38.

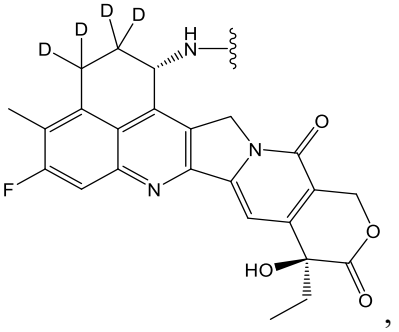
[0031] In some embodiments, the antibody drug conjugate has a structure represented by a general formula Ab-Un, wherein Ab represents a targeting moiety, U represents a linker-payload moiety, n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n represents the number of U linked to Ab, and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and Ab is covalently linked to the payload via the linker.

[0032] In some embodiments, Ab of the antibody drug conjugate is linked to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 payloads via one or more linkers.

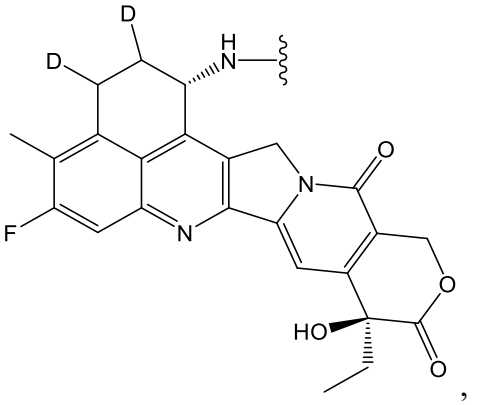
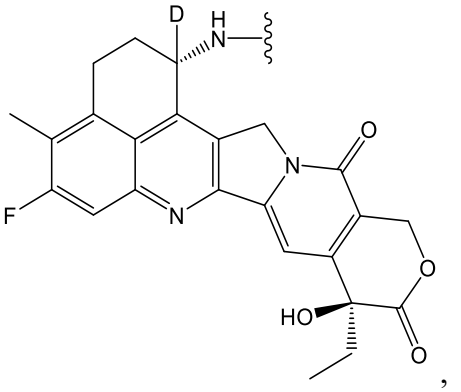
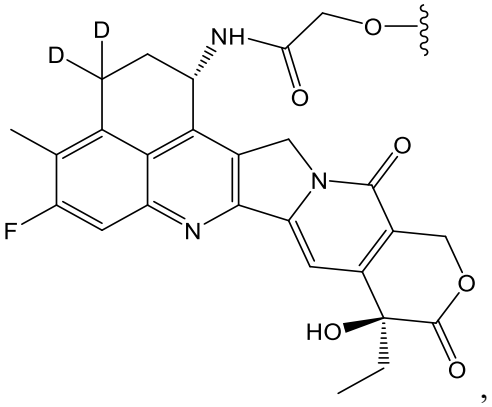
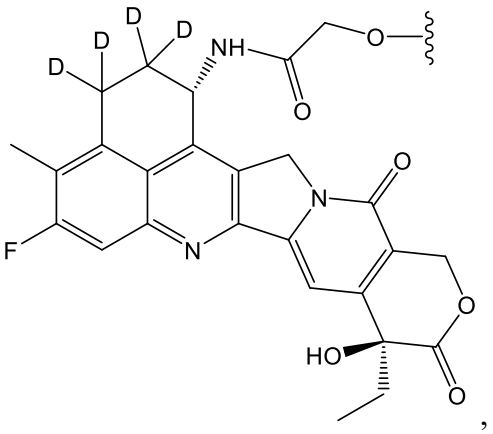
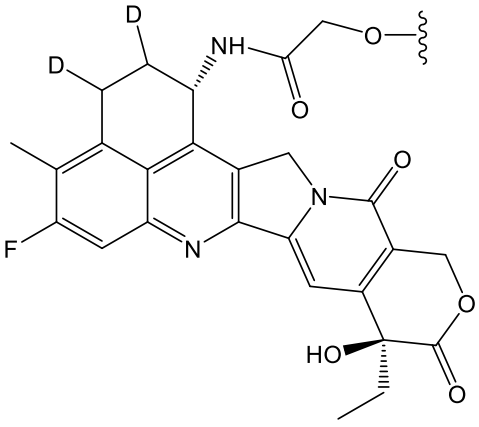
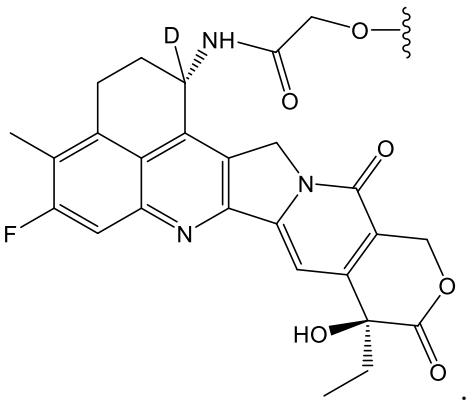
[0033] In some embodiments, for the antibody drug conjugate, the payload is selected from the group consisting of:



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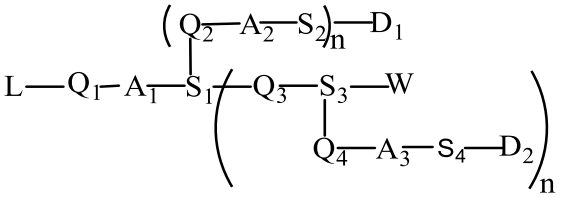


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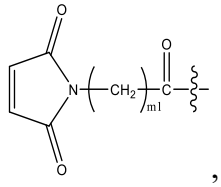
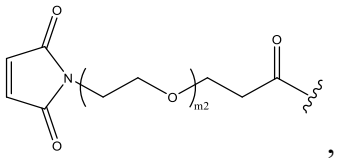
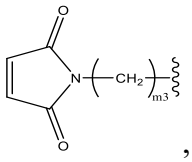
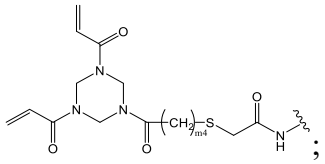
[0034] "〰" is a site where the payload and the linker are linked.

[0035] In some embodiments, for the antibody drug conjugate, the linker-payload moiety is represented by formula (I):



formula (I)

[0036] wherein,

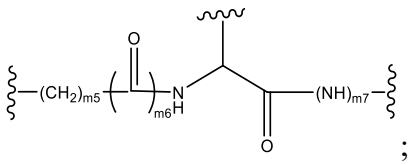
[0037] L is a linker linked to an antibody, and has a structure selected from the group consisting of , , , and ;

[0038] Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -(NHCH_2C(O))-O-, -NH(CH_2CH_2O)p-CH_2CH_2C(O)- , -(CH_2CH_2O)p-CH_2CH_2C(O)-, -CH_2-Y-(CH_2CH_2O)p-CH_2CH_2C(O)-, -NHCH_2-Y-(CH_2CH_2O)p-CH_2CH_2C(O)-, -(CH_2)-Y- and -NHCH_2C(O)CH_2C(O)-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{1-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

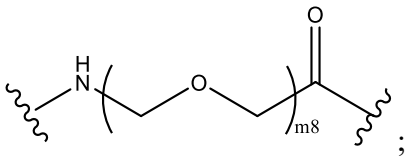
[0039] A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[0040] A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[0041] A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

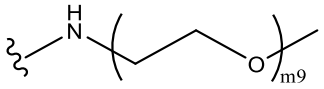
[0042] S_1 and S_3 are independently ;

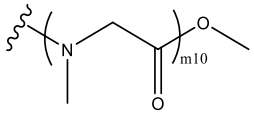
[0043] S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and



;

[0044] D_1 is a first drug unit , D2 is a second drug unit;

[0045] W is selected from the group consisting of 

and 

;

[0046] m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0047] m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0048] m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0049] m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0050] m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0051] m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0052] m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

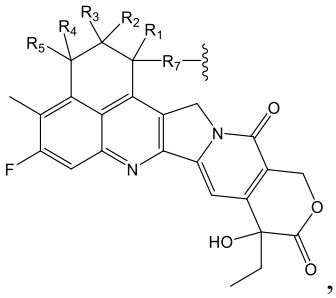
[0053] m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[0054] m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

[0055] m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

[0056] n is 0 or 1.

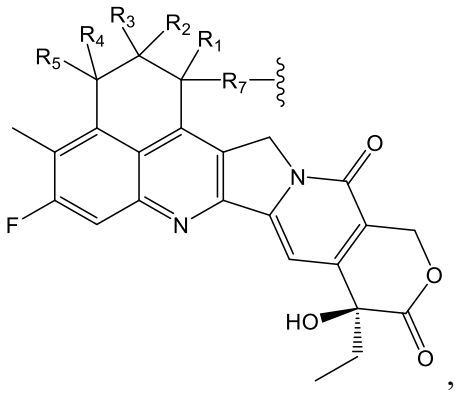
[0057] In some embodiments, the payload independently has a structure of



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[0058] "〰" is a site where the payload and the linker are linked; wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of H, D, and T, and at least one of R_1, R_2, R_3, R_4 and R_5 is D, R_7 is independently selected from the group consisting of -NH- and -NH-C(O)-CH_2-O-, and the drug unit is linked to the linker via either a hydroxyl or an amine group present.

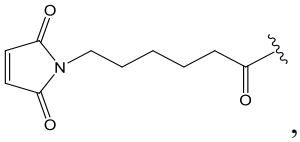
[0059] In some embodiments, the payload independently has a structure of

[0060] 

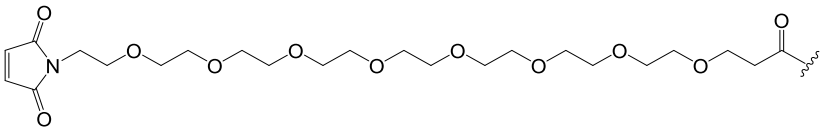
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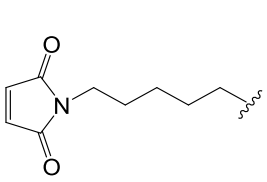
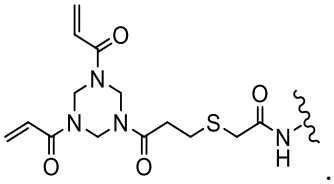
[0061] "〰" is a site where the payload and the linker are linked; wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H), deuterium (D), and tritium (T), and at least one, two, three, or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_7 is independently selected from the group consisting of -NH- and -NH-C(O)-CH_2-O-.

[0062] In some embodiments, L has a structure selected from the group consisting of:



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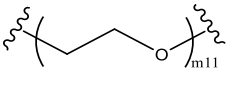

and 
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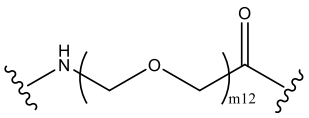
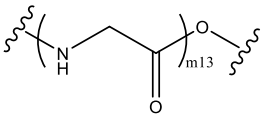
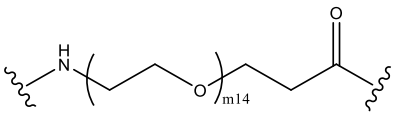
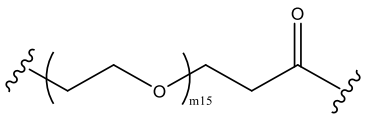
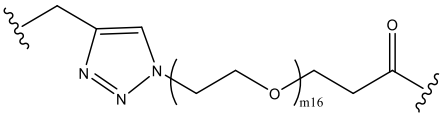
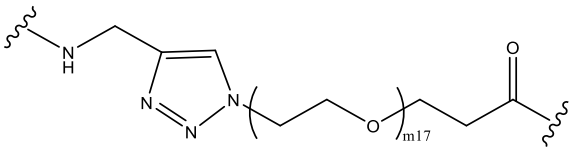
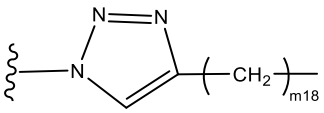
[0063] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[0064] Preferably, A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[0065] Preferably, A_1, A_2 and A_3 independently comprises a structure selected from the group consisting of:

[0066] a single bond, -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably the linker has a structure of -Val-Ala-.

[0067] In some embodiments, Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond, 
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and

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[0068] wherein,

[0069] m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[0070] m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[0071] m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

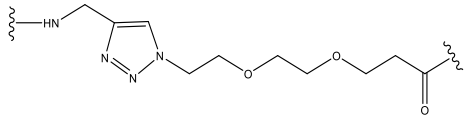
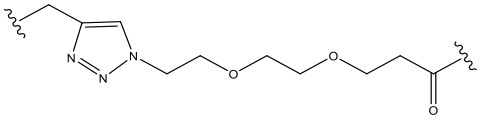
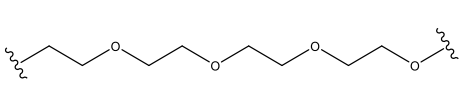
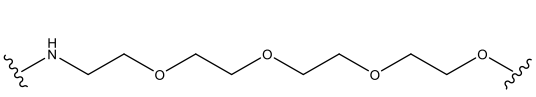
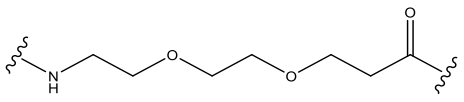
[0072] m14 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

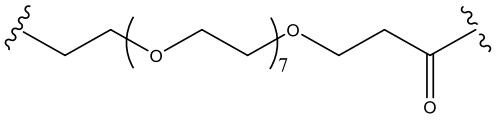
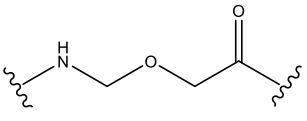
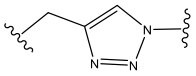
[0073] m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

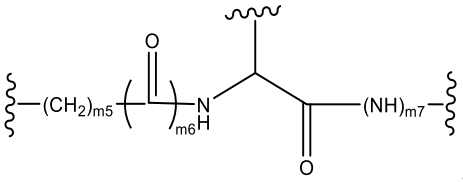
[0074] m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

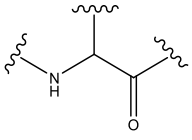
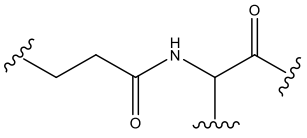
[0075] m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

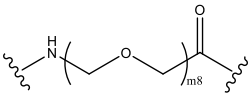
[0076] m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

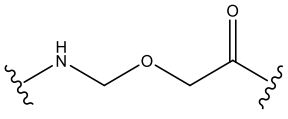
[0077] Preferably, Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

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,  and .

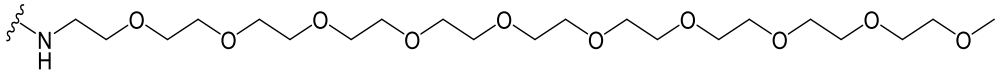
[0078] In some embodiments, S_1 and S_3 independently have a structure of: ; wherein m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

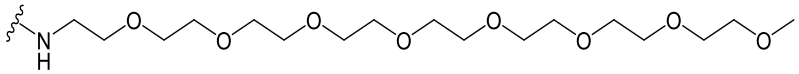
[0079] Preferably, S_1 and S_3 independently have a structure selected from the group consisting of  and .

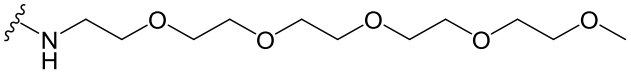
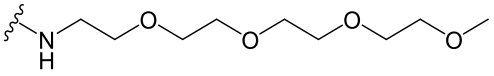
[0080] In some embodiments, S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and ; wherein m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

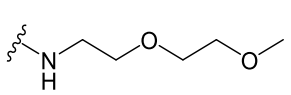
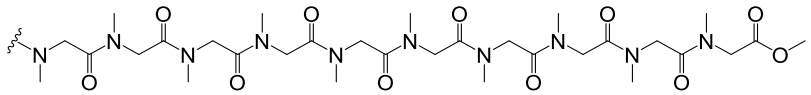
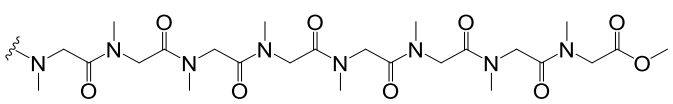
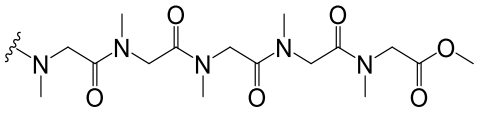
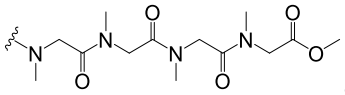
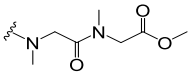
[0081] Preferably, S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and .

[0082] In some embodiments, W has a structure selected from the group consisting of:

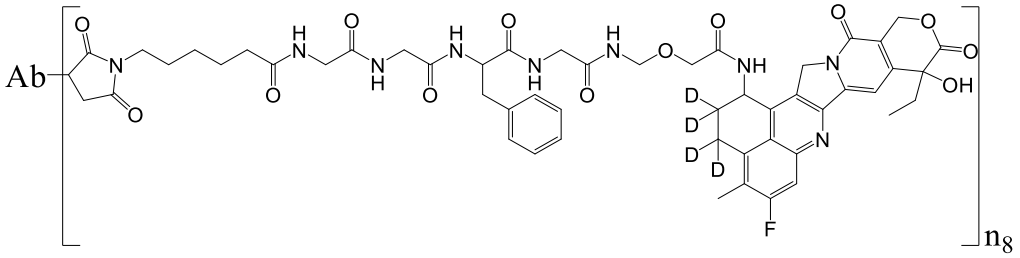
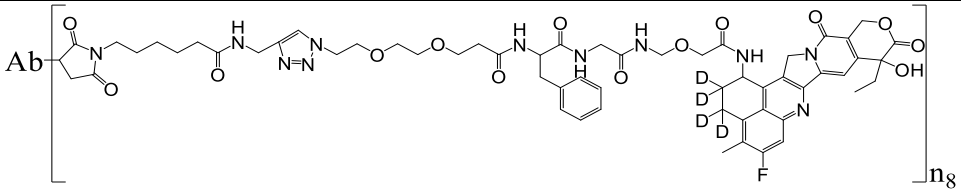
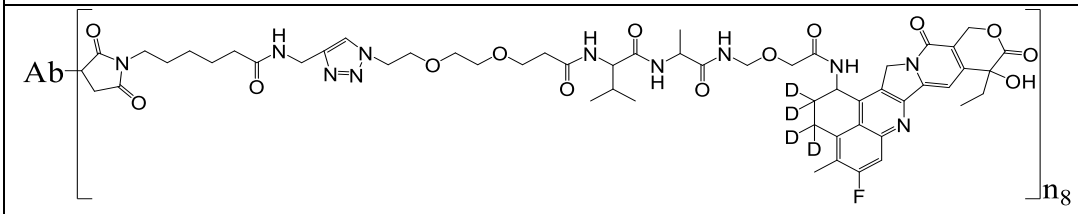
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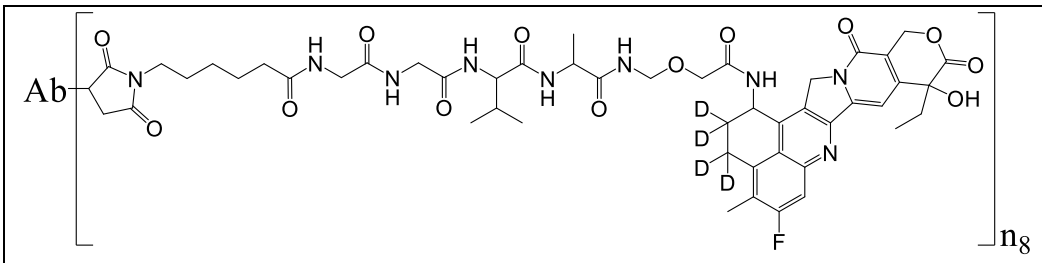
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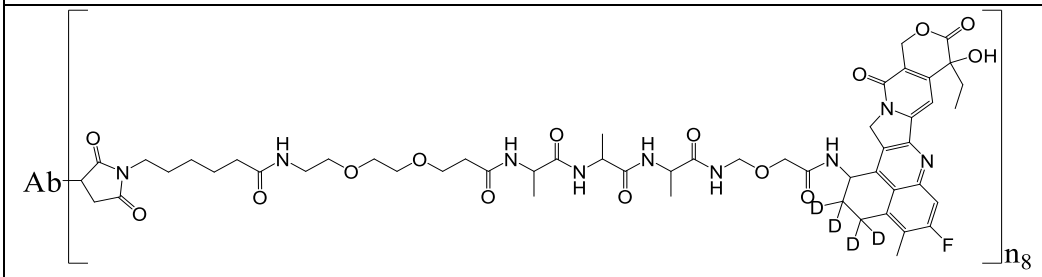

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[0083] In some embodiments, the antibody drug conjugate has a structure selected from the group consisting of:

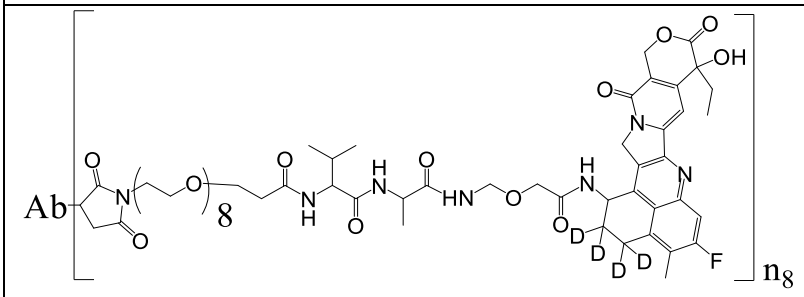
ADC-1

ADC-2

ADC-6

ADC-5



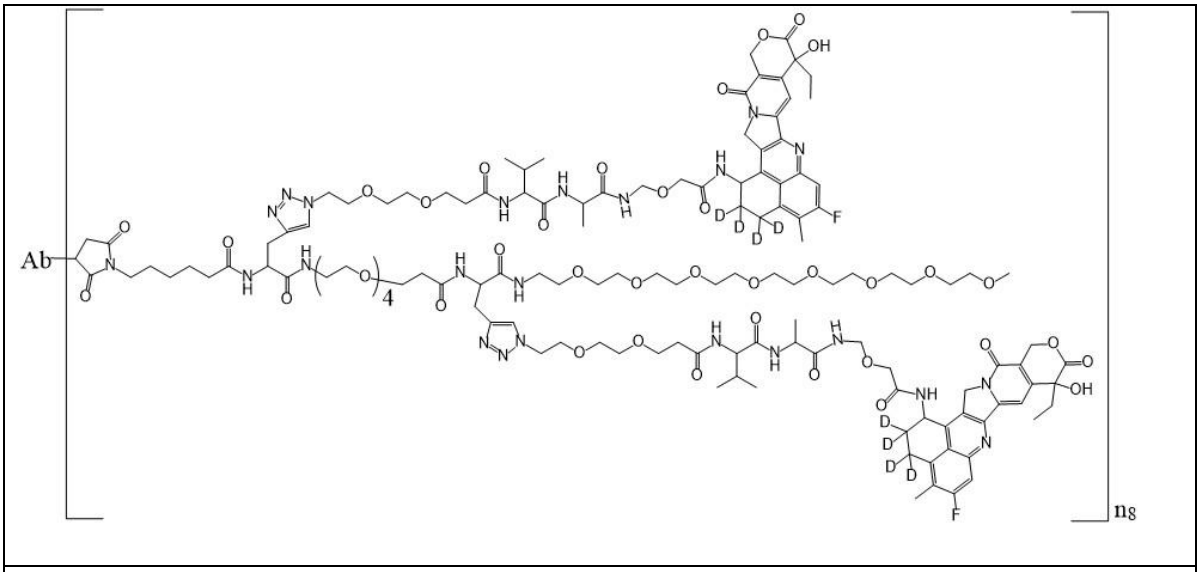
ADC-7



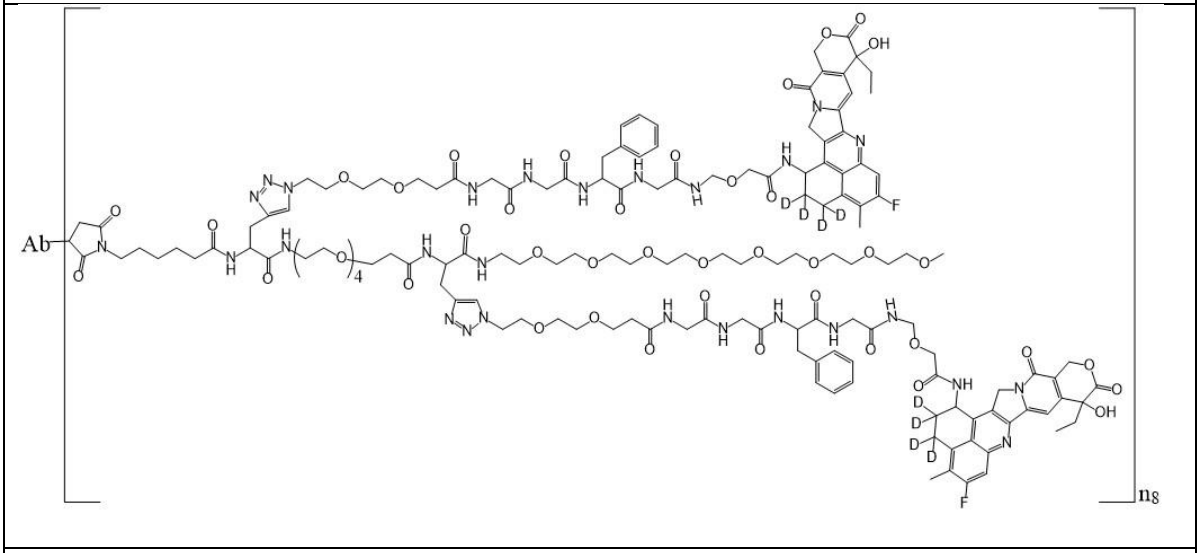
ADC-14



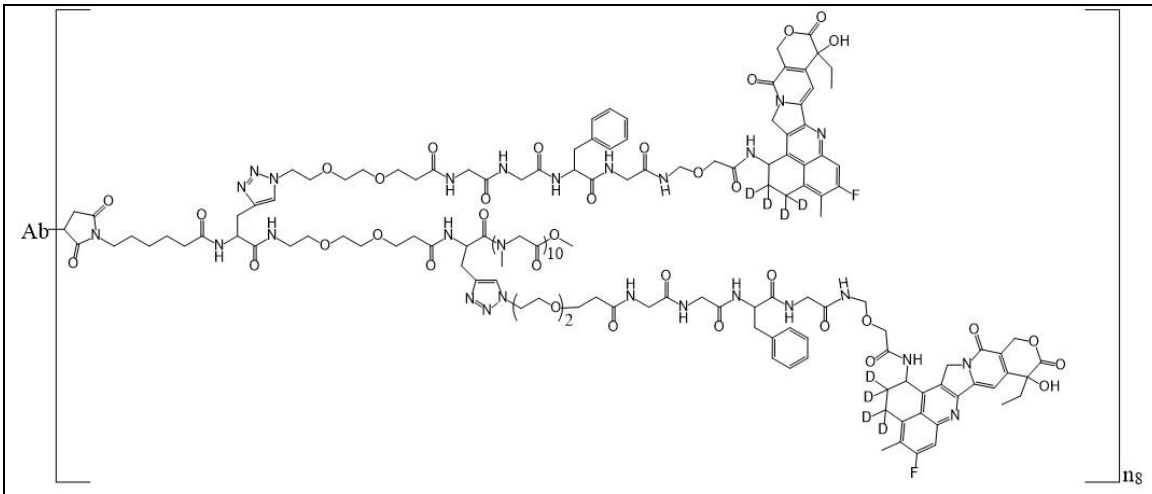
ADC-4



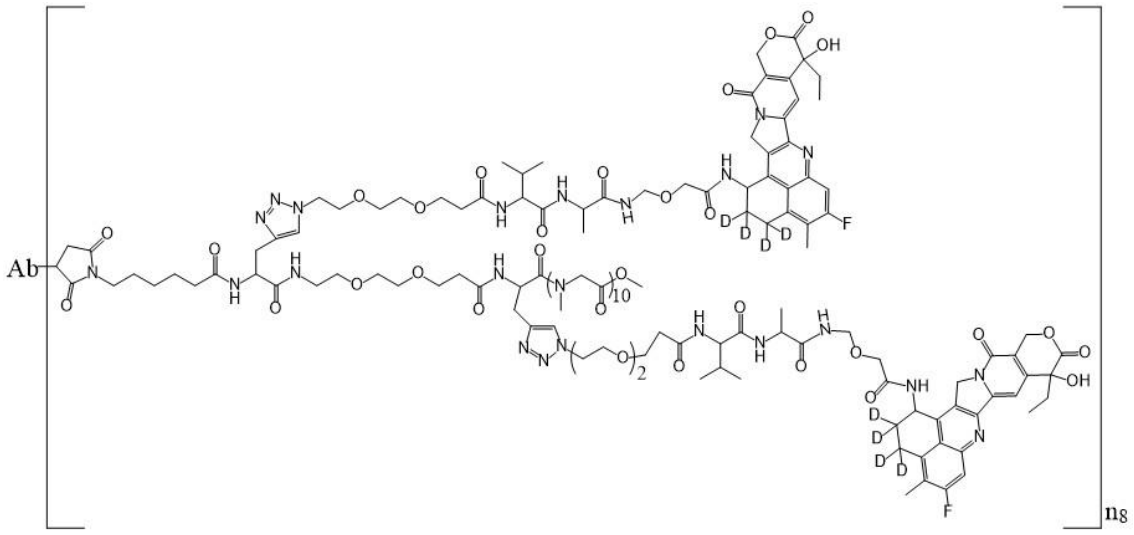
ADC-3



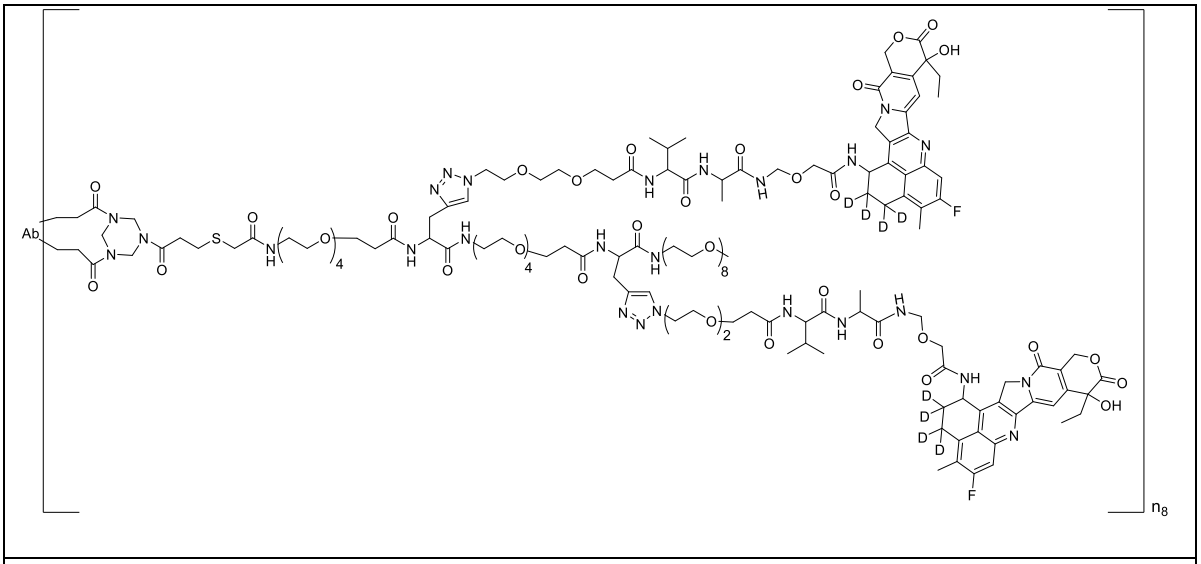
ADC-8



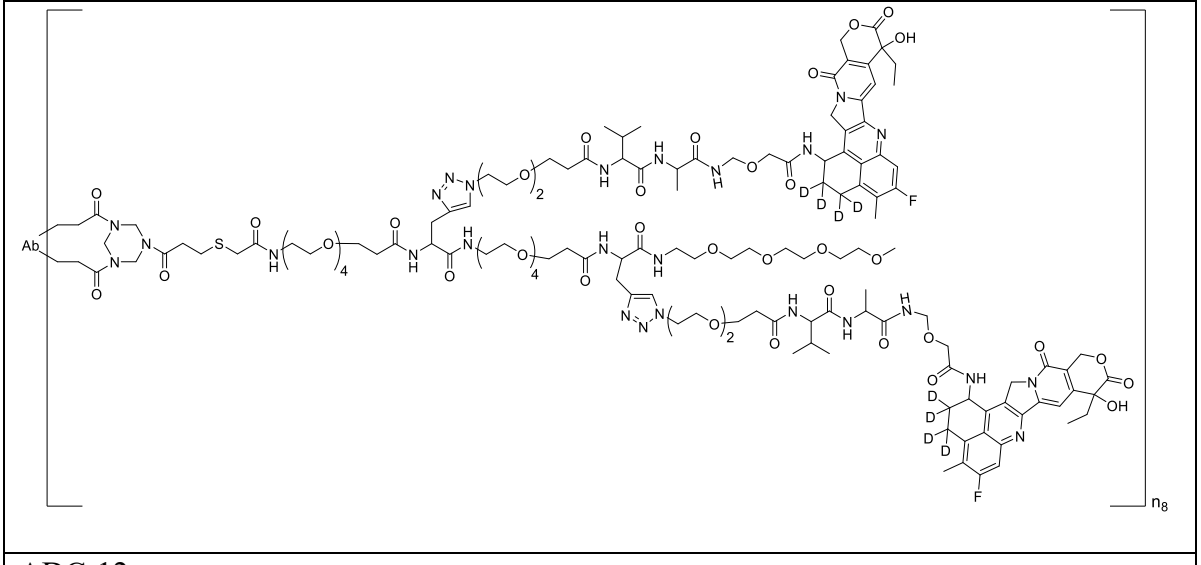
ADC-9



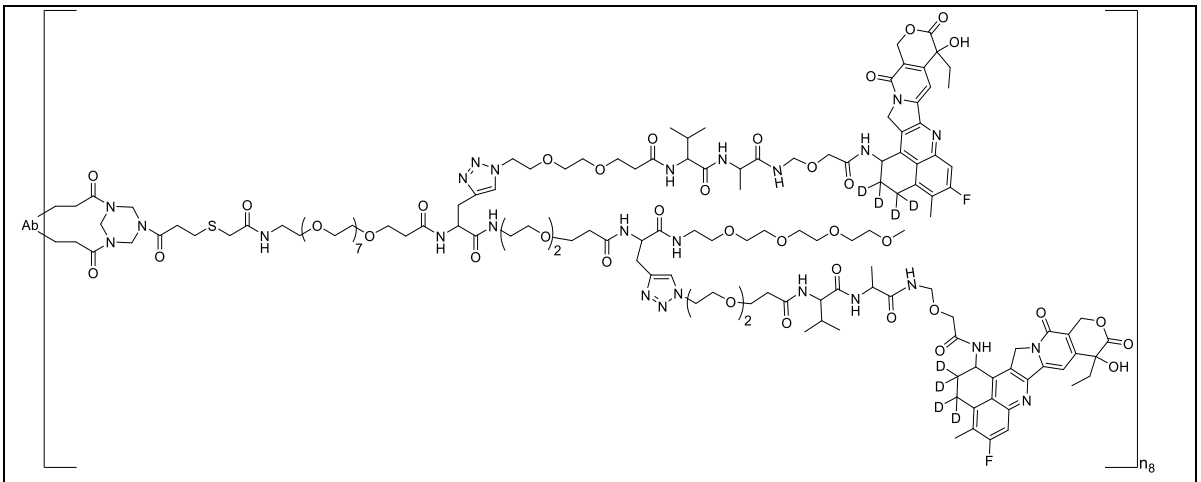
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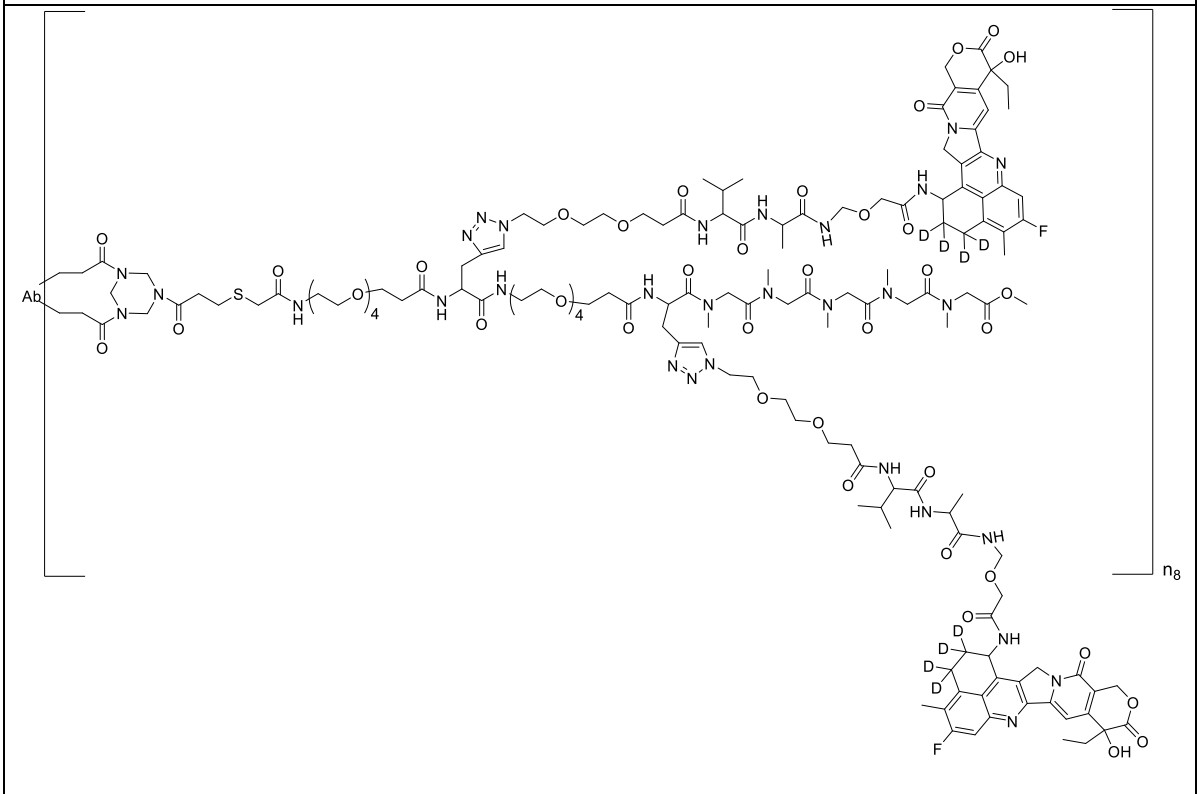
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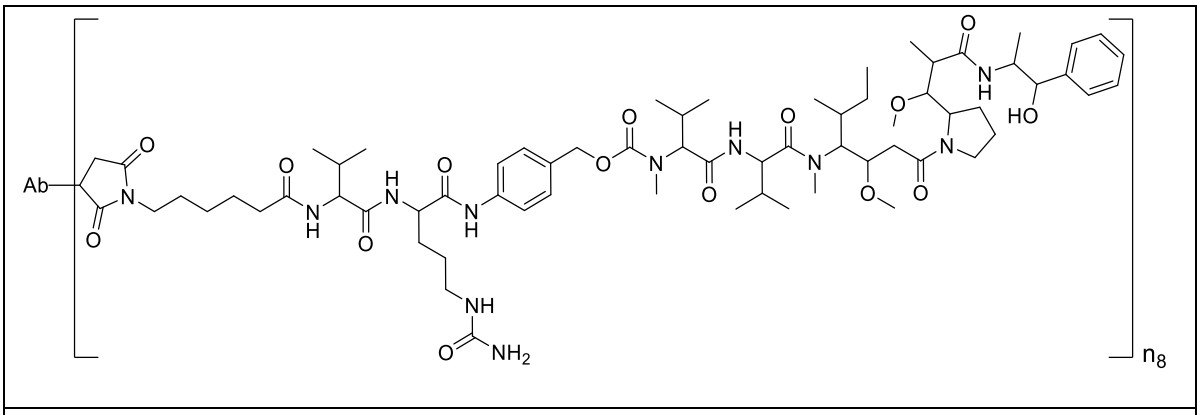
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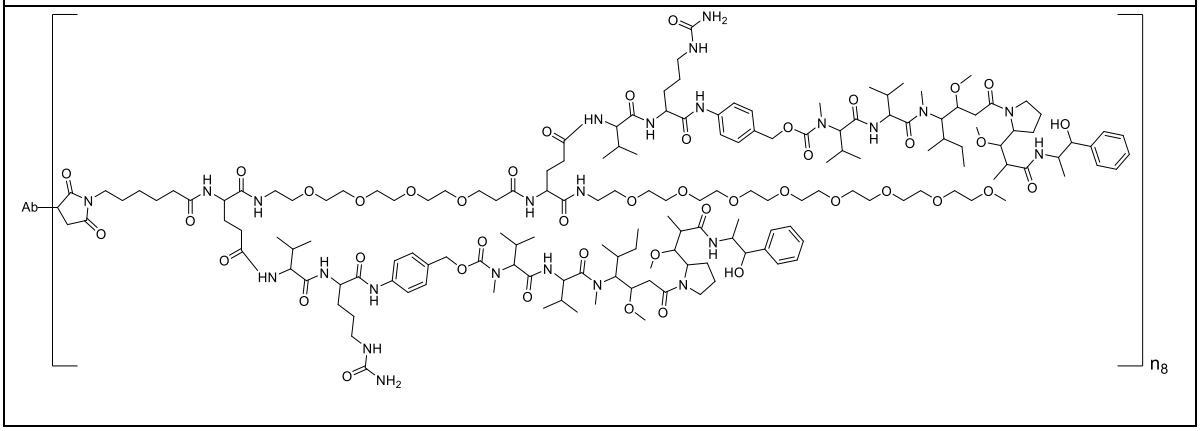
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ADC-15



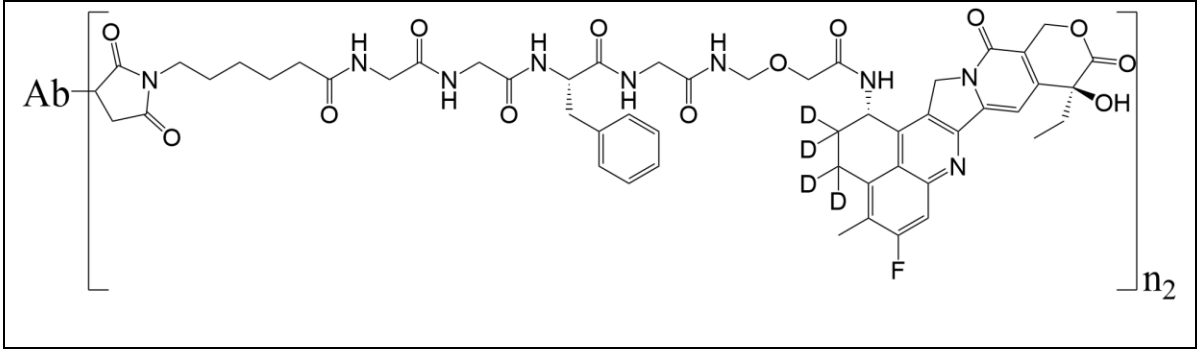
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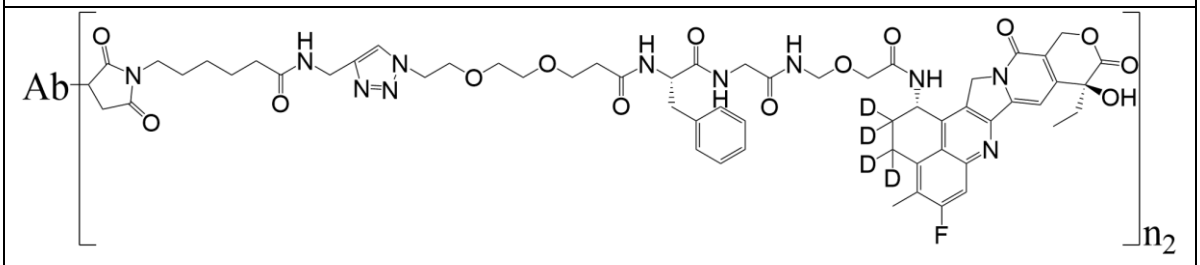
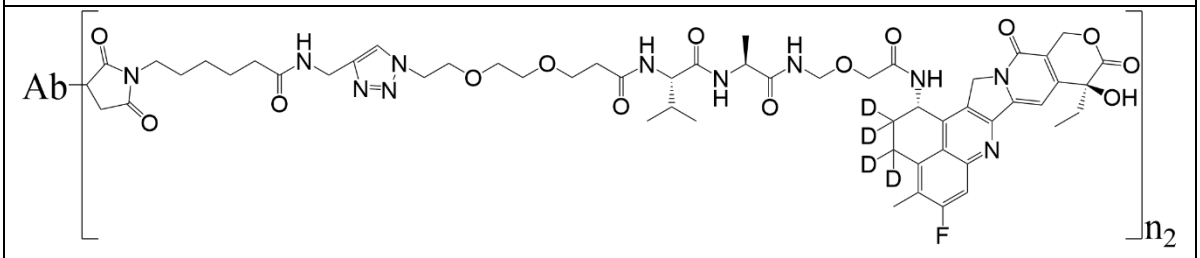
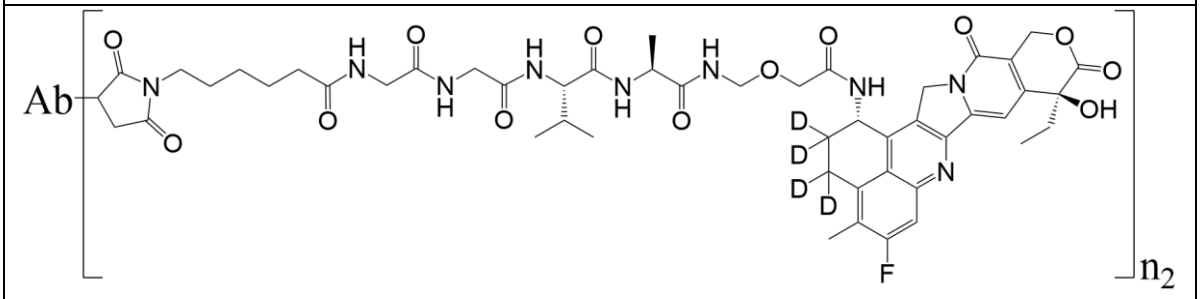
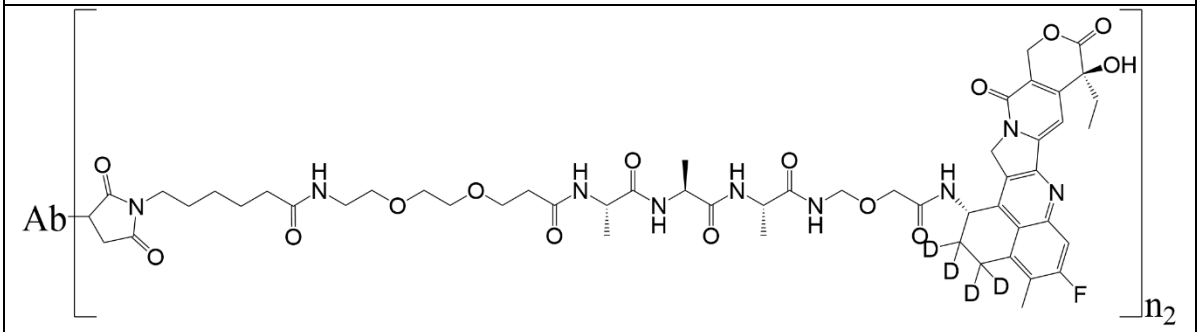


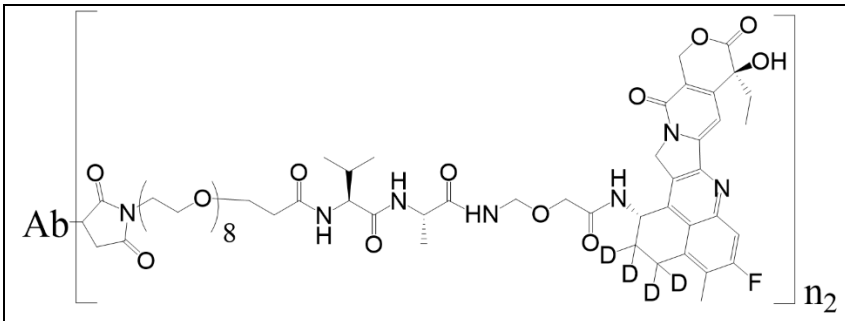
[0084] wherein n_8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_8 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and the antibody is linked to the linker-payload moiety via disulfide bond formed by cysteine of the antibody.

[0085] In some embodiments, the antibody drug conjugate has a structure selected from the group consisting of:

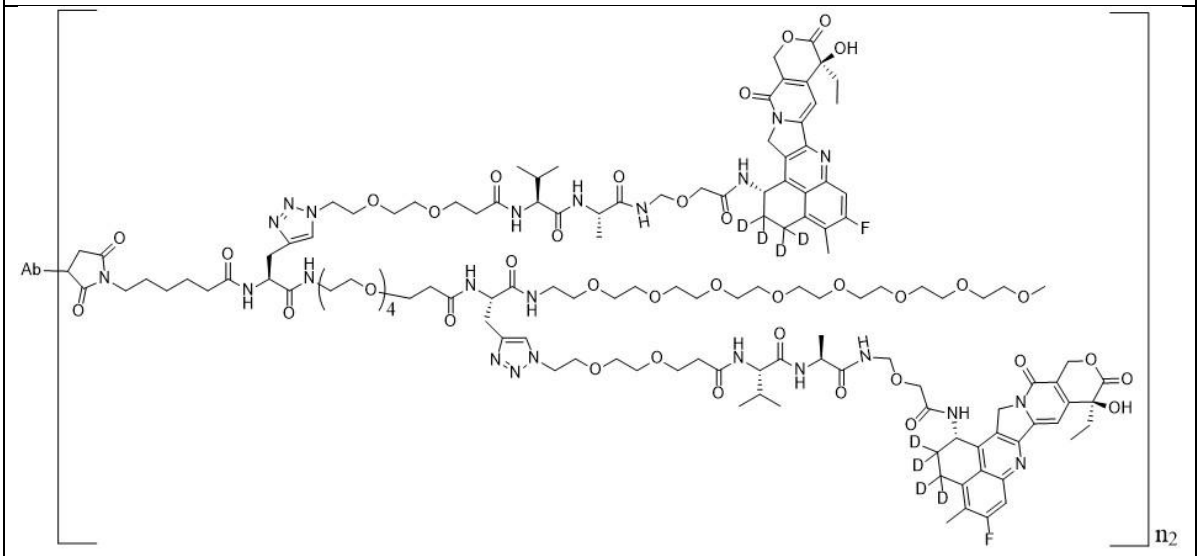
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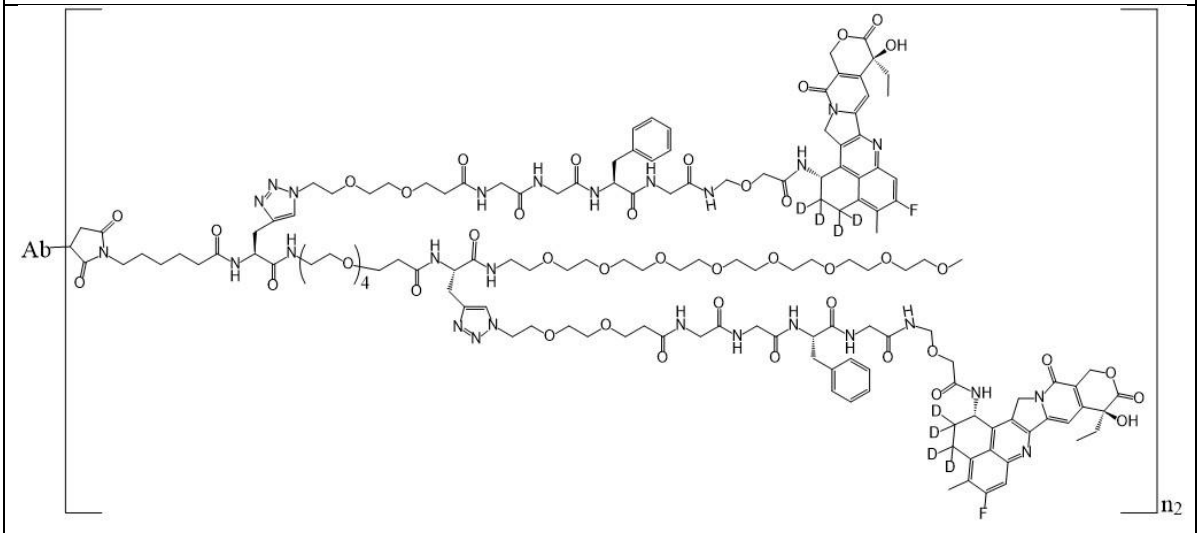
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ADC-6a

ADC-5a

ADC-7a

ADC-14a



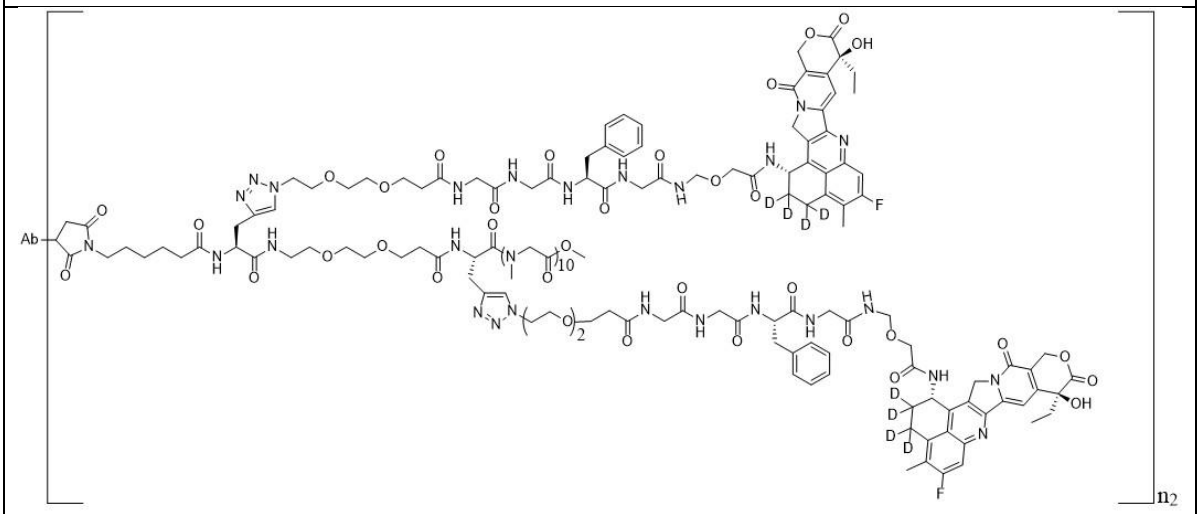
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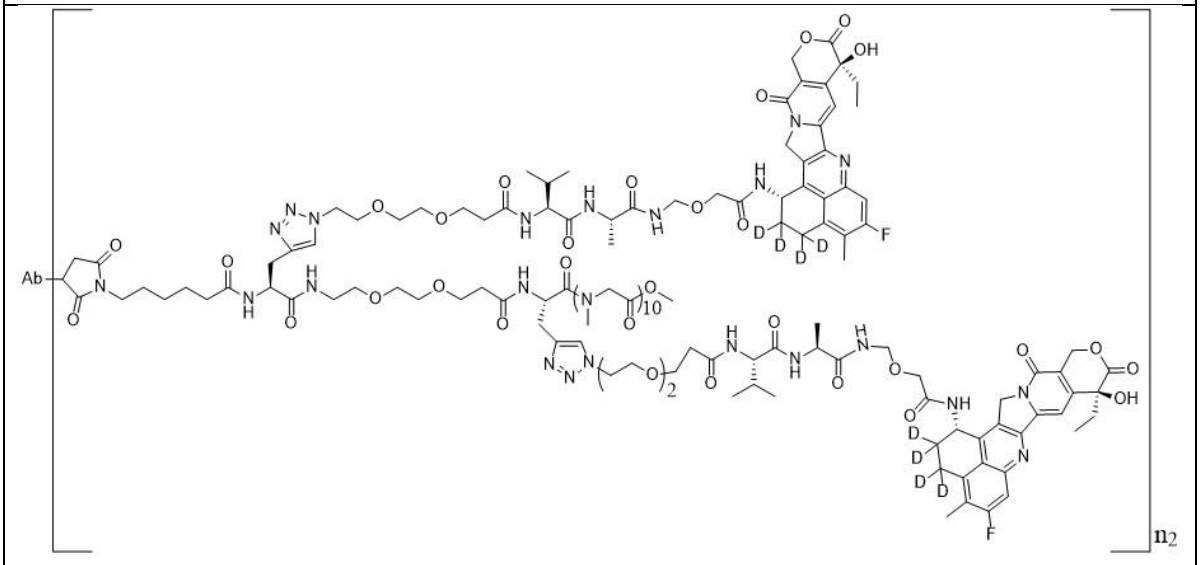
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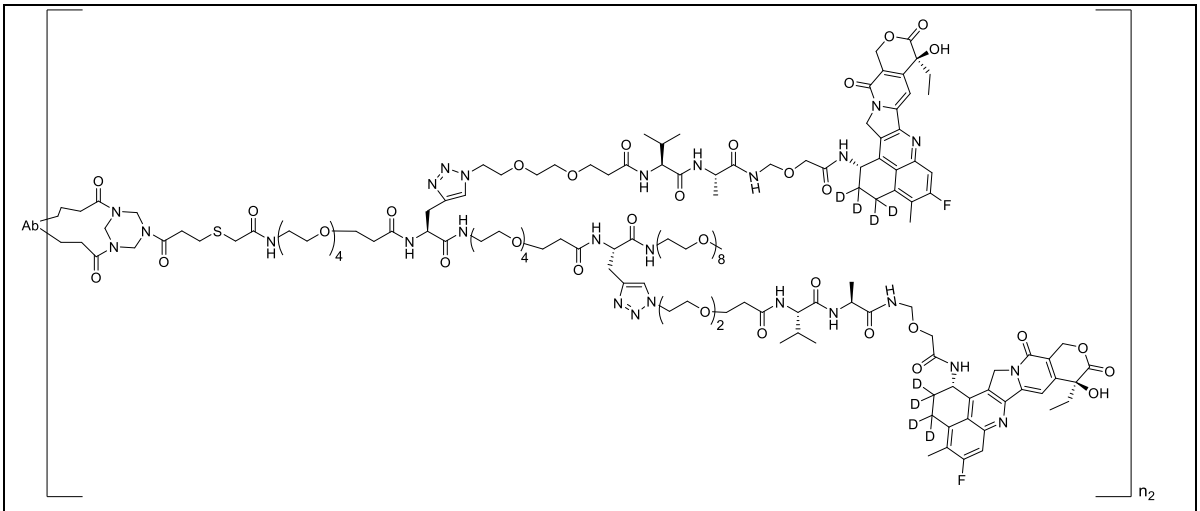
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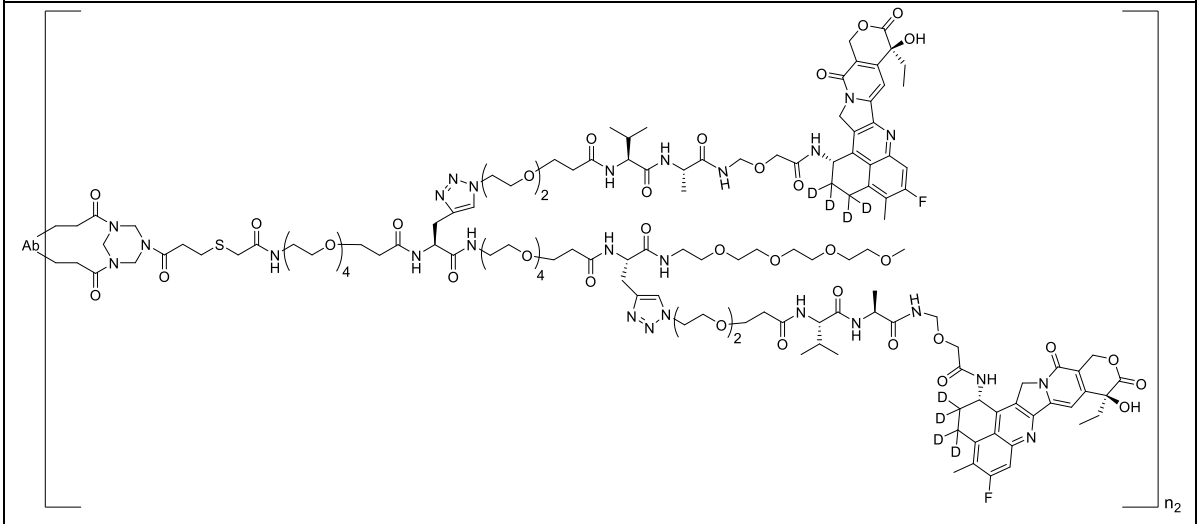
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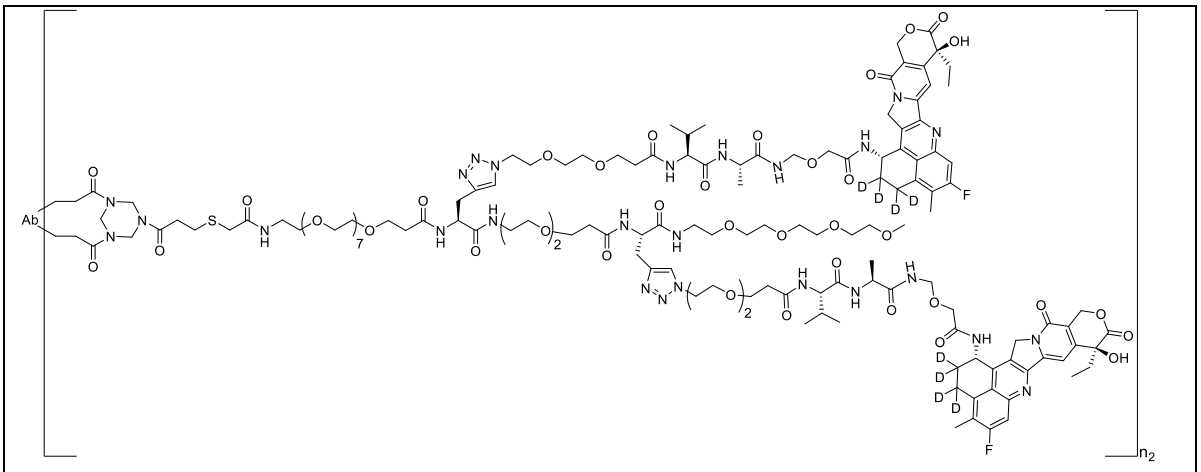
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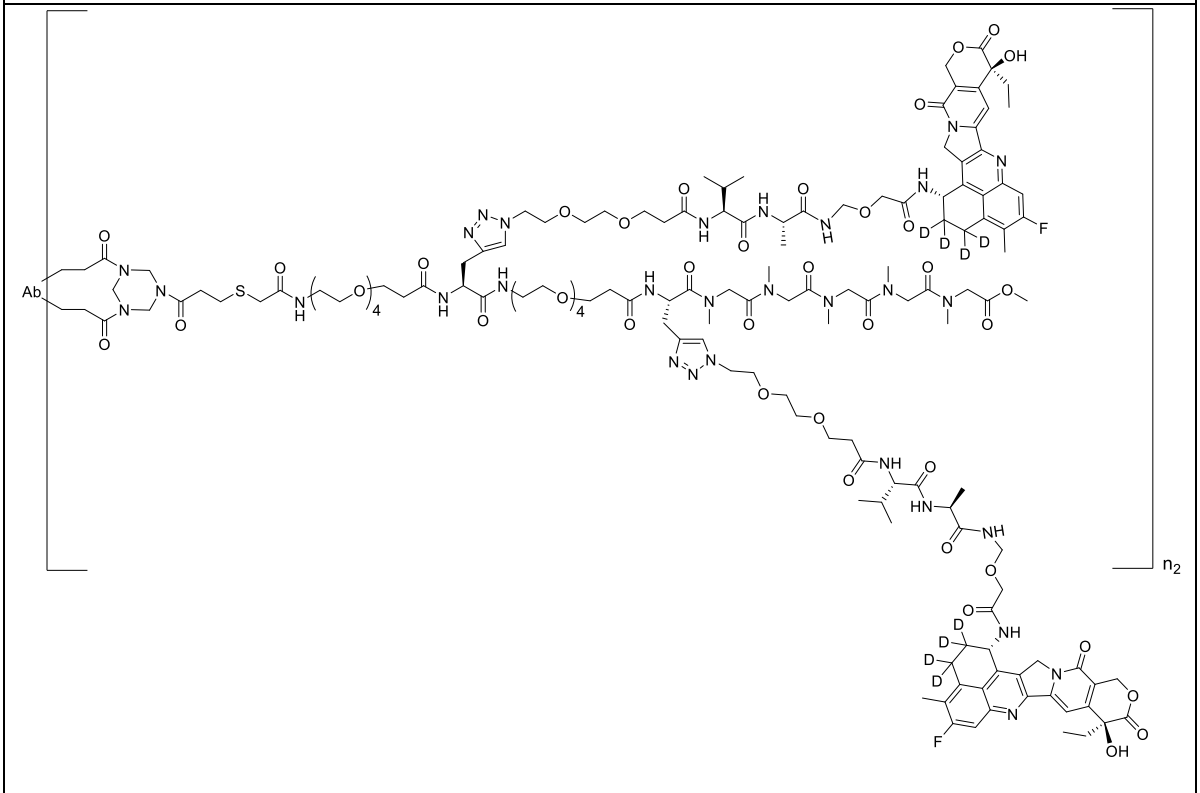
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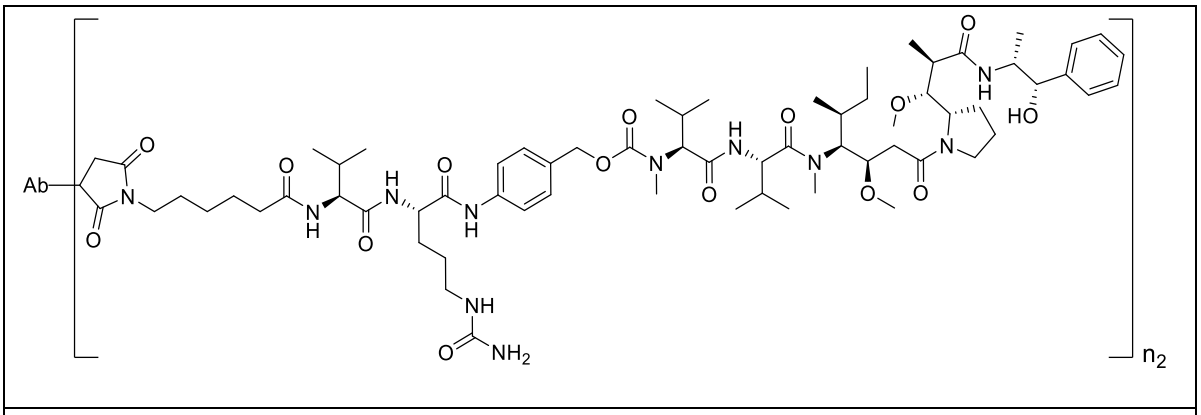
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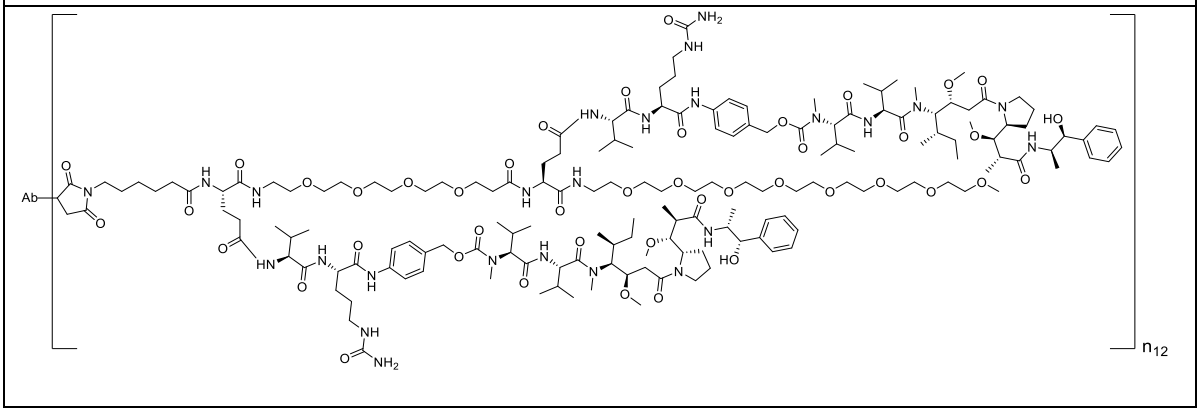
ADC-13a



ADC-15a



ADC-16a



[0086] wherein n_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_2 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and the antibody is linked to the linker-payload moiety via disulfide bond formed by cysteine of the antibody.

[0087] In an aspect, provided is a method for treating a tumor, comprising administering an effective amount of the antibody drug conjugate according to a subject in need thereof described in any one of the above.

[0088] In some embodiments, the tumor is CDCP-1 positive.

[0089] In some embodiments, the tumor is lung cancer or pancreatic cancer, further preferably lung squamous cell carcinoma, lung adenocarcinoma, non-small cell lung cancer or pancreatic adenocarcinoma.

[0090] In an aspect, provided is an antibody or an antigen binding fragment thereof targeting CDCP1, comprising a heavy chain variable region (VH) and a light chain variable region (VL),

wherein:

[0091] (1) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 19, CDR-H2 set forth in SEQ ID NO: 20, and CDR-H3 set forth in SEQ ID NO: 21, and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 22, CDR-L2 set forth in SEQ ID NO: 23, and CDR-L3 set forth in SEQ ID NO: 24; or

[0092] (2) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 29, CDR-H2 set forth in SEQ ID NO: 30, and CDR-H3 set forth in SEQ ID NO: 31, and

[0093] the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 32, CDR-L2 set forth in SEQ ID NO: 33, and CDR-L3 set forth in SEQ ID NO: 34; and

[0094] the CDRs are defined according to IMGT numbering system.

[0095] In some embodiments, the antibody or the antigen binding fragment thereof is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a multispecific antibody, a recombinant protein comprising the antigen binding fragment, Fab fragment, F(ab') fragment, $F(ab')_2$ fragment, Fv fragment, dAb, Fd and single-chain variable fragment (scFv).

[0096] In some embodiments, the antibody or the antigen binding fragment thereof further comprises a constant region of an immunoglobulin, wherein the immunoglobulin is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4.

[0097] In some embodiments, the antibody or the antigen binding fragment thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL are selected from the group consisting of (1) and (2):

[0098] (1) the heavy chain variable region has a sequence set forth in SEQ ID NO: 25 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 25 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 25, and the light chain variable region has a sequence set forth in SEQ ID NO: 26 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 26 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 26;

[0099] (2) the heavy chain variable region has a sequence set forth in SEQ ID NO: 35 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 35 and having greater than 80%, 85%,

90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 35, and the light chain variable region has a sequence set forth in SEQ ID NO: 36 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 36 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 36.

[00100] In some embodiments, the antibody or the antigen binding fragment thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL are selected from the group consisting of (1) and (2):

[00101] (1) the heavy chain variable region has a sequence set forth in SEQ ID NO: 25, and the light chain variable region has a sequence set forth in SEQ ID NO: 26;

[00102] and

[00103] (2) the heavy chain variable region has a sequence set forth in SEQ ID NO: 35, and

[00104] the light chain variable region has a sequence set forth in SEQ ID NO: 36;

[00105] In some embodiments, the antibody or the antigen binding fragment thereof comprises a heavy chain and a light chain, wherein the VH and VL are selected from the group consisting of (1) and (2):

[00106] (1) the heavy chain comprises a sequence set forth in SEQ ID NO: 27 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 27, and

[00107] the light chain comprises a sequence set forth in SEQ ID NO: 28 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 28;

[00108] and

[00109] (2) the heavy chain comprises a sequence set forth in SEQ ID NO: 37 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 37, and

[00110] the light chain comprises a sequence set forth in SEQ ID NO: 38 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 38.

[00111] In some embodiments, the antibody or the antigen binding fragment thereof comprises a

heavy chain and a light chain, wherein the VH and VL are selected from the group consisting of (1) and (2):

[00112] (1) the heavy chain has an amino acid sequence set forth in SEQ ID NO: 27, and the light chain has an amino acid sequence set forth in SEQ ID NO: 28,

[00113] (2) the heavy chain has an amino acid sequence set forth in SEQ ID NO: 37, and the light chain has an amino acid sequence set forth in SEQ ID NO: 38.

[00114] In an aspect, provided is a fusion protein comprising the antibody or the antigen binding fragment thereof described in any one of the above.

[00115] In an aspect, provided is an isolated polynucleotide encoding the antibody or the antigen-binding fragment thereof or the fusion protein described in any one of the above.

[00116] In an aspect, provided is a nucleic acid construct comprising the polynucleotide described above.

[00117] In some embodiments, the nucleic acid construct is an expression vector, wherein the polynucleotide is operably linked to a regulatory sequence allowing expression of peptide encoded by the polypeptide in a host cell or a cell-free expression system.

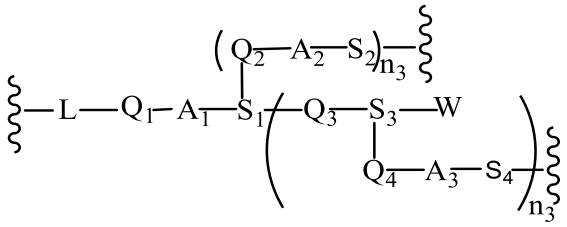
[00118] In an aspect, provided is a host cell comprising the polynucleotide or the nucleic acid construct described above, wherein the host cell is preferably selected from a group consisting of a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell and an *Escherichia coli* cell, more preferably the host cell is selected from a group consisting of a CHO cell, a NS0 cell, a Sp2/0 cell and a BHK cell.

[00119] In an aspect, provided is a method for producing the antibody or the antigen-binding fragment thereof described above, or the fusion protein, comprising culturing the host cell described above under a condition allowing the nucleic acid construct described above to express a protein, and harvesting the protein from a culture.

[00120] In an aspect, provided is use of the antibody or the antigen binding fragment thereof, the fusion protein, or the polynucleotide described above in the manufacture of a medicament.

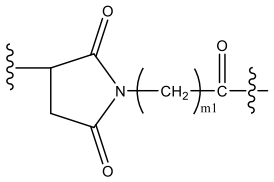
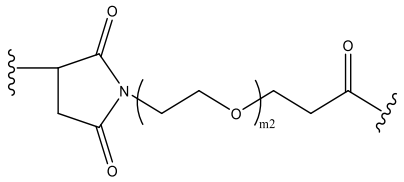
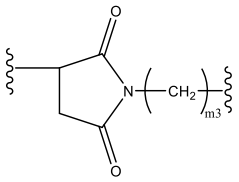
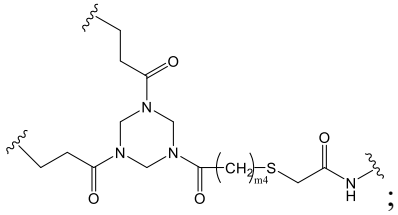
[00121] Preferably, the medicament includes a monospecific antibody, a bispecific antibody, a multispecific antibody, an antibody drug conjugate or an antibody-radionuclide conjugate.

[00122] In an aspect, provided is a linker represented by formula (II):



formula (II)

[00123] wherein, L is a linker linked to an antibody, and has a structure selected from the group consisting of:

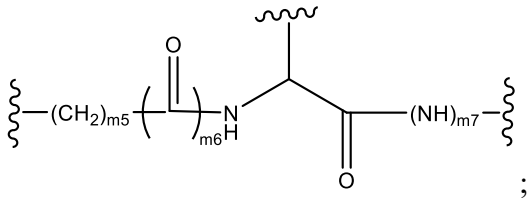

, 
, 
and 
;

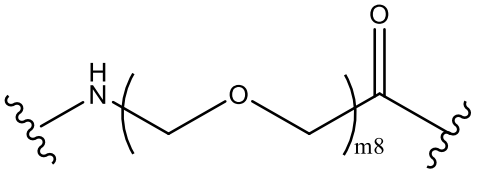
[00124] wherein, Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -($NHCH_2C(O)$)p-O-, -NH(CH_2CH_2O)p-$CH_2CH_2C(O)$- , -(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -CH_2-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -$NHCH_2$-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -(CH_2)-Y- and -$NHCH_2C(O)CH_2C(O)$-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{3-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

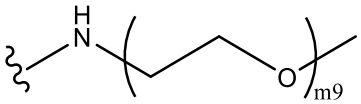
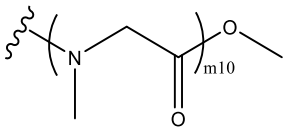
[00125] A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[00126] A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[00127] A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[00128] S_1 and S_3 are independently ;

[00129] S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and ;

[00130] W is selected from the group consisting of  and ;

[00131] m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00132] m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00133] m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00134] m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00135] m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00136] m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00137] m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

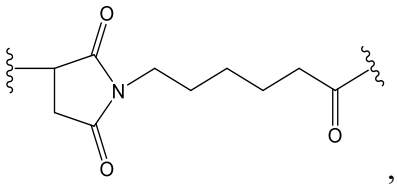
[00138] m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00139] m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

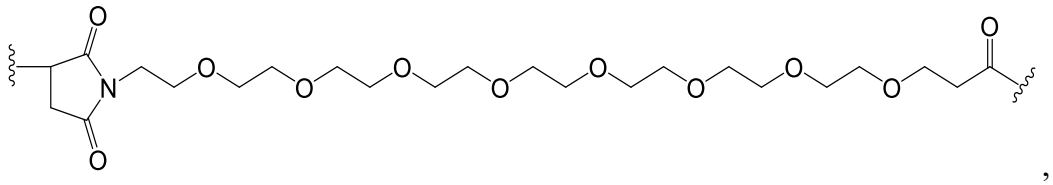
[00140] m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

[00141] n_3 is 0 or 1.

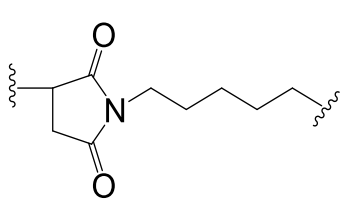
[00142] In some embodiments, L has a structure selected from the group consisting of:



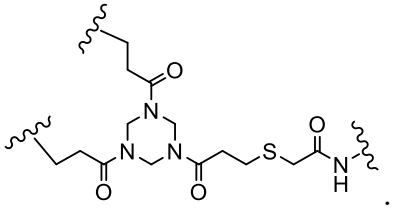
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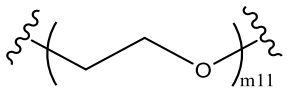
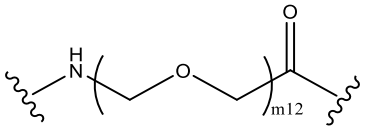
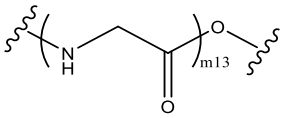
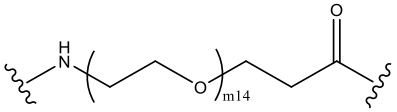
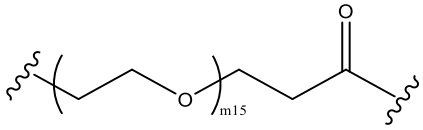
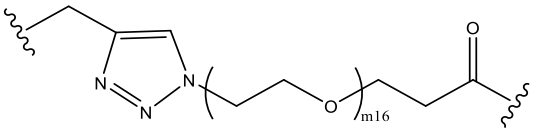
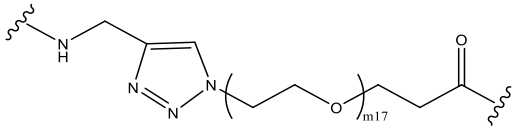
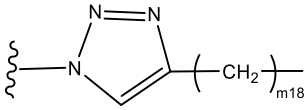
[00143] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[00144] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[00145] In some embodiments, A_1, A_2 and A_3 independently comprises a structure selected from the group consisting of:

[00146] a single bond, -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -

Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably the linker has a structure of -Val-Ala-.

[00147] In some embodiments, Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond, , , , , , , , and ; wherein

[00148] m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00149] m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00150] m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00151] m14 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

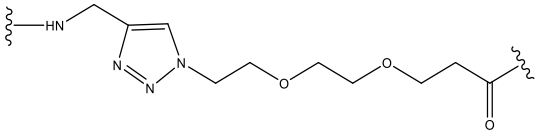
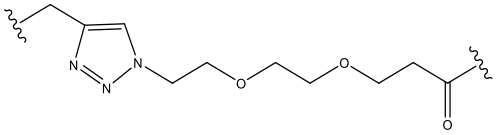
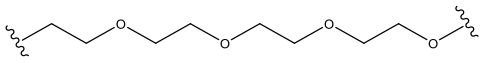
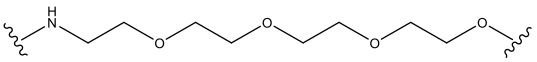
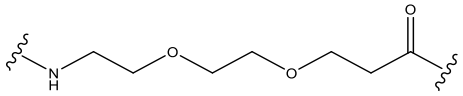
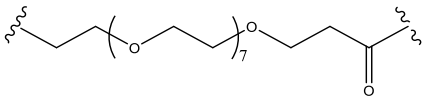
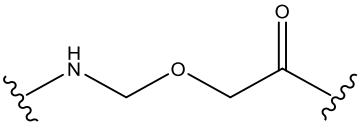
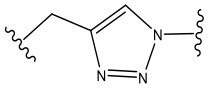
[00152] m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00153] m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

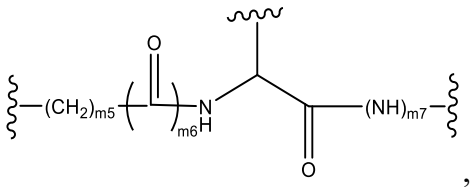
[00154] m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

[00155] m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

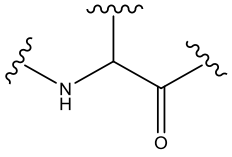
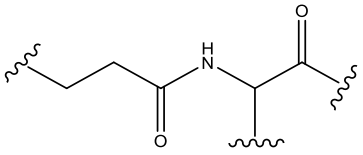
[00156] In some embodiments, Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

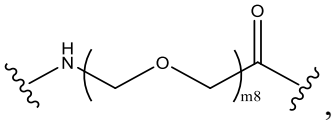

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and

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[00157] In some embodiments, S_1 and S_3 independently have a structure of


, wherein,

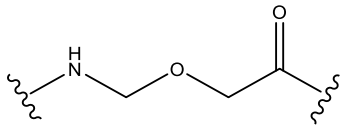
[00158] m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[00159] In some embodiments, S_1 and S_3 independently have a structure selected from the group consisting of

and

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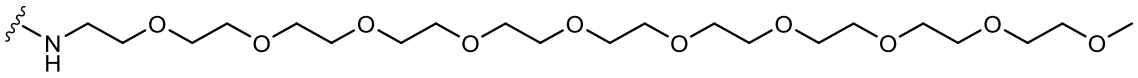
[00160] In some embodiments, S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and

, wherein,

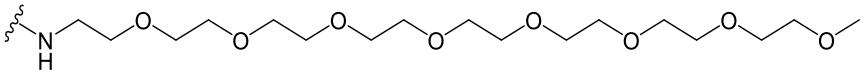
[00161] m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

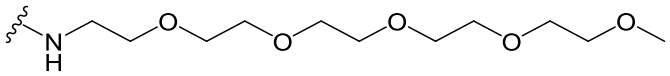
[00162] In some embodiments, S_2 and S_4 independently have a structure selected from the group

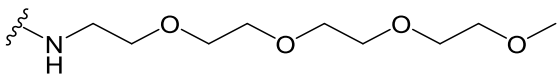
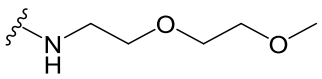
consisting of -NH-CH_2- and .

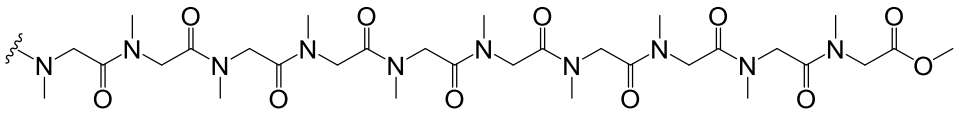
[00163] In some embodiments, W has a structure selected from the group consisting of:

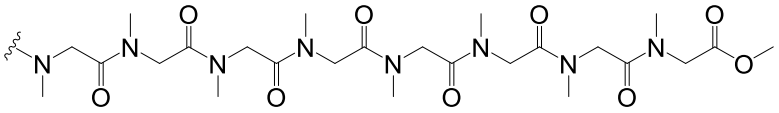
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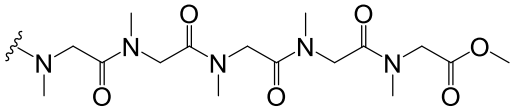
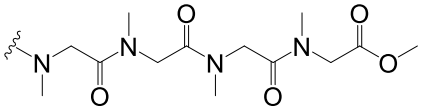
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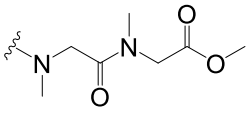
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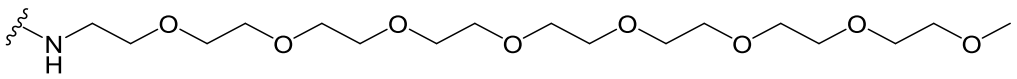
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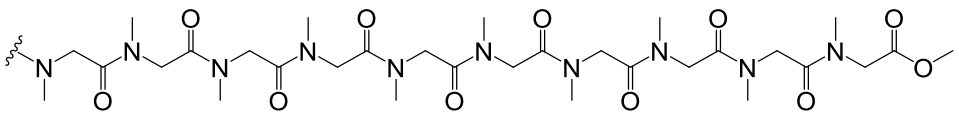
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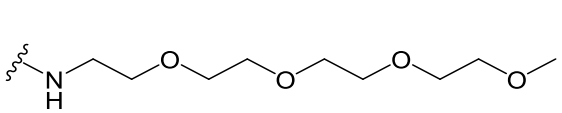
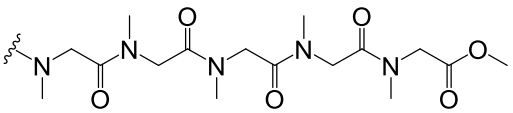
,  and

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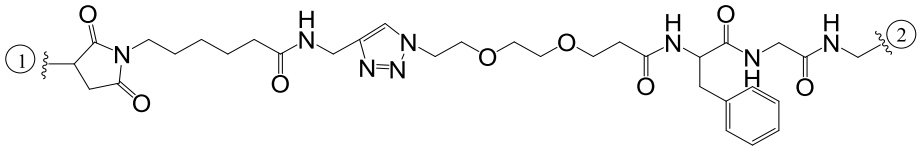
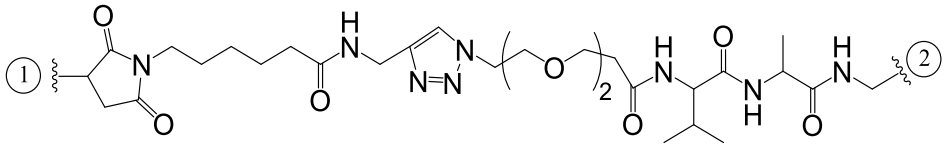
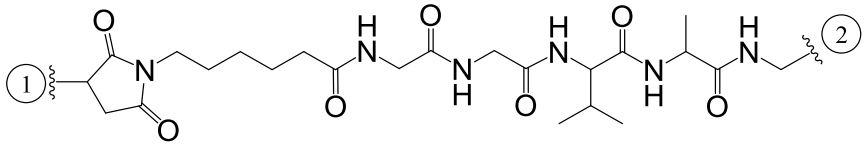
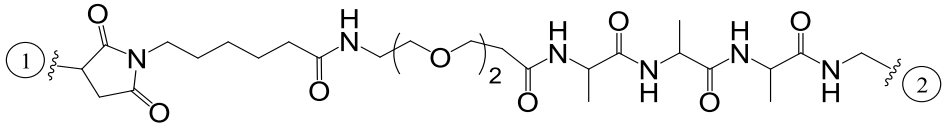
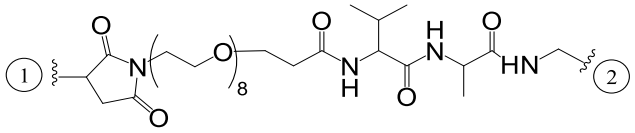
[00164] In some embodiments, W has a structure selected from the group consisting of:

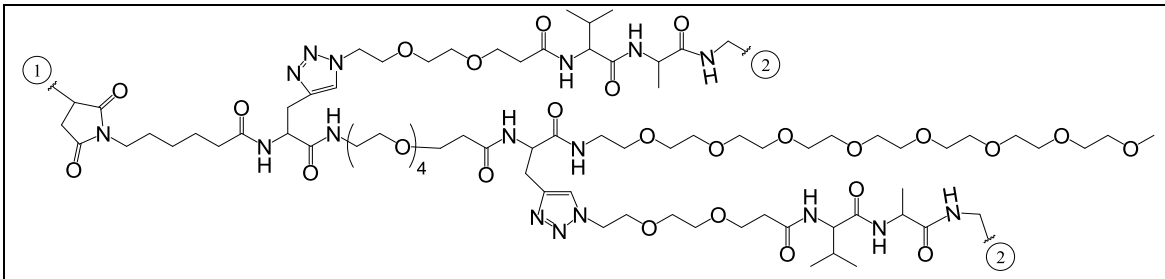
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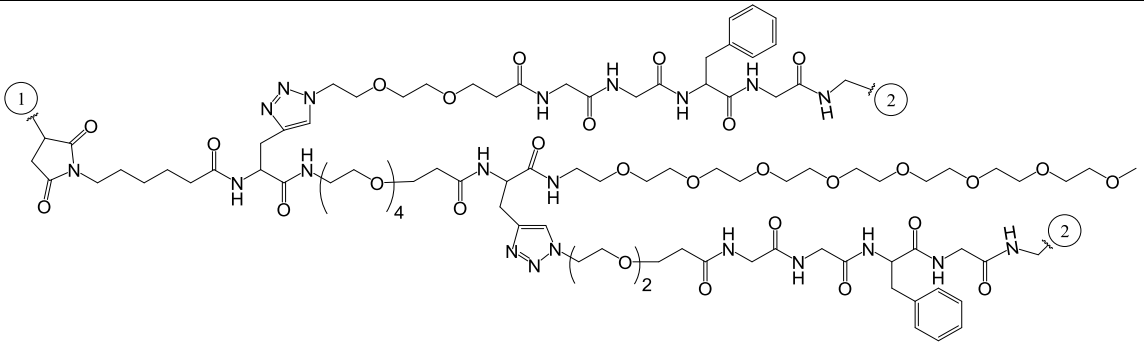
 and .

[00165] In some embodiments, the linker has a structure selected from the group consisting of:

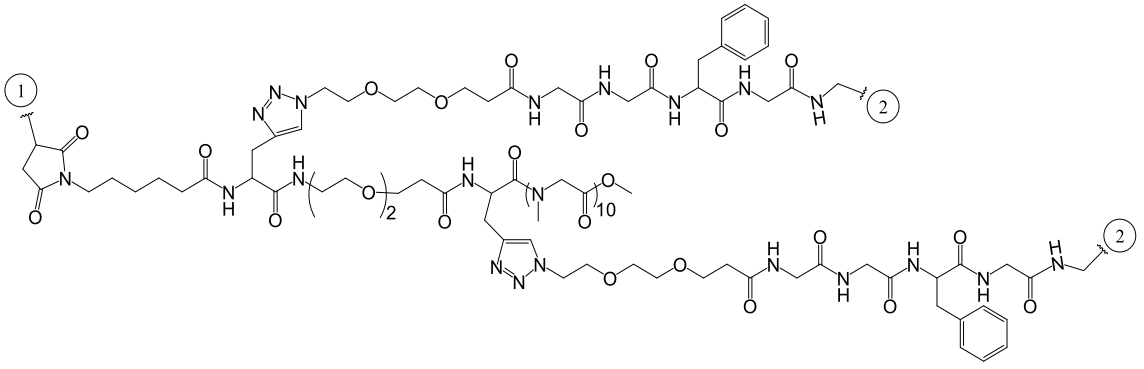
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DL-06

DL-05

DL-07

DL-14

DL-04



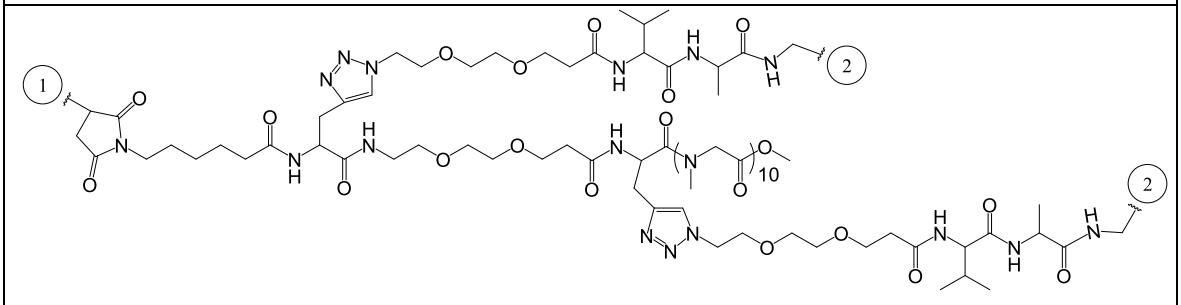
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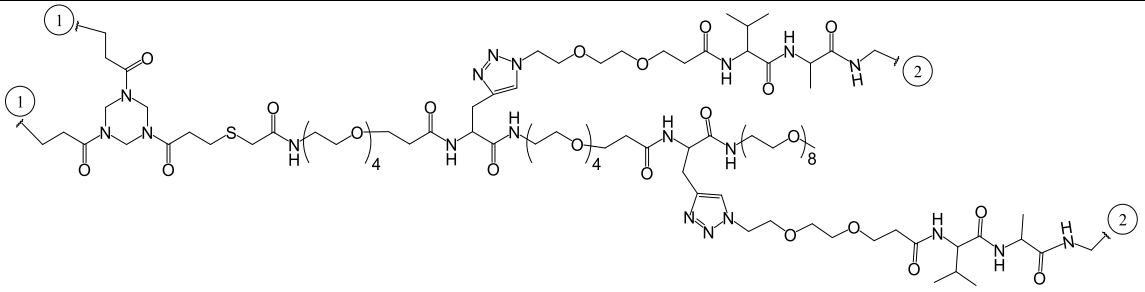
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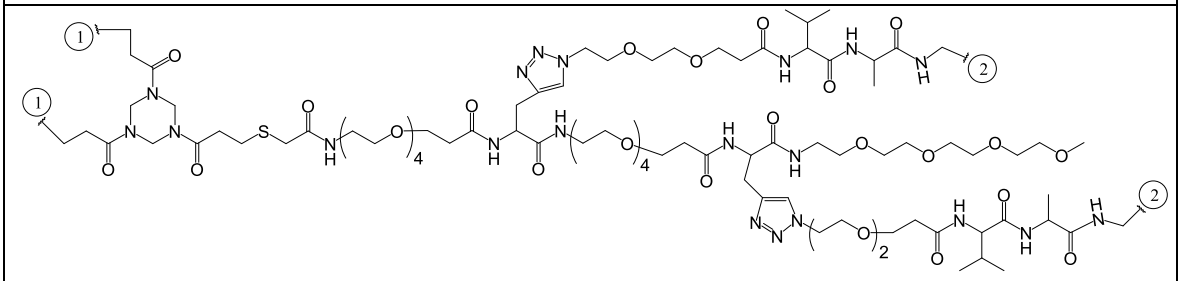
DL-09



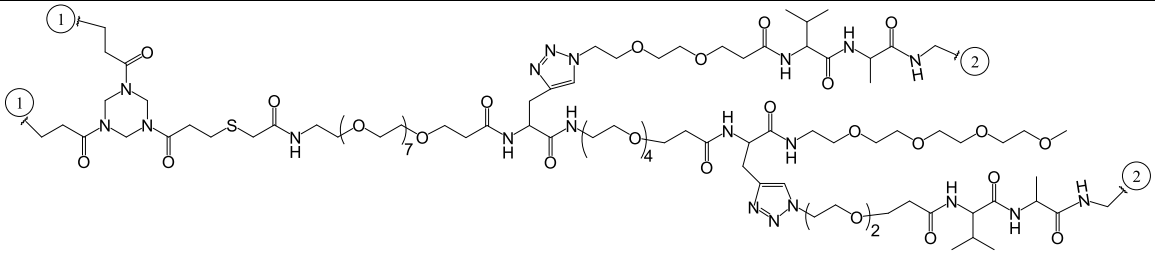
DL-10



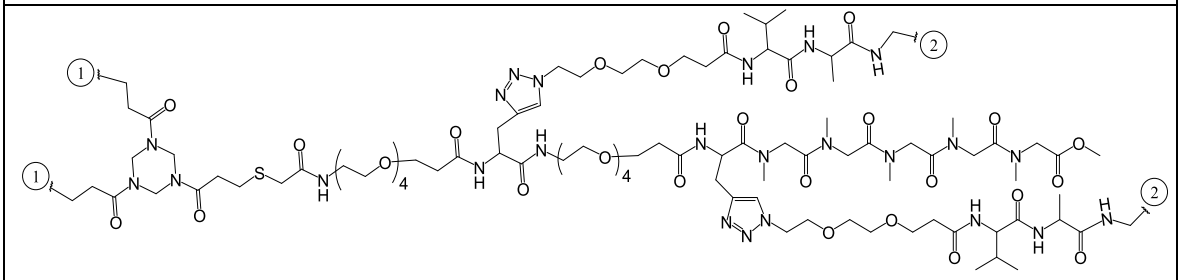
DL-11



DL-12

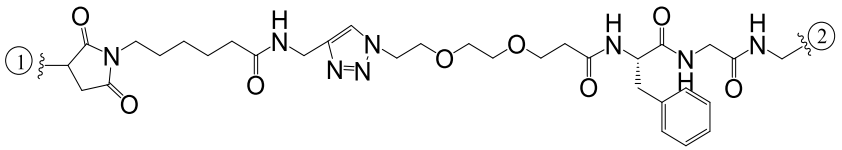
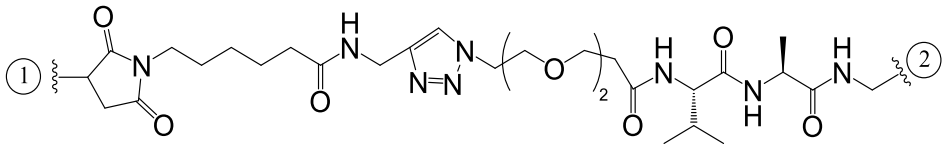
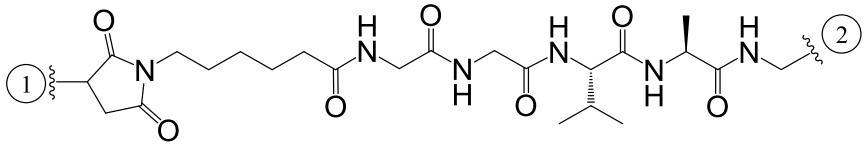
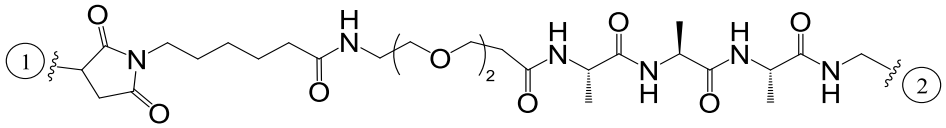
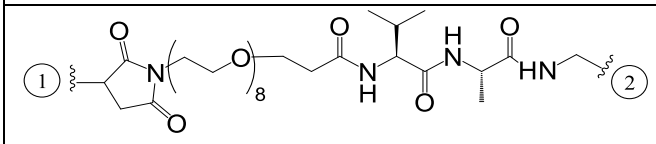
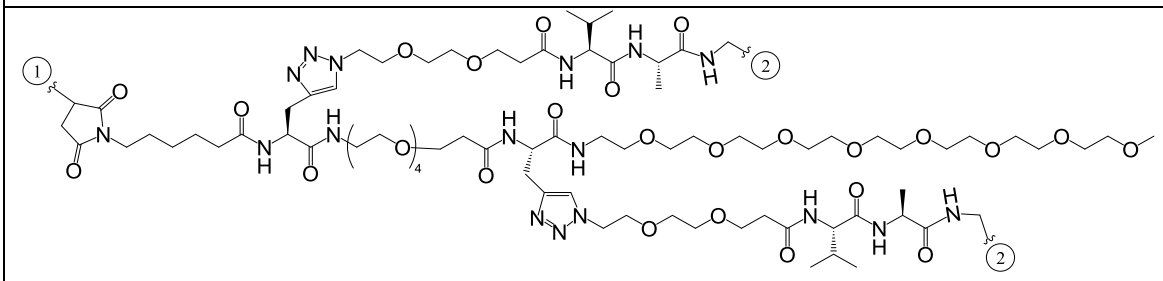


DL-13

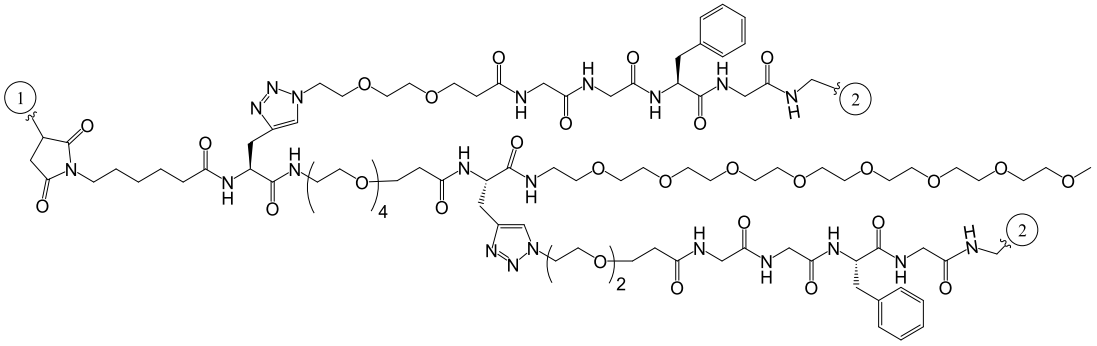


[00166] wherein, "① ~~~" is linked to an antibody; and "② ~~~" is linked to a drug unit.

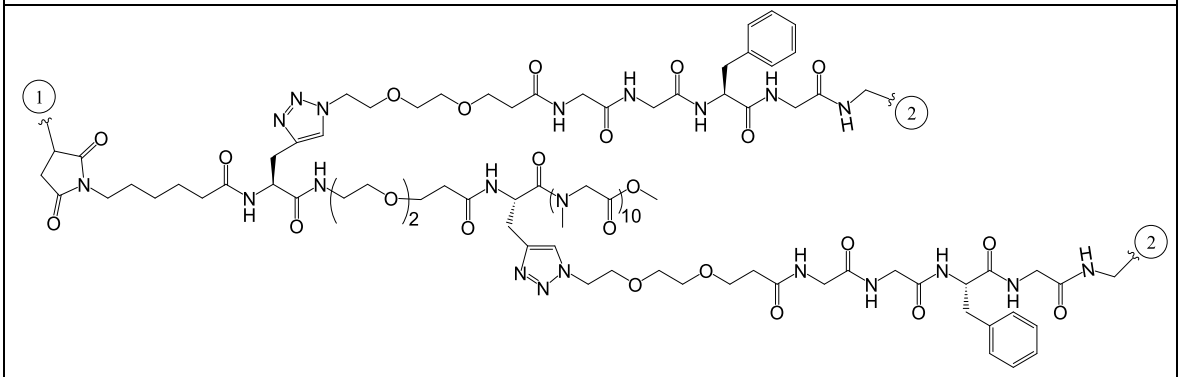
[00167] In some embodiments, the linker has a structure selected from the group consisting of

DL-02a

DL-06a

DL-05a

DL-07a

DL-14a

DL-04a


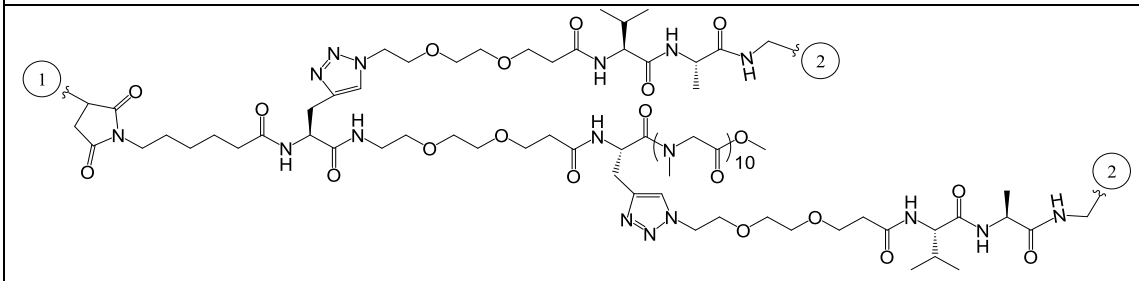
DL-03a



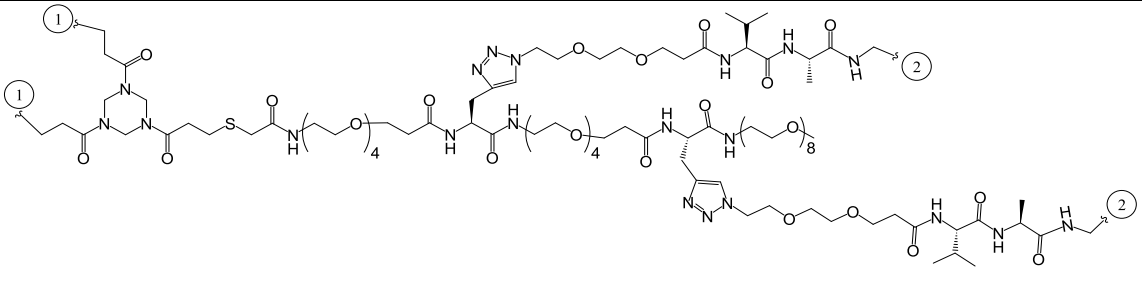
DL-08a

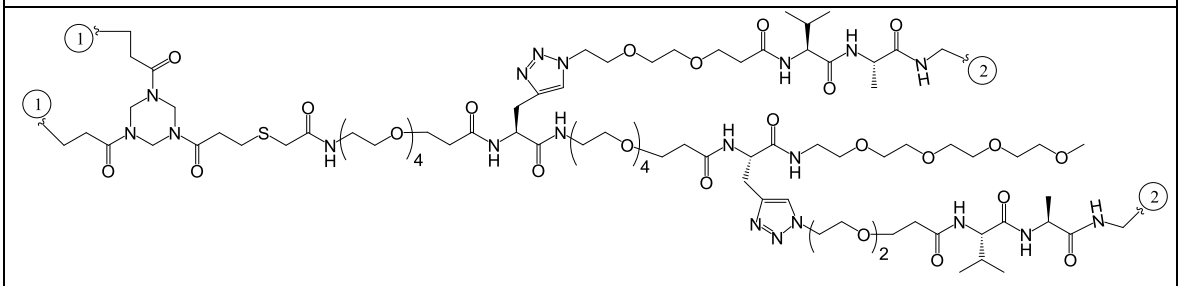
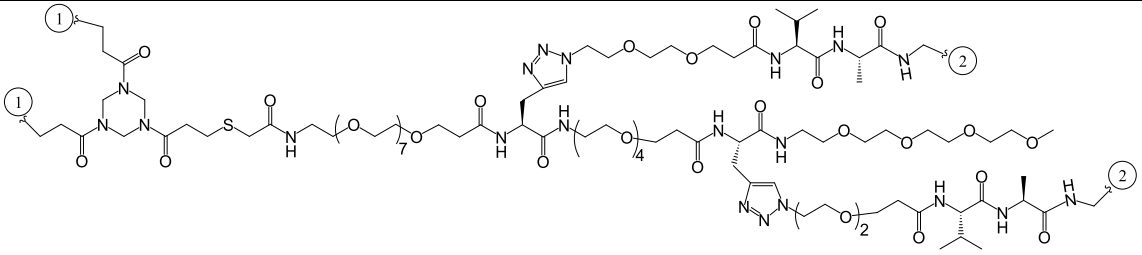
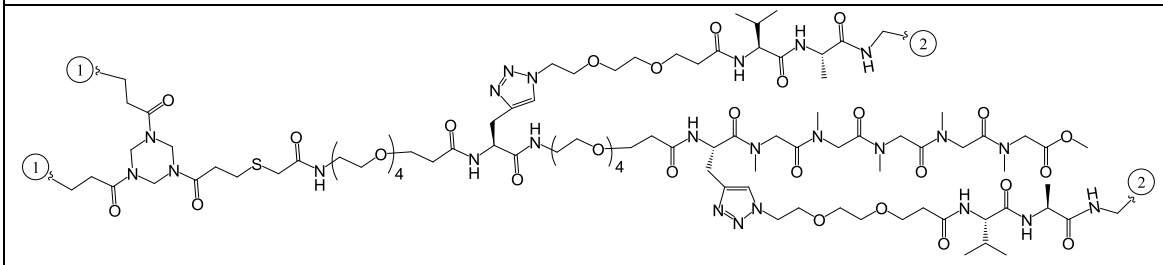


DL-09a



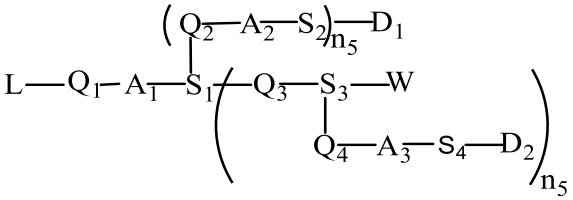
DL-10a



DL-11a

DL-12a

DL-13a


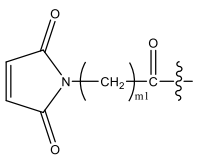
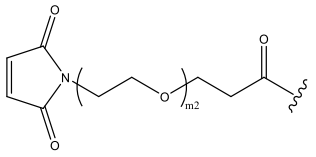
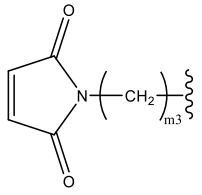
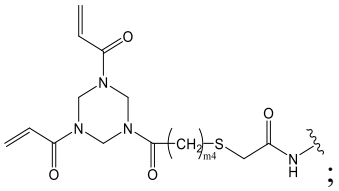
[00168] wherein, "① ~~~" is linked to an antibody; and "② ~~~" is linked to a payload compound.

