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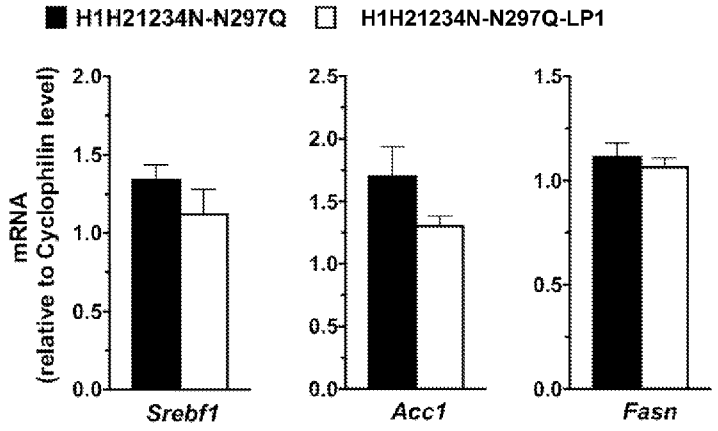

FIG. 21

(57) Abstract: Provided herein are antibodies and antigen-binding fragments that bind MSR1 and methods of use thereof. According to certain embodiments, the antibodies bind human MSR1 with high affinity. In certain embodiments, the antibodies bind MSR1 without blocking, or blocking less than 90%, of modified LDL binding to MSR1. In some embodiments, the antibodies bind cell surface expressed-MSR1 and are internalized. The antibodies of the invention may be fully human antibodies. The invention includes anti-MSR1 antibodies, or antigen-binding fragments thereof, conjugated to drugs or therapeutic compounds.

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[Continued on next page]

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ANTI-MSR1 ANTIBODIES AND METHODS OF USE THEREOF

[0001] This application claims priority to U.S. Provisional Application No. 62/669,276, filed May 9, 2018, U.S. Provisional Application No. 62/678,200, filed May 30, 2018, U.S. Provisional Application No. 62/769,946, filed November 20, 2018, U.S. Provisional Application No. 62/789,987, filed January 8, 2019, and U.S. Provisional Application No. 62/821,362, filed March 20, 2019, which are incorporated herein by reference in their entireties for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies, and antigen-binding fragments thereof, as well as antibody-drug conjugates of such antibodies, which specifically bind the trimeric membrane glycoprotein receptor (MSR1) and modulate MSR1 signal transduction, and methods of use thereof.

SEQUENCE LISTING

[0003] An official copy of the sequence listing is submitted concurrently with the specification via EFS-Web as a paper copy of an ASCII formatted sequence listing with a file name of 114581.00244_ST25.TXT, a creation date of May 30, 2018, and a size of about 165 kilobytes. The sequence listing contained in this paper copy of the ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

[0004] Macrophage scavenger receptor 1 (MSR1) is a single-pass, trimeric type II transmembrane glycoprotein pattern recognition receptor that mediates uptake of a series of negatively charged/polyanionic ligands, including modified low density lipoproteins (LDL) (Krieger, M. 1994. *Annu. Rev. Biochem.* 63:601-637; Platt, N. and S. Gordon. 2001. *J Clin Invest.* 108(5):649-654) and advanced glycation end products of bovine serum albumin (AGE--BSA) (Smedsrød *et al.* 1997. *Biochem J.* 322(Pt 2):567-573.) MSR1 receptors have been implicated in many macrophage-associated physiological and pathological processes including atherosclerosis, Alzheimer's disease, and host defense.

[0005] MSR1 expression was originally considered to be macrophage-specific. However, it has recently been demonstrated to be present on different classes of dendritic cells (Herber et al. 2010. *Nat. Med.* 16(8): 880–886). In addition, MSR1 appears to be expressed in endothelial cells and smooth muscle cells. It is internalized via coated pits at the cell surface and releases its ligand at acidic pH before being recycled back to the cell surface from the trans-Golgi

apparatus (Doi *et al.* 1994. *Journal of Biological Chemistry;* Mori, T. 1994. *Lab Invest.*). It promotes conversion of monocyte-derived macrophages into foam cells, which is a critical step for atherosclerosis progression.

[0006] MSR1 isoforms type 1 and type 2 are functional receptors and are able to mediate endocytosis of modified low density lipoproteins (LDLs). MSR1 isoform type 3 can bind modified LDL (acetyl-LDL) but appears to be incapable of internalizing the bound complex. However, MSR1 also binds to a wide variety of ligands other than modified LDLs. These ligands include beta-amyloid protein, molecular chaperones, extracellular matrix protein, advanced glycation end products, apoptotic cells, and activated B-cells.

[0007] Liver X Receptor (LXR) includes LXRα and LXRβ which are ligand-dependent transcription factors that control the expression of genes involved in cholesterol, lipid and glucose homeostasis, inflammation, and innate immunity. LXRα is highly expressed in liver, intestine, adipose tissue, and differentiated macrophages; and LXRβ is ubiquitously expressed. LXRs have various biological functions including (i) stimulating the expression of cholesterol transporters, for example, ABCA1 and ABCG1, both of which mediate cellular cholesterol efflux; and (ii) negatively regulating macrophage inflammatory gene expression via repression of NF-kB activation. LXRs have also been implicated in atherosclerosis, proliferative disorders, neurodegenerative disorders, and inflammation. Proliferative disorders include melanomas, lung cancer, oral squamous carcinoma, and prostate cancer. (Pencheva *et al.* 2004; Wu *et al.* 2015; Kaneko *et al.* 2015; Chuu *et al.* 2006) Neurodegenerative disorders include Alzheimer's disease and myelin gene expression. (Terwel *et al.* 2011; Sandoval-Hernandez *et al.* 2016; Meffre *et al.* 2014) Inflammation includes inflammatory bowel disease, ulcerative colitis, Crohn's disease, and arthritis. (Anderson *et al.* 2011; Huang *et al.* 2015; Cui *et al.* 2012). Macrophage LXRs are known to include anti-atherogenic activity. LXR agonists are believed to be capable of (i) inhibiting the initiation and delay the progression of atherosclerosis; (ii) mitigating atherosclerosis and stabilizing established atherosclerotic lesions; and (iii) reducing lesion macrophage content by apoptosis.

[0008] The therapeutic potential of small molecule LXR modulators is limited by, for example, undesired modulation of LXR at non-target cells and/or low bioavailability. Modulation of LXR at non-target cells can lead to undesirable side effects, and low bioavailability may manifest for myriad reasons including, without limitation, low solubility that further exacerbates poor therapeutic windows for treatment. The development of ADCs comprising LXR modulators would allow for target-specific modulation of LXR, thereby avoiding side-effects caused by off-target modulation of LXR. Furthermore, such ADCs would provide improved modulation of biological targets, improved bioavailability, and improved therapeutic window. Therefore, there

is a continuing need for effective treatments of, for example, metabolic diseases using antibody-drug conjugates of LXR modulators.

[0009] Glucocorticoids (GCs) are small molecule steroids that bind to glucocorticoid receptors (GRs) and are widely utilized in anti-inflammatory and immunosuppressive therapies. However, due to the ubiquitous expression of glucocorticoid receptors in many cell types, glucocorticoid treatments are compromised by toxicities to most organ systems. Thus, there is need for both novel glucocorticoids as well as novel therapies that minimize the side effects arising from glucocorticoid administration, particularly those arising from activating glucocorticoid receptors in non-target cells.

[0010] Rifamycins form a subclass of the ansamycin antibiotics family with an activity spectrum against gram-positive and gram-negative bacteria, and are commonly prescribed antimycobacterial drugs for the treatment of tuberculosis, leprosy and/or mycobacterium avium complex (MAC). The rifamycin group of antibiotics includes the "classic" rifamycin drugs as well as rifamycin derivatives such as rifampicin (or rifampin), rifabutin, rifapentine, rifalazil and rifaximin. The increasing occurrence of antibiotic resistant strains of bacteria (e.g., methicillin resitant *S. aureus* (MRSA), vancomycin resistant *S. aureus* (VRSA)), multi-drug resistant *M. tuberculosis*), particularly in nosocomial settings, is an ongoing problem. There is a need for more effective analogs of rifamycins. Rifamycins are moderate to potent inducers of the cytochrome P450 enzyme system (notably CYP3A4), which can lead to reduced bioavailability and enhanced clearance of some coadministered drugs undergoing metabolism by the cytochrome P450 enzyme system (notably CYP3A4). Such interactions may be delayed in onset but persist beyond rifamycin coadministration. Rifampin may also induce P-glycoprotein (P-gp) multidrug efflux transporters. There is a need for novel therapies that minimize the side effects arising from administration of rifamycins, particularly those arising from activation of the cytochrome P450 enzyme system.