[00169] In an aspect, provided is a linker-payload represented by formula (III):



formula (III)

[00170] wherein, L is a linker linked to an antibody, and has a structure selected from the group

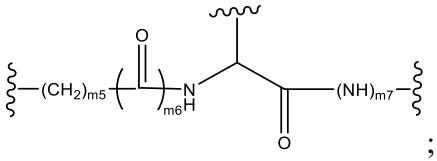
consisting of:  ,  ,  and ;

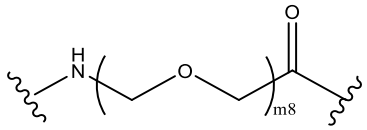
[00171] wherein, Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -$(NHCH_2C(O))$p-O-, -NH(CH_2CH_2O)p-$CH_2CH_2C(O)$- , -(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -CH_2-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -$NHCH_2$-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -(CH_2)-Y- and -$NHCH_2C(O)CH_2C(O)$-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{3-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00172] A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

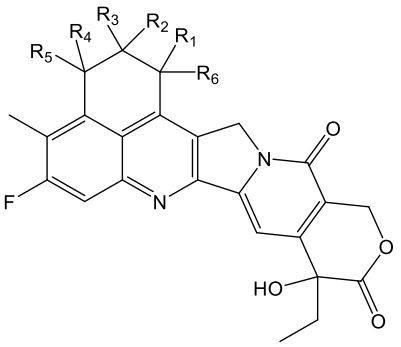
[00173] A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[00174] A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

[00175] S_1 and S_3 are independently ;

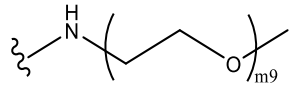
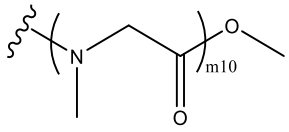
[00176] S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and ;

[00177] D_1 is a first payload compound, D_2 is a second payload compound, D_1 and D_2 are independently a compound represented by formula (IV):



formula (IV)

[00178] wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H) and deuterium (D), at least one, two, three or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_6 is selected from the group consisting of $-NH_2$ and $-NH-C(O)-CH_2-OH$; D_1 and D_2 are independently linked to S_1, S_2 or S_4 via either a hydroxyl or an amine group present;

[00179] W is selected from the group consisting of  and  ;

[00180] m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00181] m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00182] m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00183] m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00184] m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00185] m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

[00186] m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

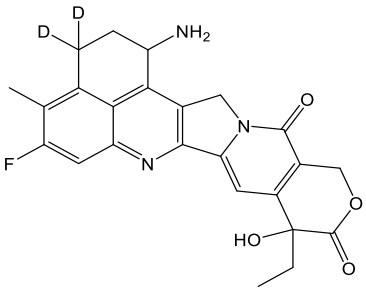
[00187] m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

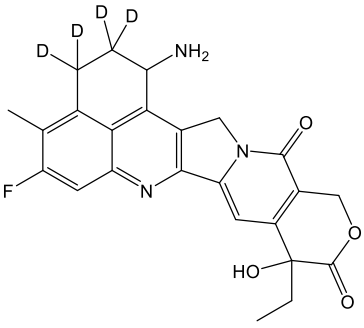
[00188] m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

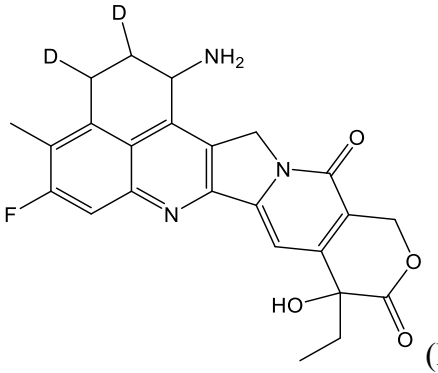
[00189] m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

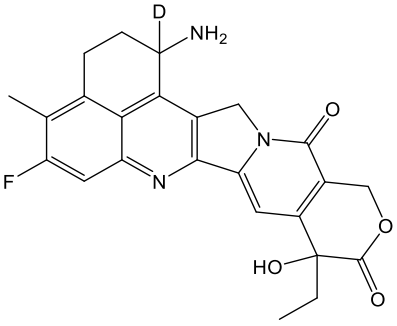
[00190] n_5 is 0 or 1.

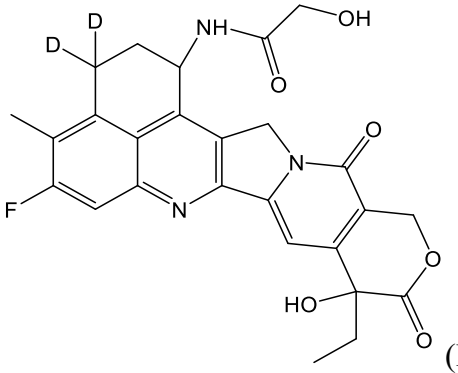
[00191] In some embodiments, for the linker-payload, D_1 and D_2 independently have a structure selected from the group consisting of:

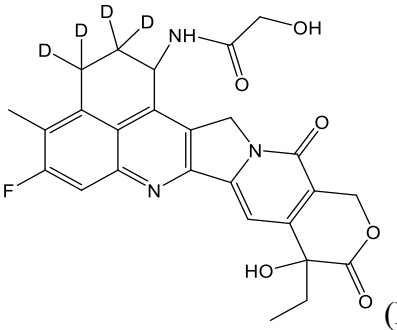

(I-1),

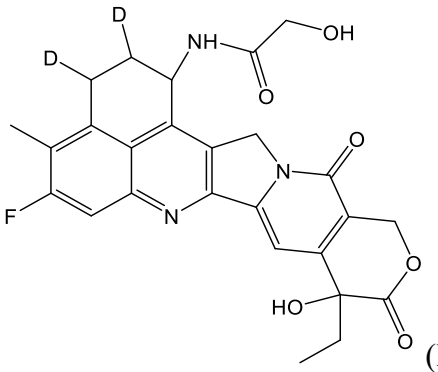
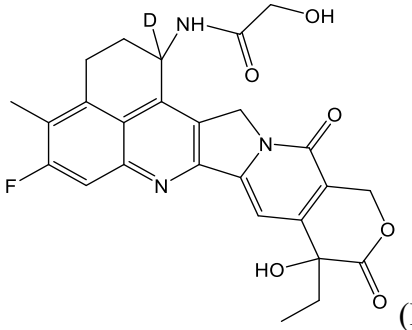

(I-2),


(I-3),


(I-4),

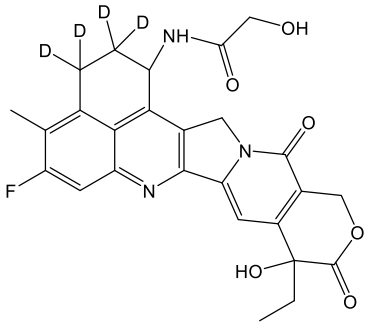
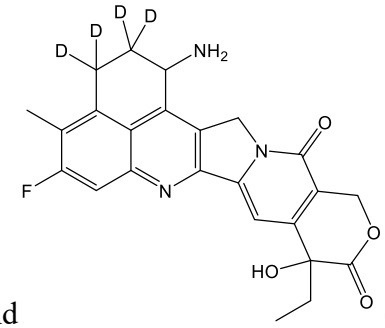

(I-5),


(I-6),

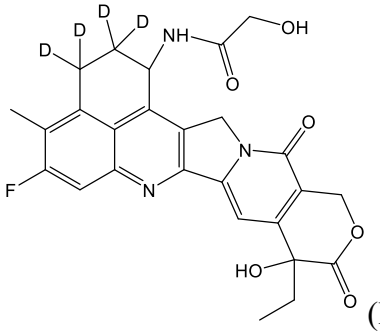



(I-7) and (I-8).

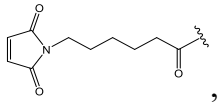
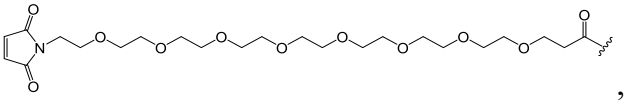
[00192] In some preferred embodiments, D_1 and D_2 independently have a structure selected from the group consisting of:

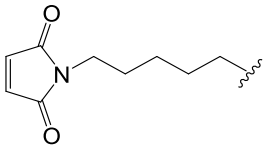
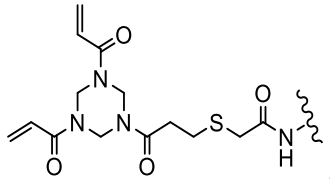
[00193]  (I-6) and  (I-2).

[00194] In some more preferred embodiments, D_1 and D_2 have a structure of

 (I-6).

[00195] In some embodiments, for the linker-payload, -L has a structure selected from the group consisting of

, ,

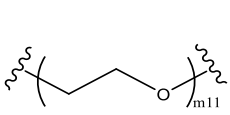
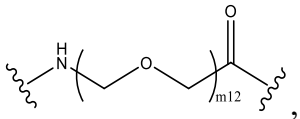
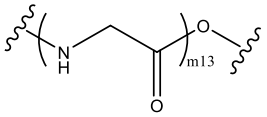
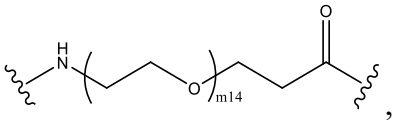
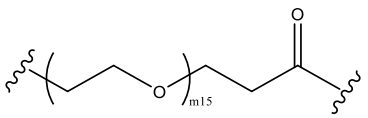
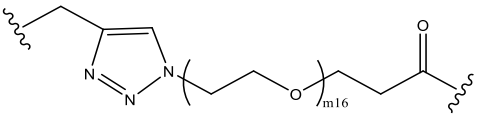

and .

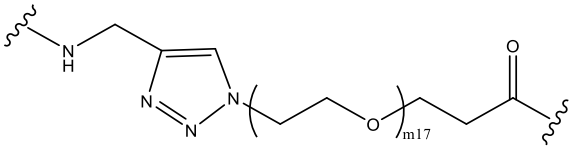
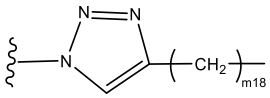
[00196] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[00197] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[00198] In some embodiments, A_1, A_2 and A_3 independently have a structure selected from the group consisting of: a single bond, -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably, the linker has a structure of -Val-Ala-.

[00199] In some embodiments, Q_1, Q_2, Q_3 and Q_4 as a linking group of the linker-payload independently have a structure selected from the group consisting of a single bond,

,
,
,
,
,
,

 and  ; wherein,

[00200] m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00201] m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00202] m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

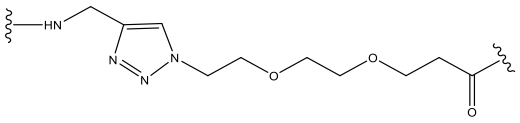
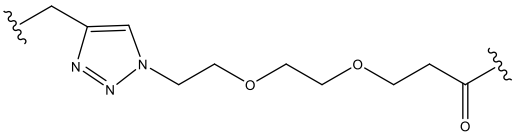
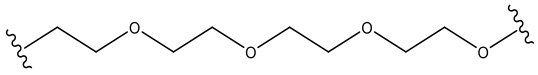
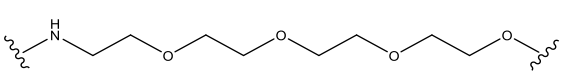
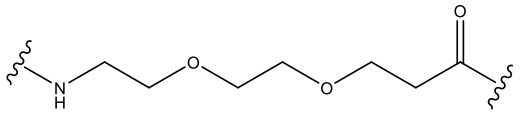
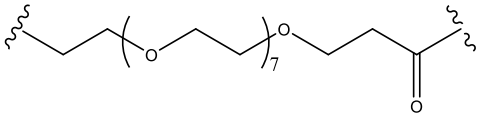
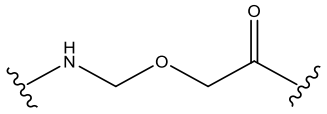
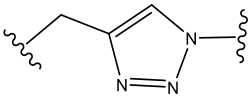
[00203] m14 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

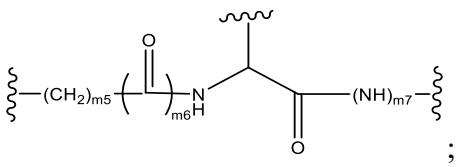
[00204] m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00205] m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00206] m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

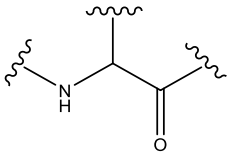
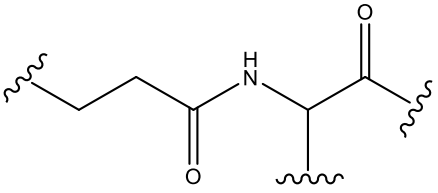
[00207] m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

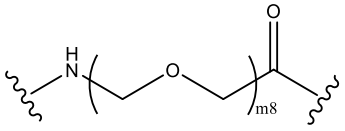
[00208] In some embodiments, Q_1, Q_2, Q_3 and Q_4 as a linking group of the linker-payload independently have a structure selected from the group consisting of a single bond, , , , , , ,  and .

[00209] In some embodiments, S_1 and S_3 of the linker-payload independently have a structure of ;

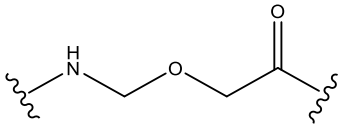
[00210] wherein m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[00211] In some embodiments, S_1 and S_3 of the linker-payload independently have a structure of

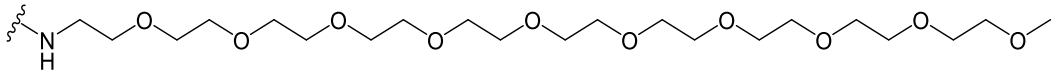
51.  and .

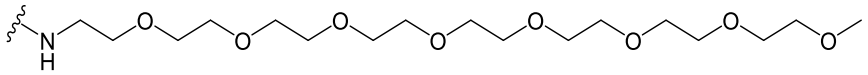
[00212] In some embodiments, S_2 and S_4 of the linker-payload independently have a structure selected from the group consisting of -NH-CH_2- and ;

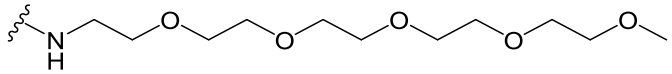
[00213] wherein m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

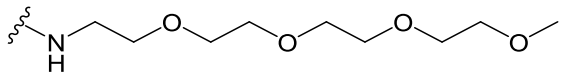
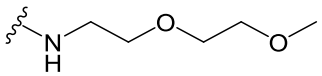
[00214] In some embodiments, S_2 and S_4 of the linker-payload independently have a structure selected from the group consisting of -NH-CH_2- and .

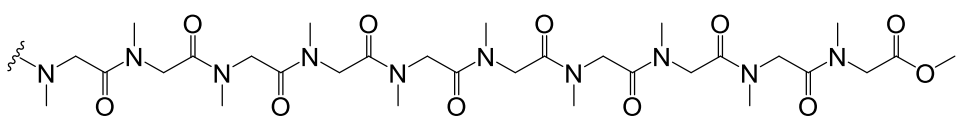
[00215] In some embodiments, W of the linker-payload has a structure selected from the group consisting of:

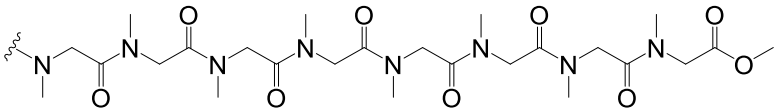
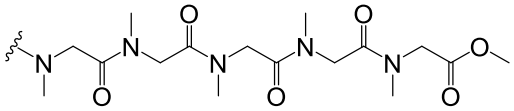
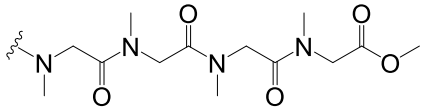
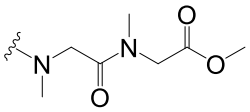
,

,

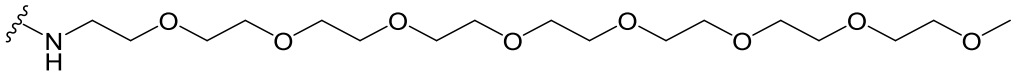
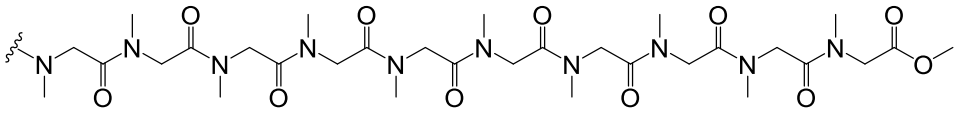
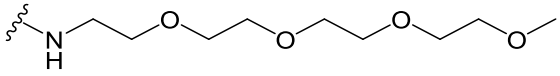
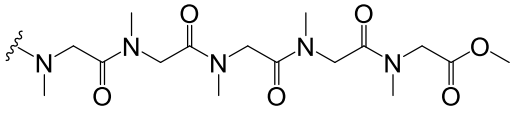
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, ,

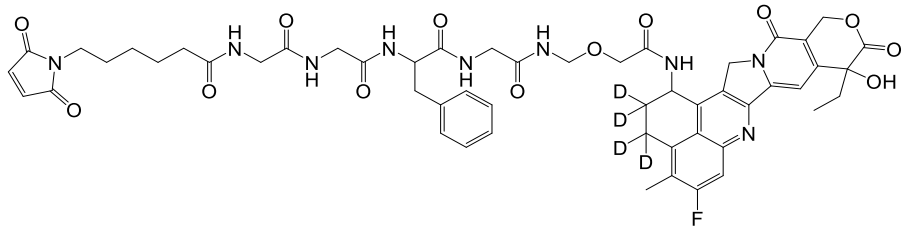
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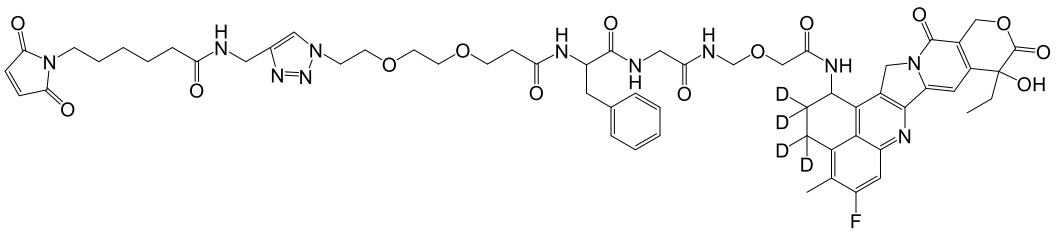

,

,

and

.

[00216] In some embodiments, W of the linker-payload has a structure selected from the group consisting of:

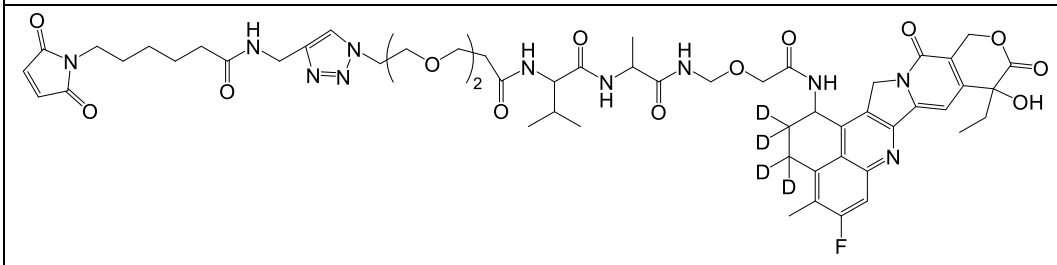

,

,

and

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[00217] In some embodiments, the linker-payload has a structure selected from the group consisting of:

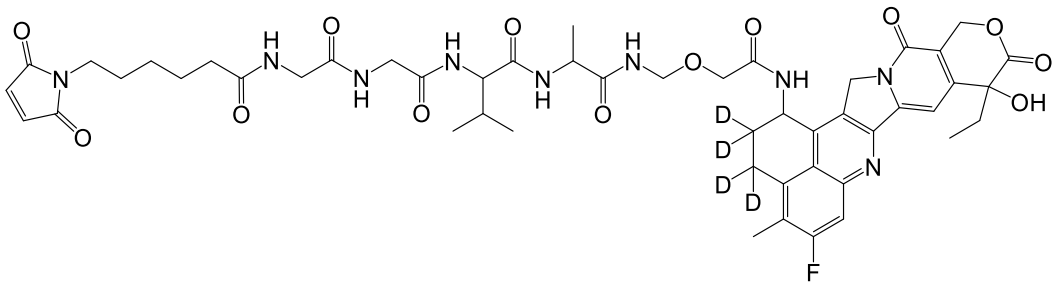
DL01-dDxd

DL02-dDxd



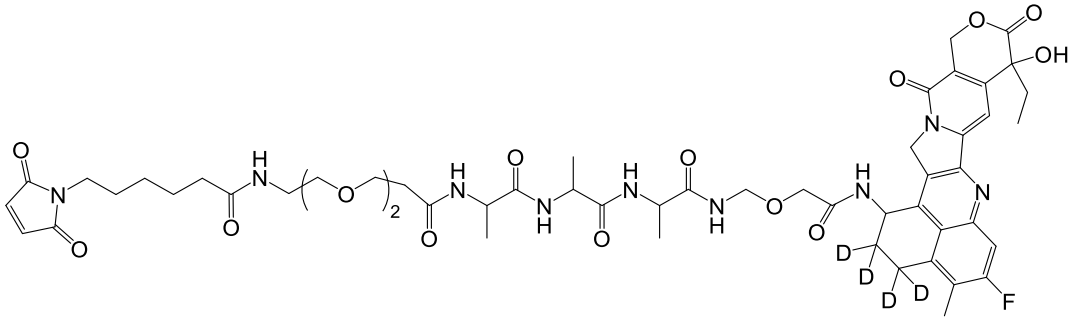
DL06-dDxd



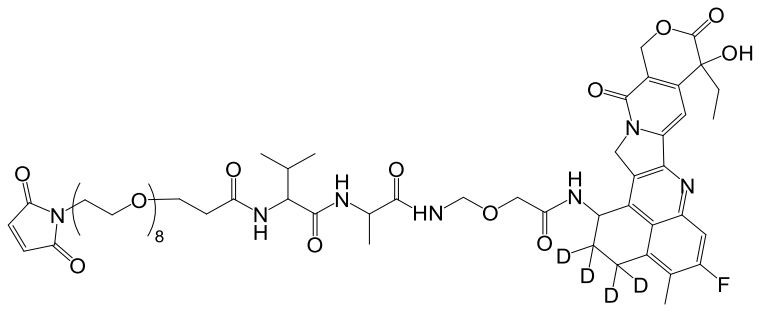
DL05-dDxd



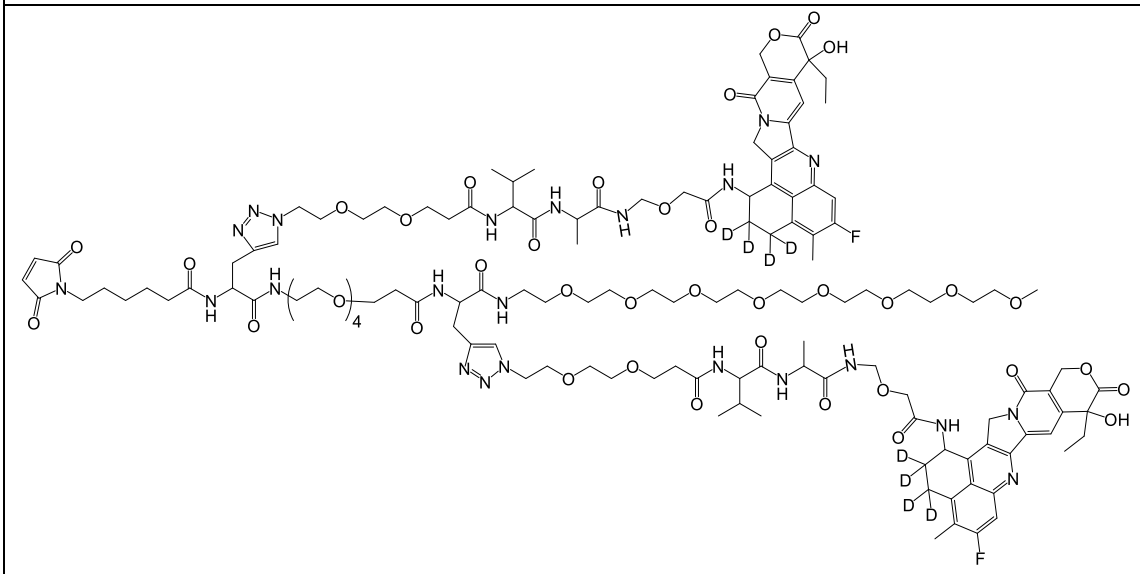
DL07-dDxd



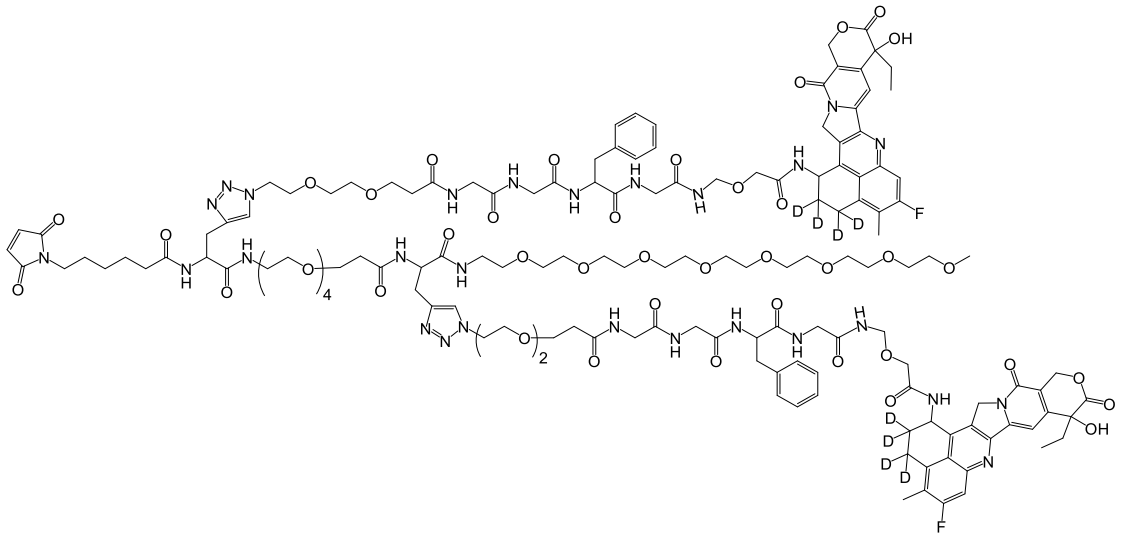
DL14-dDxd

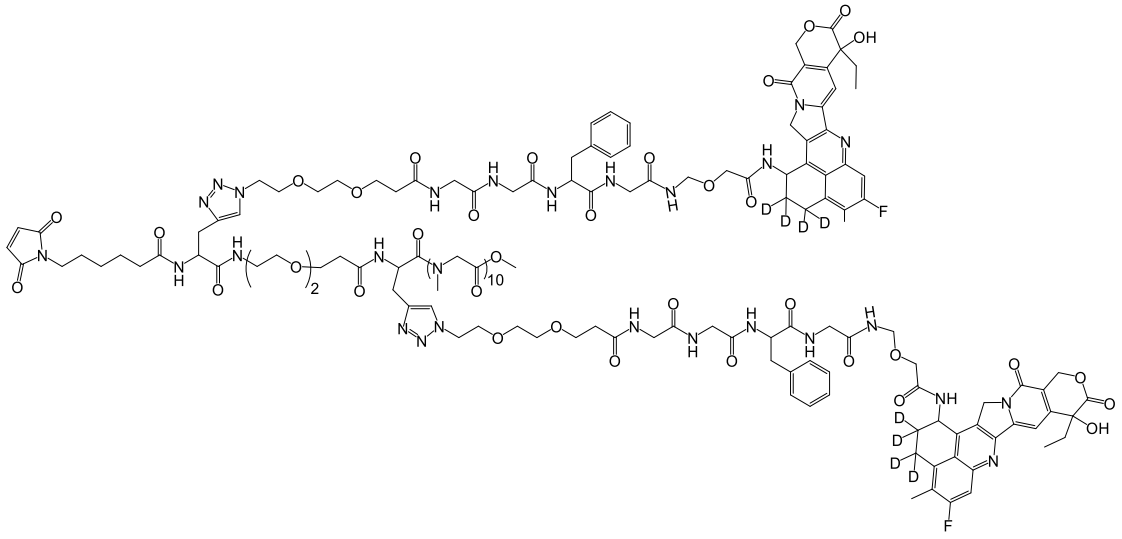
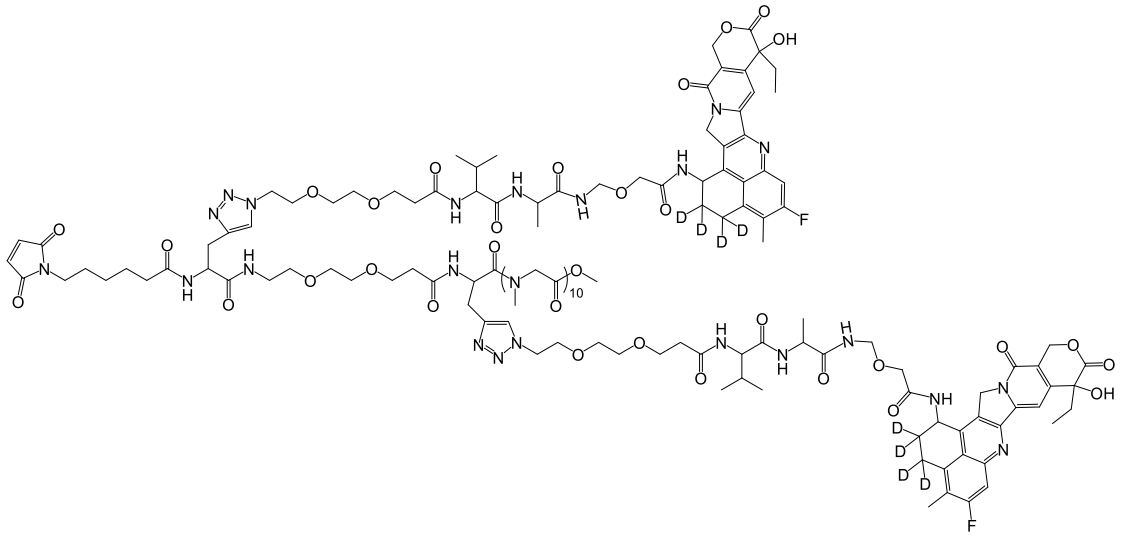


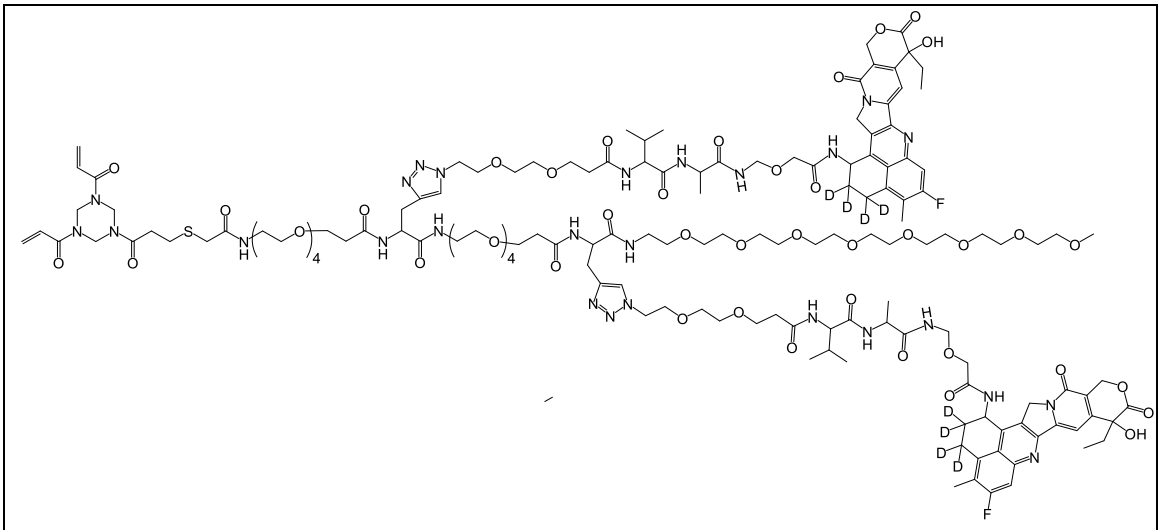
DL04-dDxd



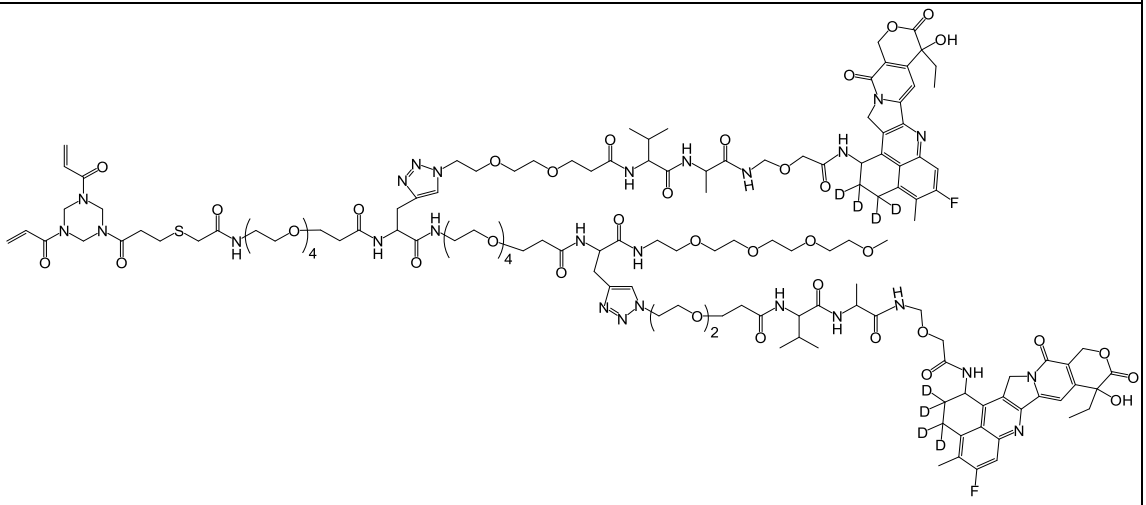
DL03-dDxd



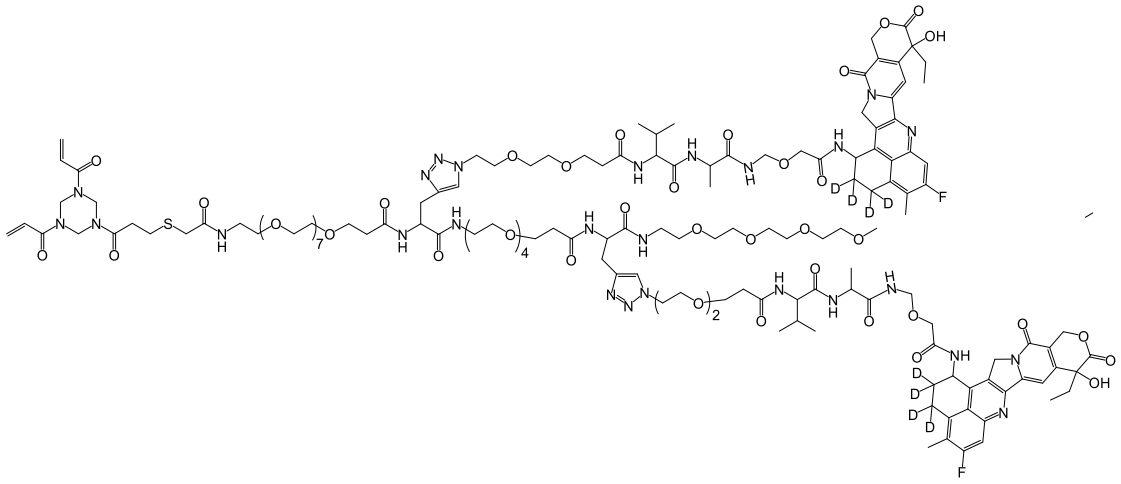
DL08-dDxd

DL09-dDxd

DL10-dDxd



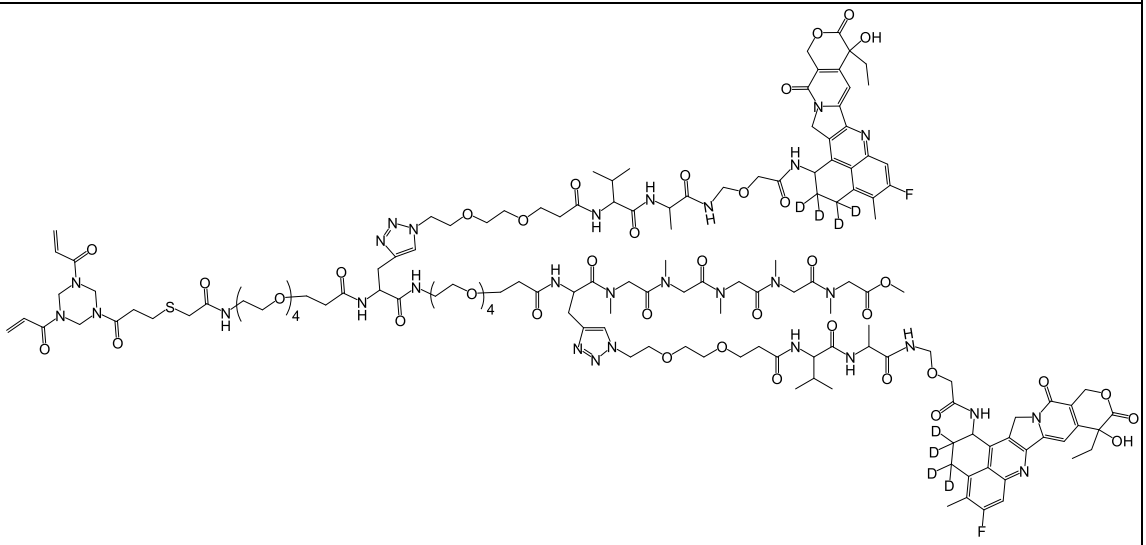
DL11-dDxd



DL12-dDxd

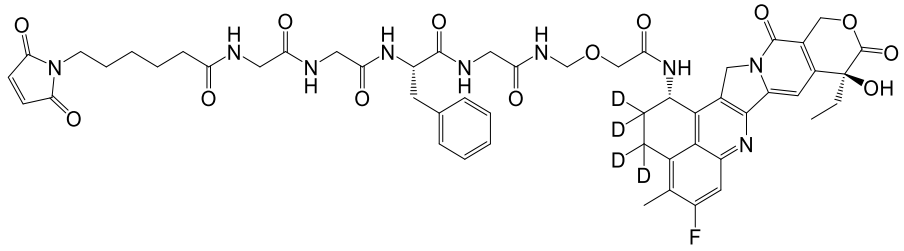


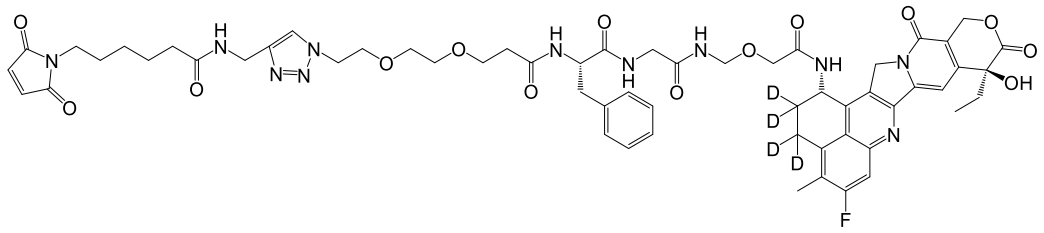
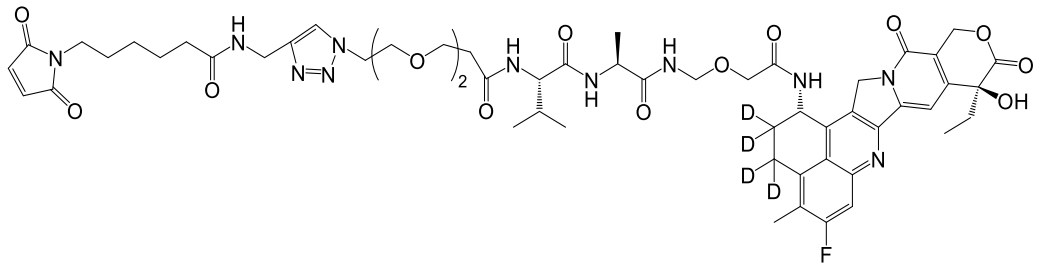
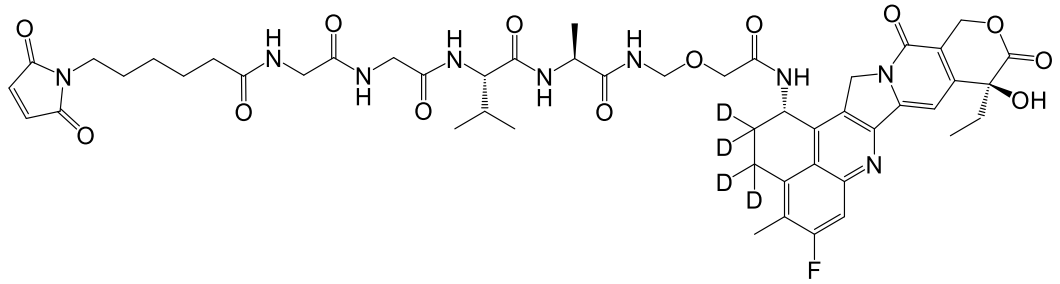
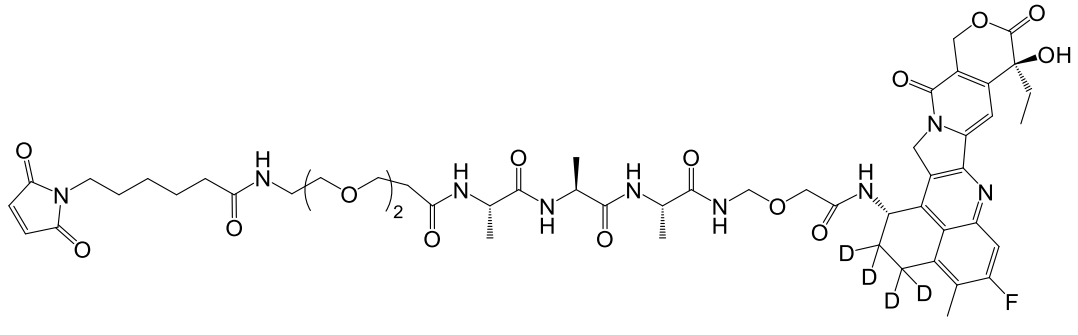
DL13-dDxd

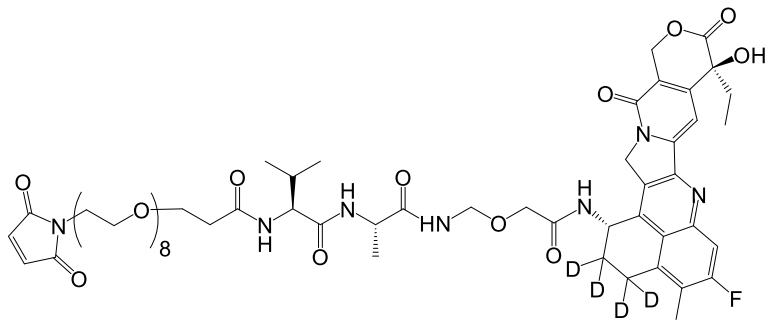


[00218] In some preferred embodiments, the linker-payload has a structure selected from the group consisting of:

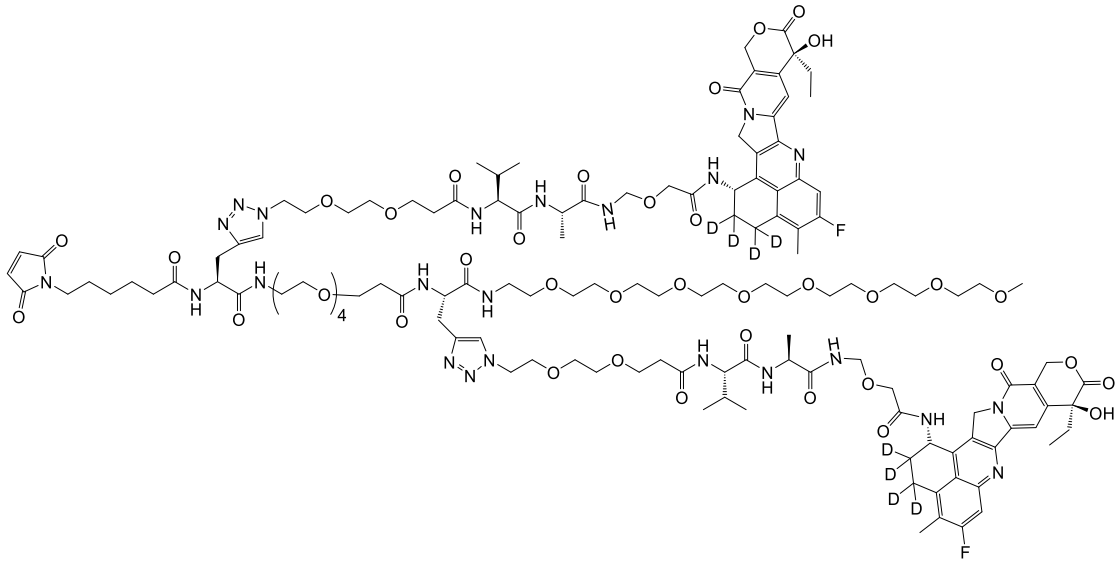
DL01-dDxd-1



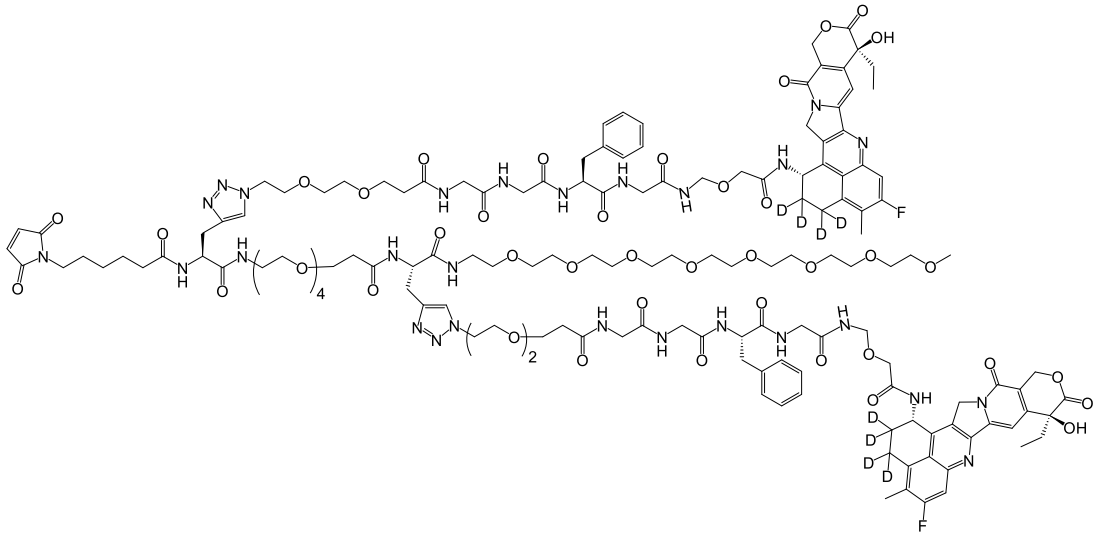
DL02-dDxd-1

DL06-dDxd-1

DL05-dDxd-1

DL07-dDxd-1

DL14-dDxd-1

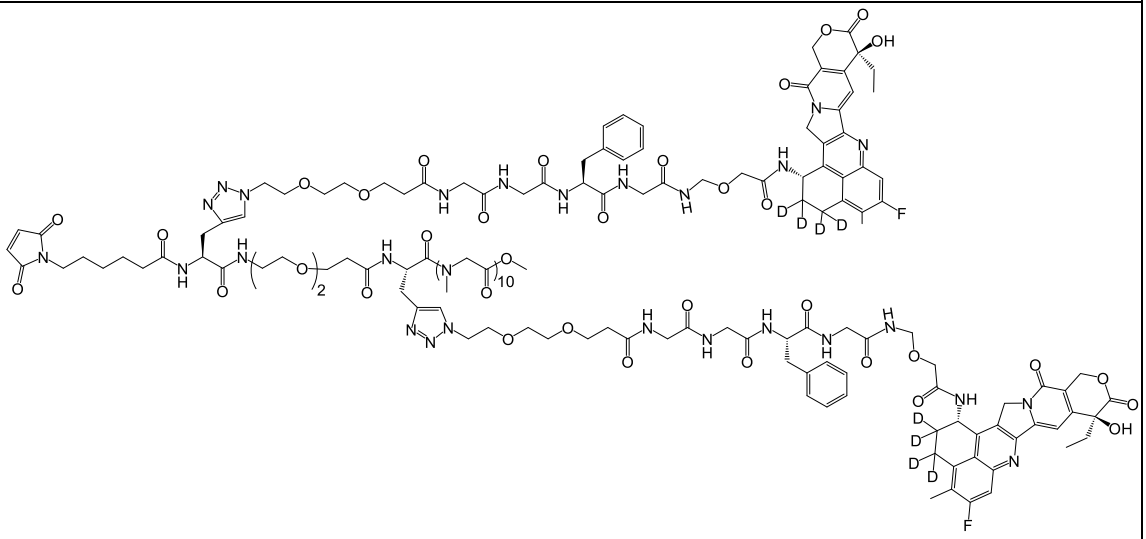
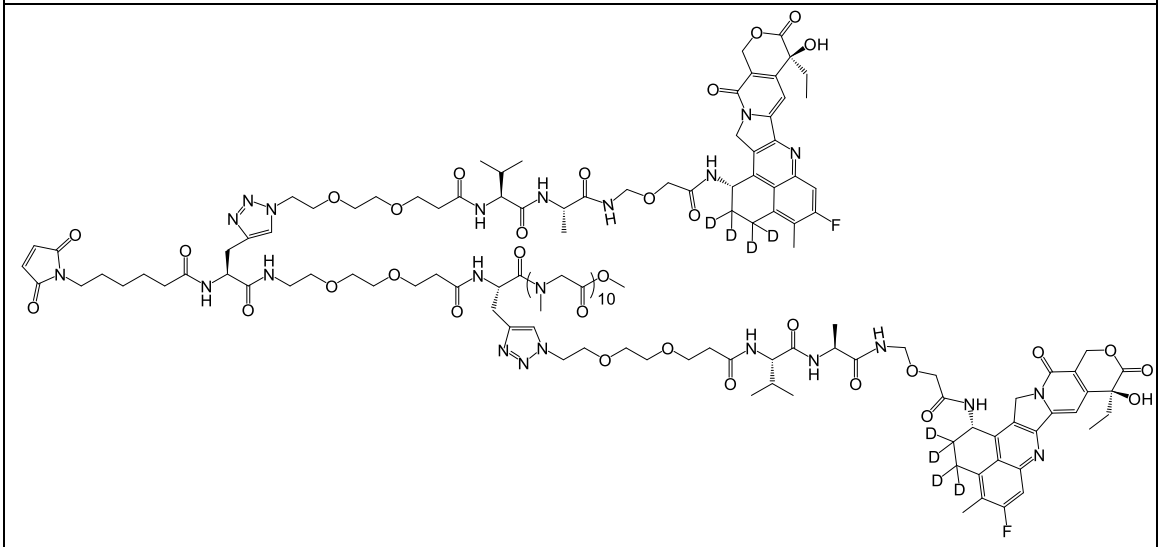


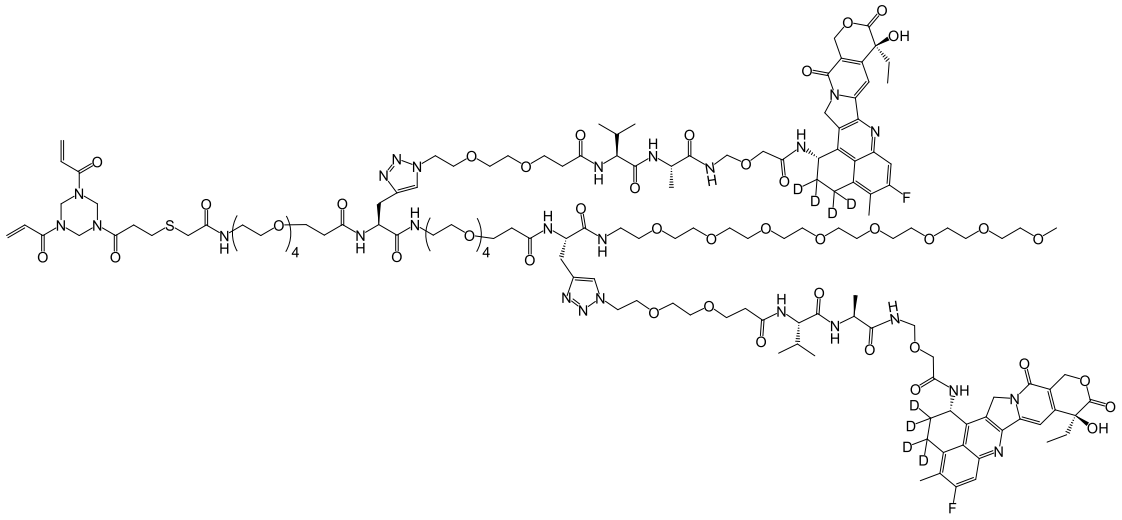
DL04-dDxd-1



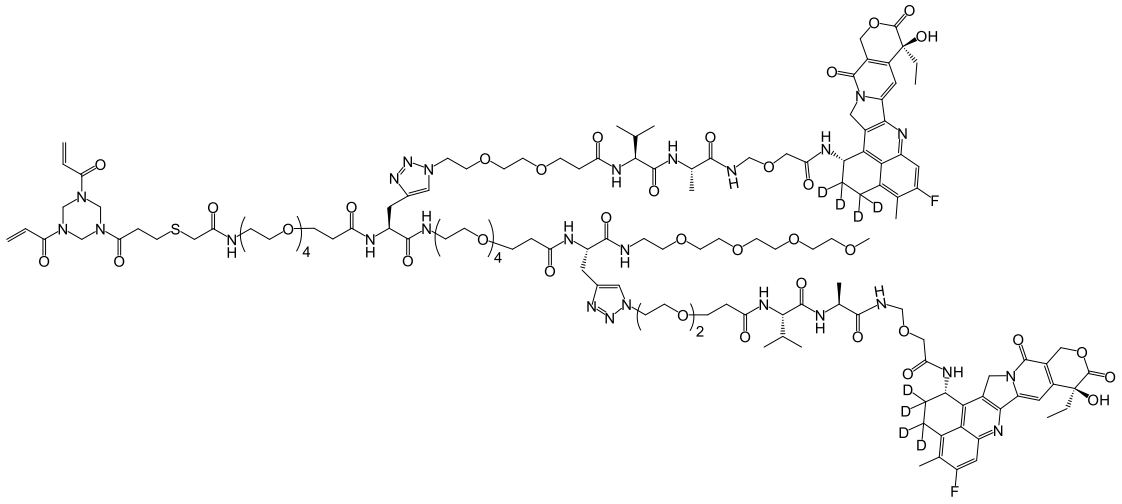
DL03-dDxd-1



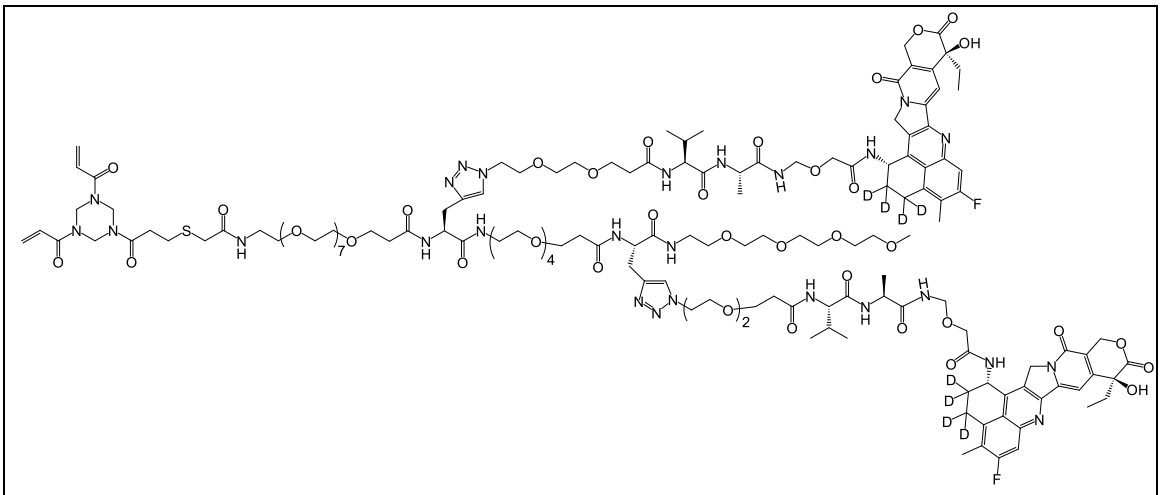
DL08-dDxd-1

DL09-dDxd-1

DL10-dDxd-1



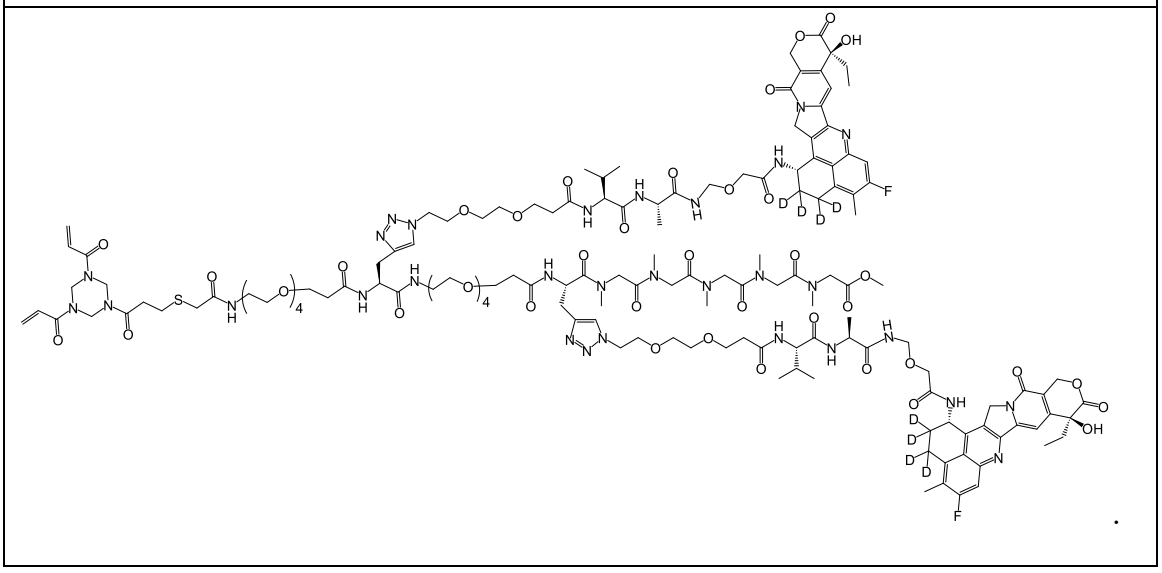
DL11-dDxd-1



DL12-dDxd-1

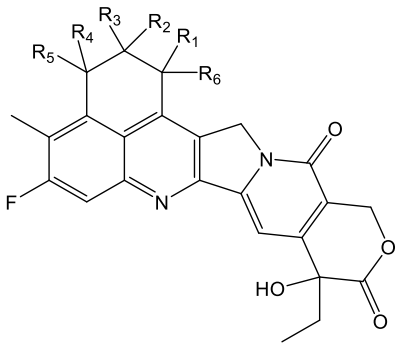


DL13-dDxd-1



[00219] In an aspect, provided is a linker-payload, which is a stereoisomer, pharmaceutically acceptable salt or solvate of the linker-payload described above.

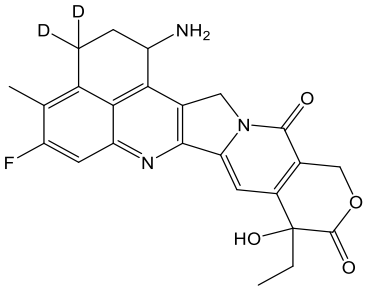
[00220] In an aspect, provided is deuterated camptothecin compound represented by formula (V):

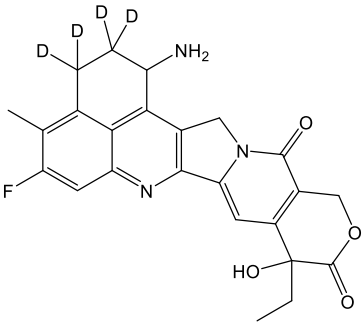


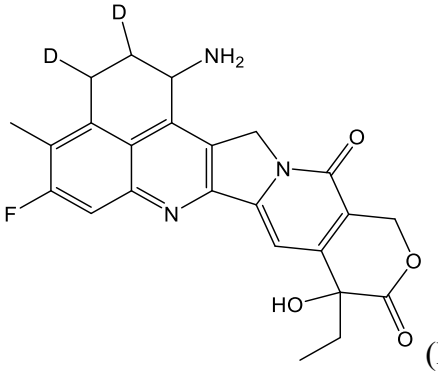
formula (V)

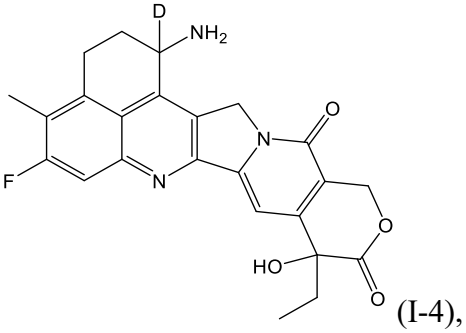
[00221] wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H) and deuterium (D), at least one, two, three, or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_6 is selected from the group consisting of $-NH_2$ and $-NH-C(O)-CH_2-OH$.

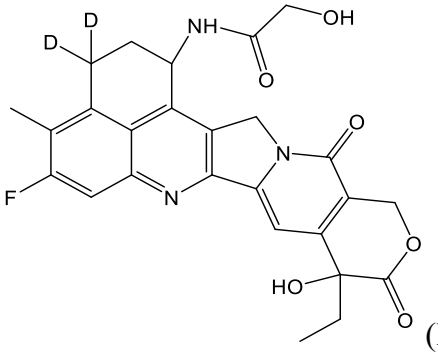
[00222] In some embodiments, the structure represented by formula (V) is selected from the group consisting of:

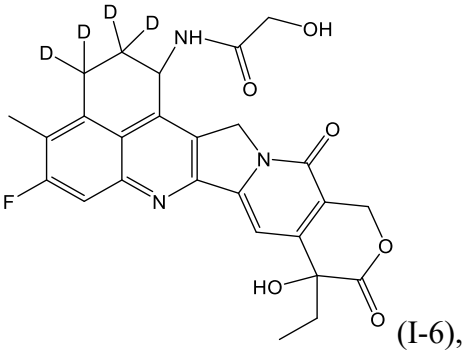

(I-1),

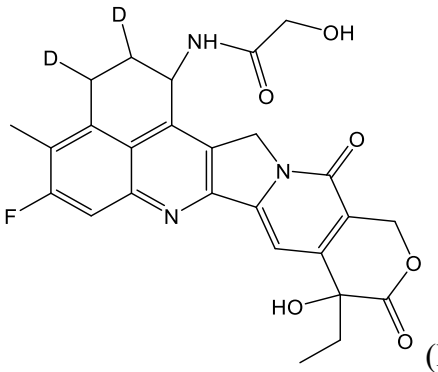
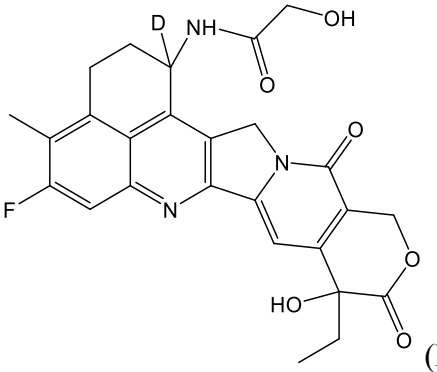

(I-2),

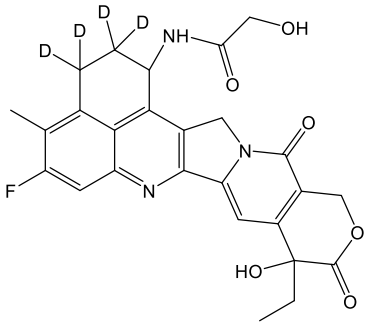
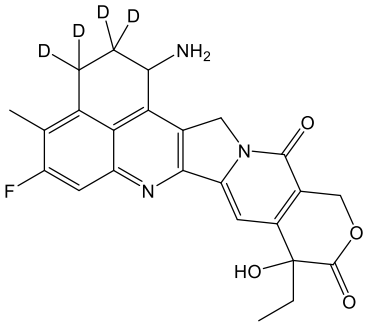
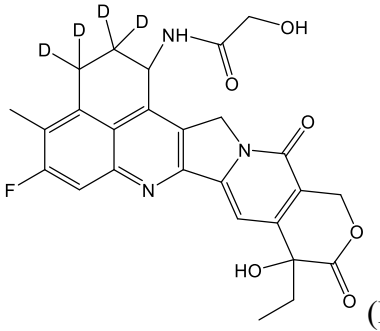

(I-3),


(I-4),

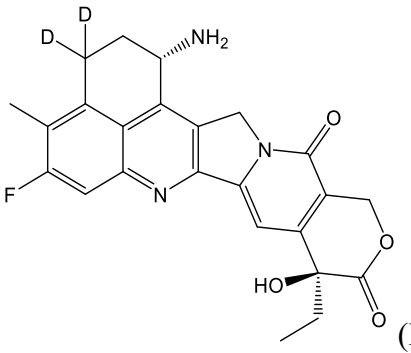

(I-5),

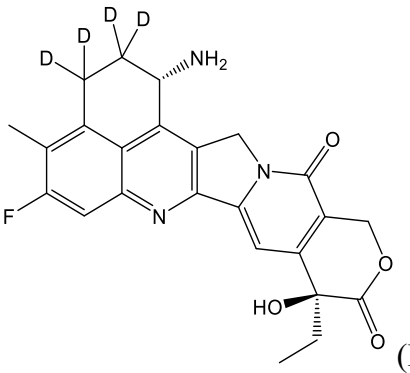

(I-6),

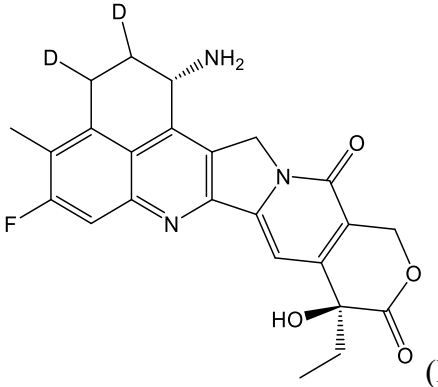

(I-7) and 
(I-8).

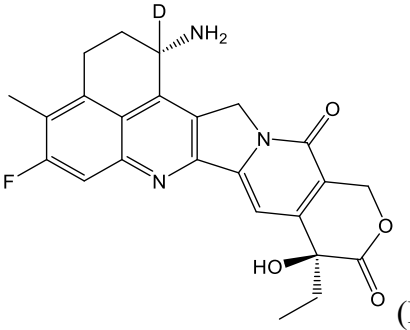
[00223] In some preferred embodiments, the structure represented by formula (V) is 
(I-6) or 
(I-2). In some more preferred embodiments, the structure represented by formula (V) is 
(I-6).

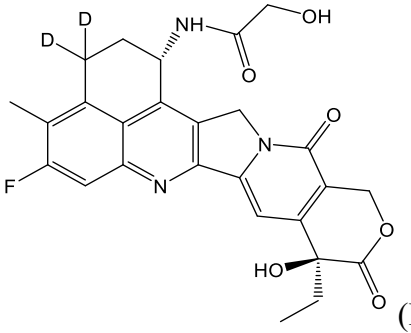
[00224] In some embodiments, for the deuterated camptothecin compound, the structure represented by formula (V) is selected from the group consisting of:

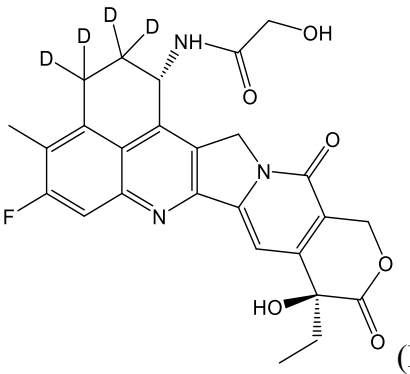

(I-1a),

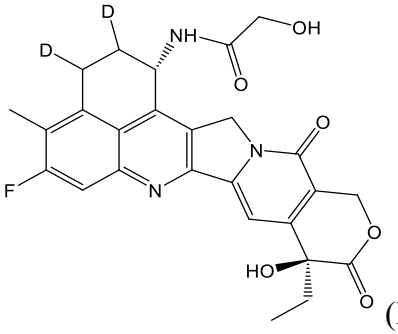

(I-2a),

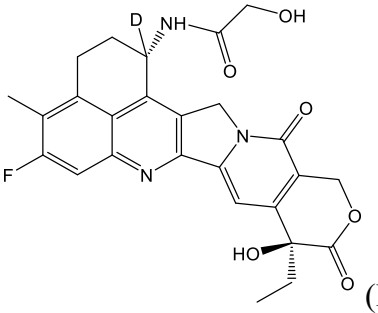

(I-3a),


(I-4a),

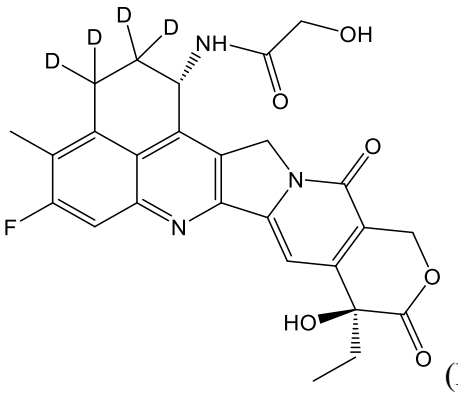
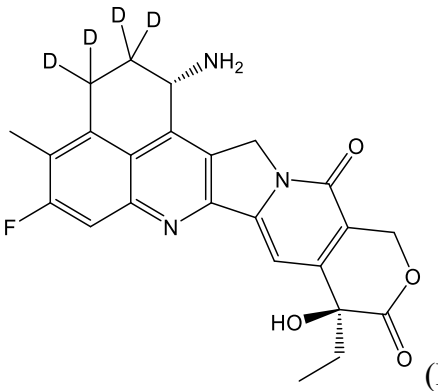

(I-5a),


(I-6a),

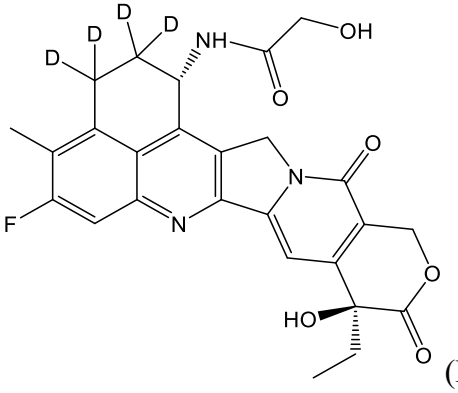

(I-7a) and


(I-8a).

[00225] In some preferred embodiments, the structure represented by formula (V) is


(I-6a) or 
(I-2a).

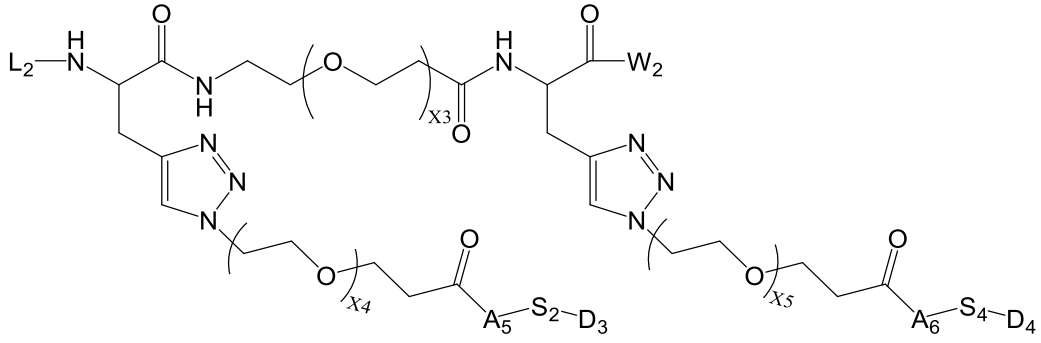
[00226] In some more preferred embodiments, the structure represented by formula (V) is


(I-6a).

[00227] In an aspect, provided is a compound, which is a stereoisomer, pharmaceutically acceptable salt or solvate of any one of compounds I-1 to I-8 and I-1a to I-8a.

[00228] In an aspect, provided is a compound represented by formula (IIIa):

[00229]



formula (IIIa)

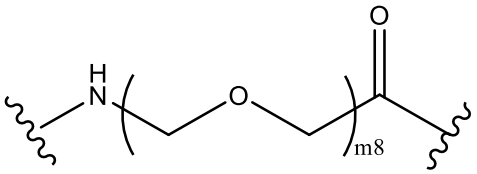
[00230] L_2 is a linker linked to an antibody,

[00231] W_2 is an optional group,

[00232] A_5 is a peptide consisting of 1, 2, 3 and 4 amino acid residues,

[00233] A_6 is a peptide consisting of 1, 2, 3 and 4 amino acid residues,

[00234] S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and



;

[00235] D_3 is a first payload compound,

[00236] D_4 is a second payload compound,

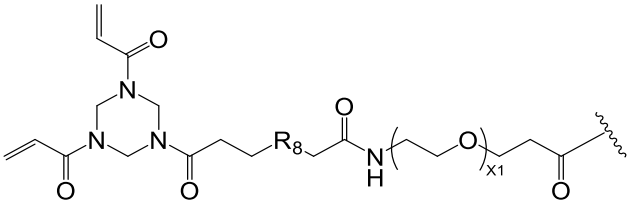
[00237] m_8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

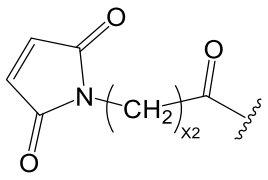
[00238] X_3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

[00239] X_4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

[00240] X_5 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[00241] In some embodiments, L_2 has a structure selected from the group consisting of:

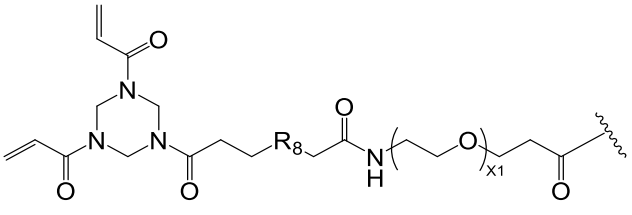


and


,

[00242] wherein R_8 is selected from the group consisting of N, O and S; X_1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and X_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

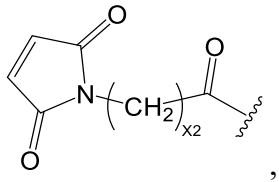
[00243] In some preferred embodiments, L_2 has a structure of



,

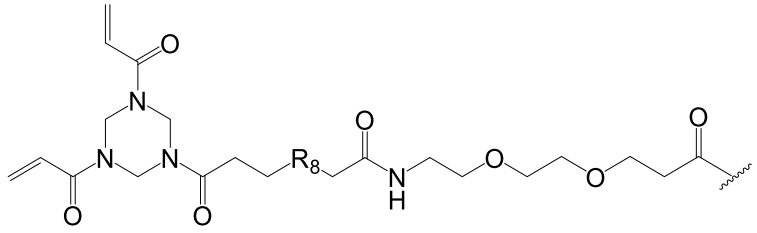
[00244] wherein R_8 is selected from the group consisting of N, O and S, and X_1 is selected from

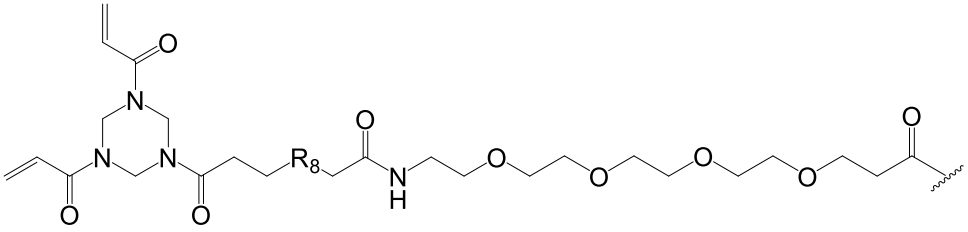
the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

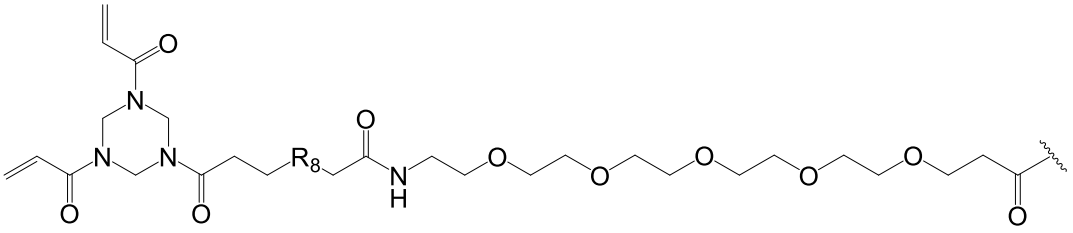
[00245] In some preferred embodiments, L_2 has a structure of ,

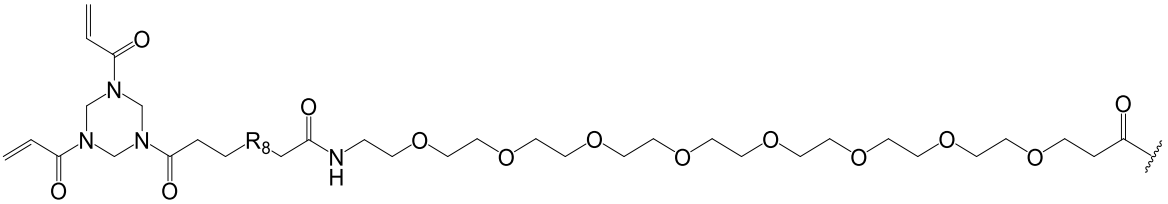
[00246] wherein X_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

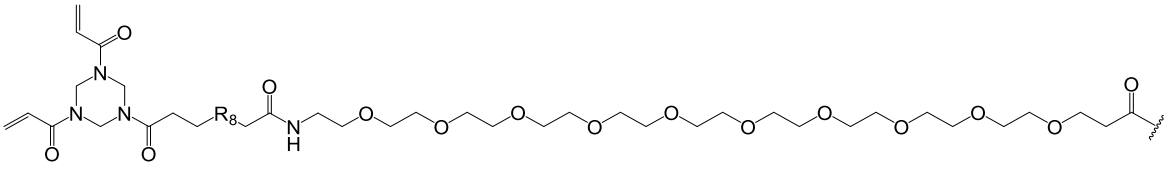
[00247] In some preferred embodiments, L_2 has a structure selected from the group consisting of:

[00248] ,

,

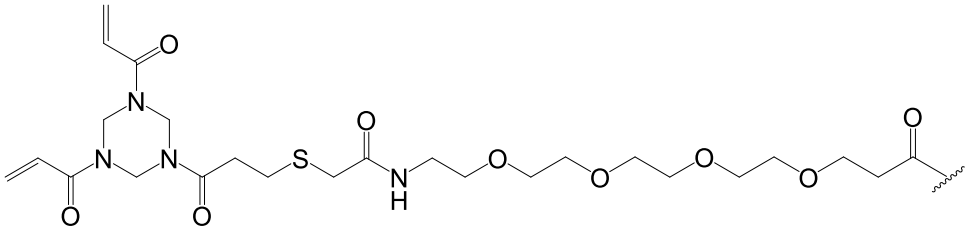
,

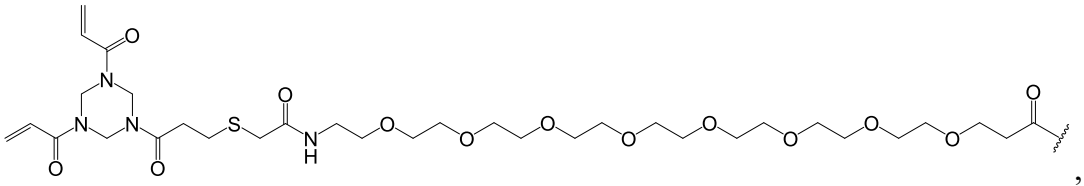
and

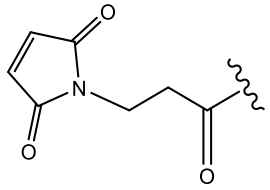
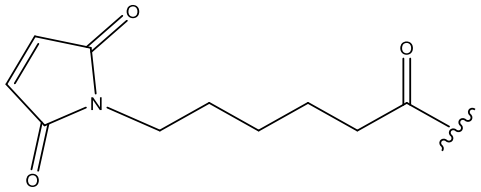
,

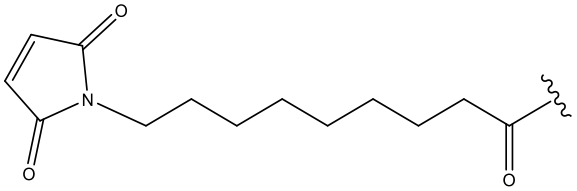
wherein R_8 is selected from the group consisting of N, O and S.

[00249] In some preferred embodiments, L_2 has a structure selected from the group consisting of:

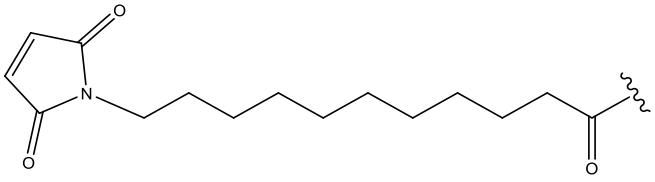
[00250] ,

,

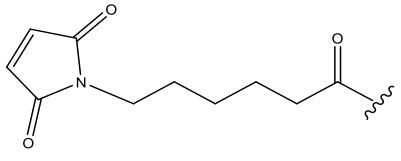
[00251] , ,

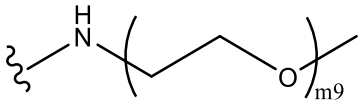
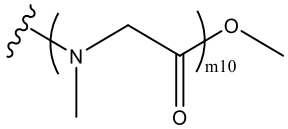


and

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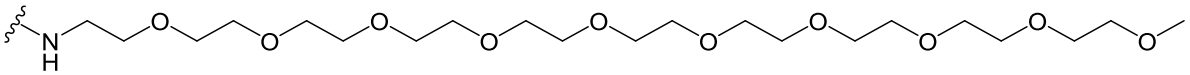
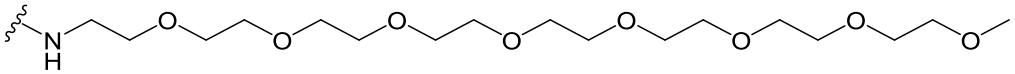
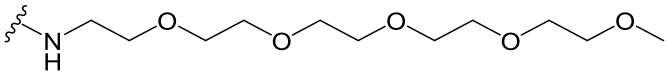
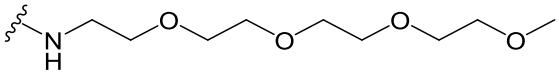
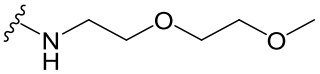
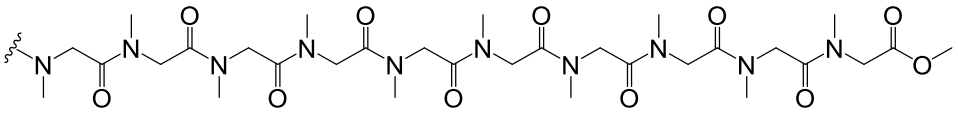
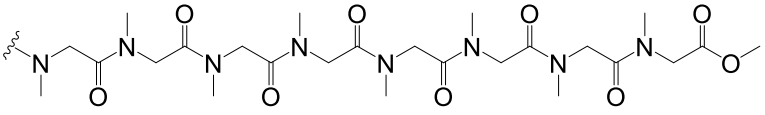
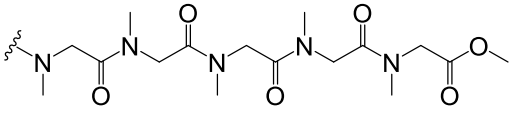
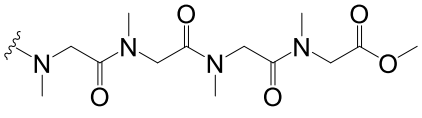
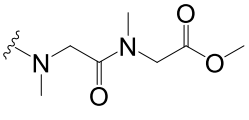
[00252] In some preferred embodiments, L_2 has a structure of:

[00253] .

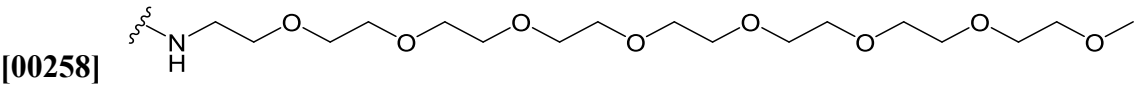
[00254] In some embodiments, W has a structure selected from the group consisting of  and , wherein M9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and M10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

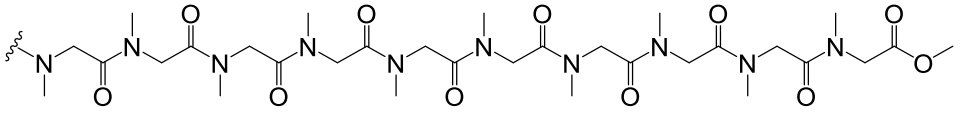
[00255] In some preferred embodiments, W has a structure selected from the group consisting of

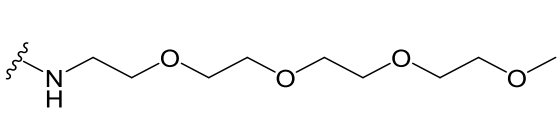
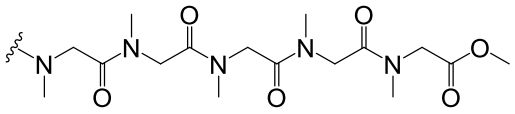
[00256]

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,  and
.

[00257] In some preferred embodiments, W has a structure selected from the group consisting of

[00258] ,

,

 and .

[00259] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[00260] In some embodiments, A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[00261] In some embodiments, A_1, A_2 and A_3 independently have a structure selected from the group consisting of:

[00262] a single bond, -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably, the linker has a structure of -Val-Ala-.

[00263] In some embodiments, the first payload compound and the second payload compound are independently selected from the group consisting of a cytotoxic molecule, an immune enhancer

and a radioactive isotope,

wherein the cytotoxic molecule includes, but is not limited to a tubulin inhibitor and a DNA damaging agent,

further preferably, the tubulin inhibitor includes, but is not limited to dolastatins, auristatin cytotoxic molecules, and maytansine cytotoxic molecules,

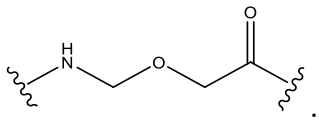
the DNA damaging agent includes, but is not limited to calicheamicins, duocarmycins, and anthramycin derivative pyrrolobenzodiazepines (PBDs), camptothecins, camptothecin derivatives, SN-38, and Dxd,

further preferably, the auristatin cytotoxic molecules include, but are not limited to monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), and derivatives thereof,

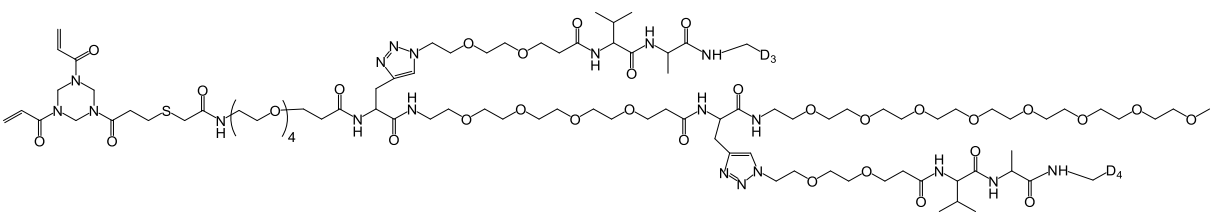
the maytansine cytotoxic molecules include, but are not limited to DM1, DM4, and derivatives thereof,

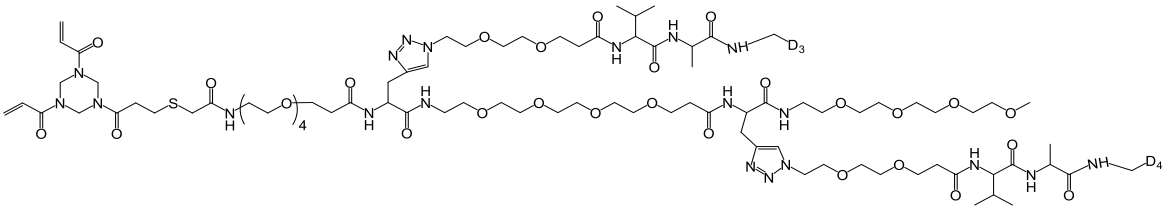
further preferably, the camptothecin derivative is the compound according to claim 65 or 66, wherein the compound is attached to S_2 or S_4 through removal of a hydrogen atom from either a hydroxyl or an amine group present.

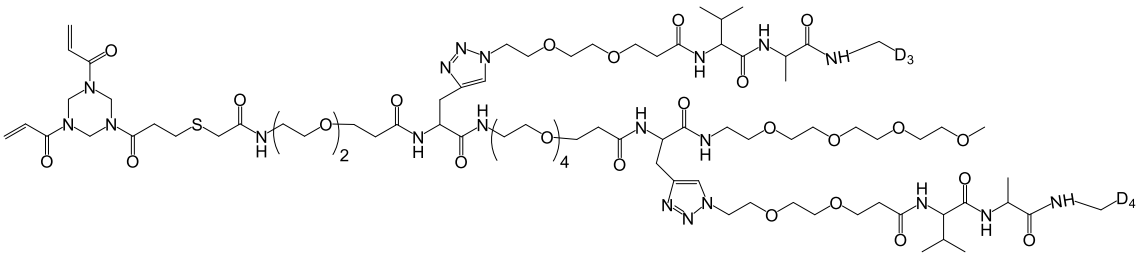
[00264] In some embodiments, S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and

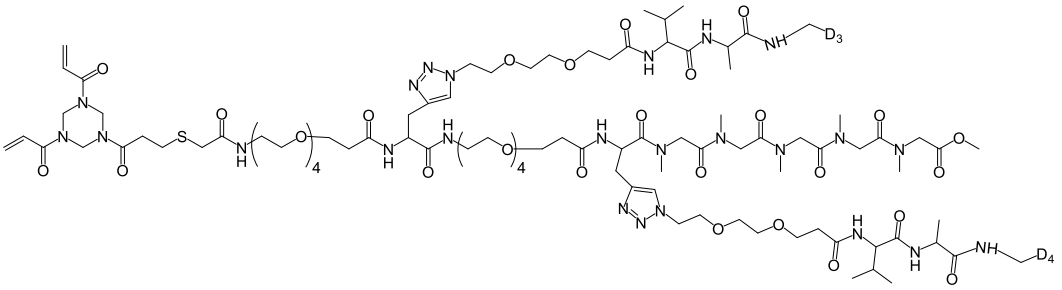
[00265] .

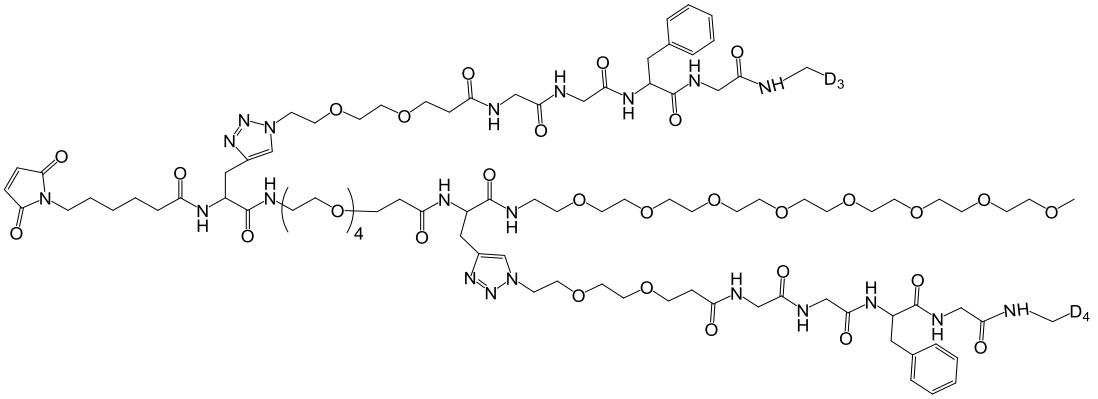
[00266] In some embodiments, the compound is selected from the group consisting of:

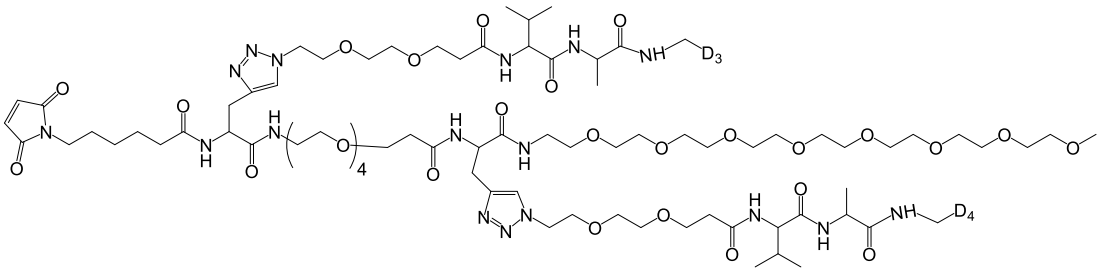
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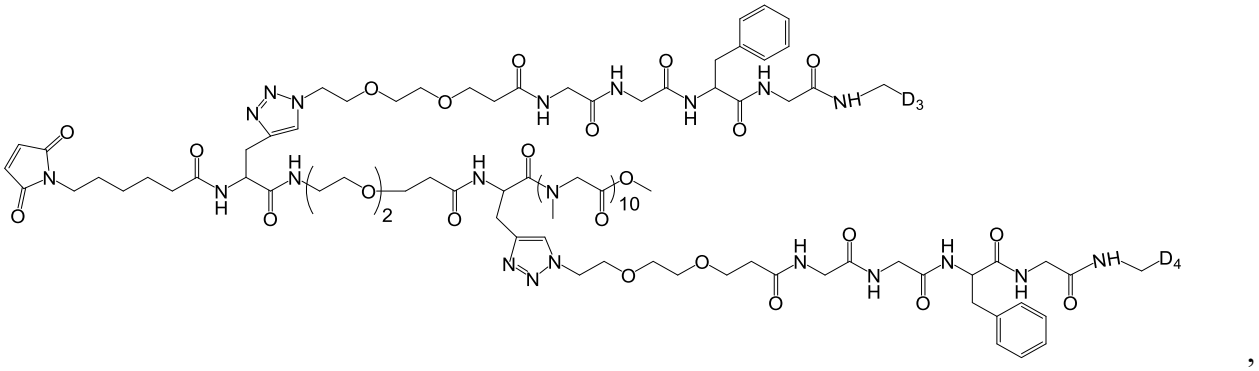

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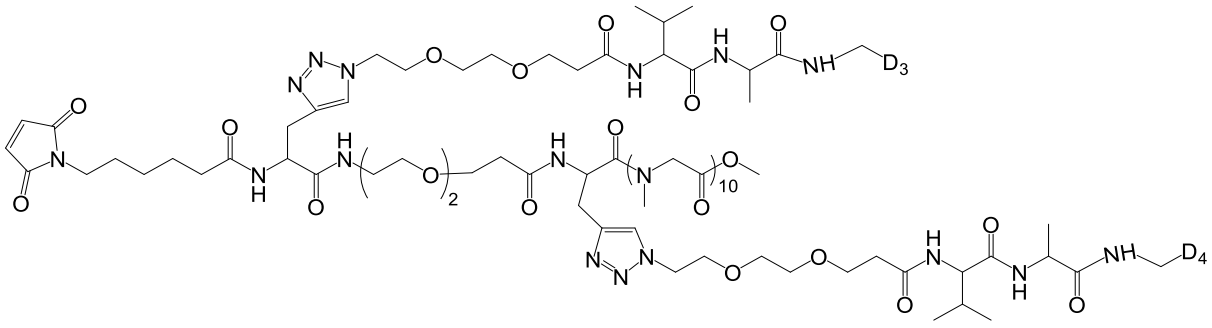

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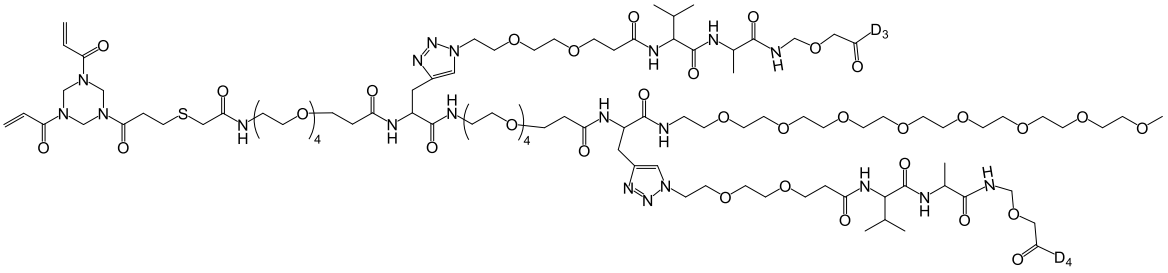

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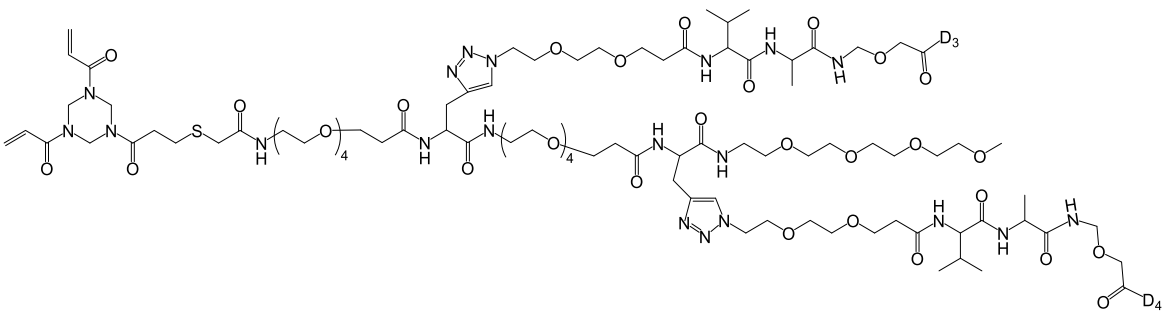

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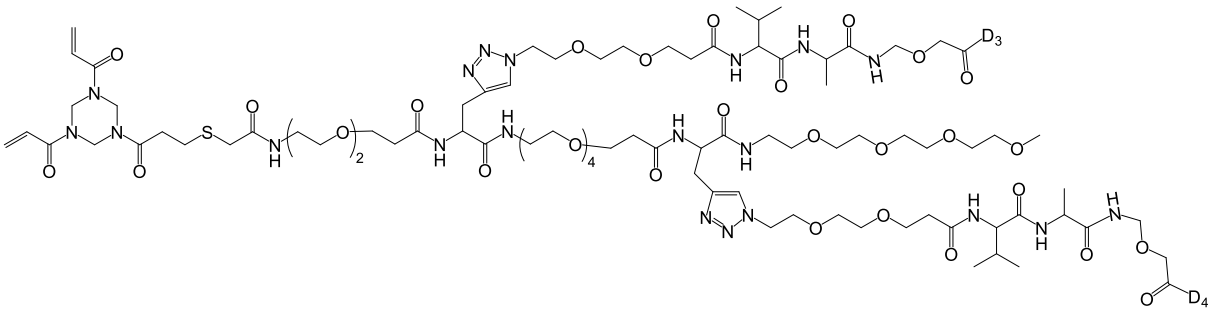

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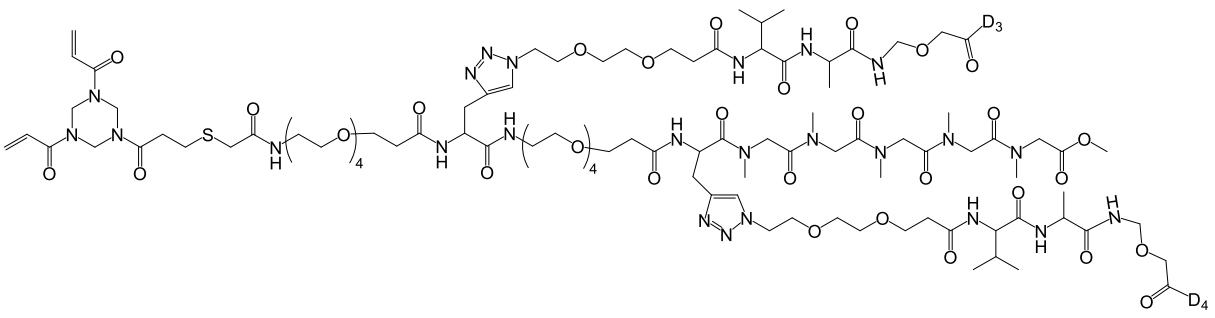

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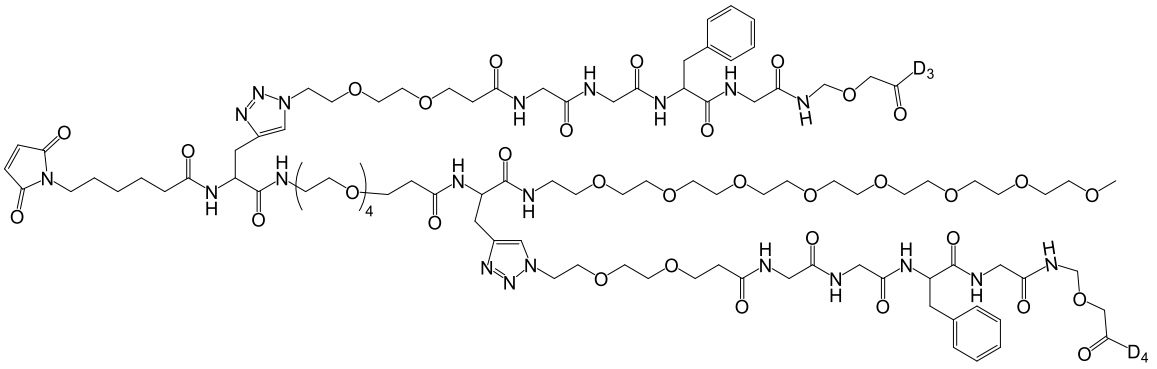

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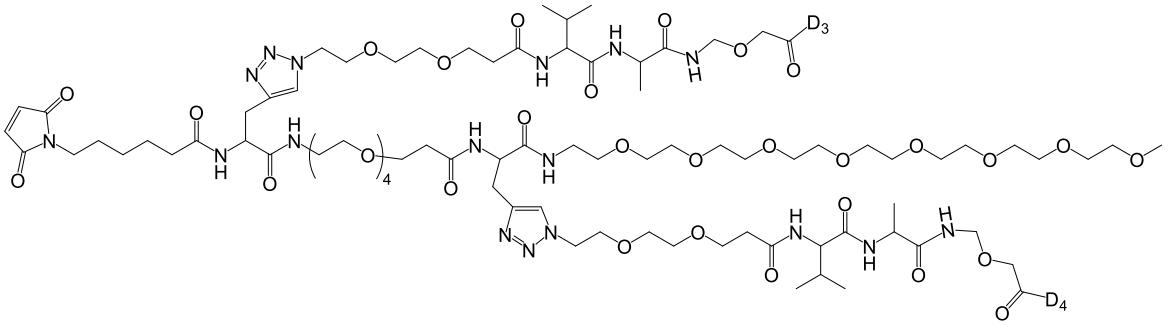

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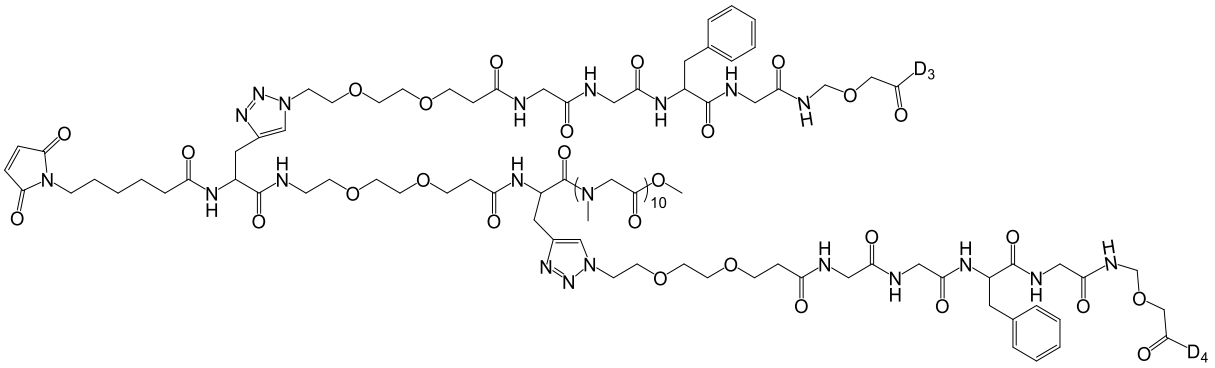

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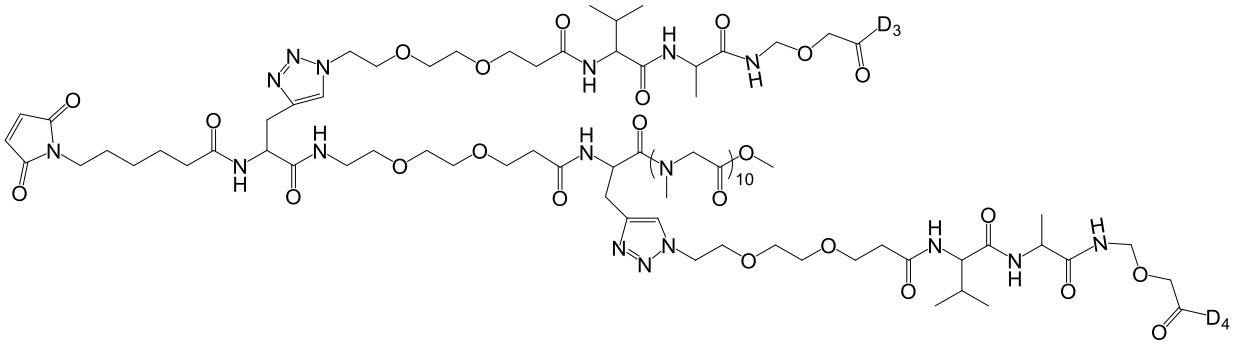

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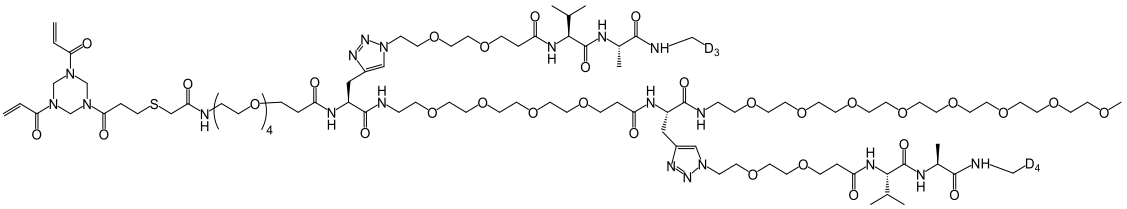
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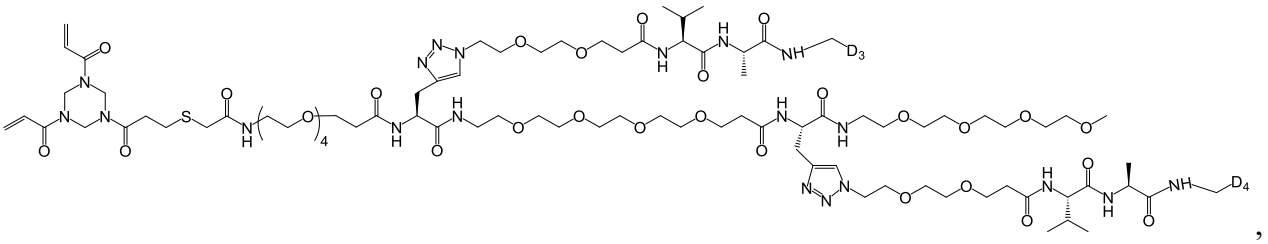
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wherein, D_3 is a first payload compound, and D_4 is a second payload compound.

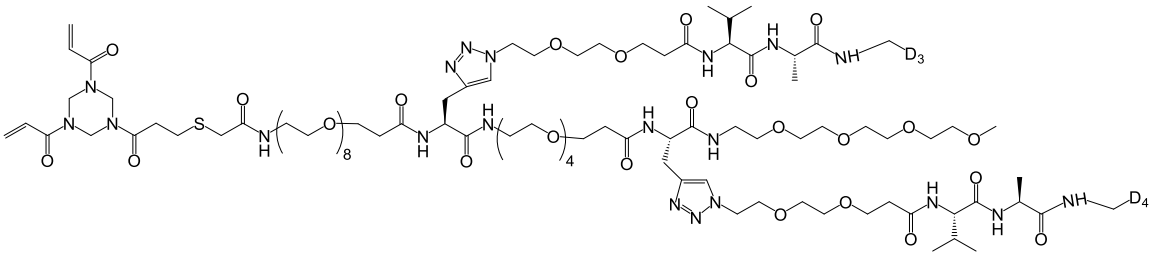
[00267] In some preferred embodiments, the compound is selected from the group consisting of



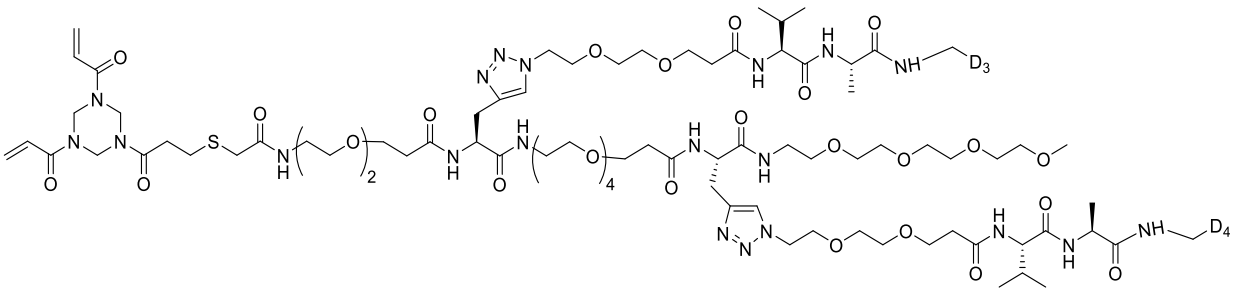
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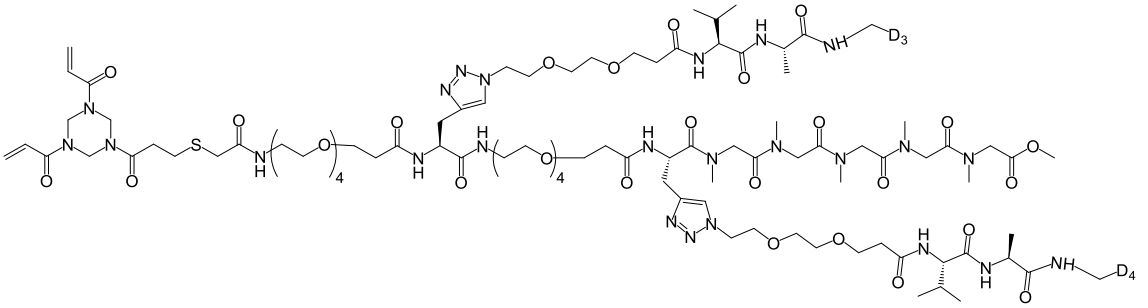
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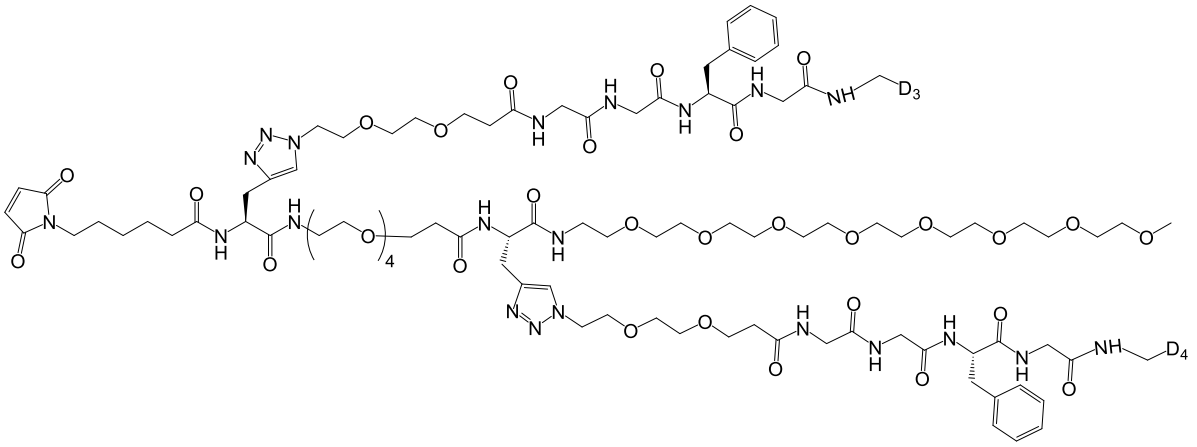
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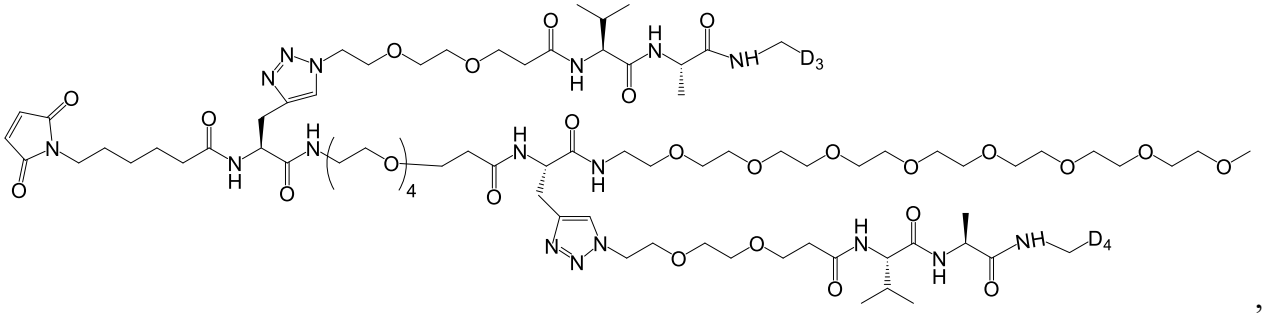
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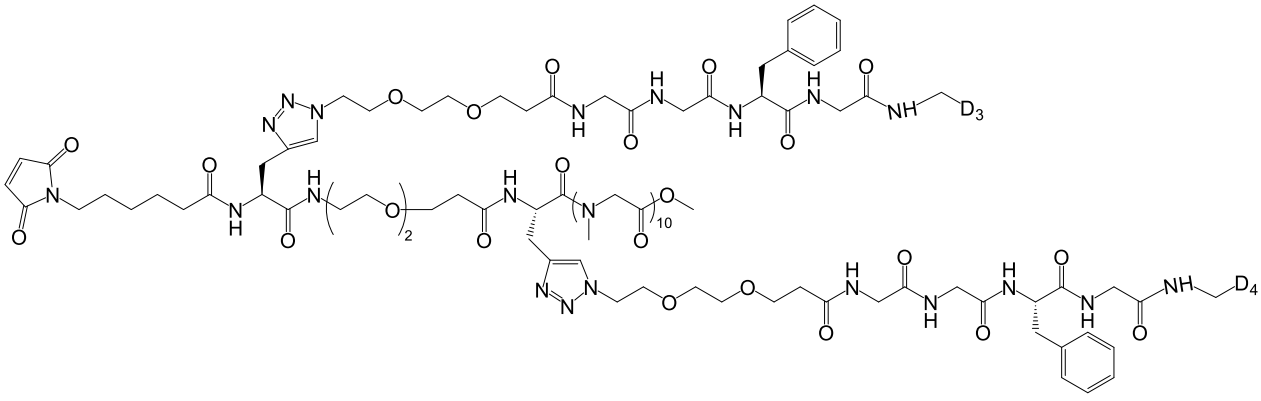
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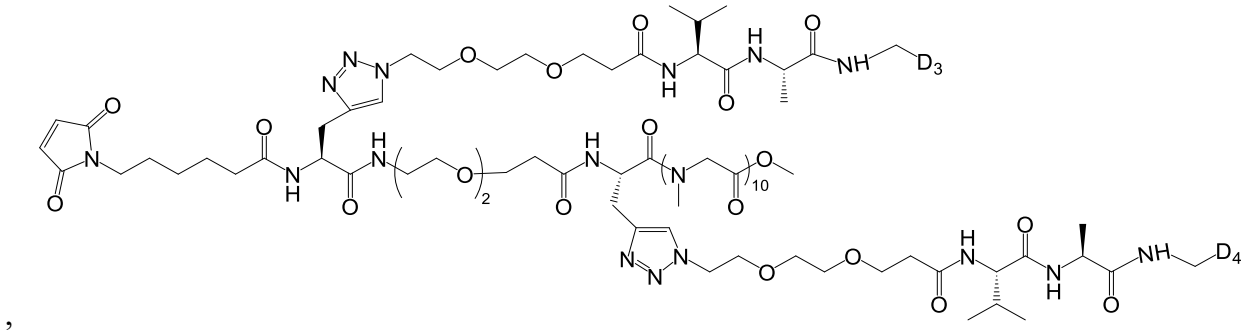


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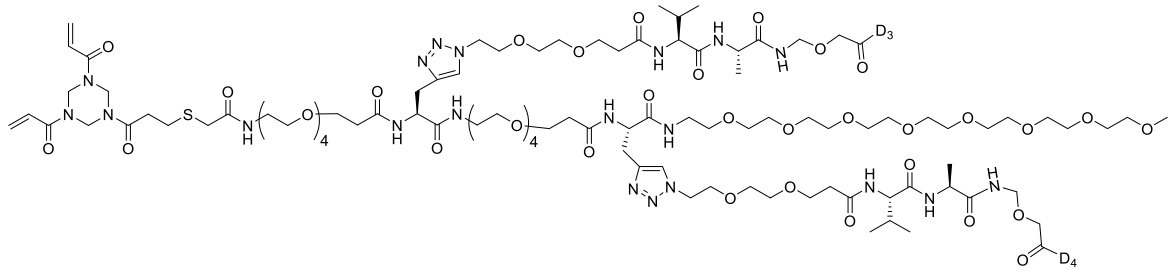


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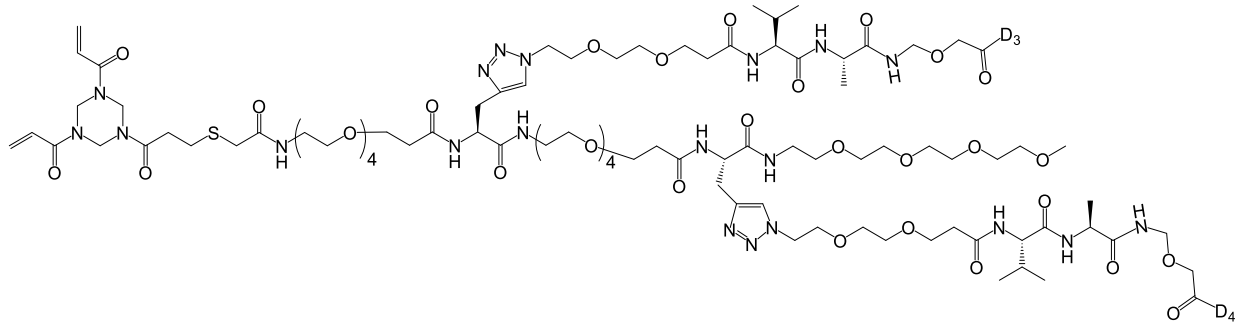




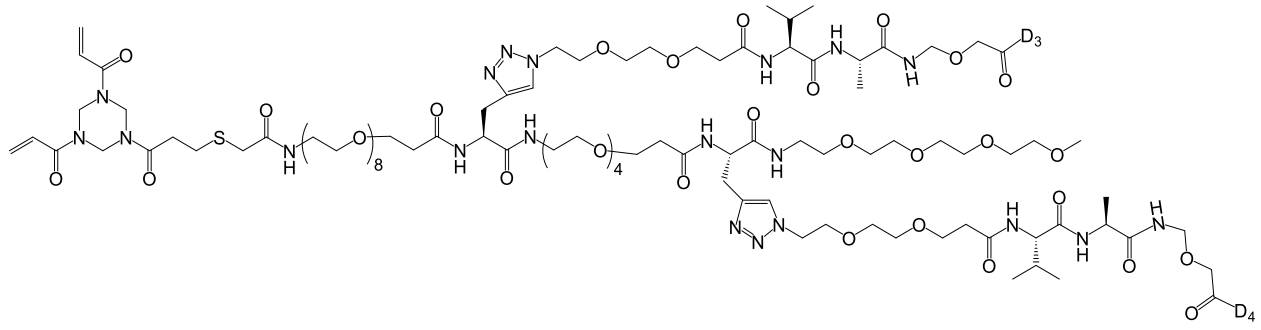
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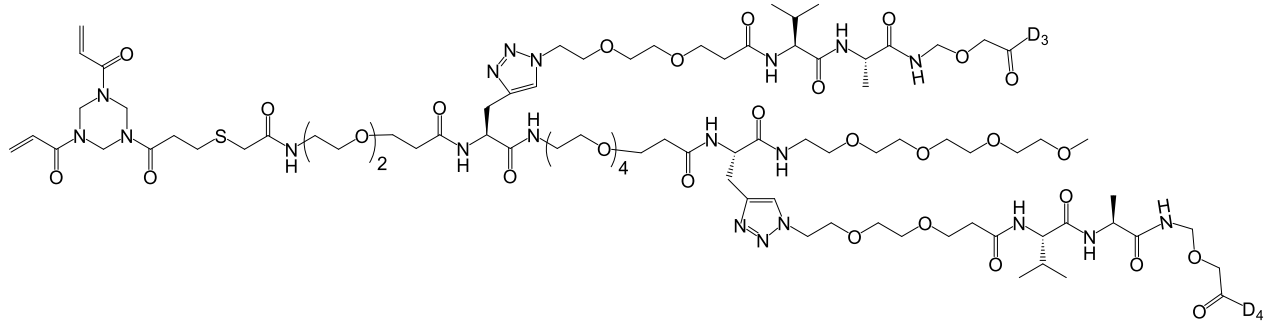


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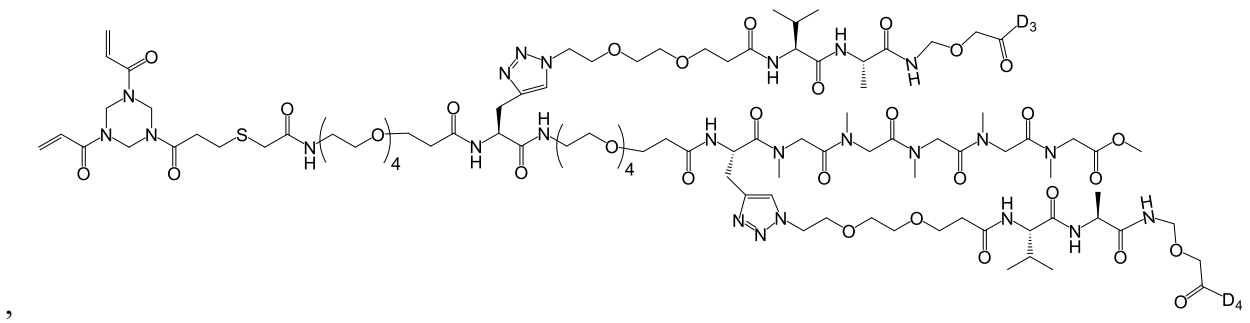


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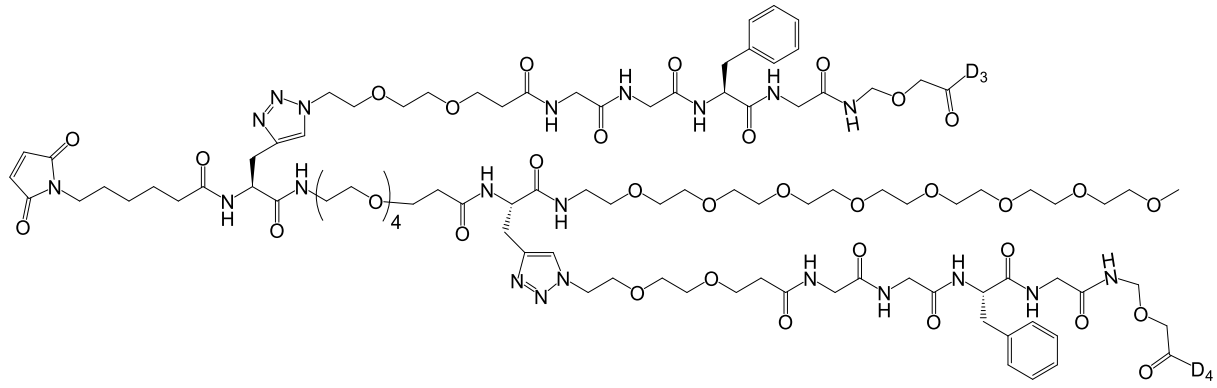




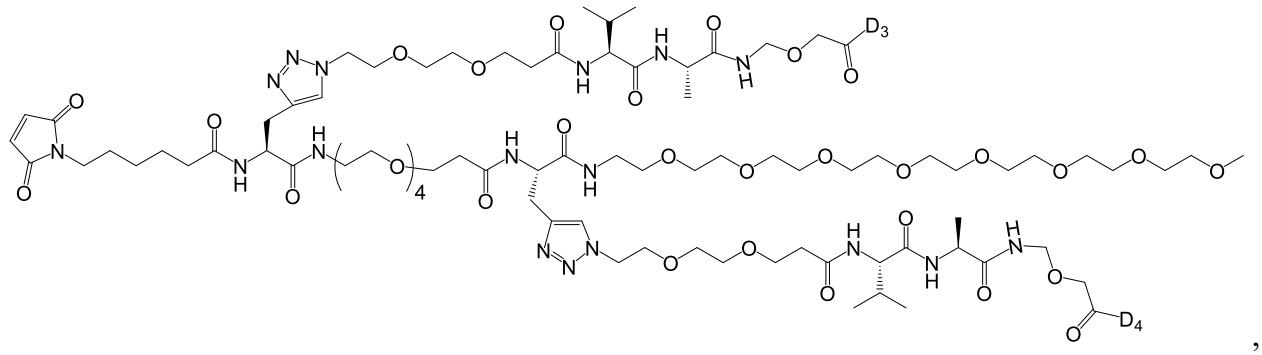
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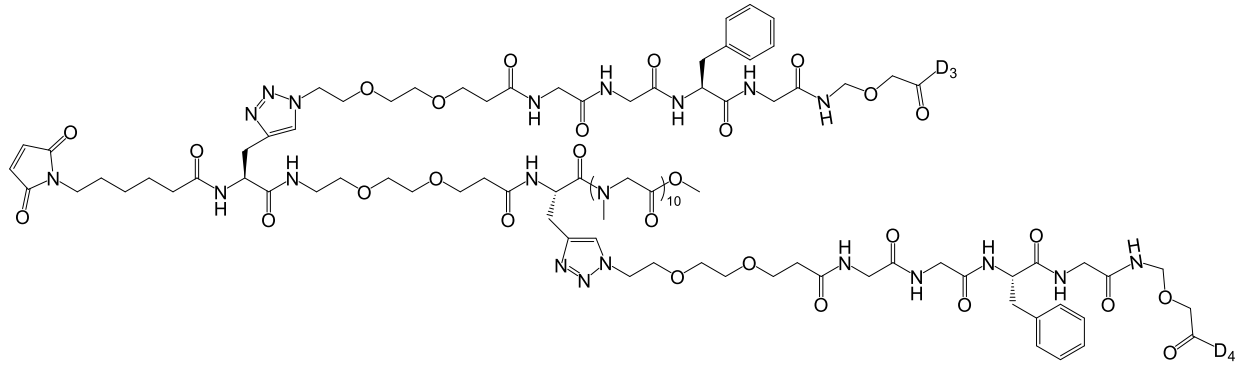
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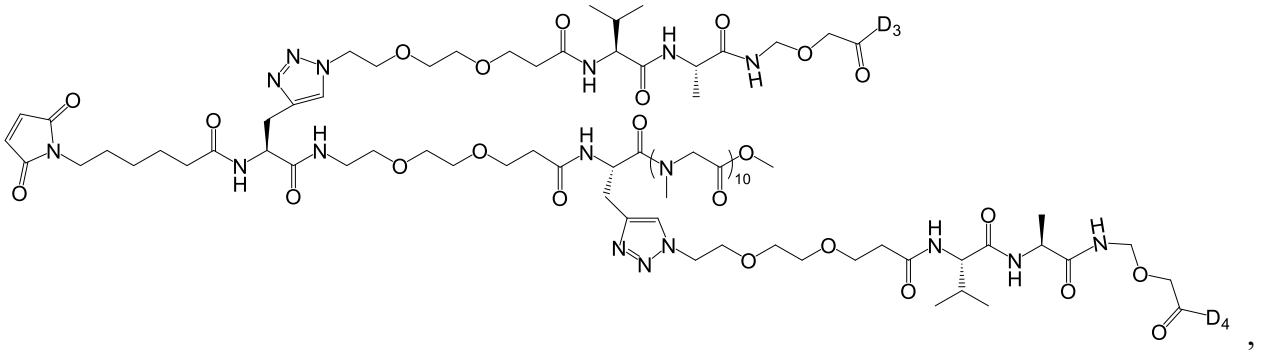
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and



wherein, D_3 is a first payload compound, and D_4 is a second payload compound.

[00268] In an aspect, provided is a stereoisomer, pharmaceutically acceptable salt, or solvate of the compound described above.

[00269] In an aspect, provided is use of the linker described above in the manufacture of an antibody drug conjugate.

[00270] In an aspect, provided is use of the linker-payload described above in the manufacture of an antibody drug conjugate.

[00271] In an aspect, provided is use of the compound described above in the manufacture of an antibody drug conjugate.

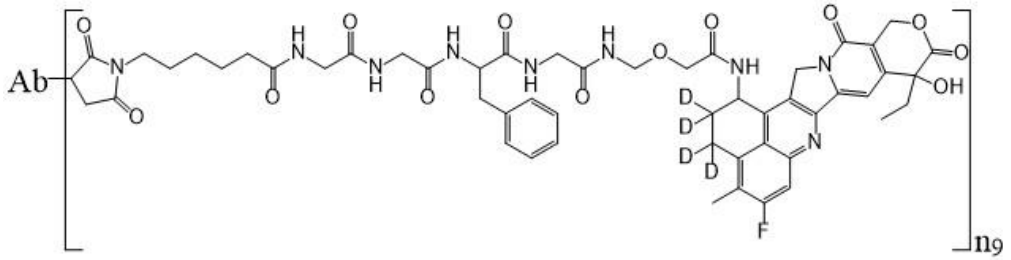
[00272] In an aspect, provided is an antibody drug conjugate comprising a targeting moiety, a linker and a payload, wherein the linker is selected from the linker described in any one of the above, and/or the payload is selected from the compound described in any one of the above.

[00273] In some embodiments, the targeting moiety of the antibody drug conjugate is the antibody or the antigen binding fragment thereof described in any one of the above.

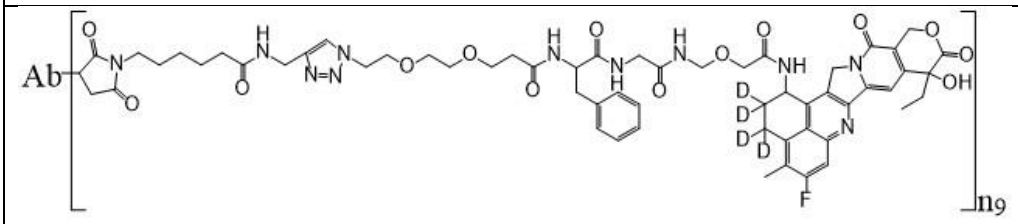
[00274] In some embodiments, the linker-payload of the antibody drug conjugate is the compound described in any one of the above.

[00275] In some embodiments, the antibody drug conjugate has a structure selected from the group consisting of:

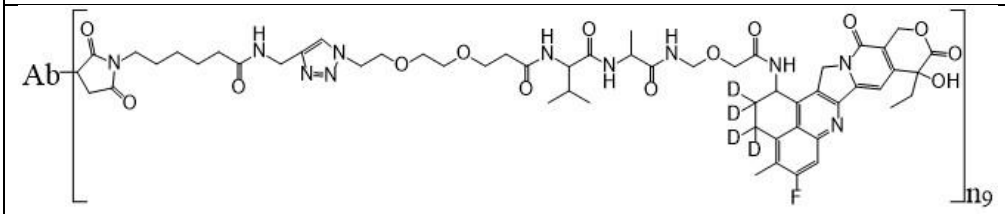
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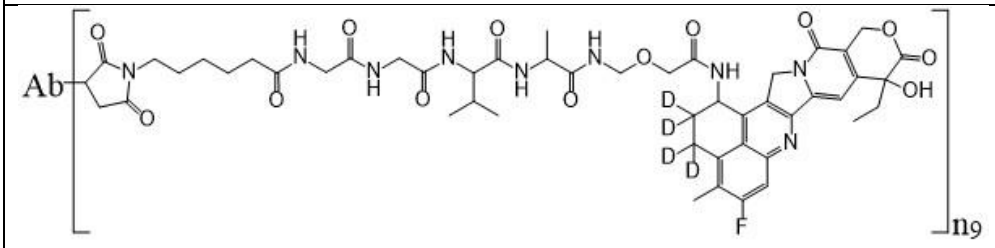
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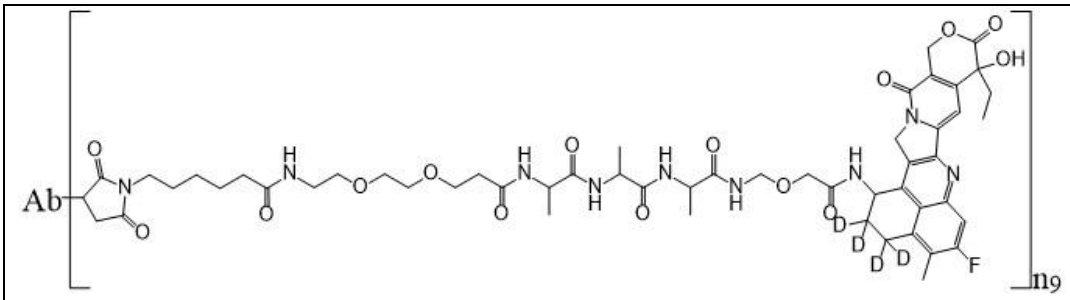
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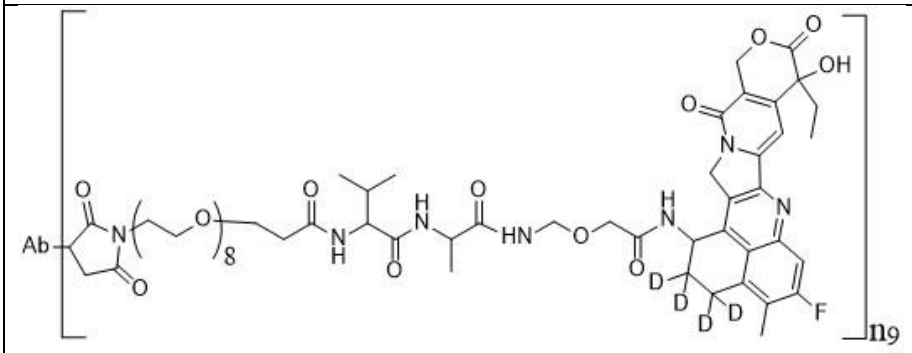
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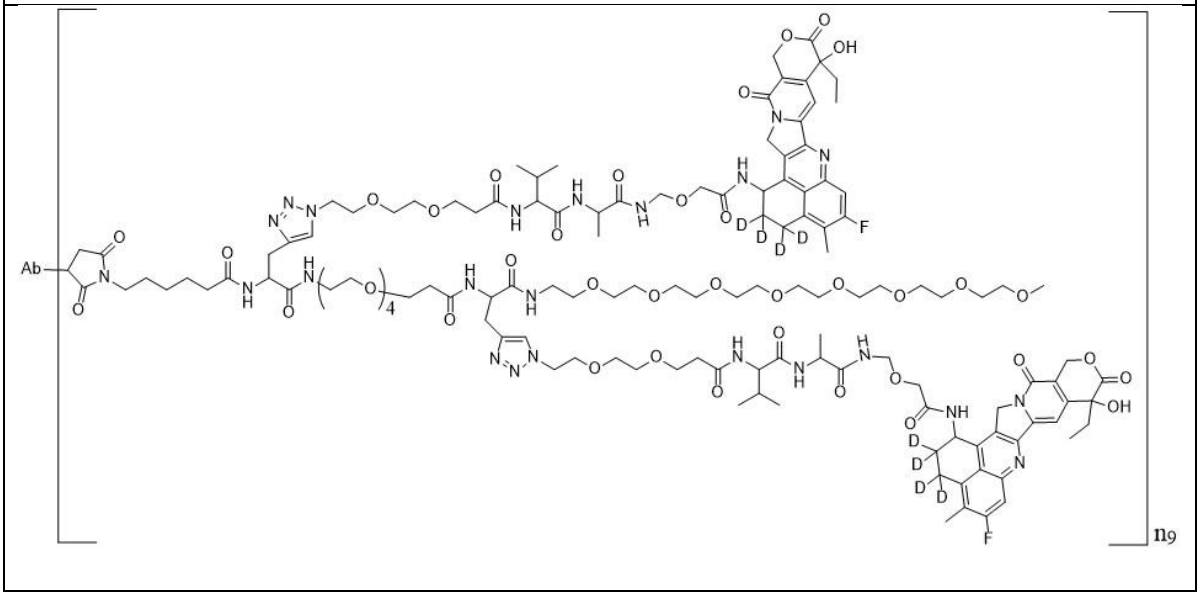
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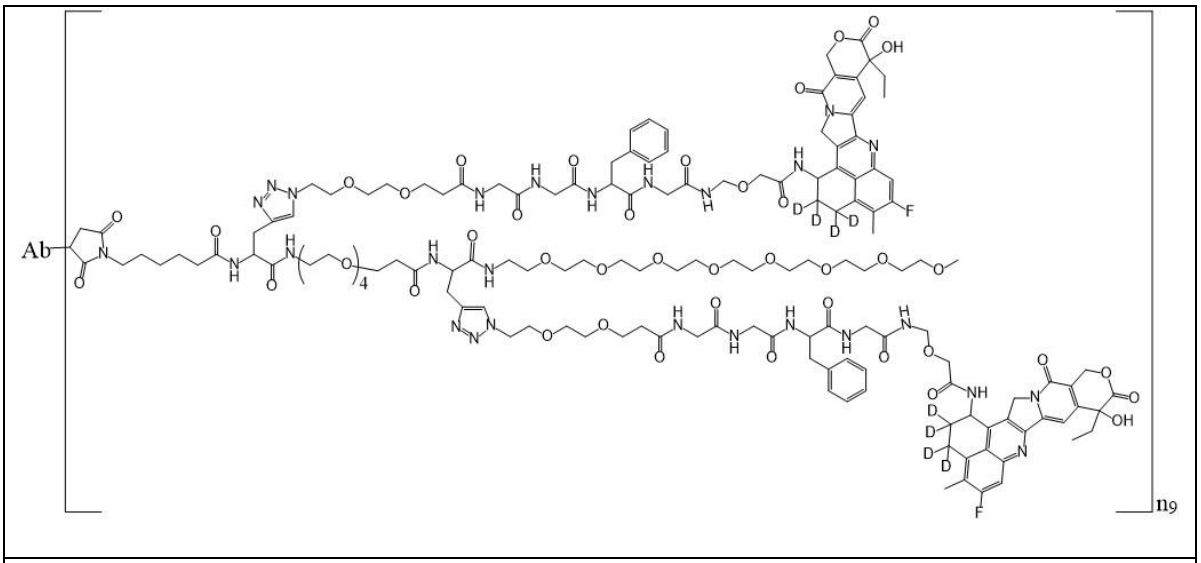
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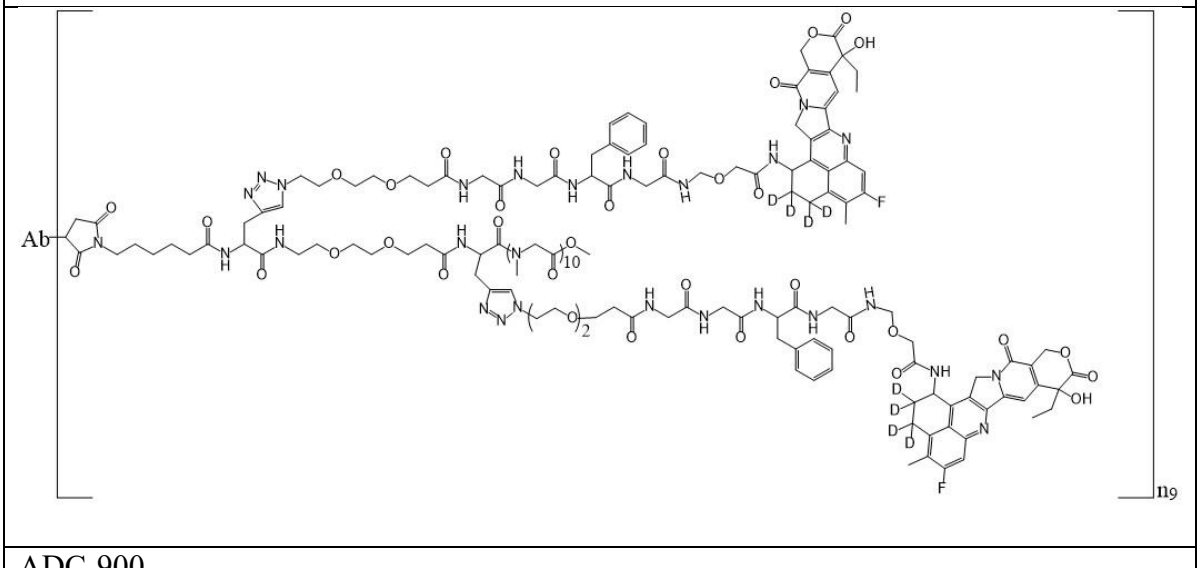
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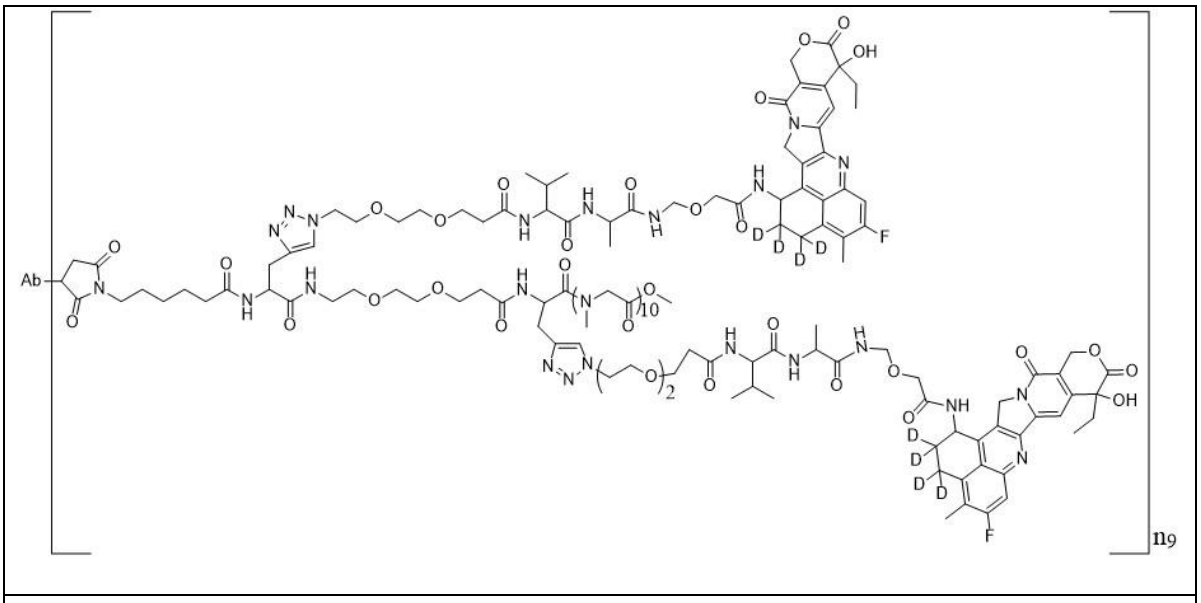
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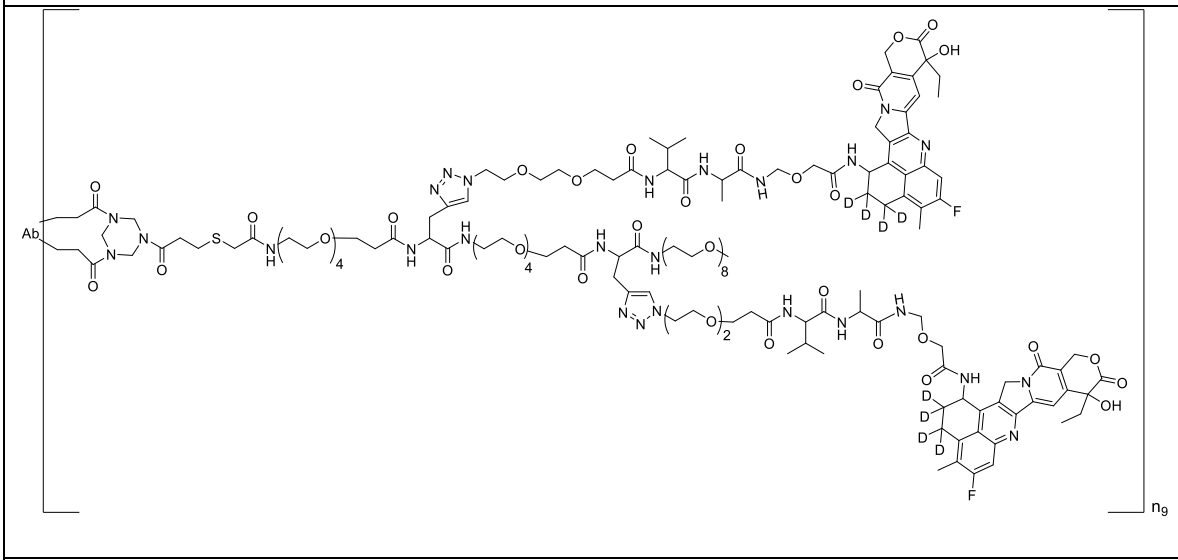
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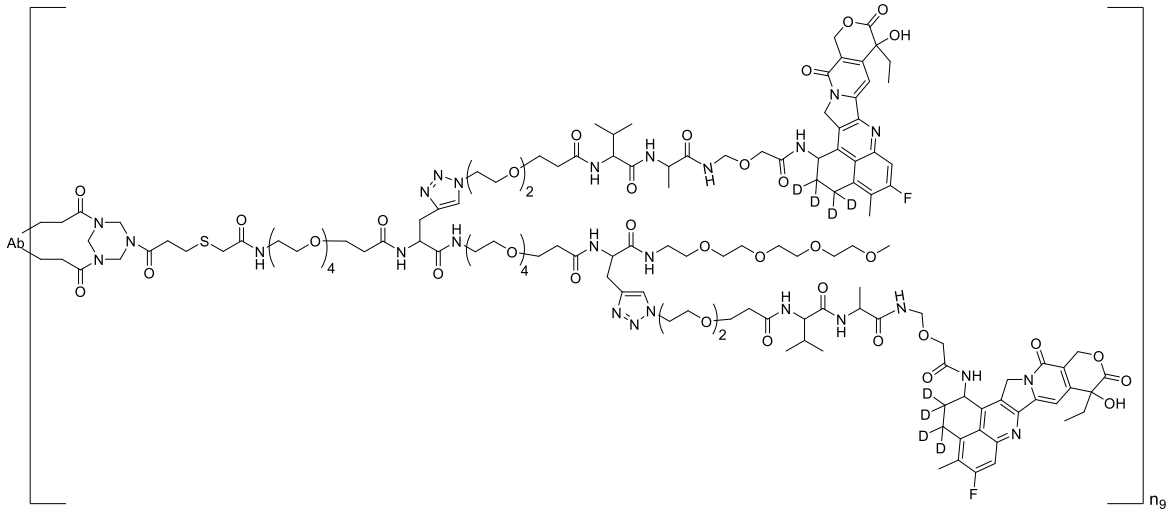
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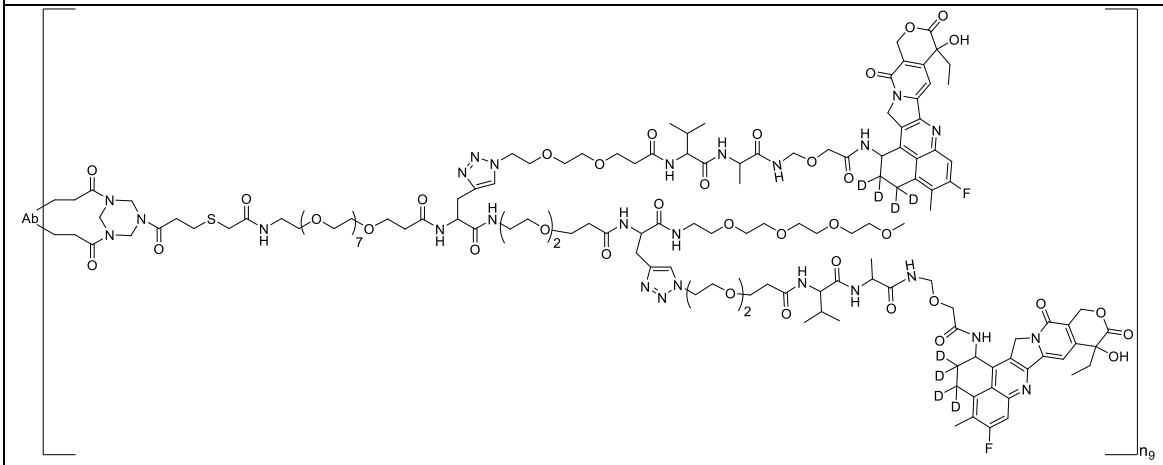
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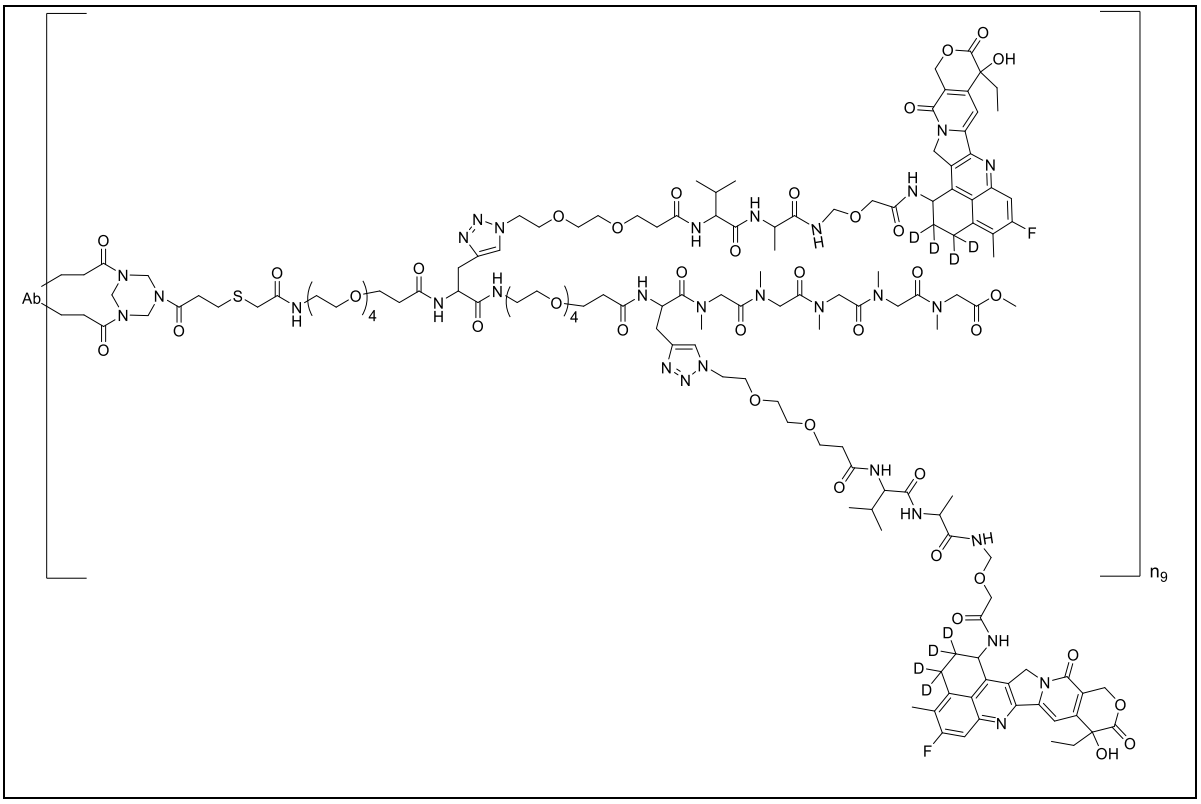
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ADC-1200

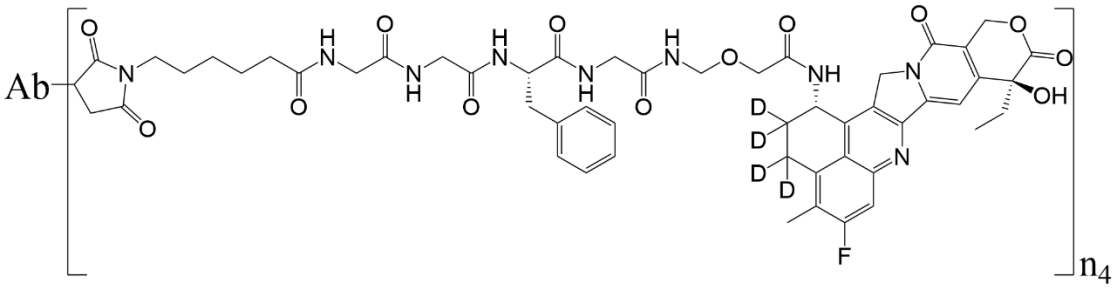


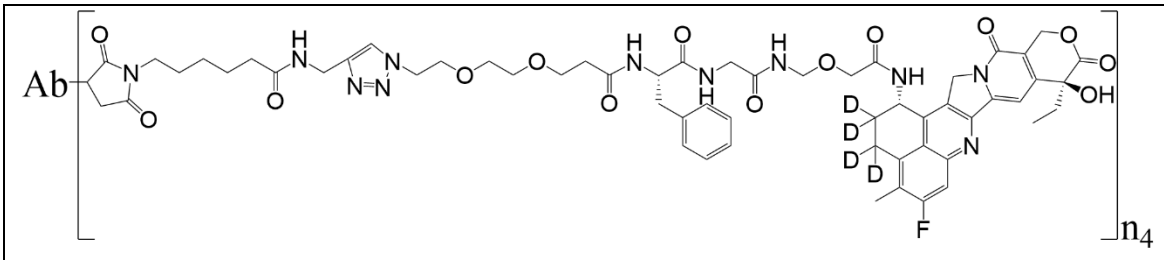
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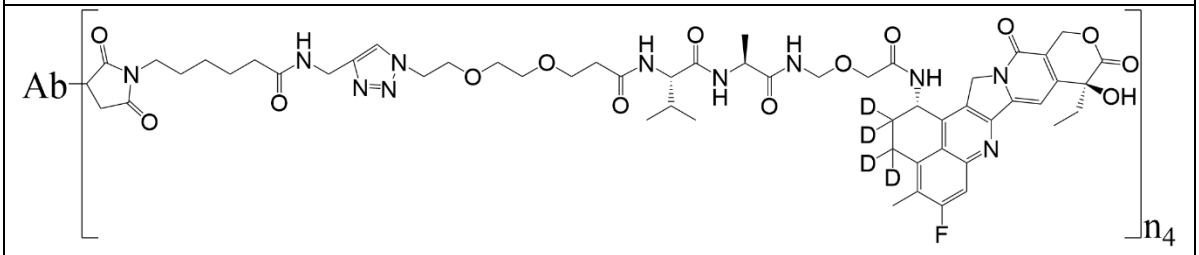
[00276] wherein Ab is a targeting moiety, and n_9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8.

[00277] In some embodiments, the antibody drug conjugate has a structure selected from the group consisting of:

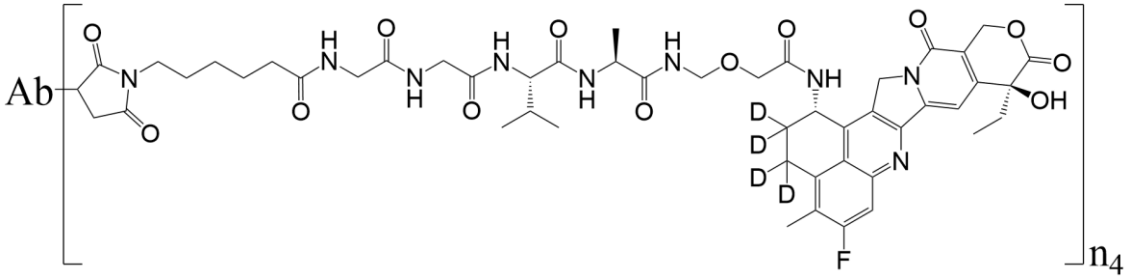
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ADC-200a



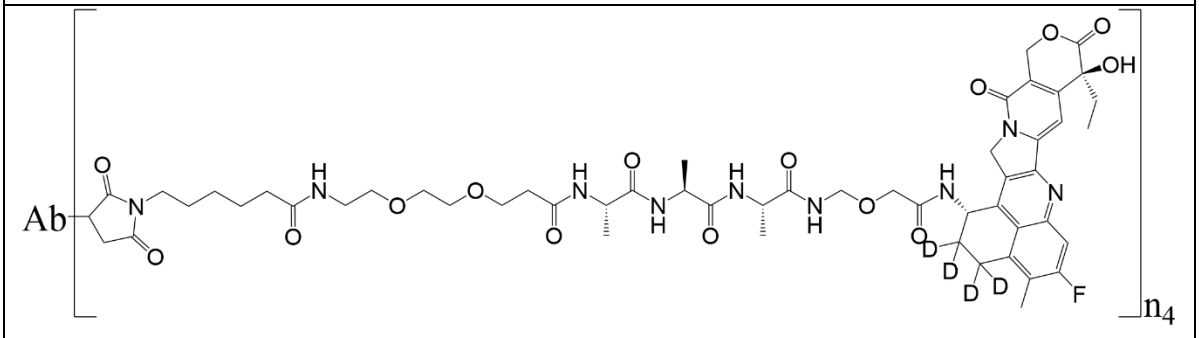
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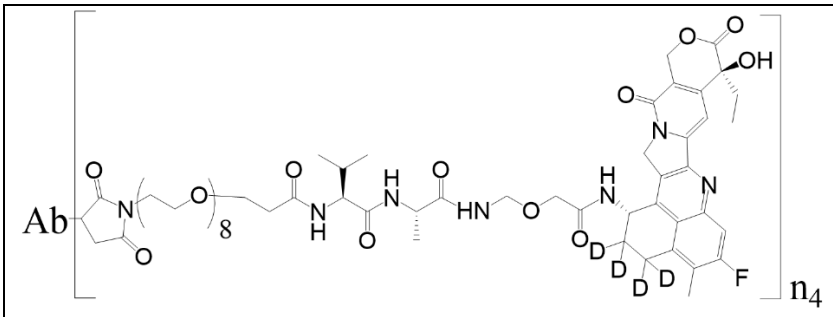
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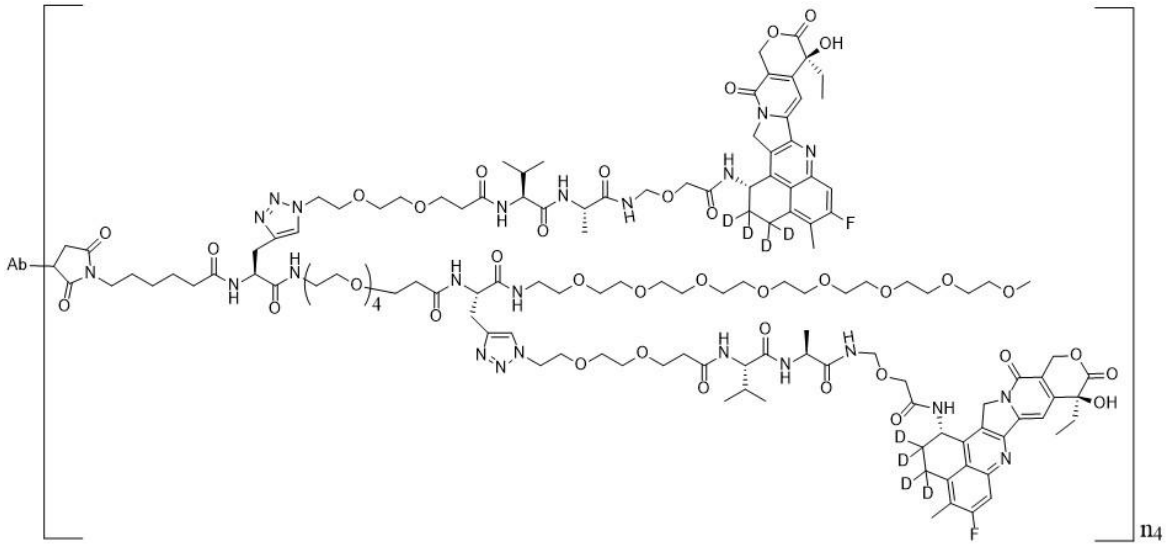
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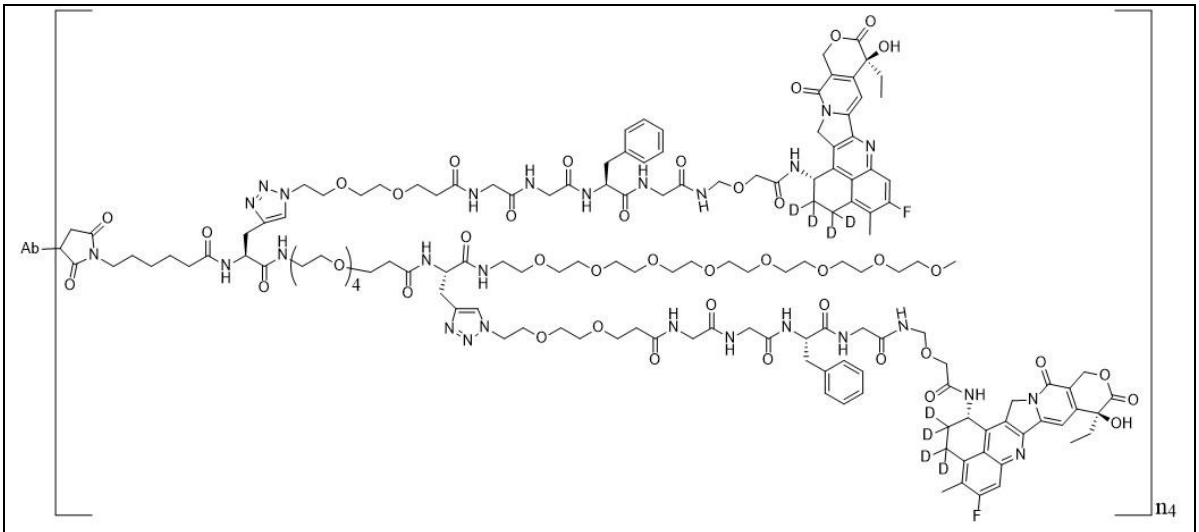
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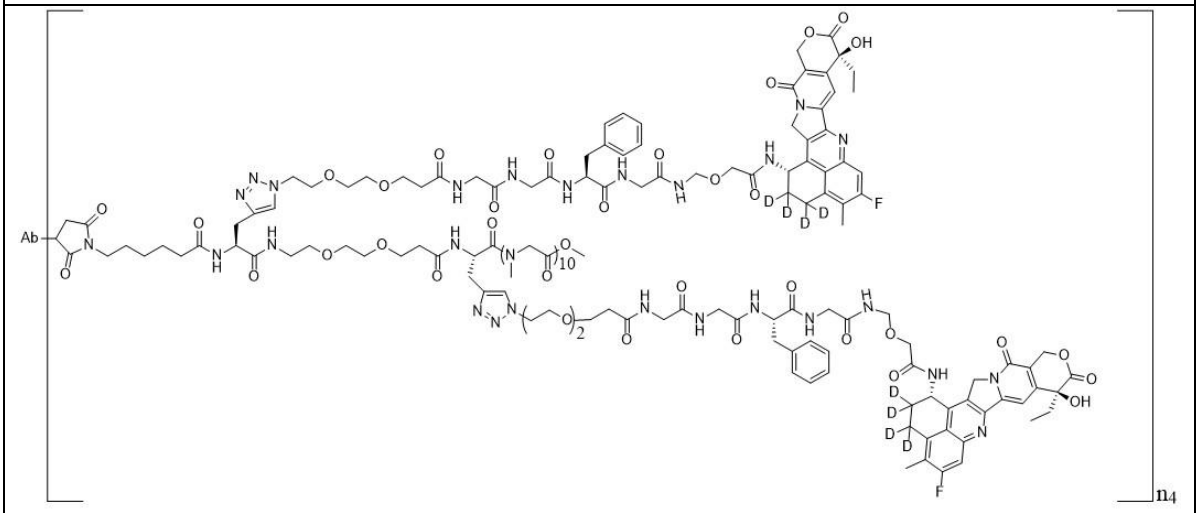
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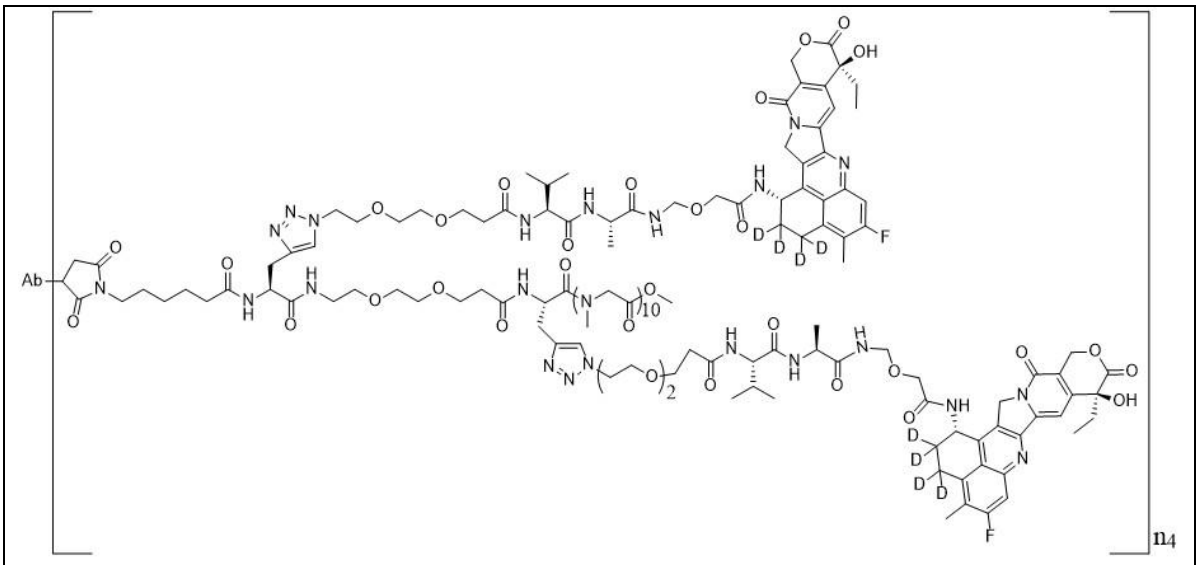
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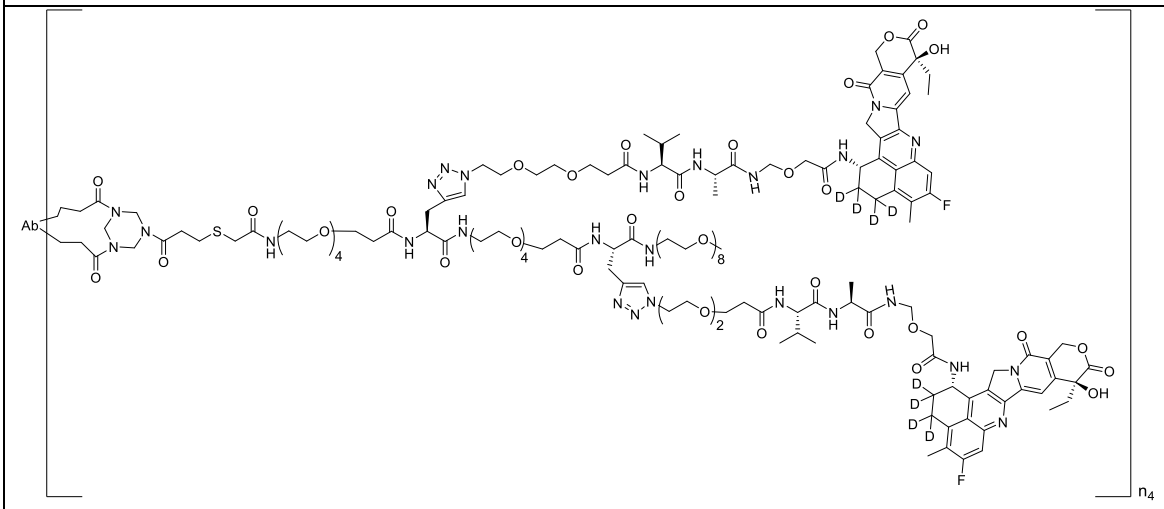
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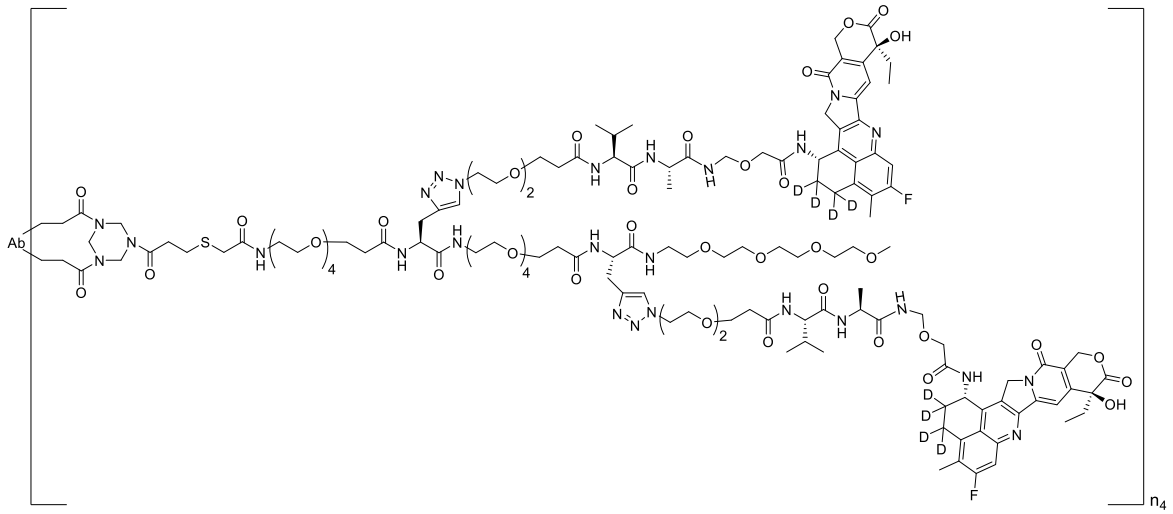
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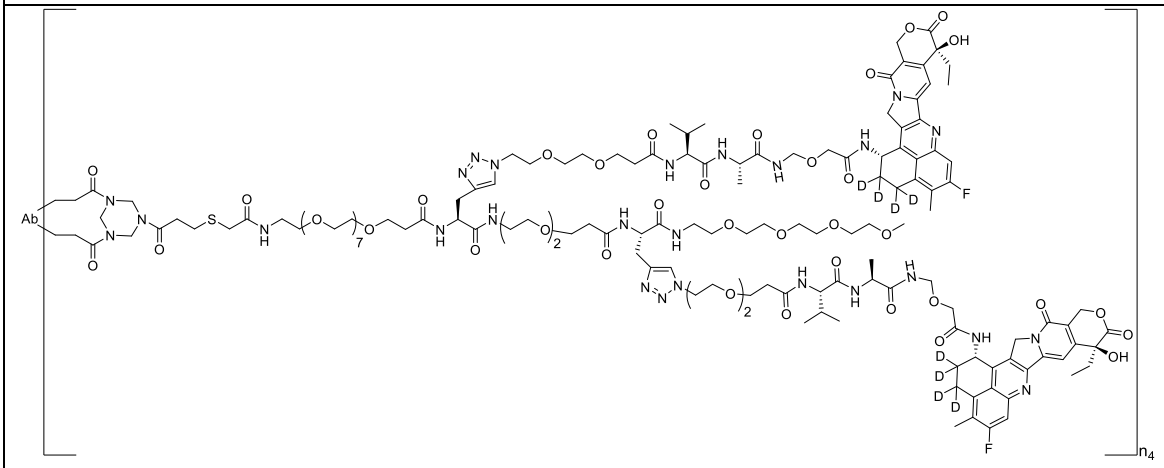
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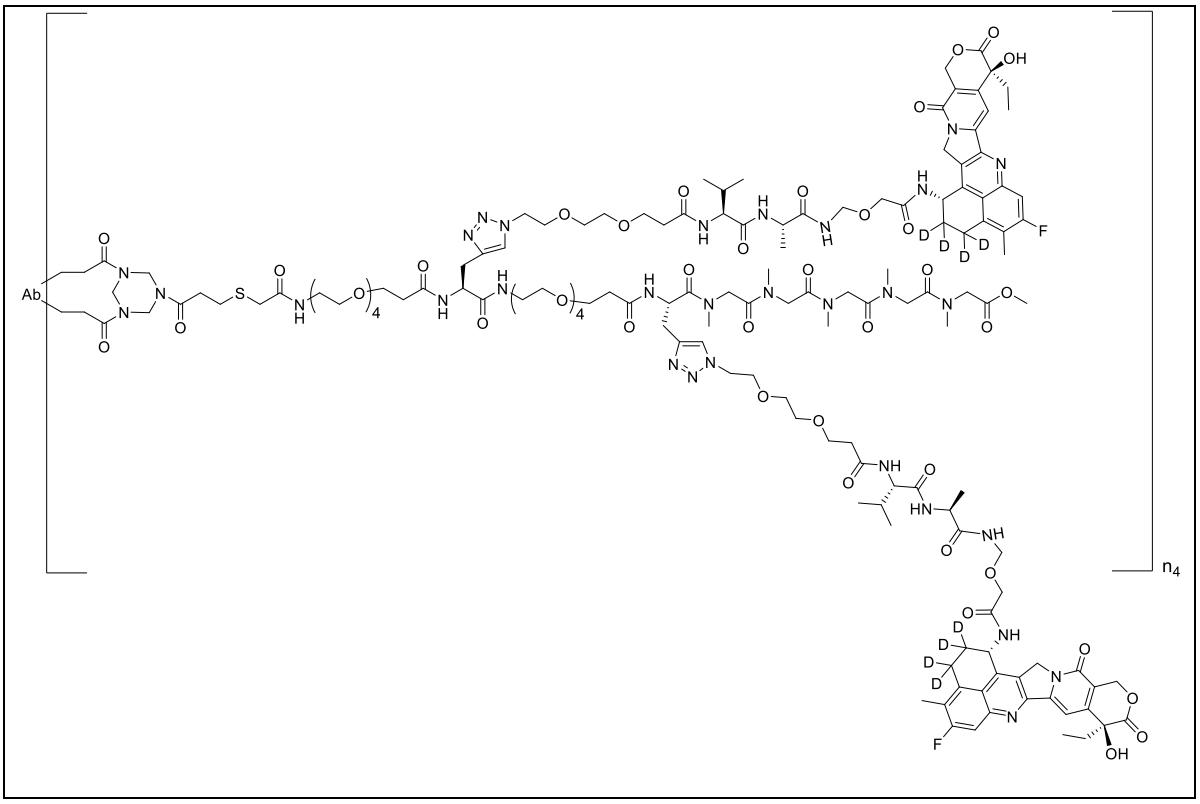
ADC-1100a



ADC-1200a



ADC-1300a



[00278] wherein Ab is a targeting moiety, and n_4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_4 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7, or 8.

[00279] In some preferred embodiments, the antibody drug conjugate has a structure of ADC-1300a, and the targeting moiety Ab is an antibody or antigen binding fragment thereof targeting CDCP1. In some more preferred embodiments, the targeting moiety Ab is the antibody or the antigen binding fragment thereof described in any one of the above described in any one of the above.

[00280] In an aspect, provided is a pharmaceutical composition comprising the antibody drug conjugate described in any one of the above and/or the antibody or the antigen binding fragment thereof described in any one of the above, and a pharmaceutically acceptable carrier.

[00281] In an aspect, provided is use of the antibody or the antigen binding fragment thereof described in any one of the above, the fusion protein described in any one of the above, the polynucleotide described in any one of the above, the nucleic acid construct described in any one of the above, the antibody drug conjugate described in any one of the above, or the pharmaceutical

composition described in any one of the above in the manufacture of a medicament for treating or preventing a cancer.

[00282] In some embodiments, the cancer is a CDCP1 positive cancer.

[00283] In some embodiments, the CDCP1 positive cancer is lung cancer or pancreatic cancer. In some preferred embodiments, the CDCP1 positive cancer is lung squamous cell carcinoma, lung adenocarcinoma, non-small cell lung cancer or pancreatic adenocarcinoma.

[00284] In an aspect, provided is a recombinant protein comprising the antibody or the antigen binding fragment thereof described in any one of the above.

[00285] In some embodiments, the recombinant protein is a bispecific antibody or multispecific antibody.

[00286] In an aspect, provided is use of the antibody or the antigen binding fragment thereof described in any one of the above in the manufacture of a recombinant protein.

[00287] All references cited herein, including patent documents, patent applications, and various publications, are hereby incorporated by reference in their entireties, to the same extent as if each reference is individually and specifically indicated to be incorporated by reference and is set forth in its entirety herein.

[00288] The citation and incorporation of patent documents herewith is made for convenience only and does not reflect any view of the validity, patentability, and/or enforceability of such patent documents.

[00289] All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way.

[00290] The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illustrate the present disclosure and does not pose a limitation on the scope of the present disclosure. Unless otherwise specified, no language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present disclosure.

[00291] The present disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law.

BRIEF DESCRIPTION OF DRAWINGS

[00292] FIG. 1 shows the internalization efficiency of ADCs using Dxd versus dDxd as payloads in EBC-1 cells (human lung adenocarcinoma cell line), with both conjugates containing the same antibody (Ab1) and linker (DL01).

[00293] FIG. 2 shows the internalization efficiency of ADCs using Dxd versus dDxd as payloads in BxPC-3 cells (human orthotopic pancreatic cancer cell line), with both conjugates containing the same antibody (Ab2) and linker (DL01).

[00294] FIG. 3 shows data of tumor growth inhibition by anti-CDCP1 antibody drug conjugates (with MMAE as payload) in the EBC-1 (human lung adenocarcinoma cell line) xenograft model.

[00295] FIG. 4 shows data of tumor growth inhibition by anti-CDCP1 antibody drug conjugates (with MMAE as payload) in the BxPC-3 (human orthotopic pancreatic cancer cell line) xenograft model.

[00296] FIG. 5 shows data of tumor growth inhibition by anti-CDCP1 antibody drug conjugates (with a camptothecin payload) at a dose of 5 mg/kg in the EBC-1 (human lung adenocarcinoma cell line) xenograft model.

[00297] FIG. 6 shows data on tumor growth inhibition by anti-CDCP1 antibody drug conjugates (with a camptothecin payload) at a dose of 2.5 mg/kg in the EBC-1 (human lung adenocarcinoma cell line) xenograft model.

[00298] FIG. 7 shows data of toxicity tolerance of anti-CDCP1 antibody drug conjugates (with dDxd or Dxd as payload).

DETAILED DESCRIPTION

Definition

[00299] Unless otherwise specifically stated, the practice of the present disclosure will be carried out using conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA technology within the technical scope of the art or as generally understood by

those of ordinary skill in the art to which the present disclosure belongs. Many of these are described below for illustrative purposes, and such techniques are fully described in the literatures.

[00300] Unless otherwise indicated, the term "alkyl" as used herein, by itself or as part of another term, refers to a substituted or unsubstituted linear or branched, saturated or unsaturated hydrocarbon having the specified number of carbon atoms (for example, -C_1-C_8 alkyl refers to an alkyl group with 1 to 8 carbon atoms). Representative linear "-C_1-C_8 alkyl" includes but is not limited to -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl; -n-heptyl and -n-octyl, and branched -C_3-C_8 alkyl includes but is not limited to -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isoamyl, and -2-methylbutyl; unsaturated -C_2-C_8 alkyl includes but is not limited to -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutenyl, -1-isopentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, -1-hexenyl, -2-hexenyl, -3-hexenyl, -ethynyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl and-3-methyl-1-butynyl. The above alkyl groups may be unsubstituted, or may be substituted by one or more groups.

[00301] Unless otherwise indicated, the term "C_1-C_8 heteroalkyl" as used herein, by itself or as part of another term, means fully saturated or, containing from 1 to 3 degrees of unsaturation, stable linear or branched hydrocarbons or combinations thereof comprising the stated number of carbon atoms and 1 to 8, preferably 1 to 3 heteroatoms selected from the group consisting of O, N and S, wherein the nitrogen and sulfur atoms may be optionally oxidized, and the nitrogen heteroatoms may optionally be quaternized. The heteroatoms O, N, and S may be located at any internal position of the heteroalkyl group or at the position of the alkyl group attaching with the rest moiety of the molecule. Typical heteroalkyl includes but is not limited to: -O-CH_3, -CH_2-O-CH_3, -CH_2-CH_2-O-CH_3, -NH-CH_3, -CH_2-NH-CH_3, -CH_2-N(CH_3)-CH_3, -S-CH_3, -CH_2-S-CH_3 and -CH_2-CH_2-S-CH_3. Typically, C_1 to C_4 heteroalkyl has 1 to 4 carbon atoms and 1 to 2 heteroatoms.

[00302] Unless otherwise indicated, the term "C_3-C_8 cycloalkyl" as used herein, by itself or as part of another term, is a monovalent, including but not limited to 3-, 4-, 5-, 6-, 7- or 8-membered, substituted or unsubstituted, saturated or unsaturated non-aromatic monocyclic or bicyclic carbocyclic ring obtained by removing one hydrogen atom from a ring atom of the parent ring system. Representative C_3-C_8 cycloalkyl includes but is not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, 1,3-cyclohexadienyl, 1,3,5-cycloheptatrienyl, cyclooctyl and cyclooctadienyl.

[00303] Unless otherwise indicated, the term "C_3-C_8 heterocycloalkyl" as used herein, by itself or as part of another term, refers to a monovalent, substituted or unsubstituted, aromatic or non-aromatic, monocyclic or bicyclic ring system having 3 to 8 carbon atoms (also called ring members) and one to four heteroatom ring members independently selected from the group consisting of N, O, P, and S, obtained by removing one hydrogen atom from a ring atom of the parent ring system. A heterocycle in which all ring atoms are involved in the structure of an aromatic compound is called a heteroaryl, otherwise it is called a heterocarbocycle.

[00304] Unless otherwise indicated, "any functional group" in the term "a group substituted by any functional group" as used herein includes but is not limited to the following groups: -halogen, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -NO_2, -N_3, -NH_2, -NH(R'), - N(R')$_2$ and -CN, wherein R' is each independently selected from the group consisting of -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl and -aryl, wherein -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), and -aryl may be further optionally substituted by one or more groups, including but not limited to: -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R", -OC(O)R", -C(O)OR", -C(O)NH_2, -C(O)NHR", -C(O)N(R")$_2$, -NHC(O)R", -SR", -SO_3R", -S(O)$_2$R", -S(O)R", -OH, -N_3, -NH_2, -NH(R"), -N(R")$_2$ and -CN, wherein R" is each independently selected from the group consisting of -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl and -aryl.

[00305] The term "amino acid" as used herein includes natural amino acids, molecules containing N and carboxylic acids capable of forming amide bonds, molecules of the general formula NH2-CHR-COOH, or molecules with residues within peptides of the parent amino acids, where "R" is one of many different side chains. "R" can be a substituent found in natural amino acids. "R" may also refer to substituents that are not in natural amino acids.

[00306] The term "amino acid residue" as used herein refers to a moiety of an amino acid that remains after the loss of a water molecule when the amino acid is connected to another amino acid.

[00307] The term "stereoisomer" as use herein refers to compounds that have the same chemical composition and connectivity but have different positions of their atoms in space that cannot be

converted into each other by rotating around a single bond.

[00308] The term "pharmaceutically acceptable salt" as used herein refers to a pharmaceutically acceptable organic or inorganic salt of a compound (linker-drug or its ligand drug conjugate). Exemplary salts include, but are not limited to, sulfate, trifluoroacetate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, tartrate, oleate, bitartrate, ascorbate, succinate, maleate, gentisate, fumarate, gluconate, glucuronate, sucrose salt, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamate. Pharmaceutically acceptable salts may involve the introduction of another molecule such as an acetate ion, a succinate ion, or other counterion which may be any organic or inorganic moiety that will stabilize the charge on the parent compound. Additionally, pharmaceutically acceptable salts may have more than one charged atoms in their structure. In cases where multiple charged atoms are part of a pharmaceutically acceptable salt, there may be multiple counterions. Therefore, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterions.

[00309] The term "antibody drug conjugate" as used herein refers to a substance obtained by connecting a biologically active molecule (drug molecule) to a targeting unit. In some embodiments of the present disclosure, the biologically active molecule is connected to the targeting unit through a linker that can be cleaved in a specific environment (such as hydrolase and/or pH environment within the tumor) and under a specific action (such as the action of lysosomal proteases), thereby separating the biologically active molecule from the targeting unit. In some embodiments of the present disclosure, the linker comprises cleavable or non-cleavable units, such as peptides or disulfide bonds. In some embodiments of the present disclosure, the biologically active molecule is directly connected to the targeting unit through a covalent bond, which can be broken under specific circumstances or effects, thereby separating the biologically active molecule from the targeting unit.

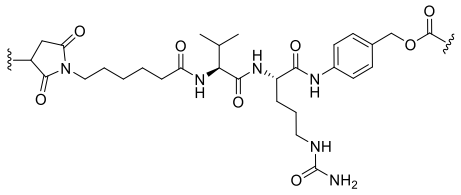
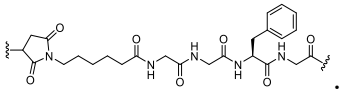
[00310] The term "targeting moiety" as used herein refers to a structure that binds or associates with a biological part or a fragment thereof, including but not limited to antibody fragments or functional fragments, substitutes, variants, protein ligands, protein scaffolds, RNA, DNA, RNA or DNA fragments, small molecule ligands or antigen-binding fragments.

[00311] The term "monoclonal antibody" as used herein includes, but is not limited to, human monoclonal antibodies, humanized monoclonal antibodies, or chimeric human-mouse (or other species) monoclonal antibodies. Antibodies include full-length antibodies and antigen-binding fragments thereof. Human monoclonal antibodies can be prepared by any of a variety of techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. USA. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; and Olsson et al., 1982, Meth. Enzymol. 92:3-16).

[00312] The term "antibody" as used herein may be a functionally active fragment, derivative or analog of an antibody that will immunospecifically bind to a target cell (e.g., a cancer cell antigen, a viral antigen, or a microbial antigen), or other antibodies that bind to tumor cells or matrix, wherein the "functionally active" refers to the ability of the fragment, derivative or analog to immunospecifically bind to target cells. Other available antibodies include, but are not limited to, fragments of antibodies, such as, but not limited to, $F(ab')_2$ fragments, Fab fragments, Fvs, single-chain antibodies, double-chain bodies, triple-chain antibodies, quadruple-chain antibodies, scFv, scFv-FV, bispecific antibodies, multispecific antibodies, or any other molecule with the same specificity as an antibody. Antibodies include modified analogs and derivatives, i.e., analogs and derivatives modified by covalent attachment of any type of molecule, as long as such covalent attachment allows the antibody to retain its antigen-binding immunospecificity. For example, derivatives and analogs of antibodies include but are not limited to those that have been further modified, e.g., by glycosylation, acetylation, PEGylation, phosphorylation, amidation, derivatization with known protecting/blocking groups, proteolytic cleavage, attachment to cellular antibody units or other proteins, and the like. Any of numerous chemical modifications can be performed by known techniques, including but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, and the like. Antibodies immunospecific for cancer cell antigens are commercially available or produced by any method known to those skilled in the art, such as recombinant expression techniques. Nucleotide sequences encoding antibodies immunospecific for cancer cell antigens can be obtained, for example, from the GenBank database or similar databases, literature publications, or by routine cloning and sequencing.

[00313] The term "antigen-binding fragment" as used herein primarily refers to the functional fragment of an antibody that can specifically bind to an antigen, such as scFv (single chain

antibody), Fv, Fab, F(ab')2, Fab', scFv-Fc, single domain antibody, antibody analog, bispecific antibody, multispecific antibody, or any antibody fragment through chemical modification.

[00314] The term "linker" as used herein has two reactive ends, with one end for linking or connecting a biological moiety or a fragment thereof, such as a targeting moiety, and the other end for coupling, for example, a drug. Unless otherwise indicated, the linker "-MC-VC-PAB-" of the present disclosure has a structure of . Unless otherwise indicated, the linker "-MC-GGFG-" of the present disclosure has a structure of .

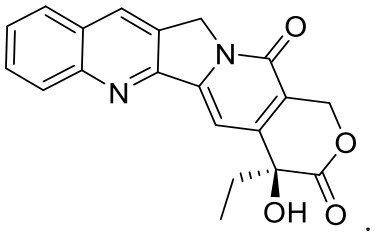
[00315] The term "cancer" as used herein refers to or describes a physiological condition or disorder in mammals that is typically characterized by uncontrolled cell growth, and "tumor" includes one or more cancer cells.

[00316] The term "therapeutic agent" as used herein refers to a cytotoxic agent, an immune enhancer, a radioactive isotope, a cell growth inhibitor, a tubulin inhibitor or a DNA damaging agent, specifically including but not limited to a dolastatin and an auristatin cytotoxic molecule, a maytansine cytotoxic molecule, a calicheamicin, a duocarmycin, and an anthramycin derivative pyrrolobenzodiazepine (PBD), camptothecin and a camptothecin derivative, SN-38, Dxd, etc.

[00317] The present disclosure also includes compounds containing various radioactive or non-radioactive isotope atoms. The atoms constituting the compounds of the present disclosure may contain unnatural proportions of atomic isotopes or may be substituted with atomic isotopes. Examples of atomic isotopes include deuterium (^{2}H), tritium (^{3}H), and carbon-14 (^{14}C).

[00318] Unless otherwise indicated, "〰" as used herein refers to the site where the structural fragment is linked to other parts of the molecule.

[00319] Unless otherwise indicated, the terms "may be substituted by", "may comprise", "comprise" and other variants thereof as used herein are inclusive or open-ended and do not exclude other elements or method steps not listed.

[00320] Unless otherwise defined, the camptothecin derivative in the present disclosure refers to a compound containing the parent structure of camptothecin substituted by any functional group, wherein the parent structure of camptothecin is .

[00321] Unless otherwise indicated, the terms "independently selected" in the present disclosure means that each substituent is selected independently of the other substituents. Therefore, each substituent can be the same as or different from the other substituents.

[00322] Unless otherwise indicated, the term "linker" as used herein has two reactive ends, with one end for linking or connecting a biological moiety or a fragment thereof, such as a targeting moiety, and the other end for coupling, for example, a payload.

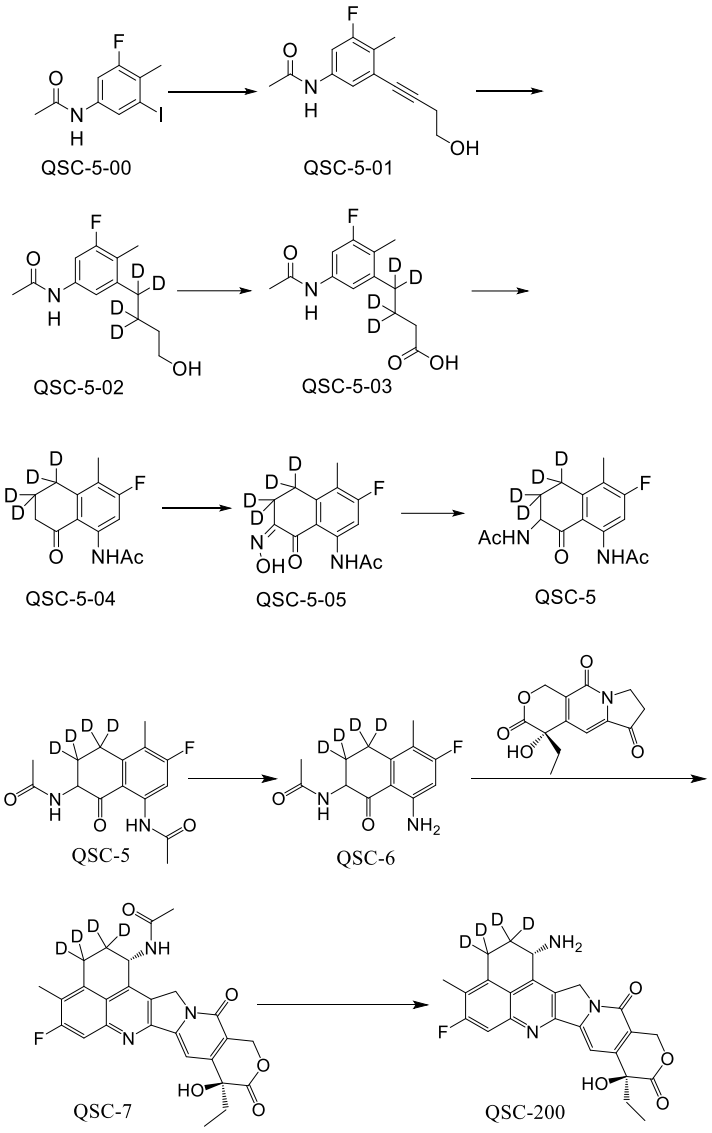
[00323] Unless otherwise indicated, the term "treating/treatment" as used herein means alleviating or eliminating the disease or condition targeted. If a subject receives a therapeutic amount of the compound or pharmaceutically acceptable form thereof of the present disclosure or the pharmaceutical composition of the present disclosure, and the subject exhibits observable and/or detectable remission and/or improvement in at least one indicator or symptom, the subject has been successfully "treated". It should be understood that treatment includes not only complete treatment, but also treatment that is less than complete but achieves some biologically or medically relevant results.

[00324] The embodiments of the present disclosure will be described in detail below in conjunction with examples, but those skilled in the art will appreciate that the following examples are only used to illustrate the present disclosure and should not be construed as limiting the scope of the present disclosure.

Examples

[00325] 1. Preparation of deuterated camptothecin compound

[00326] Example 1 Synthesis of compound QSC-200



[00327] Synthesis of QSC-5-01: N-(3-fluoro-5-iodo-4-methylphenyl)acetamide (60.0 g, 0.20 mol) and 3-butyn-1-ol (28.7 g, 0.41 mol) were added with 300 mL of DMF (N,N-dimethylformamide), stirred and dissolved. TEA (Triethylamine) (82.7 g, 0.82 mol) was added and stirred for 10 min. $PdCl_2(PPh_3)_2$ (3.6 g, 0.005 mol) and CuI (3.9 g, 0.02 mol) were then added and stirred for 4 h of reaction. The reaction solution was added dropwise to 3 L of water. After the dropwise addition was completed, the mixture was stirred for 1 h, and filtered. The resulting filter cake was transferred to a 2 L single-neck flask and dissolved in 1 L of EA. 12 g of silica gel was added and stirred for 30 min. The mixture was subjected to color removal by rapid silica gel column chromatography. The resulting mixture was concentrated by rotary evaporation to approximately 500 mL, slurried

with 1 L of Heptane for 16 h, and filtered. The resulting filter cake was dried to obtain 33.8 g of QSC-5-01, with a yield of 70.3%. LC-MS(ESI+)235.10 $[M+H]^+$.

[00328] Synthesis of QSC-5-02: QSC-5-01 (30.0 g, 0.13 mol) and PtO_2 (3.0 g, 10% w/w) were weighed and dissolved in 1200 mL of THF (tetrahydrofuran) and 300 mL of D_2O. After 16 h of reaction at room temperature under deuterium atmosphere, 2 L of EA (ethyl acetate), 1 L of H_2O and 5.0 g activated carbon were added and stirred for 0.5 h. The reaction system was filtered under assist of diatomite. The filtrate was subjected to phase-separation, and the organic phase was concentrated to obtain a white solid. The white solid was slurried with 500 mL of MTBE (Methyl tert-Butyl Ether): Heptane=1:1 for 2 h, and filtered. The resulting filter cake was dried to obtain 29.3 g of QSC-5-02, with a yield of 94.5%. LC-MS(ESI+)243.16 $[M+H]^+$.

[00329] Synthesis of QSC-5-03: QSC-5-02 (28.9 g, 0.12 mol) was weighed and dispersed in 600 mL of acetone under stirring. 58 mL of $CrO_3 \cdot H_2SO_4$ was slowly added dropwise at 0°C. After the dropwise addition was completed, the reaction was performed continuously for 10-30 min. The reaction system was added with 50 mL of isopropyl alcohol, stirred for 20 min, and filtered. The resulting filter cake was rinsed with 200 mL of EA. The filtrate was subjected to rotary evaporation to remove acetone, and added with 1 L of EA and 500 mL of H_2O, and stirred. After phase separation, the organic phase was washed twice with purified water, 500 mL each time to obtain the organic phase. NaOH solution (7.2 g NaOH was dissolved in 300 mL of purified water under stirring to prepare the NaOH solution) was added to the organic phase and stirred for 5 min. After phase separation, the aqueous phase was obtained, washed twice with EA (300 mL each time), and adjusted to pH 2-3 with 6 N hydrochloric acid. A large amount of solid was precipitated. The aqueous phase was extracted with EA three times, 300 mL each time. The organic phases were combined and concentrated. The resulting solid was stirred in Heptane for 2 h, filtered and dried to obtain 18.0 g of QSC-5-03, with a yield of 58.9%. LC-MS(ESI+)257.14 $[M+H]^+$.

[00330] Synthesis of QSC-5-04: QSC-5-03 (18.0 g, 0.07 mol) was weighed and added with 33 mL of TFA (trifluoroacetic acid), stirred and cooled to 0°C. 29 mL of TFAA (trifluoroacetic anhydride) was added dropwise for 30 min of reaction. The reaction solution was dropwise added to 500 mL of H_2O. After the dropwise addition was completed, the mixture was stirred for 30 min and filtered. The filter cake was rinsed with purified water, transferred to a 2L flask, dissolved in 500 mL of EA, added with 250 mL of purified water, and stirred for 10 min. After phase separation,

the organic phase was washed with 250 mL of saturated Na_2CO_3 solution, dried over anhydrous sodium sulfate, and subjected to rotary evaporation to obtain a solid. The solid was slurried with 250 mL of Heptane for 2 h, filtered and dried to obtain 14.2 g of QSC-5-04, with a yield of 85.0%. LC-MS(ESI+)239.13 $[M+H]^+$.

[00331] Synthesis of QSC-5-05: To a 500 mL flask, 400 mL of THF and 140 mL of tert-butanol were added and cooled to 0°C. 19.7 g of potassium tert-butoxide (19.7 g, 0.17 mol) was added and stirred for 10 min. QSC-5-04 (14.0 g, 0.06 mol) was dissolved in 130 mL of THF and slowly added to the reaction system dropwise. After the dropwise addition was completed, the reaction system was stirred for 10 min. Butyl nitrite (9.6 g, 0.09 mol) was dissolved in 30 mL of THF, and slowly added to the reaction system dropwise. After the dropwise addition was completed, the reaction system was reacted for 30 min. 200 mL of saturated NH_4Cl solution was added to the reaction system and stirred for 10 min. After the phase separation, 1L of ethyl acetate and 400 mL of purified water were added to the organic phase and stirred for 10 min. After phase separation, the organic phase was washed once with 400 mL of H_2O, and subjected to rotary evaporation to obtain a solid. The obtained solid was slurried with 200 mL of Heptane for 2 h, filtered and dried to obtain 13.7 g of QSC-5-05, with a yield of 87%. LC-MS(ESI+)268.12 $[M+H]^+$.

[00332] Synthesis of QSC-5: QSC-5-05 (13.5 g, 0.05 mol) was weighed, to which 810 mL of THF, Pt/C (1.4 g, 10% w/w) and acetic anhydride (13.5 g, 0.10 mol) were added. After 16 h of reaction under hydrogen atmosphere, the reaction system was filtered under assist of diatomite. The organic phase was concentrated by rotary evaporation to obtain a solid. The solid was slurried with 200 mL of EA for 2 h and filtered. The filter cake was dissolved in 400 mL of DCM (dichloromethane), added with 5 g of activated carbon, stirred for 1 h, and filtered under assist of diatomite. The organic phase was collected and subjected to rotary evaporation to obtain a solid. The solid was slurried with 200 mL of Heptane for 1 h, filtered and dried to obtain 9.0 g of QSC-5, with a yield of 60.4%. LC-MS(ESI+)296.15 $[M+H]^+$.

[00333] Synthesis of QSC-6: QSC-5 (8.01 g, 27.0 mmol) was dissolved in 2N hydrochloric acid (320 mL) for 2 h of reaction at 35°C. After the reaction was completed, the reaction solution was adjusted to pH 7-8 with 0.5 N $NaCO_3$ solution and extracted with dichloromethane three times. The organic phase was dehydrated, dried by rotary evaporation, and dried under vacuum to obtain 6.76 g of QSC-6, with a yield of 98.5%. LC-MS(ESI+): 255.1 $[M+H]^+$.

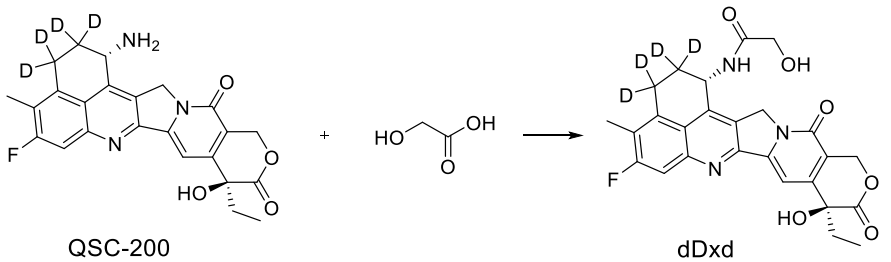
[00334] Synthesis of QSC-7: QSC-6 (6.76 g, 26.6 mmol), (S)-4-ethyl-4-hydroxy-7,8-dihydro-1H-pyranO[3,4-F]indolazine-3,6,10(4H)-one (8.57 g, 32.6 mmol) and p-toluenesulfonic acid monohydrate (2.53 g, 13.3 mmol) were dissolved in a solvent of 272 of mL toluene and 27 mL of DMF to perform a reaction under reflux to remove water at 130°C for 10 h. After the reaction was completed, the reaction system was cooled to room temperature, added with MTBE and n-heptane, and filtered to obtain a crude product. The crude product was dried under vacuum to obtain 15.36 g of QSC-7 with a yield of 120.4%. LC-MS(ESI+): 482.2$[M+H]^+$.

[00335] Synthesis of QSC-200: Crude QSC-7 (15.36 g) was weighed, placed in a reaction flask, dissolved in 150 mL of purified water and 75 mL of methanesulfonic acid, and stirred at 110°C for 5 h of reaction. After the reaction was completed, the reaction system was filtered while hot to obtain a liquid, to which 750 mL of absolute ethanol was added. The mixture was filtered and dried under vacuum to obtain 5.4 g of a crude product. The crude product was dissolved in 81 mL of purified water and 81 mL of methanesulfonic acid, and 324 mL of anhydrous ethanol was then slowly added dropwise for crystallization. The mixture was filtered and dried under vacuum to obtain 4.46 g of QSC-200, with a yield of 30.9%. LC-MS(ESI+): 439.2$[M+H]^+$.

[00336] Example 2 Synthesis of compounds dDxd and d1Dxd

[00337] 1. Synthesis of compound dDxd

[00338]



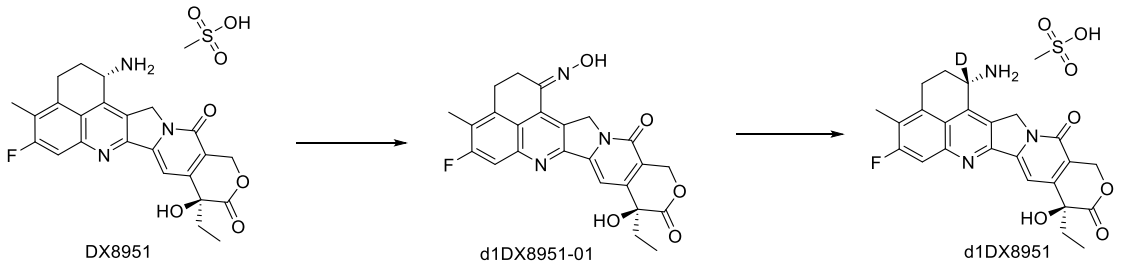
QSC-200 dDxd

[00339] QSC-200 (43.9 mg, 0.1 mmol), glycollic acid (15.1 mg, 0.2 mmol) and HATU (76.1 mg, 0.2 mmol) were dissolved in 2 mL of DMF, and DIPEA (49.7 μL, 0.3 mmol) was then added. The mixture was stirred at room temperature for 3 h, separated and purified by preparative liquid chromatography, and lyophilized to obtain 25.9 mg of dDxd, with a yield of 52.1 %. LC-MS(ESI+): 498.5 $[M+H]^+$.

[00340] 2. Synthesis of compound d1Dxd

[00341] 1) Synthesis of compound d1DX8951

[00342]

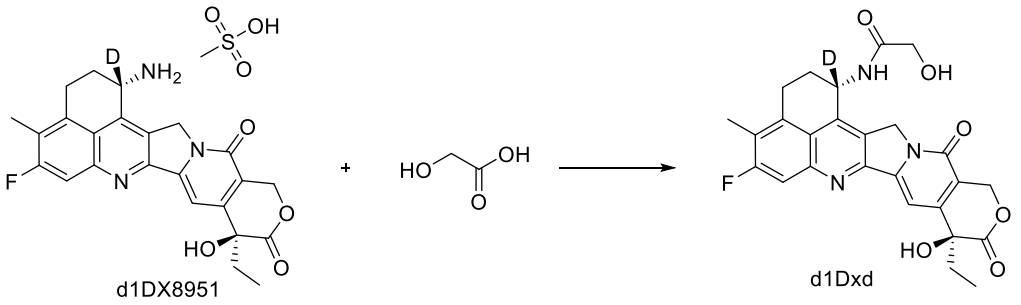


[00343] 50 mL of ethanol and 40 mL of water were added to compound DX8951 (5 g, 9.4 mmol) and stirred, and then triethylamine (1.9 g, 18.8 mmol) and sodium tungstate dihydrate (3.4 g, 10.3 mmol) were sequentially added. 25 mL of hydrogen peroxide was added dropwise and stirred at 50°C for 8 h. 100 mL of purified water was added, and the system was stirred for 1 h and then filtrated. The filter cake was slurried with 250 mL of N,N-dimethylformamide for 4 h, and filtrated to obtain 3.1 g of target compound d1DX8951-01, with a yield of 73%. LC-MS (ESI+) 450.15 $[M+H]^+$.

[00344] Compound d1DX8951-01 (2.0 g, 4.4 mmol), methane sulfonic acid (856 mg, 8.8 mmol) and 100 mg of 5% palladium on carbon (1 mg, 0.005 mmol) were dispersed in 60 mL of tetrahydrofuran and 30 mL of deuterium oxide under stirring. Deuterium gas was introduced for 4 h of reaction. The tetrahydrofuran was removed by rotary evaporation, and the residue was dissolved in 30 mL of methane sulfonic acid. 300 mL of ethanol was added for crystallization, and the mixture was filtered to obtain 1.1 g of target compound d1DX8951, with a yield of 46%. LC-MS (ESI+) 437.18 $[M+H]^+$.

[00345] 2) Synthesis of compound d1Dxd

[00346]



[00347] d1DX8951 (53.2 mg, 0.1 mmol), glycollic acid (15.0 mg, 0.2 mmol) and HATU (76.0 mg, 0.2 mmol) were dissolved in 2 mL of DMF, and DIPEA (N,N-diisopropylethylamine) (50.1 μL, 0.3 mmol) was then added. The system was stirred at room temperature for 3 h, separated and purified by preparative liquid chromatography, and lyophilized to obtain 27.3 mg of target compound d1Dxd, with a yield of 57.2%. LC-MS(ESI+) 495.5$[M+H]^+$.

[00348] Example 3 Elimination half-life and AUC assay

[00349] 1. Evaluation of liver microsomal half-life in vitro

[00350] The incubation mixed solutions for different species were prepared with PBS (pH=7.4), liver microsomal proteins from different species (with a final concentration of 0.5 mg/mL) and compound (with a final concentration of 0.5 μg/mL). 180 μL of each incubation solution was placed in a 37°C water bath for 5 min of pre-incubation. 20 μL of NADPH (nicotinamide adenine dinucleotide phosphate) was then added at a final concentration of 1 mM to initiate the reaction. The reaction was terminated at 0, 5, 10, 20, 30, and 60 minutes by adding 0.8 mL of ice-cold acetonitrile, respectively. The organic solvent content in the incubation system was controlled below 1%.

[00351] In this experiment, the liver microsomes from cynomolgus monkey and human were incubated with Dxd and dDxd *in vitro*, respectively. The contents of Dxd and dDxd remaining were measured by high performance liquid chromatography. The concentration of the test compound at 0 min was set as 100%. The percentage remaining at each time point was calculated as the concentration of the test compound at each time point relative to the 0-min concentration. A linear regression was performed on the natural logarithm of percentage remaining of the test compound versus incubation time and the slope (k) was calculated. Based on the corresponding formula, the elimination half-life ($T_{1/2}$) in the liver microsomes of each species was calculated.

Table 2 Half-life of Dxd and dDxd in liver microsomes in vitro

Compound	Species	$T_{1/2}$ (h)
Dxd	Cynomolgus Monkey	2.51
	Human	7.70
dDxd	Cynomolgus Monkey	1.89
	Human	7.22

[00352] The data of the metabolic stability evaluation of dDxd and Dxd in liver microsomes are shown in Table 2. The half-life of dDxd is shorter than that of Dxd in the liver microsomal proteins of cynomolgus monkey and human in vitro, indicating dDxd has faster liver metabolism and better safety.

[00353] 2. In vivo elimination half-life and AUC (Area Under Curve) assay

[00354] The quarantine-qualified SD rats were grouped, with 6 rats in each group, half male and half female. The test compounds obtained in examples were prepared on the day of administration, using a solvent of 5% dimethyl sulfoxide (DMSO) + 10% polyoxyl castor oil (solutol) + 85% saline. Body weights were measured before administration, and the administration dose was calculated based on body weight. The test compounds were administered via intravenous injection at an administration dose of 1 mg/kg, with an administration volume of 5 mL/kg. Blood samples (approximately 0.2 mL each) were collected from the jugular vein of rat before administration and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after administration to the rat. The heparin sodium was used as an anticoagulant. The collected blood samples were placed on ice, and centrifuged within 1 h to separate plasma (centrifugation conditions: 6800×g, 6 min, 2°C-8°C). 40 μL of plasma was mixed with 160 μL of acetonitrile for precipitation, and centrifuged at 3500×g for 5-20 min. 100 μL of the supernatant was taken for LC/MS/MS analysis to measure the concentration of the test compound. Pharmacokinetic parameters were calculated using Phoenix WinNonlin 7.0 based on the plasma-drug concentrations at various time points, yielding AUC_{0-t}, $T_{1/2}$, and corresponding mean values and standard deviations.

[00355] The pharmacokinetic test results of dDxd and Dxd in rats are shown in Table 3. Compared to Dxd, dDxd has a shorter half-life. Dxd exhibited an AUC_{0-24h} of 174.99±87.65 h*ng/mL, whereas dDxd exhibited an AUC_{0-24h} of 90.84±12.23 h*ng/mL, indicating that dDxd had a lower exposure in vivo and better safety.

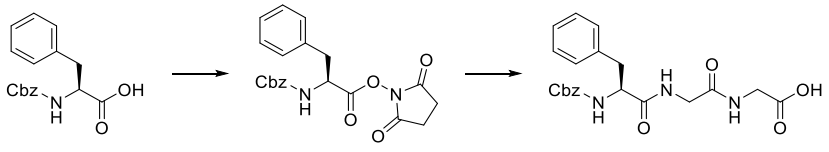
Table 3 Elimination half-life and AUC of Dxd and dDxd in rats

Compound	$T_{1/2}$ (h)	AUC_{0-24h} (h*ng/mL)
Dxd	0.46±0.03	174.99±87.65
dDxd	0.39±0.06	90.84±12.23

[00356] The properties of the d1Dxd compound were tested, and the results show that the d1Dxd compound had surprisingly high metabolic stability, good pharmacokinetic properties, potent cytotoxicity and good safety.