[0011] As disclosed in this application, MSR antibodies may provide a means for specific targeting of therapeutic molecules such as LXR agonists, steroids and rifamycins to minimize unwanted side effects arising from systemic administration of such compounds.

SUMMARY

[0012] Provided herein are antibodies, antigen-binding fragments of antibodies, and antibody-drug conjugates thereof that bind the membrane glycoprotein receptor known as MSR1. The antibodies are useful, *inter alia*, for targeting cells that express MSR1, such as macrophage cells. The anti-MSR1 antibodies, and antigen-binding portions thereof, can be used alone in unmodified form, or can be included as part of an antibody-drug conjugate.

[0013] The antibodies disclosed herein can be full-length (for example, an IgG1 or IgG4 antibody) or can comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933).

[0014] Embodiments of anti-MSR1 antibodies disclosed herein are listed in Tables 4 and 5. Table 4 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-MSR1 antibodies. Table 5 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-MSR1 antibodies.

[0015] Provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0016] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0017] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 4 paired with any of the LCVR amino acid sequences listed in Table 4. Certain embodiments relate to antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-MSR1 antibodies listed in Table 4. In some embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of: 2/10, 23/42, 50/58; 90/98, and 282/290.

[0018] Provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0019] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0020] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0021] Provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0022] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0023] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 4 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0024] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 4 paired with any of the LCDR3 amino acid sequences listed in Table 4. Certain embodiments relate to antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-MSR1 antibodies listed in Table 4. In some embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of: 8/16, 40/48, 56/64; 96/104, and 288/296.

[0025] Provided herein are antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-MSR1 antibodies listed in Table 4. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set is selected from the group consisting of: 4-6-8-12-14-16; 36-38-40-44-46-48; 52-54-56-60-62-64; 92-94-96-100-102-104, and 284-286-288-292-294-296.

[0026] In a related embodiment, provided herein are antibodies, or antigen-binding fragments thereof that specifically bind MSR1, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-MSR1 antibodies listed in Table 4. For example, the present invention includes antibodies or antigen-binding fragments thereof that specifically bind MSR1, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of: 2/10, 23/42, 50/58, 90/98, and 282/290. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0027] Also provided herein are nucleic acid molecules encoding anti-MSR1 antibodies or portions thereof. For example, provided herein are nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0028] Also provided herein are nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0029] Also provided herein are nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0030] Also provided herein are nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0031] Also provided herein are nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0032] Also provided herein are nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0033] Also provided herein are nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0034] Also provided herein are nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 4; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0035] Also provided herein are nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-MSR1 antibodies listed in Table 4.

[0036] Also provided herein are nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-MSR1 antibodies listed in Table 4.

[0037] Also provided herein are nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 4, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 4. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 5, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-MSR1 antibody listed in Table 4.

[0038] Also provided herein are recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-MSR1 antibody. For example, embodiments include recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 4. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0039] Provided herein are anti-MSR1 antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites, may be useful for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In some embodiments, modification to provide an antibody lacking a fucose moiety present on the oligosaccharide chain, may be useful for example, to increase antibody dependent cellular cytotoxicity (ADCC) function. In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0040] In another aspect, provided herein is a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds MSR1 and a pharmaceutically acceptable carrier. In a related aspect, embodiments relate to a composition

which is a combination of an anti-MSR1 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-MSR1 antibody. Also provided herein are antibody-drug conjugates (ADCs) comprising an anti-MSR1 antibody conjugated to a drug or a therapeutic agent. Exemplary combination therapies, co-formulations, and ADCs involving the anti-MSR1 antibodies are disclosed elsewhere herein.

[0041] Also provided herein are antibody-drug conjugates (ADCs) comprising an anti-MSR1 antibody, or an MSR1 antigen-binding fragment thereof, conjugated to a drug or a therapeutic agent. Also provided herein are reactive linker-payloads useful for making the ADCs. Further provided herein are modified anti-MSR1 antibodies and modified MSR1 antigen-binding fragments useful for making the ADCs.

[0042] Also provided herein are therapeutic methods comprising administration of an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof, to a subject in need thereof. The therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof to the subject. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by targeting MSR1. In some embodiments, the disease or condition is a proliferative disease, a metabolic disease, inflammation, a neurodegenerative disease, or disease, disorder, or condition associated with glucocorticoid receptor signaling. In some embodiments the disease or condition is atherosclerosis. In some embodiments, the disease, disorder, or condition associated with glucocorticoid receptor signaling is an inflammatory disease, disorder, or condition. In some of such embodiments, the side effects associated with administration of the unconjugated steroid payload of said compound are reduced. Also provided herein are therapeutic methods comprising administration an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof, for the treatment and/or prevention of bacterial infection in a subject.

[0043] Provided herein is the use of an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof, described herein, for the treatment of any disease disorder or condition described herein.

[0044] Also provided herein are therapeutic methods for treating, attenuating, or ameliorating atherosclerosis, comprising administration of an anti-MSR1 antibody, an antigen-binding portion

of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof, to a subject in need thereof. The therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof to the subject.

[0045] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0046] **Figure 1** provides a scheme for the synthesis of **P1 and P2B**.

[0047] **Figure 2** provides a scheme for the synthesis of **LP1**.

[0048] **Figure 3** provides a scheme for the synthesis of **LP2**.

[0049] **Figure 4** provides a scheme for the synthesis of **LP5**.

[0050] **Figure 5** provides a scheme for the synthesis of **LP6**.

[0051] **Figure 6** provides a scheme for the synthesis of **LP18**.

[0052] **Figure 7** provides a scheme for the synthesis of **LP4**.

[0053] **Figure 8** provides a scheme for the synthesis of **LP11**.

[0054] **Figure 9** provides a scheme for the synthesis of **LP9**.

[0055] **Figure 10** provides a scheme for the synthesis of **LP12**.

[0056] **Figure 11** provides a scheme for the synthesis of **P3** and **P4**.

[0057] **Figure 12** provides a scheme for the synthesis of **LP3**.

[0058] **Figure 13** provides a scheme for the synthesis of **LP13**.

[0059] **Figure 14** provides a scheme for the synthesis of **LP14**.

[0060] **Figure 15** provides a scheme for the synthesis of **LP15**.

[0061] **Figure 16** provides a scheme for the synthesis of antibody-drug conjugates (ADCs).

[0062] **Figure 17** is a line graph illustrating percentage of dose-dependent cholesterol efflux in THP-1 macrophages for an exemplary MSR1 antibody-LXR conjugate, its unconjugated counterpart, an isotype control-steroid conjugate, and the corresponding free payload.

[0063] **Figure 18** provides a series of bar graphs illustrating the effect of an exemplary MSR1 antibody-LXR agonist conjugate and its unconjugated counterpart on serum lipid levels in a mouse model of atherosclerosis.

[0064] **Figure 19** provides a series of bar graphs illustrating the effect of an exemplary MSR1 antibody-LXR agonist conjugate and its unconjugated counterpart on lesion lipid area and macrophage (CD68) content in a mouse model of atherosclerosis.

[0065] **Figure 20** provides a series of bar graphs illustrating the effect of an exemplary MSR1 antibody-LXR agonist conjugate and its unconjugated counterpart on hepatic triglyceride and cholesterol levels in a mouse model of atherosclerosis.

[0066] **Figure 21** provides a series of bar graphs illustrating the effect of an exemplary MSR1 antibody-LXR agonist conjugate and its unconjugated counterpart on *de novo* lipogenesis in a mouse model of atherosclerosis.

[0067] **Figure 22** provides a scheme for the synthesis of Budesonide-spacers containing the reactive groups, suc-acid (compound 1c), carbamate analogues (1d, 1e), THP-analogues (1g and 1h), glucose analogues (1i and 1j), phosphate analogues (1k and 1l), and a commercial phosphate analogue (1m).

[0068] **Figure 23** provides a scheme for the synthesis of payloads, compounds, and bis-octahydrophenanthrene carboxamides **P3B – P9B**.

[0069] **Figure 24** provides a scheme for the synthesis of payloads, compounds, and bis-octahydrophenanthrene carboxamides **P10B – P11B**.

[0070] **Figure 25** provides a scheme for the synthesis of linkers and linker payloads **LP1B – LP5B**.

[0071] **Figure 26** provides a scheme for the synthesis of linkers and linker payload **LP6B**.

[0072] **Figure 27** provides a scheme for the synthesis of linkers and linker payload **LP7B**.

[0073] **Figure 28** provides a scheme for the synthesis of linkers and linker payload **LP8B**.

[0074] **Figure 29** provides a scheme for the synthesis of linkers and linker payload **LP9B**.