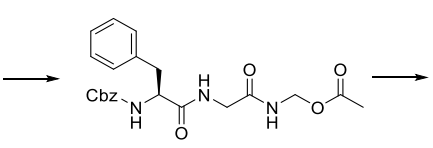
[00357] Preparation of linker-payload compounds

[00358] Example 4 Synthesis of compound INT-1

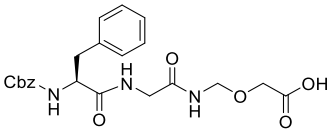


INT-1-1

INT-1-2



INT-1-3



INT-1

[00359] N-benzyloxycarbonyl-L-phenylalanine (150 g, 0.50 mol) and N-hydroxysuccinimide (69.21 g, 0.60 mol) were weighed and dissolved in 750 mL of tetrahydrofuran. The reaction solution was cooled to 0°C. DCC (N,N'-dicyclohexylcarbodiimide) (124.08 g, 0.60 mol) was weighed and dissolved in 310 mL of tetrahydrofuran under ultrasonication to obtain a clear solution of DCC in THF, which was added dropwise to the reaction solution at a temperature of 0-5°C. After

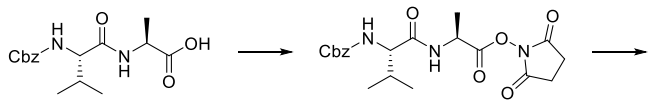
4 h of reaction at room temperature, the reaction system was cooled to 5°C and filtered. The filter cake was washed with a small amount of tetrahydrofuran. The filtrate was replaced twice with EtOH, slurried overnight, and filtered to obtain 185.42 g of INT-1-1, with a yield of 93%. LC-MS(ESI+)397.27 $[M+H]^+$.

[00360] Compound INT-1-1 (185.42 g, 0.47 mol) was dissolved in 1390 mL of tetrahydrofuran. glycylglycine (92.70 g, 0.70 mol) and sodium bicarbonate (47.16 g, 0.56 mol) were weighed and dissolved in 705 mL of water under ultrasonication. The aqueous solution of glycylglycine in sodium bicarbonate was dropwise added at room temperature. After 2 h of reaction at room temperature, the reaction solution was acidified to pH ≤ 2, extracted three times with DCM, washed once with 1 N HCl, dried and filtered. The filter cake was rinsed with DCM. The filtrate was dried by rotary evaporation until foaming solid occurred, and then EA: Hep.(1-pyridin-2-yl-ethanol) = 2:1 was added. The system was slurried overnight and filtered. The filter cake was rinsed three times with EA and Hep., respectively, and dried to obtain 162.02 g of target compound INT-1-2, with a yield of 93.78%. LC-MS(ESI+)414.34 $[M+H]^+$.

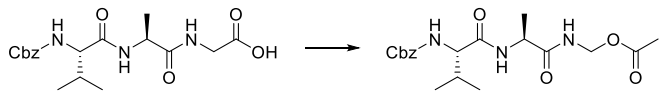
[00361] Compound INT-1-2 (20.00 g, 48.38 mmol) was dissolved in 240 mL of tetrahydrofuran, and lead tetraacetate (27.05 g, 128.68 mmol) was added at room temperature. After overnight reaction at room temperature, the reaction solution was filtered. The filter cake was rinsed with a small amount of tetrahydrofuran. The filtrate was dried by rotary evaporation and passed through a column to obtain 14.55 g of the target compound INT-1-3, with a yield of 70.36%. LC-MS(ESI+)450.34 $[M+Na]^+$.

[00362] Compound INT-1-3 (14.00 g, 32.7 mmol) was dissolved in 280 mL of DCM, and pyridinium p-toluenesulfonate (PPTS) (1.62 g, 0.033 mmol) and glycollic acid (3.73 g, 49 mmol) were then added at room temperature. The mixture was refluxed for overnight reaction and filtered. The filter cake was rinsed with a small amount of dichloromethane, and passed through a column to obtain 8.75 g of the target compound INT-1, with a yield of 60.25%. LC-MS(ESI+)444.34 $[M+H]^+$.

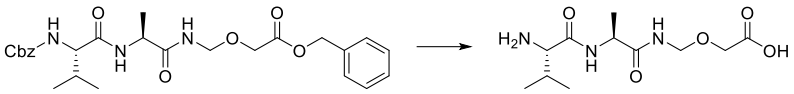
[00363] Example 5 Synthesis of compound INT-2



INT-2-1



INT-2-2 INT-2-3



INT-2-4 INT-2

[00364] Benzyloxycarbonyl-L-valyl-L-alanine (160 g, 500 mmol) and N-hydroxysuccinimide (85.71 g, 750 mol) were dissolved in 2000 mL of dichloromethane, and cooled to 0°C. Under a temperature controlled within 0-10°C, 2,4,6-collidine (180.64 g, 1490 mmol) was added and stirred under a maintained temperature for 1 h. Under a temperature kept between 0-10°C, 300 mL of TFAA (156.54 g, 750 mmol) in dichloromethane was added and stirred under a maintained temperature for 3 h. The reaction solution was filtered to obtain 199 g of INT-2-1, with a yield of 95.0%. LC-MS(ESI+)420.4 $[M+H]^+$.

[00365] Compound INT-2-1 (199 g, 470 mmol) was dissolved in 1600 mL of tetrahydrofuran, and an aqueous solution of glycine (42.74 g, 570 mmol) in 5% sodium bicarbonate (1000 mL), and 400 mL of DME (1,2-dimethoxyethane) were added. After 5 h of reaction at 40°C, the reaction solution was added with 350 mL of 2N hydrochloric acid and extracted with 2L of dichloromethane. The organic phase was collected and dried by rotary evaporation to obtain 150.0 g of the target compound INT-2-2, with a yield of 84.0%. LC-MS(ESI+)380.3 $[M+H]^+$.

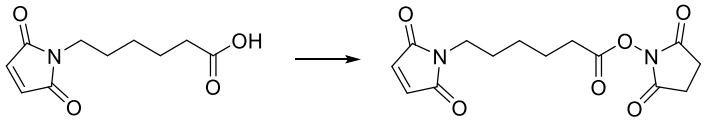
[00366] Compound INT-2-2 (20.0 g, 52.71 mmol) was dissolved in 800 mL of tetrahydrofuran, and then 5 mL of glacial acetic acid was added. The lead tetraacetate (35.0 g, 118.13 mmol) was added under rapid stirring. The reaction solution was stirred at room temperature for 3 h and centrifuged. The supernatant was concentrated, added with 600 mL of dichloromethane, washed twice with 1N hydrochloric acid (100 mL each time), washed once with 100 mL of saturated saline, dried over anhydrous sodium sulfate for 1 h, and filtered. The filtrate was concentrated to obtain

14.3 g of compound INT-2-3 as a white solid, with a yield of 68.9%. LC-MS (ESI+)395.3 $[M+H]^+$.

[00367] Compound INT-2-3 (7.0 g, 17.8 mmol) was dissolved in 200 mL of dichloromethane, and benzyl glycolate (29.57 g, 178 mmol) and PPTS (0.45 g, 0.18 mmol) were added and refluxed with stirring overnight. The reaction solution was concentrated to obtain 7.1 g of INT-2-4 as a light-yellow oil, with a yield of 80.2%. LC-MS (ESI+)500.3 $[M+H]^+$.

[00368] Compound INT-2-4 (7.1 g, 14.2 mmol) was dissolved in 150 mL of methanol, and 10% palladium on carbon (2.5 g, 0.35 w) under a hydrogen balloon. The reaction solution was stirred at room temperature for 3 h, added with 70 mL of water, and filtered. The filtrate was concentrated to obtain 3.7 g of compound INT-2 as a white solid, with a yield of 95.3%. LC-MS (ESI+)276.1 $[M+H]^+$.

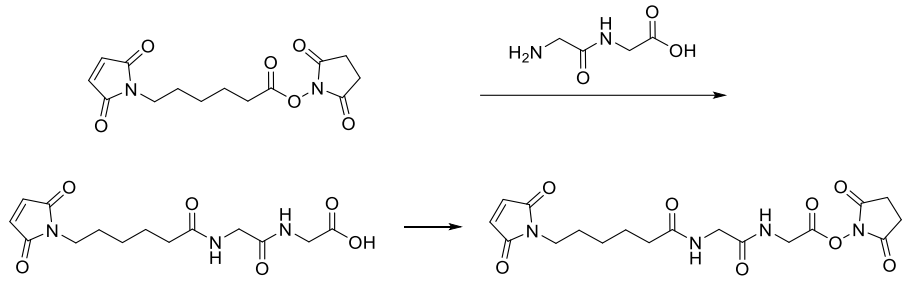
[00369] Example 6 Synthesis of compound INT-3



INT-3

[00370] Maleimidecaproic acid (2112.1 mg, 10 mmol) and N-hydroxysuccinimide (1.38 g, 12 mmol) were dissolved in 50 mL of tetrahydrofuran. The reaction solution was cooled to 0°C. DCC (2.48 g, 12 mmol) was weighed and dissolved in 40 mL of tetrahydrofuran under ultrasonication. The solution of DCC in THF was added dropwise at a temperature controlled within 0 to 5°C. After 4 h of reaction at room temperature, the reaction system was cooled to 5°C and filtered. The filter cake was rinsed with a small amount of tetrahydrofuran. The filtrate was replaced twice with EtOH, slurried overnight, and filtered to obtain 2.86 g of INT-3, with a yield of 93%. LC-MS(ESI+)331.3 $[M+Na]^+$.

[00371] Example 7 Synthesis of compound INT-4

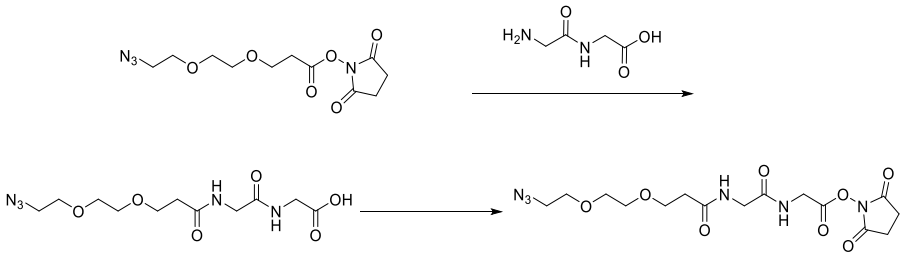


INT-4-01 INT-4

[00372] INT-3 (616.3 mg, 2.00 mmol) and glycylglycine (264.2 mg, 2.00 mmol) were dissolved in 10 mL of dichloromethane, and then TEA (415.9 μl, 3.00 mmol) was added. The mixture was stirred at room temperature for 3 h. The reaction solution was concentrated under reduced pressure. The resulting crude solid was purified by preparative liquid chromatography to obtain 572.2 mg of INT-4-01, with a yield of 88%. LC-MS(ESI+)326.1 $[M+H]^+$.

[00373] Compound INT-4-01 (572.2 mg, 1.75 mmol) was dissolved in 10 mL of tetrahydrofuran, and N-hydroxysuccinimide (402.2 mg, 3.50 mmol) and DCC (722.1 mg, 3.50 mmol) were added at room temperature. After overnight reaction at room temperature, the reaction system was cooled to 5°C and filtered. The filter cake was rinsed with a small amount of tetrahydrofuran. The filtrate was dried by rotary evaporation and slurried with ethanol to obtain crude target compound INT-4. LC-MS(ESI+)423.2 $[M+H]^+$.

[00374] Example 8 Synthesis of compound INT-5



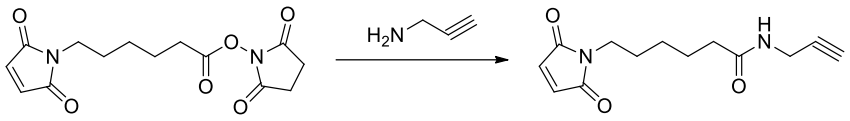
INT-5-1 INT-5

[00375] N3-PEG2-NHS (10 g, 33 mmol) was dissolved in 100 mL of tetrahydrofuran, and an aqueous solution of glycylglycine (5.28 g, 40 mmol) in 5% sodium bicarbonate (100 mL), and 40 mL of DME were added. After 5 h of reaction at 40°C, 35 mL of 2N hydrochloric acid was added. The reaction solution was extracted with 200 mL of dichloromethane. The organic phase was collected and dried by rotary evaporation to obtain 9.3 g of the target compound INT-5-1, with a

yield of 88.0%. LC-MS(ESI+)318.3 $[M+H]^+$.

[00376] INT-5-1 (9 g, 28.4 mmol) and N-hydroxysuccinimide (3.59 g, 31.2 mmol) were dissolved in 80 mL of tetrahydrofuran. The reaction solution was cooled to 0°C. DCC (6.43 g, 31.2 mmol) was weighed and dissolved in 20 mL of tetrahydrofuran under ultrasonication. The solution of DCC in THF was added dropwise at a temperature controlled within 0-5°C. After 4 h of reaction at room temperature, the reaction system was cooled to 5°C and filtered. The filter cake was rinsed with a small amount of tetrahydrofuran. The filtrate was replaced twice with EtOH, slurried overnight, and filtered to obtain 10.58 g of INT-5, with a yield of 90%. LC-MS(ESI+)415.2 $[M+H]^+$.

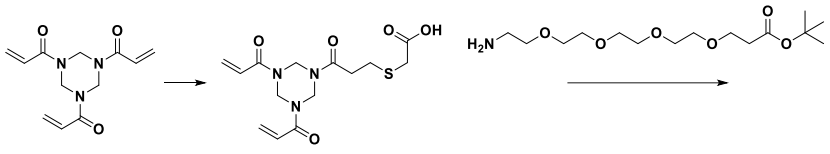
[00377] Example 9 Synthesis of compound INT-6



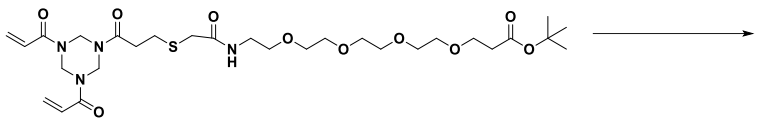
INT-3 INT-6

[00378] INT-3 (380.3 mg, 1.0 mmol) was dissolved in 5 mL of dichloromethane, and propargylamine (55.1 mg, 1.0 mmol) and N,N-diisopropylethylamine (66.1 mg, 1.2 mmol) were then added at room temperature. The reaction solution was stirred overnight at room temperature. The reaction system was dried by rotary evaporation under reduced pressure and purified by preparative liquid chromatography to obtain 181.8 mg of the crude target compound INT-6, with a yield of 73.2%. LC-MS(ESI+)249.3 $[M+H]^+$.

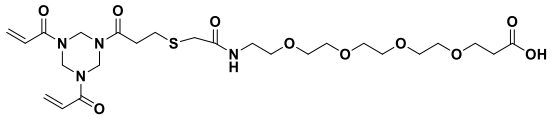
[00379] Example 10 Synthesis of compound INT-7



INT-7-1



INT-7-2



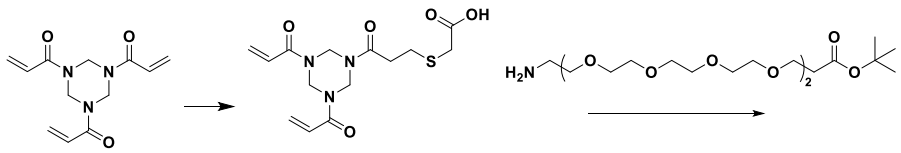
INT-7

[00380] 1,3,5-triacryloylhexahydro-1,3,5-triazine (25 g, 100 mmol) was dissolved in 500 mL of dichloromethane, and TEA (4159 μl, 30 mmol) and thioglycolic acid (2.8 g, 30 mmol) were added and stirred at room temperature for 3 h. The reaction solution was concentrated under reduced pressure. The resulting crude solid was purified through a rapid preparative column to obtain 3.1 g of INT-7-1, with a yield of 30%. LC-MS(ESI+)342.1 $[M+H]^+$.

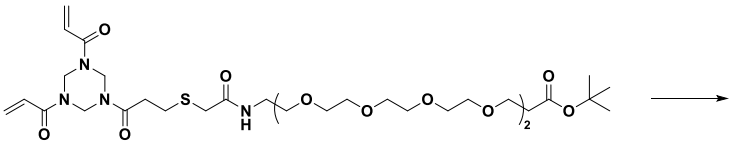
[00381] Compound INT-7-1 (1000 mg, 2.86 mmol) was dissolved in 10 mL of DMF, and 2-(7-azabenzotriazole)-N,N,N',N'-tetramethylurea hexafluorophosphate (HATU) (1100 mg, 2.89 mmol) and amino-PEG4-t-butyl ester (919 mg, 2.86 mmol) were then added at room temperature. N,N-diisopropylethylamine (DIPEA) (370 mg, 2.86 mmol) was added dropwise under stirring at room temperature. After 2 h of reaction at room temperature, the reaction solution was dried by rotary evaporation and column chromatography (eluent: DCM/MeOH=30:1) to obtain 1.04 g of the target compound INT-7-2, with a yield of 56.6%. LC-MS(ESI+)645 $[M+H]^+$.

[00382] Compound INT-7-2 (1000 mg, 1.55 mmol) was weighed and added with 20 mL of DCM. 4 mL of trifluoroacetic acid was added under stirring. The reaction solution was stirred at room temperature overnight, dried by rotary evaporation, and dissolved in 20 mL of DCM, washed three times with water (5 mL each time), and washed once with 5 mL of saturated saline. The organic phase was dried by rotary evaporation to obtain 0.91 g of the target compound INT-7 as a pale yellow oil, with a yield of 100%, LC-MS (ESI+) 589 $[M+H]^+$.

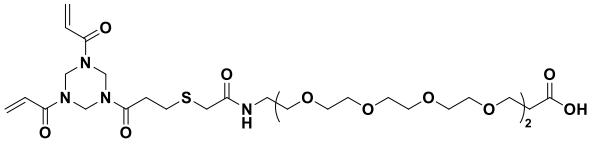
[00383] Example 11 Synthesis of compound INT-8



INT-8-1



INT-8-2



INT-8

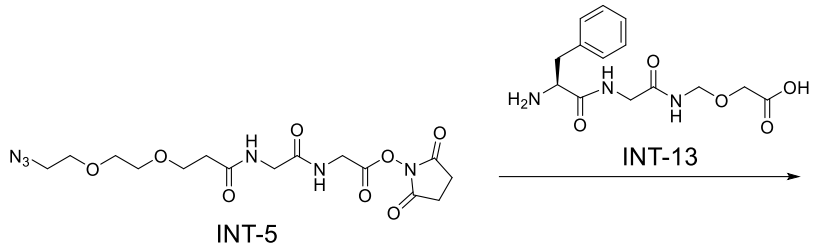
[00384] 1,3,5-triacryloylhexahydro-1,3,5-triazine (16 g, 64 mmol) was dissolved in 320 mL of dichloromethane, and TEA (2662 μl, 19 mmol) and thioglycolic acid (1.8 g, 19 mmol) were then added and stirred at room temperature for 3 h. The reaction solution was concentrated under reduced pressure. The resulting crude solid was purified through a rapid preparative column to obtain 2.1 g of INT-8-1, with a yield of 30%. LC-MS(ESI+)342.1 $[M+H]^+$.

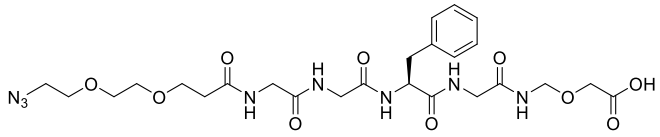
[00385] Compound INT-8-1 (2 g, 5.86 mmol) was dissolved in 40 mL of DCM, and 2-(7-azabenzotriazole)-N,N,N',N'-tetramethylurea hexafluorophosphate (HATU) (2.67 g, 7.03 mmol) and amino-PEG4-tert-butyl ester (3.21 g, 6.44 mmol) were then added at room temperature. DIPEA (N,N-Diisopropylethylamine) (908.6 mg, 7.03 mmol) was added dropwise under stirring at room temperature. After 2 h of reaction at room temperature, the reaction solution was dried by rotary evaporation and subjected to column chromatography (eluent: DCM/MeOH=80:1) to obtain 900 mg of the target compound INT-7-2, with a yield of 49.5%. LC-MS(ESI+)822 $[M+H]^+$.

[00386] Compound INT-8-2 (900 mg, 1.10 mmol) was weighed and added with 8 mL of DCM. 2 mL of trifluoroacetic acid was then added under stirring. The reaction solution was stirred at room temperature overnight, dried by rotary evaporation, dissolved in 20 mL of DCM, washed three times with water (5 mL each time), and washed once with 5 mL of saturated saline. The organic

phase was dried by rotary evaporation to obtain 750 mg of the target compound INT-7 as a pale yellow oil, with a yield of 100%, LC-MS (ESI+) 764 $[M+H]^+$.

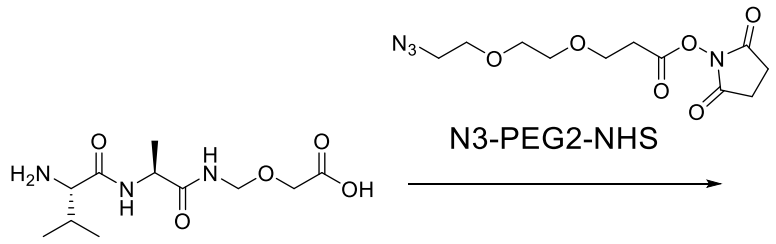
[00387] Example 12 Synthesis of compound INT-9

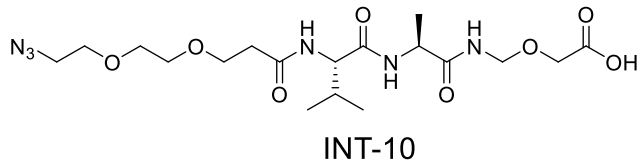




[00388] INT-5 (7.7 g, 18.6 mmol) was dissolved in 70 mL of DMF, and INT-13 (6.9 g, 22.3 mmol) and DIPEA (4.8 g, 37.2 mmol) were added. After 5 h of reaction at room temperature, the reaction solution was concentrated and purified by Flash chromatography, and lyophilized to obtain 9.3 g of INT-9, with a yield of 84.5%. LC-MS(ESI+)609.6 $[M+H]^+$.

[00389] Example 13 Synthesis of compound INT-10

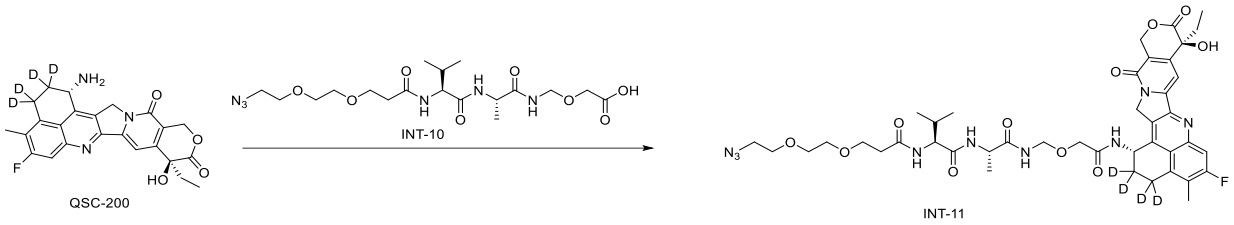




[00390] INT-2 (1 g, 3.6 mmol) and N3-PEG2-NHS (1.2 g, 4.0 mmol) were dissolved in 10 mL of DMF, and then DIPEA (0.56 g, 4.4 mmol) was added. After 24 h of reaction at room temperature,

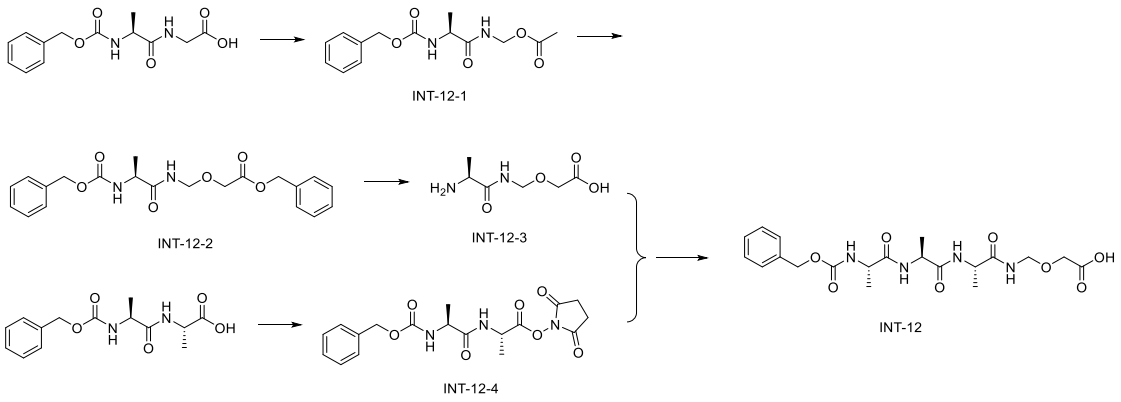
the reaction solution was concentrated and purified by Flash chromatography, and lyophilized to obtain 1.28 g of INT-10, with a yield of 80%. LC-MS(ESI+)461.2 $[M+H]^+$.

[00391] Example 14 Synthesis of compound INT-11



[00392] INT-10 (594 mg, 1.29 mmol), QSC-200 (515 mg, 1.17 mmol) and HATU (535 mg, 1.41 mmol) were weighed and placed in a reaction flask. DMF was added at room temperature and stirred well. DIPEA (454 mg, 3.51 mmol) was added dropwise. After 2 h of reaction, the reaction was terminated. The reaction solution was purified by using a reverse phase preparative column to obtain INT-11, with a yield of 73%. LC-MS(ESI+): 882.3$[M+H]^+$.

[00393] Example 15 Synthesis of Compound INT-12



[00394] Benzyloxycarbonyl-L-alanine (30 g, 110 mmol) was dissolved in 900 mL of tetrahydrofuran, and 9 mL of glacial acetic acid was added. The lead tetraacetate (104.0 g, 240 mmol) was added under rapid stirring. The reaction solution was stirred at 40°C for 4 h and filtered. The filtrate was concentrated to obtain 27.72 g of INT-12-1 as an off-white solid compound, with a yield of 88.1%. LC-MS (ESI+)295.3 $[M+H]^+$.

[00395] Compound INT-12-1 (12 g, 40.8 mmol) was dissolved in 120 mL of dichloromethane, and benzyl glycolate (67.7 g, 408 mmol) and PPTS (Pyridinium p-Toluenesulfonate) (1.02 g, 4.08 mmol) were added. The reaction solution was refluxed with stirring overnight, and concentrated to

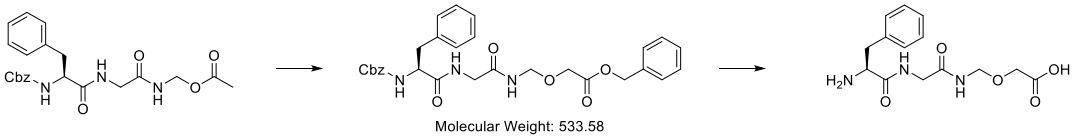
obtain 13.8 g of INT-12-2 as a pale yellow oil, with a yield of 85.2%. LC-MS (ESI+)401.5 $[M+H]^+$.

[00396] Compound INT-12-2 (10 g, 25 mmol) was dissolved in 300 mL of methanol, and 10% palladium on carbon (3.5 g, 0.35 w) was added. Under hydrogen, the reaction solution was stirred at room temperature for 3 h, added with 100 mL of water, and filtered. The filtrate was concentrated to obtain 3.5 g of compound INT-12-3 as a white solid, with a yield of 80.1%. LC-MS (ESI+)177.2 $[M+H]^+$.

[00397] Benzyloxycarbonyl-L-alanyl-alanine (10 g, 34.0 mmol) and N-hydroxysuccinimide (4.3 g, 37.3 mmol) were dissolved in 60 mL of tetrahydrofuran. The reaction solution was cooled to 0°C. At a temperature controlled within 0-10°C, 20 mL of DCC (7.71 g, 37.4 mmol) in tetrahydrofuran was added and stirred at room temperature for 3 h. The reaction solution was filtered. The filtrate was concentrated and slurried with ethanol, and filtered to obtain 12.1 g of INT-12-4, with a yield of 91.0%. LC-MS(ESI+)392.4 $[M+H]^+$.

[00398] Compound INT-12-4 (4.00 g, 10.2 mmol) and compound INT-12-3 (1.98 g, 11.2 mmol) were dissolved in 40 mL of DMF, and DIPEA (1.32 g, 10.23 mmol) was added. After 24 h of reaction at room temperature, the reaction solution was concentrated and purified by Flash chromatography, and lyophilized to obtain 2.8 g of INT-12, with a yield of 61%. LC-MS(ESI+)453.2 $[M+H]^+$.

[00399] Example 16 Synthesis of compound INT-13



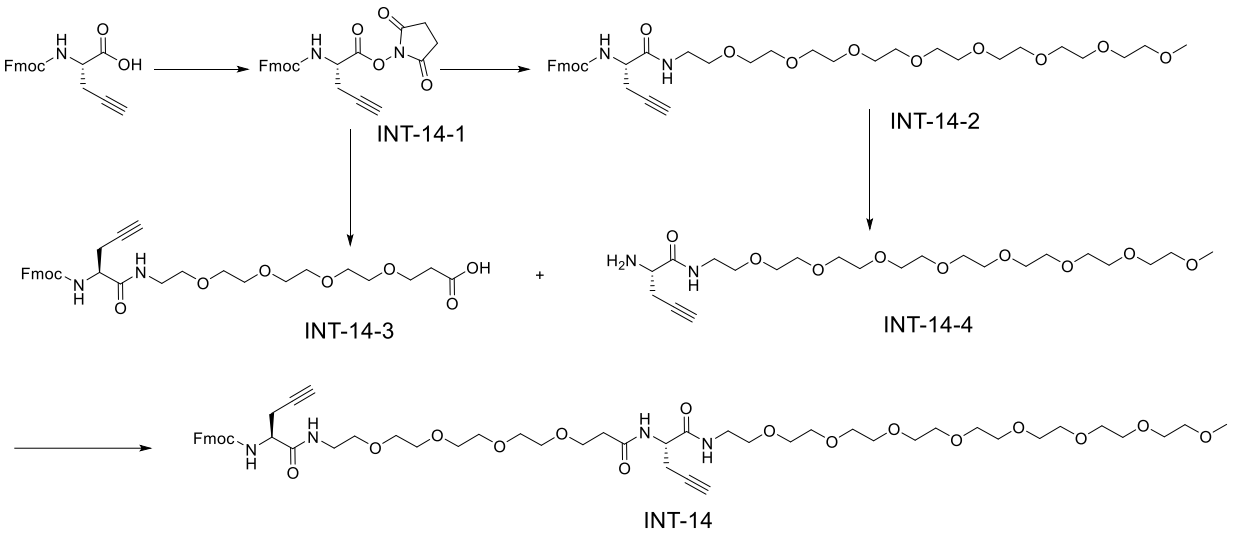
INT-1-3 INT-13-1 INT-13

[00400] Compound INT-1-3 (14.00 g, 32.7 mmol) was dissolved in 280 mL of DCM, and PPTS (1.62 g, 0.033 mmol) and benzyl glycolate (5.69 g, 49 mmol) were added at room temperature. The reaction solution was refluxed for reaction overnight, and filtered. The filter cake was rinsed with a small amount of dichloromethane, and passed through a column to obtain 13.65 g of the target compound INT-13-1, with a yield of 78.31%. LC-MS(ESI+)534.22$[M+H]^+$.

[00401] INT-13-1 (13.00, 24.4 mmol) and palladium on carbon (2.60 g, 20%) were dissolved in

130 mL of tetrahydrofuran, and stirred at room temperature for 2 h under hydrogen. After the reaction was completed, the reaction mixture was filtered to remove palladium on carbon. The filter cake was rinsed with methanol, and the filtrate was dried by rotary evaporation to obtain 6.19 g of the target compound INT-13, with a yield of 82.15%. LC-MS(ESI+)332.32$[M+Na]^+$.

[00402] Example 17 Synthesis of compound INT-14



[00403] Fmoc-L-propargylglycine (503 mg, 1.5 mmol) was dissolved in 10 mL of tetrahydrofuran, and NHS (N-Hydroxy succinimide) (190 mg, 1.65 mmol) and DCC (340 mg, 1.65 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, and filtered. The filter cake was washed three times with 1 mL of tetrahydrofuran, and the filtrate was dried by rotary evaporation under reduced pressure to obtain 940 mg of the crude target compound INT-14-1. LC-MS(ESI+)455.5 $[M+Na]^+$.

[00404] The crude compound INT-14-1 (470 mg, 0.75 mmol) was dissolved in 8 mL of dichloromethane, and $mPEG_8$-NH_2 (288 mg, 0.75 mmol) and N,N-diisopropylethylamine (96 mg, 0.75 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, dried by rotary evaporation under reduced pressure, and purified by preparative liquid chromatography to obtain 368 mg of the crude target compound INT-14-2, with a yield of 70%. LC-MS(ESI+)702.1 $[M+H]^+$.

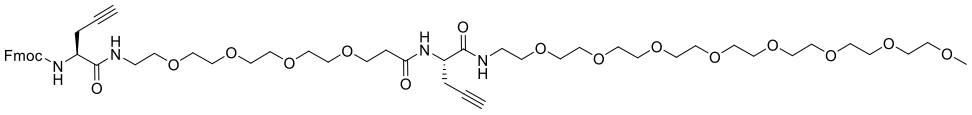
[00405] The crude compound INT-14-1 (470 mg, 0.75 mmol) was dissolved in 8 mL of dichloromethane, and NH_2-PEG_4-COOH (200 mg, 0.75 mmol) and N,N-diisopropylethylamine (96

mg, 0.75 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, dried by rotary evaporation under reduced pressure, and purified by preparative liquid chromatography to obtain 247 mg of the crude target compound INT-14-3, with a yield of 56%. LC-MS(ESI+)584.3 $[M+H]^+$.

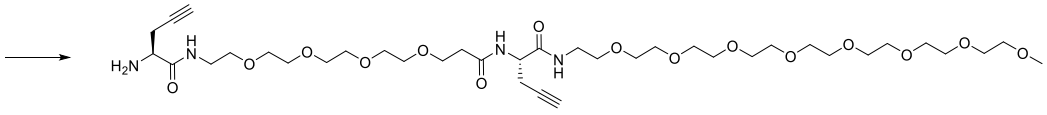
[00406] Compound INT-14-2 (200 mg, 0.29 mmol) was dissolved in 5 mL of dichloromethane, and diethylamine (1 mL) was added at room temperature. The reaction system was stirred at room temperature overnight, dried by rotary evaporation under reduced pressure, dissolved in 5 mL of dichloromethane, and dried by rotary evaporation under reduced pressure. This process was repeated five times to obtain the crude target compound INT-14-4. LC-MS(ESI+)479.9 $[M+H]^+$.

[00407] The crude compound INT-14-4 was dissolved in 5 mL of dichloromethane, and compound INT-14-3 (183 mg, 0.31 mmol), HOBT (1-Hydroxybenzotriazole) (58 mg, 0.43 mmol) and EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (82 mg, 0.43 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, dried by rotary evaporation under reduced pressure, and purified by preparative liquid chromatography to obtain 167 mg of the target compound INT-14, with a yield of 56%. LC-MS(ESI+)1044.5 $[M+H]^+$.

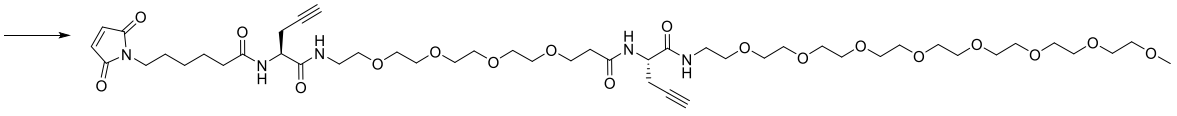
[00408] Example 18 Synthesis of compound INT-15



INT-14



INT-15-1



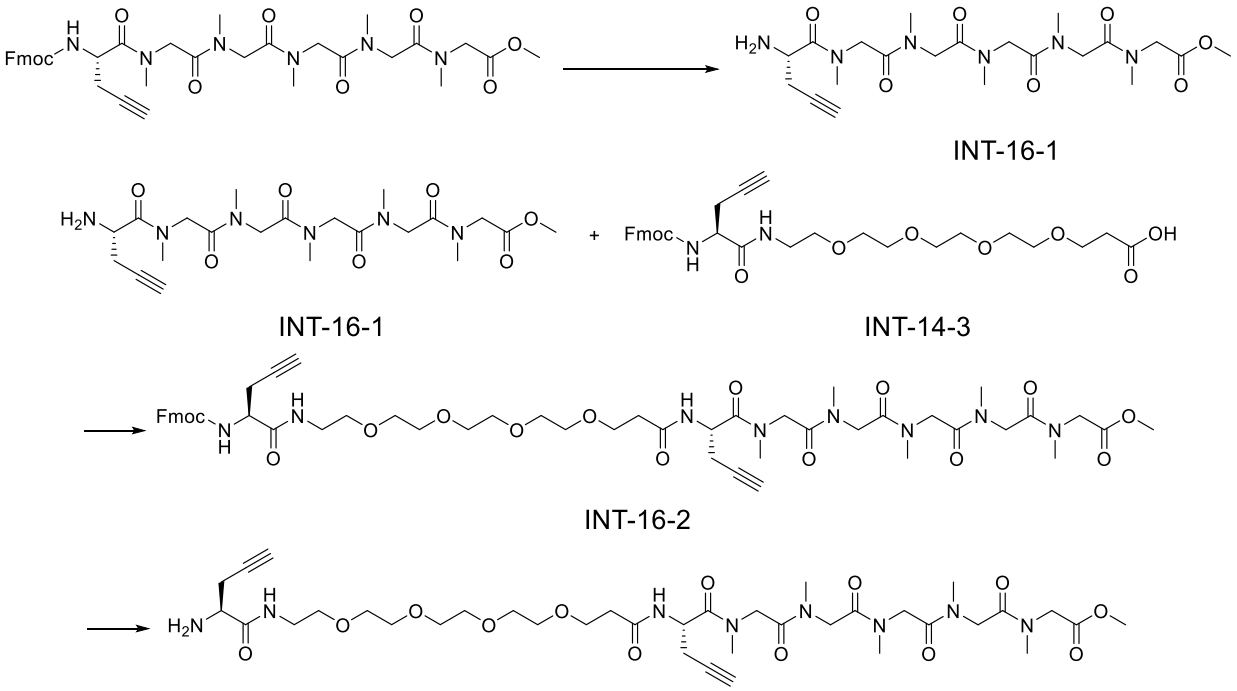
INT-15

[00409] Compound INT-14 (146 mg, 0.14 mmol) was dissolved in 4 mL of dichloromethane, and diethylamine (0.4 mL) was added at room temperature. The reaction system was stirred at room

temperature for 2 h, dried by rotary evaporation under reduced pressure, dissolved in 5 mL of dichloromethane, and dried by rotary evaporation under reduced pressure. This process was repeated five times to obtain the crude target compound INT-15-1. LC-MS(ESI+)820.5 $[M+H]^+$.

[00410] The crude compound INT-15-1 was dissolved in 2 mL of N,N-dimethylformamide, and succinimidyl 6-(maleimido)hexanoate (65 mg, 0.21 mmol) and N,N-diisopropylethylamine (36 mg, 0.28 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, and purified by preparative liquid chromatography to obtain the target compound INT-15, with a two-step yield of 62%. LC-MS(ESI+)1014.5 $[M+H]^+$.

[00411] Example 19 Synthesis of compound INT-16

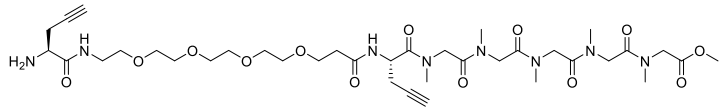


[00412] Fmoc-Pra-5Sar-OMe (705 mg, 1 mmol) was dissolved in 10 mL of dichloromethane, and diethylamine (2 mL) was added at room temperature. The reaction system was stirred at room temperature for 3 h, and dried by rotary evaporation to remove solvent and obtain the crude target compound INT-16-1, which was directly used in the next step without purification. LC-MS(ESI+)483.26 $[M+H]^+$.

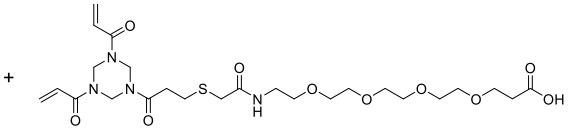
[00413] Compound INT-14-3 (583 mg, 1 mmol) was dissolved in 15 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (194 mg, 1.5 mmol) and HATU (456 mg, 1.2 mmol) were added and stirred at room temperature for 1 h. The crude compound INT-16-1 was added and stirred at room temperature overnight. The reaction system was purified by preparative liquid chromatography to obtain 456 mg of the target compound INT-16-2, with a yield of 44%. LC-MS(ESI+)1048.41 $[M+H]^+$.

[00414] Compound INT-16-2 (456 mg, 0.44 mmol) was dissolved in 6 mL of dichloromethane, and diethylamine (1.2 mL) was added at room temperature. The reaction system was stirred at room temperature for 3 h, and dried by rotary evaporation to remove solvent and obtain the crude target compound INT-16, which was directly used in the next step without purification. LC-MS(ESI+)825.64 $[M+H]^+$.

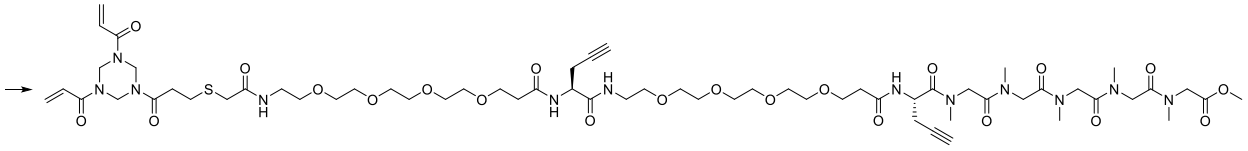
[00415] Example 20 Synthesis of compound INT-17



INT-16



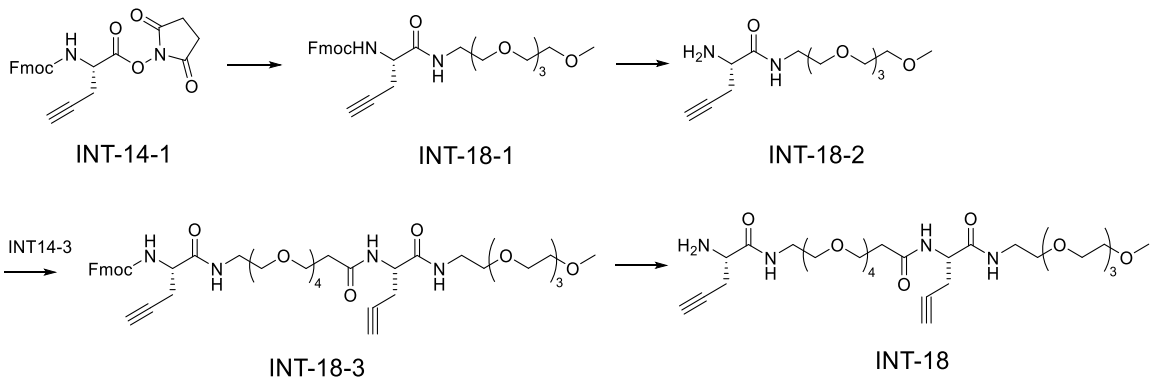
INT-7



INT-17

[00416] Compound INT-7 (306 mg, 0.52 mmol) was dissolved with 10 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (84 mg, 0.65 mmol) and HATU (456 mg, 0.52 mmol) were added and stirred at room temperature for 1 h. The crude compound INT-16 was added and stirred at room temperature overnight. The reaction system was purified by preparative liquid chromatography to obtain 200 mg of the target compound INT-17, with a yield of 33%. LC-MS(ESI+)1048.41 $[M+H]^+$.

[00417] Example 21 Synthesis of compound INT-18



[00418] INT-14-1 (300 mg, 694 μmol) was weighed and placed in a 50 mL single-neck flask, dissolved in 10 mL of dichloromethane, and stirred at room temperature. NH_2-PEG_4-OMe (143.79 mg, 694 μmol) was weighed and added. 145 μL of DIPEA was added dropwise for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was subjected to rotary evaporation to remove solvent and purified by preparative reverse phase flash chromatography to obtain 267 mg of the target product INT-18-1, with a purity of 99.79% and a yield of 71.71%. ESI-MS(m/z): 526.24$[M+H]^+$.

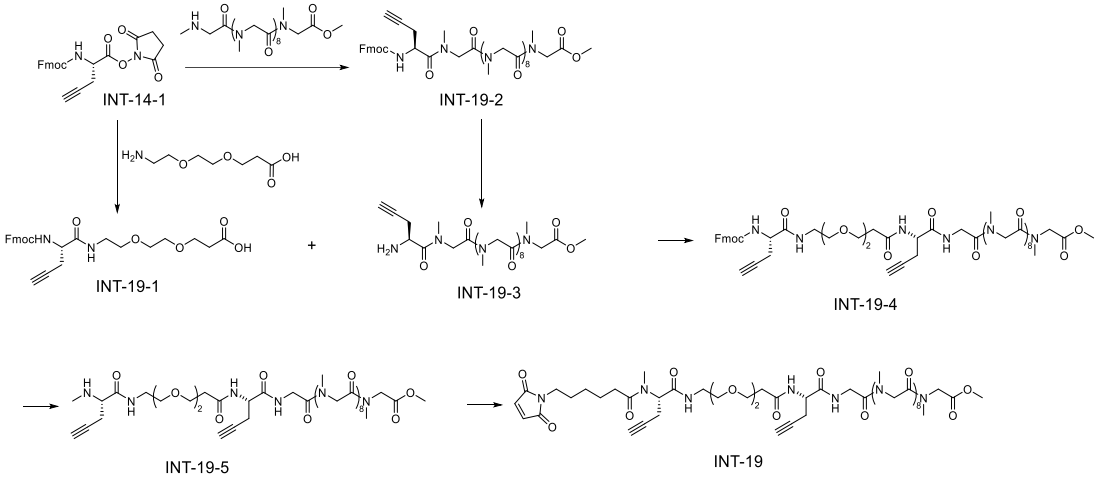
[00419] INT-18-1 (267 mg, 509 μmol) was weighed and placed in a 10 mL single-neck flask, dissolved in 3 mL of dichloromethane, and stirred at room temperature. 600 μL of diethylamine was added dropwise for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was dried by rotary evaporation, dissolved in 5 mL of dichloromethane, and dried by rotary evaporation. This process was repeated 3 times to obtain the crude INT-18-2, which was directly used in the next step.

[00420] INT-14-3 (294.82 mg, 506 μmol) was weighed into a 25 mL single-neck flask, dissolved in 6 mL of dichloromethane, and stirred at room temperature. HATU (288.60 mg, 759 μmol) was weighed and added. 177 μL of DIPEA was added dropwise to the reaction system, and stirred at room temperature for 30 min. INT-18-2 (153 mg, 506 μmol) was weighed and added for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction system was subjected to rotary evaporation to remove solvent, and purified by preparative reverse phase Flash chromatography to obtain 263.0 mg of the target product INT-18-3, with a

purity of 99% and a yield of 59.95%. LC-MS(ESI+): 868.67$[M+H]^+$.

[00421] INT-18-3 (511 mg, 589.38 μmol) was weighed and placed in a 50 mL single-neck flask, dissolved in 20 mL of dichloromethane, and stirred at room temperature. 3 mL of diethylamine was added dropwise to the reaction system for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was dried by rotary evaporation, dissolved in 20 mL of dichloromethane, dried by rotary evaporation, and washed with n-hexane. This process was repeated three times to obtain 158 mg of crude INT-18. LC-MS(ESI+)588.7$[M+H]^+$.

[00422] Example 22 Synthesis of compound INT-19



[00423] INT-14-1 (432.4 mg, 1.0 mmol) and NH_2PEG_2-COOH (177.3 mg, 1.0 mmol) were weighed and placed in a 25 mL single-neck flask and dissolved in 10 mL of dichloromethane. 50 μL of DIPEA was added dropwise, and stirred at room temperature overnight. After the reaction was completed, as monitored by LC-MS, the reaction solution was subjected to rotary evaporation to remove solvent and purified by preparative reverse phase Flash chromatography to obtain 441.5 mg of the target product INT-19-1, with a yield of 89.3%. LC-MS(ESI+): 495.5$[M+H]^+$.

[00424] INT-14-1 (432.4 mg, 1.0 mmol) and 10-Sar-COOHMe (742.8 mg, 1.0 mmol) were weighed and placed in a 50 mL single-neck flask, and dissolved in 15 mL of dichloromethane. 145 μL of DIPEA was added dropwise, and stirred at room temperature overnight. After the reaction was completed, as monitored by LC-MS, the reaction solution was subjected to rotary evaporation to remove solvent and purified by preparative reverse phase Flash chromatography to obtain 983.7

mg of the target product INT-19-2, with a yield of 92.7%. LC-MS(ESI+): 1061.2$[M+H]^+$.

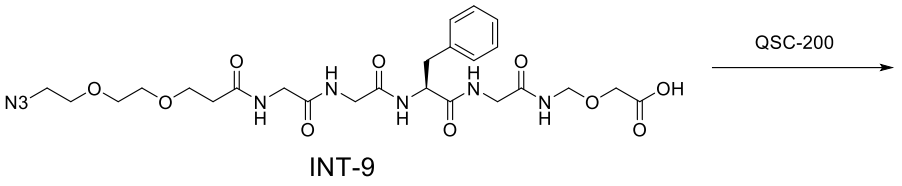
[00425] INT-19-2 (503.2 mg, 0.5 mmol) was weighed and placed in a 25 mL single-neck flask, dissolved in 5 mL of dichloromethane, and stirred at room temperature. 1 mL of diethylamine was added dropwise to the reaction system for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was dried by rotary evaporation, dissolved in 5 mL of dichloromethane, dried by rotary evaporation, and washed with n-hexane. This process was repeated three times to obtain 707.1 mg of crude INT-19-3, with a yield of 89.6%. LC-MS(ESI+)852.9$[M+H]^+$.

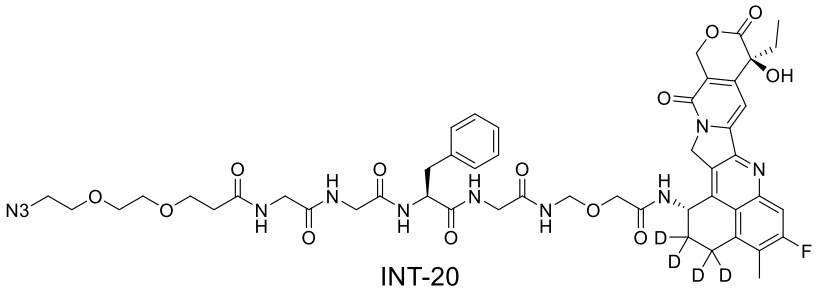
[00426] INT-19-1 (247.2 mg, 0.5 mmol), INT-19-3 (425.7 mg, 0.5 mmol) and HATU (288.60 mg, 759 μmol) were weighed and placed in a 25 mL single-neck flask, dissolved in 6 mL of DMF and 177 μL of DIPEA, and stirred at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was purified by preparative reverse phase Flash chromatography to obtain 497.9 mg of the target product INT-19-4, with a yield of 76.6%. LC-MS(ESI+): 1301.4$[M+Na]^+$.

[00427] INT-19-4 (497.9 mg, 0.38 mmol) was weighed and placed in a 25 mL single-neck flask, dissolved in 8 mL of dichloromethane, and stirred at room temperature. 1.5 mL of diethylamine was added dropwise to the reaction system for 3 h of reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction solution was dried by rotary evaporation, dissolved in 5 mL of dichloromethane, dried by rotary evaporation, and washed with n-hexane. This process was repeated three times to obtain 378.9 mg of crude INT-19-5, with a yield of 91.3%. LC-MS(ESI+)1093.2$[M+H]^+$.

[00428] INT-3 (95.1 mg, 0.25 mmol) and INT-19-5 (189.3 mg, 0.175 mmol) were weighed and placed in a 25 mL single-neck flask, added with 10 mL of dichloromethane, and stirred at room temperature overnight. After the reaction was completed, as monitored by LC-MS, the reaction solution was purified by preparative reverse phase Flash chromatography to obtain 165.2 mg of the target product INT-19, with a yield of 82.3%. LC-MS(ESI+)1286.4$[M+H]^+$.

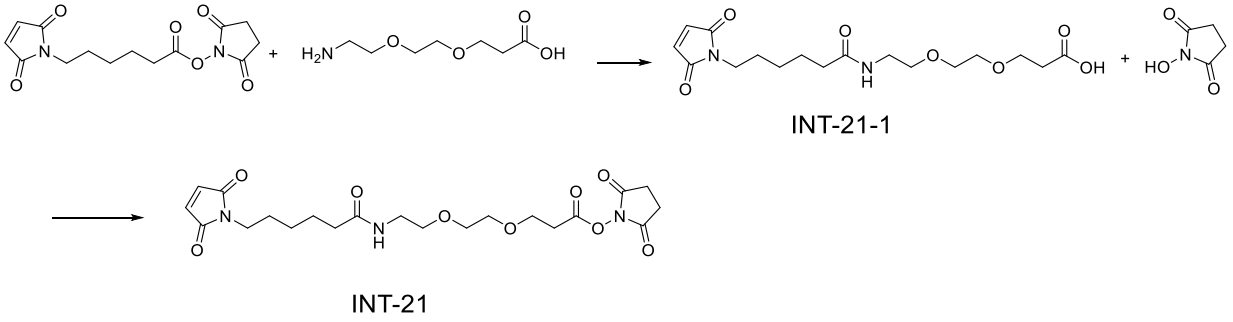
[00429] Example 23 Synthesis of compound INT-20





[00430] INT-9 (304 mg, 0.5 mmol), QSC-200 (219.2 mg, 0.5 mmol) and HATU (184.9 mg, 0.6 mmol) were weighed and placed in a 25 mL single-neck flask, added with 10 mL of DMF and 125 μL of DIPEA, and stirred at room temperature overnight. After the reaction was completed, as monitored by LC-MS, the reaction solution was purified by preparative liquid chromatography to obtain 427.9 mg of the target product INT-20 with a yield of 83.1%. LC-MS(ESI+)1031.1$[M+H]^+$.

[00431] Example 24 Synthesis of compound INT-21



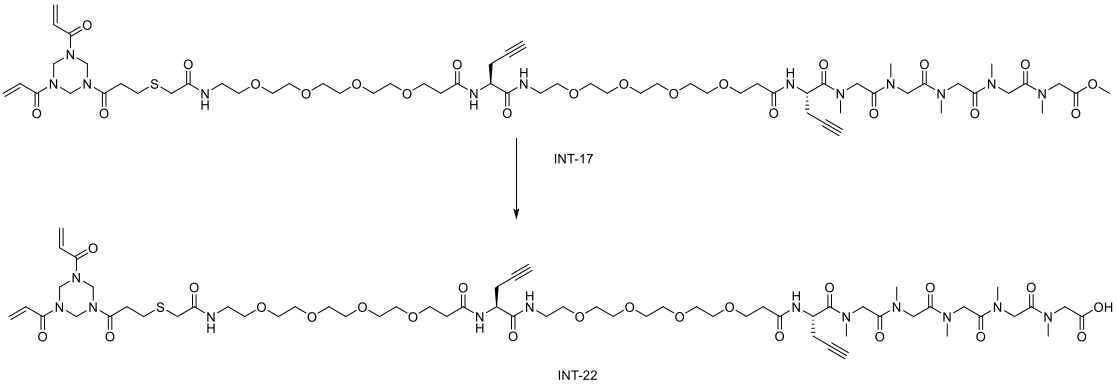
[00432] Compound INT-3 (2.0 g, 6.49 mmol) and 3-[2-(2-aminoethoxy)ethoxy]-propionic acid (1.1 g, 6.49 mmol) were dissolved in 40 mL of N,N-dimethylformamide, added with N,N-diisopropylethylamine (1.3 g, 9.7 mmol), and stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 2.1 g of the target compound INT-21-1, with a yield of 88%. LC-MS(ESI+)371.2$[M+H]^+$.

[00433] INT-21-1 (400 mg, 1.08 mmol), N-hydroxysuccinimide (150 mg, 1.32 mmol) and DCC (268 mg, 1.3 mmol) were dissolved in 10 mL of tetrahydrofuran, and stirred at room temperature

for 4 h. After the reaction was completed, the reaction mixture was filtered to remove the insoluble solid to obtain 452 mg of the target compound INT-21, with a yield of 90%. LC-MS(ESI+)468.2 $[M+H]^+$.

[00434] Example 25 Synthesis of compound INT-22

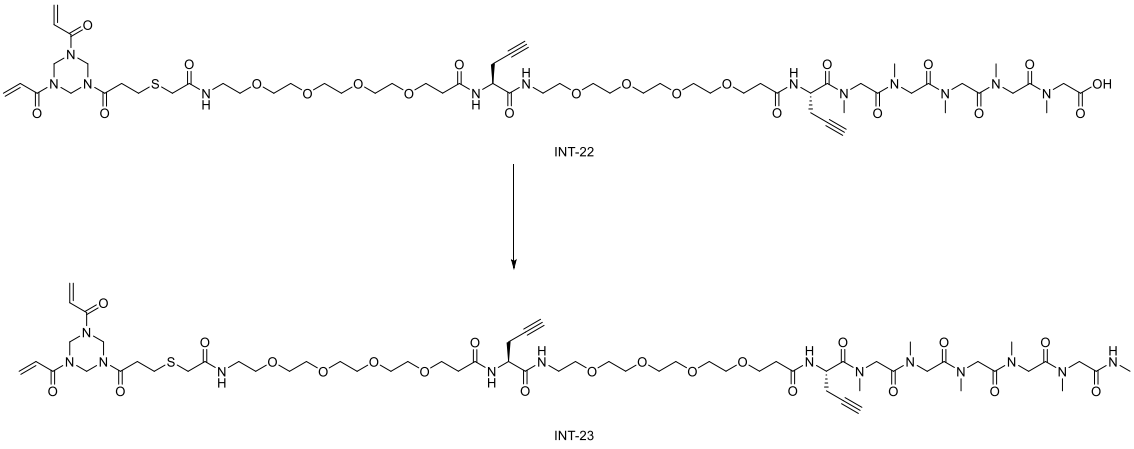
[00435]



[00436] INT-17 (500.00 mg, 0.36 mmol) was weighed and dissolved in 10 mL acetonitrile and 10 mL purified water under stirring, and DIPEA was added. The reaction mixture was adjusted to approximately pH 10, and stirred at room temperature for 2 h. After the reaction was completed, as monitored by LC-MS, the reaction solution was concentrated under reduced pressure to obtain 481.36 mg of the target compound INT-22, with a yield of 96.93%. LC-MS(ESI+)1379.6 $[M-H]^-$.

[00437] Example 26 Synthesis of compound INT-23

[00438]

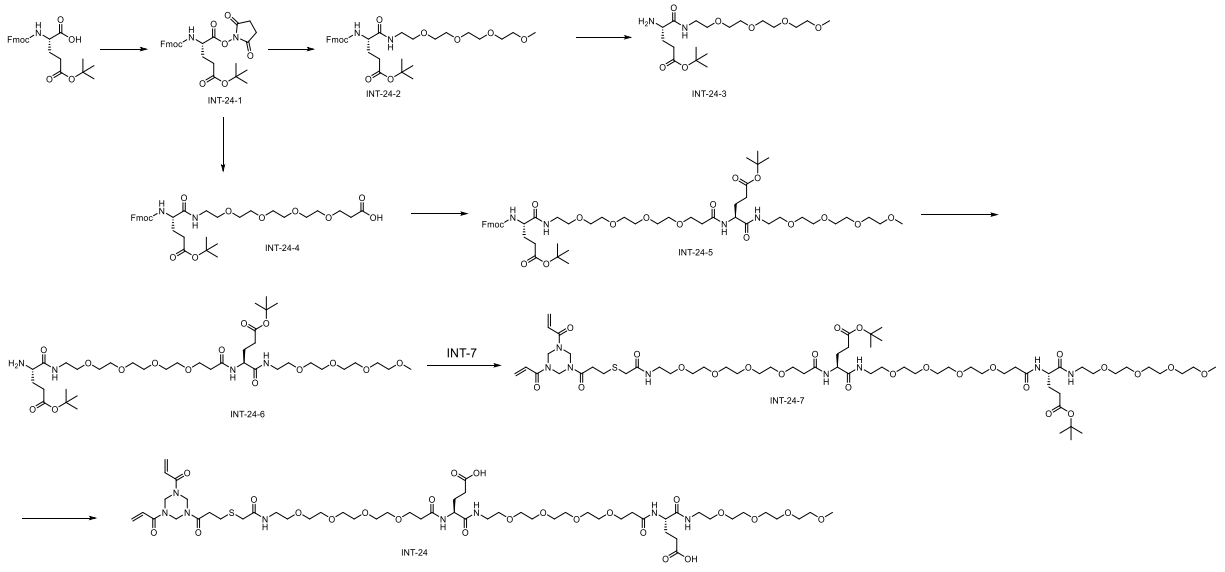


[00439] INT-22 (481.36 mg, 0.35 mmol), methylamine hydrochloride (28.23 mg, 0.42 mmol) and

HATU (198.72 mg, 0.52 mmol) were placed in a reaction flask. 5 mL of DMF was added at room temperature and stirred well, and DIPEA (112.58 mg, 0.87 mmol) was added dropwise. After 1 h of reaction, the reaction was terminated. The reaction solution was purified by preparative reverse phase chromatography to obtain 276.37 mg of INT-23, with a yield of 56.88%. LC-MS(ESI+)1394.7$[M+H]^+$.

[00440] Example 27 Synthesis of compound INT-24

[00441]



[00442] Fmoc-O-tert-butyl-L-glutamic acid (2 g, 4.7 mmol) was dissolved in 40 mL of tetrahydrofuran, and N-hydroxysuccinimide (594 mg, 4.17 mmol) and DCC (1067 mg, 5.17 mmol) were added at room temperature. After overnight reaction at room temperature, the reaction system was filtered, and the filter cake was rinsed with a small amount of tetrahydrofuran. The filtrate was dried by rotary evaporation to obtain the crude target compound INT-24-1, which was used directly in the next step without purification. LC-MS(ESI+)523.2 $[M+H]^+$.

[00443] Compound INT-24-1 (110 mg, 0.21 mmol) was dissolved in 3 mL of dichloromethane, and TEA (21 mg, 0.21 mmol) and $mPEG_4$-NH_2 (40 mg, 0.21 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, dried by rotary evaporation to remove solvent, and purified by preparative liquid chromatography to obtain 132 mg of the target compound INT-24-2, with a yield of 80%. LC-MS(ESI+)615.4 $[M+H]^+$.

[00444] Compound INT-24-2 (110 mg, 0.139 mmol) was dissolved in 1 mL of dichloromethane, and diethylamine (200 μL) was added at room temperature. The reaction solution was stirred at room temperature for 3 h, and then dried by rotary evaporation to remove solvent to obtain the crude target compound INT-24-03, which was used directly in the next step without purification. LC-MS(ESI+)393.4 $[M+H]^+$.

[00445] Compound INT-24-1 (220 mg, 0.42 mmol) was dissolved in 5 mL of dichloromethane, and TEA (42 mg, 0.42 mmol) and NH_2-PEG_4-COOH (111 mg, 0.42 mmol) were added at room temperature. The reaction solution was stirred at room temperature overnight, then dried by rotary evaporation to remove solvent, and purified by preparative liquid chromatography to obtain 221 mg of the target compound INT-24-4, with a yield of 78%. LC-MS(ESI+)673.4 $[M+H]^+$.

[00446] Compound INT-24-4 (85 mg, 0.13 mmol) was dissolved in 1.5 mL of DMF, and DIPEA (25 mg, 0.19 mmol) and TSTU (46 mg, 0.15 mmol) were added and stirred at room temperature for 1 h. The crude INT-24-03 was added and stirred at room temperature overnight. The reaction solution was purified by preparative liquid chromatography to obtain 72 mg of the target compound INT-24-5, with a yield of 53%. LC-MS(ESI+)1047.7 $[M+H]^+$.

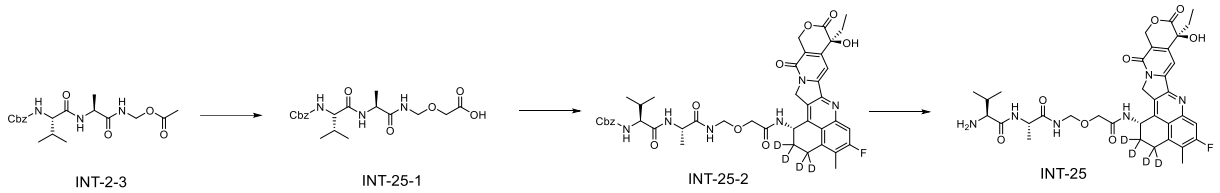
[00447] Compound INT-24-5 (72 mg, 0.06 mmol) was dissolved in 4 mL of dichloromethane, and diethylamine (800 μL) was added at room temperature. The reaction system was stirred at room temperature for 1 h, and then dried by rotary evaporation under reduced pressure to obtain the crude target compound INT-24-6. LC-MS(ESI+)825.7 $[M+H]^+$.

[00448] Crude INT-24-6 was dissolved in 4 mL of dichloromethane, and TEA (18 mg, 0.18 mmol), INT-7 (28 mg, 0.09 mmol) and HATU (68.35 mg, 0.18 mmol) were added at room temperature. The reaction system was stirred at room temperature overnight, then dried by rotary evaporation under reduced pressure, and purified by preparative liquid chromatography to obtain 27 mg of the target compound INT-24-7, with a two-step yield of 38%. LC-MS(ESI+)1394.8 $[M+H]^+$.

[00449] Compound INT-24-7 (10 mg, 0.08 mmol) was dissolved in 1 mL of dichloromethane, and TFA (200 μL) was added at room temperature. The reaction system was stirred at room temperature for 2 h and then dried by rotary evaporation under reduced pressure to obtain the crude target compound INT-24 (10 mg). LC-MS(ESI+)1283.6 $[M+H]^+$.

[00450] Example 28 Synthesis of compound INT-25

[00451]



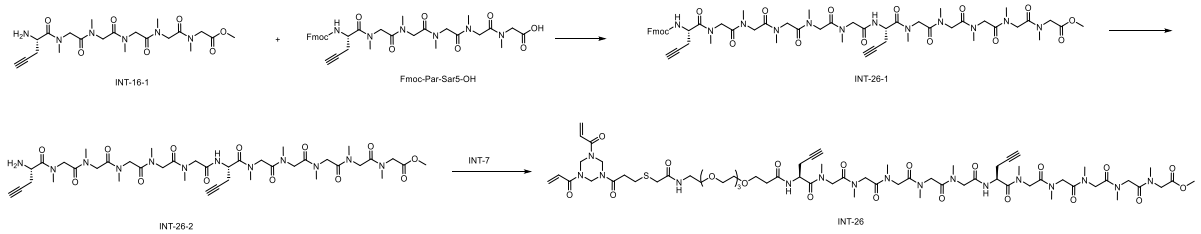
[00452] Compound INT-2-3 (14.00 g, 32.7 mmol) was dissolved in 280 mL of DCM, and PPTS (1.62 g, 0.033 mmol) and glycolic acid (3.73 g, 49 mmol) were added at room temperature. The mixture was refluxed overnight for reaction and filtered. The filter cake was rinsed with a small amount of dichloromethane and passed through a column to obtain 10.53 g of the target compound INT-25-1, with a yield of 78.31%. LC-MS(ESI+)410.2$[M+H]^+$.

[00453] INT-25-1 (410.3 mg, 1 mmol), QSC-200 (440.5 mg, 1 mmol) and HATU (571.3 mg, 1.5 mmol) were weighed and placed in a 25 mL single-neck flask, and 5 mL of DMF and 125 μL of DIPEA were added and stirred at room temperature overnight. After the reaction was completed, as monitored by LC-MS, the reaction solution purified by preparative liquid chromatography to obtain 665.3 mg of the target product INT-25-2, with a yield of 80.1%. LC-MS(ESI+)831.4$[M+H]^+$.

[00454] INT-25-2 (415.0, 0.5 mmol) and palladium on carbon (83.2 mg, 20%) were dissolved in 10 mL of tetrahydrofuran and stirred at room temperature for 2 h under hydrogen atmosphere. After the reaction was completed, the reaction solution was filtered to remove palladium on carbon, and the filter cake was rinsed with methanol. The filtrate was dried by rotary evaporation to obtain 335 mg of the target compound INT-25, with a yield of 95%. LC-MS(ESI+)697.3$[M+H]^+$.

[00455] Example 29 Synthesis of compound INT-26

[00456]



[00457] Compound INT-16-1 (337 mg, 0.7 mmol), Fmoc-Pra-Sar5-COOH (483 mg, 0.7 mmol) and HATU (418 mg, 1.1 mmol) were dissolved in 15 mL of N,N-dimethylformamide, and N,N-

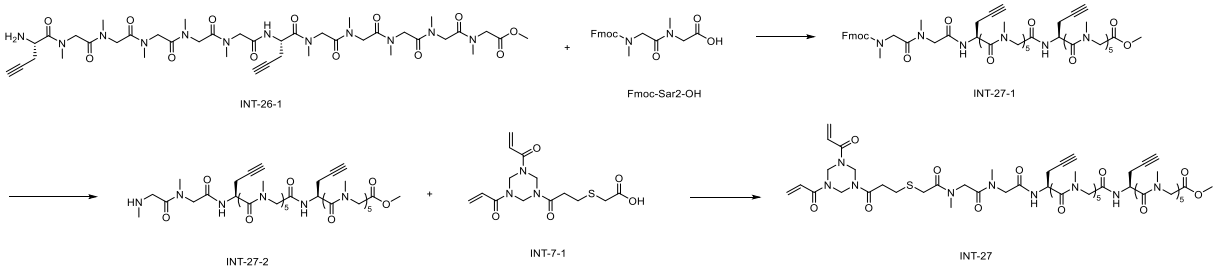
diisopropylethylamine (108 mg, 0.84 mmol) was added. The reaction system was stirred at room temperature for 3 h and then purified by preparative liquid chromatography to obtain 380 mg of the target compound INT-26-1, with a yield of 58.2%. LC-MS (ESI+) 1155.57 $[M+H]^+$.

[00458] Compound INT-26-1 (462 mg, 0.4 mmol) was dissolved in 6 mL of dichloromethane, and diethylamine (1.2 mL) was added at room temperature. The reaction system was stirred at room temperature for 3 h, dried by rotary evaporation to remove solvent and lyophilized to obtain 370 mg of the crude target compound INT-26-2, which was used directly in the next step without purification. LC-MS (ESI+) 933.47 $[M+H]^+$.

[00459] Compounds INT-26-2 (205 mg, 0.22 mmol), INT-7 (118 mg, 0.2 mmol), and HATU (114 mg, 0.3 mmol) were dissolved in 5 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (31 mg, 0.24 mmol) was added. The reaction system was stirred at room temperature for 3 h and then purified by preparative liquid chromatography to obtain 83 mg of the target compound INT-26, with a yield of 70.5%. LC-MS (ESI+) 1503.7 [M+H]+.

[00460] Example 30 Synthesis of compound INT-27

[00461]



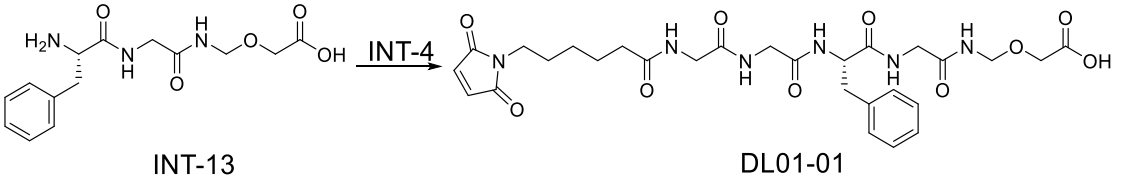
[00462] Compound INT-26-1 (160 mg, 0.17 mmol), Fmoc-Sar2-COOH (61 mg, 0.16 mmol), and HATU (73 mg, 0.19 mmol) were weighed, added to a 25 mL round-bottom reaction flask and dissolved in 6 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (62 mg, 0.48 mmol) was added. The reaction system was stirred at room temperature for 3 h and then purified by preparative liquid chromatography to obtain 157 mg of the target compound INT-27-1, with a yield of 71.2%. LC-MS (ESI+) 1298.61 $[M+H]^+$.

[00463] Compound INT-27-1 (156 mg, 0.12 mmol) was dissolved in 6 mL of dichloromethane, and diethylamine (1.2 mL) was added at room temperature. The reaction system was stirred at room

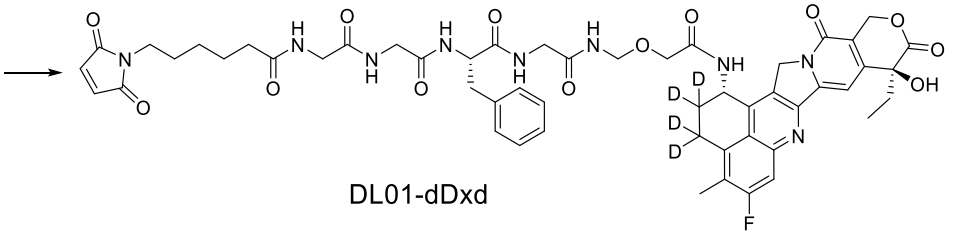
temperature for 3 h, dried by rotary evaporation to remove solvent and then lyophilized to obtain 129 mg of the crude target compound INT-27-2, which was used directly in the next step without purification. LC-MS (ESI+) 1075.55 $[M+H]^+$.

[00464] Compound INT-27-2 (117 mg, 0.11 mmol), INT-7-1 (53 mg, 0.11 mmol), and HATU (49 mg, 0.13 mmol) were weighed, added to a 25 mL round-bottom reaction flask and dissolved in 6 mL of N,N-dimethylformamide, and then N,N-diisopropylethylamine (22 mg, 0.17 mmol) was added. The reaction system was stirred at room temperature for 3 h and then purified by preparative liquid chromatography to obtain 65 mg of the target compound INT-27, with a yield of 40.9%. LC-MS (ESI+) 1398.64 $[M+H]^+$.

[00465] Example 31 Synthesis of compound DL01-dDxd



INT-13 DL01-01

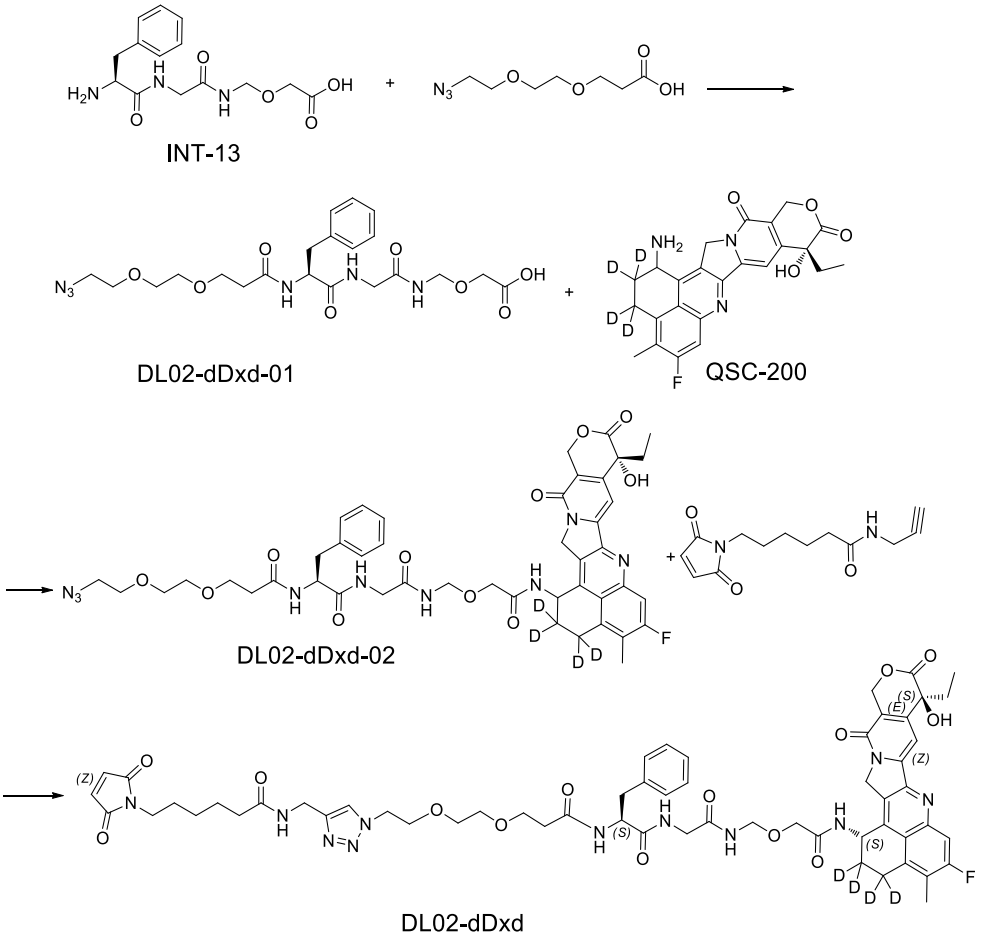


DL01-dDxd

[00466] Intermediates INT-13 (105 mg, 0.34 mmol) and INT-4 (215 mg, 0.51 mmol) were dissolved in 6.5 mL of N,N-dimethylformamide and stirred at room temperature for 2 h. The reaction system was purified by preparative liquid chromatography to obtain 186.4 mg of the target compound DL01-01, with a yield of 89.3%. LC-MS (ESI+) 615.34 $[M+H]^+$.

[00467] Compound DL01-01 (52 mg, 0.085 mmol) was dissolved in 5 mL of N,N-dimethylformamide, and QSC-200 (25 mg, 0.057 mmol), EDCI (32 mg, 0.17 mmol) and HOBT (23 mg, 0.17 mmol) were added at room temperature. The reaction system was stirred at room temperature for 2 h and then purified by preparative liquid chromatography to obtain 19.4 mg of the target compound DL01-dDxd, with a yield of 22%. LC-MS (ESI+) 1038.58 $[M+H]^+$.

[00468] Example 32 Synthesis of compound DL02-dDxd



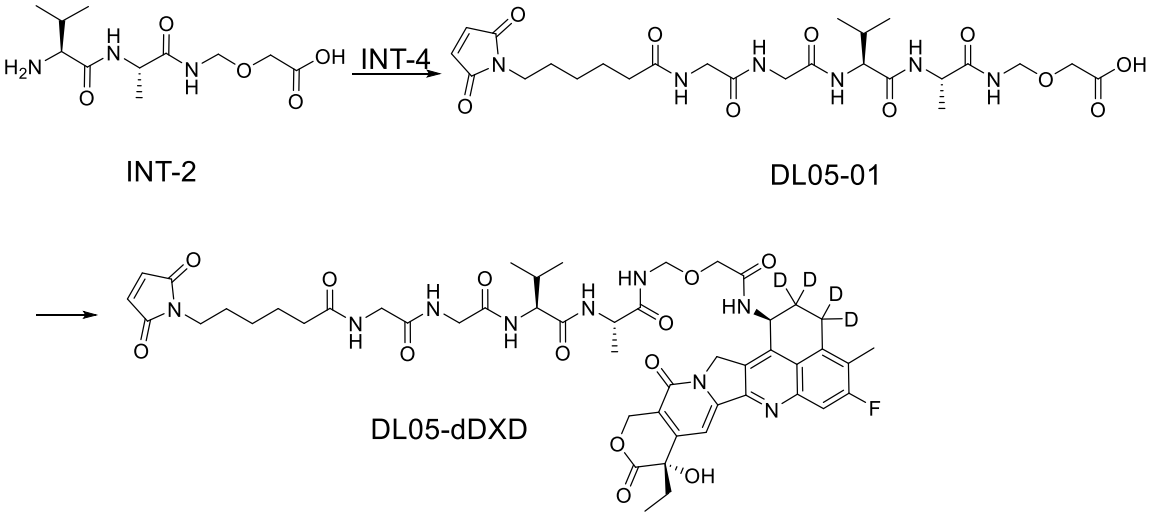
[00469] Azide-ethylene glycol-acetic acid (238 mg, 1.17 mmol) and HATU (326 mg, 0.86 mmol) were dissolved in 15 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (201 mg, 1.56 mmol) was added and pre-stirred at room temperature for 1 h. INT-13 (240 mg, 0.78 mmol) was then added to the reaction solution for 2 h of reaction. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 93.9 mg of the target compound DL02-dDxd-01, with a yield of 24%. LC-MS (ESI+) 495.4 $[M+H]^+$.

[00470] DL02-dDxd-01 (25 mg, 0.051 mmol), HATU (19 mg, 0.051 mmol) and QSC-200 (15 mg, 0.034 mmol) were dissolved in 15 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (8.8 mg, 0.068 mmol) was added and stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 6.3 mg of the target compound DL02-dDxd-02, with a yield of 14%. LC-MS (ESI+) 916.8 [M+H]+.

[00471] Compound DL02-dDxd-02 (6.3 mg, 0.0069 mmol) was dissolved in 1 mL of acetone.

INT-6 (2.5 mg, 0.01 mmol) and copper sulfate pentahydrate (2.5 mg, 0.01 mmol) were added at room temperature. Under nitrogen protection, the reaction solution was transferred to a low-temperature bath and stirred at 10°C. Sodium ascorbate (2.0 mg, 0.01 mmol) was dissolved in 1 mL of water and added dropwise to the above reaction solution. The reaction system was stirred at 10°C for 1 h, and the reaction was quenched with 1 mL of acetonitrile. The reaction system was purified by preparative liquid chromatography to obtain 5.2 mg of the target compound DL02-dDxd, with a yield of 65%. LC-MS (ESI+) 1164.8 $[M+H]^+$.

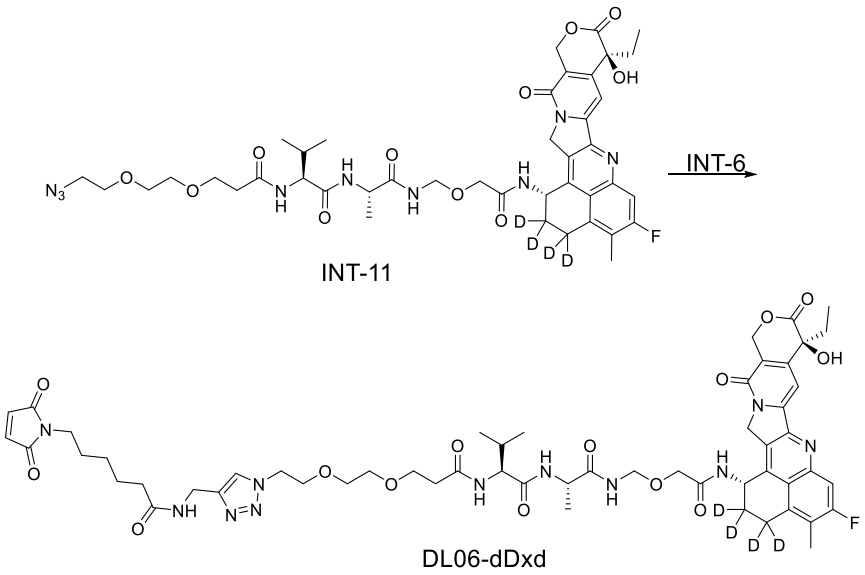
[00472] Example 33: Synthesis of Compound DL05-dDxd



[00473] Intermediates INT-2 (60 mg, 0.218 mmol) and INT-4 (138 mg, 0.327 mmol) were dissolved in 5 mL of N,N-dimethylformamide. The reaction system was stirred at room temperature for 2 h and then purified by preparative liquid chromatography to obtain 104 mg of the target compound DL05-01, with a yield of 81.9%. LC-MS (ESI+) 583.29 $[M+H]^+$.

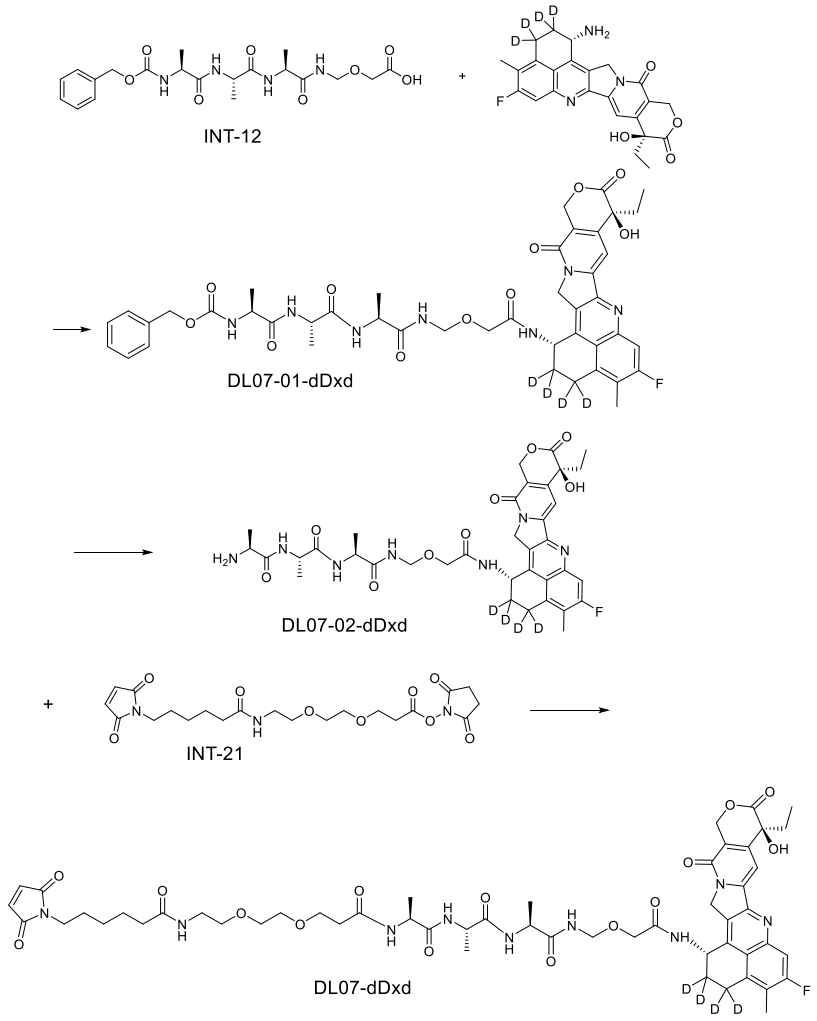
[00474] Compound DL05-01 (50 mg, 0.086 mmol) was dissolved in 5 mL of N,N-dimethylformamide, and QSC-200 (38 mg, 0.072 mmol), EDCI (41 mg, 0.21 mmol), and HOBT (29 mg, 0.21 mmol) were added at room temperature. The reaction system was stirred at room temperature for 2 h and then purified by preparative liquid chromatography to obtain 64.6 mg of the target compound DL05-dDxd, with a yield of 74.9%. LC-MS (ESI+) 1004.28 $[M+H]^+$.

[00475] Example 34: Synthesis of compound DL06-dDxd



[00476] Compound INT-11 (100 mg, 0.113 mmol) was dissolved in 4 mL of acetone. INT-6 (28 mg, 0.113 mmol) and copper sulfate pentahydrate (42.5 mg, 0.17 mmol) were added at room temperature. The reaction solution was then transferred to a low-temperature bath and stirred at 10°C. Sodium ascorbate (33.7 mg, 0.17 mmol) was dissolved in 2 mL of water and added dropwise to the above reaction solution. The reaction system was stirred at 10°C for 1 h, and the reaction was quenched with 2 mL of acetonitrile. The reaction system was purified by preparative liquid chromatography to obtain 76.8 mg of the target compound DL06-dDxd, with a yield of 60.2%. LC-MS (ESI+) 1130.93 $[M+H]^+$.

[00477] Example 35: Synthesis of Compound DL07-dDxd



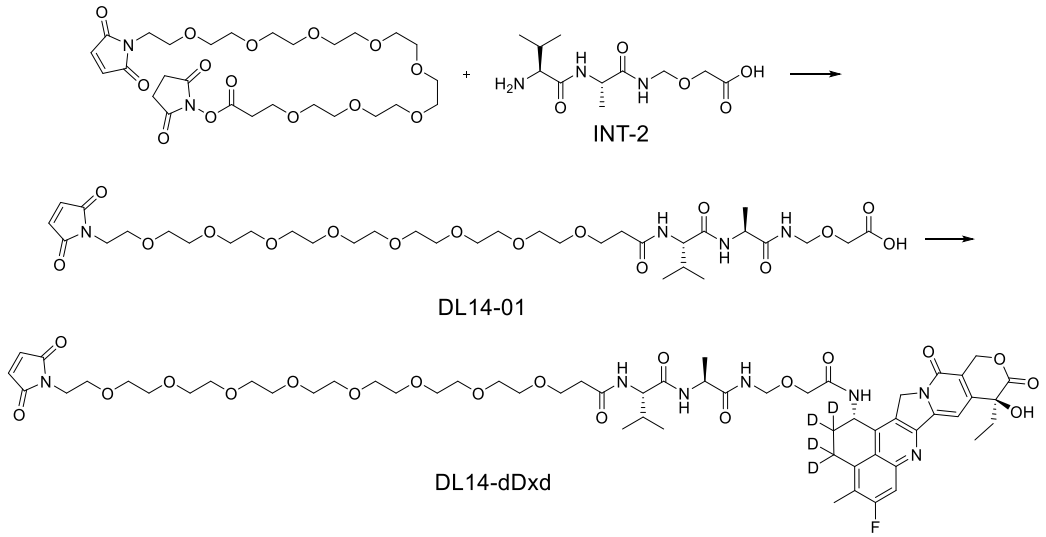
[00478] INT-12 (500 mg, 1.1 mmol), HATU (501 mg, 1.32 mmol) and QSC-200 (531 mg, 1.21 mmol) were dissolved in 30 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (213 mg, 1.65 mmol) was added. The reaction solution was stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 672 mg of the target compound DL07-01-dDxd, with a yield of 70%. LC-MS (ESI+) 874.5 $[M+H]^+$.

[00479] Compound DL07-01-dDxd (672 mg, 0.77 mmol) was dissolved in 20 mL of methanol, and palladium on carbon (200 mg, 30%) was added at room temperature. The reaction system was subjected to hydrogen replacement three times, stirred at room temperature for 2 h and filtered. Palladium on carbon was washed with 5 mL of methanol three times. The filtrate was dried by rotary evaporation to obtain crude DL07-02-dDxd, which was used directly in the next step. LC-

MS (ESI+) 740.3 $[M+H]^+$.

[00480] Compounds DL07-02-dDxd (150 mg, 0.20 mmol) and INT-21 (112 mg, 0.24 mmol) were dissolved in 10 mL of N,N-dimethylformamide, and N,N-diisopropylethylamine (39 mg, 0.3 mmol) was added and stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 75 mg of the target compound DL07-dDxd, with a yield of 34%. LC-MS (ESI+) 1092.8 $[M+H]^+$.

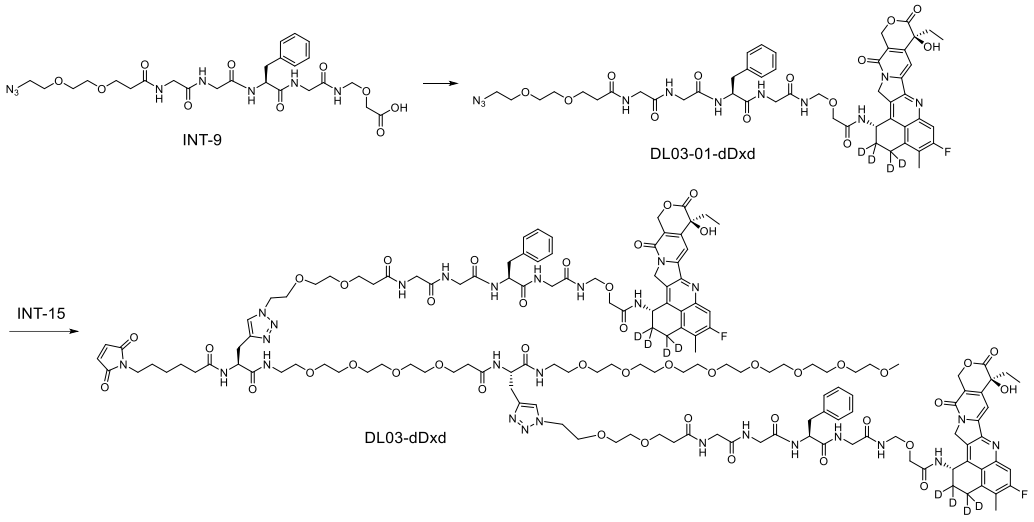
[00481] Example 36 Synthesis of compound DL14-dDxd



[00482] Maleimide-octapolyethylene glycol-succinimide acrylate (200 mg, 0.32 mmol) and INT-2 (89 mg, 0.32 mmol) were weighed and placed in a 25 mL single-necked flask. 5 mL of DMF and 60 μL of TEA were added. The reaction solution was stirred at room temperature overnight and then purified by preparative liquid chromatography to obtain 127.8 mg of DL14-01, with a yield of 50.9%. LC-MS (ESI+) 779.4 $[M+H]^+$.

[00483] To DL14-01 (127.2 mg, 0.16 mmol), EDCI (34.6 mg, 0.18 mol), HOBT (11.1 mg, 0.082 mmol) and QSC-200 (96.4 mg, 0.18 mmol), 5 mL of DMF and 41 μL of DIPEA were added. The reaction solution was stirred at room temperature for 6 h and then purified by preparative liquid chromatography to obtain 95.5 mg of DL14-dDxd, with a yield of 48.5%. LC-MS (ESI+) 1201.3 $[M+H]^+$.

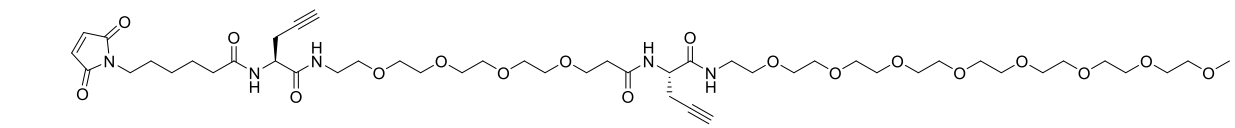
[00484] Example 37 Synthesis of compound DL03-dDxd



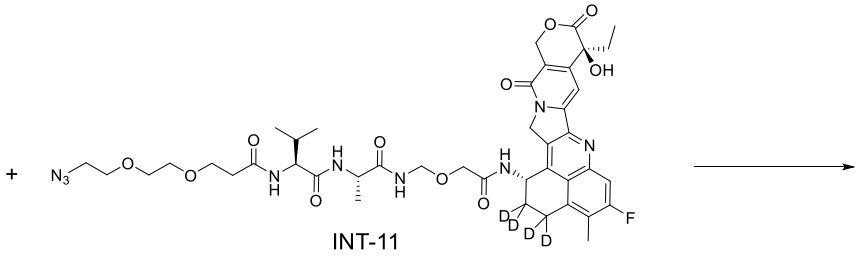
[00485] INT-9 (218 mg, 0.36 mmol) was dissolved in 5 mL of N,N-dimethylformamide, and QSC-200 (192 mg, 0.36 mmol), HATU (150 mg, 0.39 mmol), and N,N-diisopropylethylamine (95 mg, 0.72 mmol) were added at room temperature. The reaction system was stirred at room temperature for 1 h and then purified by Flash chromatography to obtain 274 mg of the target compound DL03-01-dDxd, with a yield of 74%. LC-MS (ESI+) 1029.9 $[M+H]^+$.

[00486] Compound INT-15 (178 mg, 173 μmol) was dissolved in 8 mL of acetone and 4 mL of water. Compound DL03-01-dDxd (360 mg, 344 μmol) and copper sulfate pentahydrate (134 mg, 536 μmol) were added at room temperature. The reaction system was placed in a low-temperature bath and stirred at 10°C. An aqueous solution (4 mL) containing sodium ascorbate (106.7 mg, 536 μmol) was slowly added dropwise to the reaction solution. The reaction system was stirred at 10 °C for 1 h, and the reaction was quenched with 2 mL of acetonitrile. The reaction system was purified by preparative liquid chromatography to obtain 361 mg of the target compound DL03-dDxd, with a yield of 68%. LC-MS (ESI+) 1537.1 $[M/2+H]^+$.

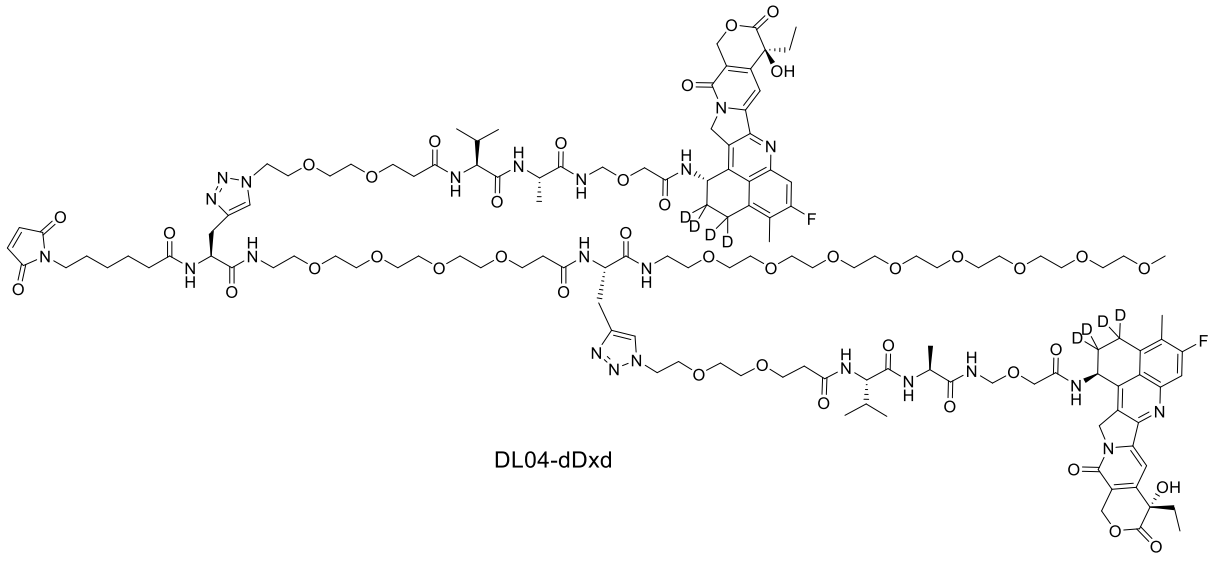
[00487] Example 38 Synthesis of compound DL04-dDxd



INT-15



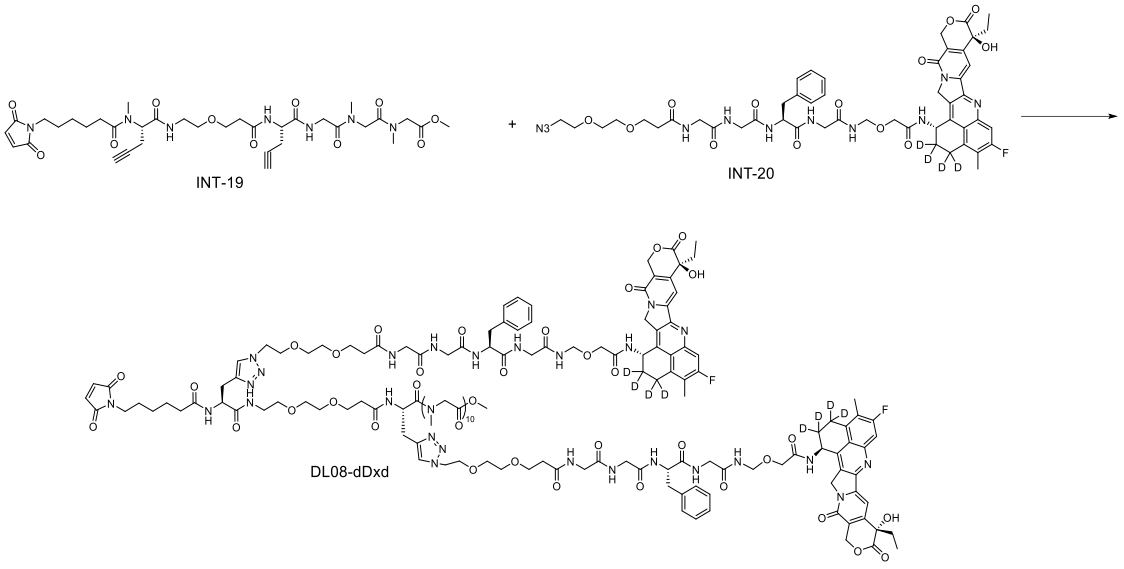
INT-11



DL04-dDxd

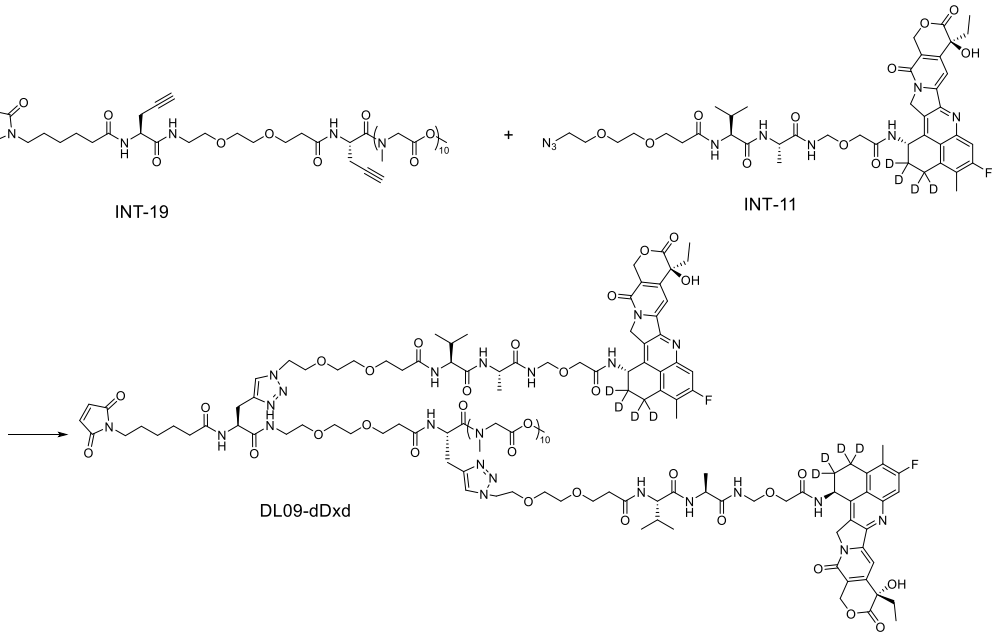
[00488] INT-15 (404.5 mg, 0.40 mmol), INT-11 (723.0 mg, 0.82 mmol), and copper sulfate pentahydrate (237.5 mg, 0.95 mmol) were dissolved in 6 mL of acetone, and 1 mL of glacial acetic acid was added. The reaction solution was stirred at 10°C under argon atmosphere. Sodium L-ascorbate (239.3 mg, 1.20 mmol) was dissolved in 2 mL of water and then added dropwise to the reaction solution. The reaction solution was stirred at 10°C for 1 h. After the reaction was completed, 2 mL of acetonitrile was added to quench the reaction. The reaction solution was purified by preparative liquid chromatography to obtain 242.5 mg of DL04-dDxd, with a yield of 22%. LC-MS (ESI+) 2778.8 $[M+H]^+$.

[00489] Example 39 Synthesis of compound DL08-dDxd



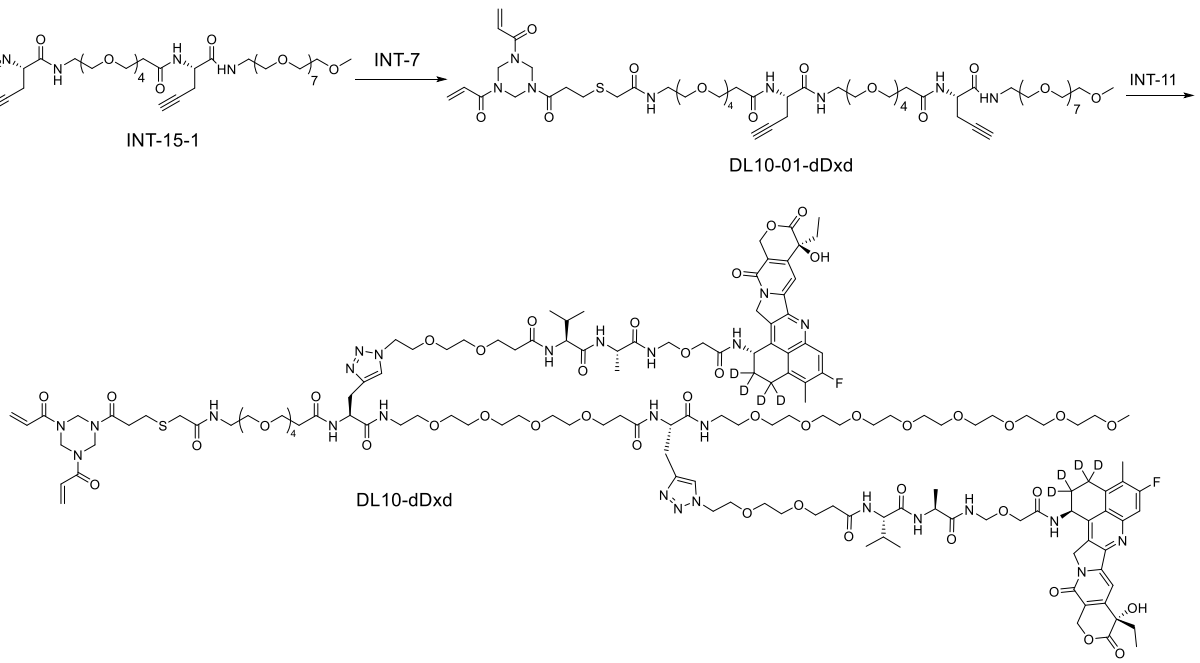
[00490] INT-19 (40 mg, 31.12 μmol) was weighed, placed in a 25 mL three-neck flask and completely dissolved in 2 mL of acetone and 2 mL of purified water. The mixture was stirred at 0°C, to which INT-20 (70.52 mg, 68.46 μmol) was weighed and added. Then, copper sulfate (14.9 mg, 93.35 μmol) was dissolved in 1 mL of water and slowly added dropwise. Then, sodium ascorbate (19.73 mg, 99.58 μmol) was dissolved in 1 mL of water and slowly added dropwise. The reaction system was stirred at 0°C for 30 min. After the reaction was completed, as monitored by LC-MS, 100 μL of ACN (acetonitrile) was added dropwise to quench the reaction. The reaction system was purified by preparative reverse phase liquid chromatography to obtain 51 mg of the target product DL08-dDxd, with a purity of 97.41% and a yield of 48.99%. ESI-MS(m/z): 1672.78$[M/2+H]^+$.

[00491] Example 40 Synthesis of compound DL09-dDxd



[00492] INT-19 (40 mg, 31.12 μmol) was weighed, placed in a 25 mL three-neck flask and completely dissolved in 2 mL of acetone and 2 mL of purified water. The mixture was stirred at 0°C, to which INT-11 (60.38 mg, 68.46 μmol) was weighed and added. Then, copper sulfate pentahydrate (23.31 mg, 93.35 μmol) was dissolved in 1 mL of water and slowly added dropwise. Then, sodium ascorbate (19.73 mg, 99.58 μmol) was dissolved in 1 mL of water and slowly added dropwise. The reaction system was stirred at 0°C for 30 min. After the reaction was completed, as monitored by LC-MS, 100 μL of ACN was added dropwise to quench the reaction. The reaction system was purified by preparative reverse phase liquid chromatography to obtain 49 mg of the target product DL09-dDxd, with a purity of 98.11% and a yield of 51.64%. ESI-MS(m/z): 1524.56$[M/2+H]^+$.

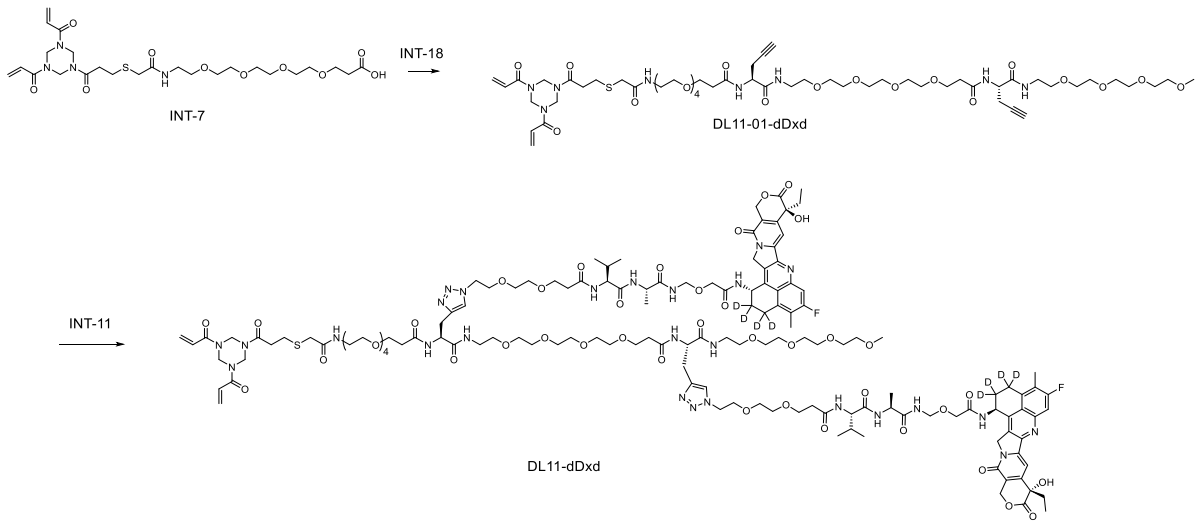
[00493] Example 41 Synthesis of compound DL10-dDxd



[00494] Compounds INT-15-1 (830 mg, 1 mmol) and INT-7 (590 mg, 1 mmol) were dissolved in 10 mL of DMF, and HATU (380 mg, 10 mmol) was added. DIPEA (130 mg, 10 mmol) was added under stirring at room temperature. The reaction solution was stirred at room temperature for 2 h of reaction, diluted with acetonitrile-water, filtered through a 0.45 μm filter membrane and purified by preparative high-pressure liquid chromatography to obtain 97 mg of DL10-01-dDxd, with a yield of 69.8%. LC-MS (ESI+) 1392 $[M+H]^+$

[00495] Compounds DL10-01-dDxd (97 mg, 0.07 mmol), INT-11 (129.3 mg, 0.15 mmol), and copper sulfate (18.5 mg, 0.21 mmol) were dissolved in 5 mL of acetone and stirred at 10°C for 10 min. Sodium ascorbate (13.8 mg, 0.21 mmol) was weighed, dissolved in 5 mL of water and added to the reaction solution. The reaction solution was stirred at 10°C for 1 h, subjected to rotary evaporation to remove acetone, diluted with acetonitrile-water and then purified by preparative high-pressure liquid chromatography to obtain 65 mg of DL10-dDxd, with a yield of 30%. LC-MS (ESI+) 1758.3 $[M/2]^+$.

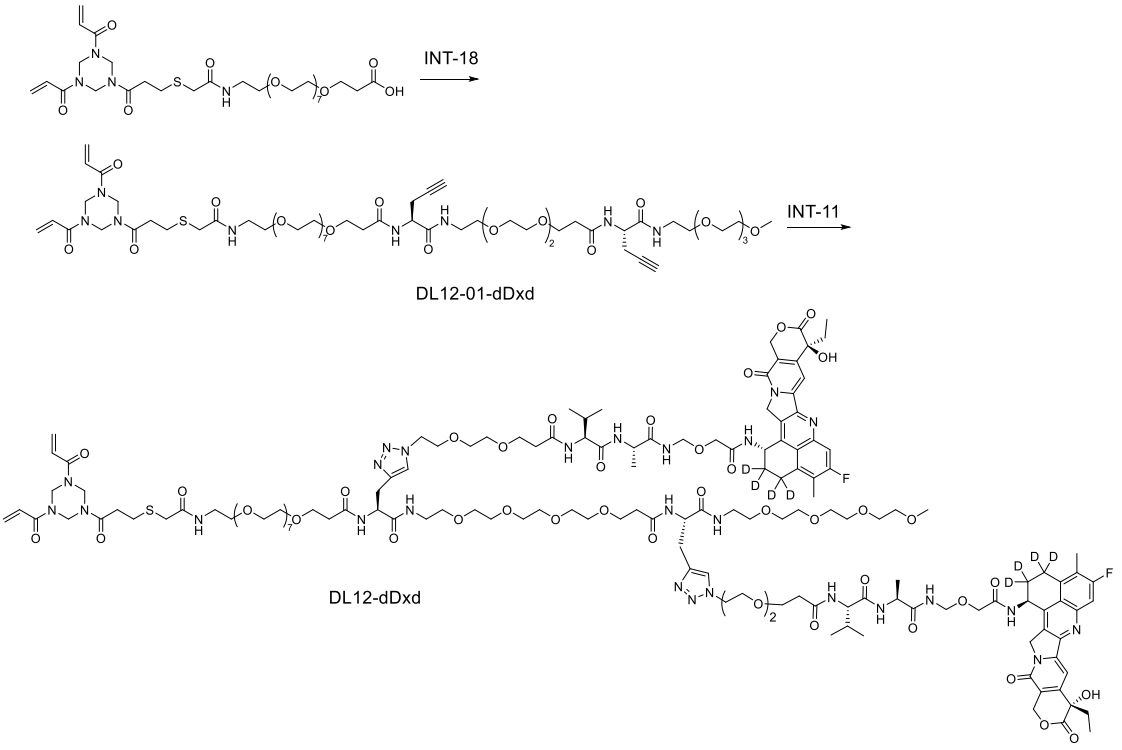
[00496] Example 42 Synthesis of compound DL11-dDxd



[00497] INT-7 (403.3 mg, 0.68 mmol) and HATU (195.2 mg, 0.51 mmol) were dissolved in 10 mL of N,N-dimethylformamide, and 90.3 μL of N,N-diisopropylethylamine was added. The mixture was pre-stirred at room temperature for 1 h. Then, INT-18 (221.4 mg, 0.34 mmol) was added for 2 h of reaction. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to obtain 147.6 mg of the target compound DL11-01-dDxd, with a yield of 36%. LC-MS (ESI+) 1216.3 $[M+H]^+$.

[00498] DL11-01-dDxd (147.6 mg, 0.12 mmol), INT-11 (236.8 mg, 0.27 mmol), and copper sulfate pentahydrate (99.8 mg, 0.40 mmol) were dissolved in 6 mL of acetone and stirred at 10°C under argon atmosphere. Then, sodium L-ascorbate (84.7 mg, 0.43 mmol) was dissolved in 2 mL of water and added dropwise. The reaction solution was stirred at 10°C for 1 h. After the reaction was completed, 2 mL of acetonitrile was added to quench the reaction. The reaction solution was purified by preparative liquid chromatography to obtain 57.3 mg of DL11-dDxd, with a yield of 16%. LC-MS (ESI+) 2978.8 $[M+H]^+$.

[00499] Example 43 Synthesis of compound DL12-dDxd

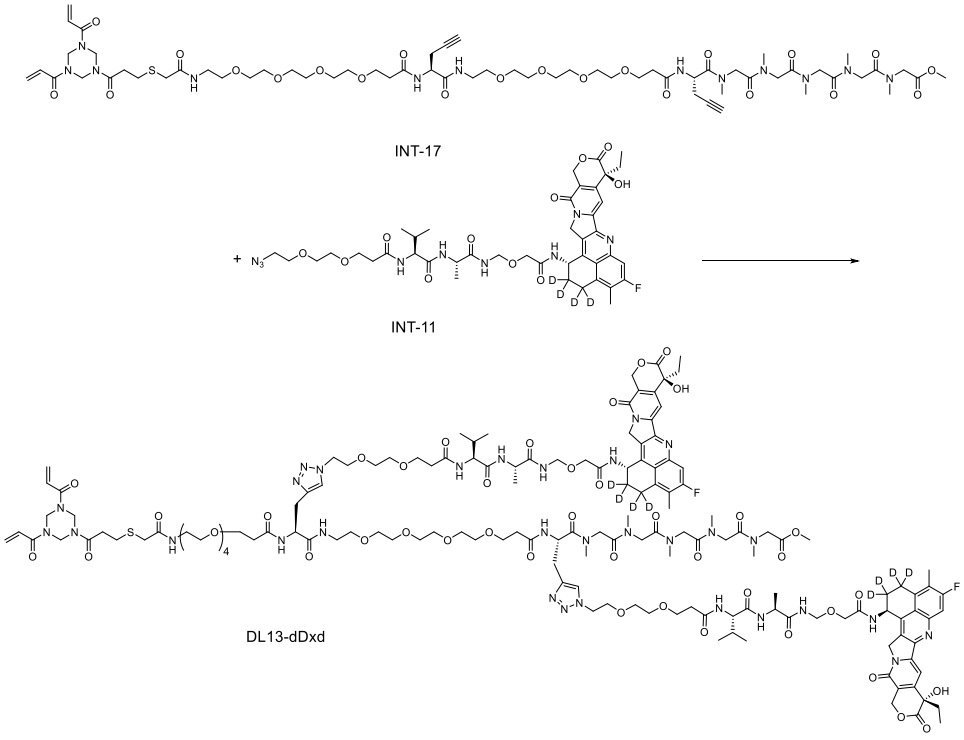


[00500] INT-9 (188 mg, 245.79 μmol) was weighed, placed in a 50 mL single-necked flask, dissolved in 10 mL of DMF, and stirred at room temperature, and then HATU (102.8 mg, 270 μmol) was weighed and added. Then, 52 μL of DIPEA was added dropwise to the reaction system and stirred at room temperature for 50 min. INT-18 (158 mg, 245.8 μmol) was weighed and added for overnight reaction at room temperature. After the reaction was completed, as monitored by LC-MS, the reaction system was purified by preparative reverse phase high-pressure liquid chromatography to obtain 185 mg of the target product DL12-01-dDxd, with a purity of 98% and a yield of 59.95%. LC-MS (ESI+): 1392.59 $[M+H]^+$.

[00501] DL12-01-dDxd (121 mg, 86.95 μmol) was weighed, placed in a 50 mL three-neck flask and completely dissolved in 5 mL of acetone and 1 mL of purified water. The mixture was stirred at 0°C, to which INT-11 (153.37 mg, 173.9 μmol) was weighed and added. Then, copper sulfate pentahydrate (54.27 mg, 217.37 μmol) was dissolved in 2 mL of water and slowly added dropwise. Then, sodium ascorbate (51.67 mg, 260.85 μmol) was dissolved in 2 mL of water and slowly added dropwise. The reaction system was stirred at 0°C for 30 min. After the reaction was completed, as monitored by LC-MS, 100 μL of ACN was added dropwise to quench the reaction. The reaction system was purified by preparative reverse phase liquid chromatography to obtain 89 mg of the

target product DL12-dDxd, with a yield of 32.44%. ESI-MS(m/z)：1578.76$[M+2H]^{2+}$.

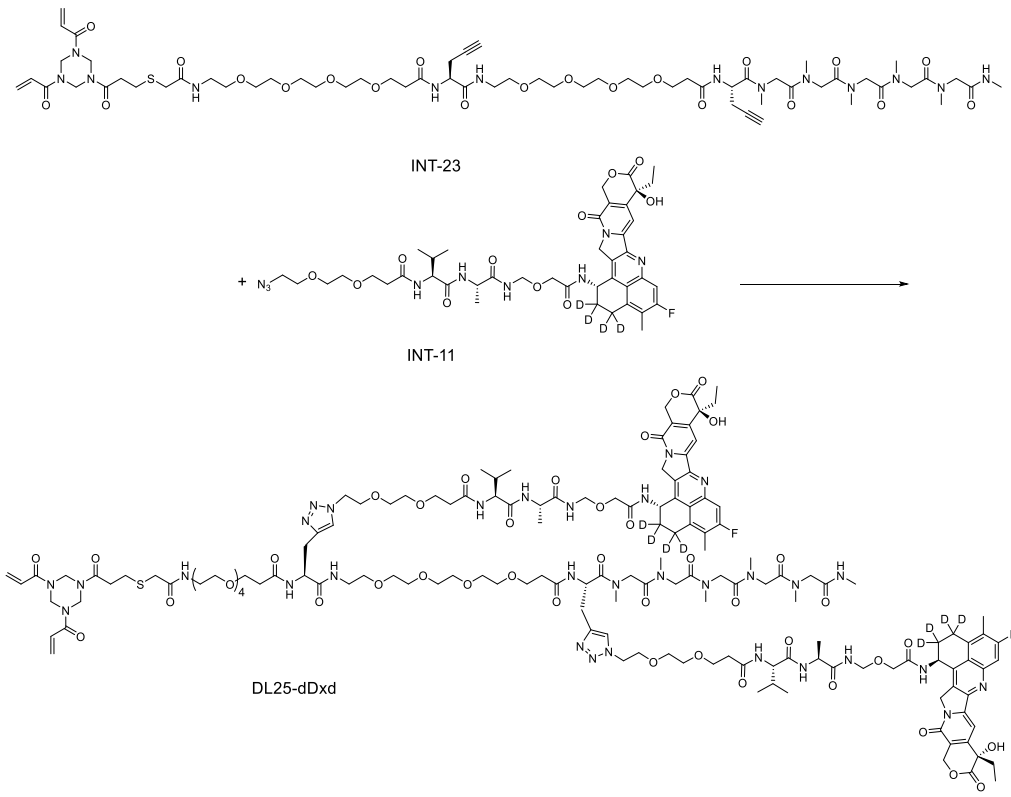
[00502] Example 44 Synthesis of compound DL13-dDxd



[00503] Compound INT-17 (200 mg, 0.14 mmol) was dissolved in 10 mL of acetone, and INT-11 (256 mg, 0.29 mmol), copper sulfate pentahydrate (105 mg, 0.42 mmol), and sodium ascorbate (83 mg, 0.42 μmol) were added at room temperature. The mixture was stirred at room temperature for 1 h. The reaction was quenched with acetonitrile (5.0 mL). The reaction system was purified by preparative liquid chromatography to obtain 181 mg of the target compound DL13-dDxd, with a yield of 41%. LC-MS (ESI+) 1579.76 $[M/2+H]^{+}$.

[00504] Example 45 Synthesis of compound DL25-dDxd

[00505]

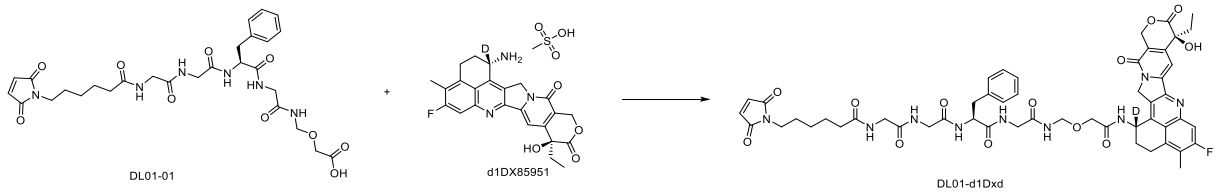


[00506]

[00507] INT-23 (51.00 mg, 0.04 mmol) and INT-11 (63.11 mg, 0.07 mmol) were weighed and dissolved in acetone (1.5 mL) and purified water (0.5 mL) under stirring. The nitrogen replacement was carried out three times. Copper sulfate pentahydrate (8.87 mg, 0.04 mmol) and sodium ascorbate (7.05 mg, 0.04 mmol) were separately weighed and dissolved with purified water (0.2 mL each) for later use. The solution of copper sulfate pentahydrate and the solution of sodium ascorbate were added sequentially and stirred at room temperature for 0.5 h of reaction. After the reaction was completed, as monitored by LC-MS, the reaction solution was purified by preparative liquid chromatography to obtain 60.13 mg of DL25-dDxd, with a yield of 54.02%. LC-MS (ESI+) 3157.5 $[M+H]^+$.

[00508] Example 46 Synthesis of compound DL01-d1Dxd

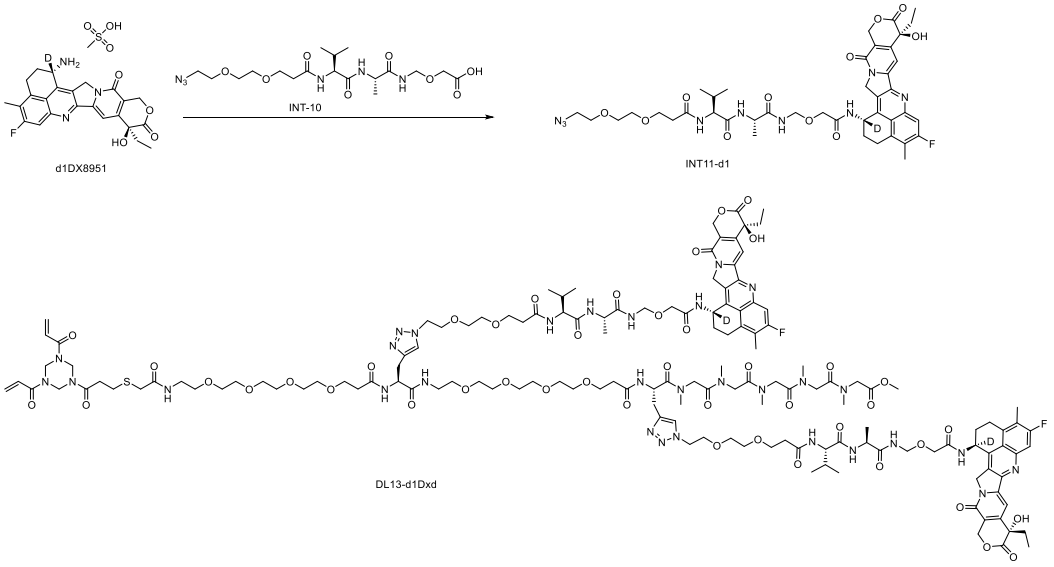
[00509]



[00510] Compound DL01-01 (850 mg, 1.4 mmol) and d1DX85951 (620 mg, 1.2 mmol) were dissolved in 20 mL of N,N-dimethylformamide, and HATU (684 mg, 1.8 mmol) and N,N-diisopropylethylamine (387 mg, 3 mmol) were added. The reaction system was stirred at room temperature for 2 h and then purified by preparative liquid chromatography to obtain 868 mg of the target compound DL-d1Dxd, with a yield of 72%. LC-MS (ESI+) 1035.40 $[M+H]^+$.

[00511] Example 47 Synthesis of compound DL13-d1Dxd

[00512]



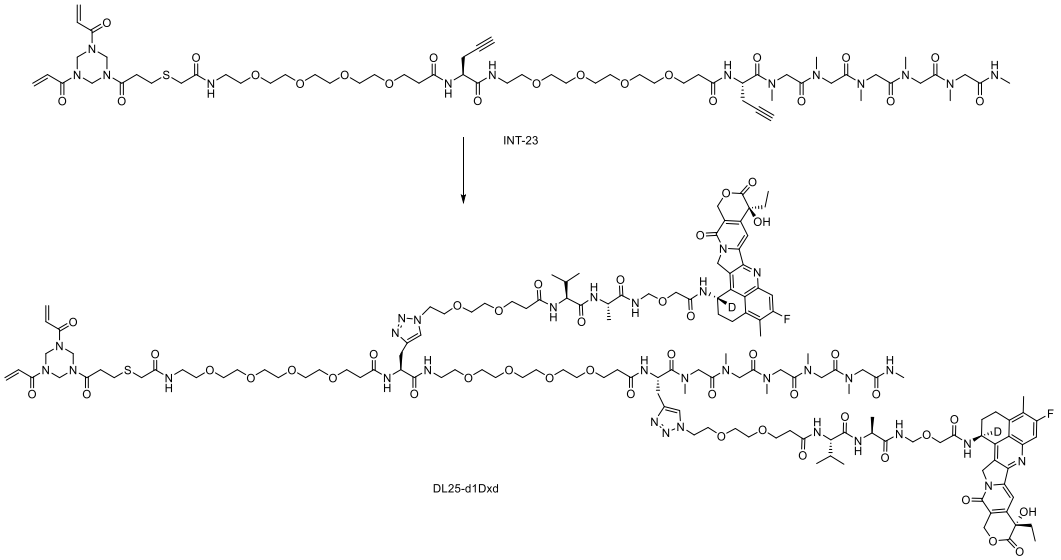
[00513] INT-10 (594.0 mg, 1.29 mmol), d1DX8951 (507.24 mg, 1.16 mmol), and HATU (535 mg, 1.41 mmol) were weighed and placed in a reaction flask, and 10 mL of DMF was added and stirred well at room temperature. DIPEA (454 mg, 3.51 mmol) was added dropwise. After 2 h of reaction, the reaction was quenched and the reaction system was purified by preparative reverse phase liquid chromatography to obtain 649.25 mg of INT-11-d1, with a yield of 57.27%. LC-MS (ESI+) 879.3 [M+H]+.

[00514] INT-17 (50.00 mg, 0.04 mmol) and INT-11-d1 (63.02 mg, 0.07 mmol) were weighed and dissolved in acetone (1.5 mL) and purified water (0.5 mL) under stirring. The nitrogen replacement was carried out three times. Copper sulfate pentahydrate (8.96 mg, 0.04 mmol) and sodium ascorbate (7.10 mg, 0.04 mmol) were separately weighed and dissolved with purified water (0.2 mL each) for later use. The solution of copper sulfate pentahydrate and the solution of sodium ascorbate were added sequentially and stirred at room temperature for 0.5 h of reaction. After the

reaction was completed, as monitored by LC-MS, the reaction solution was purified by preparative liquid chromatography to obtain 50.86 mg of DL13-d1Dxd, with a yield of 45.01%. LC-MS(ESI+)3152.4$[M+H]^+$.

[00515] Example 48 Synthesis of compound DL25-d1Dxd

[00516]



[00517] INT-23 (50.00 mg, 0.04 mmol) and INT-11-d1 (63.02 mg, 0.07 mmol) were weighed and dissolved in acetone (1.5 mL) and purified water (0.5 mL) under stirring. The nitrogen replacement was carried out three times. Copper sulfate pentahydrate (8.96 mg, 0.04 mmol) and sodium ascorbate (7.10 mg, 0.04 mmol) were separately dissolved with purified water (0.2 mL each) for later use. The solution of copper sulfate pentahydrate and the solution of sodium ascorbate were sequentially added and stirred at room temperature for 0.5 h of reaction. After the reaction was completed, as monitored by LC-MS, the reaction mixture was purified by preparative liquid chromatography to obtain 53.79 mg of DL25-d1Dxd, with a yield of 47.59%. LC-MS(ESI+)3151.4$[M+H]^+$.

[00518] 3. Preparation of antibody drug conjugate

[00519] Example 49 Preparation of antibody drug conjugate by general conjugation method

[00520] The antibody drug conjugate was prepared by a general conjugation method, as described below. The reducing agent (1-20 mM TCEP (Tris-2-carboxyethyl-phosphine)) and the protecting

agent (1-20 mM DTPA (Diethylene triamine pentacetate acid)) were separately prepared with purified water. The reducing agent, used at an amount varying within a certain concentration range according to the desired conjugation rate, was mixed with the monoclonal antibody at a certain concentration (e.g., 5-30 mg/mL) to achieve a final molar ratio of TCEP to antibody of 8:1 to 14:1. The mixture was stirred at 25°C for 1 h of reaction. The antibody reduced by TCEP can be directly used for conjugation.

[00521] The linker-active drug compound was dissolved in dimethyl sulfoxide (DMSO) to a certain concentration (5 mM). The drug was slowly added to the antibody at a molar ratio of drug to sulfhydryl of 1.2:1 to 1.5:1. The mixture was stirred at 25°C for 1-4 h of reaction. After the reaction was completed, the reaction mixture was purified through centrifugal ultrafiltration using HIS buffer to remove residual unreacted drugs and free small molecules such as DMSO. The conjugation was detected by liquid chromatography-mass spectrometry (LC-MS), and the purity of the conjugated sample was measured by size exclusion chromatography (SEC).

[00522] Example 50 Preparation of antibody drug conjugate by engineered cysteine-based conjugation method

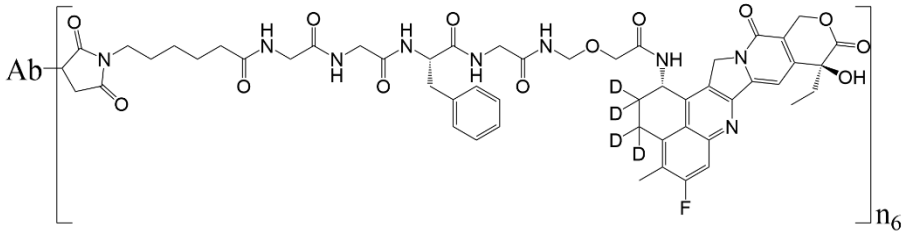
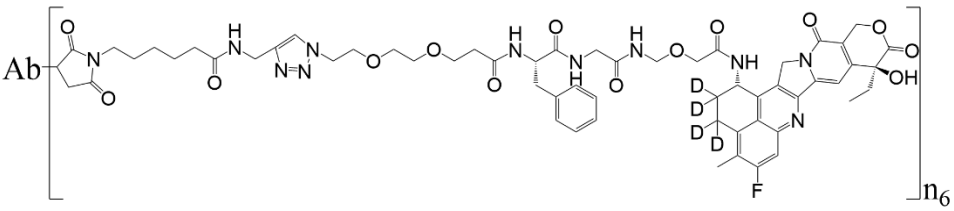
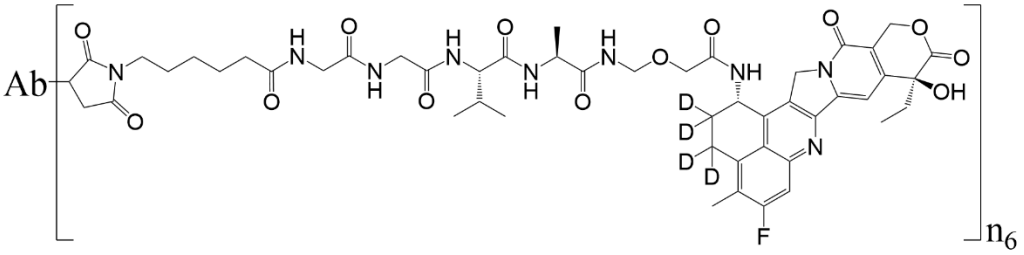
[00523] The antibody drug conjugate was prepared by an engineered cysteine-based conjugation method, as described below. The reducing agent (1-100 mM TCEP) was prepared with purified water, and the oxidizing agent (1-100 mM dehydroacetic acid (DHAA)) was prepared with DMSO. TCEP was mixed with the monoclonal antibody at a certain concentration (e.g., 1-20 mg/mL) to achieve a final molar ratio of TCEP to antibody of 10:1 to 100:1. The mixture was stirred at 25°C for 2-18 h of reaction. After the reduction was completed, extra TCEP was removed through centrifugal ultrafiltration using Tris-HCl buffer. DHAA was added to the reaction system at a final molar ratio of DHAA to antibody of 10:1 to 100:1, and the mixture was stirred at 25°C for 1-4 h of reaction. After the reaction was completed, extra DHAA was removed through centrifugal ultrafiltration using Tris-HCl buffer. The linker-active drug compound was dissolved in DMSO to a certain concentration (5 mM). The drug was slowly added to the antibody at a molar ratio of drug to sulfhydryl of 1.2:1 to 2:1. The mixture was stirred at 25°C for 1-4 h of reaction. After the reaction was completed, residual unreacted drugs and free small molecules such as DMSO were removed through centrifugal ultrafiltration using Tris-HCl buffer. The conjugation was detected by liquid chromatography-mass spectrometry (LC-MS), and the purity of the conjugated sample was

measured by size exclusion chromatography (SEC).

[00524] Example 51 Preparation of Trop2-targeting antibody drug conjugate (random conjugation) and quality examination

[00525] The linker-payload compounds DL01-dDxd, DL02-dDxd, DL05-dDxd, DL06-dDxd, DL07-dDxd, DL05-Dxd, DL06-Dxd and DL07-Dxd were conjugated with antibody Ab (Ab: RD219A1 antibody, a self-expressed Trop2-targeting antibody) by the general conjugation method of Example 49. The average drug-to-antibody ratio (DAR) and purity of conjugates were detected by liquid chromatography-mass spectrometry (LC-MS) and size-exclusion chromatography (SEC), respectively.

Table 4 Structures of antibody drug conjugates prepared by the general conjugation method

Number of ADC	Structure of ADC
RD219A1-DL01-dDxd	
RD219A1-DL02-dDxd	
RD219A1-DL05-dDxd	

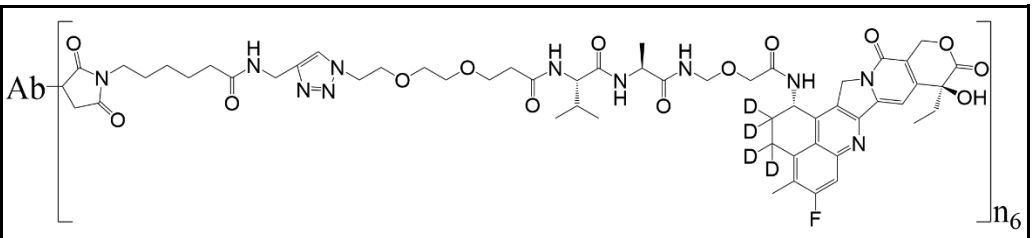
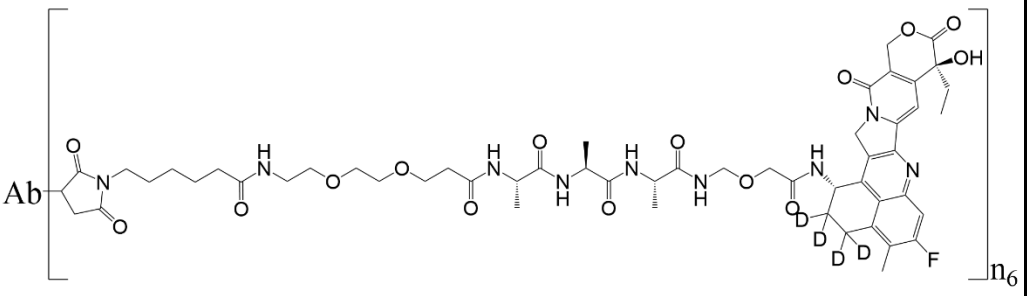
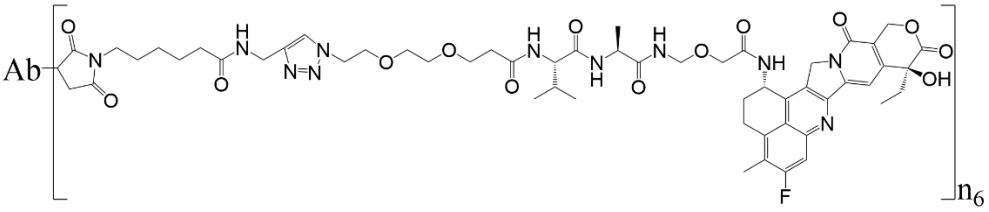
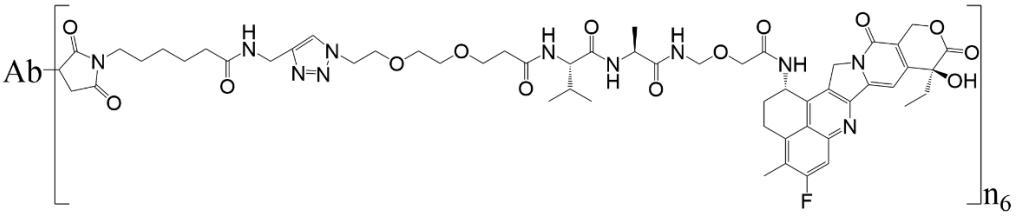
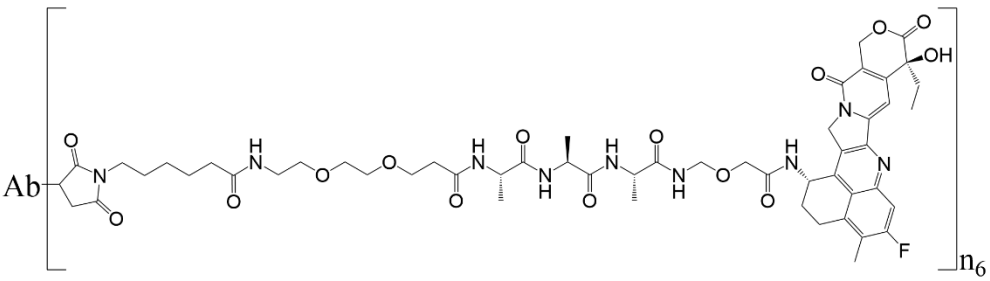
RD219A1-DL06-dDxd	
RD219A1-DL07-dDxd	
RD219A1-DL05-Dxd	
RD219A1-DL06-Dxd	
RD219A1-DL07-Dxd	

Table 5 Results of detection of antibody drug conjugates prepared by the general conjugation method

Number of ADC	DAR	SEC%

RD219A1-DL01-dDxd	8.14	96.5
RD219A1-DL02-dDxd	7.86	96.9
RD219A1-DL05-dDxd	8.40	96.0
RD219A1-DL06-dDxd	8.30	97.6
RD219A1-DL07-dDxd	8.05	96.6
RD219A1-DL05-Dxd	7.92	96.8
RD219A1-DL06-Dxd	7.96	96.2
RD219A1-DL07-Dxd	7.97	96.4

Number of ADC consists of three parts linked via two dashes ("-"), representing the targeting moiety, linker and payload moiety in sequence. For example, RD219A1-DL01-dDxd represents that the targeting moiety is RD219A1 antibody, the linker is DL01, and the payload is dDxd compound.

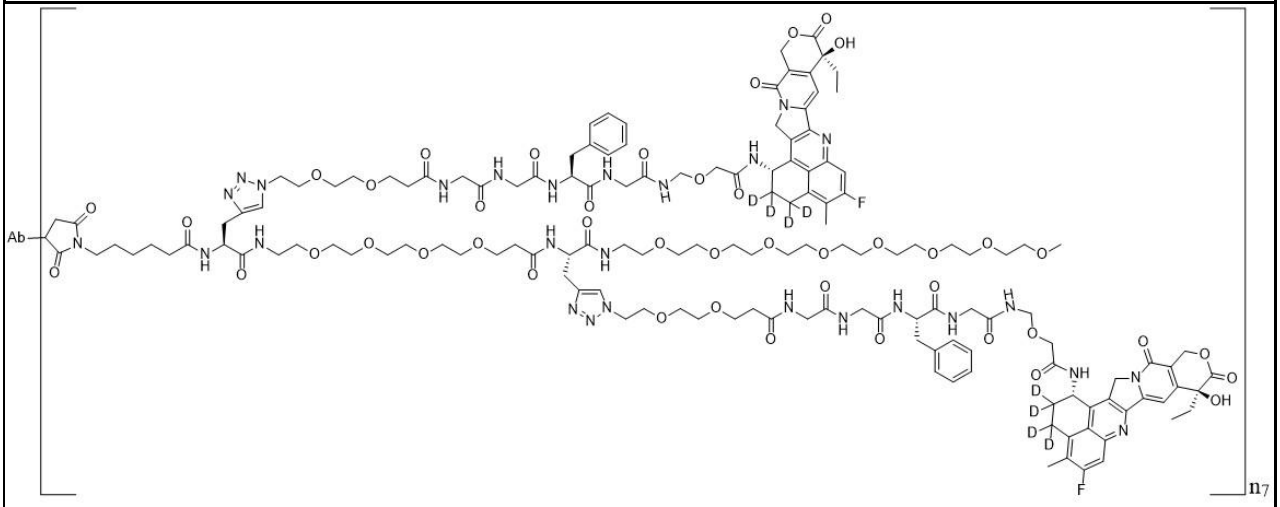
[00526] Using a validated antibody for ADC target (Trop2), the antibody drug conjugate was prepared and subjected to quality examination. Results of quality examination (Table 5) show that the linker or linker-payload structures of the present disclosure can enable the production of the antibody drug conjugate with high DAR value and high purity.

[00527] Example 52 Preparation of Trop2-targeting antibody drug conjugate (site-specific conjugation) and quality examination

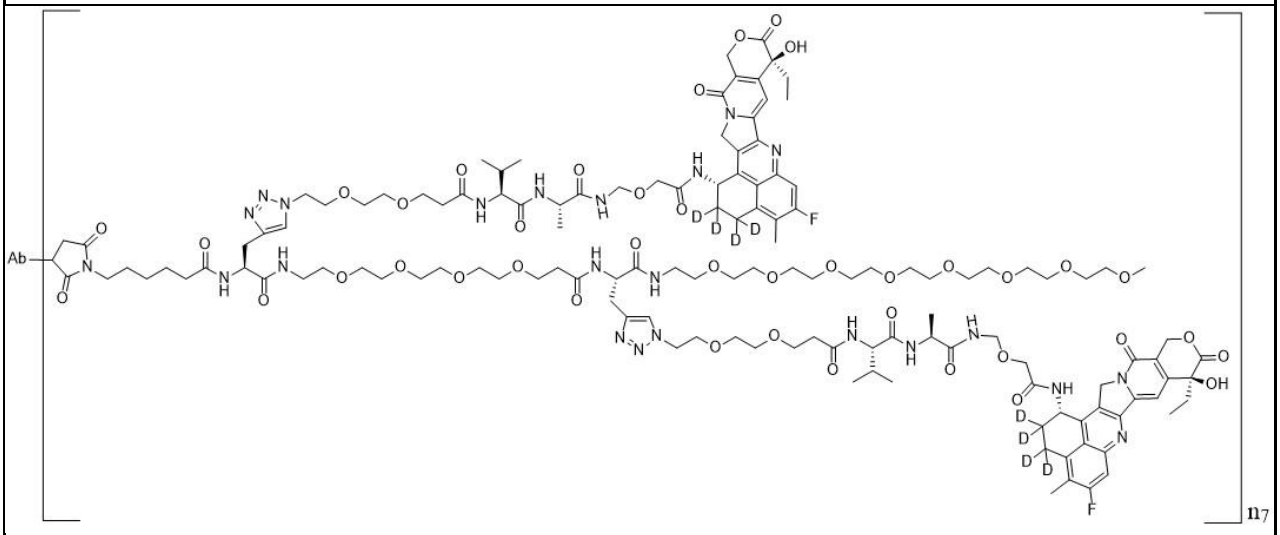
[00528] The linker-payload compounds DL03-dDxd, DL04-dDxd, DL08-dDxd, DL09-dDxd, DL03-Dxd and DL04-Dxd were conjugated with an engineered antibody Ab (Ab: RD219B1 antibody, a Trop2-targeting antibody with site-specific cysteine mutations) by the engineered cysteine-based conjugation method of Example 50. The average drug-to-antibody ratio (DAR) and purity of conjugates were detected by liquid chromatography-mass spectrometry (LC-MS) and size-exclusion chromatography (SEC), respectively.

Table 6 Structures of antibody drug conjugates prepared by the engineered cysteine-based conjugation method

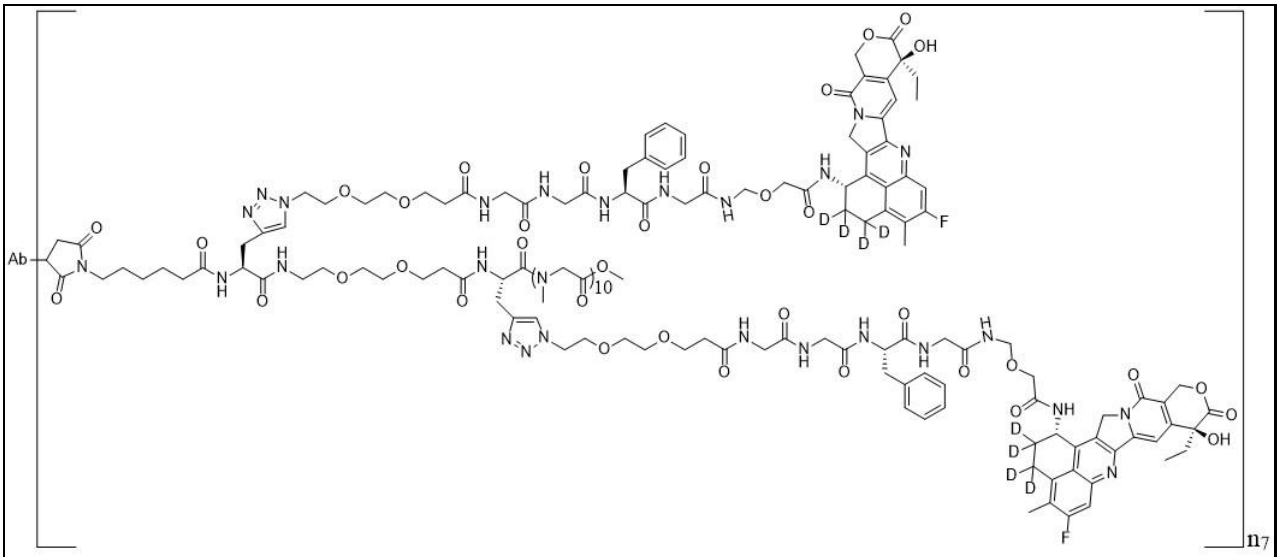
RD219B1-DL03-dDxd



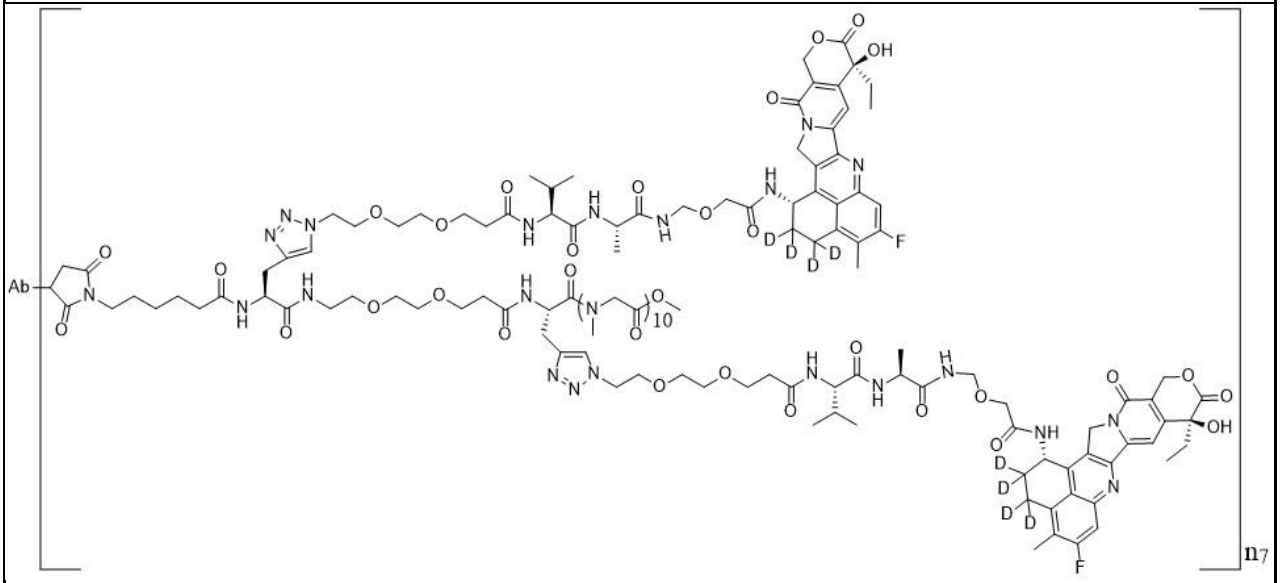
RD219B1-DL04-dDxd



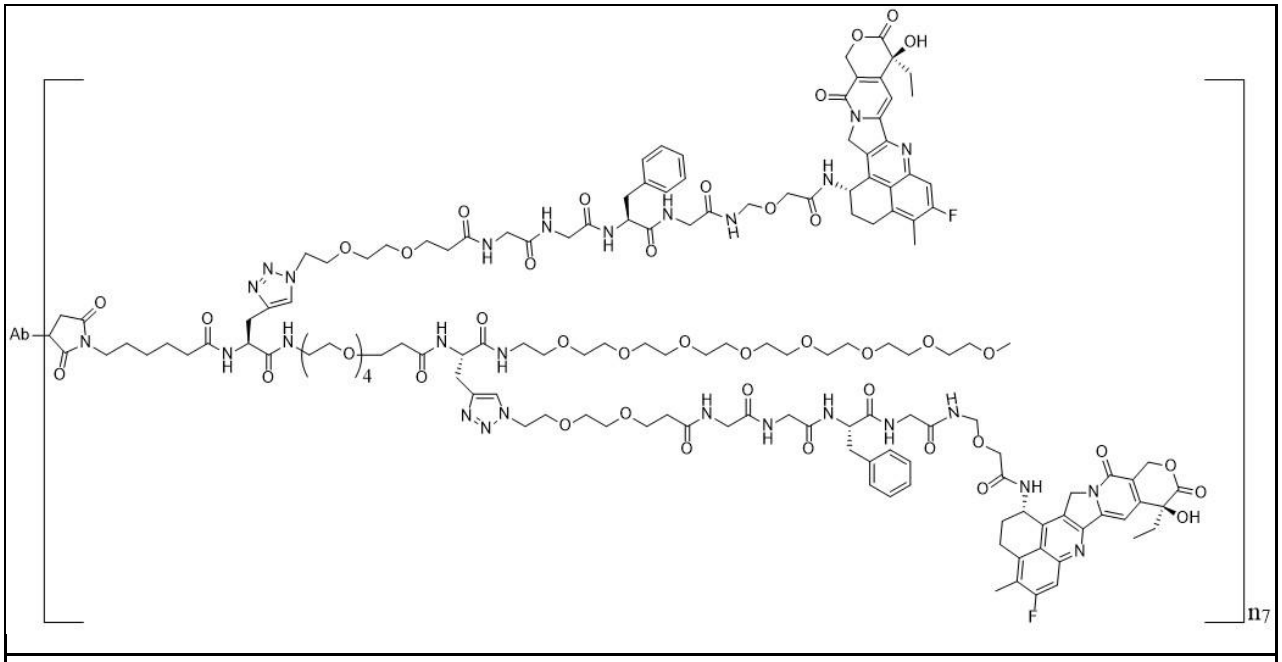
RD219B1-DL08-dDxd



RD219B1-DL09-dDxd



RD219B1-DL03-Dxd



RD219B1-DL04-Dxd

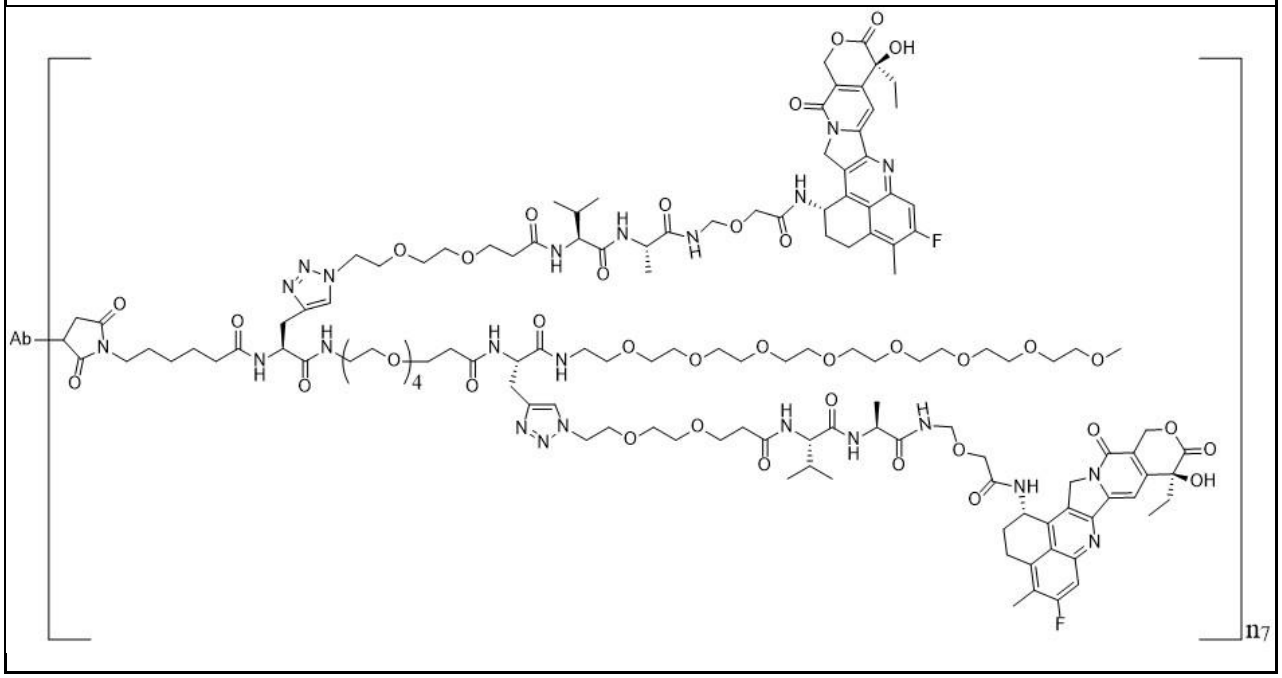


Table 7 Results of detection of antibody drug conjugates prepared by the engineered cysteine-based conjugation method

Number of ADC	DAR	SEC%

RD219B1-DL03-dDxd	7.71	94.8
RD219B1-DL04-dDxd	7.63	94.9
RD219B1-DL08-dDxd	7.69	94.6
RD219B1-DL09-dDxd	7.52	94.9
RD219B1-DL03-Dxd	7.68	94.7
RD219B1-DL04-Dxd	7.68	95.1

Number of ADC consists of three parts linked via two dashes ("-"), representing the targeting moiety, linker and payload moiety in sequence. For example, RD219B1-DL03-dDxd represents that the targeting moiety is RD219B1 antibody, the linker is DL03, and the payload is dDxd compound.

[00529] Using a validated antibody with site-specific cysteine mutations for ADC target (Trop2), the antibody drug conjugate was prepared by site-specific conjugation and subjected to quality examination. Results of quality examination (Table 7) show that the linker or linker-payload structures of the present disclosure can enable the production of the site-specific cysteine-based antibody drug conjugate with high DAR value and high purity.

[00530] 5. In vitro toxicity assay of antibody drug conjugate

[00531] Example 53: Cytotoxicity of antibody drug conjugate

[00532] BxPC-3 human orthotopic pancreatic cancer cells in the exponential growth phase were plated into a 96-well plate at 100 μL per well, with a density of 5E4-1E5/mL (scientific notation was used throughout the present disclosure), equivalent to 5×10^4-1×10^5/mL. The cells were incubated overnight in a 37°C, 5% CO_2 incubator. The antibody drug conjugates were prepared using the complete medium to gradient concentrations of 0-333 nM. 100 μL of antibody drug conjugates were then added to each well, three replicates for each concentration. A blank control group was set up. After 168±2 h of drug treatment, CCK-8 reagent was added, and cells were incubated in a 37°C, 5% CO_2 incubator for 1 to 4 h. The OD value of each well was detected at 450 nm using a microplate reader. The inhibition rate was calculated as IR (%) = (OD blank - OD drug) × 100/OD blank. IC_{50} was calculated by fitting a four-parameter logistic curve based on

inhibition rate and drug concentration.

Table 8 Cytotoxicity of antibody drug conjugates against BxPC-3 cells (human orthotopic pancreatic cancer cell line)

ADC	IC50 (nM)	MIR (%)
RD219A1-DL01-dDxd	0.068	93.27
RD219A1-DL02-dDxd	0.057	95.35
RD219B1-DL03-dDxd	0.055	97.25
RD219B1-DL04-dDxd	0.054	96.67
RD219A1-DL05-dDxd	0.082	94.64
RD219A1-DL06-dDxd	0.078	94.00
RD219A1-DL07-dDxd	0.059	96.97
RD219B1-DL08-dDxd	0.046	96.77
RD219B1-DL09-dDxd	0.053	96.31
RD219B1-DL03-DXD	0.056 /	96.62
RD219B1-DL04-DXD	0.055	96.4
RD219A1-DL05-DXD	0.052	96.32
RD219A1-DL06-DXD	0.060	95.97
RD219A1-DL07-DXD	0.056	96.39
RD219A1-MC-GGFG-DXD	0.065	96.73

Except for "RD219A1-MC-GGFG-DXD", all numbers of ADC consist of three parts linked via two dashes ("-"), representing the targeting moiety, linker and payload moiety in sequence. For example, RD219A1-DL01-dDxd represents that the targeting moiety is RD219A1 antibody, the linker is DL01, and the payload is dDxd compound. "RD219A1-MC-GGFG-DXD" represents that

the antibody is “RD219A1”, the linker is “MC-GGFG”, and the payload is DXD.

[00533] As shown in Table 8, the results show that the ADCs prepared using the linker-payload structures of the present disclosure exhibits high cytotoxicity against the BxPC-3 tumor cells (human orthotopic pancreatic cancer cell line). Compared to MC-GGFG-DXD, a clinically validated linker-payload structure in the ADC field, DL01-dDxd, DL02-dDxd, DL03-dDxd, DL04-dDxd, DL05-dDxd, DL06-dDxd, DL07-dDxd, DL08-dDxd and DL09-dDxd demonstrate superior or comparable cytotoxicity. The results of the cytotoxicity assay in this example indicate that the linker-payload structures of the present disclosure have broad application prospects.

[00534] Example 54 Development and screening of anti-CDCP1 antibody

[00535] 1. Preparation of mouse anti-CDCP-1 antibody

[00536] (1) Animal immunization protocol

[00537] Balb/C and CD1 mice were immunized with the full-length extracellular domain of CDCP-1 protein (Phe30-Thr667, Cat. No. CD1-H52H6, purchased from ACRO) as the immunogen. The protein (40 μg per dose), in combination with complete or incomplete Freund’s adjuvant, was injected subcutaneously into mice every other week, for a total of four immunizations. Seven days after the last immunization, blood was collected via retro-orbital puncture, from which serum was separated. The specific antibody titer in the serum was determined by flow cytometry (FACS). When the serum antibody titer reached the level required for conventional hybridoma preparation, the mice were given a booster immunization with 40 μg of protein three days prior to cell fusion.

[00538] (2) Preparation of hybridoma and screening

[00539] After requirement for proceeding with cell fusion was met, splenocytes were harvested from booster-immunized mice, and fused with mouse myeloma cells using PEG to prepare hybridoma cells. Specifically, the splenocytes were fused with mouse myeloma cells (SP2/0) at a 2:1 ratio in the presence of 50% PEG1500. The fused cells were suspended in medium containing HAT (hypoxanthine (H)-aminopterin (A)-thymidine(T)), plated into a 96-well plate at a density of 2-3E6 cells per well, and cultured in a 37°C, 5% CO_2 incubator. After 5 days of culture, the medium was replaced with HT medium, and the cells were cultured for another 5 days. The binding activity of antibodies secreted by hybridoma cells in supernatants to the full-length extracellular domain of

hCDCP1 was assessed by FACS using MDA-MB-231 (human breast cancer cells) as the target. Specifically, 100 μL of culture supernatants of hybridomas was added to Eppendorf tubes containing 5E5 cells each, incubated at room temperature for 1 h, centrifuged at 3000 rpm for 3 min, and washed twice with PBS. Each sample was then incubated with 100 μL of 1:2000 diluted Anti-Mouse IgG-APC antibody for 1 h at room temperature, washed twice with PBS, resuspended, and analyzed on an Invitrogen Attune NxT flow cytometer. Results of FACS analysis show that the culture supernatants of hybridomas have a good affinity for CDCP1.

[00540] Positive hybridomas exhibiting good binding affinity were subcloned to obtain the monoclonal hybridoma cells capable of producing specific mouse antibodies. To confirm the specificity of the generated antibodies, culture supernatants of hybridomas were further assessed for binding of antibody to a negative cell line (Caki-1). Culture supernatants of hybridomas exhibiting positive binding to MDA-MB-231 cells and no binding to Caki-1 cells (negative) in the detection and screening indicated the hybridomas were monoclonal cells producing specific mouse antibodies. FACS results show that the antibodies in the culture supernatants of hybridomas exhibited good specific binding.

[00541] 2. Preparation of chimeric anti-CDCP-1 antibody

[00542] (1) Preparation of chimeric antibody

[00543] Hybridomas with good specificity were subjected to RACE (Rapid Amplification of cDNA Ends) to obtain the complete variable region sequence of mouse antibody. The monoclonal hybridomas were expanded, from which genomic RNA was extracted. The reverse transcription reaction was carried out using the SMARTer RACE 5'/3' Kit (Clontech 634913) to obtain cDNA. Mouse heavy and light chain variable regions were then amplified with universal primers.

[00544] Following the protocol of SMARTer RACE 5'/3' Kit (Clontech 634913), the sequences encoding variable regions of 136 hybridoma clones were successfully obtained. The gene sequences encoding the heavy chain variable region and light chain variable region were integrated into a pcDNA3.1 expression vector containing human IgG1 heavy chain constant region and human κappa light chain constant region through homologous recombination, respectively, to obtain the plasmids expressing heavy and light chains of chimeric antibody. They were then expressed in 293T cells in a small scale. The expressed antibodies were screened by FACS binding assay.

Through expression, validation, and large-scale production, the following 19 chimeric antibodies were obtained: #2, #5, #6, #13, #31, #32, #33, #38, #66, #89, #92, #94, #96, #108, #110, #111, #118, #119 and #120. The details of chimeric antibodies #6 and #31 are shown below:

[00545] SEQ ID NO: 1 (Heavy chain variable region sequence of #6)

[00546]

EVQLQQSGAEVVRPGASAKLSCKASGYTFTDYEMHWVKQTPVHGLEWIGAIDPE TGGIADNQKFKGKATLTADKSSSTAYMELRSLTSEDSAVYYCIKGWNYAMDYWGQGTSV TVSS

[00547] SEQ ID NO: 2 (Light chain variable region sequence of #6)

[00548]

DIVMTQSLKFMSTSVGDRVSITCKASQDVSTGVAWYQQKPGQSPKLFIYWASTRHT GVPDRFTGSGSGTDFTLTISSVQAEDLALYYCQQHYSTPWTFGGGTKLEIK

[00549] SEQ ID NO: 3 (Heavy chain variable region sequence of #31)

[00550]

EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLDWVATISSGG RYTYYADSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHYDYDGVYFDVWGA GTTVTVSS

[00551] SEQ ID NO: 4 (Light chain variable region sequence of #31)

[00552]

DVVMTQSPAIMSASPGEKVTMTCSASSSVSFVHWYQQKSVTSPKRWIYDTSKLAS GVPTRFSGSGSGTSYSLTISSMEAEDAATYYCQQWNNNKPPTFGVGTKLEIK

[00553] 3. Chimeric antibody property analysis

[00554] (1) Measurement of chimeric antibody affinity

[00555] (1.1) Binding kinetics measurement-OCTET

[00556] Binding kinetics were measured using an Octet RED96e instrument (purchased from Sartorius) following specific operation and detailed methods as provided in the instrument instruction and the manufacturer's protocol. Specifically, ProA biosensor (purchased from

Sartorius) was used for affinity measurements. The chimeric anti-CDCP1 antibodies were diluted in PBS (pH 7.4) containing Tween (0.02%, v/v) to final concentrations of 400 nM, 200 nM, and 100 nM, and then loaded onto the ProA biosensor for 1 min. Subsequently, the biosensor was exposed to antigens (10 μg/L in pH 7.4 PBS containing Tween (0.02%, v/v)) for 1.5 min. The used antigens included full-length human extracellular CDCP1 protein (CD1-H52H6, ACRO, Phe30-Thr667), extracellular membrane-distal domain of human CDCP1 protein (13262-H08H, Sino Biological, Met1-Glu343), full-length mouse extracellular CDCP1 protein (51110-M08H, Sino Biological, Met1-Leu666), and full-length monkey extracellular CDCP1 protein (90296-C08H, Sino Biological, Met1-Thr667). The assotiation and dissociation between antibodies and various antigens were measured using interference wavelength shifts by Octet instrument. Data was fitted using Octet User Software to calculate association and dissociation rate constants. The affinity constant was the ratio of dissociation rate constant to association rate constant. Results show that antibodies #6 and #31 had a good binding affinity.

[00557] (1.2) Measurement of chimeric antibody affinity by FACS

[00558] Chimeric anti-CDCP1 antibodies were diluted to 20 μg/mL, 10 μg/mL, 5 μg/mL, 1 μg/mL, 0.2 μg/mL, 0.04 μg/mL, 0.004 μg/mL and 0.0004 μg/mL, and separately co-incubated with 5E5 MDA-MB-231 human breast cancer cells, HCT116 human colon cancer cells, and PC-3 cells at room temperature for 1 h. Cells were centrifuged at 3000 rpm for 3 min, washed twice with PBS, and incubated with 100 μL of Anti-Mouse IgG-APC diluted at 1:2000 at room temperature for 1 h. The cells were washed twice with PBS, resuspended, and analyzed by an Invitrogen Attune NxT flow cytometer. FACS results show that antibodies #6 and #31 had a good affinity.

[00559] Example 55: Humanization and optimization of anti-CDCP1 antibody

[00560] 1. Preparation of humanized anti-CDCP1 antibody

[00561] (1) Humanization of chimeric antibody #6

[00562] Based on the results of affinity measurement, chimeric antibody #6 was subjected to humanization. The humanization of chimeric antibody #6 resulted in sequences of variable regions of 6 heavy chains (6-H1, 6-H2, 6-H3, 6-H4, 6-H5 and 6-H6) and 6 light chains (6-L1, 6-L2, 6-L3, 6-L4, 6-L5 and 6-L6). Their amino acid sequences are as follows.

[00563] SEQ ID NO: 5 (heavy chain variable region sequence H1 of humanized #6)

[00564] EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA IDPETGGIAY AQKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00565] SEQ ID NO: 6 (heavy chain variable region sequence H2 of humanized #6)

[00566] EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA IDPETGGIAY AQKFQGRVTI TADKSTSTAY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00567] SEQ ID NO: 7 (heavy chain variable region sequence H3 of humanized #6)

[00568] EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA IDPETGGIAD NQKFQGRVTI TADKSTSTAY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00569] SEQ ID NO: 8 (heavy chain variable region sequence H4 of humanized #6)

[00570] QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYEMHWVRQA PGQGLEWMGA IDPETGGIAY AQKFQGRVTM TRDTSTSTVY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00571] SEQ ID NO: 9 (heavy chain variable region sequence H5 of humanized #6)

[00572] QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYEMHWVRQA PGQGLEWMGA IDPETGGIAY AQKFKGRVTM TADKSTSTVY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00573] SEQ ID NO: 10 (heavy chain variable region sequence H6 of humanized #6)

[00574] EVQLVQSGAE VKKPGASVKV SCKASGYTFT DYEMHWVRQA PGQGLEWMGA IDPETGGIAD NQKFKGRVTM TADKSTSTVY MELSSLRSED TAVYYCIKGWNYAMDYWGQG TLVTVSS

[00575] SEQ ID NO: 11 (light chain variable region sequence L1 of humanized #6)

[00576] DIQMTQSPSS LSASVGDRVT ITCRASQDVS TGLAWYQQKP GKAPKLLIYW ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGGGTKVEIK

[00577] SEQ ID NO: 12 (light chain variable region sequence L2 of humanized #6)

[00578] DIQMTQSPSS LSASVGDRVT ITCRASQDVS TGLAWYQQKP GKAPKLLIYW ASTRHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGGGTKVEIK

[00579] SEQ ID NO: 13 (light chain variable region sequence L3 of humanized #6)

[00580] DIVMTQSPSS LSASVGDRVT ITCRASQDVS TGVAWYQQKP GKAPKLLIYW ASTRHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGGGTKVEIK

[00581] SEQ ID NO: 14 (light chain variable region sequence L4 of humanized #6)

[00582] DIVMTQSPDS LAVSLGERAT INCKSSQDVS TGLAWYQQKP GQPPKLLIYW ASTRESGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGGGTKVEIK

[00583] SEQ ID NO: 15 (light chain variable region sequence L5 of humanized #6)

[00584] DIVMTQSPDS LAVSLGERAT INCKSSQDVS TGLAWYQQKP GQPPKLLIYW ASTRHSGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGGGTKVEIK

[00585] SEQ ID NO: 16 (light chain variable region sequence L6 of humanized #6)

[00586] DIVMTQSPDS LAVSLGERAT INCKASQDVS TGVAWYQQKP GQPPKLLIYW ASTRHSGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGGGTKVEIK

[00587] Based on the obtained amino acid sequences of the heavy and light chain variable regions, the corresponding nucleotide sequences were codon-optimized. An enzyme cutting site sequence, a sequence encoding signal peptide, and sequences encoding antibody heavy/light chain constant region were incorporated and synthesized to construct a pCDNA3.1 vector.. The complete heavy and light chain genes were prepared by plasmid in large scale. The prepared plasmids were transfected into Expi293 cells according to a cross-combination mode of heavy and light chainsfor expression in a 100 mL system, followed by purification and characterization analysis.

[00588] 2. Screening for humanized #6 antibody

[00589] (1) Binding kinetics measurement of humanized #6 antibody

[00590] Binding kinetics were measured using an Octet RED96e instrument (purchased from Sartorius) following specific operation and detailed methods as provided in the instrument instruction and the manufacturer’s protocol. Specifically, ProA biosensor (purchased from

Sartorius) was used for affinity measurements. The chimeric anti-CDCP1 antibody #6 and 36 humanized antibodies thereof were diluted in PBS (pH 7.4) containing Tween (0.02%, v/v) to final concentrations of 400 nM, 200 nM, and 100 nM, and then loaded onto the ProA biosensor for 1 min. Subsequently, the biosensor was exposed to antigens (10 μg/mL in pH 7.4 PBS containing Tween (0.02%, v/v)) for 1.5 min. The used antigens included full-length human extracellular CDCP1 protein (CD1-H52H6, ACRO, Phe30-Thr667), extracellular membrane-distal domain of human CDCP1 protein (13262-H08H, Sino Biological, Met1-Glu343), and full-length monkey extracellular CDCP1 protein (90296-C08H, Sino Biological, Met1-Thr667). The assotiation and dissociation between antibodies and various antigens were measured usinginterference wavelength shifts by Octet instrument. Data was fitted using Octet User Software to calculate association and dissociation rate constants. The affinity constant was the ratio of dissociation rate constant to association rate constant. Experimental results show that 36 humanized #6 antibodies have a good binding affinity for the three antigens above.

[00591] (2) Measurement of humanized #6 antibody affinity by FACS

[00592] 36 humanized anti-CDCP1 antibodies were diluted to 20 μg/mL, 10 μg/mL, 5 μg/mL, 1 μg/mL, 0.2 μg/mL, 0.04 μg/mL, 0.004 μg/mL and 0.0004 μg/mL, and separately co-incubated with 5E5 MDA-MB-231 human breast cancer cells and PC-3 cells at room temperature for 1 h. Cells were centrifuged at 3000 rpm for 3 min, washed twice with PBS, and incubated with 100 μL of Anti-Mouse IgG-APC diluted at 1:2000 at room temperature for 1 h. The cells were washed twice with PBS, resuspended, and analyzed by an Invitrogen Attune NxT flow cytometer. As shown in Table 9, FACS results show that multiple humanized antibodies had an excellent affinity.

Table 9 FACS measurement results of humanized #6 antibodies for binding affinity in PC-3 cells

PC-3					
H*L	EC50 (μg/mL)	H*L	EC50 (μg/mL)	H*L	EC50 (μg/mL)
H1+L1	0.1219	H3+L1	0.02621	H5+L1	Approximately 234.1

H1+L2	0.03939	H3+L2	0.02899	H5+L2	0.3341
H1+L3	0.03477	H3+L3	0.02927	H5+L3	0.4954
H1+L4	158.3	H3+L4	Approximately 0.02259	H5+L4	nd
H1+L5	0.02669	H3+L5	0.02642	H5+L5	0.6013
H1+L6	0.03202	H3+L6	0.0209	H5+L6	0.2605
H2+L1	8.181	H4+L1	4.298	H6+L1	0.02684
H2+L2	0.04465	H4+L2	0.2444	H6+L2	0.02382
H2+L3	0.02989	H4+L3	0.4751	H6+L3	0.02986
H2+L4	nd	H4+L4	2.855	H6+L4	0.03683
H2+L5	0.01297	H4+L5	0.1603	H6+L5	0.01071
H2+L6	0.03172	H4+L6	0.08725	H6+L6	Approximately 0.01874
Chimeric antibody #6	Approximately 0.02248	h14A043	0.4953	--	--

[00593] Note: "nd"= not detected.

[00594] (3) Measurement of internalization rate of humanized antibody derived from chimeric antibody #6

[00595] 18 humanized antibodies were selected and labeled according to the instructions of promega PHAb Antibody Dye Labeling Kit and the concentration of the antibods before the labeling was 2 mg/mL. The labeled antibodies were incubated with PC-3 cells (human prostate cancer cell line) and HCT116 cells (human colon cancer cell line) at 37°C. A series of sampling time points were set as follows: 0 h, 3 h, 6 h, 8 h, 16 h, and 24 h. The concentration was set at 100 nM. The fluorescence intensity was detected by a flow cytometer at each sampling point. The experimental results show that H1+L6 (humanized antibody Ab1), H5+L2, and H6+L2 antibodies

had a relatively fast internalization rate.

[00596] 3. Humanization of chimeric antibody #31

[00597] (1) screening for humanized chimeric antibody #31

[00598] Based to the results of affinity measurement of chimeric antibodies, the chimeric antibody #31 was selected for humanization. Through humanization and performance screening, the humanized antibody hAb2 was obtained and its sequence is shown below.

[00599] Variable region sequence of humanized antibody hAb2:

[00600] SEQ ID NO: 17 (heavy chain variable region sequence H1 of humanized #31)

[00601]

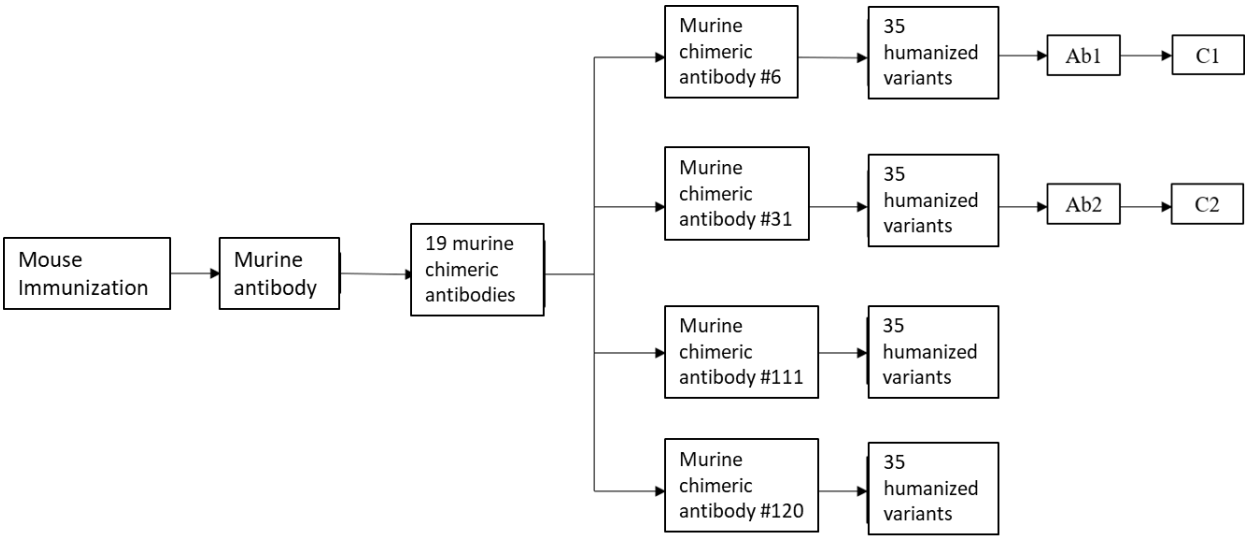
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATISSGG
RYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHYDYDGVYFDVWGQ
GTMVTVSS

[00602] SEQ ID NO: 18 (light chain variable region sequence L1 of humanized #31)

[00603]

DIQMTQSPSSLSASVGDRVTITCRASSSVSFVHWYQQKPGKAPKLLIYDTSKLQSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWNNNKPPTFGQGTKLEIK

[00604] The screening flowchart for the anti-CDCP1 antibody of the present disclosure is shown below. Ab1 and Ab2 were separately subjected to heavy chain A114C mutations (according to Kabat numbering) to obtain C1 and C2.



[00605] 4. Characterization of optimized humanized antibody

[00606] (1) Yield of anti-CDCP1 antibodies Ab1 and Ab2

[00607] Antibodies were expressed in Expi293 cells, purified from the media by a Protein A column, and further purified by a size-exclusion column. The expression titers of the antibodies were determined utilizing quantitative protocol on the Gator BLI system. The data show 400 mg/L yield for Ab1 and 400 mg/L yield for Ab2, demonstrating that both Ab1 and Ab2 had a high yield.

[00608] (2) Melting temperature of anti-CDCP1 antibodies Ab1 and Ab2

[00609] The melting point of antibody was measured on a Bio-Rad CFX Opus 96 real time PCR system by differential scanning fluorimetry (DSF). The antibody was mixed with SYPRO Orange dye and incubated at a temperature gradient from 20°C to 95°C. Fluorescence emission was monitored in real-time at 570 nm, and the melting point was calculated using Bio-Rad CFX Maestro software. The data show that the melting temperatures (Tm) of Ab1 and Ab2 were both 67.5°C, indicating good thermal stability.

[00610] (3) Purity of anti-CDCP1 antibodies Ab1 and Ab2

[00611] The purity of antibody was evaluated using ACQUITY UPLC Protein BEH SEC column (200 Å, 1.7 μm, 4.6 mm×300 mm) on the Agilent 1290 system. The mobile phase was 205 mM NaCl, 4 mM KCl, 15 mM NaH_2PO_4, 2.7 mM K_2HPO_4, pH 7.4. The data was analyzed using Agilent Chemstation software. The results show that Ab1 and Ab2 had an excellent SEC purity, both 100%.

[00612] (4) Protein binding activity of anti-CDCP1 antibodies Ab1 and Ab2

[00613] The protein binding assay was performed on Biacore T200 system. The antibodies were immobilized on CM5 chips prepared using the Anti-human Fc Capture Kit. A series of dilutions of full-length CDCP-1 were subjected to a multi-cycle kinetic experiment. The association rate constant (Ka), dissociation rate constant (Kd) and equilibrium dissociation constant (KD) were calculated using the Biacore evaluation software. As shown in Table 10, the data show that Ab1 and Ab2 had a good binding activity.

Table 10 Protein binding activity of anti-CDCP1 antibodies Ab1 and Ab2

Item	Ab1	Ab2
Association rate constant (ka, 1/Ms)	6.42E05	2.75E05
Dissociation rate constant (kd, 1/s)	1.10E-02	5.38E-03
Equilibrium dissociation constant (KD, nM)	17.07	19.6

[00614] (5) Internalization of anti-CDCP1 antibodies Ab1 and Ab2

[00615] Cells were plated into a 96-well black transparent bottom plate at a density of 20,000 cells per well and incubated overnight. Individual antibodies were labeled using the IncuCyte® FabFluor-pH Red Antibody Labeling Kit. The fluorescently labeled antibodies were added to the cell plate and the fluorescent signal was measured using IncuCyte® SX5. As shown in Table 11, the data show that Ab1 and Ab2 had good internalization efficiency.

Table 11 Internalization of anti-CDCP1 antibodies Ab1 and Ab2

Item	Ab1	Ab2

Internalization	++++ (High internalization efficiency)	++++ (High internalization efficiency)

[00616] Example 56 Preparation of anti-CDCP1 antibody and determination of amino acid sequence

[00617] According to the screening results of Example 55, the preferred anti-CDCP1 antibodies were Ab1 (humanized #6, H1+L6) and Ab2 (humanized #31, H1+L1).

[00618] Amino acid sequences of CDRs 1-3 of Ab1 heavy and light chain variable regions

[00619] Table 12 Amino acid sequence of CDR of anti-CDCP1 antibody Ab1

CDR	Amino acid sequence	Sequence Number
HCDR1	GYTFTDYE	SEQ ID NO: 19
HCDR2	IDPETGGI	SEQ ID NO: 20
HCDR3	IKGWNYAMDY	SEQ ID NO: 21
LCDR1	QDVSTG	SEQ ID NO: 22
LCDR2	WAS	SEQ ID NO: 23
LCDR3	QQHYSTPWT	SEQ ID NO: 24

Note: The CDRs were defined according to the IMGT numbering system.

[00620] Amino acid sequence of Ab1 heavy chain variable region (SEQ ID NO: 25):

EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA 50

IDPETGGIAY AQKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCIKGW 100

NYAMDYWGQG TLVTVSS 117

[00621] Amino acid sequence of Ab1 light chain variable region (SEQ ID NO: 26):

DIVMTQSPDS LAVSLGERAT INCKASQDVS TGVAWYQQKP GQPPKLLIYW 50

ASTRHSGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGG 100

GTKVEIK 107

[00622] Amino acid sequence of Ab1 heavy chain (SEQ ID NO: 27):

EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA 50

IDPETGGIAY AQKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCIKGW 100

NYAMDYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF 150

PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC 200

NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT 250

LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300

RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 350

LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 400

DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 447

[00623] Amino acid sequence of Ab1 light chain (SEQ ID NO: 28):

DIVMTQSPDS LAVSLGERAT INCKASQDVS TGVAWYQQKP GQPPKLLIYW 50

ASTRHSGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGG 100

GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150

DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG 200

LSSPVTKSFN RGEC 214

[00624] CDRs 1-3 of Ab2 heavy and light chain variable regions

Table 13 Amino acid sequence of CDRs of anti-CDCP1 antibody Ab2

CDR	Amino acid sequence	Sequence Number
HCDR1	GFTFSSYG	SEQ ID NO: 29
HCDR2	ISSGGRYT	SEQ ID NO: 30

HCDR3	ARHYDYDGVYFDV	SEQ ID NO: 31
LCDR1	SSVSF	SEQ ID NO: 32
LCDR2	DTS	SEQ ID NO: 33
LCDR3	QQWNNNKPPT	SEQ ID NO: 34

Note: The CDRs were defined according to the IMGT numbering system.

[00625] Amino acid sequence of Ab2 heavy chain variable region (SEQ ID NO: 35):

EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLEWVAT 50

ISSGGRYTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHY 100

DYDGVYFDVW GQGTMVTVSS 120

[00626] Amino acid sequence of Ab2 light chain variable region (SEQ ID NO:36):

DIQMTQSPSS LSASVGDRVT ITCRASSSVS FVHWYQQKPG KAPKLLIYDT 50

SKLQSGVPSR FSGSGSGTDF TLTISSLQPE DFATYYCQQW NNNKPPTFGQ 100

GTKLEIK 107

[00627] Amino acid sequence of Ab2 heavy chain (SEQ ID NO: 37):

EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLEWVAT 50

ISSGGRYTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHY 100

DYDGVYFDVW GQGTMVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK 150

DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT 200

YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP 250

KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 300

STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ 350

VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV 400

LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 450

[00628] Amino acid sequence of Ab2 light chain (SEQ ID NO: 38):

DIQMTQSPSS LSASVGDRVT ITCRASSSVS FVHWYQQKPG KAPKLLIYDT 50

SKLQSGVPSR FSGSGSGTDF TLTISSLQPE DFATYYCQQW NNNKPPTFGQ 100

GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150

DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG 200

LSSPVTKSFN RGEC 214

[00629] Example 57 Preparation of anti-CDCP1 antibody drug conjugate (with MMAE as payload)

[00630] The antibody drug conjugate was prepared by a general conjugation method, as described below. The reducing agent (1-20 mM TCEP (Tris-2-carboxyethyl-phosphine)) and the protecting agent (1-20 mM DTPA (Diethylene triamine pentacetate acid)) were separately prepared with purified water. The reducing agent, used at an amount varying within a certain concentration range according to the desired conjugation rate, was mixed with the monoclonal antibody at a certain concentration (e.g., 5-30 mg/mL) to achieve a final molar ratio of TCEP to antibody of 2:1 to 2.5:1. The mixture was stirred at 25°C for 1-2 h of reaction. The antibody reduced by TCEP can be directly used for conjugation.

[00631] The amino acid sequence of anti-CDCP1 antibody C1 is shown below.

[00632] Amino acid sequence of C1 heavy chain (SEQ ID NO: 26)

EVQLVQSGAE VKKPGATVKI SCKVSGYTFT DYEMHWVQQA PGKGLEWMGA 50

IDPETGGIAY AQKFQGRVTI TADTSTDTAY MELSSLRSED TAVYYCIKGW 100

NYAMDYWGQG TLVTVSSCST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF 150

PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC 200

NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT 250

LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300

RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 350

LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 400

DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 447

[00633] Amino acid sequence of C1 light chain (SEQ ID NO: 40)

```
DIVMTQSPDS LAVSLGERAT INCKASQDVS TGVAWYQQKP GQPPKLLIYW 50
ASTRHSGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSTPWTFGG 100
GTKVEIKRTV AAPSVFIFPP SEQLKSGTAS VVCLLNNFYP REAKVQWKVD 150
NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL 200
SSPVTKSFNR GEC                                        213
```

[00634] The amino acid sequence of anti-CDCP1 antibody C2 is shown below.

[00635] Amino acid sequence of C2 heavy chain (SEQ ID NO: 41)

```
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLEWVAT 50
ISSGGRYTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHY 100
DYDGVYFDVW GQGTMVTVSS CSTKGPSVFP LAPSSKSTSG GTAALGCLVK 150
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT 200
YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP 250
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 300
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ 350
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV 400
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 450
```

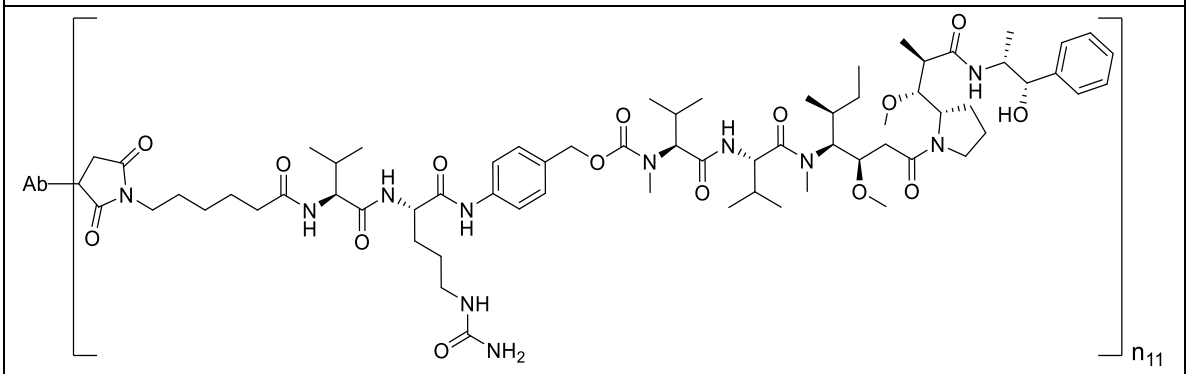
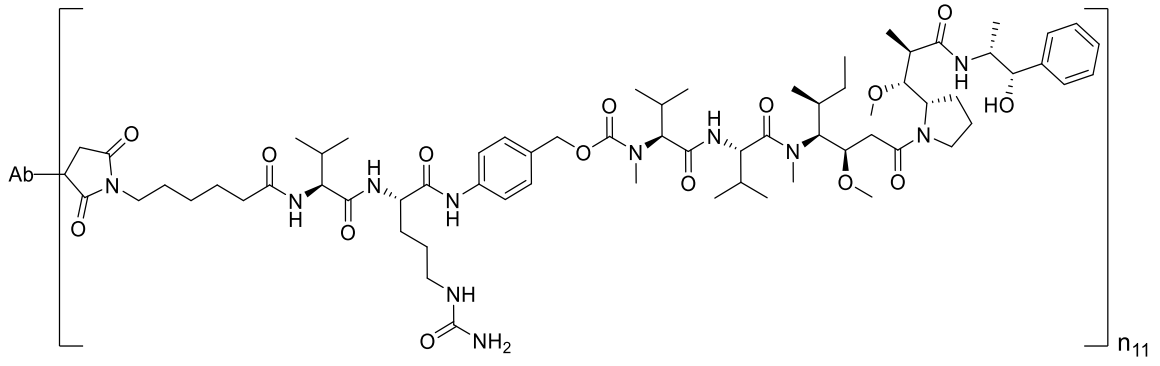
[00636] Amino acid sequence of C2 light chain (SEQ ID NO: 42)