[0075] **Figure 30** provides a scheme for the synthesis of linkers and linker payloads **LP10B**, and **LP11B**.

[0076] **Figure 31** provides a scheme for the synthesis of payloads **12B**, linkers and linker payload **LP12B**.

[0077] **Figure 32** provides a scheme for the synthesis of cyclodextrin-azide **105a**.

[0078] **Figure 33** provides a scheme for the synthesis of azido-PEG_4-taurine **105b**.

[0079] **Figure 34** provides a scheme for the synthesis of maltose-azide **105c**.

[0080] **Figure 35** is a plot of the results of the *S. aureus* growth inhibition assay conducted with rifamycin analogs.

[0081] **Figure 36** is a bar graph of the results of the *S. aureus* intracellular killing assay conducted with rifamycin analogs.

[0082] **Figure 37** is a plot of the results of the *S. aureus* intracellular killing assay conducted with rifamycin analogs.

DETAILED DESCRIPTION

[0083] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0084] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, *etc.*).

[0085] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[0086] The expressions "MSR1," "hMSR1" and the like, as used herein, refer to the human single-pass, trimeric type II transmembrane glycoprotein pattern recognition receptor comprising (i) the amino acid sequence as set forth in NCBI accession No. NP_002436.1, (ii) the amino acid sequence as set forth in NCBI accession No. NP_619729.1, and/or (iii) the amino acid sequence as set forth in NCBI accession No. NP_619730.1, which represent the various types and isoforms of class A macrophage scavenger receptors. The expression "MSR1" includes both monomeric and multimeric MSR1 molecules. As used herein, the expression "monomeric human MSR1" means a MSR1 protein or portion thereof that does not contain or possess any multimerizing domains and that exists under normal conditions as a single MSR1 molecule

without a direct physical connection to another MSR1 molecule. An exemplary monomeric MSR1 molecule is the molecule referred to herein as "His-hMSR1" comprising the amino acid sequence of SEQ ID NO: 393 (*see*, *e.g.*, Example 3, herein).

[0087] All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "MSR1" means human MSR1 unless specified as being from a non-human species, *e.g.*, "mouse MSR1," "monkey MSR1," *etc.*

[0088] As used herein, the expression "cell surface-expressed MSR1" means one or more MSR1 protein(s), or the extracellular domain thereof, that is/are expressed on the surface of a cell *in vitro* or *in vivo*, such that at least a portion of a MSR1 protein is exposed to the extracellular side of the cell membrane and is accessible to an antigen-binding portion of an antibody. A "cell surface-expressed MSR1" can comprise or consist of a MSR1 protein expressed on the surface of a cell which normally expresses MSR1 protein. Alternatively, "cell surface-expressed MSR1" can comprise or consist of MSR1 protein expressed on the surface of a cell that normally does not express human MSR1 on its surface but has been artificially engineered to express MSR1 on its surface.

[0089] As used herein, the expression "anti-MSR1 antibody" includes monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds MSR1 and a second arm that binds a second (target) antigen, wherein the anti-MSR1 arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Table 4 herein. The expression "anti-MSR1 antibody" also includes antibody-drug conjugates (ADCs) comprising an anti-MSR1 antibody or antigen-binding portion thereof conjugated to a drug or a therapeutic agent. The expression "anti-MSR1 antibody" also includes antibody-radionuclide conjugates (ARCs) comprising an anti-MSR1 antibody or antigen-binding portion thereof conjugated to a radionuclide.

[0090] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, MSR1). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises

one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-MSR1 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0091] The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, *etc.*

[0092] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0093] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework

sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0094] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0095] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0096] The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See*, *e.g.*, U.S. Patent

Nos 5,500,362 and 5,821,337, and Clynes *et al.* (1998) Proc. Natl. Acad. Sci. (USA) *95*:652-656). The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0097] In certain embodiments, the anti-MSR1 antibodies disclosed herein are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0098] The antibodies disclosed herein may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0099] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain

(half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0100] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. Embodiments disclosed herein encompass antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0101] The antibodies disclosed herein may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0102] The anti-MSR1 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. Embodiments include antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the

residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, *etc.* Antibodies and antigen-binding fragments obtained in this general manner are encompassed within embodiments disclosed herein.

[0103] Embodiments also include anti-MSR1 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, embodiments include anti-MSR1 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 4 herein.

[0104] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0105] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%,

97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0106] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0107] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such

as homologous polypeptides from different species of organisms or between a wild type protein and a mutant thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

[0108] When referring to the compounds provided herein, the following terms have the following meanings unless indicated otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. In the event that there is a plurality of definitions for a term provided herein, these Definitions prevail unless stated otherwise.

[0109] The terms "a" or "an," as used in herein means one or more, unless context clearly dictates otherwise.

[0110] As used herein, "alkyl" refers to a monovalent and saturated hydrocarbon radical moiety. Alkyl is optionally substituted and can be linear, branched, or cyclic, *i.e.*, cycloalkyl. Alkyl includes, but is not limited to, those radicals having 1-20 carbon atoms, *i.e.*, C_{1-20} alkyl; 1-12 carbon atoms, *i.e.*, C_{1-12} alkyl; 1-8 carbon atoms, *i.e.*, C_{1-8} alkyl; 1-6 carbon atoms, *i.e.*, C_{1-6} alkyl; and 1-3 carbon atoms, *i.e.*, C_{1-3} alkyl. Examples of alkyl moieties include, but are not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, i-butyl, a pentyl moiety, a hexyl moiety, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. A pentyl moiety includes, but is not limited to, n-pentyl and i-pentyl. A hexyl moiety includes, but is not limited to, n-hexyl.

[0111] As used herein, "alkylene" refers to a divalent alkyl group. Unless specified otherwise, alkylene includes, but is not limited to, 1-20 carbon atoms. The alkylene group is optionally substituted as described herein for alkyl. In some embodiments, alkylene is unsubstituted. In some embodiments, alkylene is a divalent branched alkyl group.

[0112] The term "amino" means $-NH_2$.

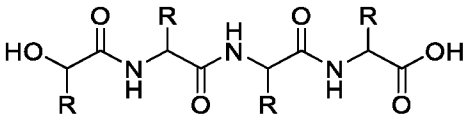
[0113] The term "alkylamino," as used herein, and unless otherwise specified, refers to the group $-NHR'$ where R' is C_{1-10}alkyl, as defined herein. In some or any embodiments, the alkylamino is C_{1-6}alkylamino.

[0114] The term "dialkylamino," as used herein, and unless otherwise specified, refers to the group –NR′R′ where each R′ is independently C_{1-10}alkyl, as defined herein. In some or any embodiments, the dialkylamino is di-C_{1-6}alkylamino.

[0115] The term "aminoalkyl," as used herein, and unless otherwise specified, refers to an alkyl group, as defined herein, which is substituted with one or more amino groups. In some or any embodiments, the aminoalkyl is an alkyl group substituted with one –NH_2 group (e.g., –R′(NH_2) where R′ is -C_{1-10}alkyl, as defined herein). In some or any embodiments, the aminoalkyl is an alkyl group substituted with two –NH_2 groups. In some embodiments, "aminoalkyl" is aminoC_{1-6}alkyl

[0116] An "alkylaminoalkyl," as used herein, and unless otherwise specified, refers to an alkyl group, as defined herein, which is substituted with one or more alkylamino groups as defined herein. In some embodiments, an "alkylaminoalkyl" is C_{1-6}alkylaminoC_{1-6}alkyl. In some embodiments, each alkyl in alkylaminoalkyl is independently selected.

[0117] A "dialkylaminoalkyl" as used herein, and unless otherwise specified, refers to an alkyl group, as defined herein, which is substituted with one or more dialkylamino groups as defined herein. In some embodiments, "dialkylaminoalkyl" is di-C_{1-6}alkylaminoC_{1-6}alkyl. In some embodiments, each alkyl in dialkylaminoalkyl is independently selected.

[0118] As used herein, the term "O-amino acid" or "HO-amino acid" designates an amino acid wherein the native amino group at the N-terminus of an amino acid or an amino acid sequence has been replaced with an oxygen or hydroxyl group, respectively. For example, "O-AAAA" or "HO-AAAA" is intended to designate an amino acid sequence (AAAA) wherein the native amino group at the N-terminus has been replaced with an oxygen or hydroxyl group, respectively (e.g.,  , where each R is an amino acid side chain). Similarly, the terms "O-amino acid residue" or "HO-amino acid residue" refers to the chemical moiety within a compound that remains after a chemical reaction. For example, "O-amino acid residue" or "HO-amino acid residue" refers to the product of an amide coupling or peptide coupling of an O-amino acid or a HO-amino acid to a suitable coupling partner; wherein, for example, a water molecule is expelled after the amide or peptide coupling of the O-amino acid or a HO-amino acid, resulting in the product having the O-amino acid residue or a HO-amino acid residue incorporated therein.