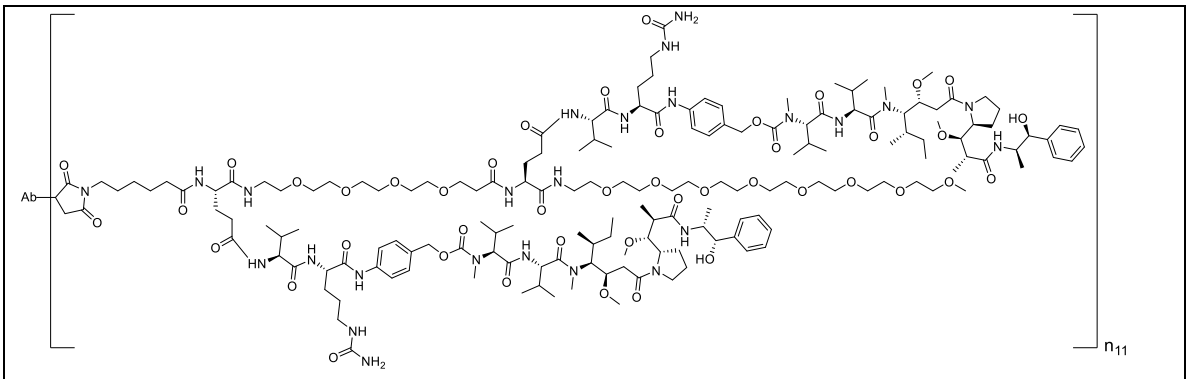
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DIQMTQSPSS LSASVGDRVT ITCRASSSVS FVHWYQQKPG KAPKLLIYDT 50
SKLQSGVPSR FSGSGSGTDF TLTISSLQPE DFATYYCQQW NNNKPPTFGQ 100
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG 200
LSSPVTKSFN RGEC                                       214
```

[00637] The linker-active drug compound was dissolved in dimethyl sulfoxide (DMSO) to a

certain concentration (5 mM). The drug was slowly added to the antibody at a molar ratio of drug to sulfhydryl of 1.2:1 to 1.5:1. The mixture was stirred at 25°C for 1-2 h of reaction. After the reaction was completed, the reaction mixture was purified by centrifugal ultrafiltration using HIS buffer to remove residual unreacted drugs and free small molecules such as DMSO. The conjugation was detected by hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC) and liquid chromatography-mass spectrometry (LC-MS), and the purity of the conjugated sample was measured by size exclusion chromatography (SEC).

Table 14 Structures of prepared antibody drug conjugates (with MMAE as payload)

Ab1-MC-VA-PAB-MMAE

Ab2-MC-VA-PAB-MMAE

C1-DL02-MMAE



C2-DL02-MMAE

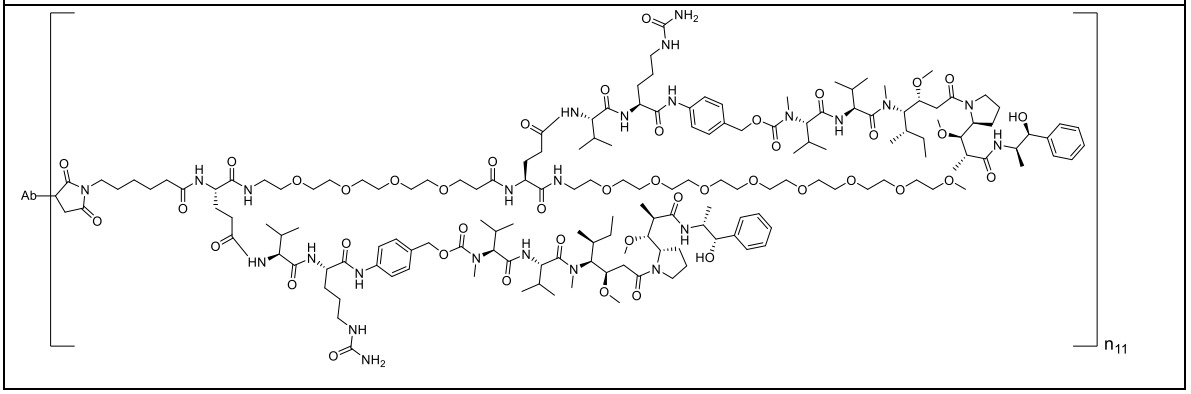


Table 15 Results of detection of antibody drug conjugates

Number of ADC	DAR	SEC%
Ab1-MC-VC-PAB-MMAE	3.95	94.42
Ab2-MC-VC-PAB-MMAE	4.07	97.25
C1-DL02-MMAE	4.12	94.62
C2-DL02-MMAE	3.92	96.28

[00638] Except for "Ab1-MC-VC-PAB-MMAE" and "Ab2-MC-VC-PAB-MMAE", all numbers of ADC consist of three parts linked via two dashes ("-"), representing the targeting moiety, linker and payload moiety in sequence. For example, C1-DL02-MMAE represents that the targeting moiety is C1 antibody, the linker is DL02, and the payload is MMAE compound. "Ab1-MC-VC-PAB-MMAE" represents that the antibody is "Ab1", the linker is "MC-VC-PAB", and the payload is MMAE. "Ab2-MC-VC-PAB-MMAE" represents that the antibody is "Ab2", the linker is "MC-VC-PAB", and the payload is MMAE.

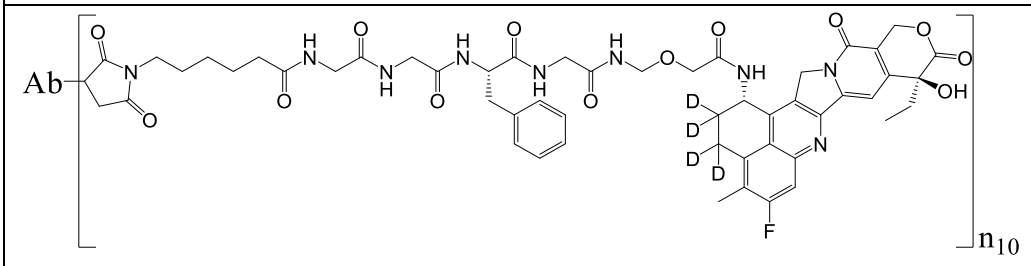
[00639] Example 58 Preparation of anti-CDCP1 antibody drug conjugate (with a camptothecin payload)

[00640] (1) General conjugation method

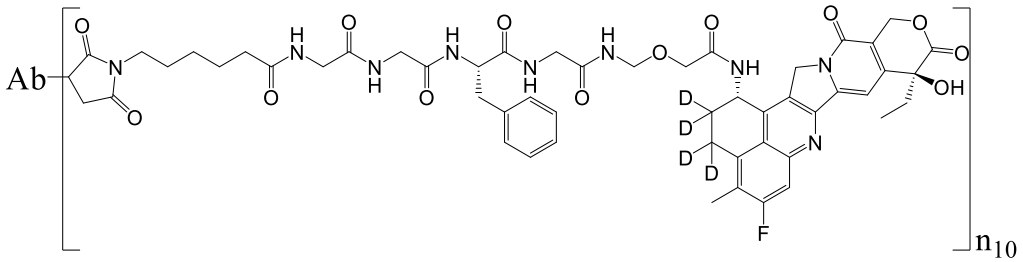
[00641] The antibody drug conjugate was prepared by a general conjugation method (based on DAR4 and DAR8), as described below. The reducing agent (1-20 mM TCEP (Tris-2-carboxyethyl-phosphine)) and the metal chelating agent (1-20 mM DTPA (Diethylene triamine pentacetate acid)) were separately prepared with purified water. The reducing agent, used at an amount varying within a certain concentration range according to the desired conjugation rate, was mixed with the monoclonal antibody at a certain concentration (e.g., 5-30 mg/mL) to achieve a final molar ratio of TCEP to antibody of 5:1 to 14:1 depending on DAR value (such as DAR4 and DAR8). The mixture was stirred at 25°C for 1 h of reaction. The antibody reduced by TCEP can be directly used for conjugation.

[00642] The linker-active drug compound was dissolved in dimethyl sulfoxide (DMSO) to a certain concentration (5 mM). The drug was slowly added to the antibody at a molar ratio of drug to sulfhydryl of 1.5:1 to 5:1 depending on DAR value (such as DAR4 and DAR8). The mixture was stirred at 25°C for 1-4 h of reaction. After the reaction was completed, the reaction mixture was purified through centrifugal ultrafiltration using ADC buffer (20 mM histidine hydrochloride, 36.5 g/L mannose, pH 6.0) to remove residual unreacted drugs and free small molecules such as DMSO. The conjugation was detected by hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC) and liquid chromatography-mass spectrometry (LC-MS), and the purity of the conjugated sample was measured by size exclusion chromatography (SEC).

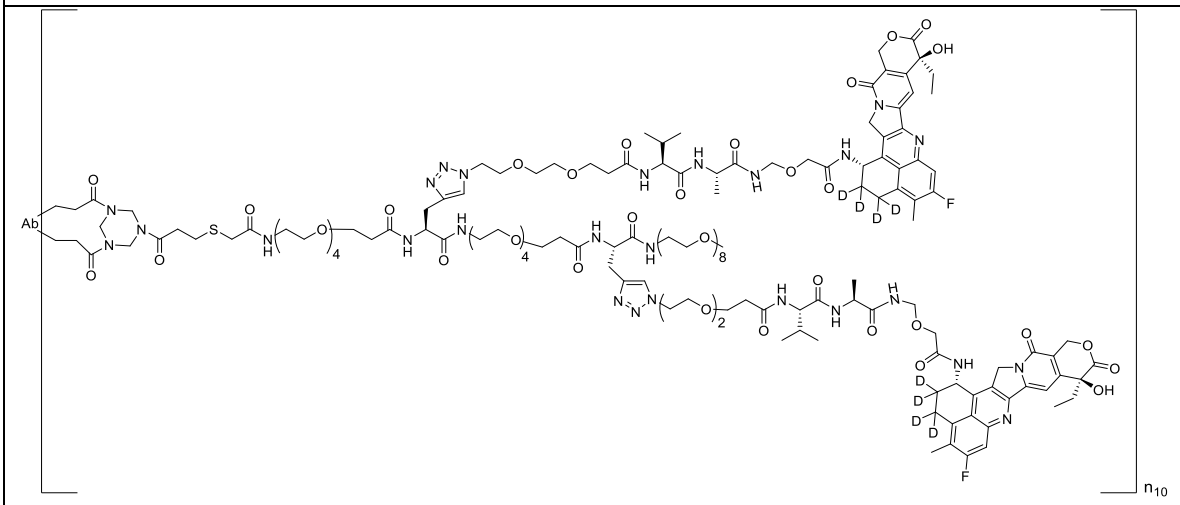
Table 16 Structures of antibody drug conjugates prepared by the general conjugation method

Ab1-DL01-dDxd


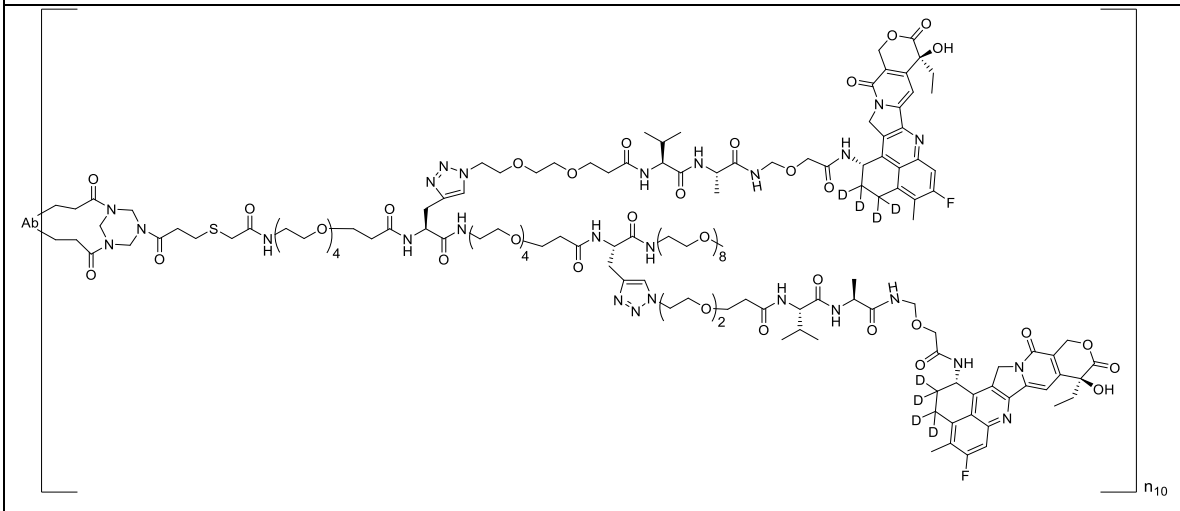
Ab2-DL01-dDxd



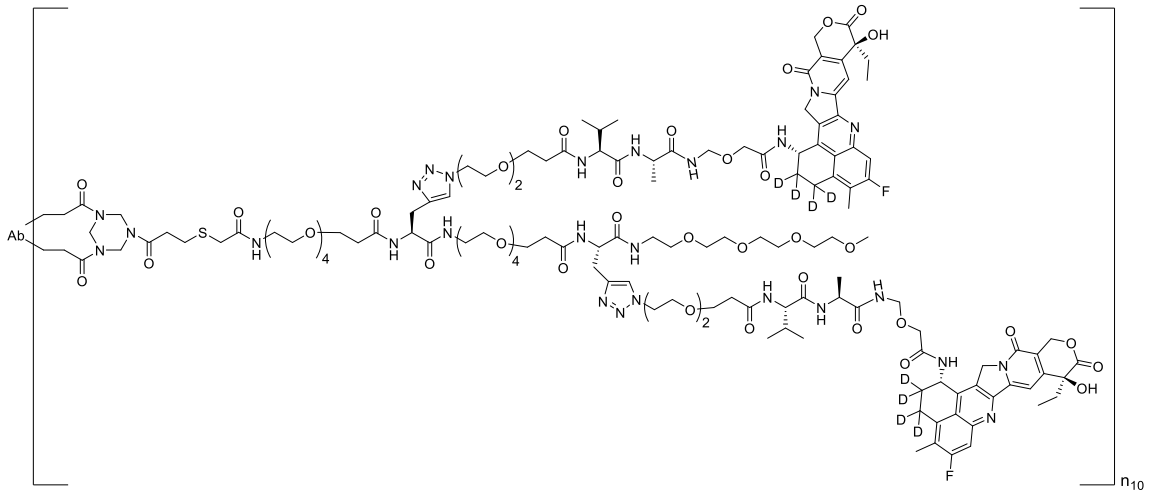
Ab1-DL10-dDxd



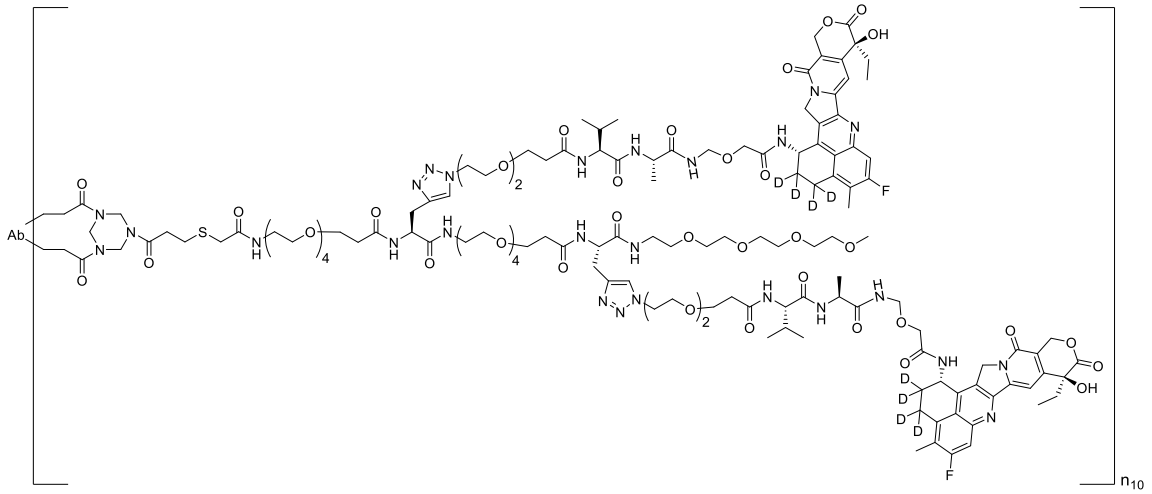
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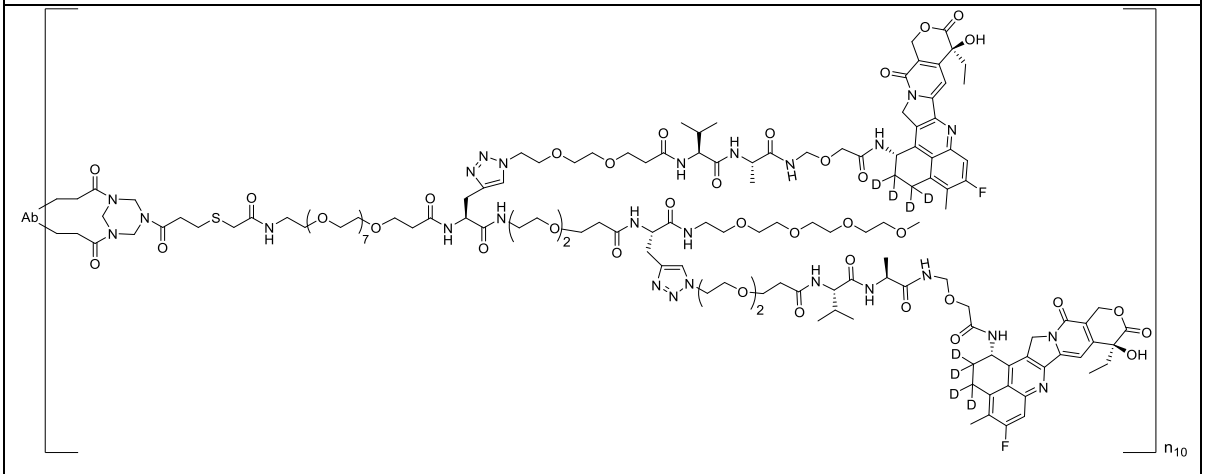
Ab1-DL11-dDxd



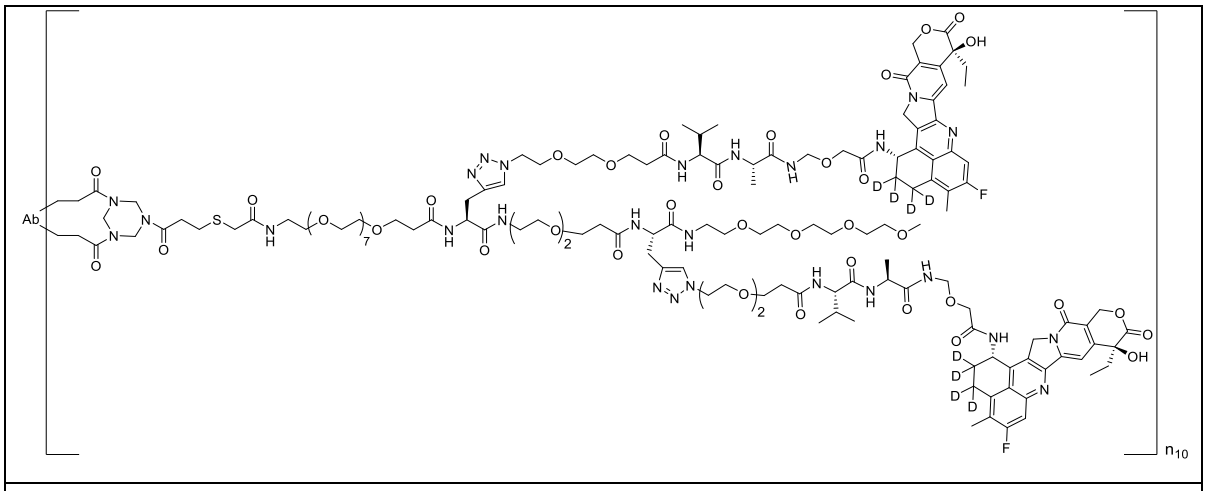
Ab2-DL11-dDxd



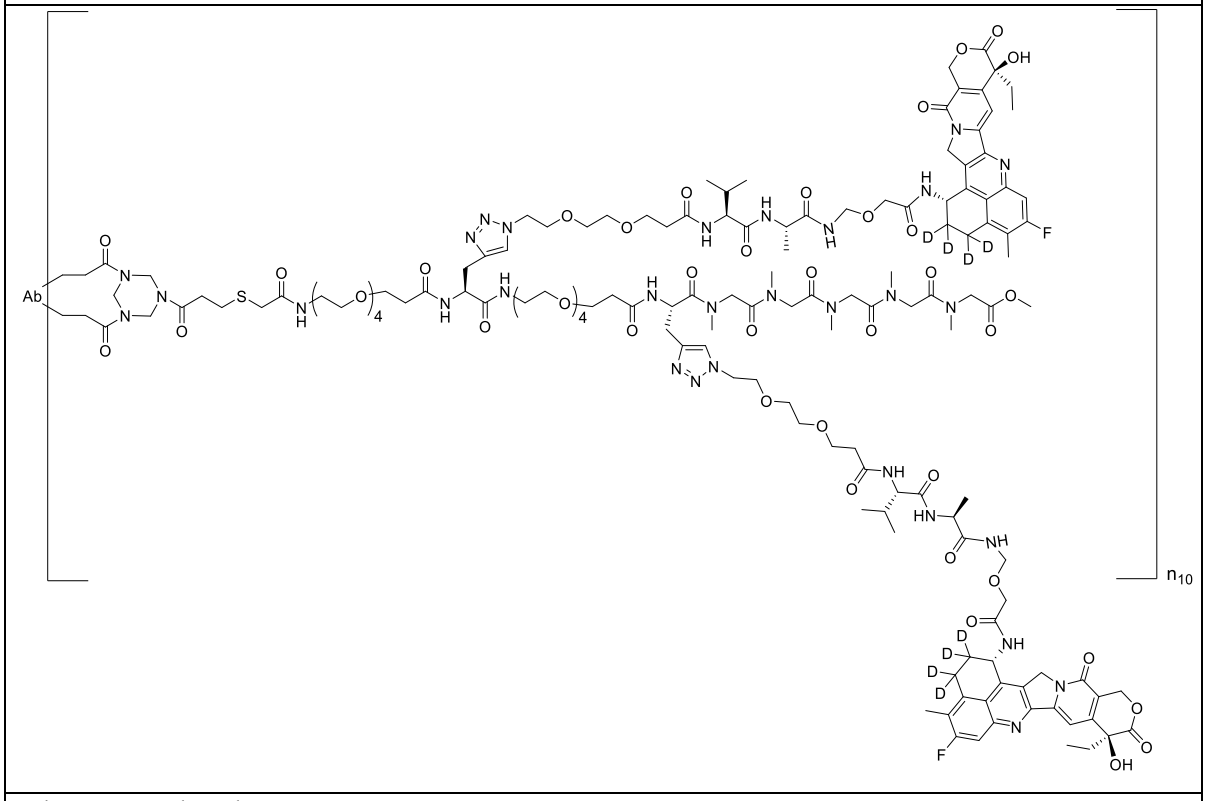
Ab1-DL12-dDxd



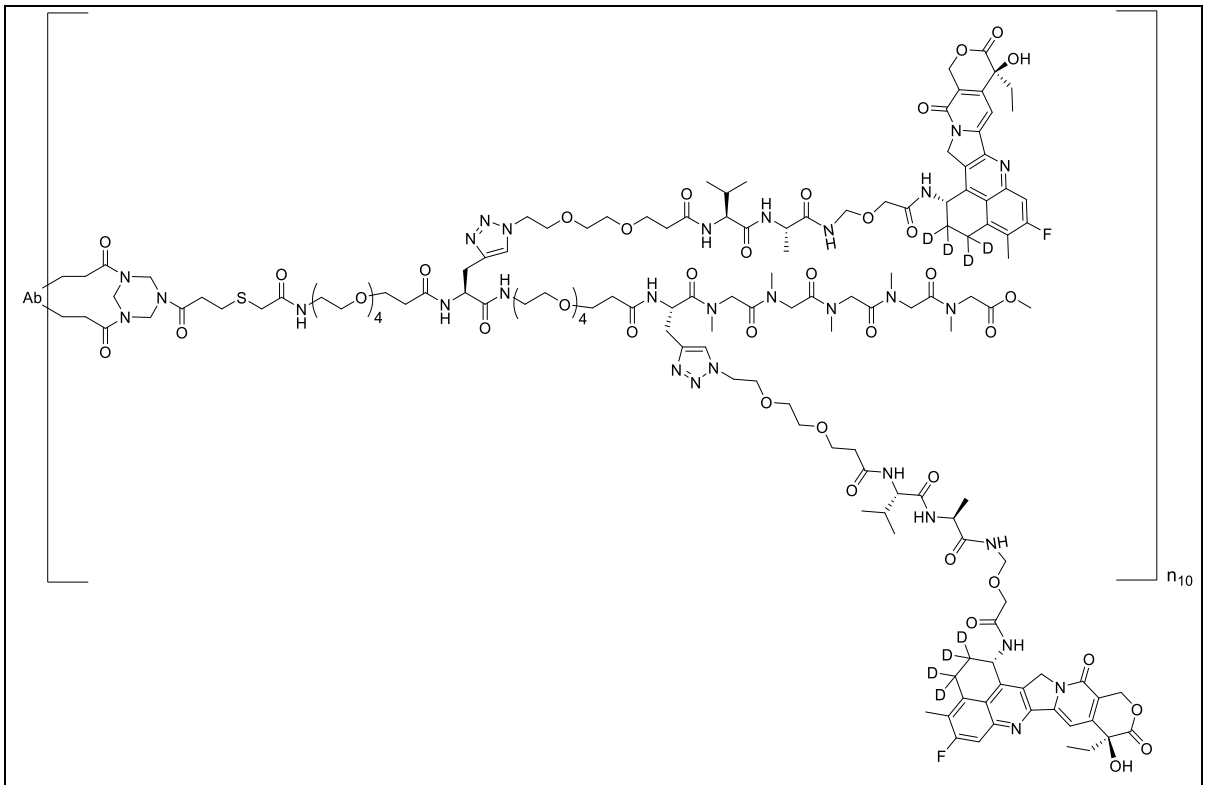
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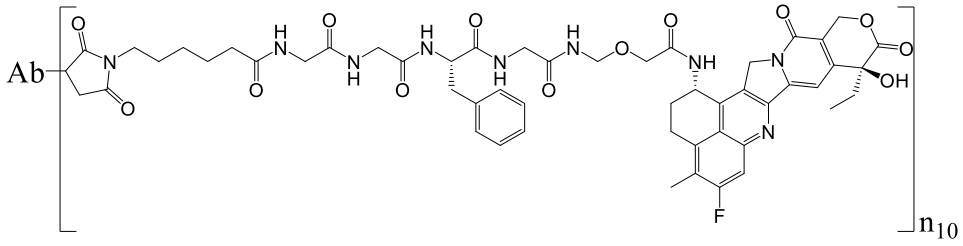
Ab1-DL13-dDxd



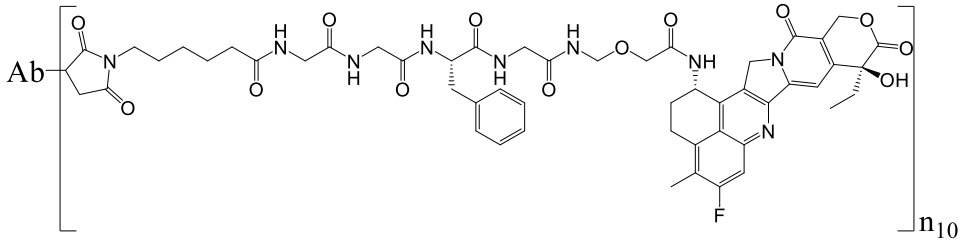
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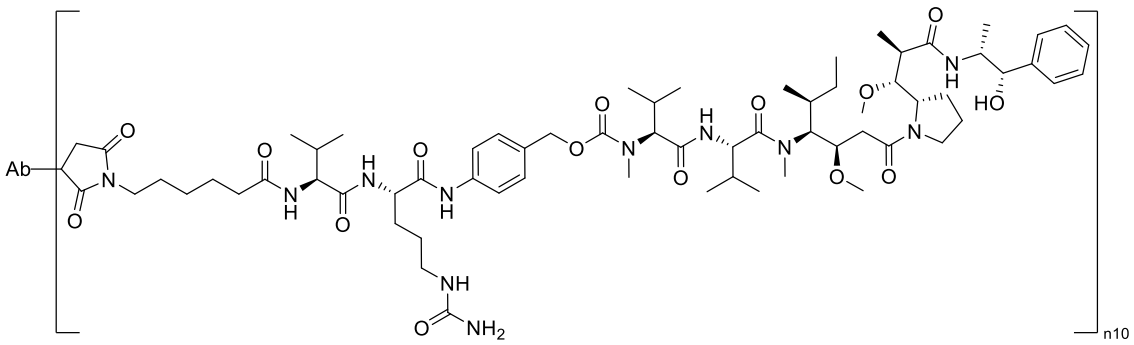
Ab1-DL01-Dxd



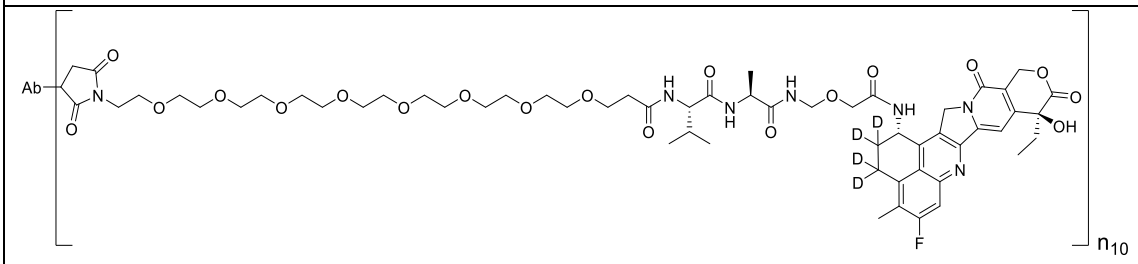
Ab2-DL01-Dxd



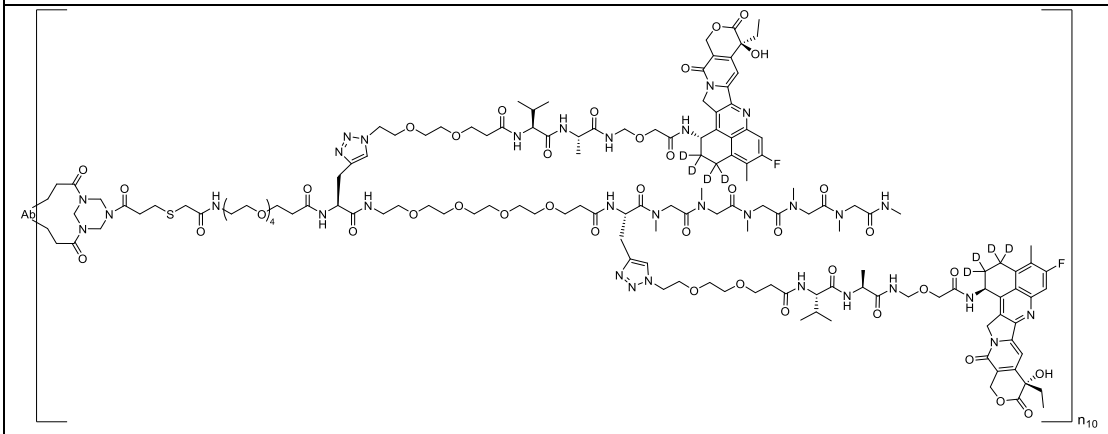
Ab1-MC-VC-PAB-MMAE



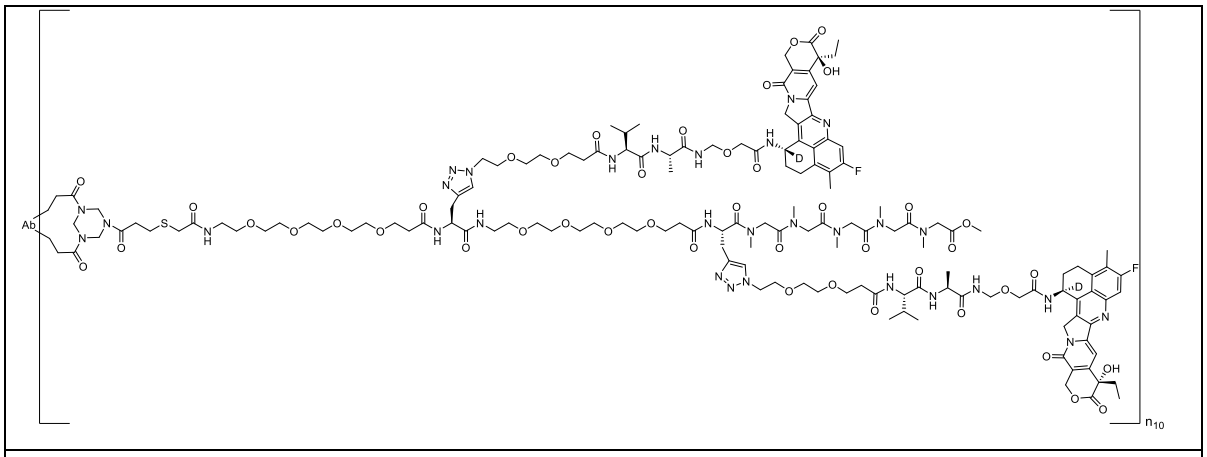
Ab1-DL14-dDxd



Ab1-DL25- dDxd



Ab1-DL13-d1Dxd



Ab1-DL25-d1Dxd

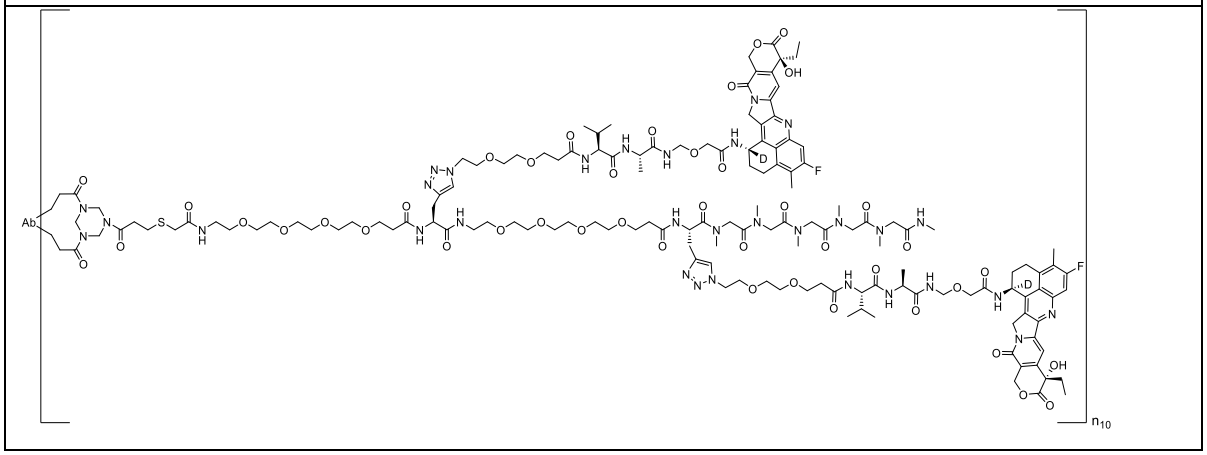


Table 17 Results of detection of antibody drug conjugates prepared by the general conjugation method

Number of ADC	DAR	SEC%
Ab1-DL01-dDxd	7.95	98.98
Ab2-DL01-dDxd	7.95	97.37
Ab1-DL10-dDxd	8.10	97.37
Ab2-DL10- dDxd	8.16	95.35
Ab1-DL11- dDxd	8.24	95.26
Ab2-DL11-dDxd	8.64	89.17
Ab1-DL12-dDxd	8.52	95.21

Ab2-DL12-dDxd	8.58	98.24
Ab1-DL13-dDxd	8.54	95.08
Ab2-DL13-dDxd	8.56	98.42
Ab1-DL01-Dxd	7.9	99.15
Ab2-DL01-Dxd	7.95	99.24
Ab1-DL13-Dxd	7.86	98.19
Ab1-MC-VC-PAB-MMAE	3.95	94.42
Ab1-DL14-dDxd	7.99	98.93
Ab1-DL25- dDxd	7.92	97.67
Ab1-DL13-d1Dxd	8.12	99.15
Ab1-DL25-d1Dxd	7.68	99.15

Except for "Ab1-MC-VC-PAB-MMAE", all numbers of ADC consist of three parts linked via two dashes ("-"), representing the targeting moiety, linker and payload moiety in sequence. For example, Ab1-DL01-dDxd represents that the targeting moiety is Ab1 antibody, the linker is DL01, and the payload is dDxd compound. "Ab1-MC-VC-PAB-MMAE" represents that the antibody is "Ab1", the linker is "MC-VC-PAB", and the payload is MMAE.

[00643] Example 59 Internalization efficiency of anti-CDCP1 antibody drug conjugate in tumor cells

[00644] The day before the experiment, EBC-1 cells (human lung adenocarcinoma cell line) in exponential growth phase were evenly plated into a 6-well plate at approximately 5E5 cells per well (2 mL). The antibody was dialyzed into 10 mM sodium bicarbonate buffer (pH 8.5), and pHAb Amine Reactive Dye was added for 1 h of reaction, followed by ultrafiltration and dialysis. According to the calculated concentration, Antibody-dye was diluted to 10 μg/mL using cell medium. 100 μL of diluted Antibody-dye was added to each well containing 2 mL of cells, and the cells were incubated at 37°C for a series of time points: 0 h, 1 h, 3 h, 5 h, 7 h and 24 h. Fluorescence intensity was measured using a flow cytometer at each time point.

[00645] Based on a shared antibody of Ab1 and linker of DL01, a comparison of internalization efficiency between Ab1-DL01-Dxd (with Dxd as the payload) and Ab1-DL01-dDxd (with dDxd as the payload) in EBC-1 human lung adenocarcinoma cells is shown in FIG. 1, and the results show that the dDxd payload confered higher internalization efficiency on the ADC compared to Dxd in EBC-1 human lung adenocarcinoma cells.

[00646] Based on a shared antibody of Ab2 and linker of DL01, a comparison of internalization efficiency between Ab2-DL01-Dxd (with Dxd as the payload) and Ab2-DL01-dDxd (with dDxd as the payload) is shown in FIG, 2, and the results show that the dDxd payload confered higher internalization efficiency on the ADC compared to Dxd in BxPC-3 human orthotopic pancreatic cancer cells.

[00647] Surprisingly, according to the various antibodies used herein, it was found that, when conjugated to the same antibody, the antibody drug conjugate prepared with the dDxd payload of the present disclosure exhibited superior internalization efficiency compared to the antibody drug conjugate prepared with the Dxd payload in certain tumor cell types.

[00648] Example 60 Inhibitory effect of anti-CDCP1 antibody drug conjugate on tumor cells

[00649] EBC-1 human lung adenocarcinoma cells and HCC827 human non-small cell lung cancer cells in the exponential growth phase were plated separately into a 96-well plate at 100 μL per well, with a density of 5,000-10,000 cells/mL. The cells were incubated overnight in a 37°C, 5% CO_2 incubator. The ADCs were prepared using the complete medium to gradient concentrations of 0-3,333.3 nM, nine concentrations in total. 100 μL of ADCs were then added to each well, three replicates for each concentration. A blank control group was set up. After 72-144 h of drug treatment, CCK-8 reagent was added, and cells were incubated in a 37°C, 5% CO_2 incubator for 1 to 4 h. The OD value of each well was detected at 450 nm using a microplate reader. The inhibition rate was calculated as IR (%) = (OD blank - OD drug) × 100/OD blank. IC_{50} was calculated by fitting a four-parameter logistic curve using GraphPad Prism based on inhibition rate and drug concentration.

Table 18 Inhibitory effect of antibody drug conjugates on EBC-1 cells (human lung adenocarcinoma cell line)

ADC	EBC-1	
	IC50 (nM)	MIR (%)
Ab1-DL01-dDxd	13.20	99.04
Ab2-DL01-dDxd	10.95	99.45
Ab1-DL10-dDxd	12.52	99.51
Ab2-DL10-dDxd	13.72	99.38
Ab1-DL11-dDxd	18.08	99.37
Ab2-DL11-dDxd	16.58	99.5
Ab1-DL12-dDxd	16.88	99.21
Ab2-DL12-dDxd	18.63	99.03
Ab1-DL13-dDxd	16.51	98.74
Ab2-DL13-dDxd	19.08	98.83
Ab1-DL01-Dxd	18.19	99.24

Table 19 Inhibitory effect of antibody drug conjugates on HCC827 cells (human non-small cell lung cancer cell line)

ADC	HCC827	
	IC50 (nM)	MIR (%)
Ab1-DL01-dDxd	1.02	98.70
Ab2-DL01-dDxd	1.25	98.28
Ab1-DL10-dDxd	1.37	99.55
Ab2-DL10-dDxd	1.55	99.24
Ab1-DL11-dDxd	1.75	99.37
Ab2-DL11-dDxd	1.70	99.16
Ab1-DL12-dDxd	1.34	99.26
Ab2-DL12-dDxd	1.74	98.93
Ab1-DL13-dDxd	1.36	99.33
Ab2-DL13-dDxd	2.09	99.15

[00650] The experimental results show that the antibody drug conjugates prepared with multiple

linker-payload (L-D) structures of the present disclosure had obvious inhibitory effects on the proliferation of tumor cells.

[00651] Example 61 Comparison of inhibitory effects of antibody drug conjugates with various payloads on cells

[00652] 1) EBC-1 cells (human lung adenocarcinoma cell line) in the exponential growth phase were plated into a 96-well plate at 100 μL per well, with a density of 5,000-10,000 cells/mL. The cells were incubated overnight in a 37°C, 5% CO_2 incubator.

[00653] 2) The ADCs were prepared using the complete medium to gradient concentrations of 0-3,333.3 nM, nine concentrations in total. 100 μL of ADCs were then added to each well, three replicates for each concentration. A blank control group was set up.

[00654] 3) After 72-144 h of drug treatment, CCK-8 reagent was added, and cells were incubated in a 37°C, 5% CO_2 incubator for 1 to 4 h. The OD value of each well was detected at 450 nm using a microplate reader. The inhibition rate was calculated as IR (%) = (OD blank - OD drug)*100/OD blank. IC_{50} was calculated by fitting a four-parameter logistic curve using GraphPad Prism based on inhibition rate and drug concentration,.

[00655] The experimental results show that the antibody drug conjugates of this example had obvious inhibitory effects on the proliferation of tumor cells. As shown in Table 20, when conjugated to the same antibody and linker, the ADC with the dDxd payload demonstrated superior inhibitory activity compared to the ADC with the Dxd payload, with comparable migration inhibition rates.

Table 20 Comparison of the inhibitory effects on EBC-1 cells

ADC	Cell	IC50 (nM)	MIR(%)
Ab1-DL01-dDxd	EBC-1 (human lung adenocarcinoma cell line)	18.19	99.24
Ab1-DL01-Dxd		56.10	100.0

[00656] Example 62 Inhibition of tumor growth by anti-CDCP1 antibody drug conjugate (with

MMAE payload) in EBC-1 (human lung adenocarcinoma cell line) xenograft model

[00657] 1) EBC-1 cells (human lung adenocarcinoma cell line) were cultured in an MEM medium (Minimum Essential Medium) containing 10% FBS (Fetal Bovine Serum) and maintained in a saturated humidity, 37°C, 5% CO_2 incubator.

[00658] 2) EBC-1 cells in the logarithmic growth phase were harvested, resuspended in MEM basic medium containing 50% Matrigel, and adjusted to a cell density of 1E8 cells/mL.

[00659] 3) Under aseptic conditions, 0.1 mL of the cell suspension was inoculated subcutaneously into the right axilla of mice at 1E7 cells/0.1 mL/mouse.

[00660] 4) Seven days after inoculation, animals bearing tumors that were excessively large, excessively small, or irregular in shape were excluded. Animals bearing regular tumors with volumes between 120.61 mm^3 and 207.69 mm^3 were selected and grouped according to an average tumor volume of each group of animals being about 146.34 mm^3, 6 mice per group. The grouping day was recorded as Day 0, and the animals were administered according to body weight.

[00661] 5) The body weight of animals and the tumor volume were measured twice weekly. The clinical symptoms of animals were observed and recorded daily.

[00662] As shown in FIG. 3, in the EBC-1 (human lung adenocarcinoma cell line) xenograft model, Ab1-MC-VC-PAB-MMAE (antibody: Ab1, linker: MC-VC-PAB, payload: MMAE) and C1-DL02-MMAE exhibited inhibitory effects on tumor at a dose of 1 mg/kg. The inhibition efficacy of Ab1-MC-VC-PAB-MMAE was significantly better than that of C1-DL02-MMAE. C1-DL02-MMAE exhibited higher efficacy as the dose increases, which was obviously dose-dependent.

[00663] Example 63 Inhibition of tumor growth by anti-CDCP1 antibody drug conjugate (with MMAE as payload) in BxPC-3 (human orthotopic pancreatic cancer cell line) xenograft model

[00664] 1) BxPC-3 cells (human orthotopic pancreatic cancer cell line) were cultured in an RPMI-1640 medium containing 10% FBS and maintained in a saturated humidity, 37°C, 5% CO_2 incubator.

[00665] 2) BxPC-3 cells (human orthotopic pancreatic cancer cell line) in the logarithmic growth phase were harvested, resuspended in MEM basic medium containing 50% Matrigel, and adjusted

to a cell density of 1E8 cells/mL.

[00666] 3) Under aseptic conditions, 0.1 mL of the cell suspension was inoculated subcutaneously into the right axilla of mice at 1E7 cells/0.1 mL/mouse.

[00667] 4) Twenty-seven days after inoculation, animals bearing tumors that were excessively large, excessively small, or irregular in shape were excluded. Animals bearing regular tumors with volumes between 100.5 mm^3 and 207.86 mm^3 were selected and grouped according to an average tumor volume of each group of animals being 145.33 mm^3, 6 mice per group. The grouping day was recorded as Day 0, and the animals were administered according to body weight.

[00668] 5) The animals were administered twice on day 0 and day 11, respectively. The body weight of animals and the tumor volume were measured twice weekly. The clinical symptoms of animals were observed and recorded daily.

[00669] The experimental results (FIG. 4) show that, in BxPC-3 (human orthotopic pancreatic cancer cell line) xenograft model, at a low dose of 2 mg/kg, Ab1-MC-VC-PAB-MMAE and C1-DL02-MMAE exhibited similar efficacy and both potently inhibited tumor growth. C1-DL02-MMAE exhibited an obvious dose-dependent efficacy, with tumor regression observed at a dose of 6 mg/kg.

[00670] Example 64 Inhibition of tumor growth by anti-CDCP1 antibody drug conjugate (with a camptothecin payload) in EBC-1 (human lung adenocarcinoma cell line) xenograft model (dose: 5 mg/kg)

[00671] 1) EBC-1 cells were cultured in an MEM medium containing 10% FBS and maintained in a saturated humidity, 37°C, 5% CO_2 incubator.

[00672] 2) EBC-1 cells in the logarithmic growth phase were harvested, resuspended in MEM basic medium containing 50% Matrigel, and adjusted to a cell density of 1E8 cells/mL.

[00673] 3) Under aseptic conditions, 0.1 mL of the cell suspension was inoculated subcutaneously into the right dorsal side of mice at 1E7 cells/0.1 mL/mouse.

[00674] 4) Seven days after the inoculation, animals bearing tumors that were excessively large, excessively small, or irregular in shape were excluded. Animals bearing regular tumors with volumes between 100.72 mm^3 and 193.56 mm^3 were selected and grouped according to an average

tumor volume of each group of animals being 134.79 mm^3, 7 mice per group. The grouping day was recorded as Day 0, and the animals were administered according to body weight on day 0 and day 24, respectively.

[00675] 5) The body weight of animals and the tumor volume were measured twice weekly throughout the experiment. The clinical symptoms of animals were observed and recorded daily.

[00676] The experimental results (FIG. 5) show that, at a dose of 5 mg/kg, the inhibitory effect of Ab1-DL13-dDxd on tumor was significantly better than that of Ab1-DL13-Dxd, indicating a better in vivo efficacy.

[00677] Example 65 Inhibition of tumor growth by anti-CDCP1 antibody drug conjugate (with a camptothecin payload) in EBC-1 (human lung adenocarcinoma cell line) xenograft model (dose: 2.5 mg/kg)

[00678] 1) EBC-1 cells were cultured in an MEM medium containing 10% FBS and maintained in a saturated humidity, 37°C, 5% CO_2 incubator.

[00679] 2) EBC-1 cells in the logarithmic growth phase were harvested, resuspended in MEM basic medium containing 50% Matrigel, and adjusted to a cell density of 1E8 cells/mL.

[00680] 3) Under aseptic conditions, 0.1 mL of the cell suspension was inoculated subcutaneously into the right dorsal side of mice at 1E7 cells/0.1 mL/mouse.

[00681] Seven days after the inoculation, animals bearing tumors that were excessively large, excessively small, or irregular in shape were excluded. Animals bearing regular tumors with volumes between 123.27 mm^3 and 213.02 mm^3 were selected and grouped according to an average tumor volume of each group of animals being 149.65 mm^3, 7 mice per group. The grouping day was recorded as Day 0, and the animals were administered according to body weight.

[00682] The body weight of animals and the tumor volume were measured twice weekly throughout the experiment. The clinical symptoms of animals were observed and recorded daily.

[00683] The experimental results (FIG. 6) show that, at a dose of 2.5 mg/kg, the inhibitory effect of Ab1-DL13-dDxd on tumor was better than that of Ab1-DL13-Dxd.

[00684] Example 66 Toxicity tolerance test

[00685] 1) The quarantine-qualified ICR mice were weighed before grouped, with female animals weighing approximately 26-31 g.

[00686] 2) According to the body weight, the mice were randomly grouped into the following groups: Ab1-DL01-dDxd 600 mg/kg and Ab1-DL01-Dxd 600 mg/kg, 4 mice per group. The mice in each group were administered with the corresponding drug solution via intravenous injection.

[00687] 3) A control group was set up, in which mice from the same batch were administered with 0.9% sodium chloride injection via tail vein at the same volume as the drug solution.The body weight was measured 4, 7, 11, 14 and 17 days after administration.

[00688] The experimental results (FIG. 7) show shat, at a dose of 600 mg/kg, 1 mouse in the Ab1-DL01-dDxd group died, and all mice in the Ab1-DL01-DXd group died under the conditions of this experiment. This indicates that Ab1-DL01-dDxd had a higher toxicity tolerance than Ab1-DL01-DXd in this mouse model, suggesting that the dDxd provides superior in vivo safety compared to DXd when used as payload in ADCs.

[00689] The experimental results of the above examples show that the anti-CDCP1 antibodies of the present disclosure have high yield, good thermal stability and protein binding stability, excellent purity, cell binding activity and internalization efficiency, and has a broad application prospect in improving the precision and effectiveness of tumor treatment. The anti-CDCP1 antibody drug conjugates of the present disclosure have high payload delivery efficiency and internalization efficiency, excellent inhibitory activity on tumor cells, and good in vivo drug efficacy and safety. The deuterated payloads of the present disclosure can greatly reduce the in-vivo toxicity of the antibody drug conjugate and significantly improve the safety of the prepared antibody drug conjugate, providing an innovative solution for optimizing drugs for tumor treatment.

[00690] Only some of the embodiments of the present disclosure are disclosed above, which do not limit the present disclosure in any form. It can be understood by those of ordinary skill in the art that the present disclosure is not limited to each specific embodiment. For those of ordinary skill in the art, several improvements and modifications can be made to the present disclosure without departing from the principle of the present disclosure, and these improvements and modifications also fall within the scope of protection of the claims of the present disclosure.

CLAIMS

[Claim 1] An antibody drug conjugate targeting CDCP1, comprising a targeting moiety targeting CDCP1, wherein the targeting moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(1) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 19, CDR-H2 set forth in SEQ ID NO: 20, and CDR-H3 set forth in SEQ ID NO: 21, and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 22, CDR-L2 set forth in SEQ ID NO: 23, and CDR-L3 set forth in SEQ ID NO: 24; or

(2) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 29, CDR-H2 set forth in SEQ ID NO: 30, and CDR-H3 set forth in SEQ ID NO: 31, and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 32, CDR-L2 set forth in SEQ ID NO: 33, and CDR-L3 set forth in SEQ ID NO: 34; and

the CDRs are defined according to IMGT numbering system.

[Claim 2] The antibody drug conjugate according to claim 1, wherein:

(1) the heavy chain variable region (VH) comprises a sequence set forth in SEQ ID NO: 25 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 25, and the light chain variable region (VL) comprises a sequence set forth in SEQ ID NO: 26 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 26; or

(2) the heavy chain variable region (VH) comprises a sequence set forth in SEQ ID NO: 35 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 35, and the light chain variable region (VL) comprises a sequence set forth in SEQ ID NO: 36 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 36.

[Claim 3] The antibody drug conjugate according to claim 1 or 2, wherein the targeting moiety is selected from the group consisting of an antibody and an antigen binding fragment thereof.

[Claim 4] The antibody drug conjugate according to any one of claims 1 to 3, wherein the antibody or the antigen binding fragment thereof is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a multispecific antibody, Fab fragment, F(ab') fragment, $F(ab')_2$ fragment, Fv fragment, dAb, Fd and single-chain variable fragment (scFv).

[Claim 5]The antibody drug conjugate according to claim 4, wherein the targeting moiety further comprises a constant region of an immunoglobulin, further preferably, the immunoglobulin is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4.

[Claim 6] The antibody drug conjugate according to any one of claims 1 to 5, wherein the targeting moiety comprises a heavy chain and a light chain, wherein,

(1) the heavy chain comprises a sequence set forth in SEQ ID NO: 27 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 27, and the light chain comprises a sequence set forth in SEQ ID NO: 28 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 28; or

(2) the heavy chain comprises a sequence set forth in SEQ ID NO: 37 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 37, and the light chain comprises a sequence set forth in SEQ ID NO: 38 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 38.

[Claim 7] The antibody drug conjugate according to any one of claims 1 to 6, wherein the antibody drug conjugate has a structure represented by a general formula Ab-Un, wherein:

Ab represents a targeting moiety,

U represents a linker-payload moiety,

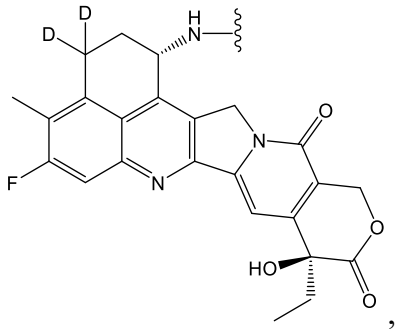
n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n represents the number of U linked to Ab, and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and

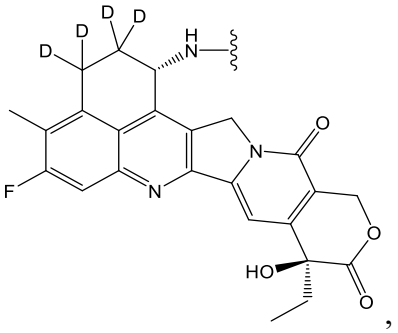
Ab is covalently linked to the payload via the linker.

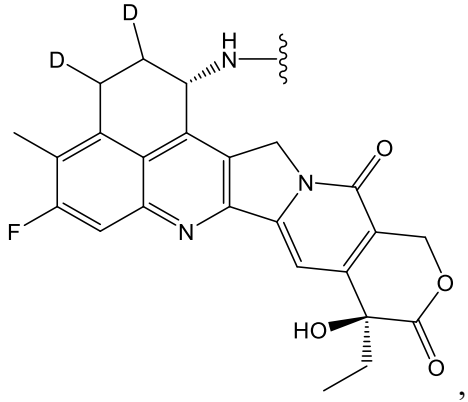
[Claim 8] The antibody drug conjugate according to claim 7, wherein Ab is linked to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 payloads via one or more linkers.

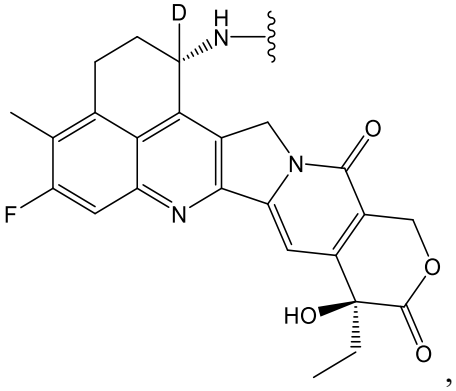
[Claim 9] The antibody drug conjugate according to claim 8, wherein the payload is selected

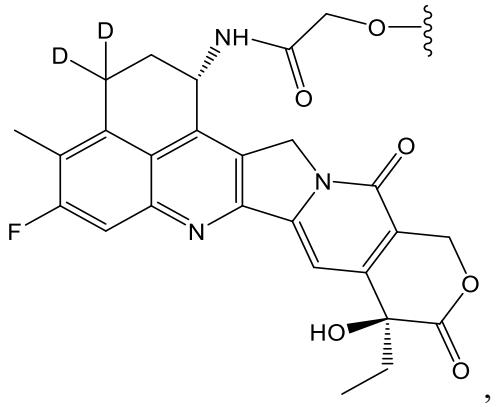
from the group consisting of:

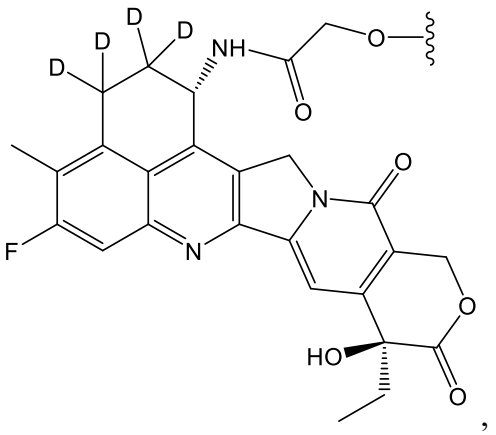

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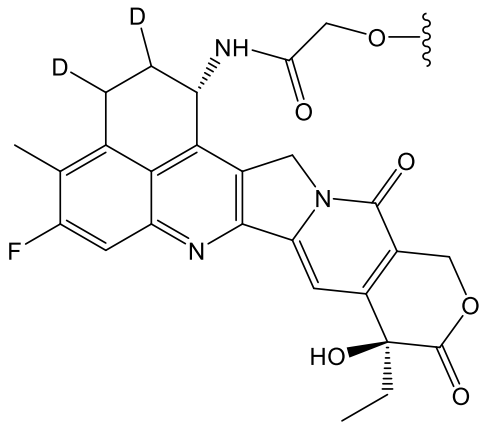

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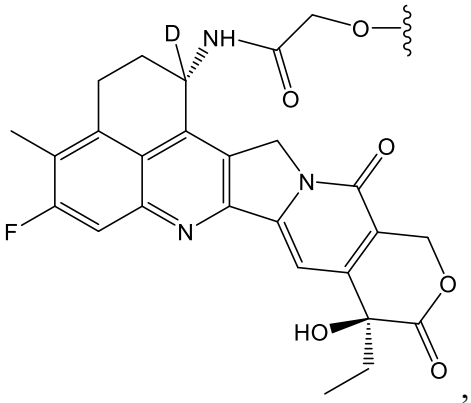

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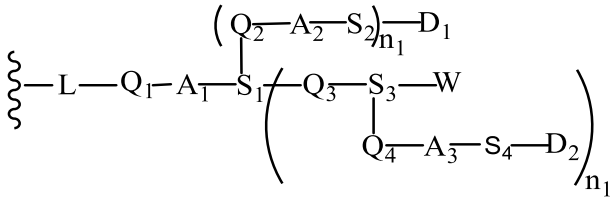
and



, and

"〰" is a site where the payload and the linker are linked.

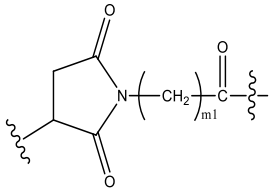