[0119] Designation of an amino acid or amino acid residue without specifying its stereochemistry is intended to encompass the L form of the amino acid, the D form of the amino acid, or a racemic mixture thereof.

[0120] As used herein, "haloalkyl" refers to alkyl, as defined above, wherein the alkyl includes at least one substituent selected from a halogen, for example, fluorine (F), chlorine (Cl), bromine (Br), or iodine (I). Examples of haloalkyl include, but are not limited to, $-CF_3$, $-CH_2CF_3$, $-CCl_2F$, and $-CCl_3$.

[0121] As used herein, "alkenyl" refers to a monovalent hydrocarbon radical moiety containing at least two carbon atoms and one or more non-aromatic carbon-carbon double bonds. Alkenyl is optionally substituted and can be linear, branched, or cyclic. Alkenyl includes, but is not limited to, those radicals having 2-20 carbon atoms, *i.e.*, C_{2-20} alkenyl; 2-12 carbon atoms, *i.e.*, C_{2-12} alkenyl; 2-8 carbon atoms, *i.e.*, C_{2-8} alkenyl; 2-6 carbon atoms, *i.e.*, C_{2-6} alkenyl; and 2-4 carbon atoms, *i.e.*, C_{2-4} alkenyl. Examples of alkenyl moieties include, but are not limited to vinyl, propenyl, butenyl, and cyclohexenyl.

[0122] As used herein, "alkynyl" refers to a monovalent hydrocarbon radical moiety containing at least two carbon atoms and one or more carbon-carbon triple bonds. Alkynyl is optionally substituted and can be linear, branched, or cyclic. Alkynyl includes, but is not limited to, those radicals having 2-20 carbon atoms, *i.e.*, C_{2-20} alkynyl; 2-12 carbon atoms, *i.e.*, C_{2-12} alkynyl; 2-8 carbon atoms, *i.e.*, C_{2-8} alkynyl; 2-6 carbon atoms, *i.e.*, C_{2-6} alkynyl; and 2-4 carbon atoms, *i.e.*, C_{2-4} alkynyl. Examples of alkynyl moieties include, but are not limited to ethynyl, propynyl, and butynyl.

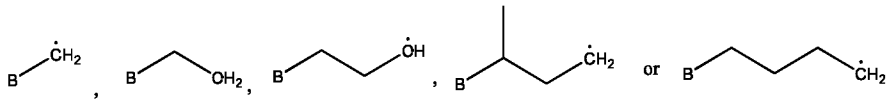
[0123] As used herein, "alkoxy" refers to a monovalent and saturated hydrocarbon radical moiety wherein the hydrocarbon includes a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*, CH_3CH_2-O· for ethoxy. Alkoxy substituents bond to the compound which they substitute through this oxygen atom of the alkoxy substituent. Alkoxy is optionally substituted and can be linear, branched, or cyclic, *i.e.*, cycloalkoxy. Alkoxy includes, but is not limited to, those having 1-20 carbon atoms, *i.e.*, C_{1-20} alkoxy; 1-12 carbon atoms, *i.e.*, C_{1-12} alkoxy; 1-8 carbon atoms, *i.e.*, C_{1-8} alkoxy; 1-6 carbon atoms, *i.e.*, C_{1-6} alkoxy; and 1-3 carbon atoms, *i.e.*, C_{1-3} alkoxy. Examples of alkoxy moieties include, but are not limited to methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, i-butoxy, a pentoxy moiety, a hexoxy moiety, cyclopropoxy, cyclobutoxy, cyclopentoxy, and cyclohexoxy.

[0124] As used herein, "haloalkoxy" refers to alkoxy, as defined above, wherein the alkoxy includes at least one substituent selected from a halogen, *e.g.*, F, Cl, Br, or I.

[0125] As used herein, "aryl" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms. Aryl is optionally substituted and can be monocyclic or polycyclic, *e.g.*, bicyclic or tricyclic. Examples of aryl moieties include, but are not limited to, those having 6 to 20 ring carbon atoms, *i.e.*, C_{6-20} aryl; 6 to 15 ring carbon atoms, *i.e.*,

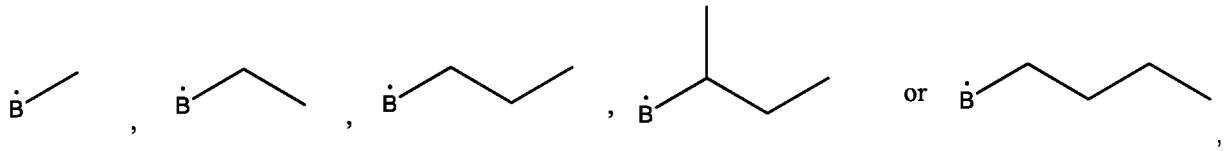
C_{6-15} aryl, and 6 to 10 ring carbon atoms, *i.e.*, C_{6-10} aryl. Examples of aryl moieties include, but are not limited to phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, and pyrenyl.

[0126] As used herein, "arylalkyl" refers to a monovalent moiety that is a radical of an alkyl compound, wherein the alkyl compound is substituted with an aromatic substituent, *i.e.*, the aromatic compound includes a single bond to an alkyl group and wherein the radical is localized on the alkyl group. An arylalkyl group bonds to the illustrated chemical structure via the alkyl group. An arylalkyl can be represented by the structure, *e.g.*,

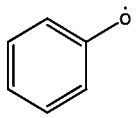


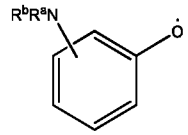
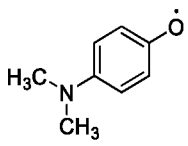
, wherein B is an aromatic moiety, *e.g.*, phenyl. Arylalkyl is optionally substituted, *i.e.*, the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of arylalkyl include, but are not limited to, benzyl.

[0127] As used herein, "alkylaryl" refers to a monovalent moiety that is a radical of an aryl compound, wherein the aryl compound is substituted with an alkyl substituent, *i.e.*, the aryl compound includes a single bond to an alkyl group and wherein the radical is localized on the aryl group. An alkylaryl group bonds to the illustrated chemical structure via the aryl group. An alkylaryl can be represented by the structure, *e.g.*,



wherein B is an aromatic moiety, *e.g.*, phenyl. Alkylaryl is optionally substituted, *i.e.*, the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of alkylaryl include, but are not limited to, toluyl.

[0128] As used herein, "aryloxy" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms and wherein the ring is substituted with an oxygen radical, *i.e.*, the aromatic compound includes a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*,  for phenoxy. Aryloxy substituents bond to the compound which they substitute through this oxygen atom. Aryloxy is optionally substituted. Aryloxy includes, but is not limited to, those radicals having 6 to 20 ring carbon atoms, *i.e.*, C_{6-20} aryloxy; 6 to 15 ring carbon atoms, *i.e.*, C_{6-15} aryloxy, and 6 to 10 ring carbon atoms, *i.e.*, C_{6-10} aryloxy. Examples of aryloxy moieties include, but are not limited to phenoxy, naphthoxy, and anthroxy.

[0129] As used herein, "R^aR^bN-aryloxy" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms and wherein the ring is substituted with at least one R^aR^bN – substituent and at least one oxygen radical, *i.e.*, the aromatic compound includes a single bond to an R^aR^bN - substituent and a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*,  . R^aR^bN -aryloxy substituents bond to the compound which they substitute through this oxygen atom. R^aR^bN -aryloxy is optionally substituted. R^aR^bN -aryloxy includes, but is not limited to, those having 6 to 20 ring carbon atoms, for example, $C_{6\text{-}20}$ (R^aR^bN)n-aryloxy, 6 to 15 ring carbon atoms, for example, $C_{6\text{-}15}$ (R^aR^bN)n-aryloxy, and 6 to 10 ring carbon atoms, for example, $C_{6\text{-}10}$ (R^aR^bN)n-aryloxy, wherein n represents the number of R^aR^bN – substituents. An example of an R^aR^bN -aryloxy moiety includes, but is not limited to 4-(dimethylamino)-phenoxy,  .

[0130] As used herein, "arylene" refers to a divalent moiety of an aromatic compound wherein the ring atoms are only carbon atoms. Arylene is optionally substituted and can be monocyclic or polycyclic, *e.g.*, bicyclic or tricyclic. Examples of arylene moieties include, but are not limited to those having 6 to 20 ring carbon atoms, *i.e.*, $C_{6\text{-}20}$ arylene; 6 to 15 ring carbon atoms, *i.e.*, $C_{6\text{-}15}$ arylene, and 6 to 10 ring carbon atoms, *i.e.*, $C_{6\text{-}10}$ arylene.