[Claim 10] The antibody drug conjugate according to any one of claims 7 to 9, wherein the linker-payload moiety is represented by formula (I):



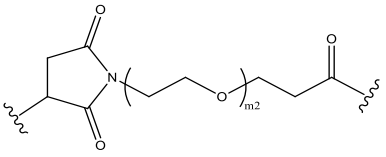
formula (I)

wherein,

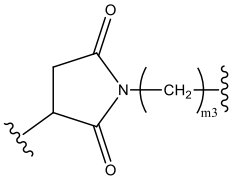
L is a linker linked to an antibody, and has a structure selected from the group consisting of



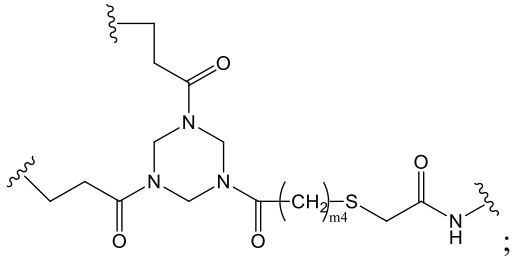
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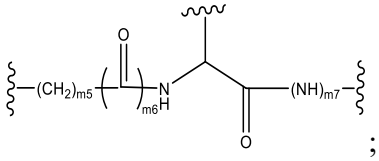
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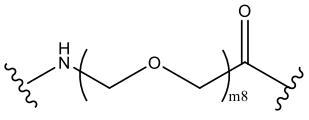
Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -$(NHCH_2C(O))$-O-, -NH(CH_2CH_2O)p-$CH_2CH_2C(O)$- , -(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -CH_2-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -$NHCH_2$-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -(CH_2)-Y- and -$NHCH_2C(O)CH_2C(O)$-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{3-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is independently selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

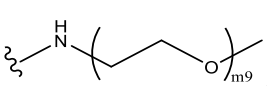
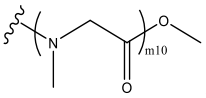
A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

S_1 and S_3 are independently ;

S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and ;

D_1 is a first payload compound, D2 is a second payload compound;

W is selected from the group consisting of  and ;

m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

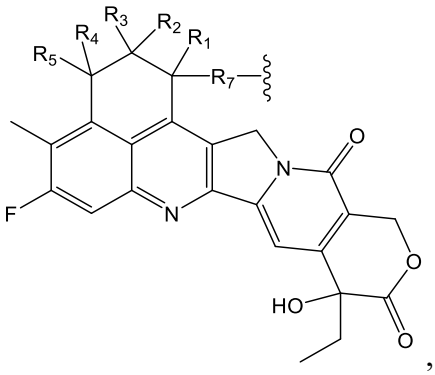
m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

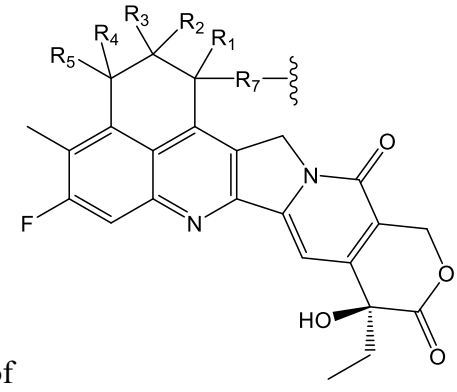
m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

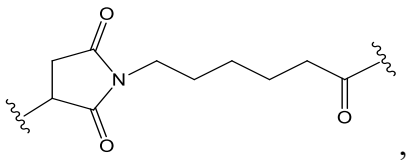
n_1 is 0 or 1.

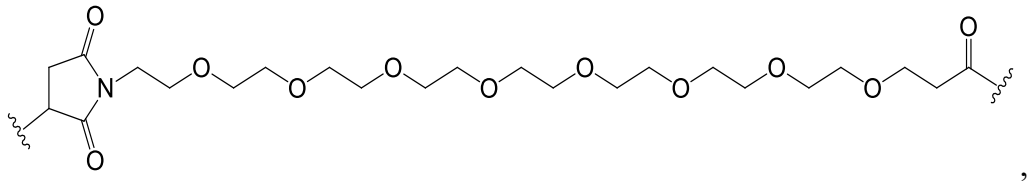
[Claim 11] The antibody drug conjugate according to any one of claims 7 to 10, wherein the payload independently has a structure of , "〰" is a site where the payload and the linker are linked; wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H), deuterium (D), and tritium (T), and at least one, two, three, or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_7 is independently selected from the group consisting of -NH- and -NH-C(O)-CH_2-O-.

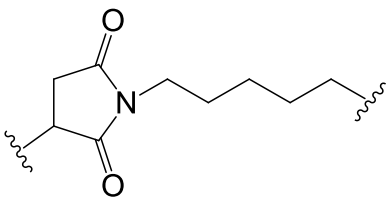
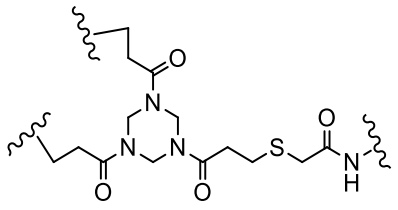
[Claim 12] The antibody drug conjugate according to claim 11, wherein the payload independently has a structure of , "〰" is a site where the payload and the linker are linked; wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H), deuterium (D), and tritium (T), and at least one, two, three,

or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_7 is independently selected from the group consisting of -NH- and -NH-C(O)-CH_2-O-.

[Claim 13] The antibody drug conjugate according to any one of claims 7 to 12, wherein -L- has a structure selected from the group consisting of:


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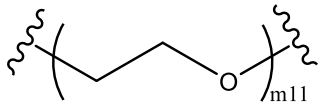
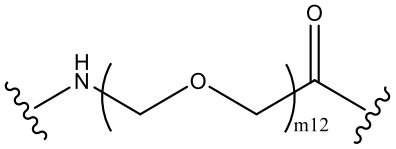
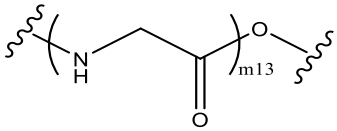
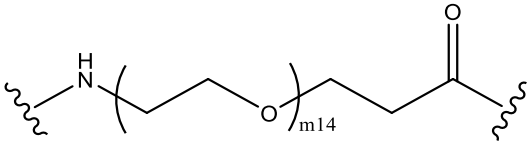
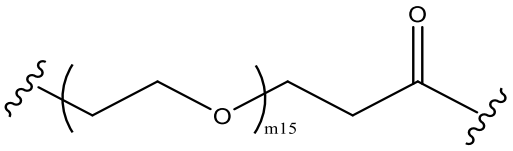
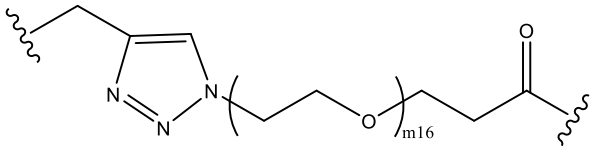
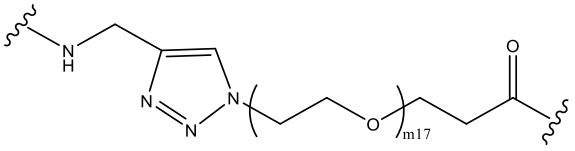
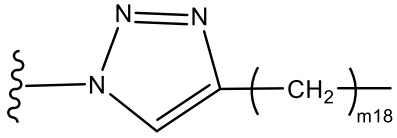

and

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[Claim 14] The antibody drug conjugate according to any one of claims 7 to 12, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[Claim 15] The antibody drug conjugate according to claim 14, wherein A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[Claim 16] The antibody drug conjugate according to claim 15, wherein A_1, A_2 and A_3 are

independently a single bond or independently comprises a structure selected from the group consisting of: -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; preferably the linker comprises a structure of -Val-Ala-.

[Claim 17] The antibody drug conjugate according to any one of claims 7 to 16, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond, , , , , , ,  and ;

wherein,

m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m14 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

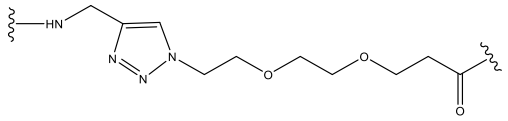
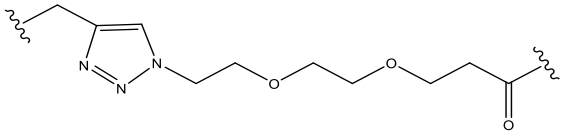
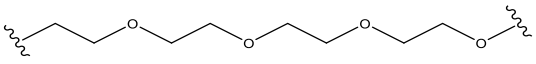
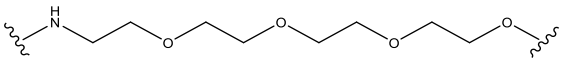
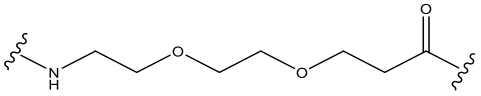
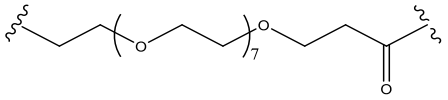
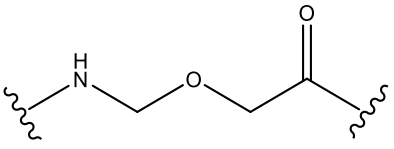
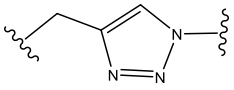
m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

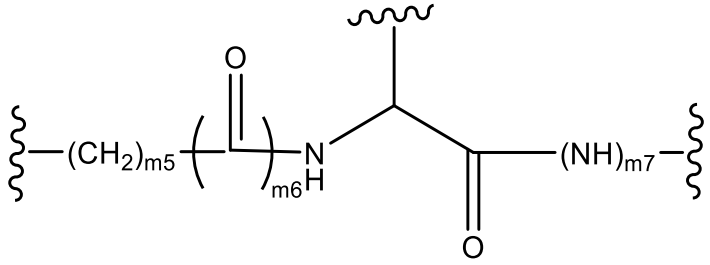
m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[Claim 18] The antibody drug conjugate according to claim 17, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

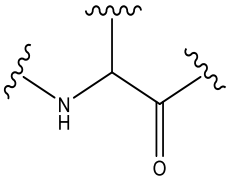
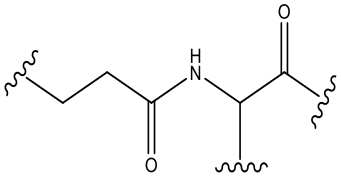

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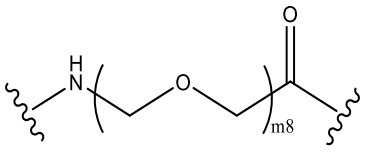
[Claim 19] The antibody drug conjugate according to any one of claims 7 to 18, wherein S_1 and S_3 independently have a structure of:

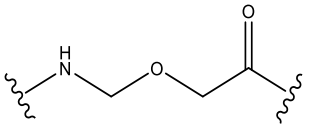

;

wherein m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

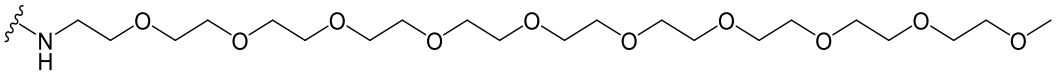
[Claim 20] The antibody drug conjugate according to claim 19, wherein S_1 and S_3

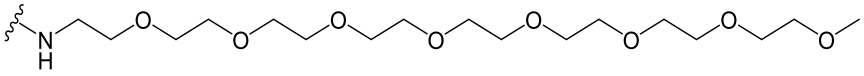
independently have a structure selected from the group consisting of  and .

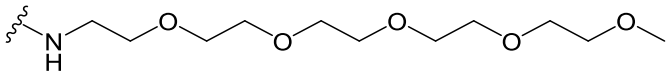
[Claim 21] The antibody drug conjugate according to any one of claims 7 to 20, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and ; wherein m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

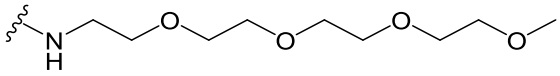
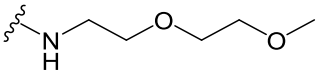
[Claim 22] The antibody drug conjugate according to claim 21, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and .

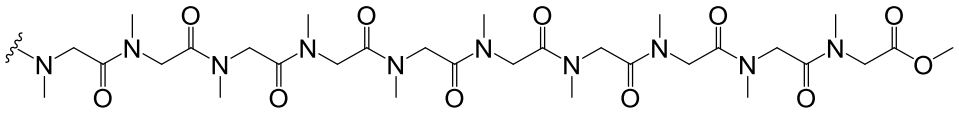
[Claim 23] The antibody drug conjugate according to any one of claims 7 to 22, wherein W has a structure selected from the group consisting of:

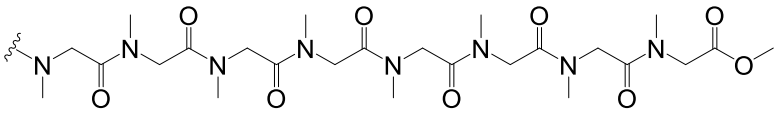
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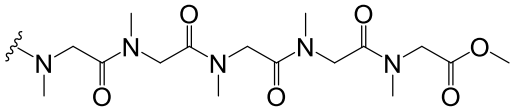
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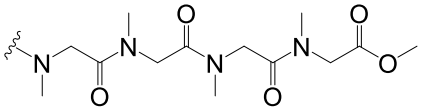
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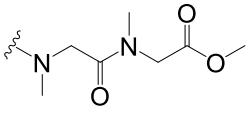
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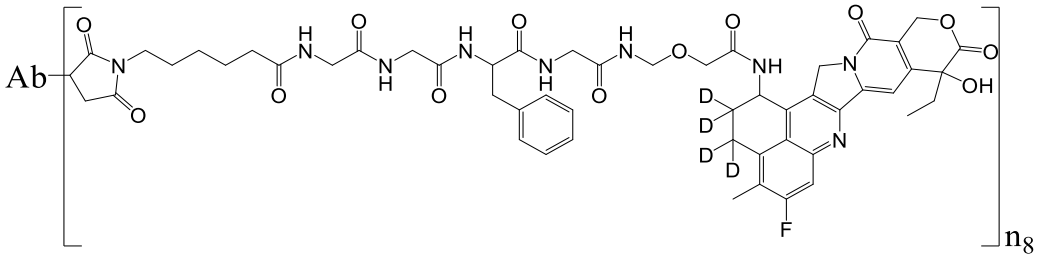
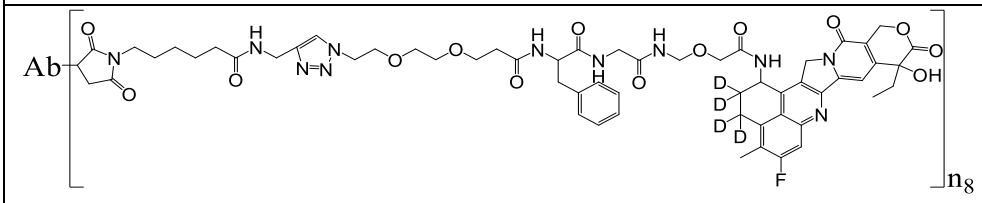

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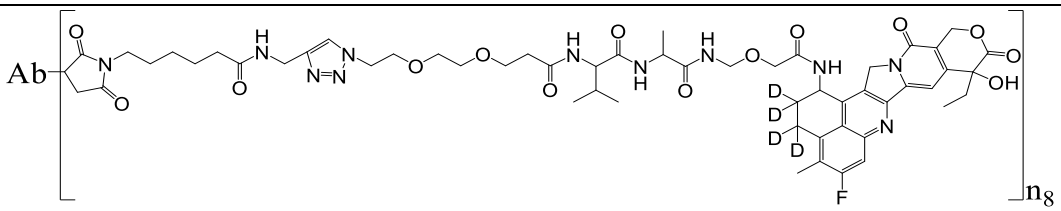

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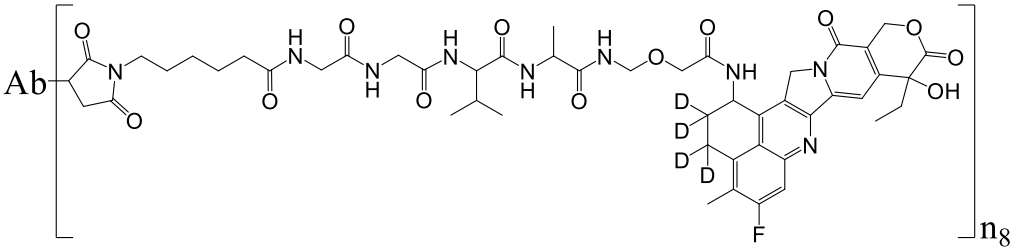

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[Claim 24] The antibody drug conjugate according to claim 7, wherein the antibody drug conjugate has a structure selected from the group consisting of:

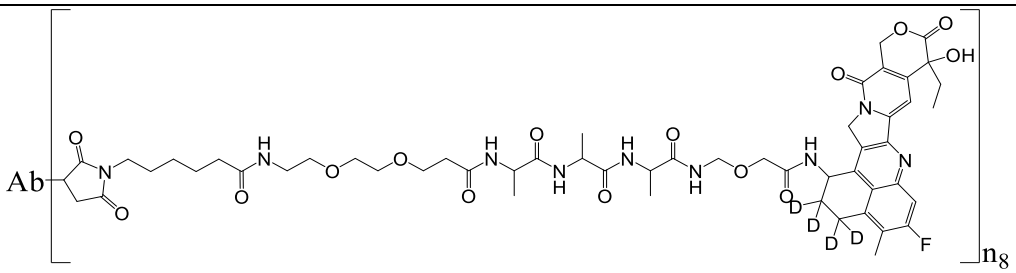
ADC-1

ADC-2

ADC-6



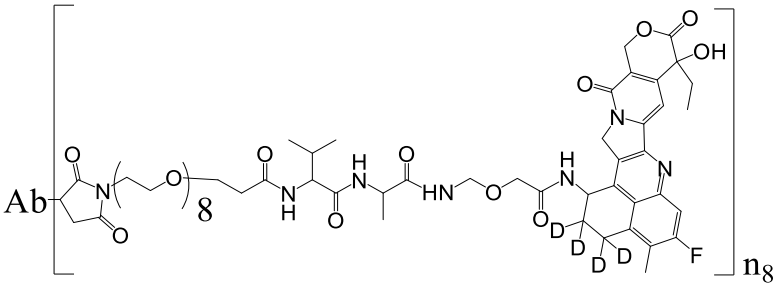
ADC-5



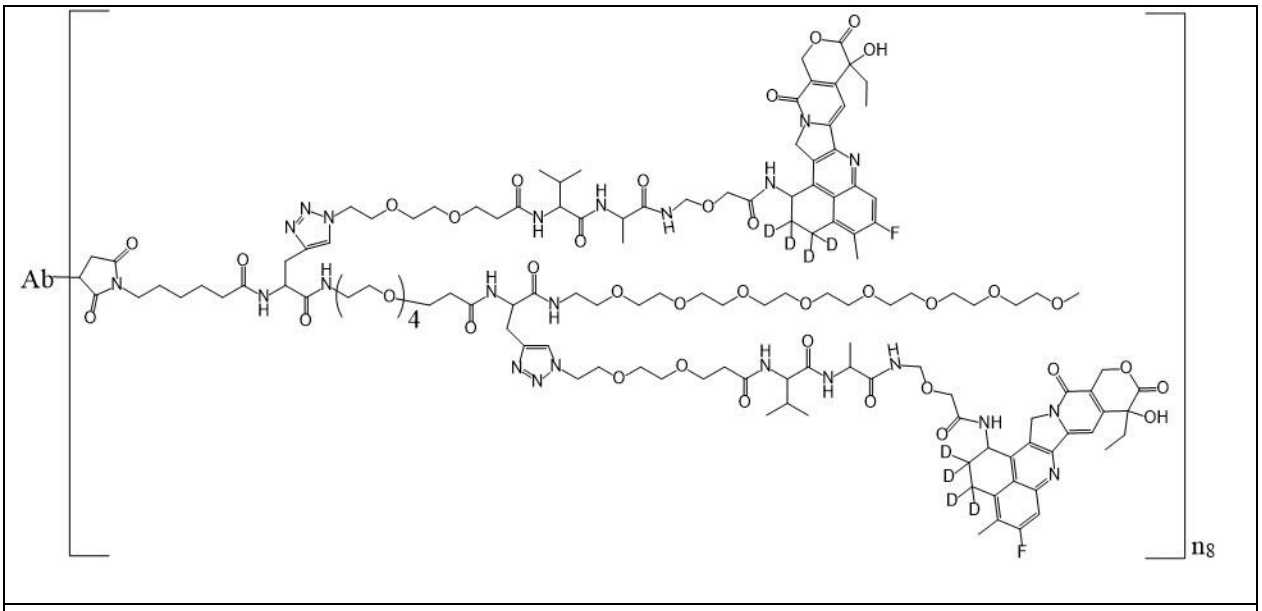
ADC-7



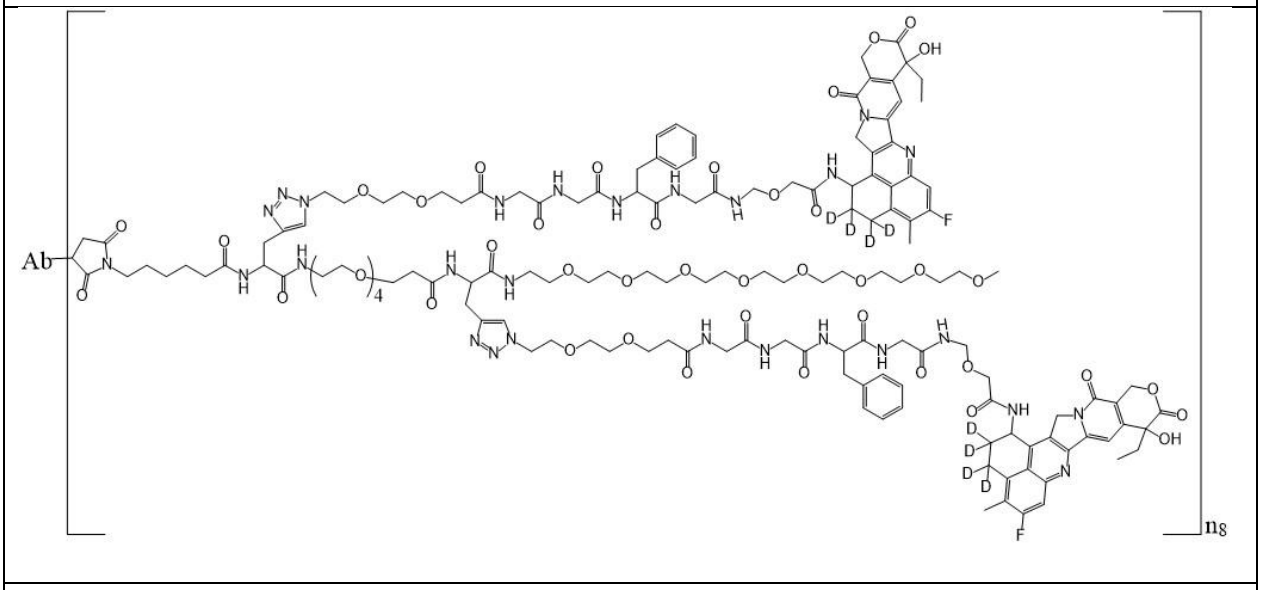
ADC-14



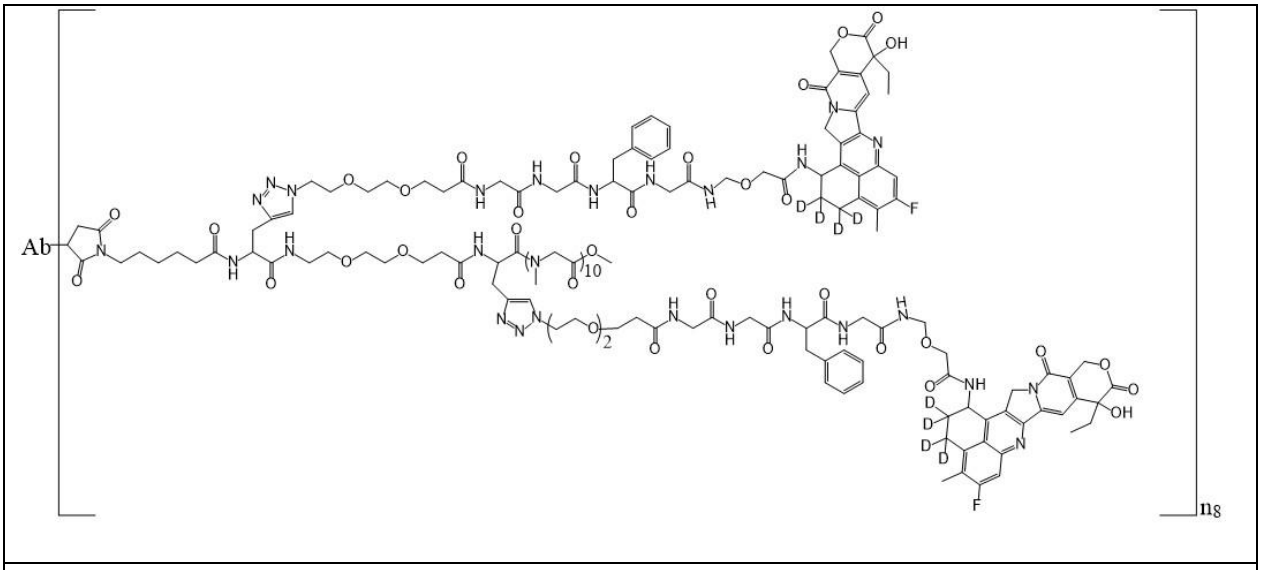
ADC-4



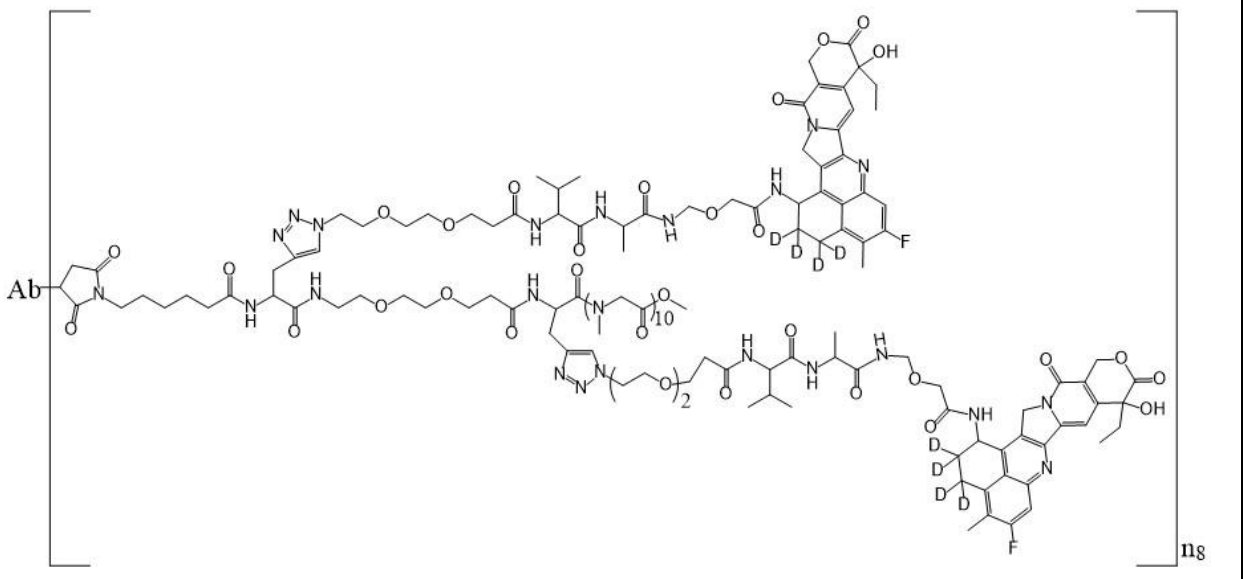
ADC-3



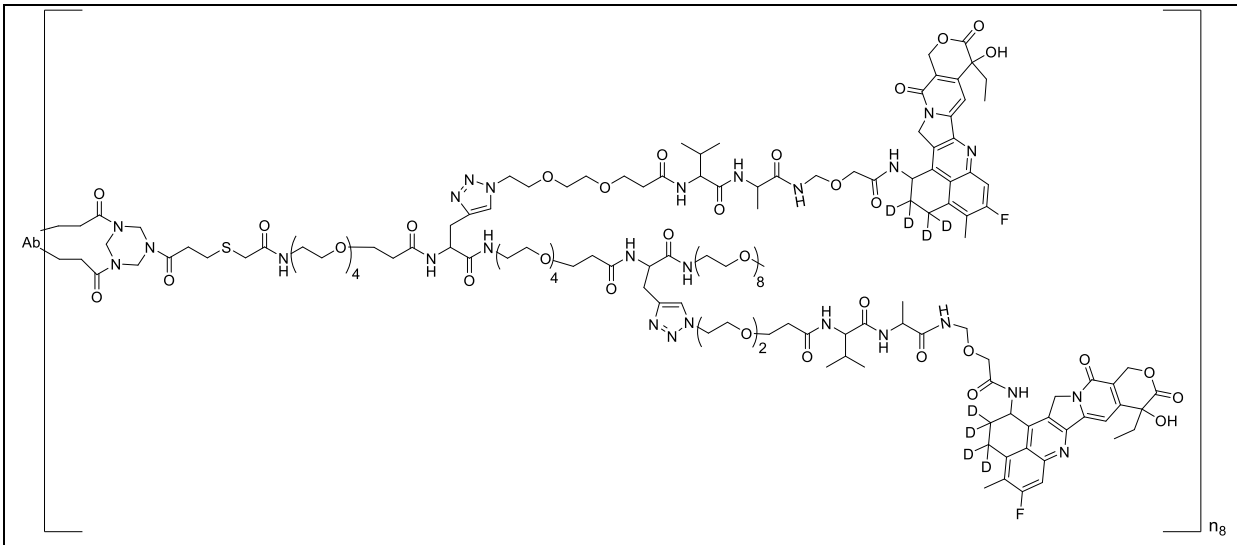
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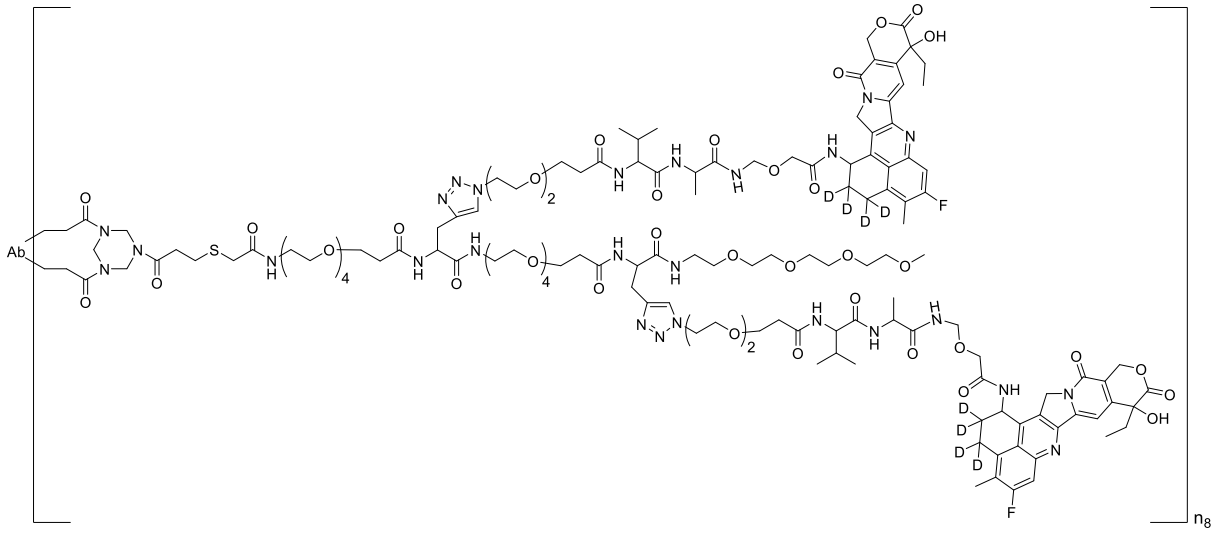
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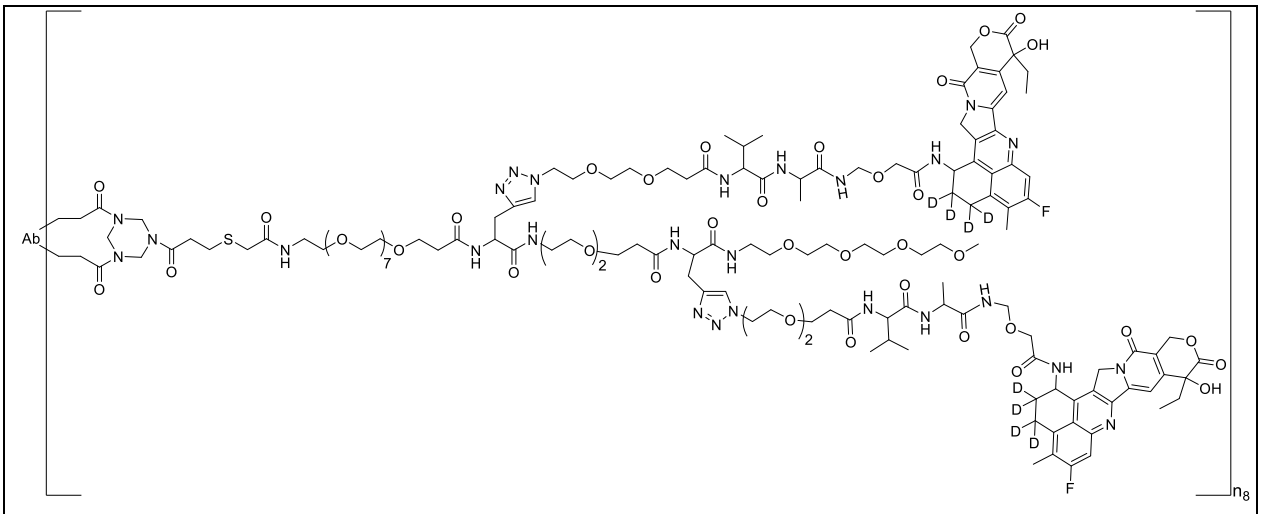
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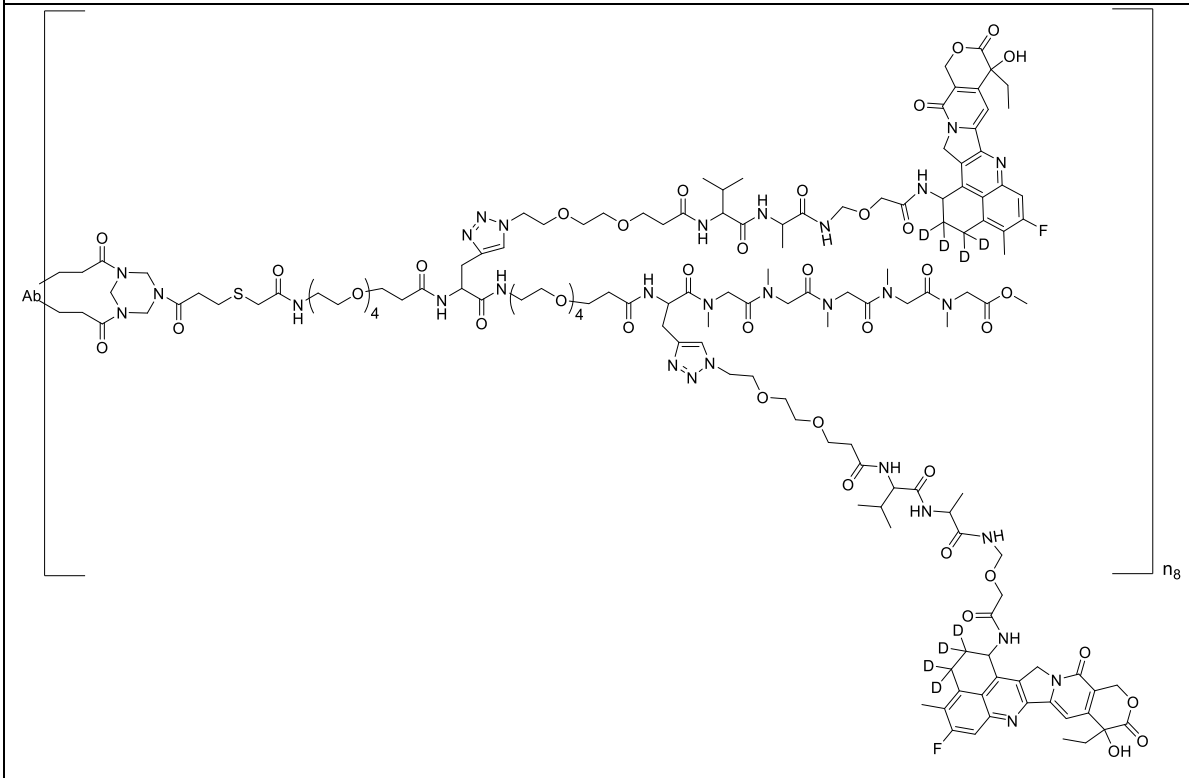
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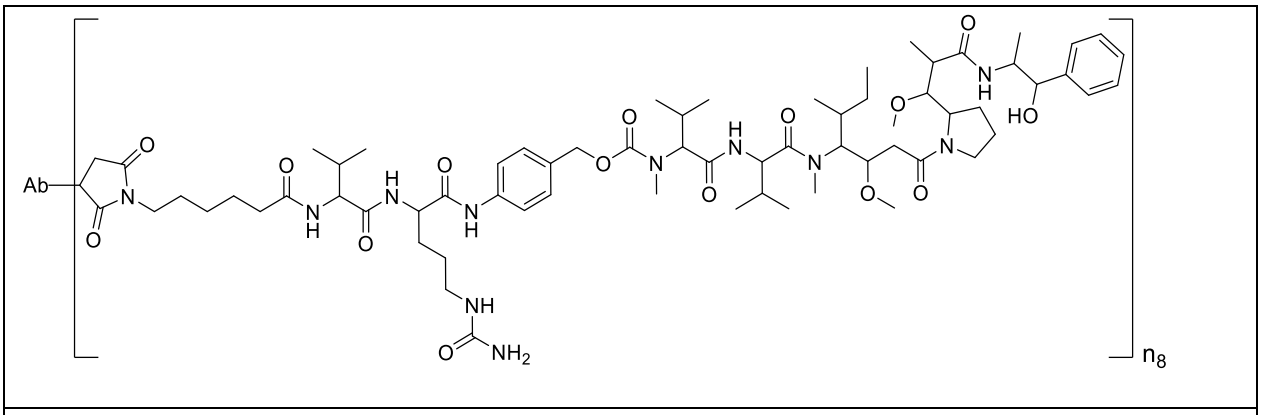
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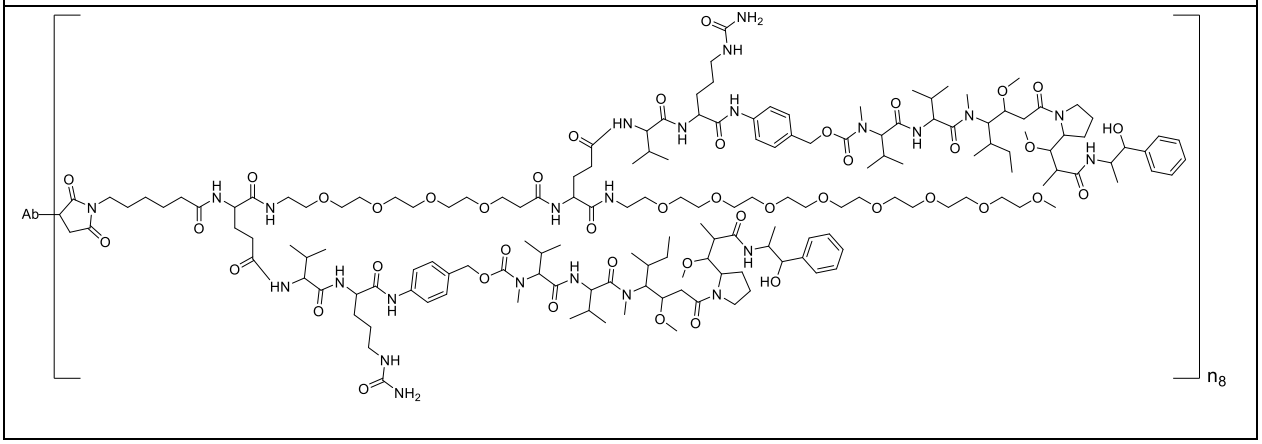
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ADC-15



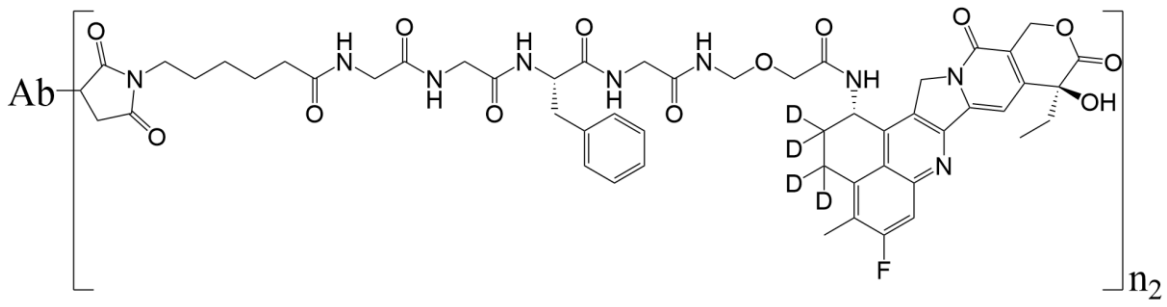
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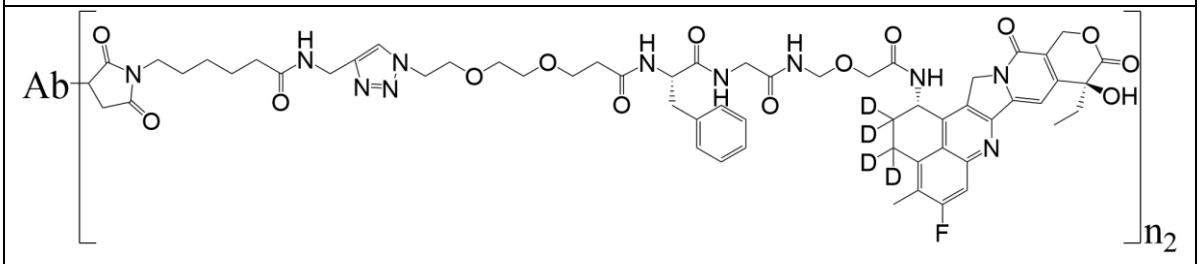
wherein n_8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_8 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and the antibody is linked to the linker-payload moiety via disulfide bond formed by cysteine of the antibody.

[Claim 25] The antibody drug conjugate according to claim 7, wherein the antibody drug conjugate has a structure selected from the group consisting of:

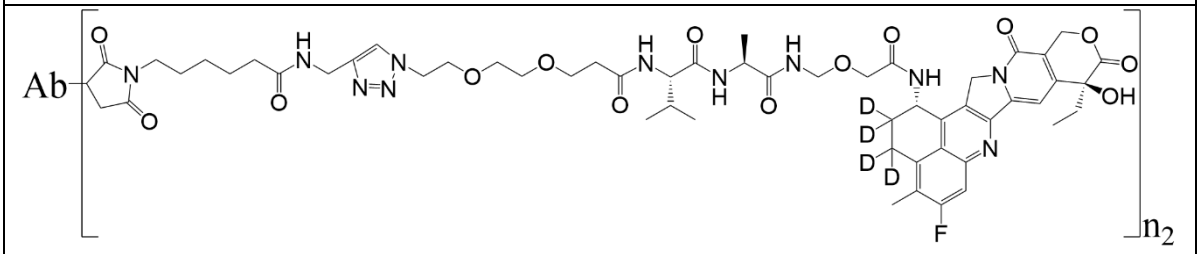
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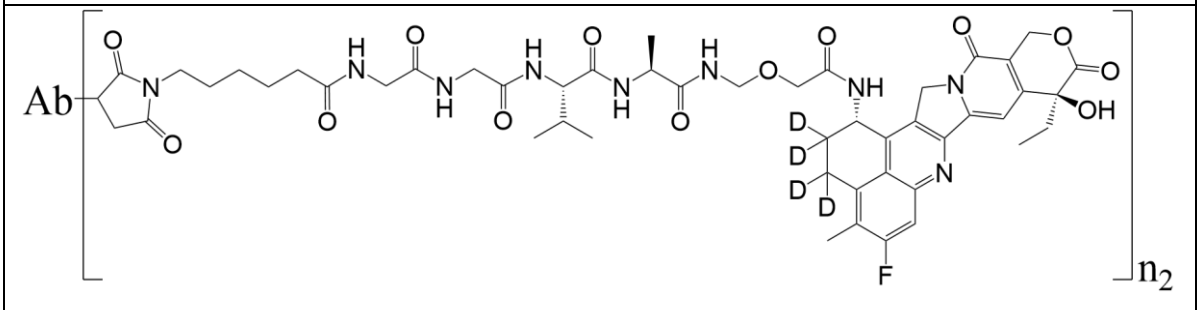
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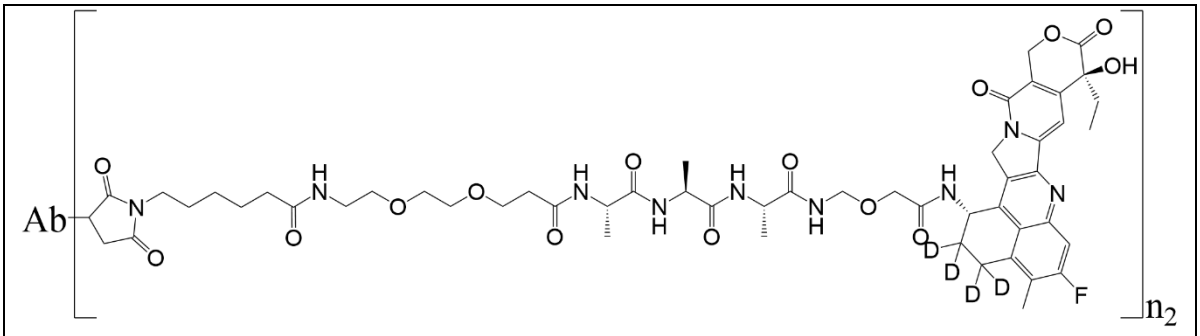
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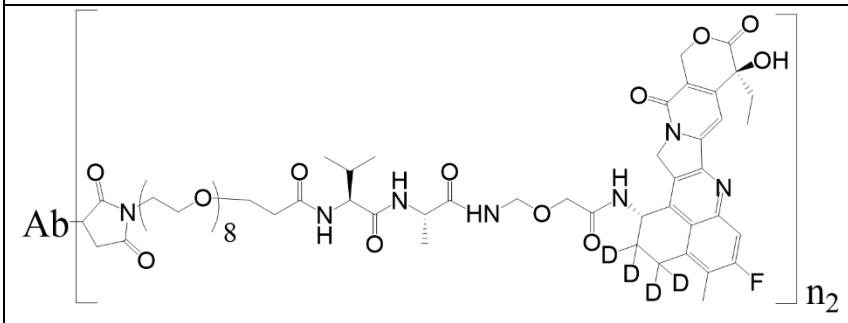
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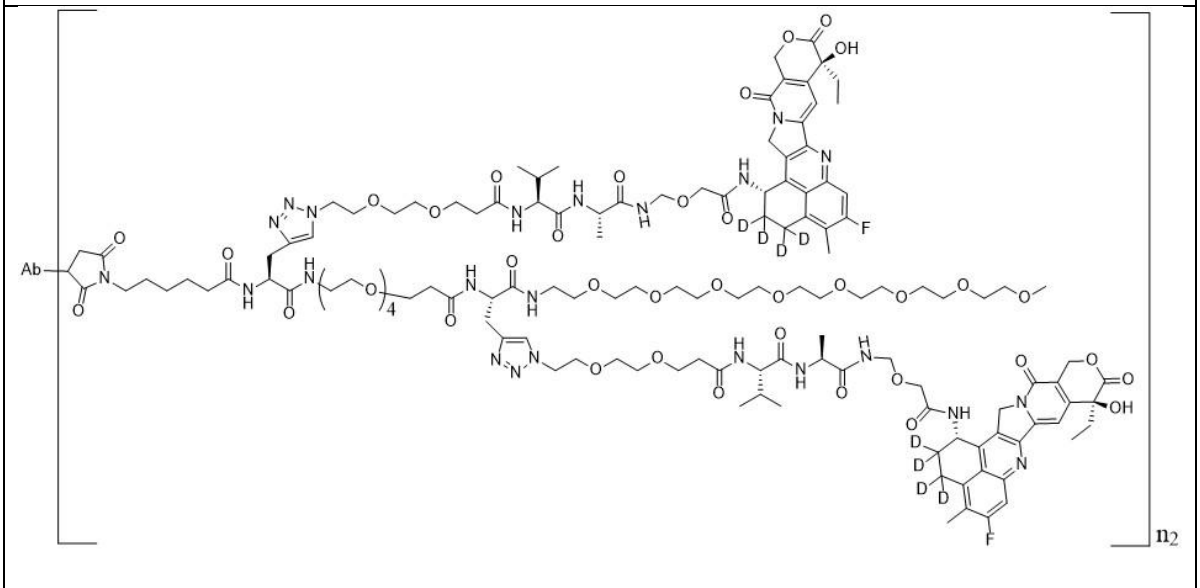
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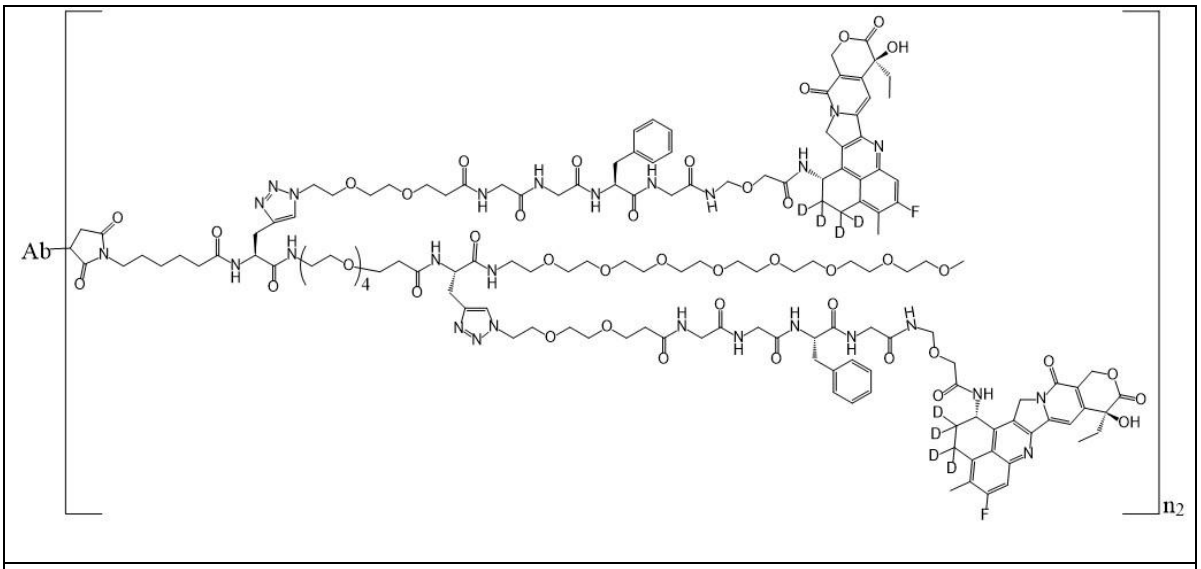
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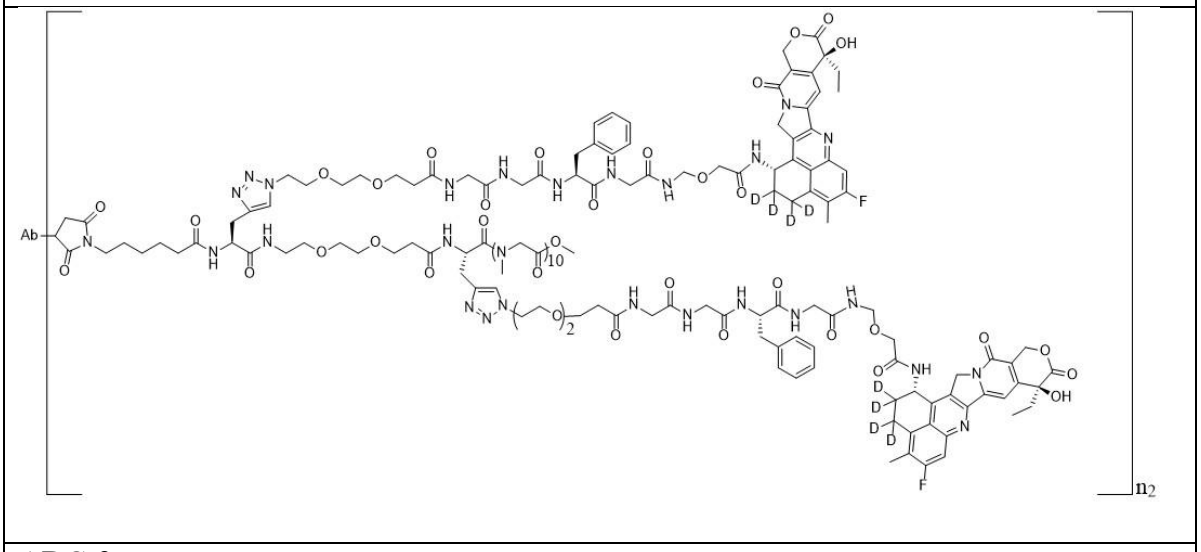
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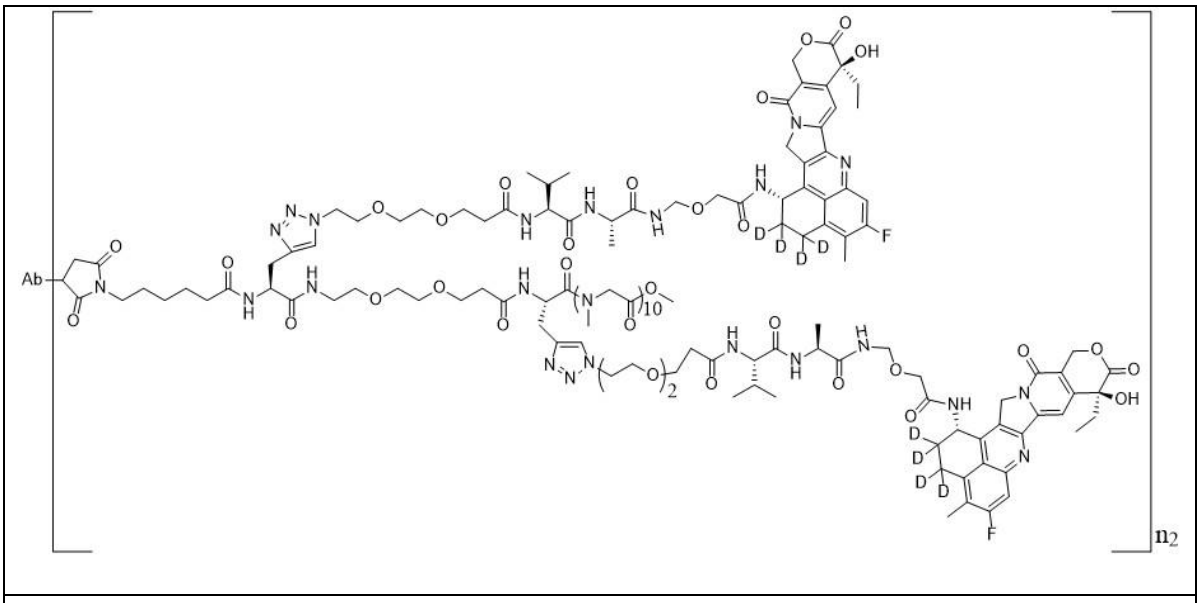
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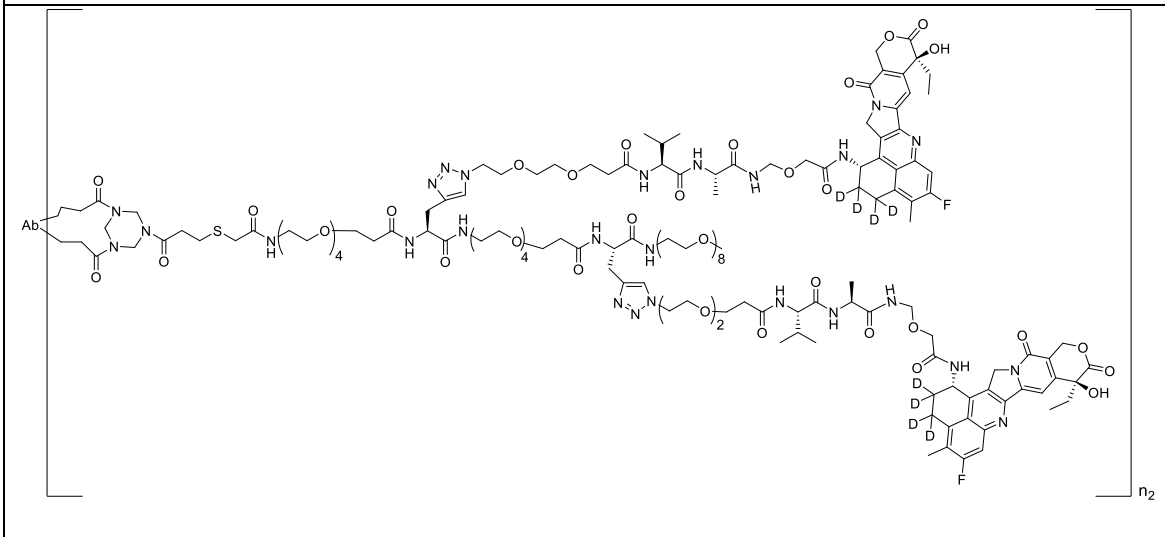
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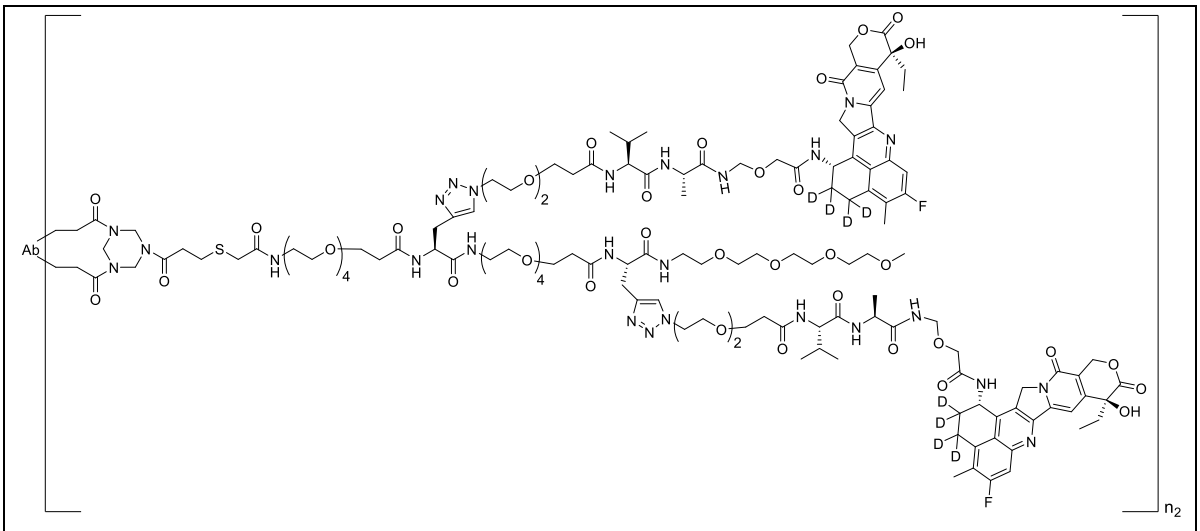
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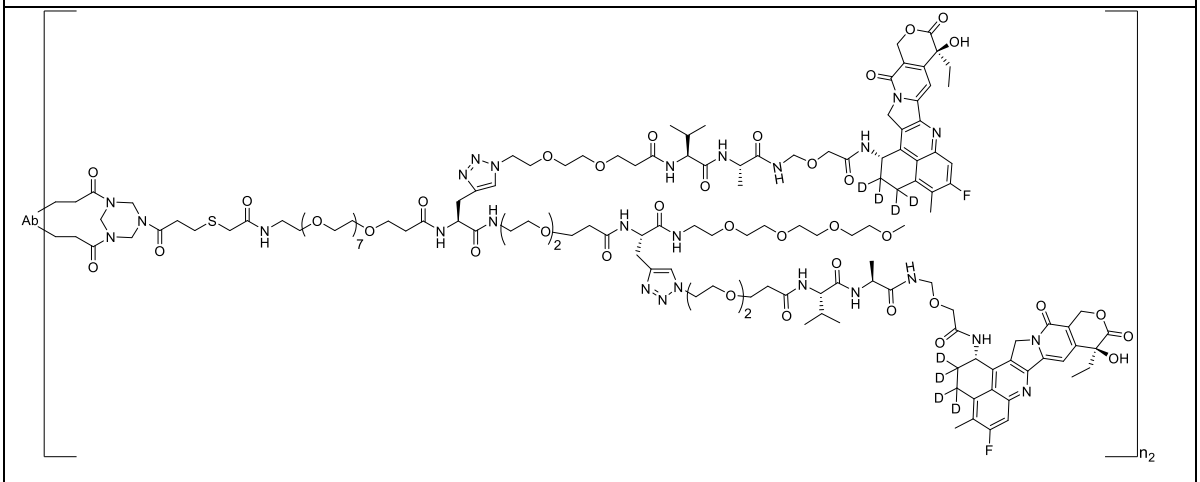
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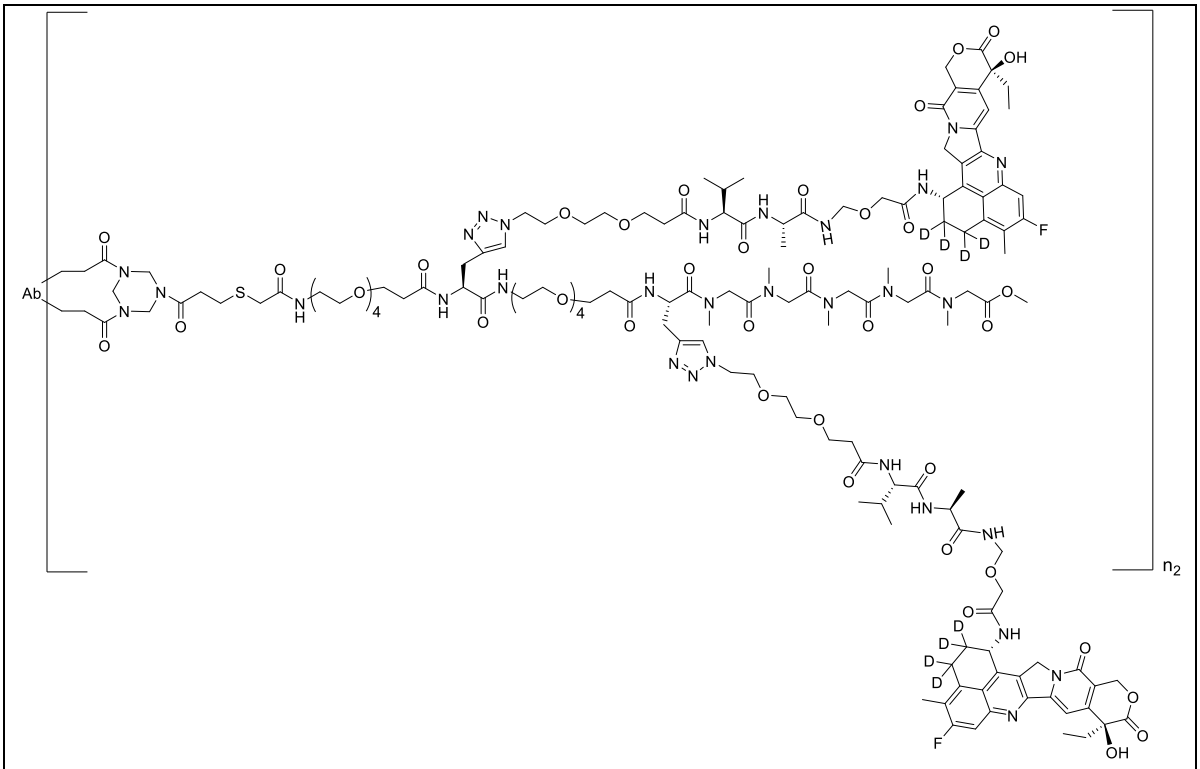
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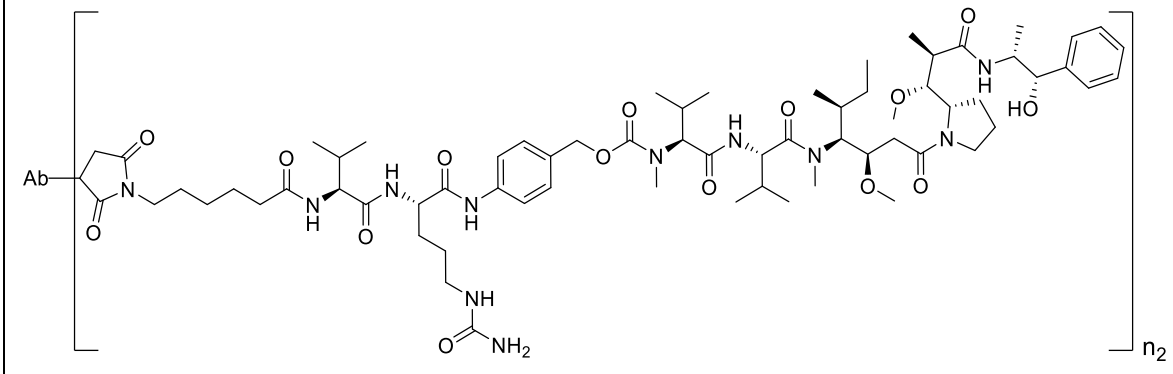
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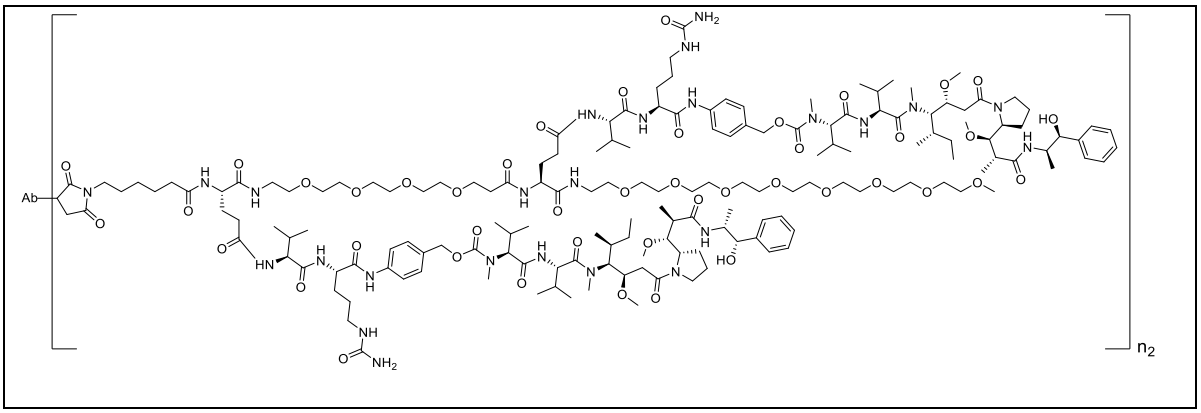
ADC-13a



ADC-15a



ADC-16a



wherein n_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_2 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7 or 8, and the antibody is linked to the linker-payload moiety via disulfide bond formed by cysteine of the antibody.

[Claim 26] A method for treating a tumor, comprising administering an effective amount of the antibody drug conjugate according to any one of claims 1 to 25 to a subject in need thereof.

[Claim 27] The method according to claim 26, wherein the tumor is CDCP-1 positive.

[Claim 28] The method according to claim 27, wherein the tumor is lung cancer or pancreatic cancer; further preferably the tumor is lung squamous cell carcinoma, lung adenocarcinoma, non-small cell lung cancer or pancreatic adenocarcinoma.

[Claim 29] An antibody or an antigen binding fragment thereof targeting CDCP1, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(1) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 19, CDR-H2 set forth in SEQ ID NO: 20, and CDR-H3 set forth in SEQ ID NO: 21, and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 22, CDR-L2 set forth in SEQ ID NO: 23, and CDR-L3 set forth in SEQ ID NO: 24; or

(2) the heavy chain variable region (VH) comprises 3 CDRs: CDR-H1 set forth in SEQ ID NO: 29, CDR-H2 set forth in SEQ ID NO: 30, and CDR-H3 set forth in SEQ ID NO: 31, and the light chain variable region (VL) comprises 3 CDRs: CDR-L1 set forth in SEQ ID NO: 32, CDR-L2 set forth in SEQ ID NO: 33, and CDR-L3 set forth in SEQ ID NO: 34; and

the CDRs are defined according to IMGT numbering system.

[Claim 30] The antibody or the antigen binding fragment thereof according to claim 29, wherein the antibody or the antigen binding fragment thereof is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a multispecific antibody, Fab fragment, F(ab') fragment, $F(ab')_2$ fragment, Fv fragment, dAb, Fd and single-chain variable fragment (scFv).

[Claim 31] The antibody or the antigen binding fragment thereof according to claim 29 or 30, wherein the antibody or the antigen binding fragment thereof further comprises a constant region of an immunoglobulin, wherein the immunoglobulin is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4.

[Claim 32] The antibody or the antigen binding fragment thereof according to any one of claims 29 to 31, wherein the antibody or the antigen binding fragment thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL are selected from the group consisting of (1) and (2):

(1) the heavy chain variable region comprises a sequence set forth in SEQ ID NO: 25 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 25 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 25, and the light chain variable region comprises a sequence set forth in SEQ ID NO: 26 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 26 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 26;

(2) the heavy chain variable region comprises a sequence set forth in SEQ ID NO: 35 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 35 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 35, and the light chain variable region comprises a sequence set forth in SEQ ID NO: 36 or a sequence comprising the same CDRs 1-3 as SEQ ID NO: 36 and having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 36.

[Claim 33] The antibody or the antigen binding fragment thereof according to claim 32, which comprises a heavy chain and a light chain, wherein

(1) the heavy chain comprises a sequence set forth in SEQ ID NO: 27 or a sequence having

greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 27, and the light chain comprises a sequence set forth in SEQ ID NO: 28 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 28; or

(2) the heavy chain comprises a sequence set forth in SEQ ID NO: 37 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 37, and the light chain comprises a sequence set forth in SEQ ID NO: 38 or a sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 38.

[Claim 34] A fusion protein comprising the antibody or the antigen binding fragment thereof according to any one of claims 29 to 33.

[Claim 35] An isolated polynucleotide encoding the antibody or the antigen-binding fragment thereof according to any one of claims 29 to 33 or the fusion protein according to claim 34.

[Claim 36] A nucleic acid construct comprising the polynucleotide according to claim 35.

[Claim 37] The nucleic acid construct according to claim 36, wherein the nucleic acid construct is an expression vector, wherein the polynucleotide is operably linked to a regulatory sequence allowing expression of peptide encoded by the polypeptide in a host cell or a cell-free expression system.

[Claim 38] A host cell comprising the polynucleotide according to claim 35 or the nucleic acid construct according to claim 36 or 37, wherein the host cell is preferably selected from a group consisting of a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell and an *Escherichia coli* cell, more preferably the host cell is selected from a group consisting of a CHO cell, a NS0 cell, a Sp2/0 cell and a BHK cell.

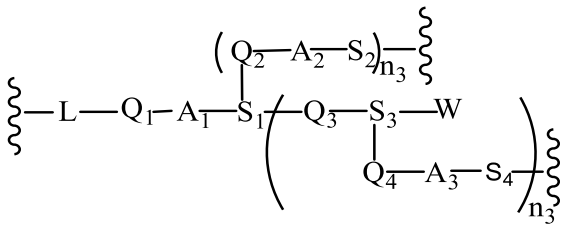
[Claim 39] A method for producing the antibody or the antigen-binding fragment thereof according to any one of claims 29 to 33 or the fusion protein according to claim 34, comprising culturing the host cell according to claim 38 under a condition allowing the nucleic acid construct according to claim 36 to express a protein, and harvesting the protein from a culture.

[Claim 40] Use of the antibody or the antigen binding fragment thereof according to any one of claims 29 to 33, the fusion protein according to claim 34 or the polynucleotide according to

claim 35 in the manufacture of a medicament.

[Claim 41] The use according to claim 40, wherein the medicament includes a monospecific antibody, a bispecific antibody, a multispecific antibody, an antibody drug conjugate or an antibody-radionuclide conjugate.

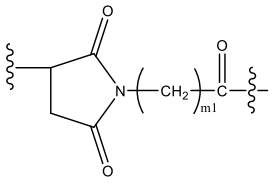
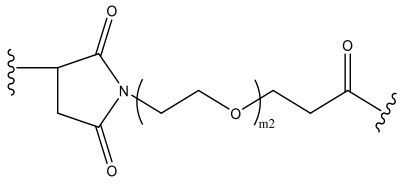
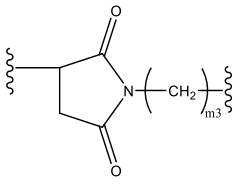
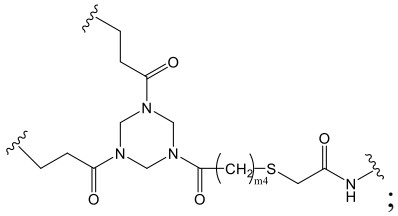
[Claim 42] A linker represented by formula (II):



formula (II),

wherein,

L is a linker linked to an antibody, and has a structure selected from the group consisting of:

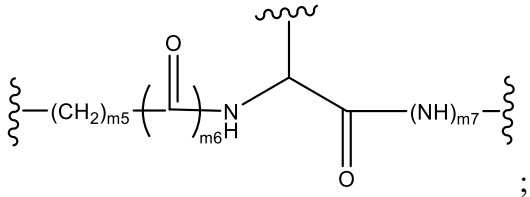

, 
, 
and 
;

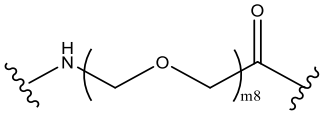
wherein, Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -$(NHCH_2C(O))$p-O-, -NH(CH_2CH_2O)p-$CH_2CH_2C(O)$- , -(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -CH_2-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -$NHCH_2$-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -(CH_2)-Y- and -$NHCH_2C(O)CH_2C(O)$-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{3-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

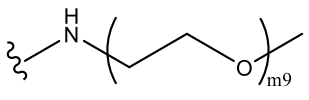
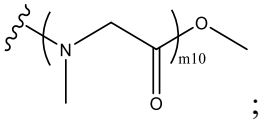
A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

S_1 and S_3 are independently ;

S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and ;

W is selected from the group consisting of  and ;

m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

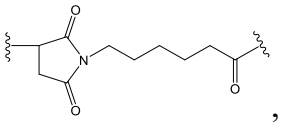
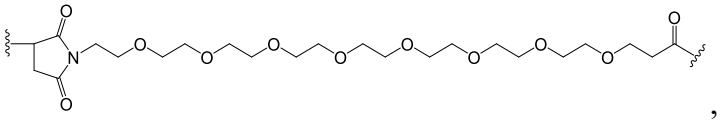
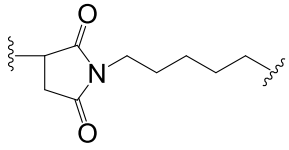
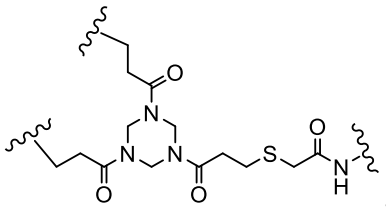
m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

n_3 is 0 or 1.

[Claim 43] The linker according to claim 42, wherein L has a structure selected from the group consisting of:

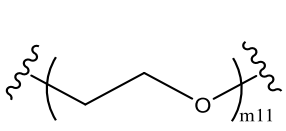

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and

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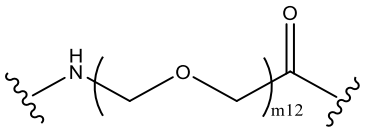
[Claim 44] The linker according to claim 43, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

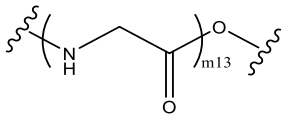
[Claim 45] The linker according to claim 44, wherein A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

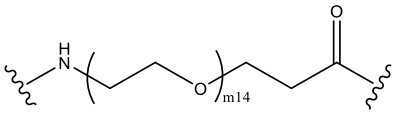
[Claim 46] The linker according to claim 45, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a structure selected from the group consisting of:, -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably the linker comprises a structure of -Val-Ala-.

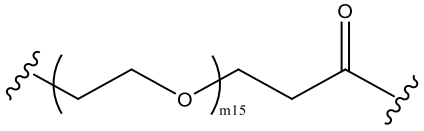
[Claim 47] The linker according to any one of claims 42 to 46, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

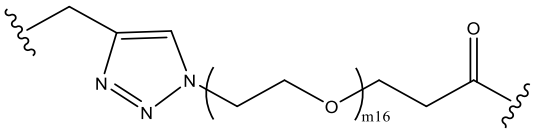


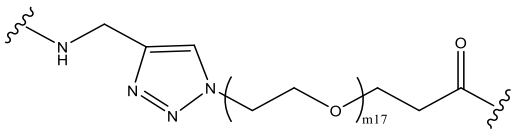
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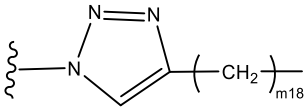
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and


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wherein,

m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m14 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

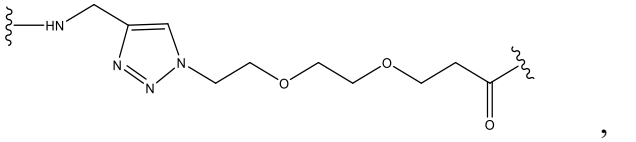
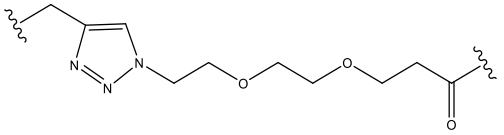
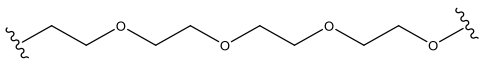
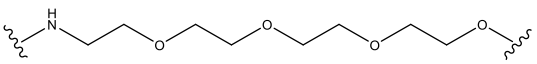
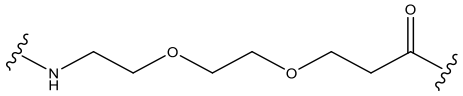
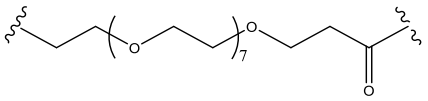
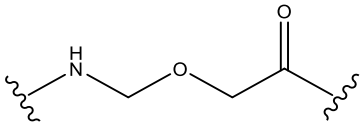
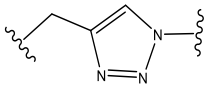
m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

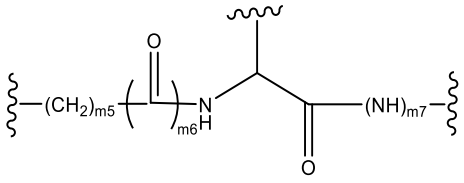
m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

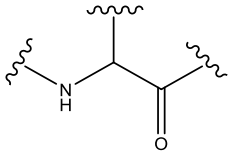
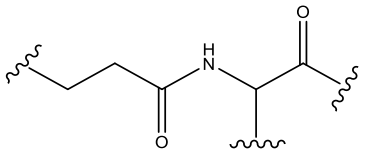
m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

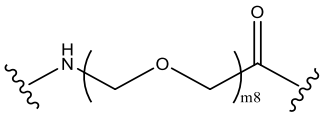
m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[Claim 48] The linker according to claim 47, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

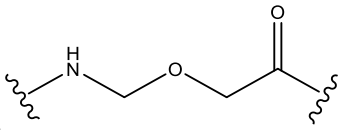

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and

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[Claim 49] The linker according to any one of claims 42 to 48, wherein S_1 and S_3 independently have a structure of

; wherein m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

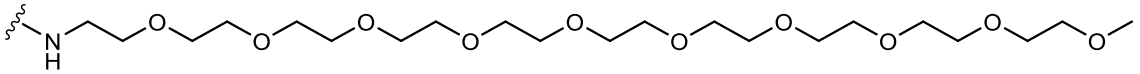
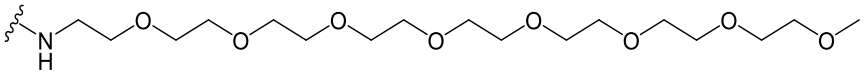
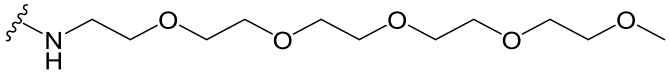
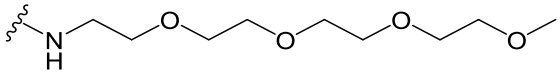
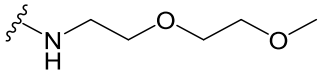
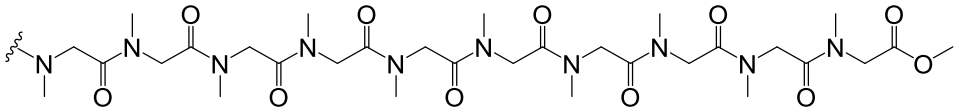
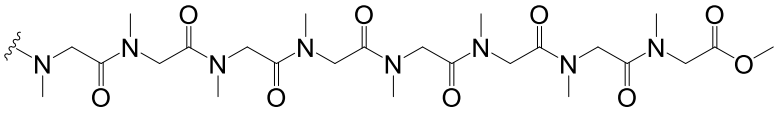
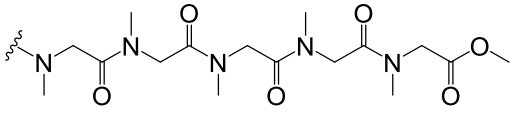
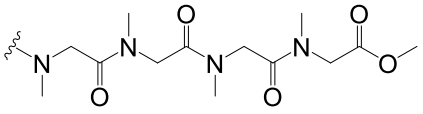
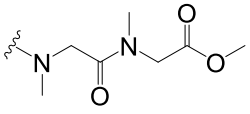
[Claim 50] The linker according to claim 49, wherein S_1 and S_3 independently have a structure selected from the group consisting of

and

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[Claim 51] The linker according to any one of claims 42 to 50, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and

; wherein m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

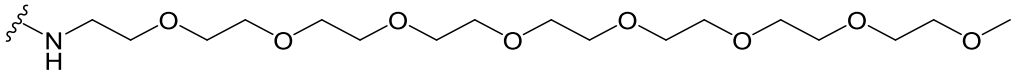
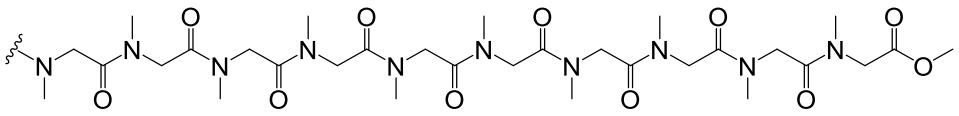
[Claim 52]The linker according to claim 51, wherein S_2 and S_4 independently have a structure

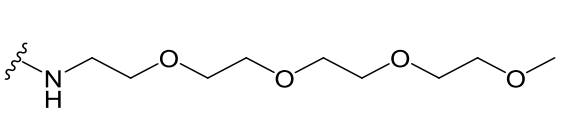
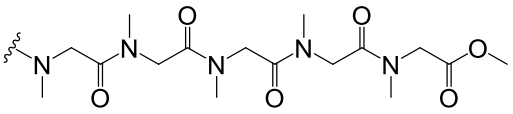
selected from the group consisting of -NH-CH_2- and  .

[Claim 53] The linker according to any one of claims 42 to 52, wherein W has a structure selected from the group consisting of

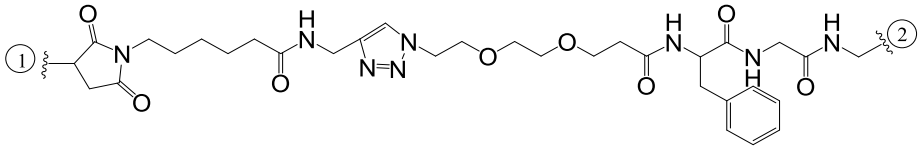
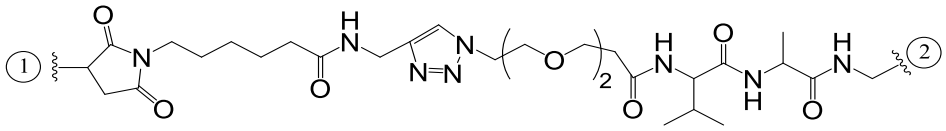
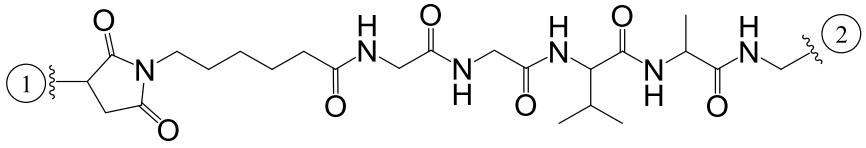
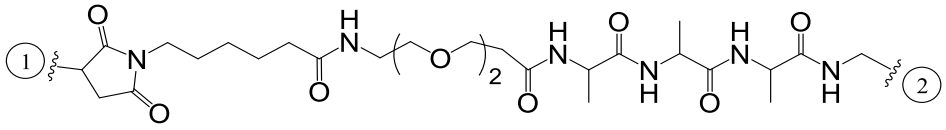
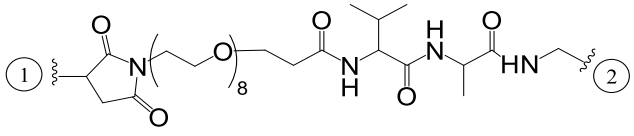
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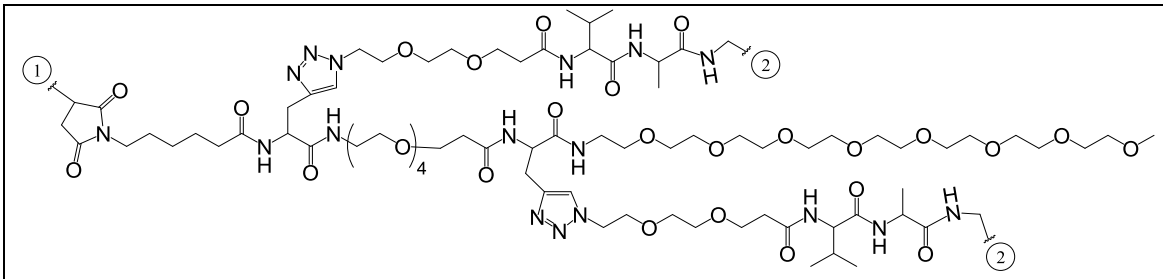
[Claim 54] The linker according to claim 53, wherein W has a structure selected from the group consisting of

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,

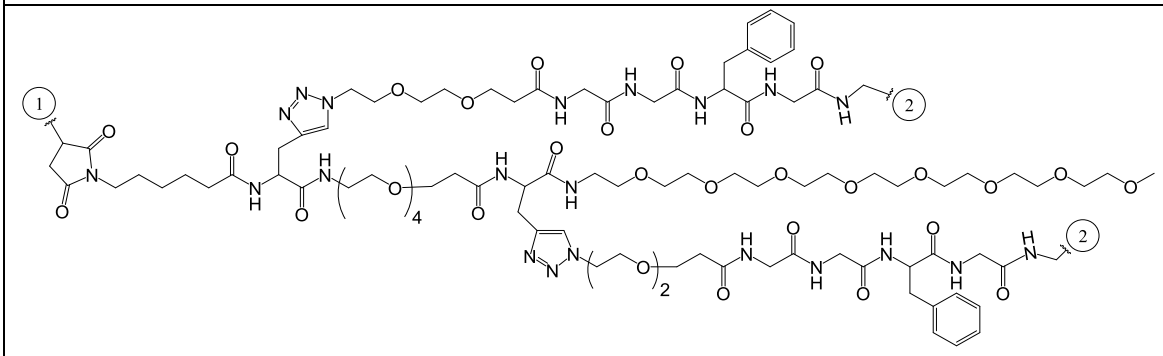
 and .

[Claim 55] The linker according to any one of claims 42 to 54, wherein the linker has a structure selected from the group consisting of:

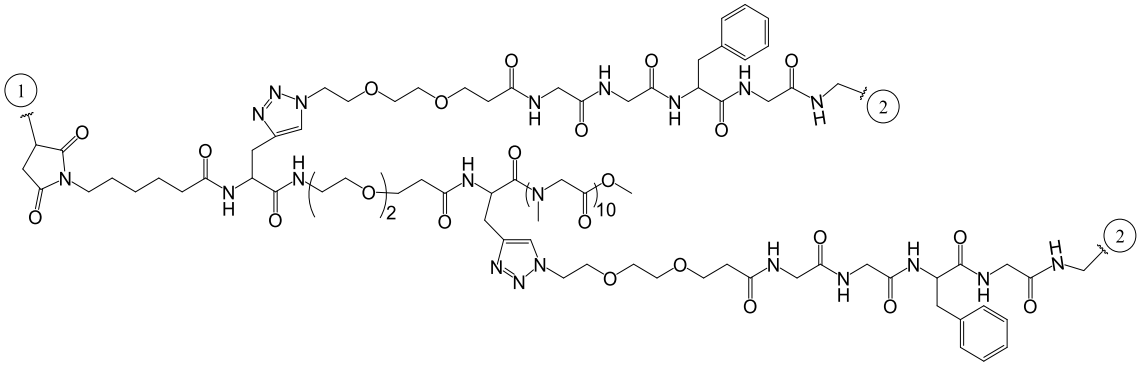
DL-02

DL-06

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DL-07

DL-14

DL-04



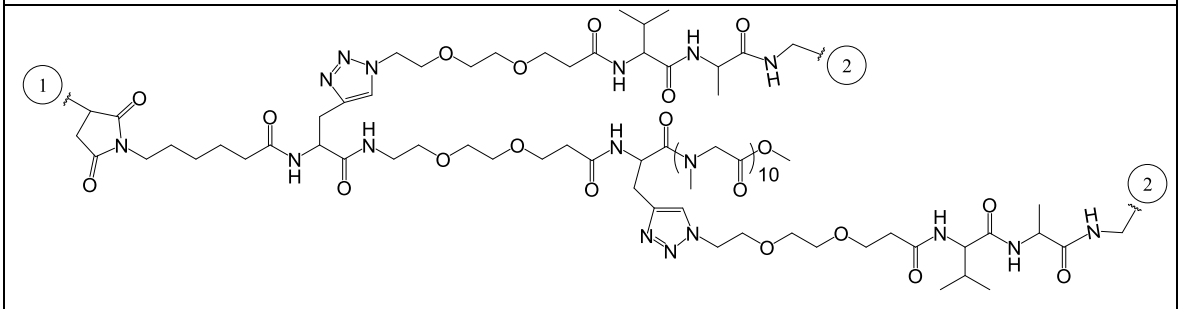
DL-03



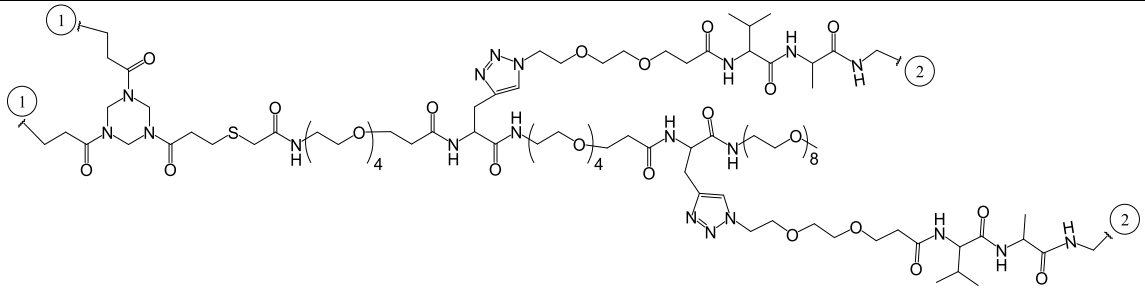
DL-08



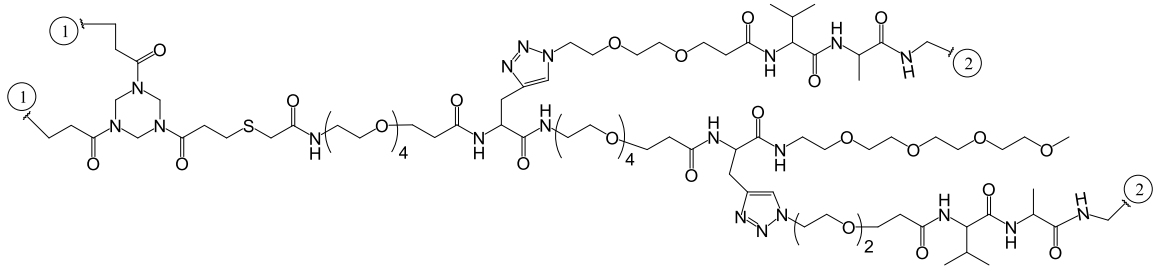
DL-09



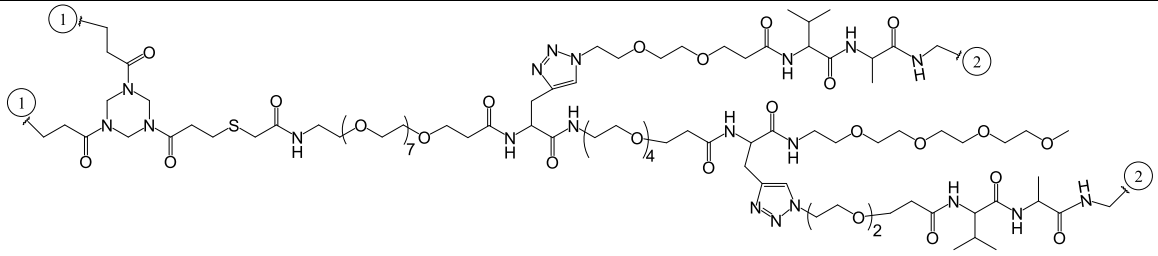
DL-10



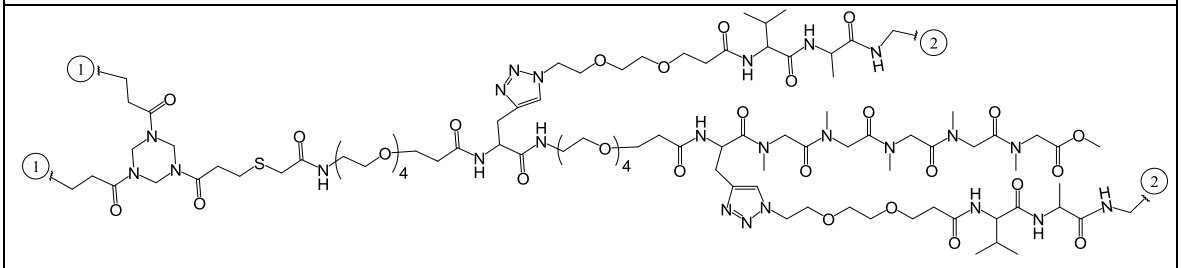
DL-11



DL-12



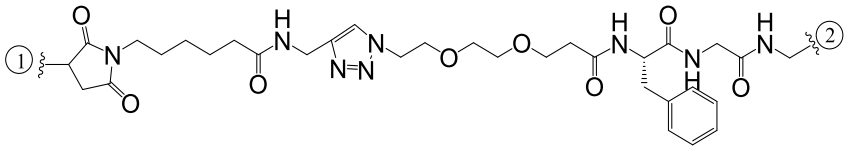
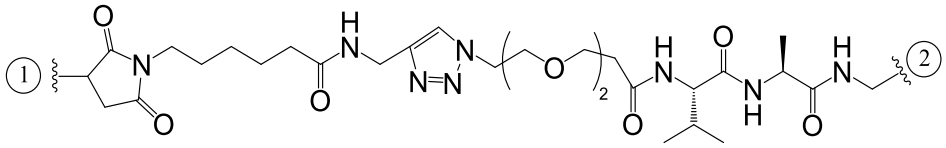
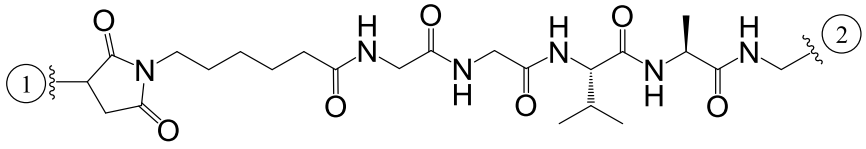
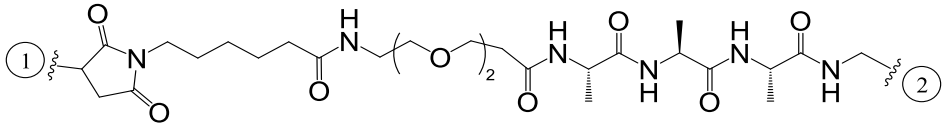
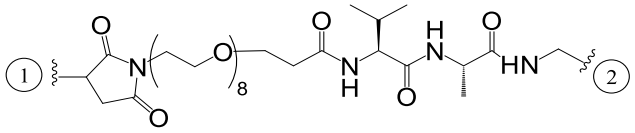
DL-13

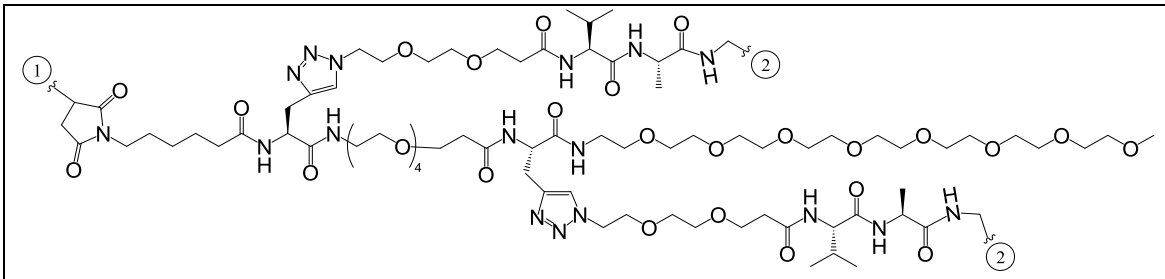


wherein, "① ~~~" is linked to an antibody; and "② ~~~" is linked to a drug unit.

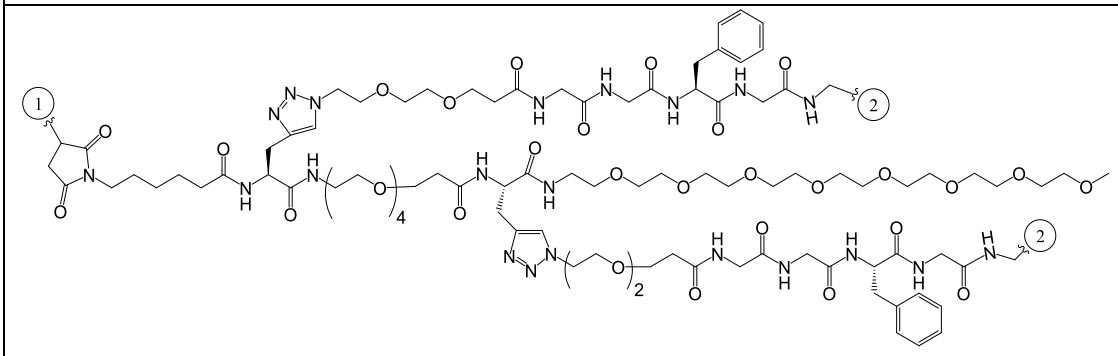
[Claim 56] The linker according to claim 55, wherein the linker has a structure selected from

the group consisting of

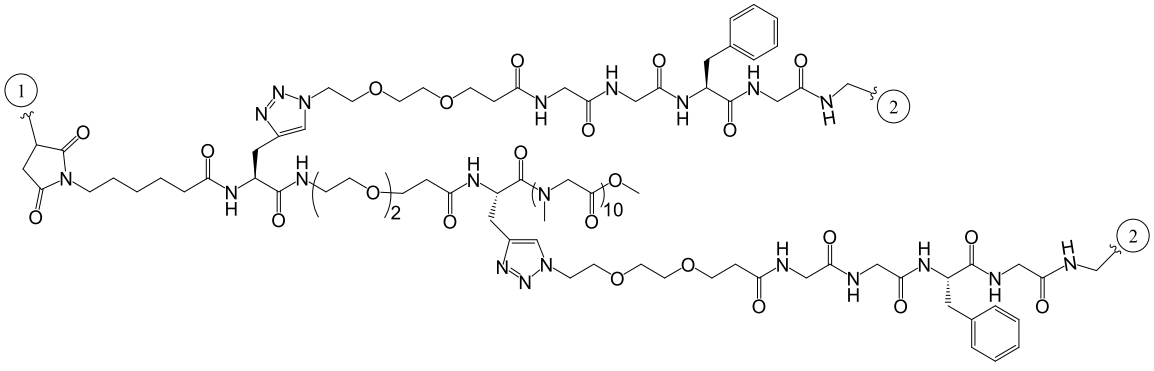
DL-02a

DL-06a

DL-05a

DL-07a

DL-14a

DL-04a



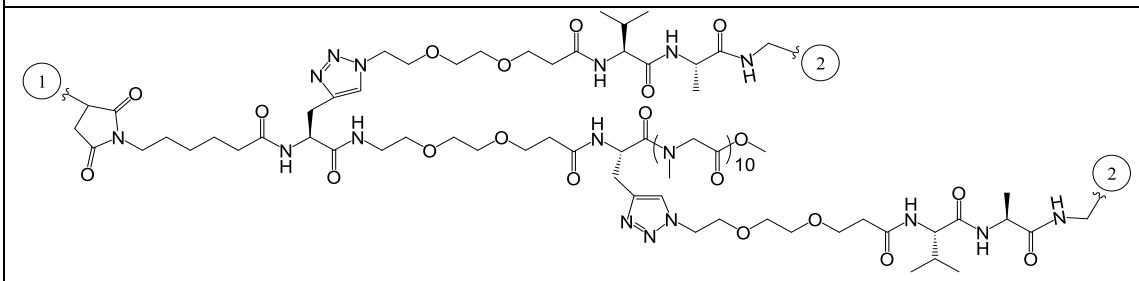
DL-03a

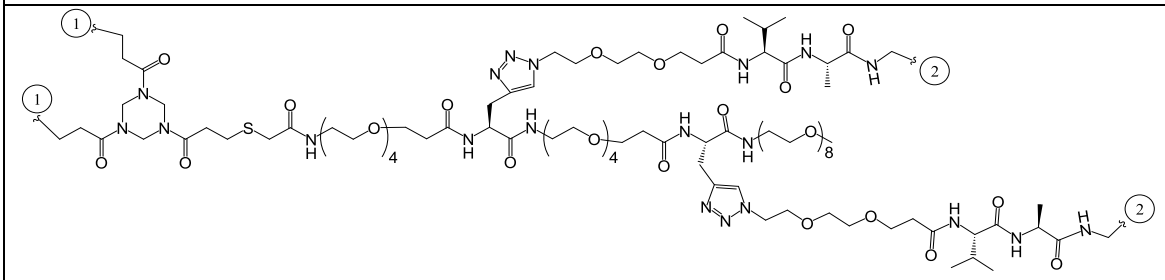
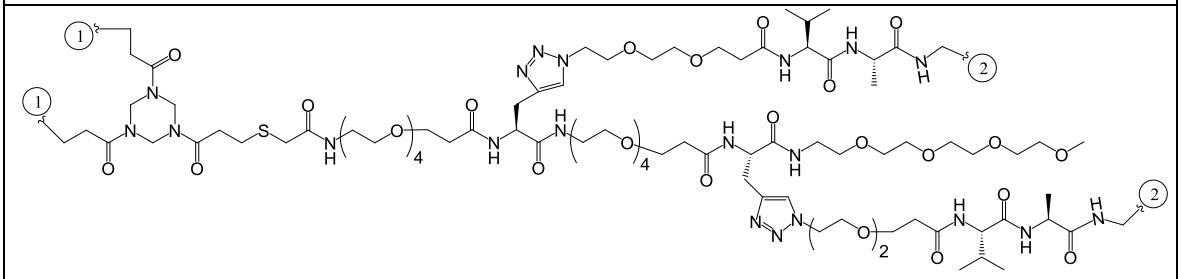
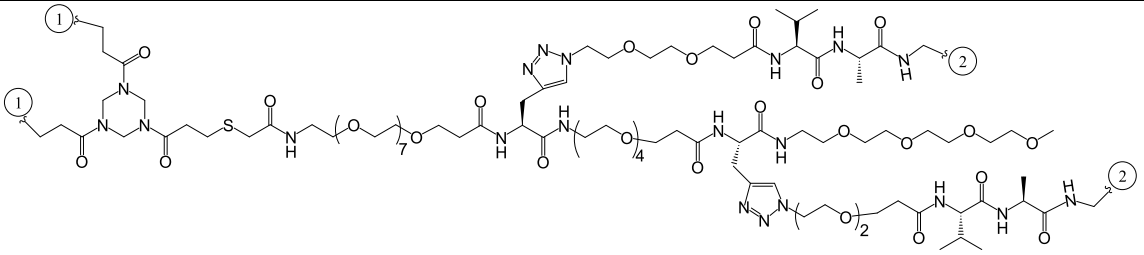
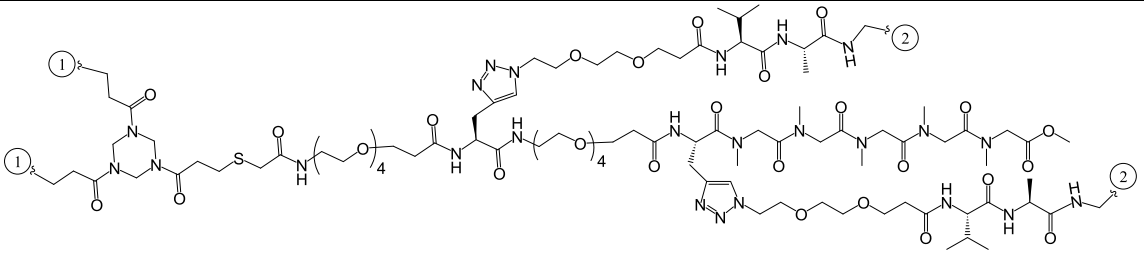


DL-08a



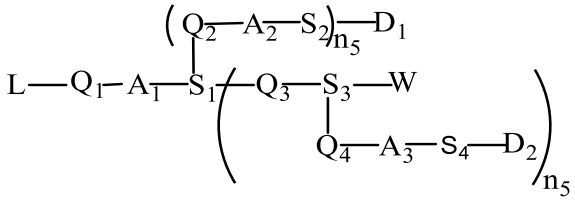
DL-09a



DL-10a

DL-11a

DL-12a

DL-13a


wherein, "" is linked to an antibody; and "" is linked to a payload compound.

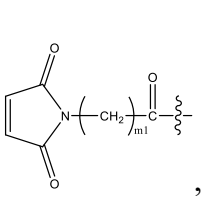
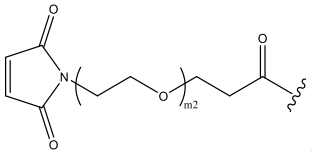
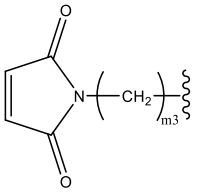
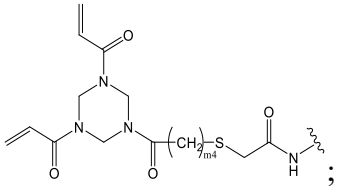
[Claim 57] A linker-payload having a structure represented by formula (III):



formula (III)

wherein,

L is a linker linked to an antibody, and has a structure selected from the group consisting of:

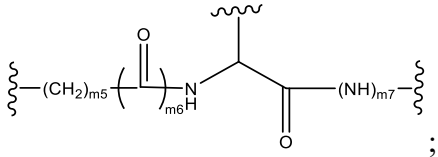
, ,  and ;

wherein, Q_1, Q_2, Q_3 and Q_4 are each independently selected from the group consisting of a single bond, -(CH_2CH_2O)p-, -NH-(CH_2OCH_2)p-C(O)-, -$(NHCH_2C(O))$p-O-, -NH(CH_2CH_2O)p-$CH_2CH_2C(O)$- , -(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -CH_2-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -$NHCH_2$-Y-(CH_2CH_2O)p-$CH_2CH_2C(O)$-, -(CH_2)-Y- and -$NHCH_2C(O)CH_2C(O)$-; wherein Y is selected from the group consisting of C_{3-8} cycloalkyl, C_{3-8} cyclic heteroalkyl and unsaturated C_{3-8} cyclic heteroalkyl; wherein the heteroalkyl contains 1-3 atoms selected from the group consisting of N, O and S, and p is independently selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

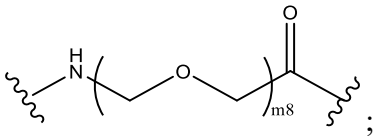
A_1 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

A_2 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

A_3 is a single bond or a peptide consisting of 1, 2, 3, or 4 amino acid residues;

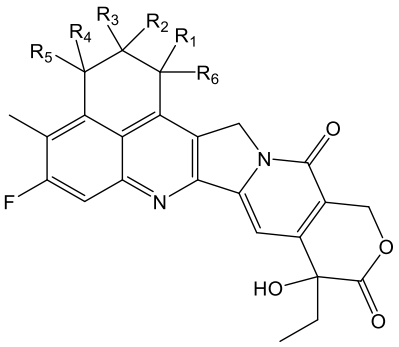
S_1 and S_3 are independently ;

S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and



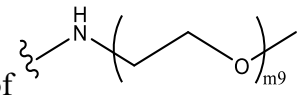
;

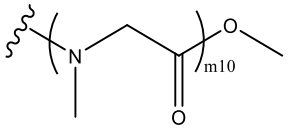
D_1 is a first payload compound, D_2 is a second payload compound, D_1 and D_2 are independently a compound represented by formula (IV), or a stereoisomer, pharmaceutically acceptable salt or solvate thereof:



formula (IV)

wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H), deuterium (D) and tritium (T), at least one, two, three or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_6 is selected from the group consisting of $-NH_2$ and $-NH-C(O)-CH_2-OH$; D_1 and D_2 are independently linked to S_1, S_2 or S_4 via either a hydroxyl or an amine group present;

W is selected from the group consisting of 

and 

;

m1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

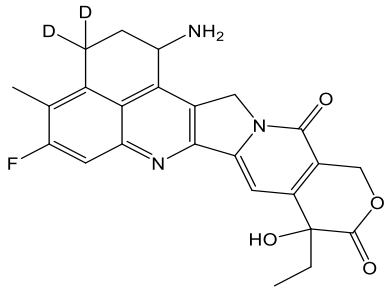
m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