[0131] As used herein, "heteroalkyl" refers to an alkyl in which one or more carbon atoms are replaced by heteroatoms. As used herein, "heteroalkenyl" refers to an alkenyl in which one or more carbon atoms are replaced by heteroatoms. As used herein, "heteroalkynyl" refers to an alkynyl in which one or more carbon atoms are replaced by heteroatoms. Suitable heteroatoms include, but are not limited to, nitrogen, oxygen, and sulfur atoms. Heteroalkyl is optionally substituted. Examples of heteroalkyl moieties include, but are not limited to, aminoalkyl, sulfonylalkyl, and sulfinylalkyl. Examples of heteroalkyl moieties also include, but are not limited to, methylamino, methylsulfonyl, and methylsulfinyl.

[0132] As used herein, "heteroaryl" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms contain carbon atoms and at least one oxygen, sulfur, nitrogen, or phosphorus atom. Examples of heteroaryl moieties include, but are not limited to those having 5 to 20 ring atoms; 5 to 15 ring atoms; and 5 to 10 ring atoms. Heteroaryl is optionally substituted.

[0133] As used herein, "heteroarylene" refers to an arylene in which one or more ring atoms of the aromatic ring are replaced with an oxygen, sulfur, nitrogen, or phosphorus atom. Heteroarylene is optionally substituted.

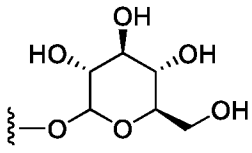
[0134] As used herein, "heterocycloalkyl" refers to a cycloalkyl in which one or more carbon atoms are replaced by heteroatoms. Suitable heteroatoms include, but are not limited to, nitrogen, oxygen, and sulfur atoms. Heterocycloalkyl is optionally substituted. Examples of heterocycloalkyl moieties include, but are not limited to, morpholinyl, piperidinyl, tetrahydropyranyl, pyrrolidinyl, imidazolidinyl, oxazolidinyl, thiazolidinyl, dioxolanyl, dithiolanyl, oxanyl, or thianyl.

[0135] As used herein, "N-containing heterocycloalkyl," refers to a cycloalkyl in which one or more carbon atoms are replaced by heteroatoms and wherein at least one heteroatom is a nitrogen atom. Suitable heteroatoms in addition to nitrogen, include, but are not limited to oxygen and sulfur atoms. N-containing heterocycloalkyl is optionally substituted. Examples of N-containing heterocycloalkyl moieties include, but are not limited to, morpholinyl, piperidinyl, pyrrolidinyl, imidazolidinyl, oxazolidinyl, or thiazolidinyl.

[0136] As used herein, "optionally substituted," when used to describe a radical moiety, for example, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted arylene, and optionally substituted heteroarylene, means that such moiety is optionally bonded to one or more substituents. Examples of such substituents include, but are not limited to, halo, cyano, nitro, optionally substituted haloalkyl, azido, epoxy, optionally substituted heteroaryl, optionally substituted heterocycloalkyl, $-OR^A$, $-SR^A$, $-NR^AR^B$, $-C(O)R^A$, $-C(O)OR^A$, $-C(O)NR^AR^B$, $-NR^CC(O)R^A$, $-C(NH)NR^AR^B$, $-NR^CC(NH)NR^AR^B$, $-S(O)R^A$, $-S(O)_2R^A$, , , or , wherein R^A, R^B, and R^C are, independently at each occurrence, a hydrogen atom, alkyl, alkenyl, alkynyl, aryl, alkylaryl, arylalkyl, heteroalkyl, heteroaryl, or heterocycloalkyl, or R^A and R^B together with the atoms to which they are bonded, form a saturated or unsaturated carbocyclic ring, wherein the ring is optionally substituted, and wherein one or more ring atoms is optionally replaced with a heteroatom. In certain embodiments, when a radical moiety is optionally substituted with an optionally substituted heteroaryl, optionally substituted heterocycloalkyl, or optionally substituted saturated or unsaturated carbocyclic ring, the substituents on the optionally substituted heteroaryl, optionally substituted heterocycloalkyl, or optionally substituted saturated or unsaturated carbocyclic ring, if they are substituted, are not substituted with substituents which

are further optionally substituted with additional substituents. In some embodiments, when a group described herein is optionally substituted, the substituent bonded to the group is unsubstituted unless otherwise specified.

[0137] As used herein, "O-glucose" refers to a monovalent moiety attached via an exocyclic glucose oxygen atom. Suitable O-glucose moieties include, without limitation,



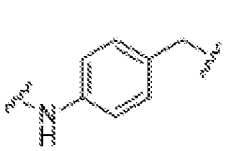
, and the like.

[0138] As used herein, "O-PEG_n" refers to a monovalent moiety attached via the terminal oxygen atom, where **n** is from 1 to 100. For example, when **n** is 1, then O-PEG**n** is –O–CH_2CH_2OH; when **n** is two, then O-PEG**n** is –O–CH_2CH_2O–CH_2CH_2OH; and when **n** is three, then O-PEG**n** is –O–CH_2CH_2O–CH_2CH_2O–CH_2CH_2OH.

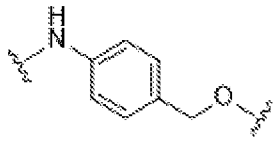
[0139] As used herein, "binding agent" refers to any molecule, *e.g.*, protein or antibody, capable of binding with specificity to a given binding partner, *e.g.*, antigen.

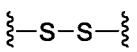
[0140] As used herein, "linker" refers to a divalent, trivalent, or multivalent moiety that covalently links the binding agent to one or more compounds described herein, for instance payload compounds and/or a hydrophilic group, as described herein.

[0141] As used herein, a "connector group" or a "connector group residue" refers to any group which may facilitate a release of a payload. Suitable connector groups facilitate the release of divalent or multivalent appended payloads back to the original unconjugated forms with little or no derivatization. Examples of connector groups include and are not limited to



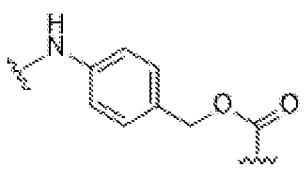
,



, or derivatives thereof. In another example, a connector group is 

.

[0142] In further examples, a connector group is a self immolative group. A "self-immolative group" refers to any such group known to those of skill in the art. In particular embodiments, the self-immolative group is *p*-aminobenzyloxycarbonyl (PAB/PABC), *i.e.*

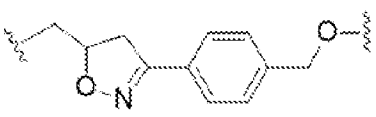


.

Those of skill will recognize that a self-immolative group is capable of carrying out a chemical reaction which releases the remaining atoms of a linker from a payload.

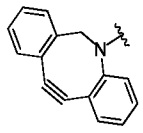
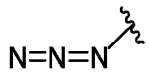
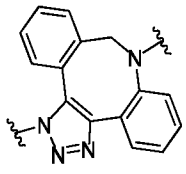
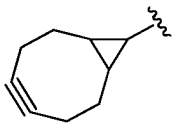
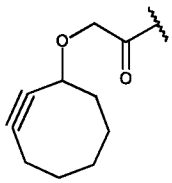
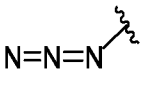
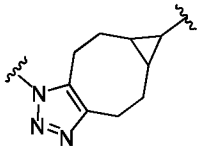
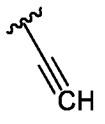
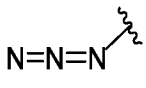
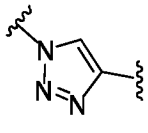
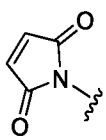
[0143] As used herein. "connecting linker" (L^2) or "connecting linkers" refers to divalent groups which are cleavable or non–cleavable. Cleavable linkers are linkers that are cleaved by intracellular metabolism following internalization, *e.g.*, cleavage via hydrolysis, reduction, or enzymatic reaction. Non–cleavable linkers are linkers that release an attached payload via lysosomal degradation of the antibody following internalization. Suitable connecting linkers include, but are not limited to, acid–labile linkers, hydrolysis–labile linkers, enzymatically cleavable linkers, reduction labile linkers, and non–cleavable linkers. Suitable linkers also include, but are not limited to, those that are or comprise glucuronides, succinimide–thioethers, polyethylene glycol (PEG) units, hydrazones, mal–caproyl units, disulfide units (*e.g.*, –S–S–, –S–C($R^{1b}R^{2b}$)–, wherein R^{1b} and R^{2b} are independently hydrogen or hydrocarbyl), carbamate units, para-amino-benzyl units (PAB), phosphate units, *e.g.*, mono-, bis-, or tris- phosphate units, and peptide units, *e.g.*, peptide units containing two, three four, five, six, seven, eight, or more amino acid residues, including but not limited to valine–citrulline residue units. As used herein, "caproyl" means a -$(CH_2)_5$-C(O)- group.