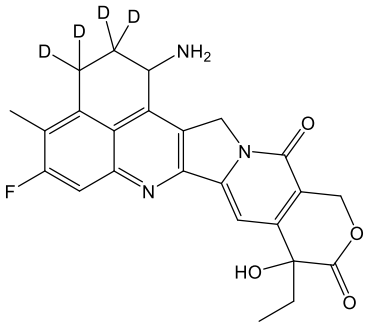
m9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15;

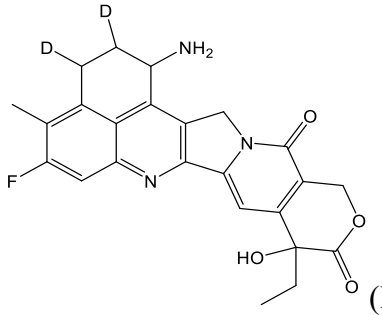
m10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15; and

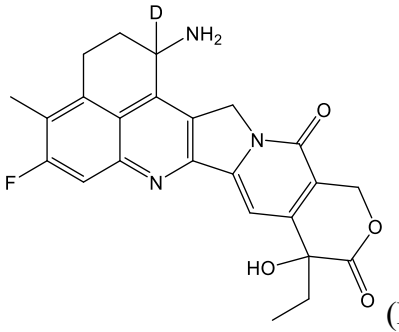
n_5 is 0 or 1.

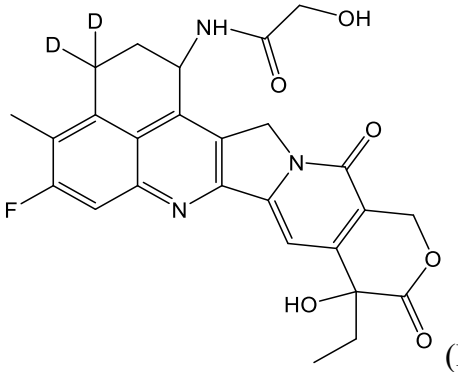
[Claim 58]The linker-payload according to claim 57, wherein the D_1 and D_2 independently have a structure selected from the group consisting of:

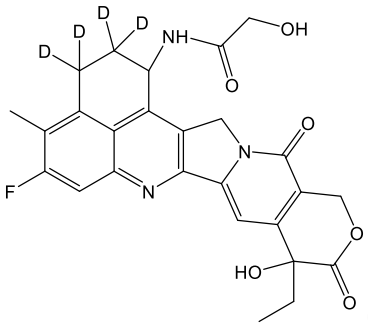

(I-1),

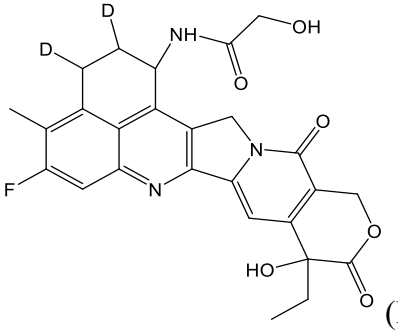

(I-2),

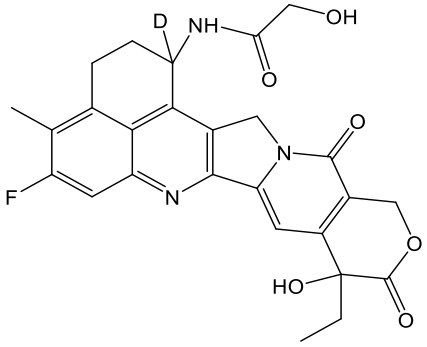

(I-3),


(I-4),

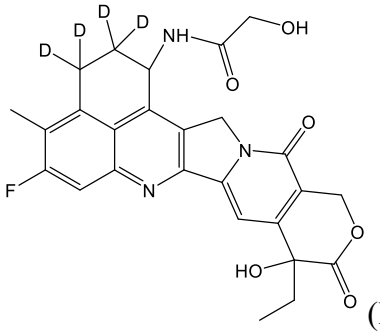

(I-5),

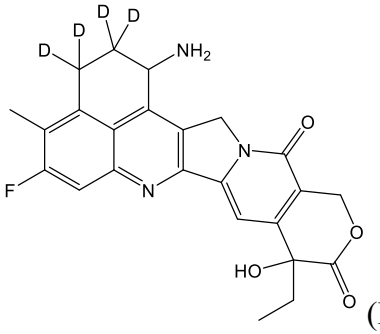

(I-6),

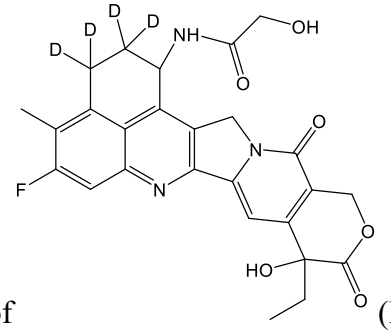

(I-7) and


(I-8);

preferably, the D_1 and D_2 independently have a structure selected from the group consisting of:

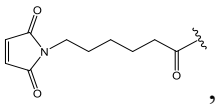
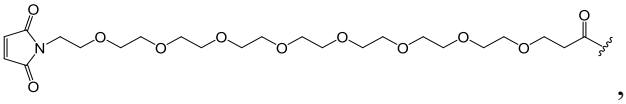
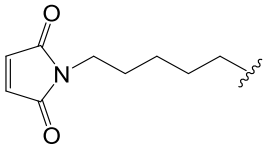
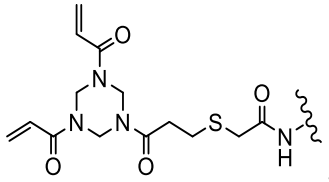

(I-6) and


(I-2);


more preferably, D_1 and D_2 have a structure of (I-6).

[Claim 59]The linker-payload according to claim 57 or 58, wherein -L has a structure selected

from the group consisting of

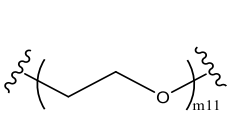
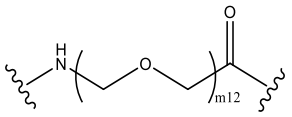
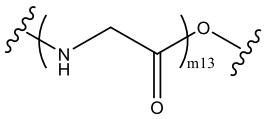
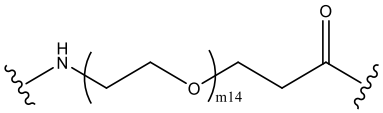
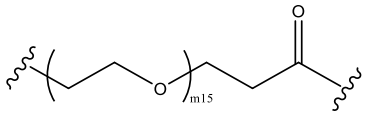
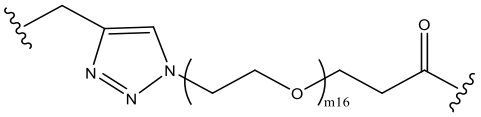
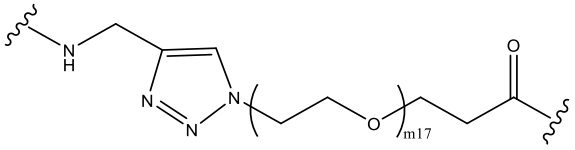
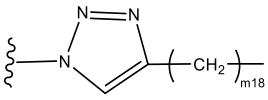

,

,

and

.

[Claim 60]The linker-payload according to claim 59, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[Claim 61]The linker-payload according to claim 60, wherein A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[Claim 62]The linker-payload according to claim 61, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a structure selected from the group consisting of: -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more

preferably, A_1 is a single bond, and A_2 and A_3 are independently -Val-Ala-.

[Claim 63] The linker-payload according to any one of claims 57 to 62, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond, , , , , , , , and ,

wherein,

m11 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m12 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m13 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

m14 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

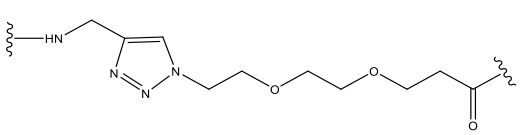
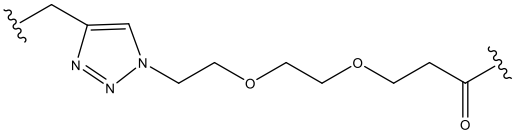
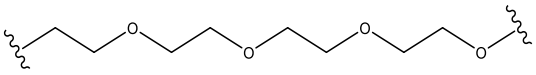
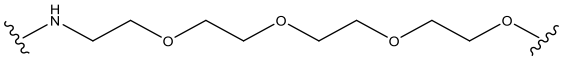
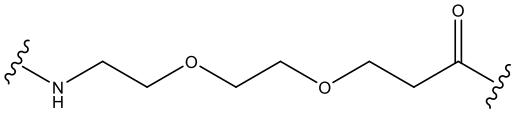
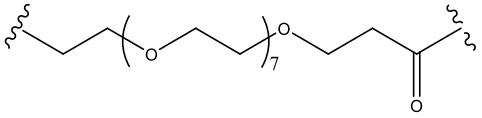
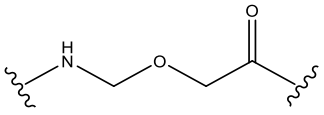
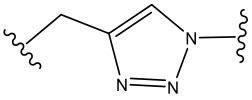
m15 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

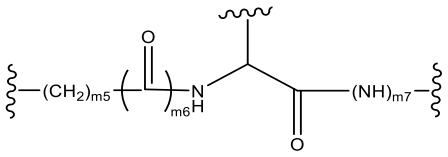
m16 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

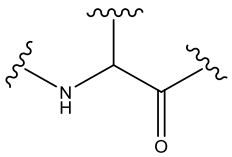
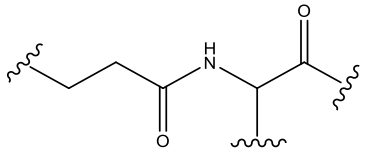
m17 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

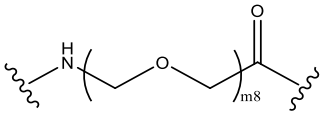
m18 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[Claim 64]The linker-payload according to claim 63, wherein Q_1, Q_2, Q_3 and Q_4 as a linking group independently have a structure selected from the group consisting of a single bond,

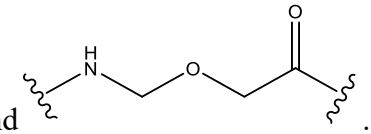

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and

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[Claim 65] The linker-payload according to any one of claims 57 to 64, wherein S_1 and S_3 independently have a structure of ; wherein m5 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; m6 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and m7 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

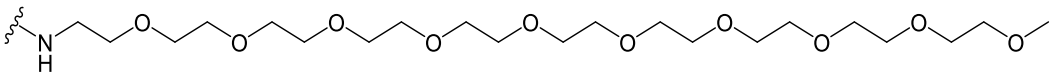
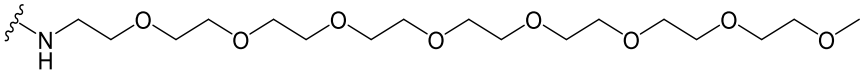
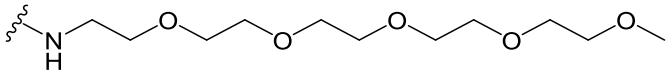
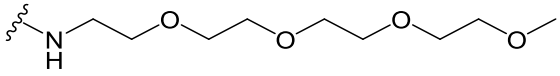
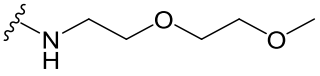
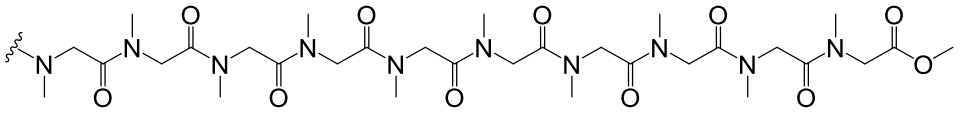
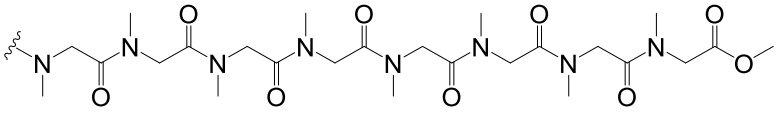
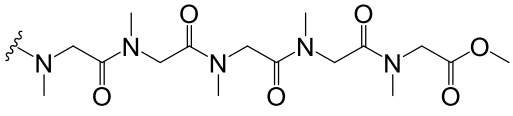
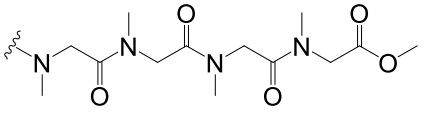
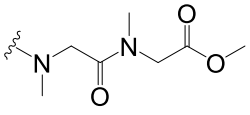
[Claim 66] The linker-payload according to claim 65, wherein S_1 and S_3 independently have a structure selected from the group consisting of  and .

[Claim 67] The linker-payload according to any one of claims 57 to 66, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and ; wherein m8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8,

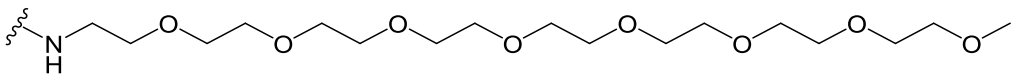
9 and 10.

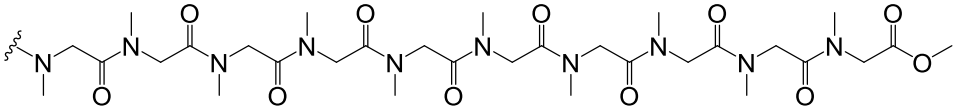
[Claim 68]The linker-payload according to claim 67, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and .

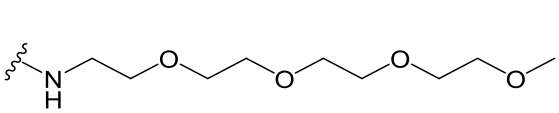
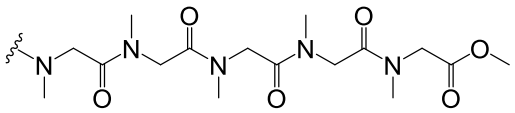
[Claim 69]The linker-payload according to any one of claims 57 to 68, wherein W has a structure selected from the group consisting of

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  and
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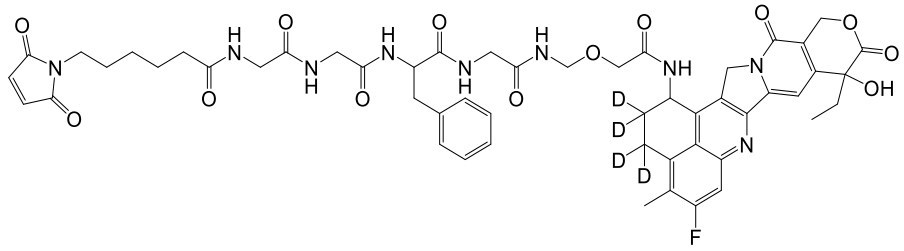
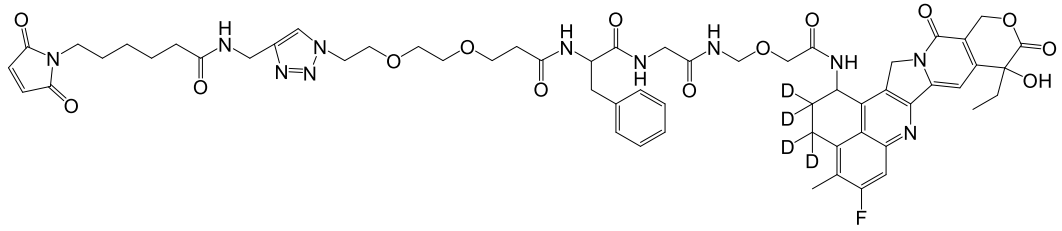
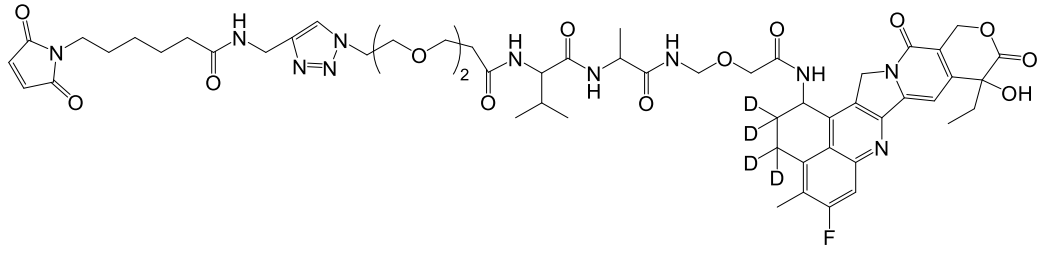
[Claim 70]The linker-payload according to claim 69, wherein W has a structure selected from the group consisting of

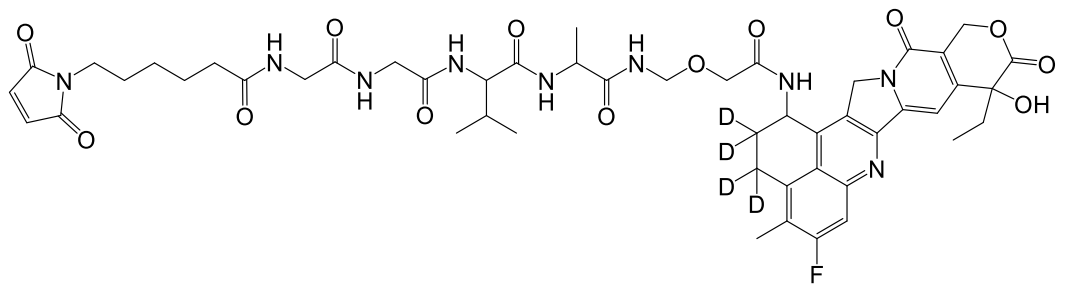
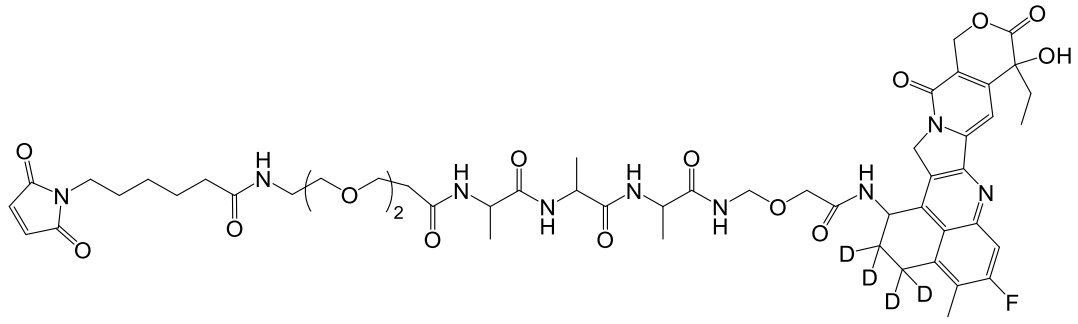
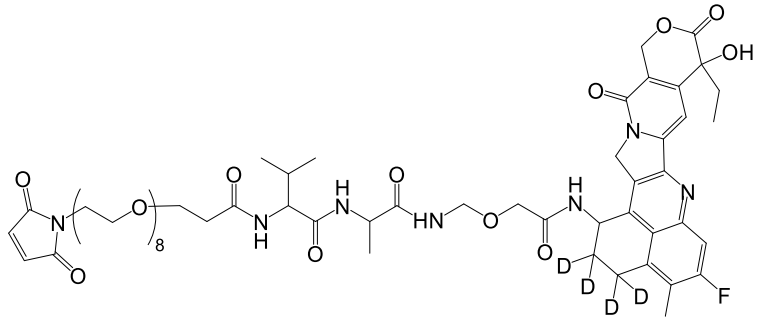
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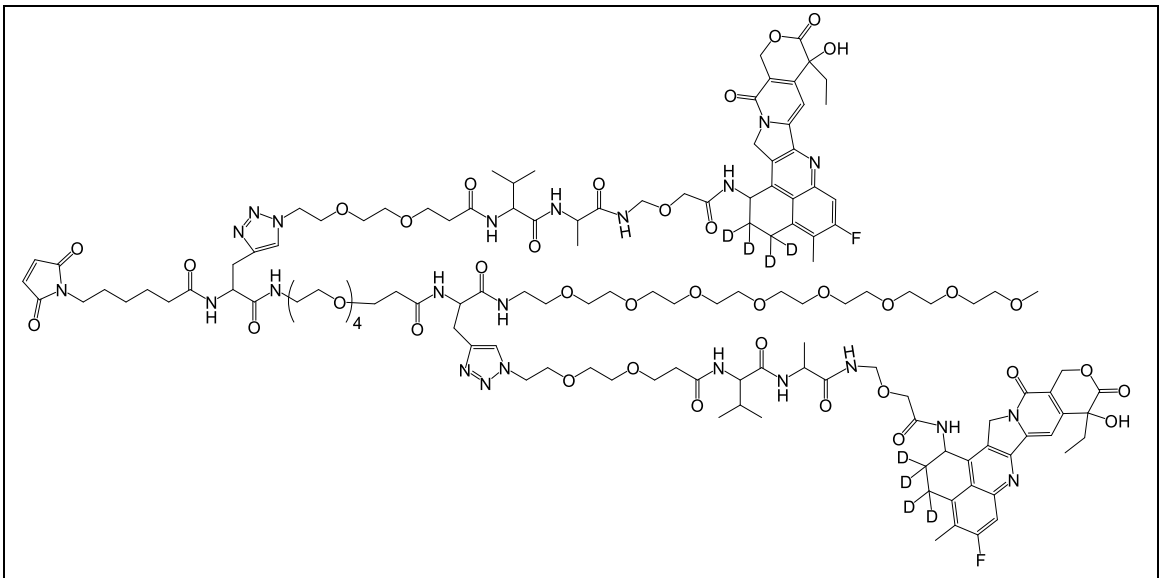

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and 
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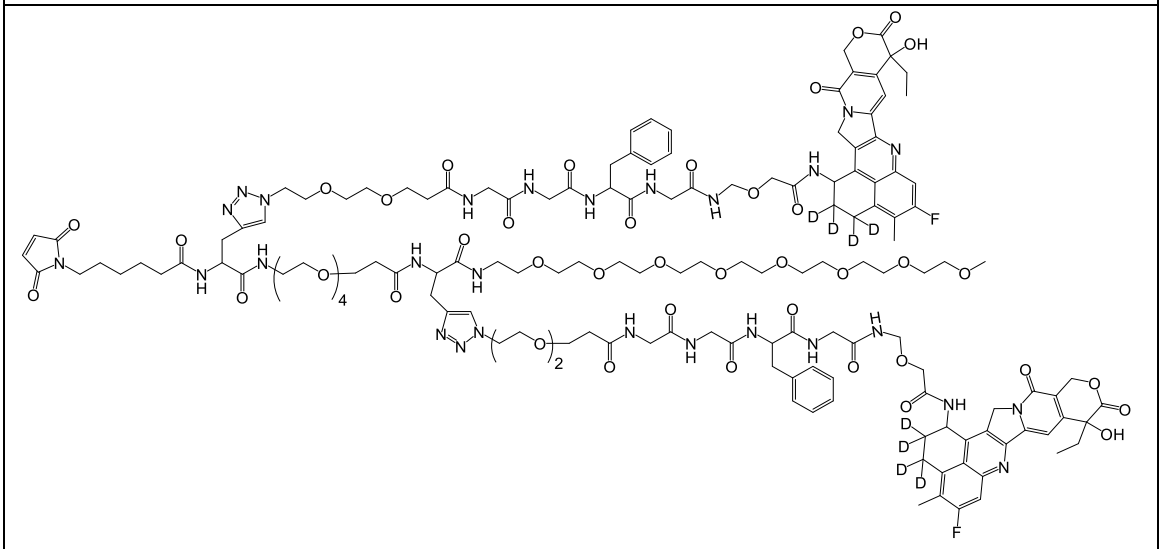
[Claim 71] The linker-payload according to any one of claims 57 to 70, wherein the linker-payload has a structure selected from the group consisting of:

DL01-dDxd

DL02-dDxd

DL06-dDxd

DL05-dDxd

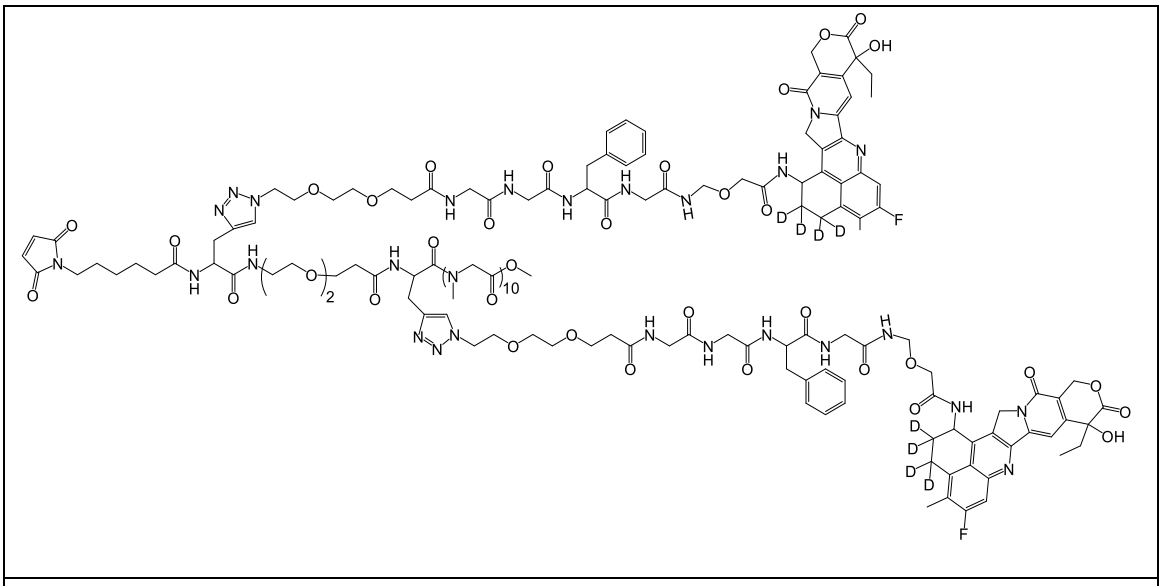

DL07-dDxd

DL14-dDxd

DL04-dDxd



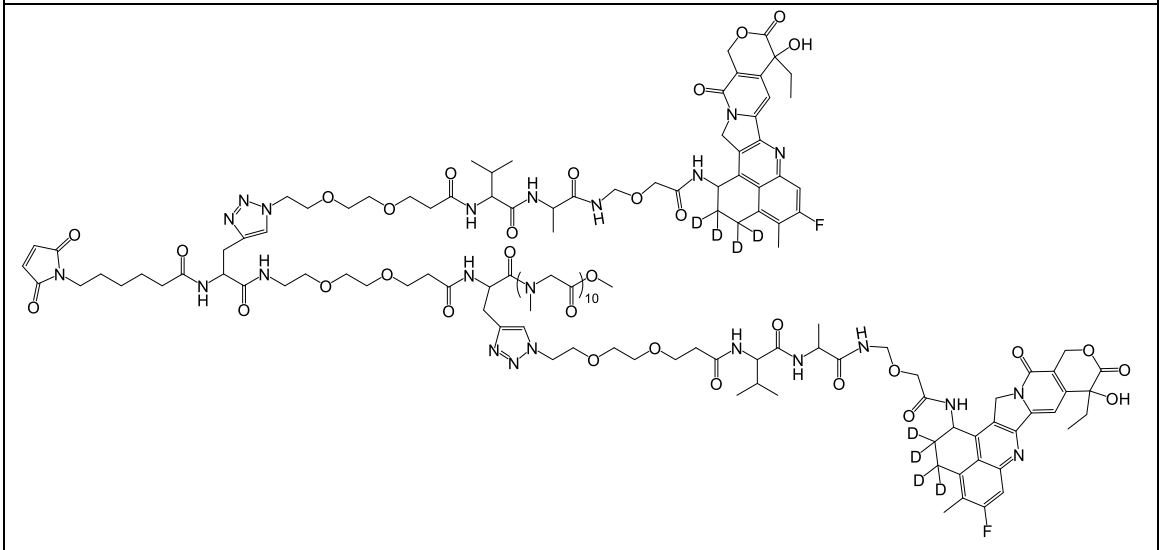
DL03-dDxd



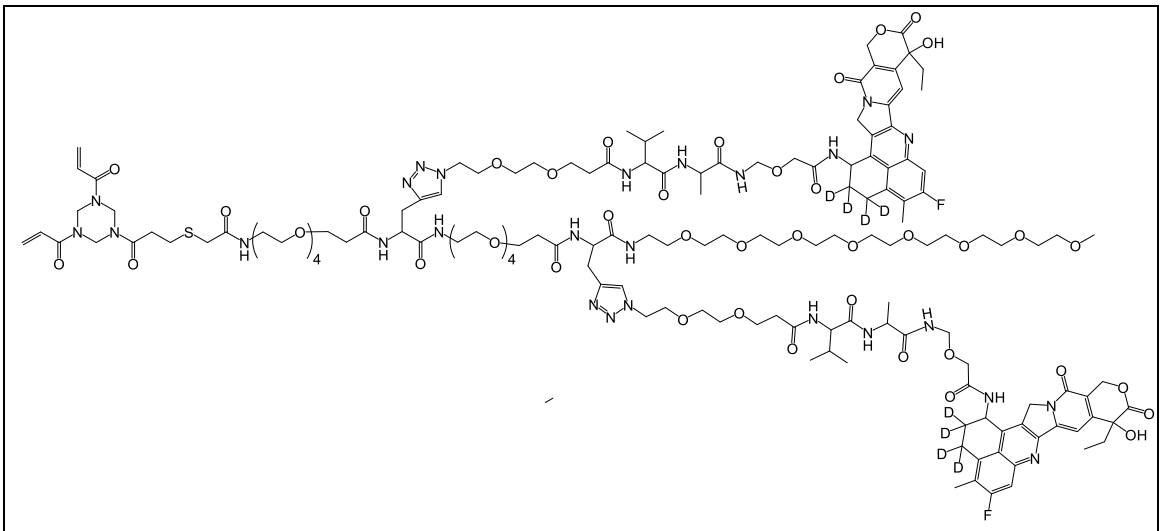
DL08-dDxd



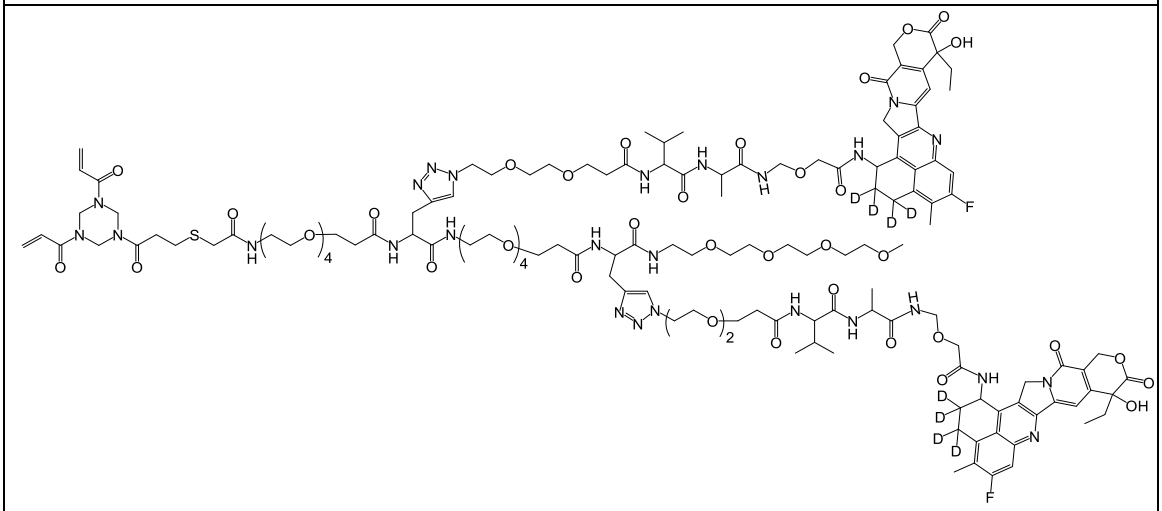
DL09-dDxd



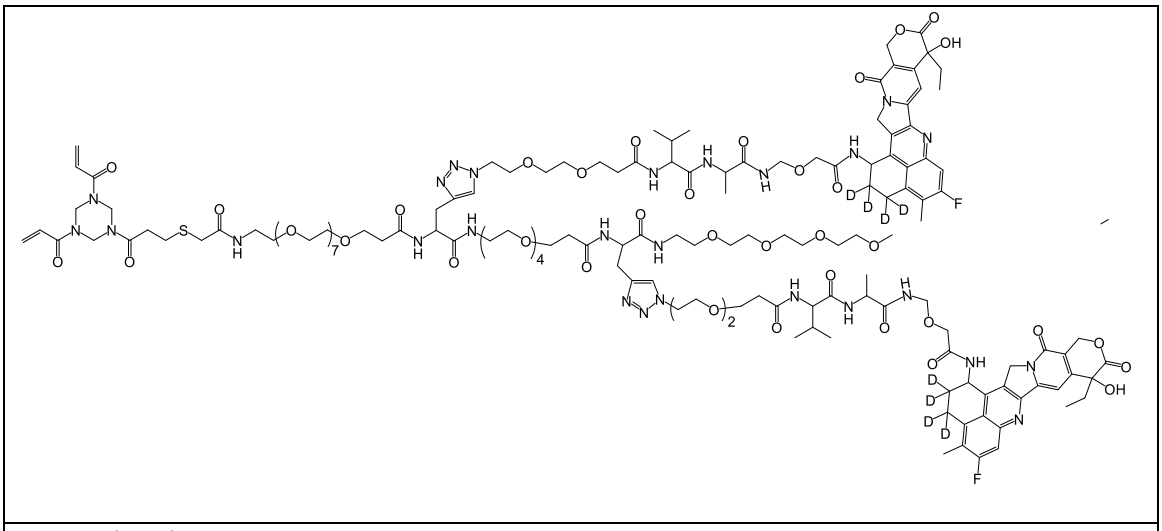
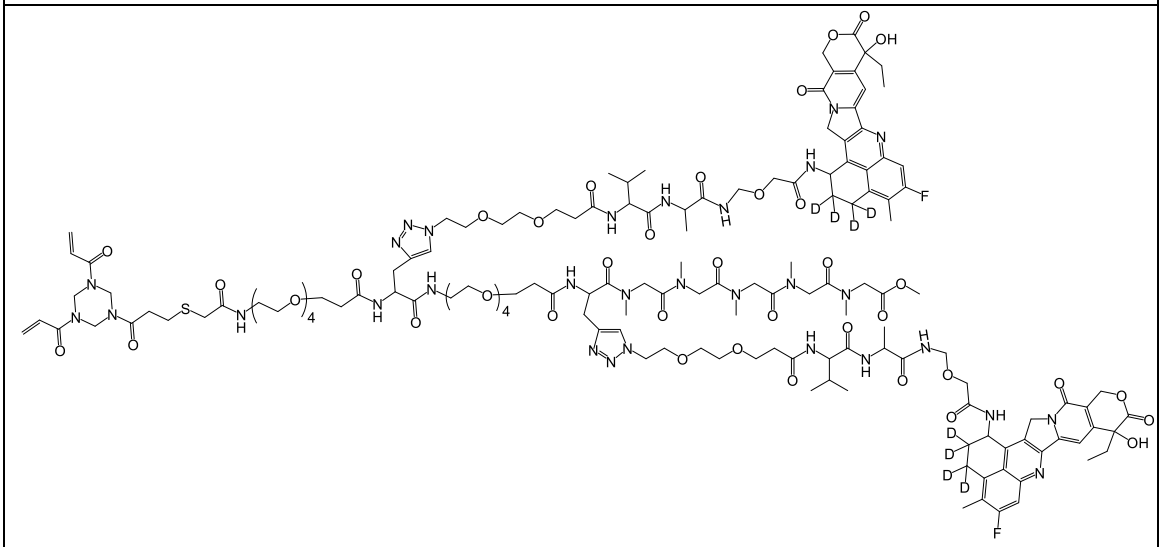
DL10-dDxd



DL11-dDxd

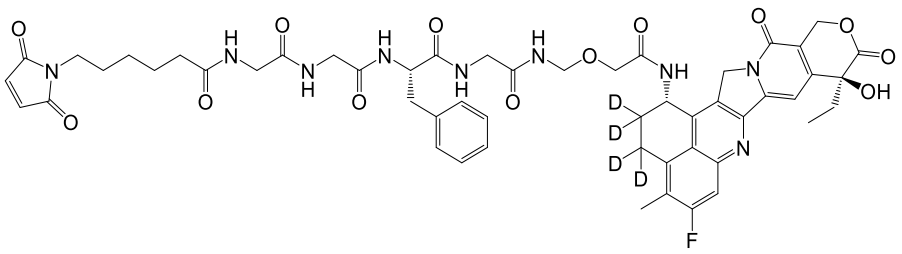


DL12-dDxd

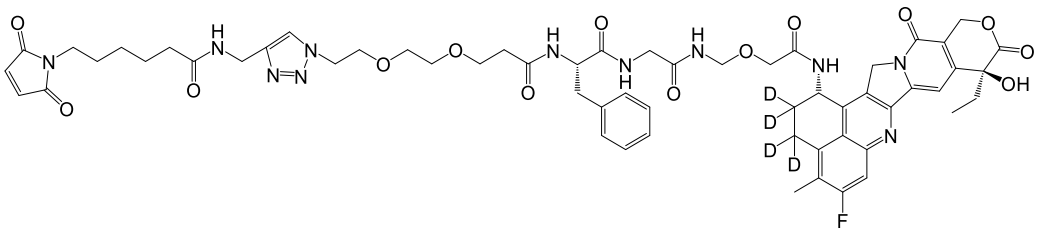

DL13-dDxd
 .

[Claim 72]The linker-payload according to claim 71, wherein the linker-payload has a structure selected from the group consisting of:

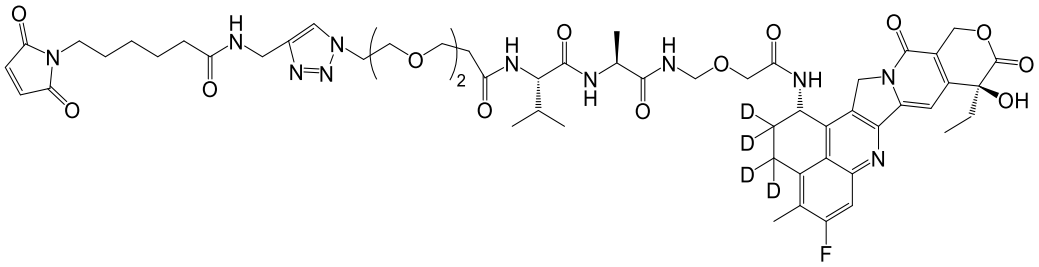
DL01-dDxd-1



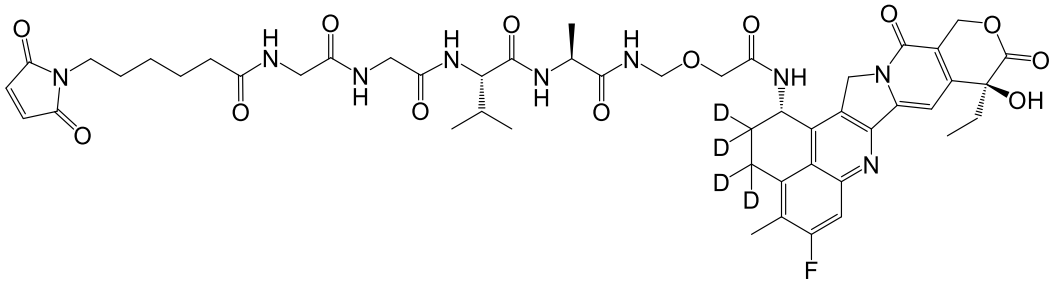
DL02-dDxd-1



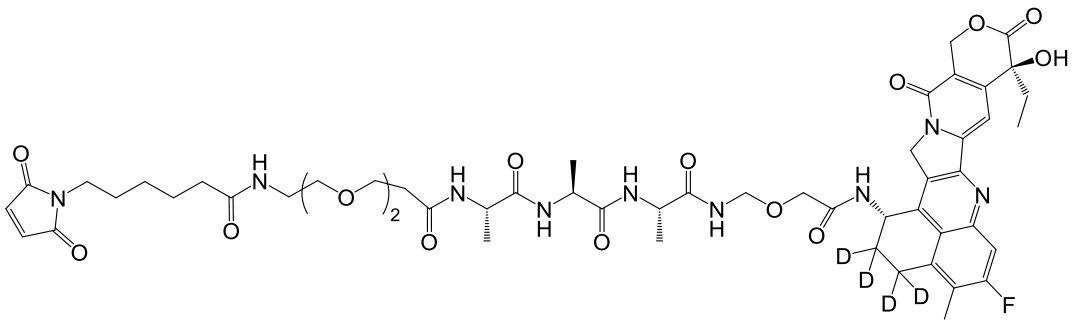
DL06-dDxd-1



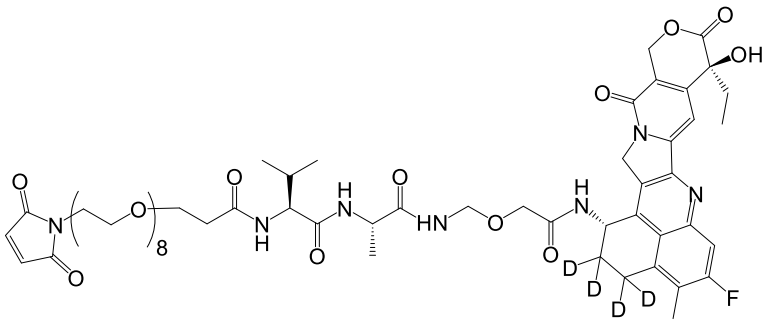
DL05-dDxd-1



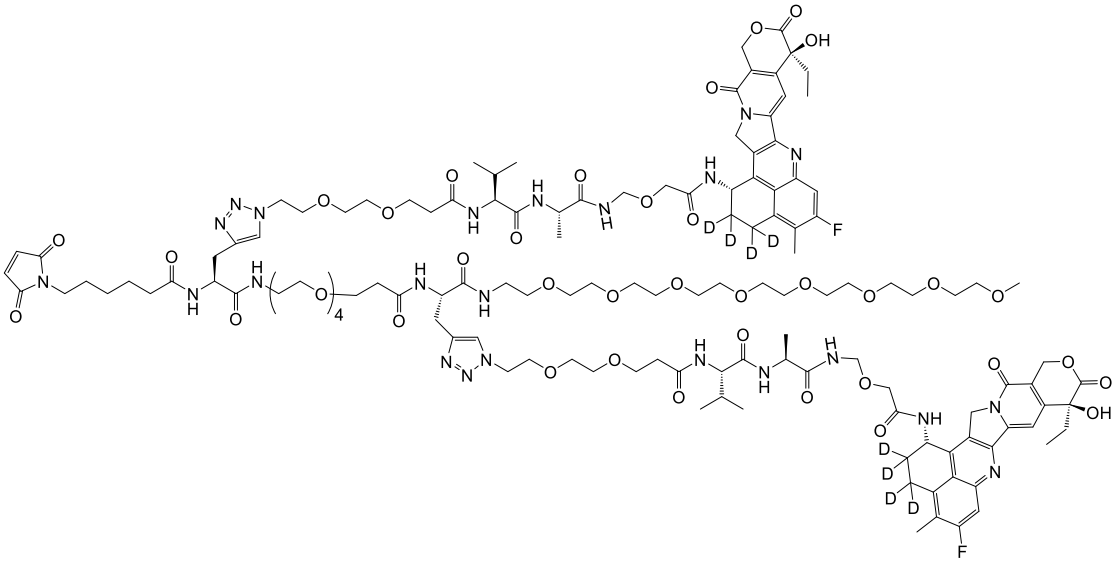
DL07-dDxd-1



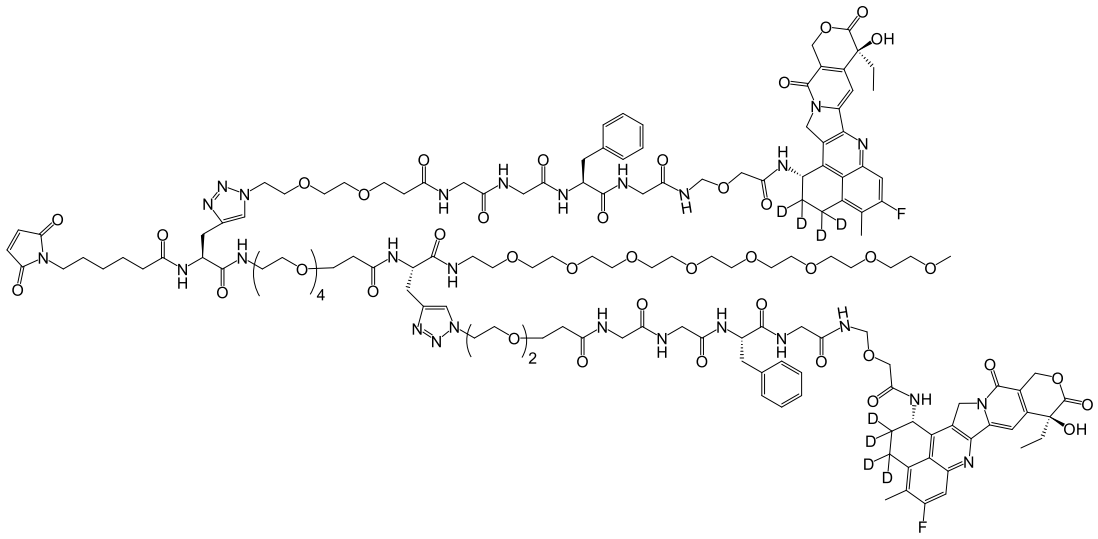
DL14-dDxd-1



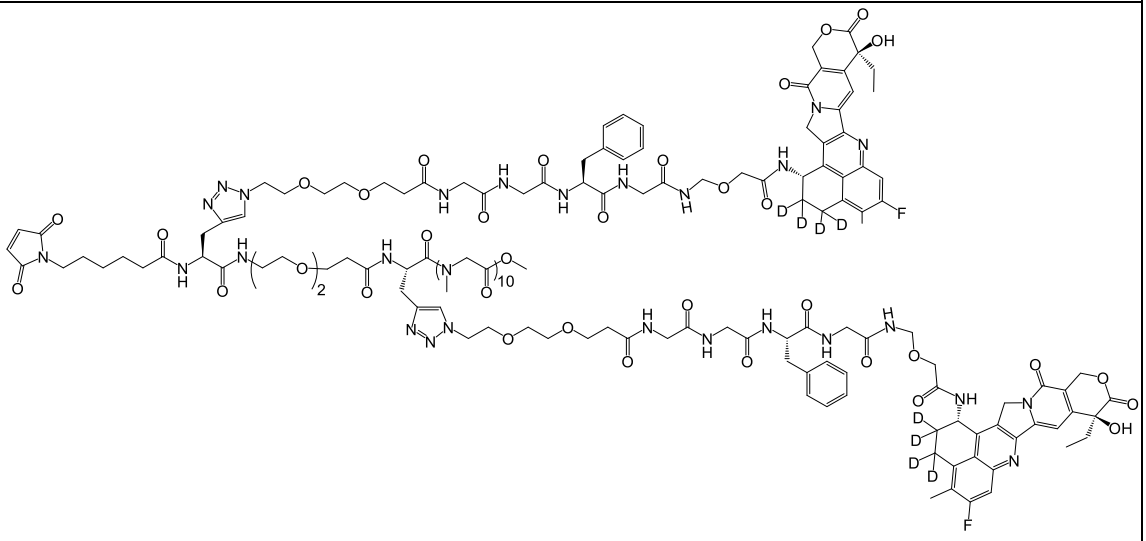
DL04-dDxd-1



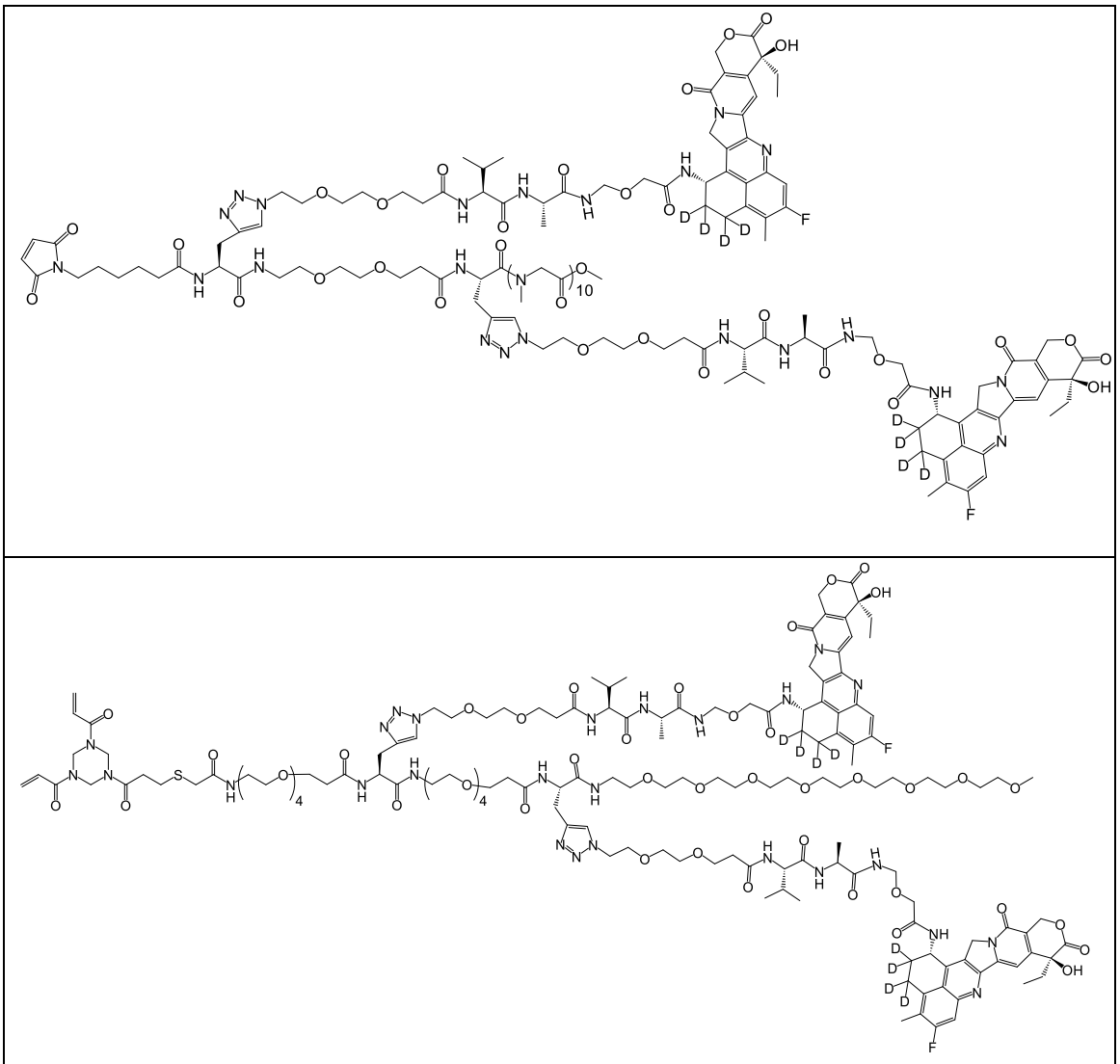
DL03-dDxd-1



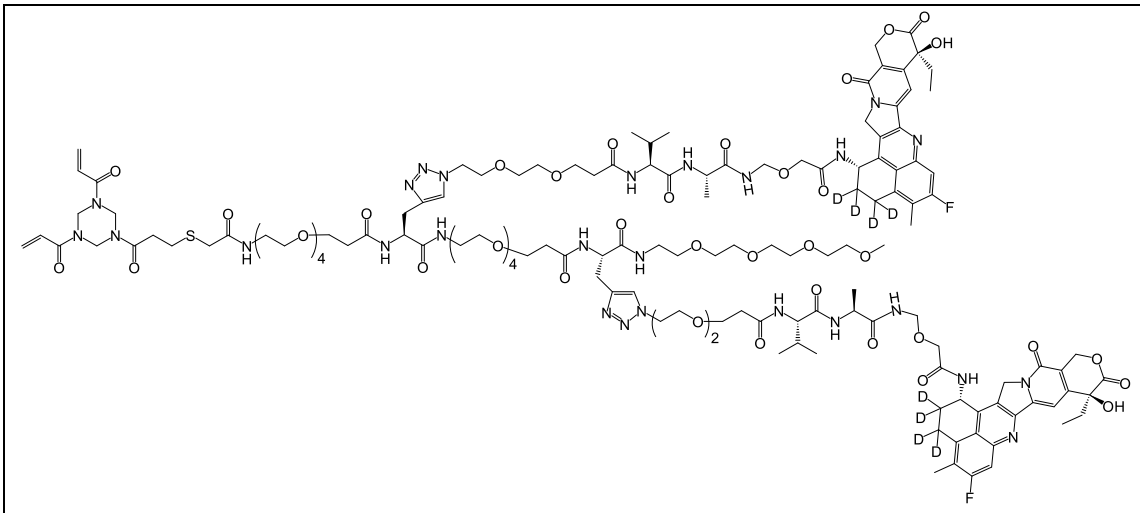
DL08-dDxd-1



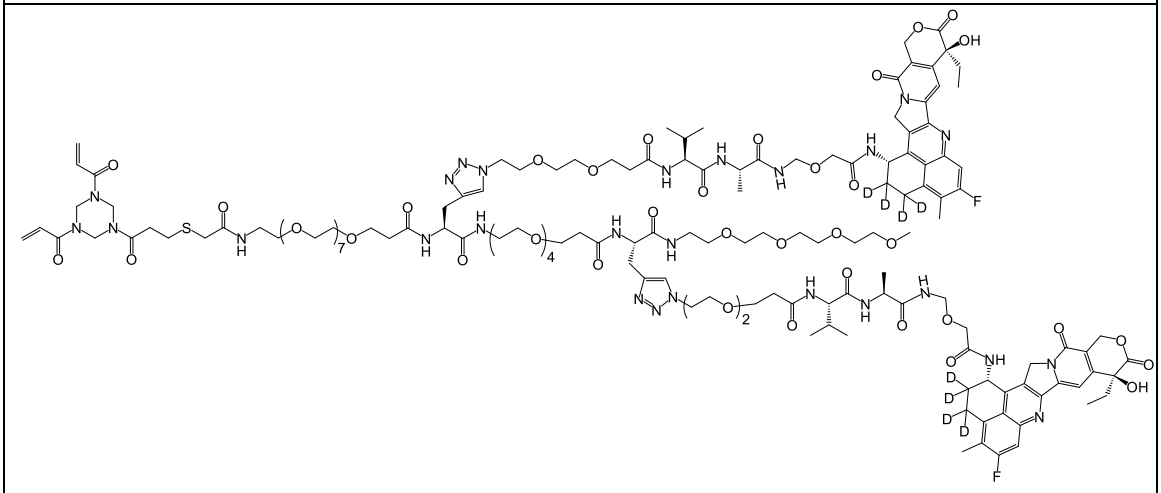
DL09-dDxd-1



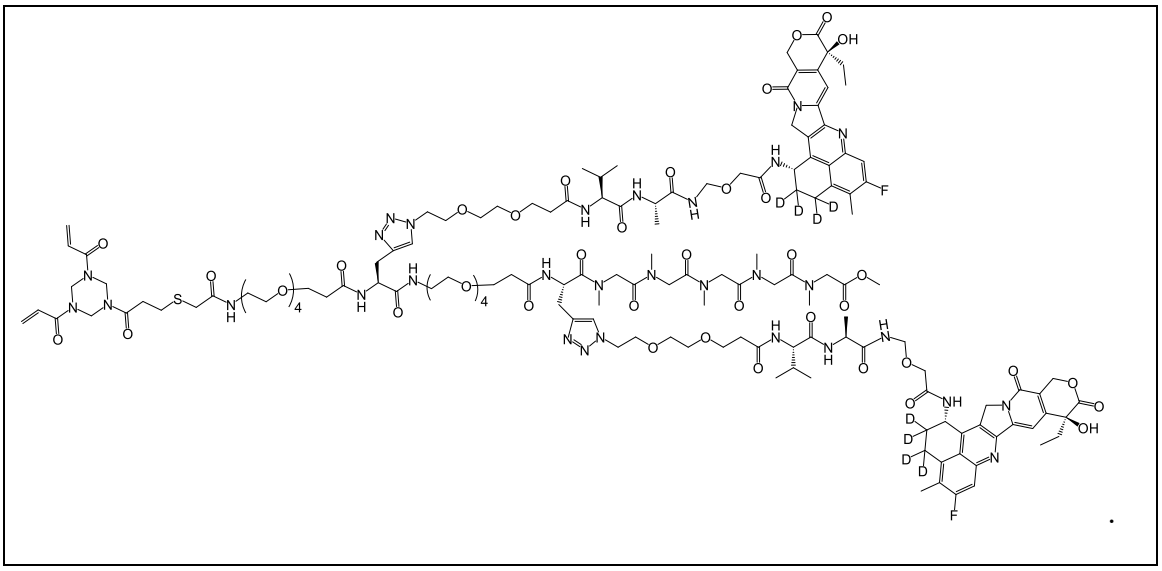
DL11-dDxd-1



DL12-dDxd-1

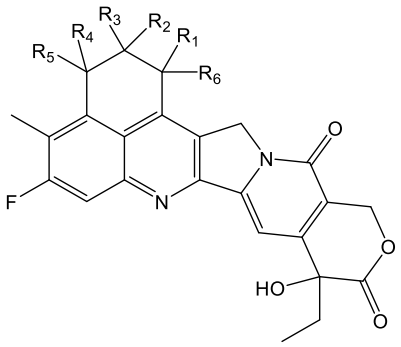


DL13-dDxd-1



[Claim 73] A linker-payload, which is a stereoisomer, pharmaceutically acceptable salt or solvate of the linker-payload according to any one of claims 57 to 72.

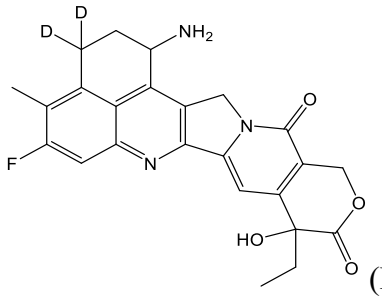
[Claim 74] A deuterated camptothecin compound represented by formula (V):

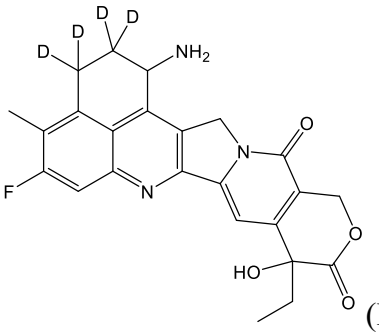


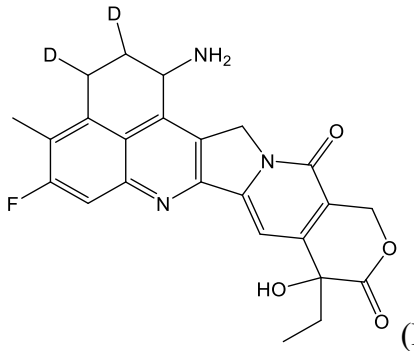
formula (V)

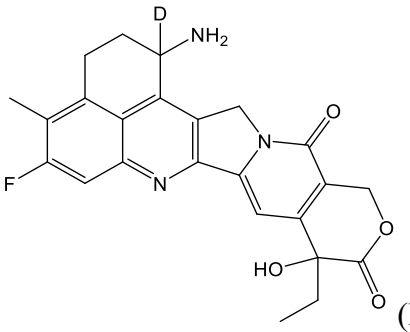
wherein: R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of hydrogen (H), deuterium (D) and tritium (T), at least one, two, three, or four of R_1, R_2, R_3, R_4 and R_5 are D, and R_6 is selected from the group consisting of $-NH_2$ and $-NH-C(O)-CH_2-OH$.

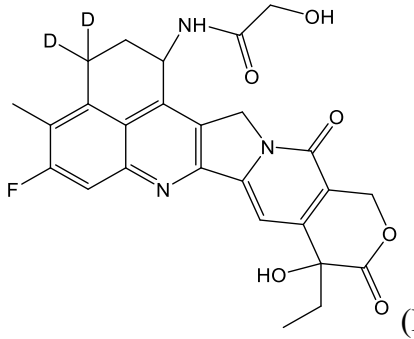
[Claim 75]The deuterated camptothecin compound according to claim 74, wherein the structure represented by formula (V) is selected from the group consisting of:

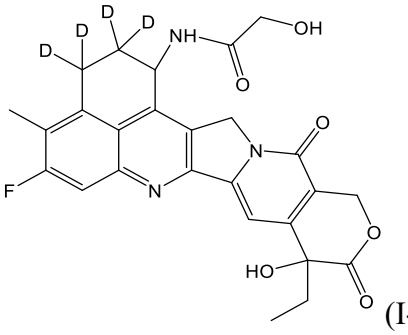

(I-1),

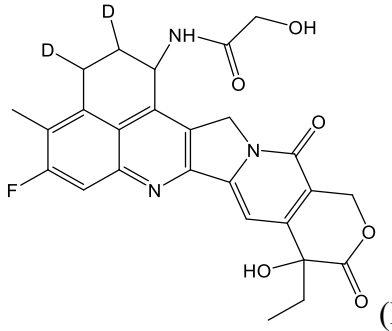

(I-2),

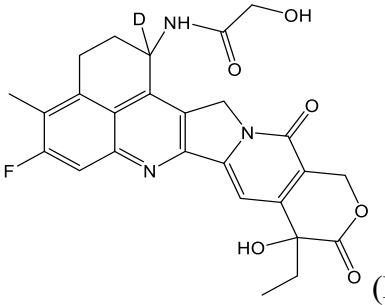

(I-3),


(I-4),

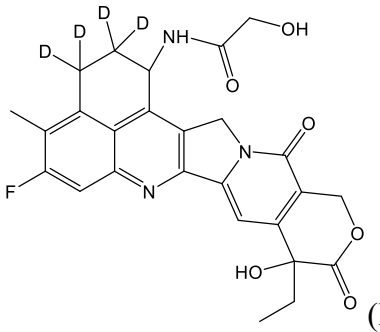
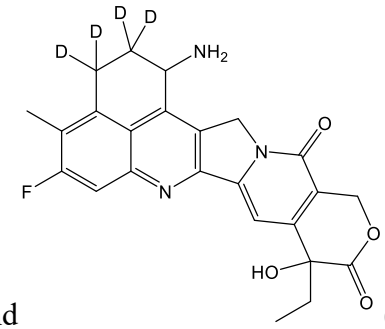

(I-5),

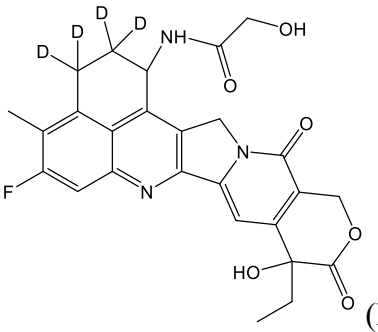

(I-6),


(I-7) and

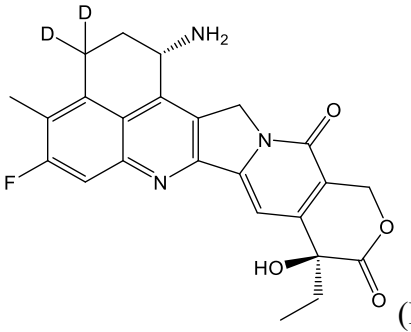
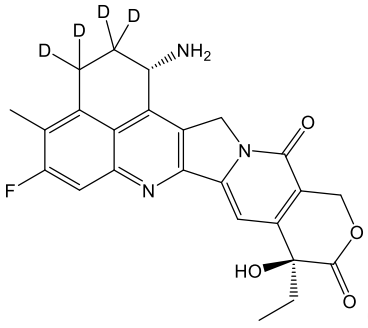

(I-8);

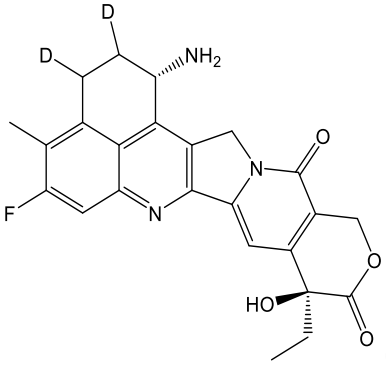
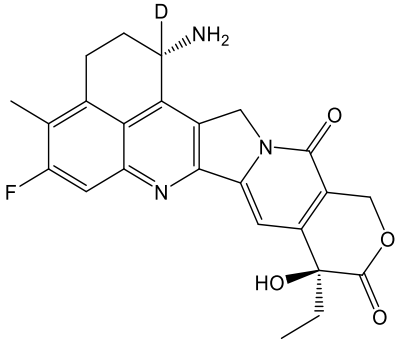
preferably the structure represented by formula (V) is selected from the group consisting of

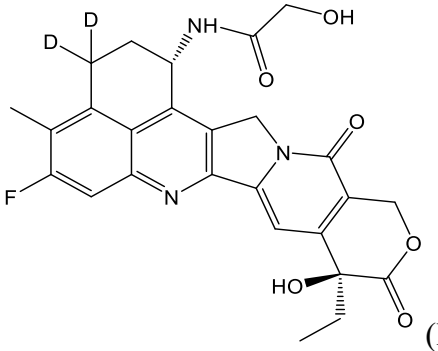
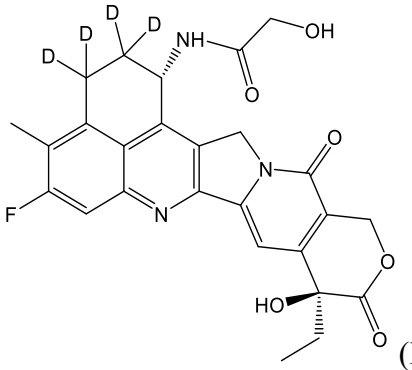
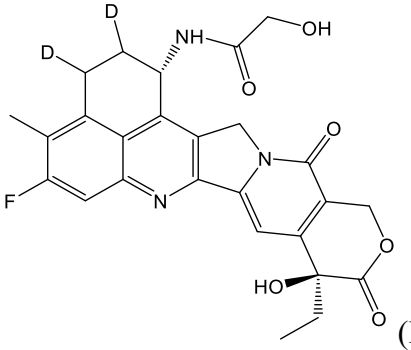
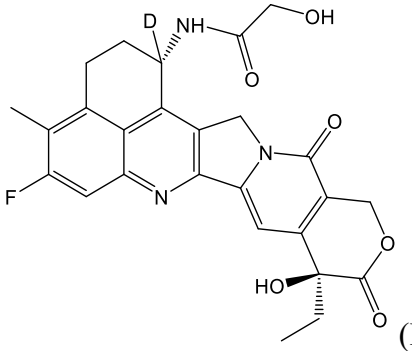

(I-6) and 
(I-2);


more preferably, the structure represented by formula (V) is (I-6).

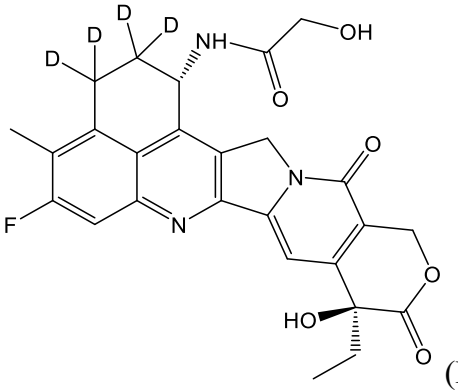
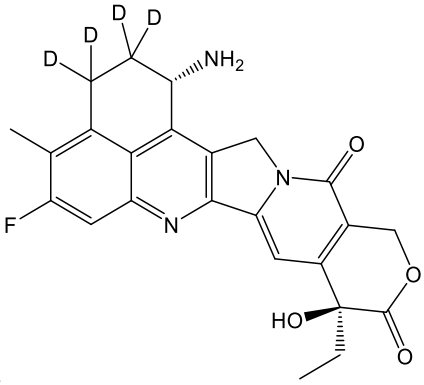
[Claim 76] The deuterated camptothecin compound according to claim 75, wherein the structure represented by formula (V) is selected from the group consisting of:


(I-1a), 
(I-2a),

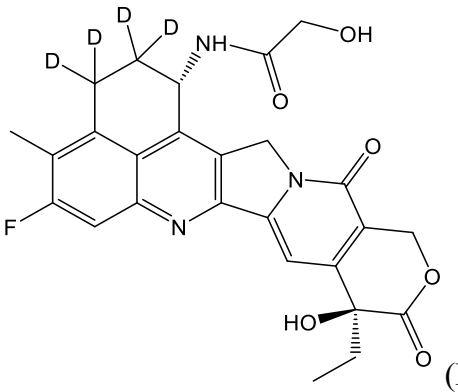

(I-3a), 
(I-4a),


(I-5a),

(I-6a),

(I-7a) and

(I-8a);

preferably the structure represented by formula (V) is selected from the group consisting of:

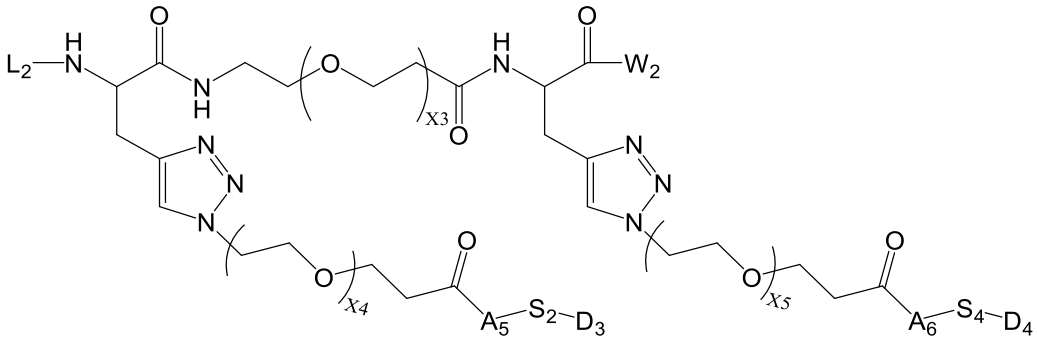

(I-6a) and

(I-2a);

more preferably, the deuterated camptothecin compound has a structure of


(I-6a).

[Claim 77] A compound, which is a stereoisomer, pharmaceutically acceptable salt or solvate of the deuterated camptothecin compound according to any one of claims 74 to 76.

[Claim 78]A compound represented by formula (IIIa):



formula (IIIa)

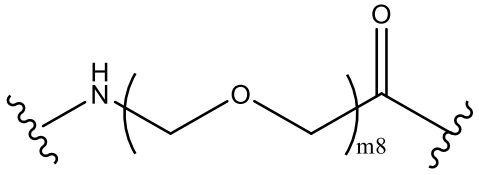
wherein, L_2 is a linker linked to an antibody,

W_2 is an optional group,

A_5 is a peptide consisting of 1, 2, 3 and 4 amino acid residues,

A_6 is a peptide consisting of 1, 2, 3 and 4 amino acid residues,

S_2 and S_4 are independently selected from the group consisting of -NH-CH_2- and


,

D_3 is a first payload compound,

D_4 is a second payload compound,

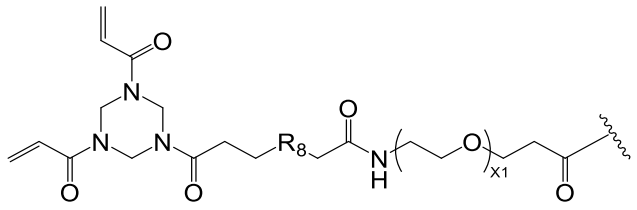
m_8 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

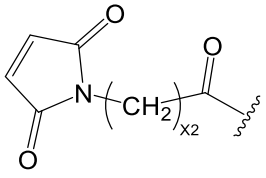
X_3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10,

X_4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

X_5 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

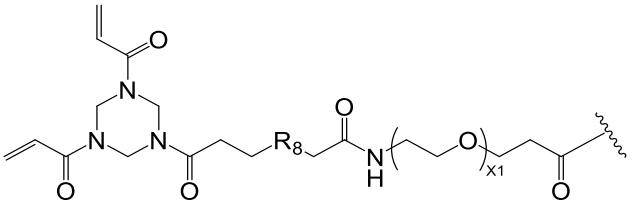
[Claim 79] The compound according to claim 78, wherein L_2 comprises a structure selected from the group consisting of:



and


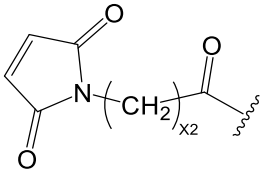
, wherein R_8 is selected from the group consisting of N, O and S; X_1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and X_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[Claim 80] The compound according to claim 79, wherein L_2 comprises a structure of



, wherein R_8 is selected from the group consisting of N, O and S, and X_1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

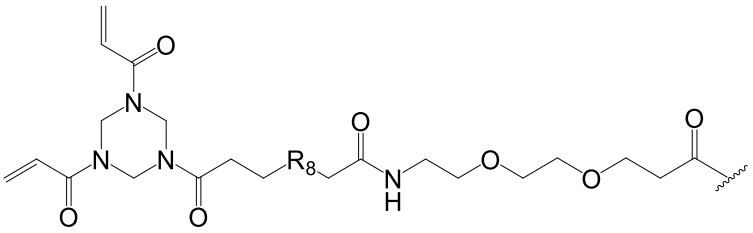
[Claim 81]The compound according to claim 80, wherein L_2 comprises a structure of

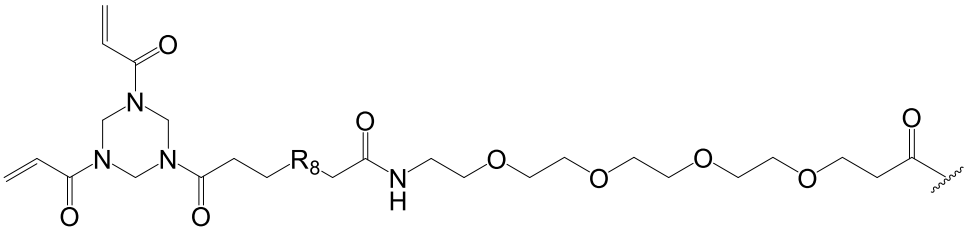


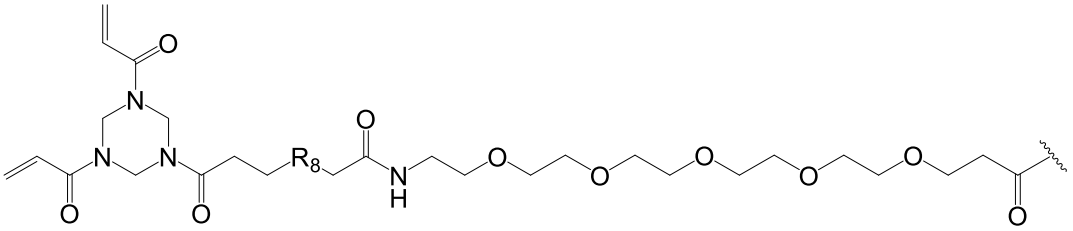
, wherein X_2 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9

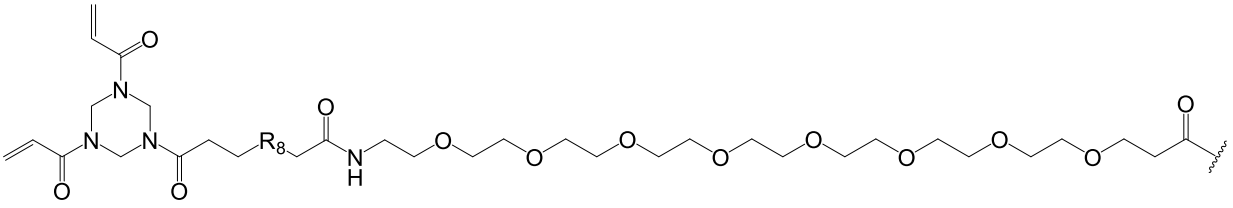
and 10.

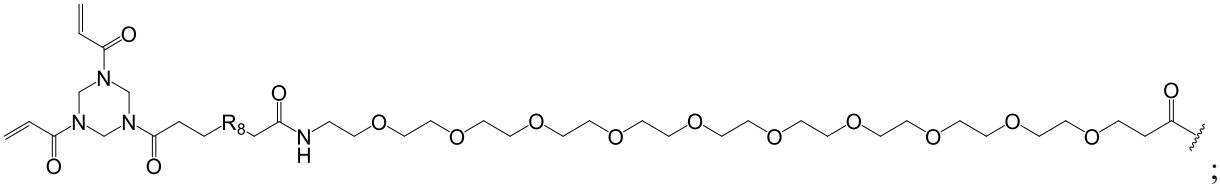
[Claim 82] The compound according to any one of claims 78 to 81, wherein L_2 comprises a structure selected from the group consisting of:


,


,

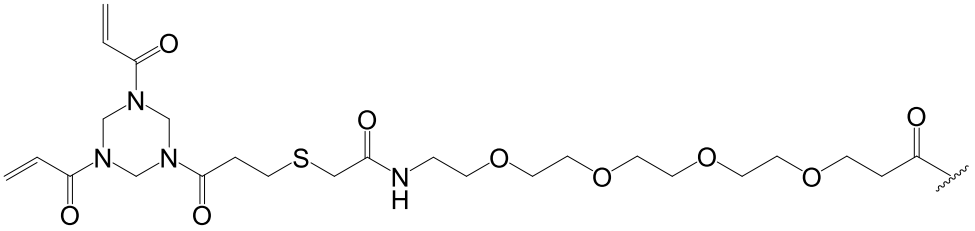

,

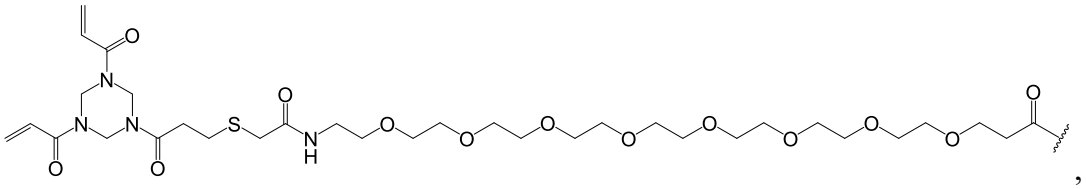

and

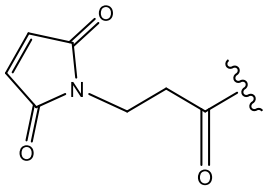

;

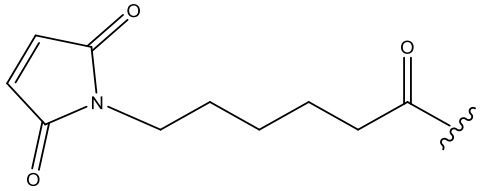
wherein R_8 is selected from the group consisting of N, O and S.

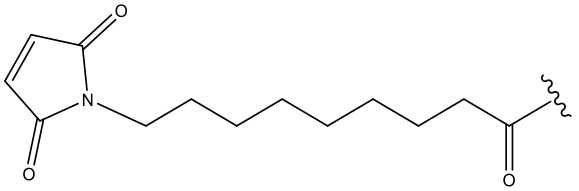
[Claim 83] The compound according to any one of claims 78 to 82, wherein L_2 comprises a structure selected from the group consisting of:

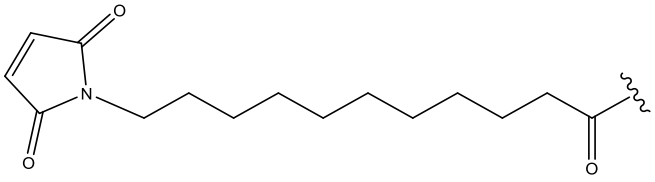

,


,

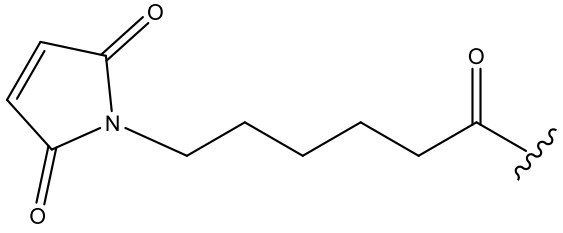

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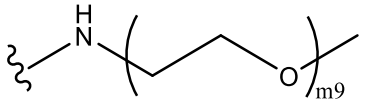

and

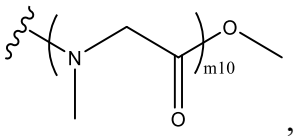

.

[Claim 84] The compound according to any one of claims 78 to 83, wherein L_2 has a structure


of .

[Claim 85] The compound according to any one of claims 78 to 84, wherein W comprises a structure selected from the group consisting of


and

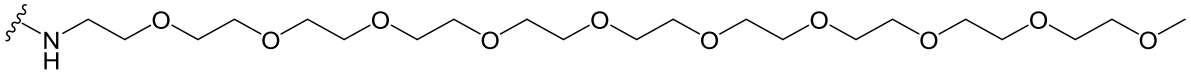
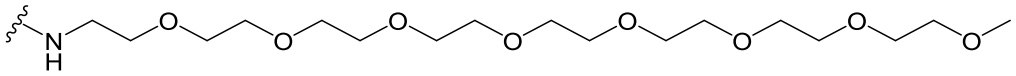
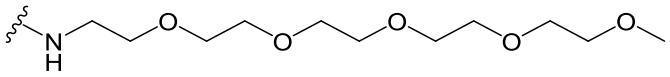
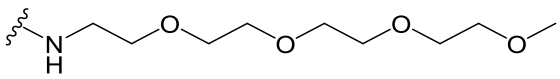
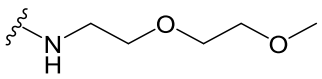
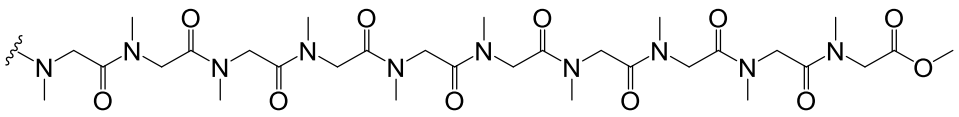
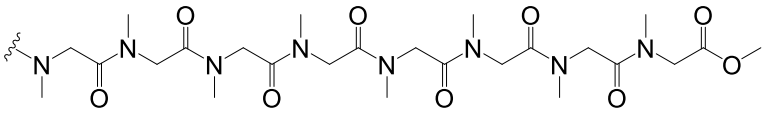
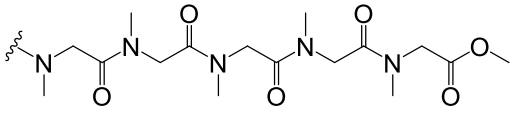
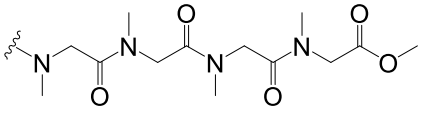
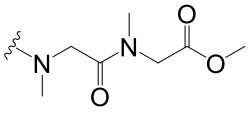


wherein,

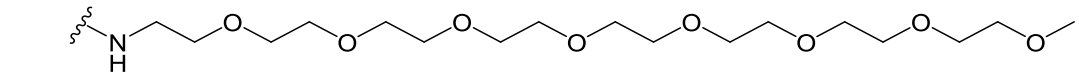
M9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

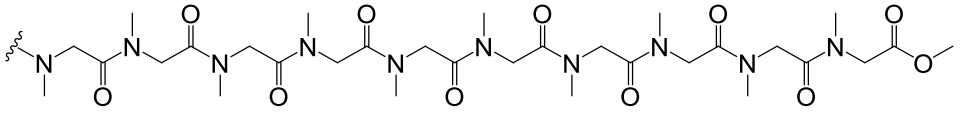
M10 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

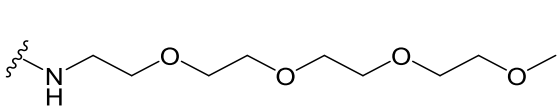
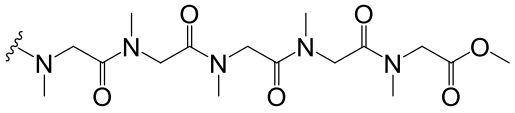
[Claim 86] The compound according to any one of claims 78 to 85, wherein W has a structure selected from the group consisting of

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[Claim 87] The compound according to any one of claims 78 to 86, wherein W has a structure selected from the group consisting of


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[Claim 88] The compound according to any one of claims 78 to 87, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a peptide, wherein the peptide consists of amino acids selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit).

[Claim 89] The compound according to claim 88, wherein A_1, A_2 and A_3 are independently a single bond or independently comprise a peptide, wherein the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), valine (Val), lysine (Lys), arginine (Arg), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), cysteine (Cys) and citrulline (Cit), preferably the peptide consists of 2-4 amino acid residues selected from the group consisting of phenylalanine (Phe), glycine (Gly), alanine (Ala) and valine (Val).

[Claim 90] The compound according to claim 89, wherein A_1, A_2 and A_3 are independently a single bond or independently comprises a structure selected from the group consisting of: -Gly-Ala-, -Ala-Gly-, -Val-Gly-, -Gly-Val-, -Val-Cit-, -Val-Ala-, -Gly-Phe-, -Phe-Gly-, -Gly-Gly-Ala-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Phe-Gly-, -Gly-Ala-Gly-Gly-, -Gly-Val-Gly-Gly-, -Gly-Phe-Gly-Gly-, -Gly-Gly-Lys-Gly-, -Gly-Gly-Ser-Gly-, -Gly-Gly-Glu-Gly-, -Gly-Lys-Gly-Gly-, -Gly-Ser-Gly-Gly-, -Gly-Glu-Gly-Gly-, -Gly-Gly-Val-Ala-, -Gly-Gly-Cit-Gly-, -Gly-Cit-Gly-Gly- and -Ala-Ala-Ala-, preferably a structure selected from the group consisting of -Gly-Gly-Phe-Gly-, -Val-Ala-, -Val-Gly-, -Phe-Gly-, -Gly-Gly-Val-Gly-, -Gly-Gly-Val-Ala- and -Ala-Ala-Ala-; more preferably, the linker comprises a structure of -Val-Ala-.

[Claim 91] The compound according to any one of claims 78 to 90, wherein the first payload

compound and the second payload compound are independently selected from the group consisting of a cytotoxic molecule, an immune enhancer and a radioactive isotope,

wherein the cytotoxic molecule includes, but is not limited to a tubulin inhibitor and a DNA damaging agent,

further preferably, the tubulin inhibitor includes, but is not limited to dolastatins, auristatin cytotoxic molecules, and maytansine cytotoxic molecules,

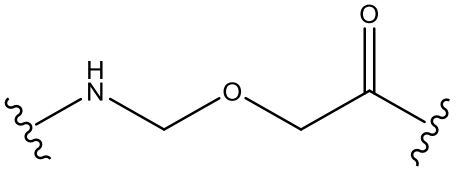
the DNA damaging agent includes, but is not limited to calicheamicins, duocarmycins, and anthramycin derivative pyrrolobenzodiazepines (PBDs), camptothecins, camptothecin derivatives, SN-38, and Dxd,

further preferably, the auristatin cytotoxic molecules include, but are not limited to monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), and derivatives thereof,

the maytansine cytotoxic molecules include, but are not limited to DM1, DM4, and derivatives thereof,

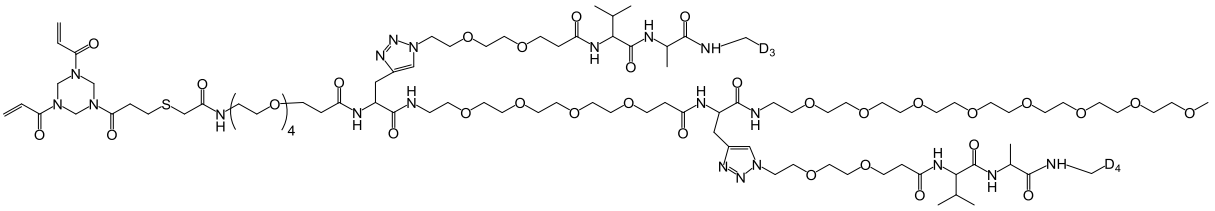
further preferably, the camptothecin derivative is the compound according to claim 65 or 66, wherein the compound is attached to S_2 or S_4 through removal of a hydrogen atom from either a hydroxyl or an amine group present.

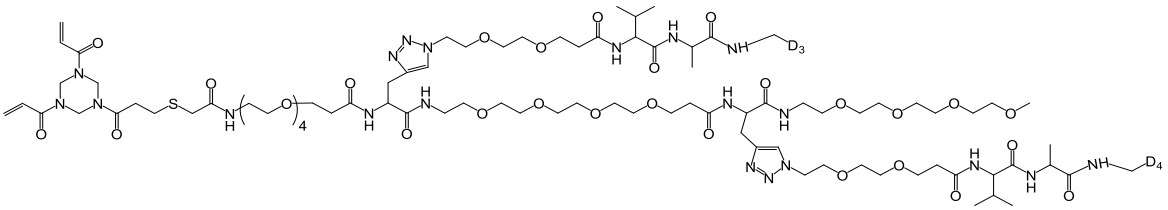
[Claim 92] The compound according to any one of claims 78 to 91, wherein S_2 and S_4 independently have a structure selected from the group consisting of -NH-CH_2- and

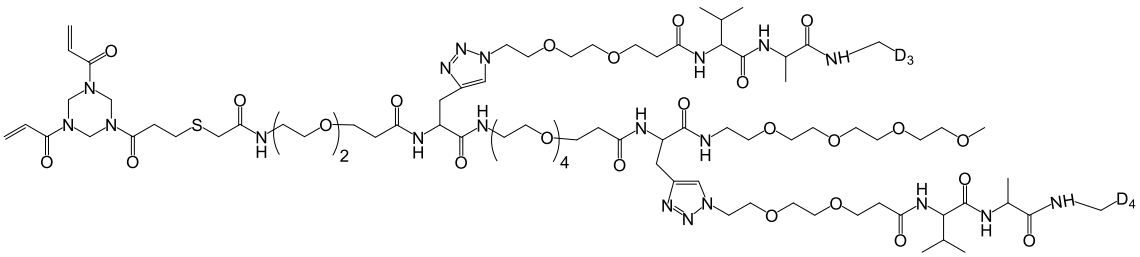


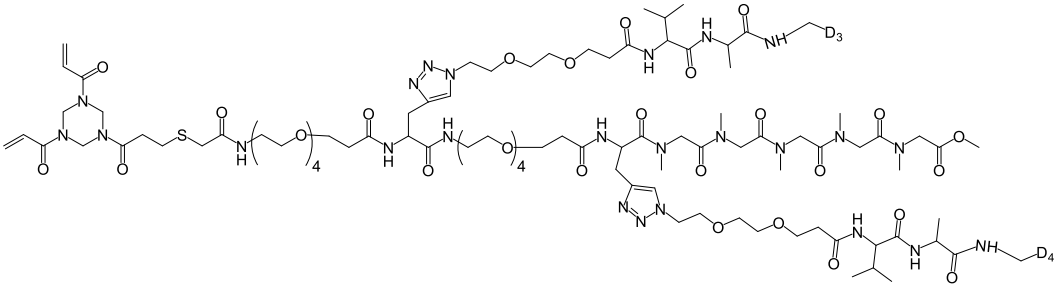
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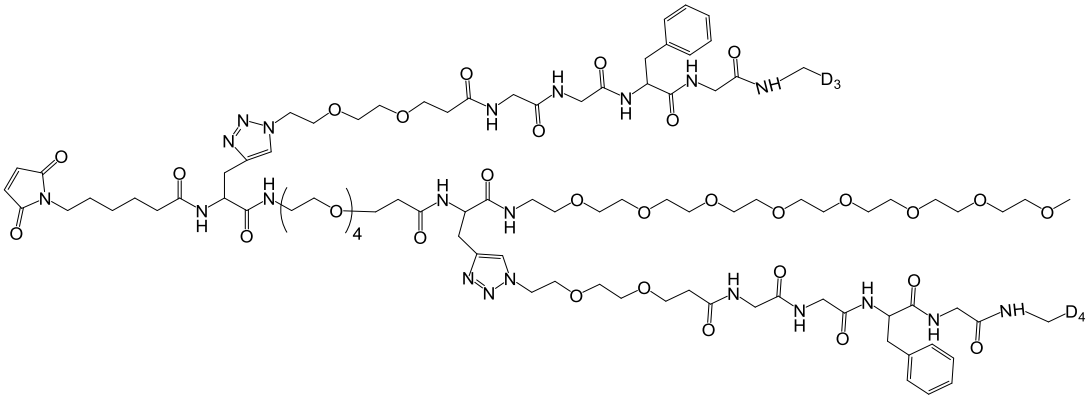
[Claim 93] The compound according to any one of claims 78 to 92, wherein the compound is selected from the group consisting of:

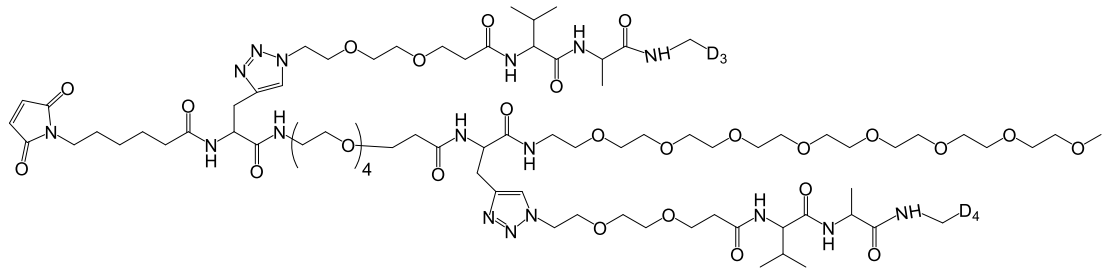

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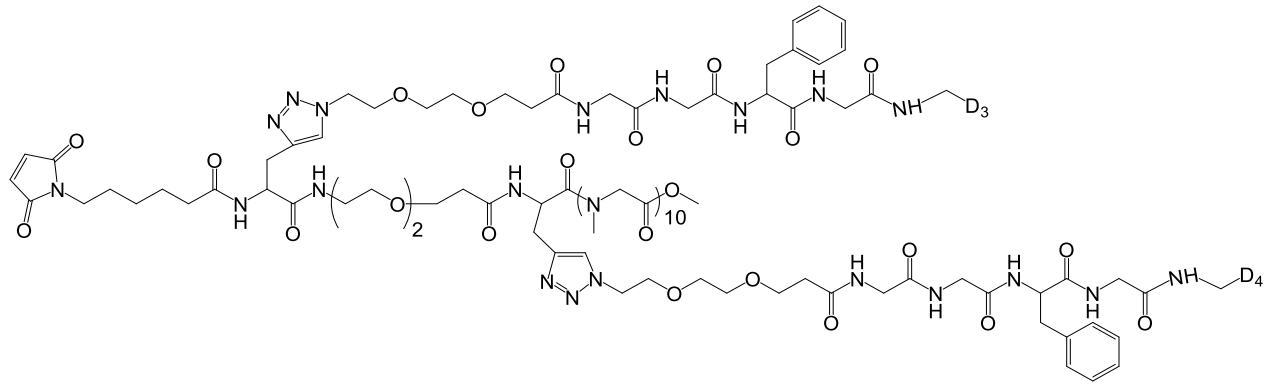

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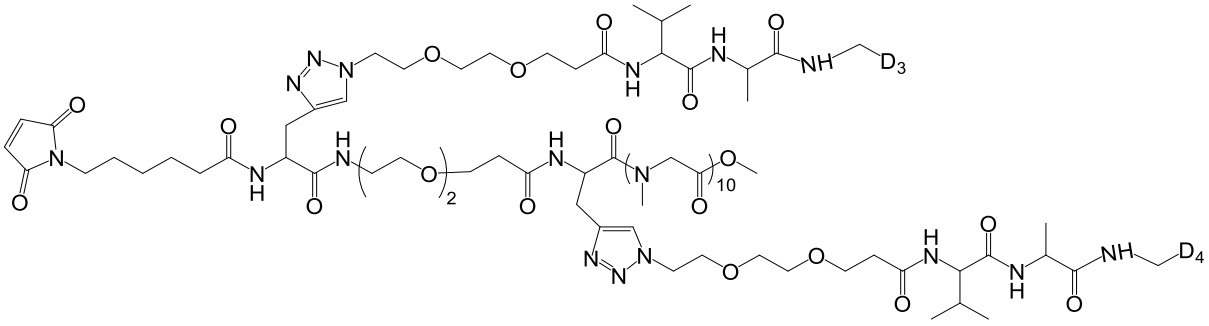

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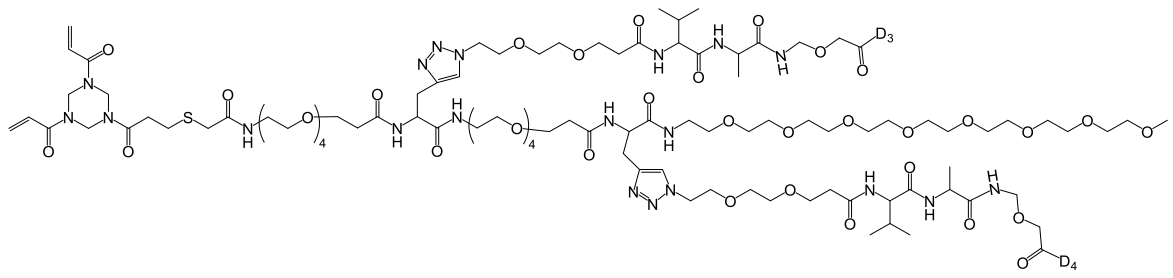
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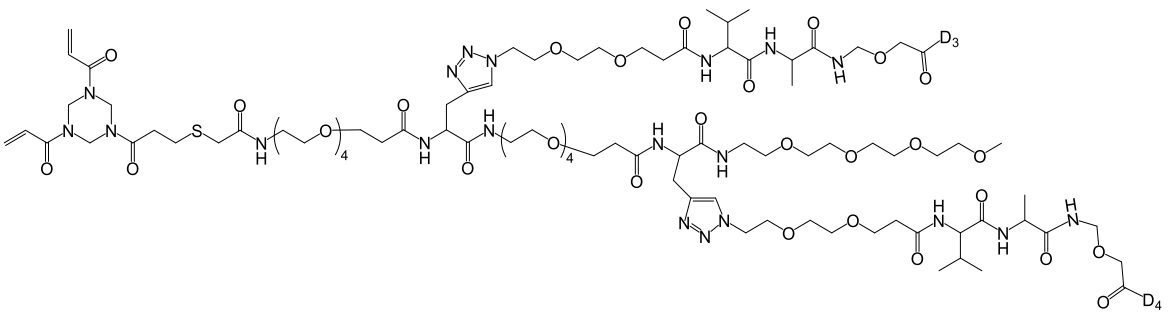


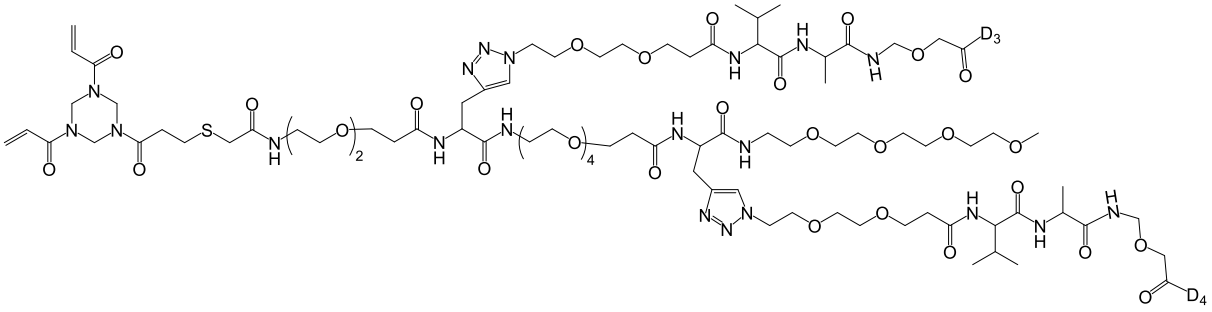
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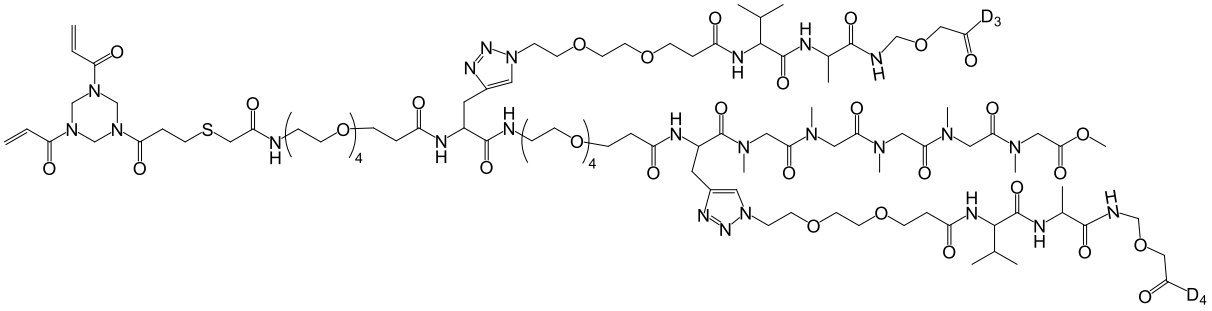
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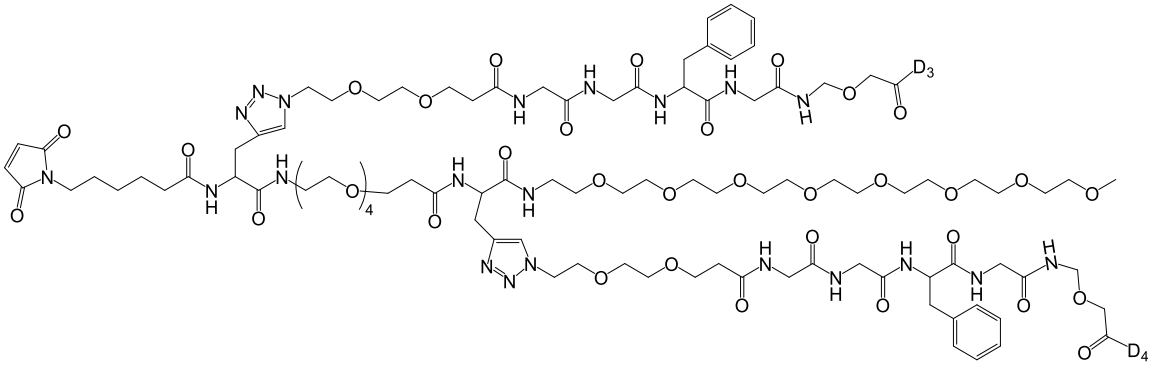


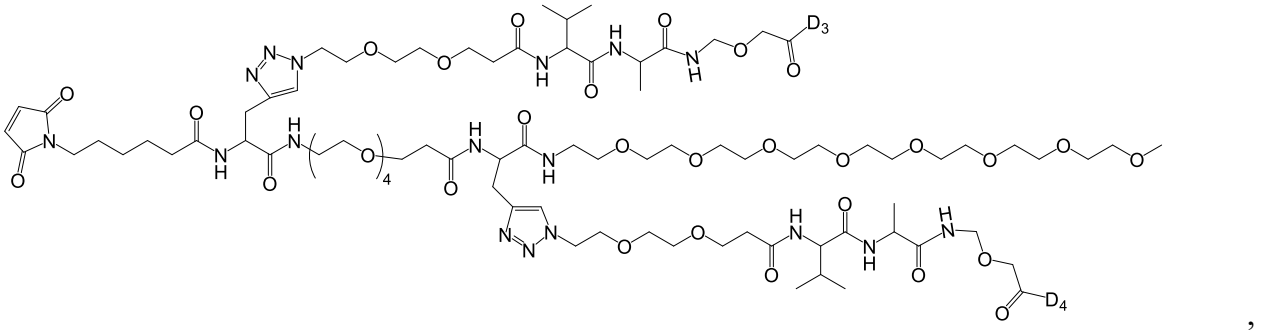
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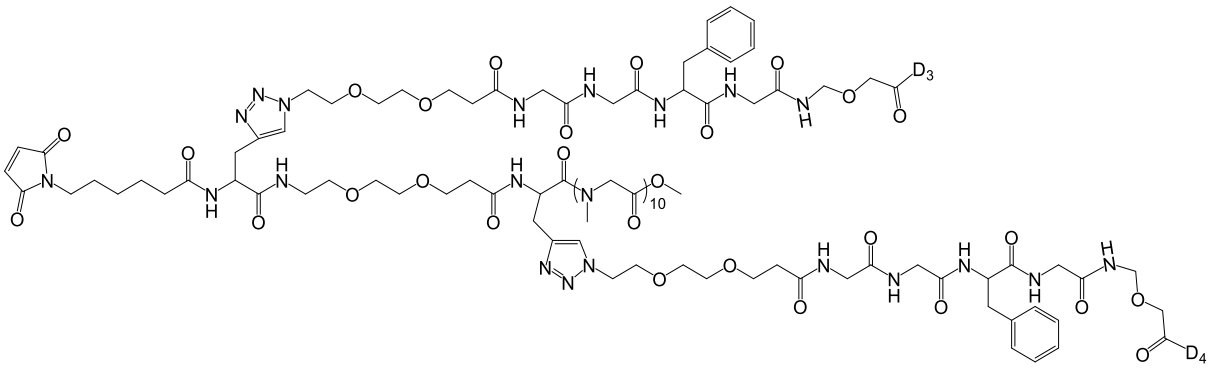

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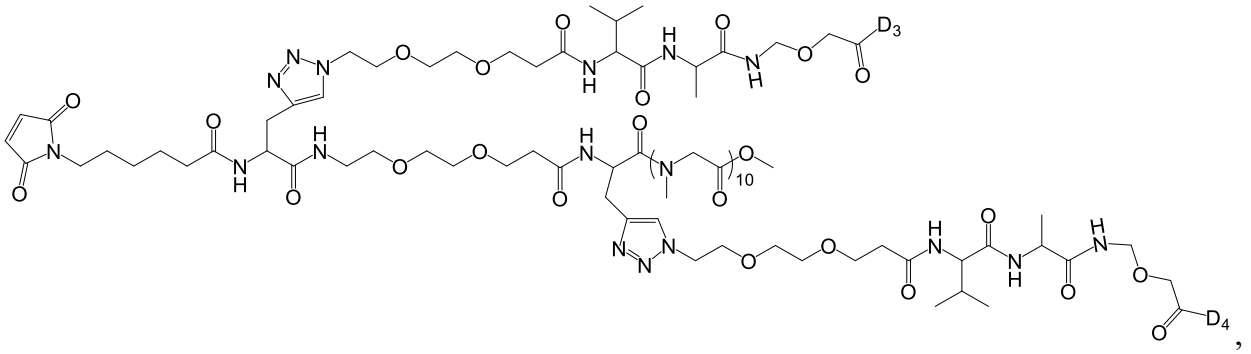

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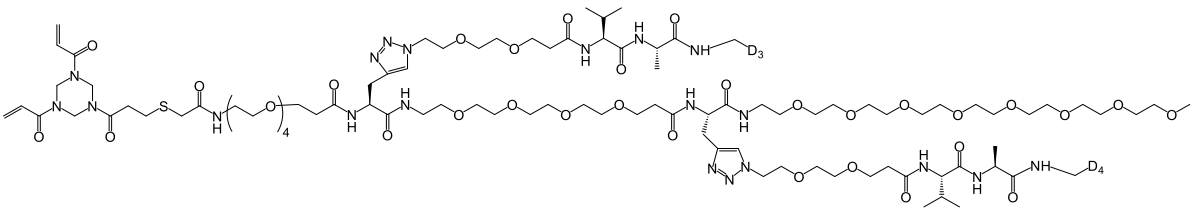
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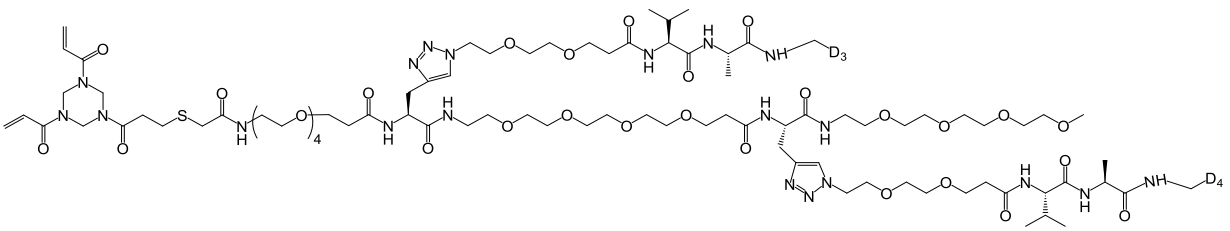
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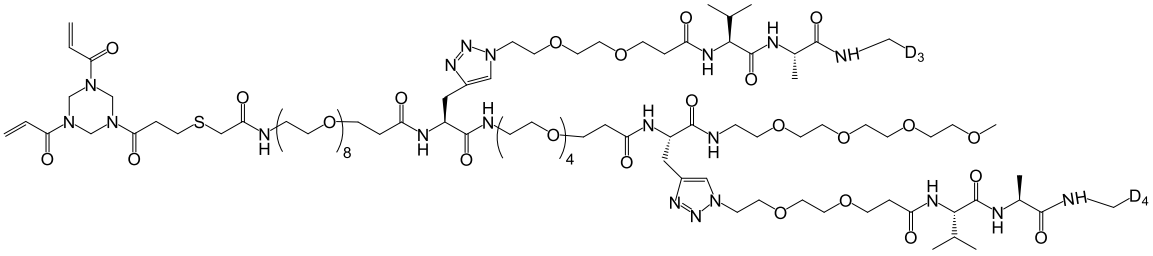
wherein, D_3 is a first payload compound, and D_4 is a second payload compound.

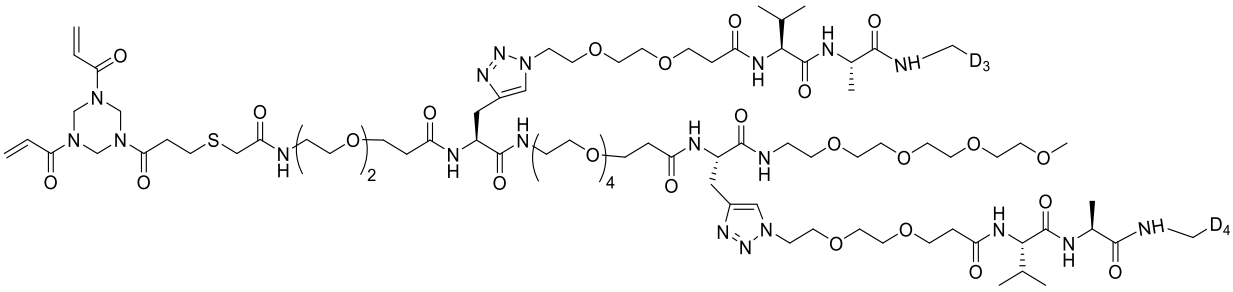
[Claim 94] The compound according to claim 93, wherein the compound is selected from the group consisting of:

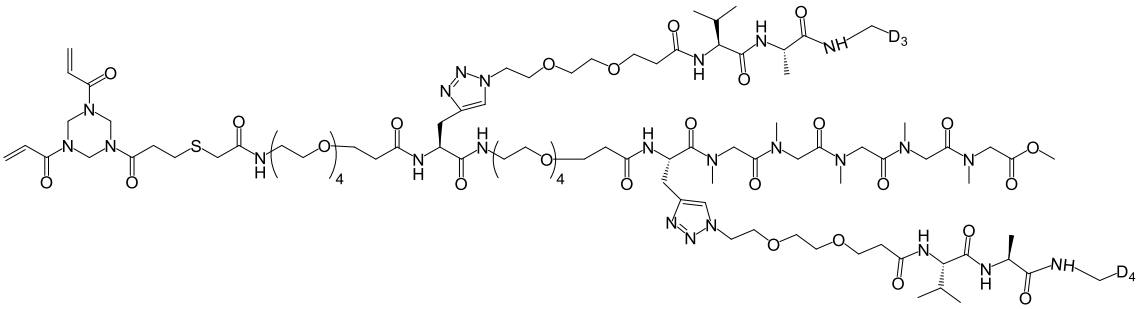


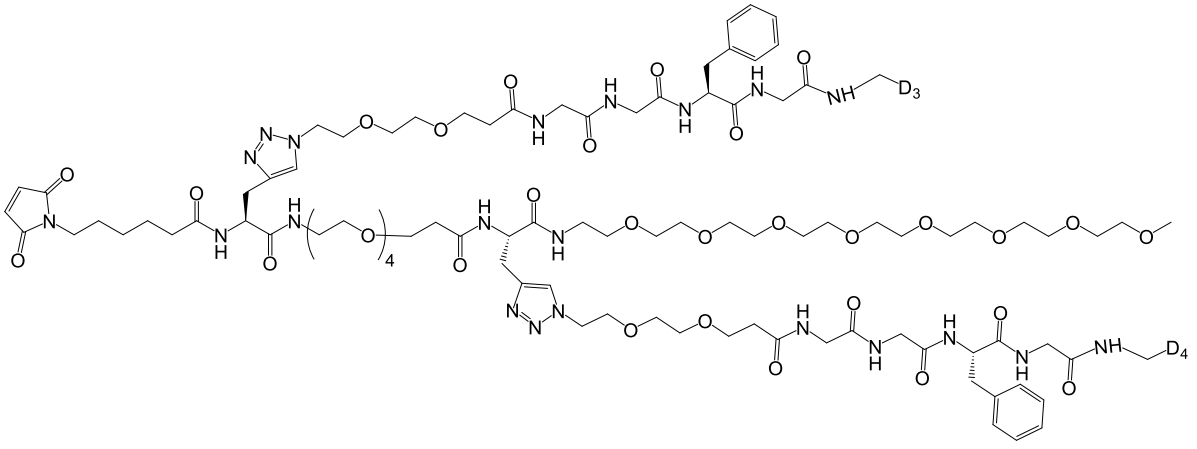
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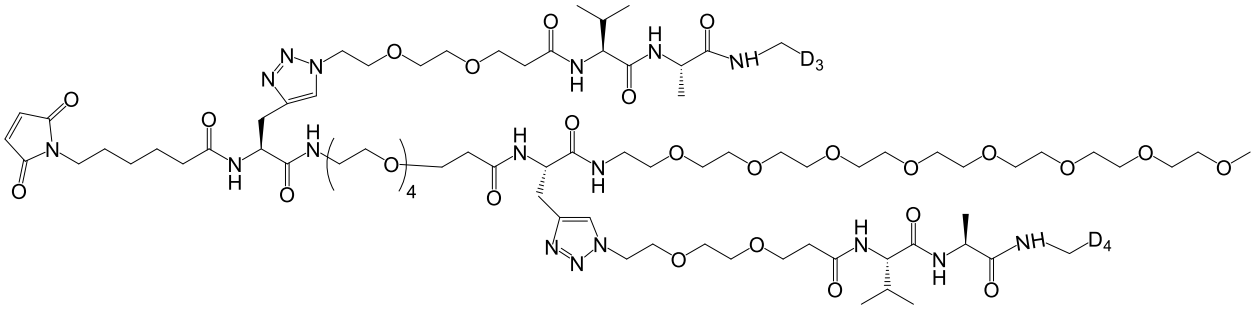
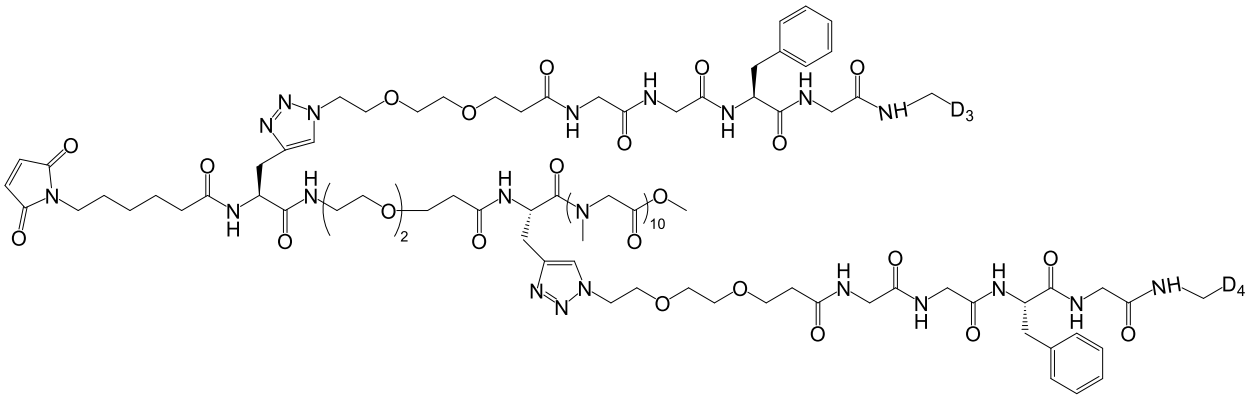
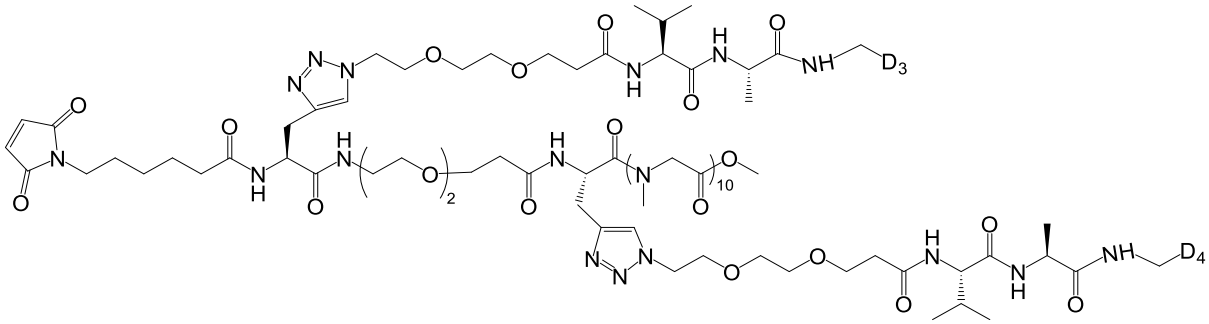
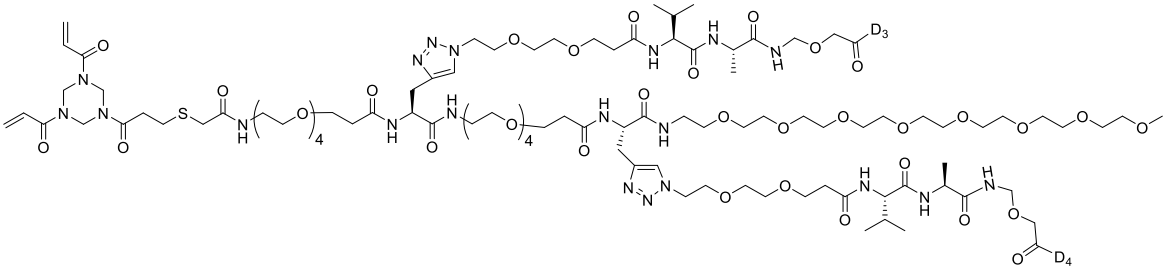

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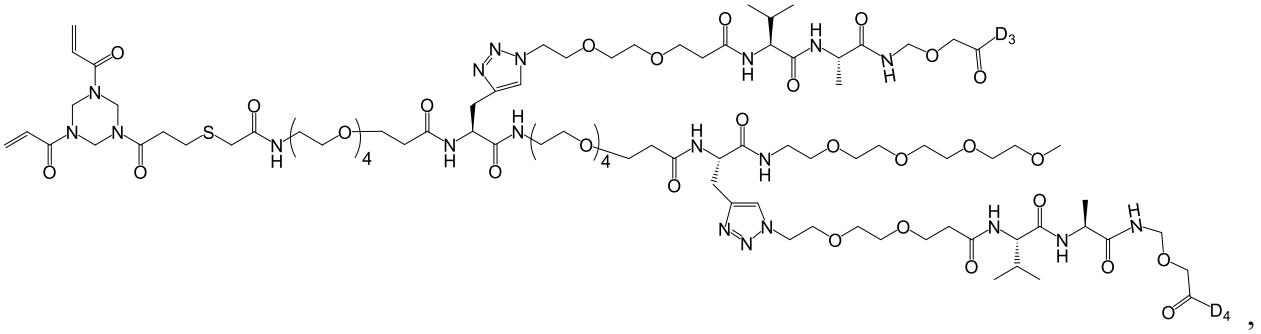

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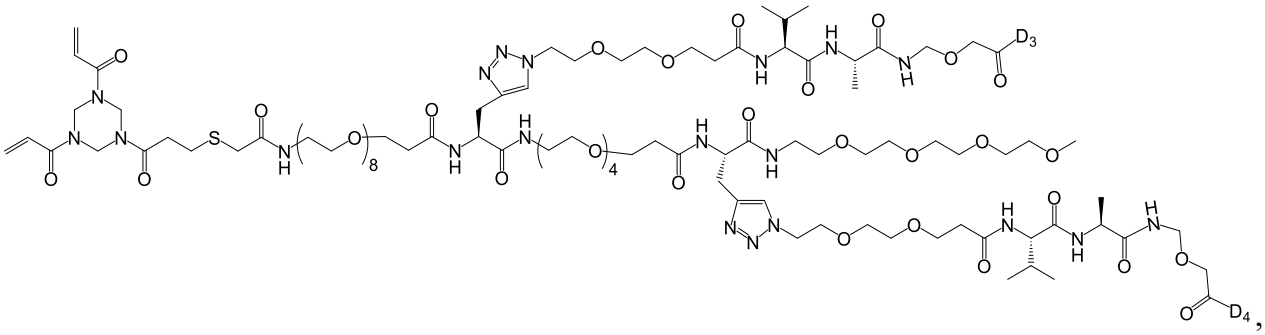

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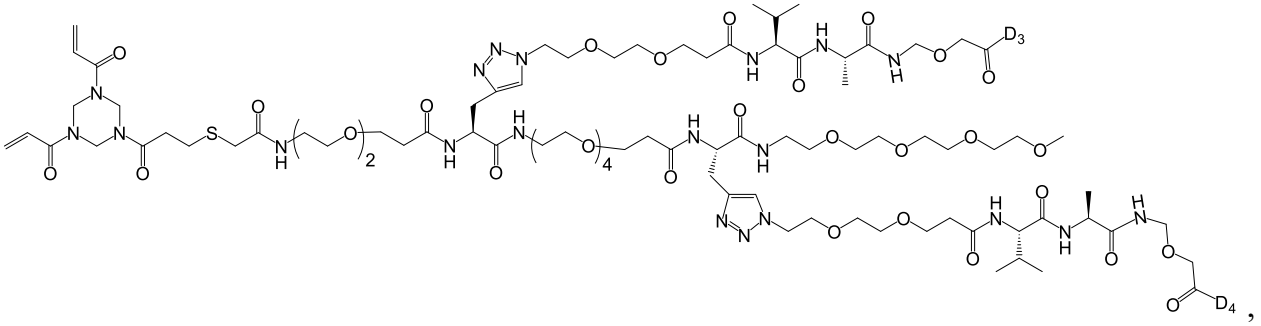

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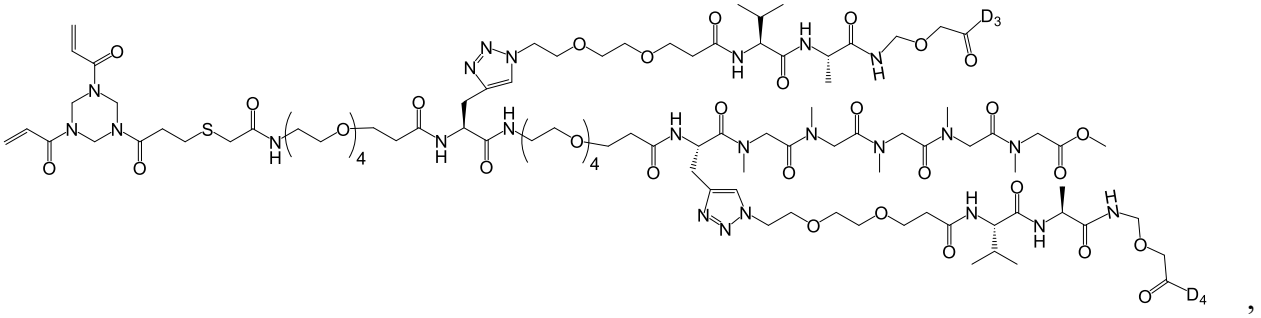
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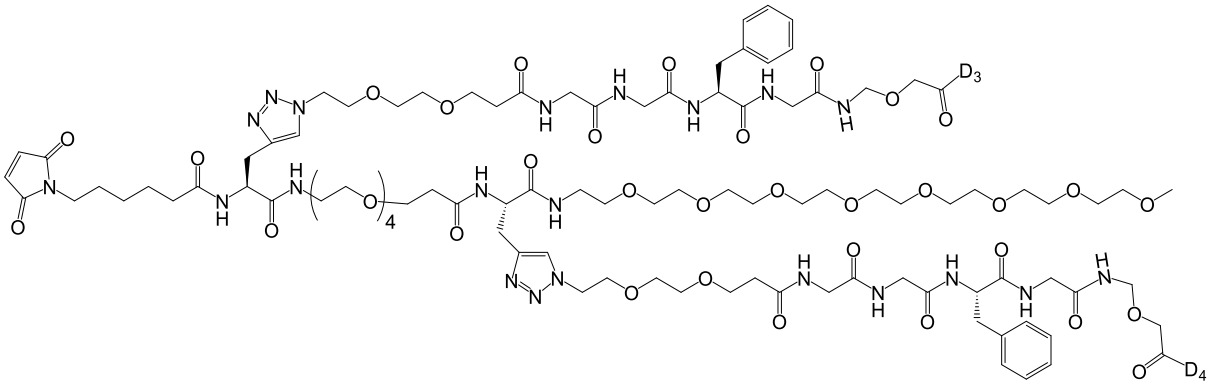
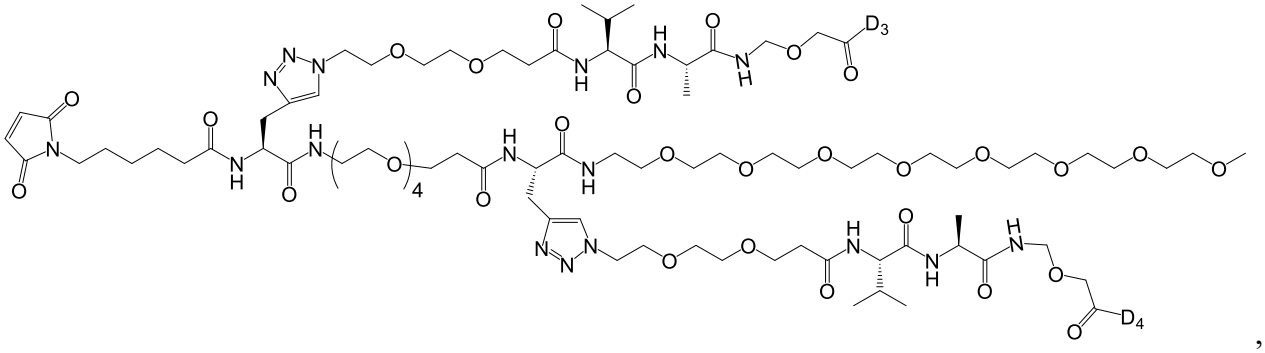
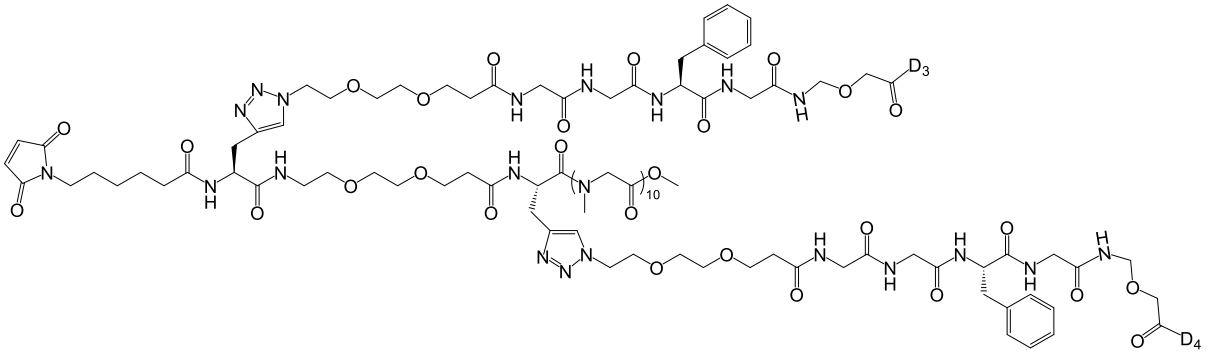
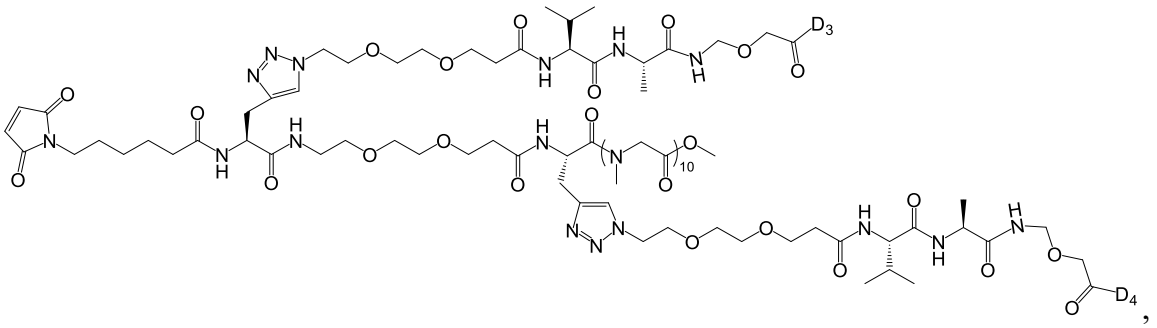
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wherein, D_3 is a first payload compound, and D_4 is a second payload compound.

[Claim 95] A compound, wherein the compound is a stereoisomer, pharmaceutically

acceptable salt, or solvate of the compound according to any one of claims 74 to 94.

[Claim 96] Use of the linker according to any one of claims 42 to 56 in the manufacture of an antibody drug conjugate.

[Claim 97] Use of the linker-payload according to any one of claims 57 to 73 or 78 to 95 in the manufacture of an antibody drug conjugate.

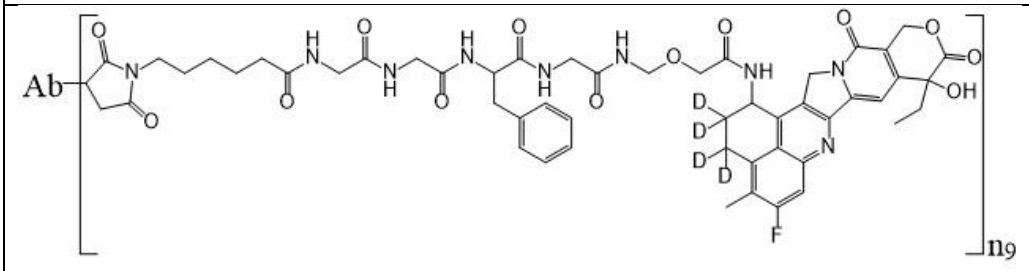
[Claim 98] Use of the compound according to any one of claims 74 to 77 in the manufacture of an antibody drug conjugate.

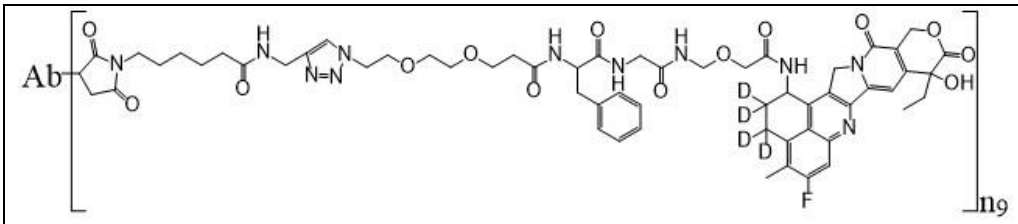
[Claim 99] An antibody drug conjugate comprising a targeting moiety, a linker and a payload, wherein the linker is selected from the linker according to any one of claims 42 to 56, and/or the payload is selected from the compound according to any one of claims 74 to 77.

[Claim 100] The antibody drug conjugate according to claim 99, wherein the targeting moiety is the antibody or the antigen binding fragment thereof according to any one of claims 29 to 33.

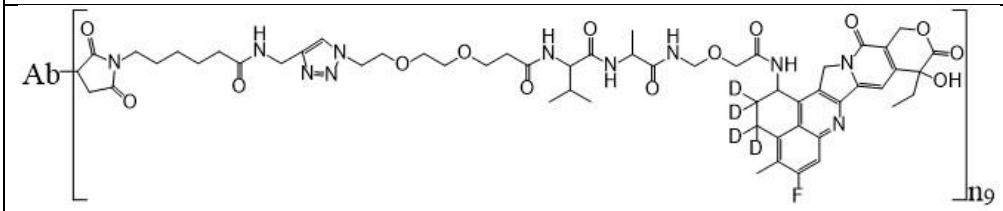
[Claim 101] The antibody drug conjugate according to claim 99, wherein the linker-payload is the compound according to any one of claims 57 to 73 or 78 to 95.

[Claim 102] The antibody drug conjugate according to any one of claims 99 to 101, wherein the antibody drug conjugate has a structure selected from the group consisting of:

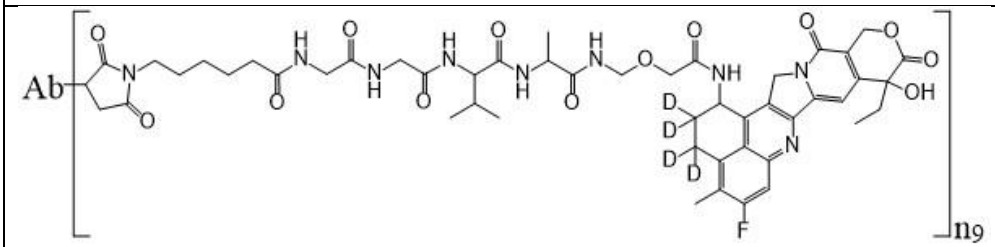
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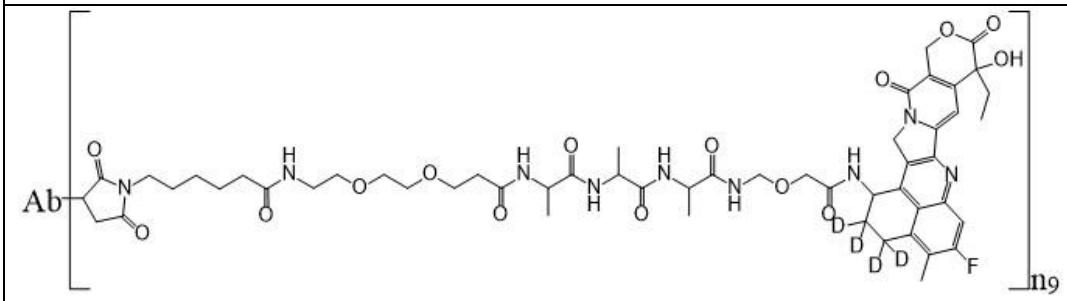
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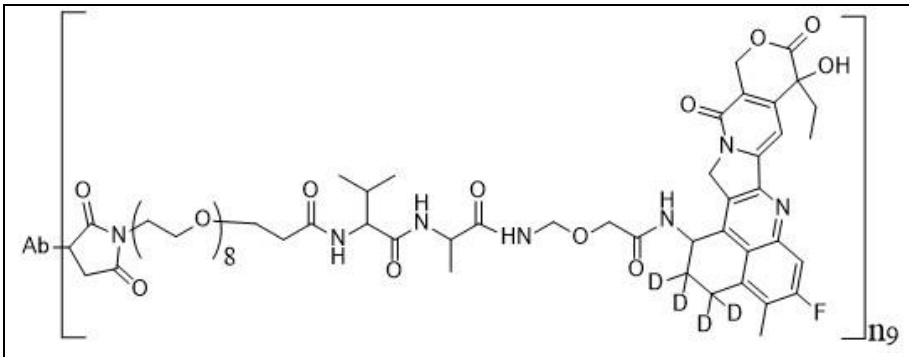
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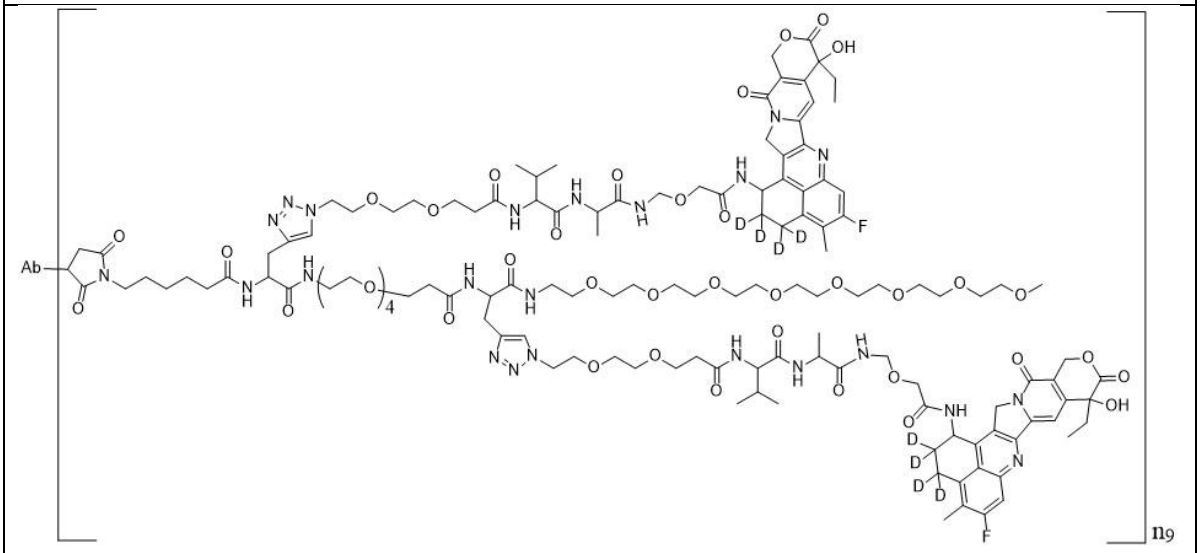
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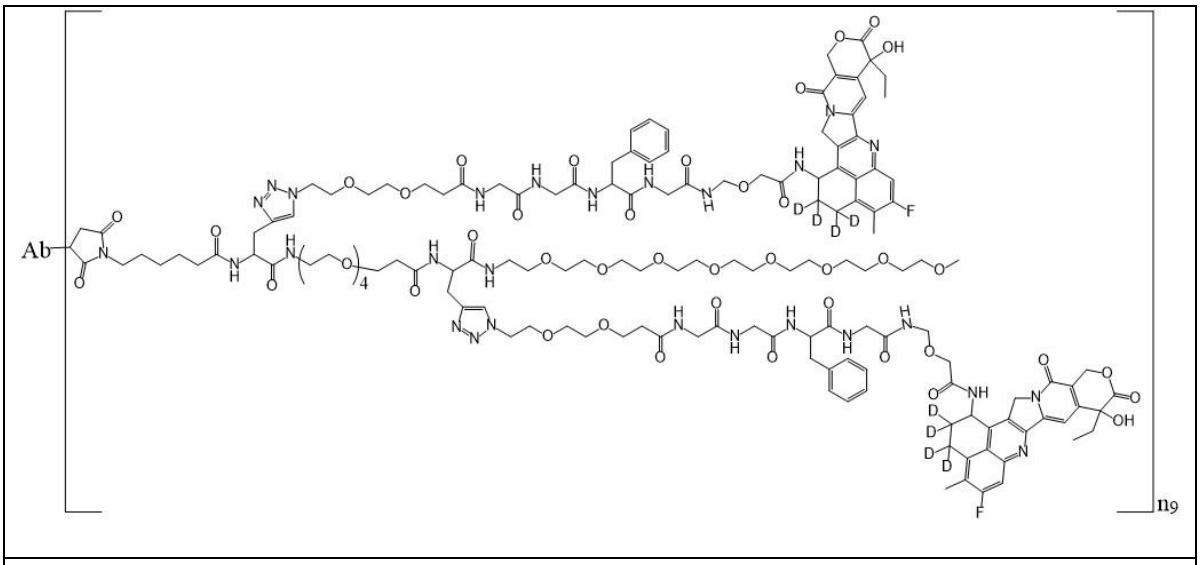
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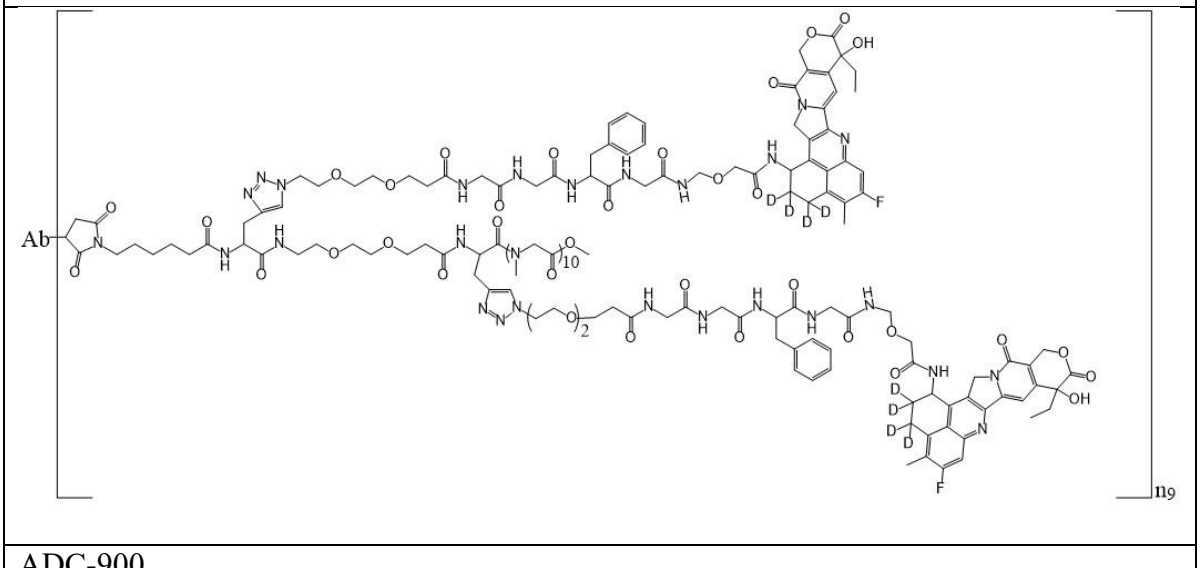
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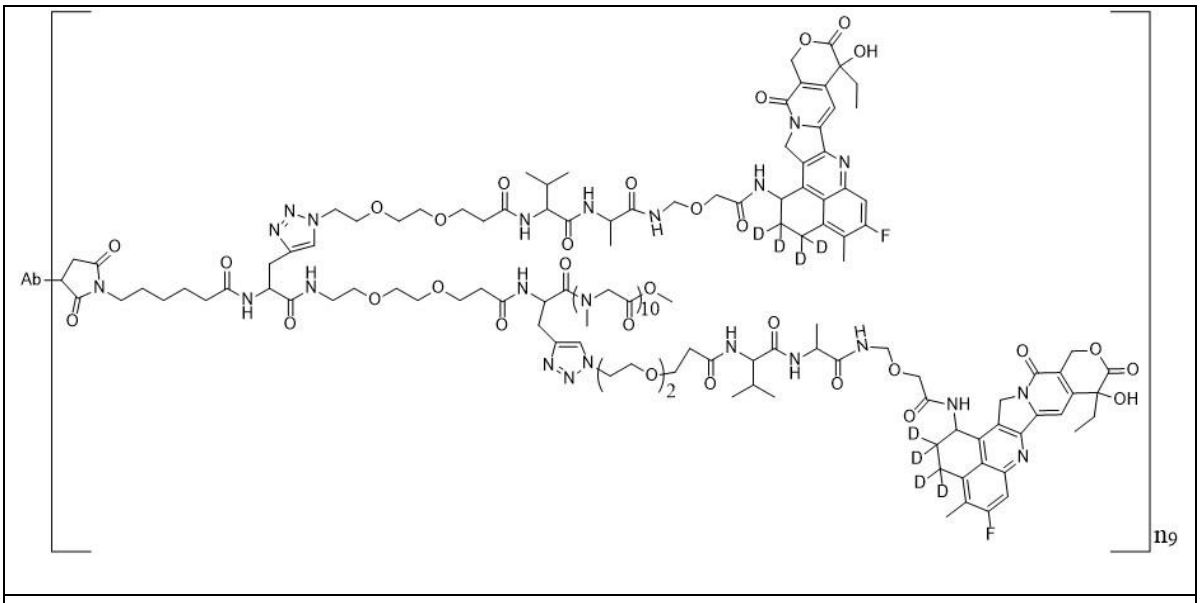
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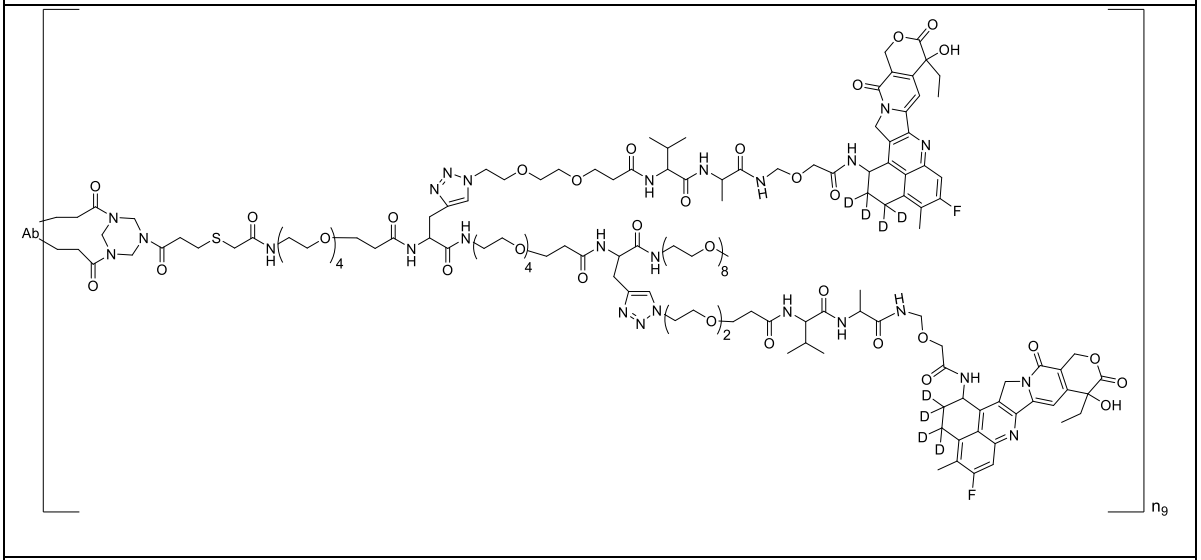
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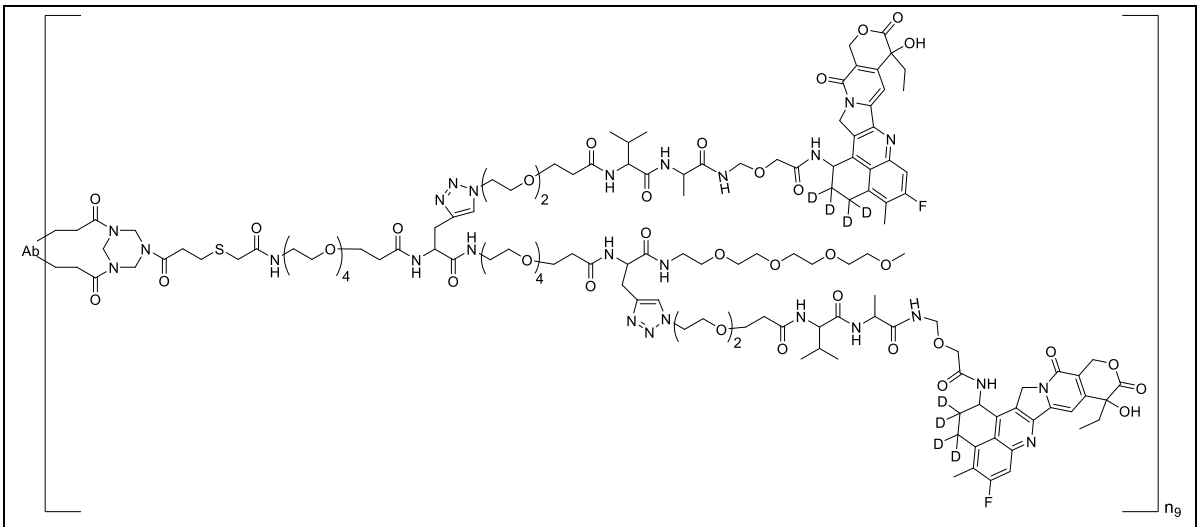
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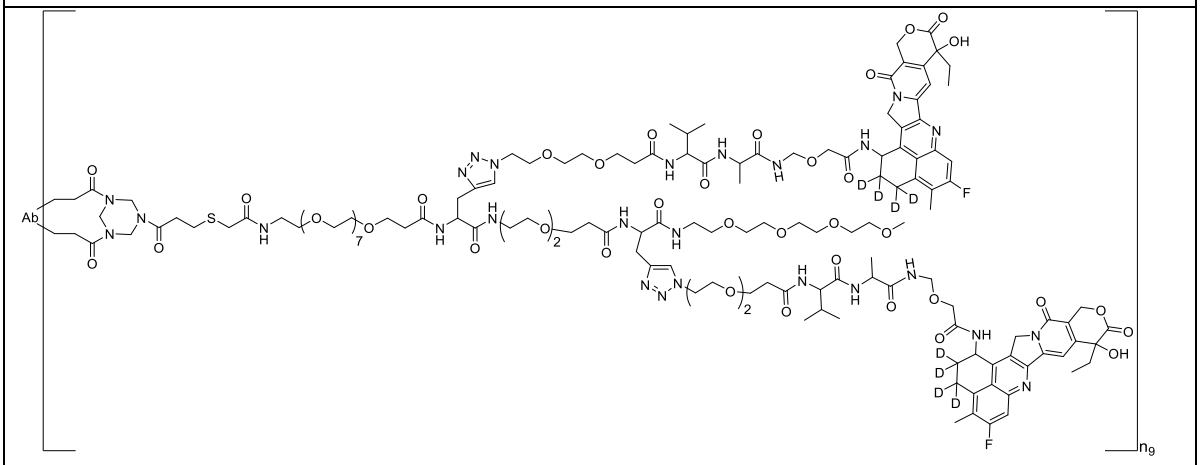
ADC-1000



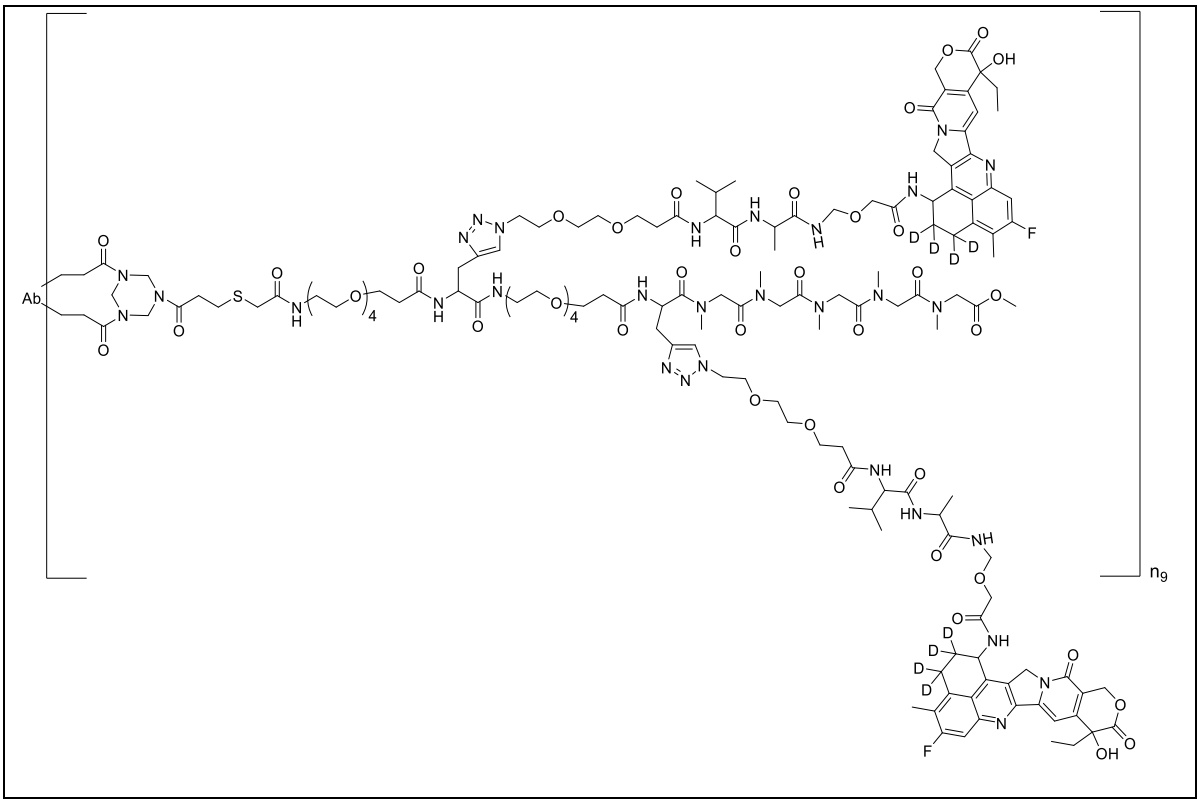
ADC-1100



ADC-1200

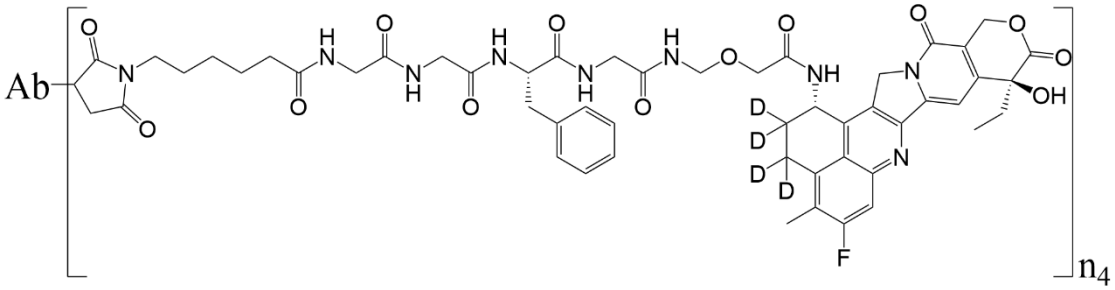


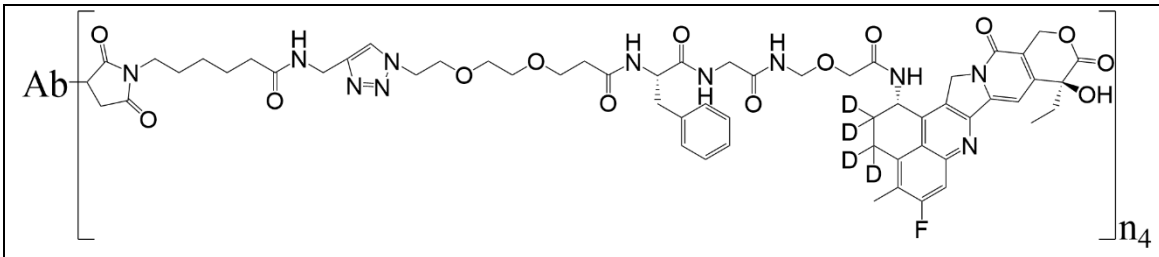
ADC-1300



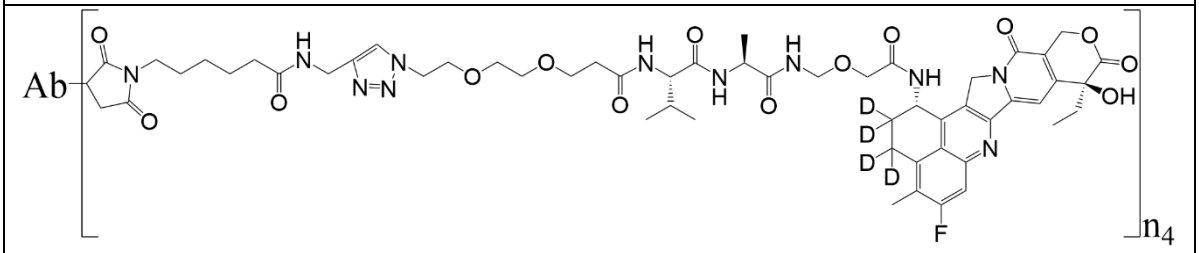
wherein Ab is a targeting moiety, and n_9 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8.

[Claim 103] The antibody drug conjugate according to claim 96, which has a structure selected from the group consisting of:

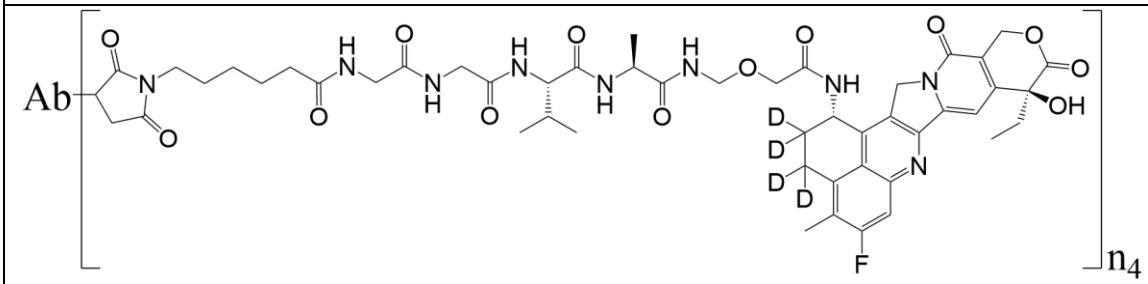
ADC-100a

ADC-200a



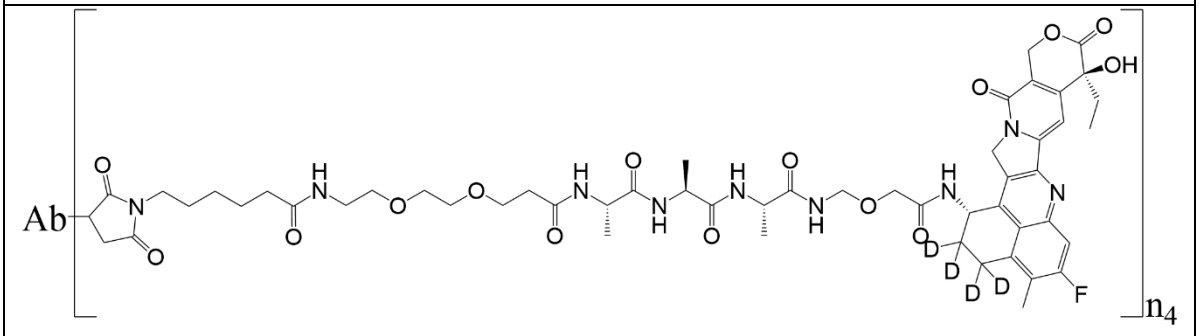
ADC-600a



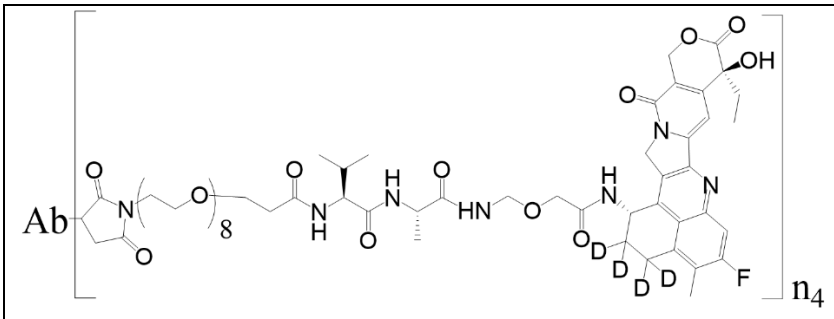
ADC-500a



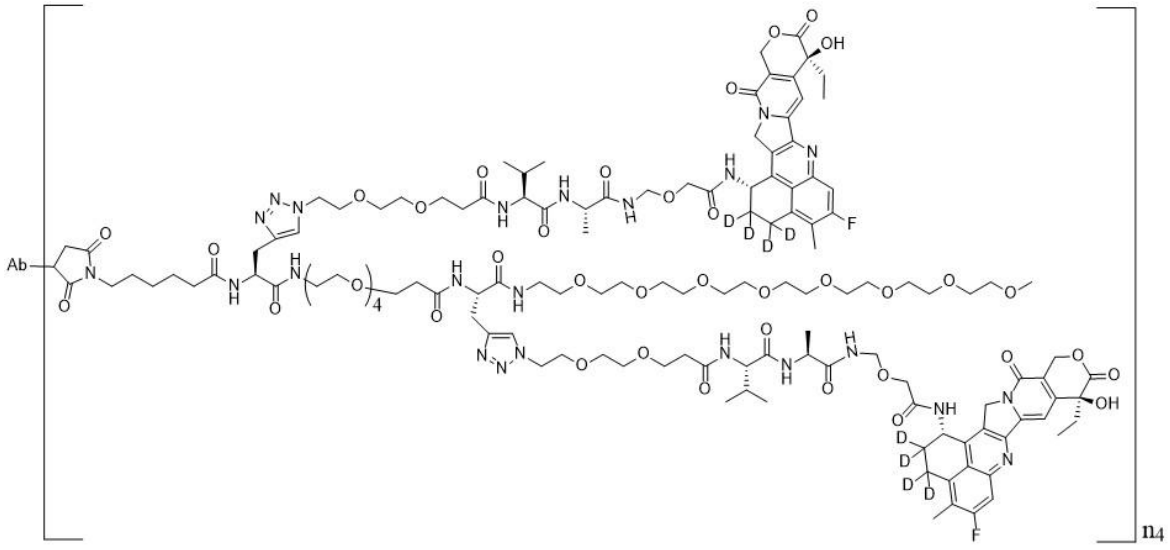
ADC-700a



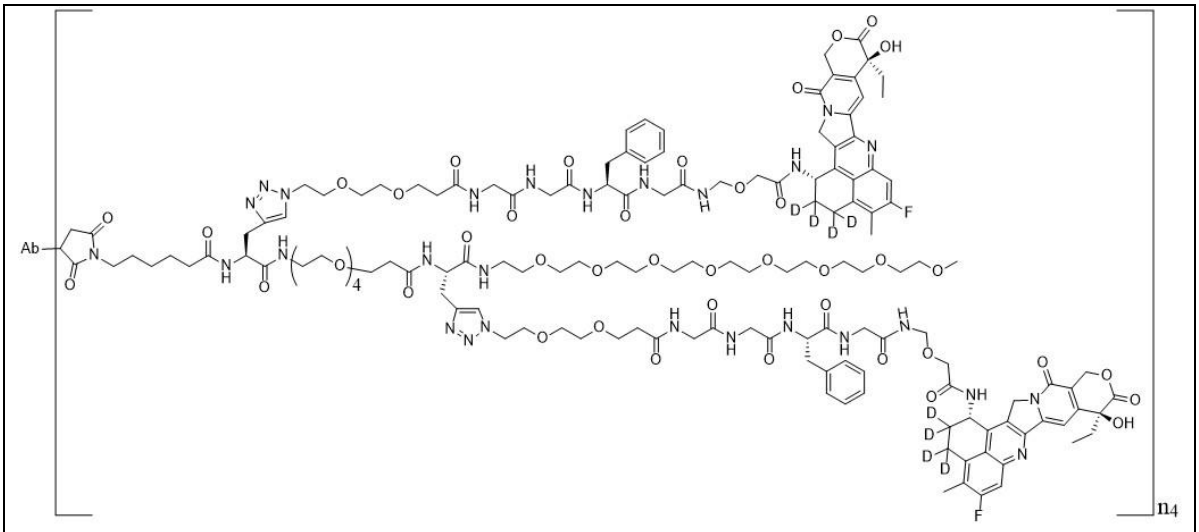
ADC-1400a



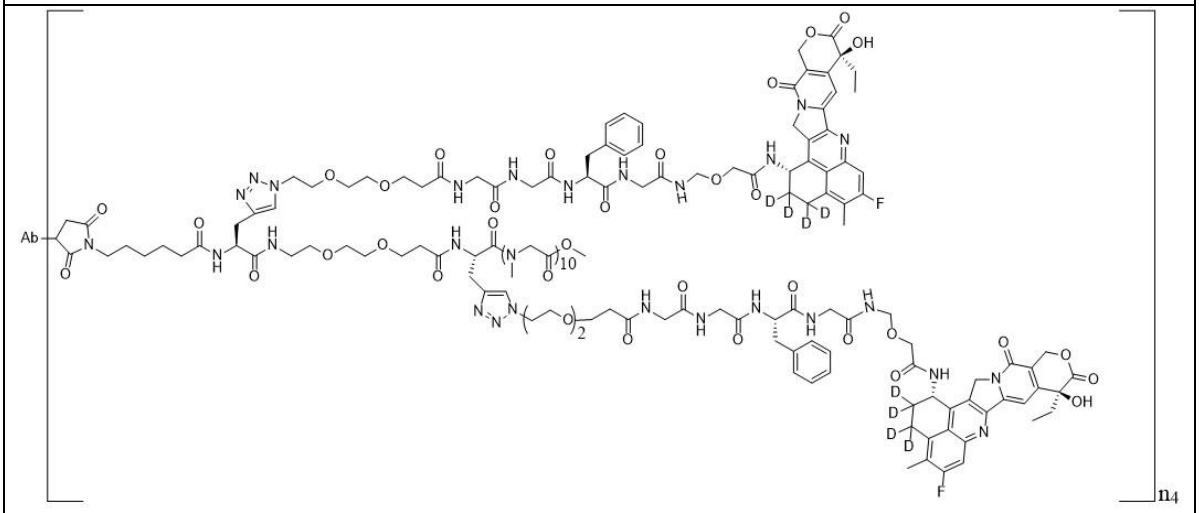
ADC-400a



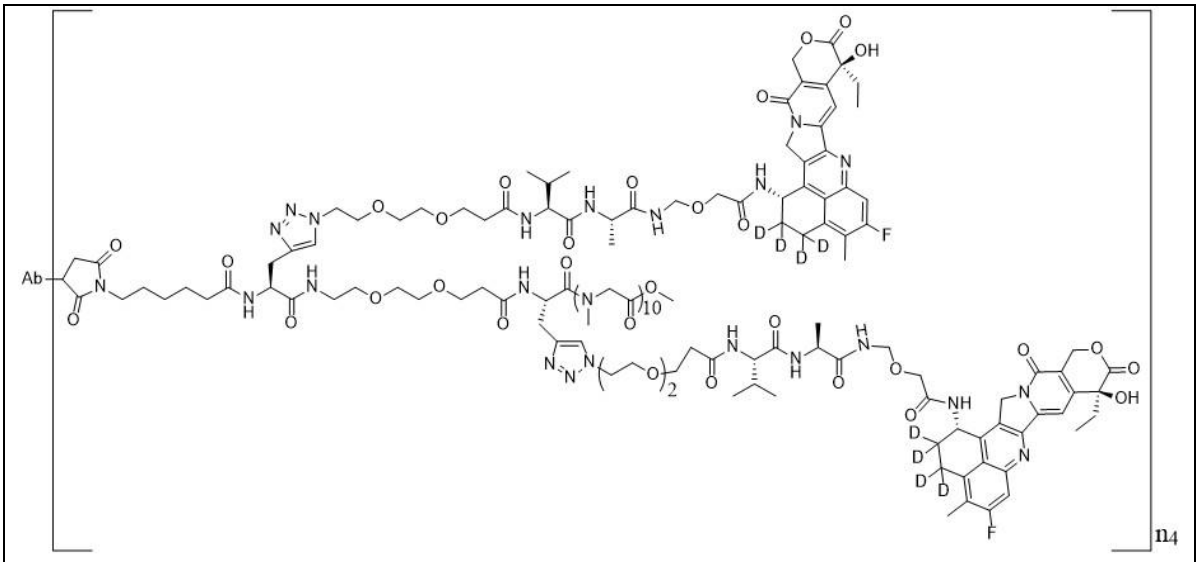
ADC-300a



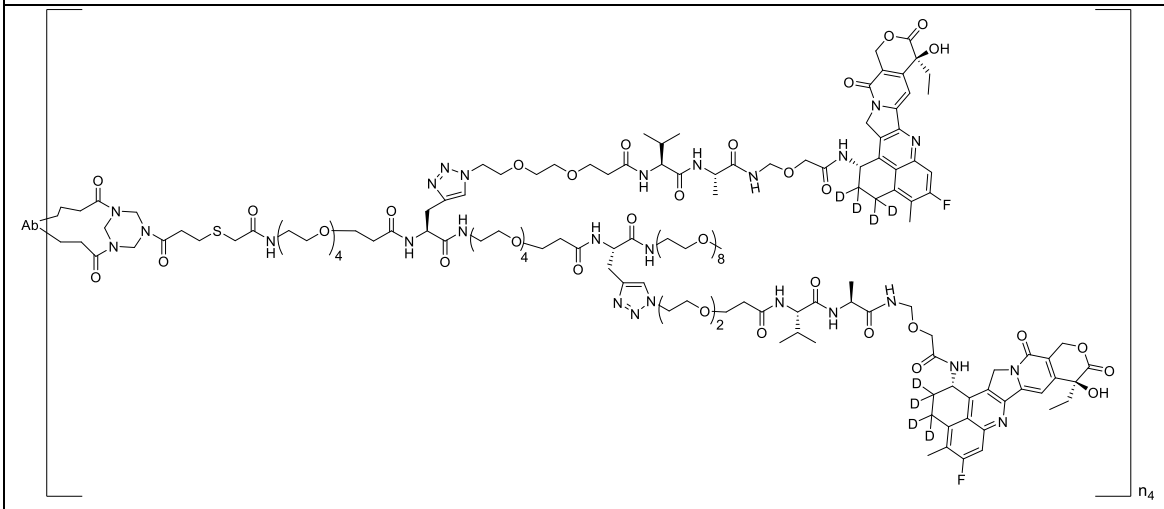
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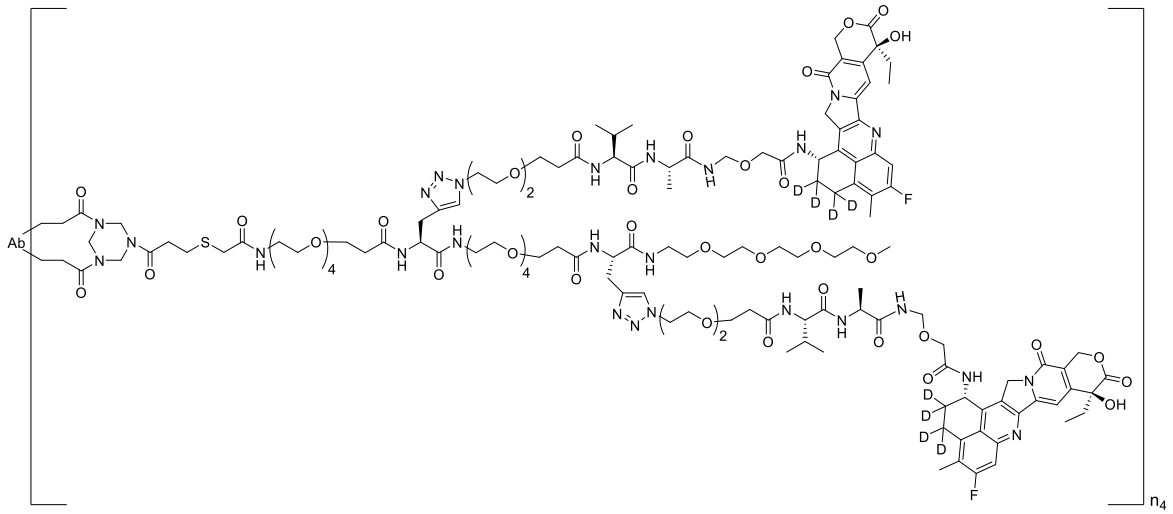
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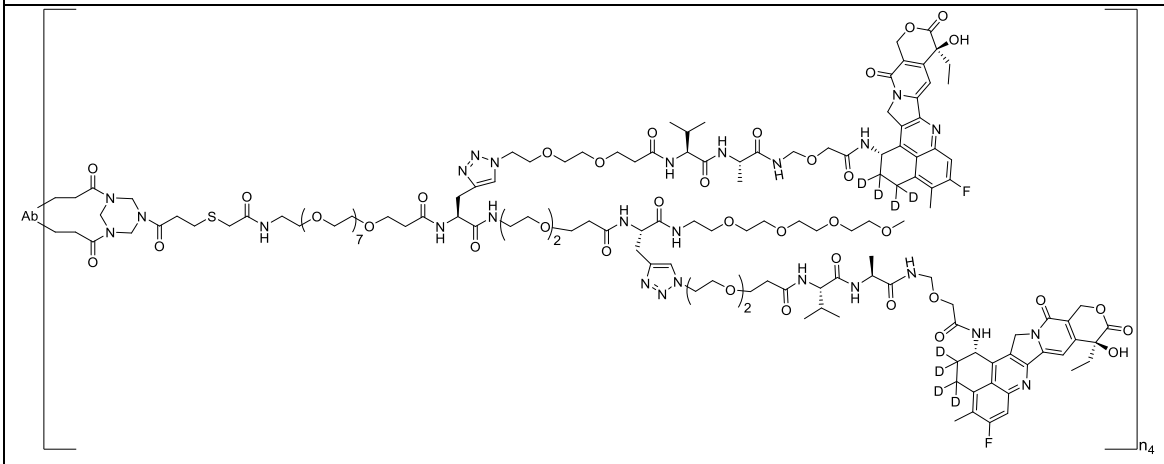
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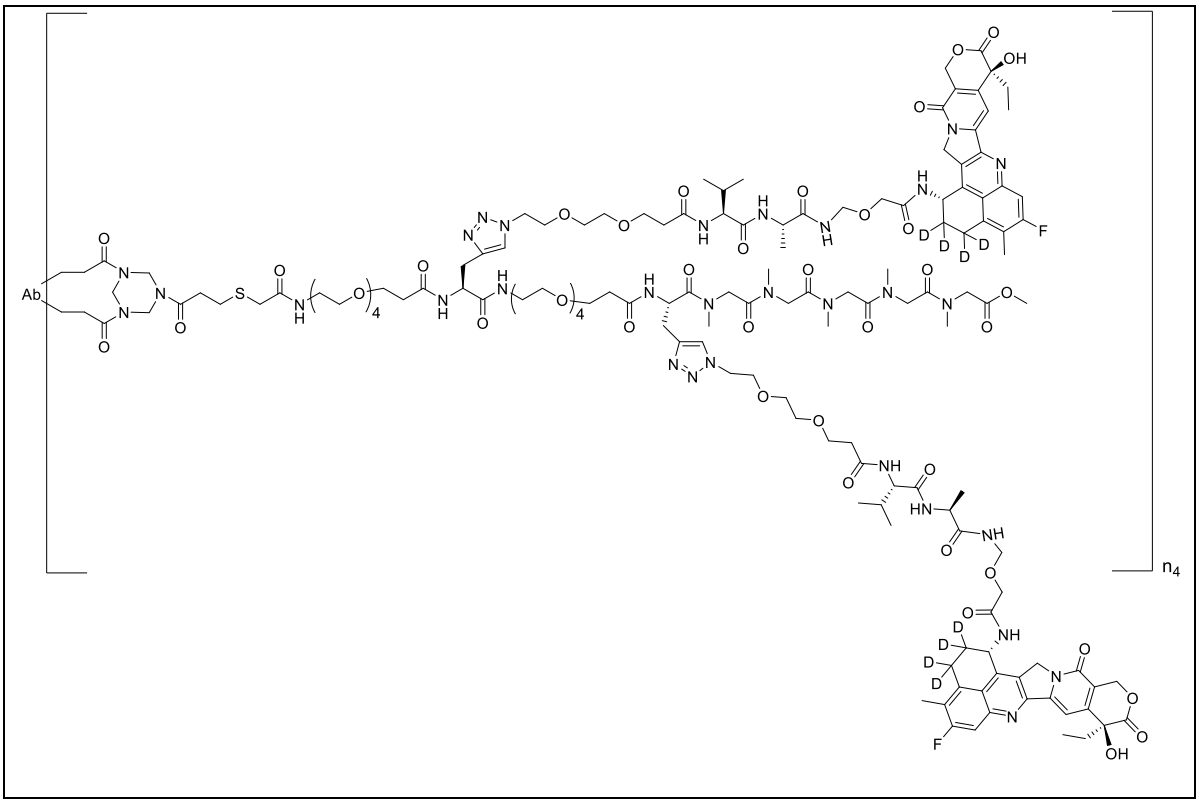
ADC-1100a



ADC-1200a



ADC-1300a



wherein Ab is a targeting moiety, and n_4 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8, wherein n_4 represents the number of the linker-payload moiety linked to Ab and the number is 1, 2, 3, 4, 5, 6, 7, or 8.

[Claim 104] The antibody drug conjugate according to claim 99, wherein the antibody drug conjugate has a structure of ADC-1300a; preferably, the targeting moiety Ab is an antibody or antigen binding fragment thereof targeting CDCP1; more preferably, the targeting moiety Ab is the antibody or the antigen binding fragment thereof according to any one according to claims 29 to 34.

[Claim 105] A pharmaceutical composition, comprising the antibody drug conjugate according to any one of claims 1 to 25 or 99 to 103 and/or the antibody or the antigen binding fragment thereof according to any one of claims 29 to 34, and a pharmaceutically acceptable carrier.

[Claim 106] Use of the antibody or the antigen binding fragment thereof according to any one of claims 29 to 34, the fusion protein according to claim 34, the polynucleotide according to claim 35, the nucleic acid construct according to claim 36 or 37, the antibody drug conjugate according to any one of claims 1 to 25 or 99 to 103, or the pharmaceutical composition according to claim

105 in the manufacture of a medicament for treating or preventing a cancer.

[Claim 107] The use according to claim 106, wherein the cancer is a CDCP1 positive cancer.

[Claim 108] The use according to claim 107, wherein the CDCP1 positive cancer is lung cancer or pancreatic cancer; further preferably the CDCP1 positive cancer is lung squamous cell carcinoma, lung adenocarcinoma, non-small cell lung cancer or pancreatic adenocarcinoma.

[Claim 109] A recombinant protein comprising the antibody or the antigen binding fragment thereof according to any one of claims 29 to 34.

[Claim 110] The recombinant protein according to claim 109, wherein the recombinant protein is a bispecific antibody or multispecific antibody.

[Claim 111] Use of the antibody or the antigen binding fragment thereof according to any one of claims 29 to 34 in the manufacture of a recombinant protein.