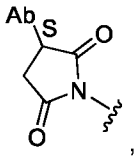
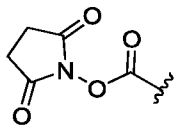
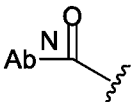
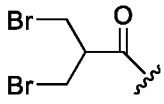
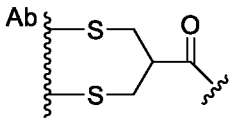
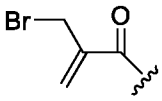
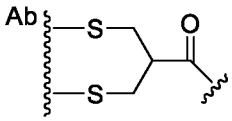
[0144] As used herein, the phrase "reactive group" ("RG"), refers to a functional group or moiety that reacts with a reactive portion of an antibody, modified antibody, or antigen binding fragment thereof. In certain embodiments, the "reactive group" is a functional group or moiety (*e.g.*, maleimide or NHS ester) that reacts with a cysteine or lysine residue of an antibody or antigen–binding fragment thereof. In certain embodiments, the "reactive group" is a functional group or moiety that is capable of undergoing a click chemistry reaction. In some embodiments of said click chemistry reaction, the reactive group comprises an alkyne that is capable of undergoing a 1,3 cycloaddition reaction with an azide. Such suitable reactive groups include, but are not limited to, strained alkynes, *e.g.*, those suitable for strain–promoted alkyne–azide cycloadditions (SPAAC), cycloalkynes, *e.g.*, cyclooctynes, benzannulated alkynes, and alkynes capable of undergoing 1,3 cycloaddition reactions with azides in the absence of copper catalysts. Suitable alkynes also include, but are not limited to, DIBAC (where the -C(O)CH_2CH_2C(O)- portion of DIBAC moiety can be represented by **L** and/or L^2), DIBO (where the -O- portion of DIBO moiety can be represented by **L** and/or L^2), BARAC (where the

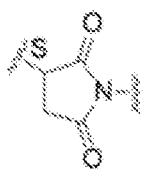


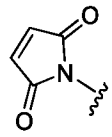
portion of BARAC moiety can be represented by **L** and/or L^2), DIFO (where the -O- portion can be represented by **L** and/or L^2), substituted alkynes, *e.g.*, fluorinated alkynes, aza–cycloalkynes, BCN, and derivatives thereof. Linker–payloads

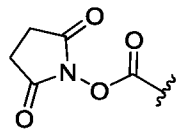
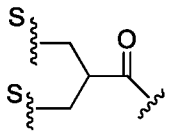
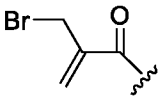
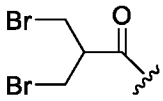
comprising such reactive groups are useful for conjugating antibodies that have been functionalized with azido groups. Such functionalized antibodies include antibodies functionalized with azido–polyethylene glycol groups. In certain embodiments, such functionalized antibody is derived by reacting an antibody comprising at least one glutamine residue, *e.g.*, heavy chain Q295 (EU numbering), with a compound according to the formula $H_2N–LL–N_3$, wherein LL is a divalent polyethylene glycol group, in the presence of the enzyme transglutaminase.

[0145] In some embodiments, the reactive group (**RG**) is an alkyne, *e.g.*,  , which can react via click chemistry with an azide, *e.g.*,  , to form a click chemistry product, *e.g.*,  , its regioisomer, or mixture thereof. In some embodiments, the reactive group is an alkyne, *e.g.*,  or  (where **L** and/or $\mathbf{L^2}$ encompasses -OCH2C(O)-), which can react via click chemistry with an azide, *e.g.*,  to form a click chemistry product, *e.g.*,  . In some embodiments, the reactive group is an alkyne, *e.g.*,  , which can react via click chemistry with an azide, *e.g.*,  , to form a click chemistry product, *e.g.*,  , its regioisomer, or mixture thereof. In some embodiments, the reactive group is a functional group, *e.g.*,  , which reacts with a cysteine residue on

an antibody or antigen–binding fragment thereof, to form a bond thereto, *e.g.*,  , wherein Ab refers to an antibody or antigen–binding fragment thereof and S refers to the S atom on a cysteine residue through which the functional group bonds to the Ab. In some embodiments, the reactive group is a functional group, *e.g.*,  , which reacts with a lysine residue on an antibody or antigen–binding fragment thereof, to form a bond thereto, *e.g.*,  , wherein Ab refers to an antibody or antigen–binding fragment thereof and N refers to the N atom on, e.g., a lysine or an amino terminus residue through which the functional group bonds to the Ab. In some instances, a reactive group is  , which reacts with two thiols (e.g., thiols on two different chains) of an antibody or antigen-binding fragment thereof, to form a bond thereto, e.g.,  . In some instances, a reactive group is  , which reacts with two thiols (e.g., thiols on two different chains) of an antibody or antigen-binding fragment thereof, to form a bond thereto, e.g.,  .

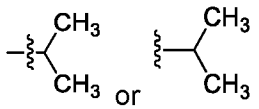
[0146] **As used herein, the phrase "reactive group residue" refers to a product of the reaction** of a functional group in the linker moiety with the reactive portion of a binding agent (**BA**), including the product of click chemistry. The reactive group residue is formed by the reaction of a reactive group on an amino acid of the binding agent, and is attached to the binding agent (e.g. antibody) and to the linker. In some embodiments, the reactive group residue is a group which comprises 1,2,3-tetrazole, *i.e.* is formed by the reaction of an alkyne with an azide. In some embodiments, the reactive group residue is  (a) wherein S refers to the S atom on a cysteine residue through which (a) bonds to the Ab, and which is formed by the

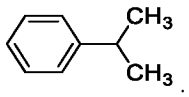
reaction of  with a cysteine residue on an antibody or antigen–binding fragment thereof.

In some embodiments, the reactive group residue is wherein N refers to the N atom on a lysine residue, and which is formed by the reaction of a functional group, *e.g.*,  , with a lysine residue on an antibody or antigen–binding fragment thereof. Those of skill in the art will recognize that portions of the reactive group residue may come from the reactive group, from the antibody, or both. In some instances the reactive group residue is  , where S refers to the S atom on a cysteine residue, and which is formed by the reaction of a functional group  or  with a cysteine residue on an antibody or antigen–binding fragment thereof.

[0147] As used herein, "pharmaceutically acceptable salt" refers to any salt suitable for administration to a patient. Suitable salts include, but are not limited to, those disclosed in. Berge *et al.*, "Pharmaceutical Salts", *J. Pharm. Sci.*, 1977, 66:1, incorporated herein by reference. Examples of salts include, but are not limited to, acid–derived, base–derived, organic, inorganic, amine, and alkali or alkaline earth metal salts, including but not limited to calcium salts, magnesium salts, potassium salts, sodium salts, salts of hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p–toluenesulfonic acid, salicylic acid, and the like. In some examples, a payload described herein (e.g., a rifamycin analog described herein) comprises a tertiary amine, where the nitrogen atom in the tertiary amine is the atom through which the payload is bonded to a linker or a linker-spacer. In such instances, bonding to the tertiary amine of the payload yields a quarternary amine in the linker-payload molecule. The positive charge on the quarternary amine can be balanced by a counter ion (e.g., chloro, bromo, iodo, or any other suitably charged moiety such as those described above).

[0148] Certain groups, moieties, substituents, and atoms are depicted with a wavy line. The wavy line can intersect or cap a bond or bonds. The wavy line indicates the atom through which the groups, moieties, substituents, or atoms are bonded. For example, a phenyl group that is substituted with a propyl group depicted as:

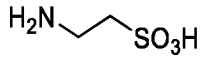
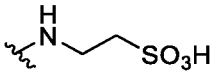


has the following structure: 
.

[0149] As used herein, "amide synthesis conditions" refers to reaction conditions suitable to effect the formation of an amide, *e.g.*, by the reaction of a carboxylic acid, activated carboxylic acid, or acyl halide with an amine. In some examples, amide synthesis conditions refer to reaction conditions suitable to effect the formation of an amide bond between a carboxylic acid and an amine. In some of these examples, the carboxylic acid is first converted to an activated carboxylic acid before the activated carboxylic acid reacts with an amine to form an amide. Suitable conditions to effect the formation of an amide include, but are not limited to, those utilizing reagents to effect the reaction between a carboxylic acid and an amine, including, but not limited to, dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP), bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP), O-(benzotriazol-1-yl)-**N,N,N′,N′**-tetramethyluronium hexafluorophosphate (HBTU), O-(benzotriazol-1-yl)-**N,N,N′,N′**-tetramethyluronium tetrafluoroborate (TBTU), 1–[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), N-ethyl-**N′**-(3-dimethylaminopropyl)carbodiimide (EDC), 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP), 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), and carbonyldiimidazole (CDI). In some examples, a carboxylic acid is first converted to an activated carboxylic ester before treating the activated carboxylic ester with an amine to form an amide bond. In certain embodiments, the carboxylic acid is treated with a reagent. The reagent activates the carboxylic acid by deprotonating the carboxylic acid and then forming a product complex with the deprotonated carboxylic acid as a result of nucleophilic attack by the deprotonated carboxylic acid onto the protonated reagent. The activated carboxylic esters for certain carboxylic acids are subsequently more susceptible to nucleophilic attack by an amine

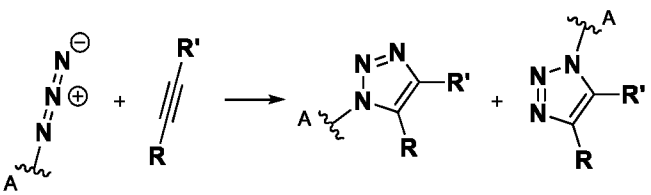
than the carboxylic acid is before it is activated. This results in amide bond formation. As such, the carboxylic acid is described as activated. Exemplary reagents include DCC and DIC.

[0150] "Amino acid" or "amino acid residue" refers, in some embodiments, to naturally occurring amino acids. In other embodiments, an amino acid or amino acid residue may be a naturally occurring amino acid and/or an unnatural amino acid (e.g., β-amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, and/or *N*-methyl amino acids, or any other commercially available unnatural amino acid (e.g., unnatural amino acids available from Sigma-Aldrich)). In other embodiments, an amino acid residue may be a residue having a side chain involved in a reaction mediated by a transglutaminase, e.g., a transglutaminase-mediated reaction of an amino acid side chain with a primary amine. For example, a reaction of an asparagine and/or glutamine side chain with a primary amine is used for preparation of certain ADCs described herein. In certain embodiments, such functionalized antibody is derived by reacting an antibody comprising at least one glutamine residue, *e.g.*, heavy chain Q295 (EU numbering), with a compound according to the formula H_2N–LL–N_3, wherein LL is a divalent polyethylene glycol group, in the presence of the enzyme transglutaminase.

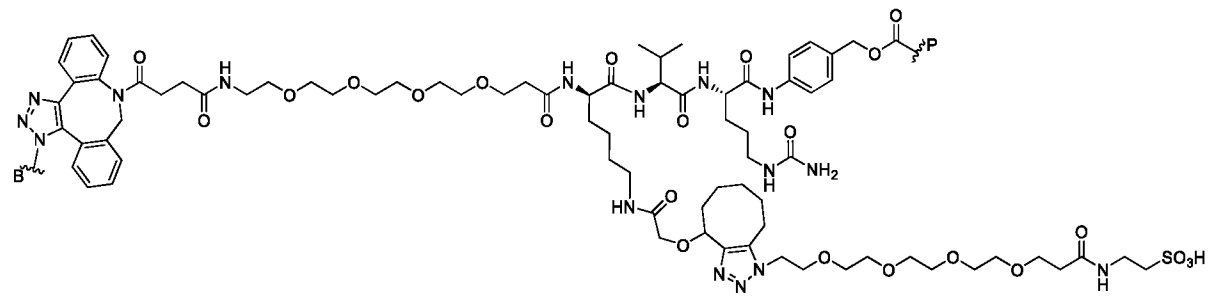
[0151] As used herein, "taurine" refers to the reagent  , or the group  where indicates the atom through which the taurine is bonded to the adjacent groups in the formula.

[0152] As used herein, "stereoisomeric form" or "stereoisomer" refers to the relative spatial orientation of different groups in a compound. Stereoisomeric forms include enantiomers, diasteromers, and/or mixtures thereof.

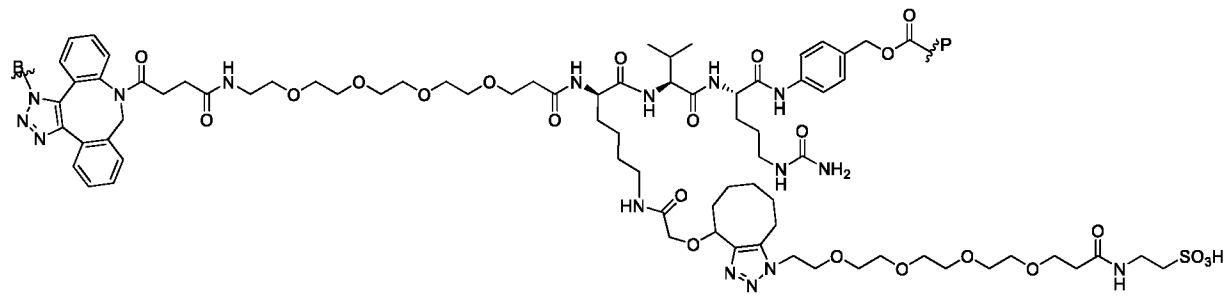
[0153] As used herein, "regioisomer," "regioisomers," or "mixture of regioisomers" refers to the product(s) of 1,3-cycloadditions or strain-promoted alkyne-azide cycloadditions (SPAACs) – otherwise known as click reactions – that derive from suitable azides (*e.g.*, –N_3, or PEG-N_3 derivitized antibodies) treated with suitable alkynes. In certain embodiments, for example, regioisomers and mixtures of regioisomers are characterized by the click reaction products shown below:



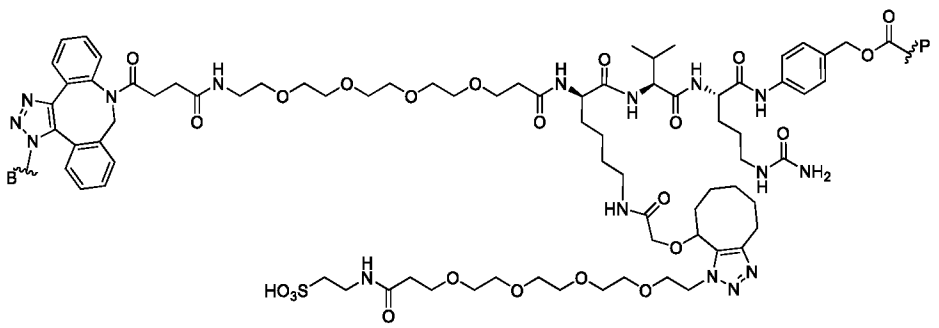
[0154] By way of example only, regioisomers of compound A1', *i.e.*, compounds A2', A3', A4', are shown below, wherein each is a bond to the binding agent; and each is a bond to the payload:



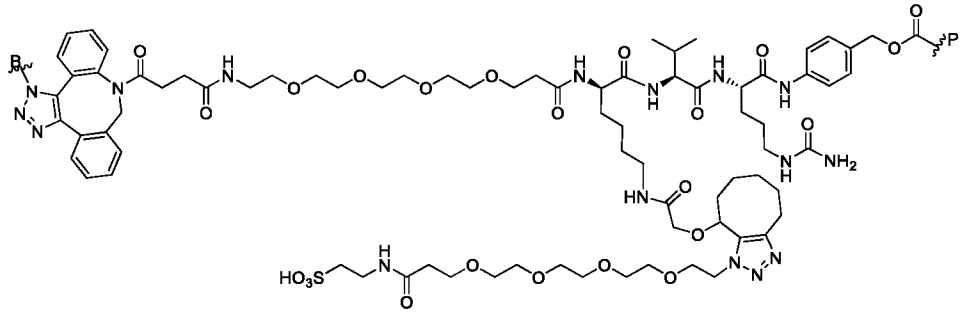
compound **A1'**



compound **A2'**;



compound **A3'**;



compound A4′.

Anti-MSR1 Antibodies Comprising Fc Variants

[0155] According to certain embodiments, anti-MSR1 antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, provided herein are anti-MSR1 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, H/L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P).

[0156] For example, embodiments include anti-MSR1 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

Biological Characteristics of the Antibodies

[0157] Embodiments include antibodies and antigen-binding fragments thereof that bind human MSR1 with high affinity. For example, the present invention includes anti-MSR1 antibodies that bind human MSR1 extracellular domain expressed with an N-terminal nonahistidine tag (*e.g.*, His9-hMSR1) with a K_D of less than about 10 nM as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that bind human MSR1 at 37ºC with a K_D of less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than

about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, or less than about 10 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. In some embodiments, the anti-MSR1 antibodies disclosed herein bind human MSR1 at 25ºC with a K_D of less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, or less than about 20 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0158] Embodiments also include antibodies and antigen-binding fragments thereof that bind monkey MSR1 with high affinity. For example, disclosed herein are anti-MSR1 antibodies that bind monkey MSR1 extracellular domain expressed with an N-terminal myc-myc-hexahistidine tag (*e.g.*, HMM-mfMSR1) with a K_D of less than about 20 nM as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that bind monkey MSR1 at 37ºC with a K_D of less than about 20 nM, less than about 18 pM, less than about 15 nM, less than about 12 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, or less than about 10 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. In some embodiments, the anti-MSR1 antibodies disclosed herein bind monkey MSR1 at 25ºC with a K_D of less than about 12 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900

pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, or less than about 20 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0159] The present invention also includes antibodies and antigen-binding fragments thereof that bind human MSR1 extracellular domain expressed with an N-terminal nonahistidine tag (*e.g.*, His9-hMSR1) with a dissociative half-life (t½) of greater than about 5 minutes as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that bind human MSR1 at 37ºC with a t½ of greater than about 4 minutes, greater than about 5 minutes, greater than about 6 minutes, greater than about 8 minutes, greater than about 10 minutes, greater than about 12 minutes, greater than about 14 minutes, greater than about 16 minutes, greater than about 18 minutes, greater than about 20 minutes, greater than about 30 minutes, greater than about 40 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, greater than about 80 minutes, greater than about 90 minutes, greater than about 120 minutes, greater than about 150 minutes, greater than about 180 minutes, greater than about 210 minutes, greater than about 240 minutes, or longer, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0160] Embodiments also include antibodies and antigen-binding fragments thereof that can bind monkey MSR1 extracellular domain expressed with an N-terminal myc-myc-hexahistidine tag (*e.g.* HMM-mfMSR1) with high affinity. For example, the present invention includes anti-MSR1 antibodies that bind HMM-mfMSR1 with a K_D of less than about 20 nM as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that bind HMM-mfMSR1 at 37ºC with a K_D of less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 150 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM,

less than about 70 pM, less than about 60 pM, or less than about 50 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. In some embodiments, the anti-MSR1 antibodies disclosed herein bind HMM-mfMSR1 at 25ºC with a K_D of less than about 12 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 150 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, or less than about 50 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0161] Embodiments also include antibodies and antigen-binding fragments thereof that bind monkey MSR1 extracellular domain expressed with an N-terminal myc-myc-hexahistidine tag (*e.g.* HMM-mfMSR1) with a dissociative half-life (t½) of greater than about 55 minutes as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that bind dimeric human MSR1 at 37ºC with a t½ of greater than about 1 minute, greater than about 2 minutes, greater than about 3 minutes, greater than about 4 minutes, greater than about 5 minutes, greater than about 6 minutes, greater than about 8 minutes, greater than about 10 minutes, greater than about 12 minutes, greater than about 14 minutes, greater than about 16 minutes, greater than about 18 minutes, greater than about 20 minutes, greater than about 30 minutes, greater than about 40 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, greater than about 80 minutes, greater than about 90 minutes, greater than about 120 minutes, greater than about 150 minutes, greater than about 180 minutes, greater than about 210 minutes, or longer, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0162] Embodiments also include antibodies and antigen-binding fragments thereof that bind engineered cell-surface expressed hMSR1 with binding ratios of engineered hMSR1-expressing cells to non-expressing cells of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, or greater, as measured by antibody binding

assay, *e.g.*, using an assay format as defined in Example 5 herein, or a substantially similar assay. In some embodiments, provided herein are antibodies that bind cells with endogenously-expressed hMSR1 with binding ratios of endogenous hMSR1-expressing cells to non-expressing cells of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, or greater at least about 12-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, or greater, as measured by antibody binding assay, *e.g.*, using an assay format as defined in Example 5 herein, or a substantially similar assay. In some embodiments, an MSR1 antibody or antigen binding fragment disclosed herein bind engineered cell-surface expressed mouse MSR1 with binding ratios of engineered mouse MSR1-expressing cells to non-expressing cells of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, or greater, as measured by antibody binding assay, *e.g.*, using an assay format as defined in Example 5 herein, or a substantially similar assay.

[0163] Embodiments also include antibodies and antigen-binding fragments thereof that bind MSR1 and exhibit maximum inhibition of uptake of modified low-density lipoprotein (LDL) in cells expressing human MSR1 of less than about 95%. For example, embodiments include anti-MSR1 antibodies that exhibit maximum inhibition of uptake of modified LDL (*e.g.*, oxidized or acetylated) in cells that express human MSR1 of less than about 95%, with an IC_{50} of less than about 6.1 nM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay. According to certain embodiments, anti-MSR1 antibodies are provided that exhibit maximum inhibition of modified LDL uptake in cells expressing human MSR1 of less than about 95%, with an IC_{50} of less than about 6.1 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1.5 nM, less than about 1.4 nM, less than about 1.3 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 900 pM, less than about 800 pM, less than about 600 pM, less than about 400 pM, less than about 200 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20 pM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay.

[0164] In some embodiments, the anti-MSR1 antibodies exhibit maximum inhibition of modified LDL uptake in cells expressing human MSR1 of less than about 90%, with an IC_{50} of less than about 6.1 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1.5 nM, less than about 1.4 nM, less than about 1.3 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 900 pM, less than about 800 pM, less than about 600 pM, less than about 400 pM, less than about 200 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20 pM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay.

[0165] In some embodiments, the anti-MSR1 antibodies exhibit maximum inhibition of modified LDL uptake in cells expressing human MSR1 of less than about 75%, with an IC_{50} of less than about 6.1 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1.5 nM, less than about 1.4 nM, less than about 1.3 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 900 pM, less than about 800 pM, less than about 600 pM, less than about 400 pM, less than about 200 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20 pM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay.

[0166] In some embodiments, the anti-MSR1 antibodies exhibit maximum inhibition of modified LDL uptake in cells expressing human MSR1 of less than about 60%, with an IC_{50} of less than about 6.1 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1.5 nM, less than about 1.4 nM, less than about 1.3 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 900 pM, less than about 800 pM, less than about 600 pM, less than about 400 pM, less than about 200 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20 pM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay.

[0167] In some embodiments, the anti-MSR1 antibodies exhibit maximum inhibition of modified LDL uptake in cells expressing human MSR1 of less than about 50%, with an IC_{50} of less than about 6.1 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1.5 nM, less than about 1.4 nM, less than about 1.3 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 900 pM, less than about 800 pM, less than about 600 pM, less than about 400 pM, less than about 200 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20

pM as measured using a ligand uptake assay, *e.g.*, using an assay format as defined in Example 8 herein, or a substantially similar assay.

[0168] Embodiments also include antibodies and antigen-binding fragments thereof that bind cell surface-expressed MSR1 and are internalized by the cells. For example, antibodies that bind to cell surface-expressed MSR1 on THP-1 cells and become internalized by the cells are provided herein. For example, the instant disclosure includes anti-MSR1 antibodies that bind to cell surface-expressed MSR1 on THP-1 cells and become internalized by the cells with a relative percentage of at least about 10%, as measured by chemiluminescence, *e.g.*, using an assay format as defined in Example 9 herein, or a substantially similar assay. In certain embodiments, anti-MSR1 antibodies that bind to cell surface-expressed MSR1 on THP-1 cells and become internalized by the cells with a relative percentage of at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, as measured by chemiluminescence, *e.g.*, using an assay format as defined in Example 9 herein, or a substantially similar assay.

[0169] The antibodies disclosed herein may possess one or more of the aforementioned biological characteristics, or any combination thereof. The foregoing list of biological characteristics of the antibodies disclosed herein is not intended to be exhaustive. Other biological characteristics of the antibodies disclosed herein will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Antibody-Drug Conjugates (ADCs)

[0170] Provided herein are antibody-drug conjugates (ADCs) comprising an anti-MSR1 antibody or antigen-binding fragment thereof conjugated to a drug or a therapeutic agent. In some embodiments, the therapeutic agent is a liver X receptor (LXR) agonist or a steroid. Also provided herein are reactive linker-payloads useful for making the ADCs. Further provided herein are modified anti-MSR1 antibodies and modified MSR1 antigen-binding fragments useful for making the ADCs.

[0171] The ADCs generally have the Formula (I): **BA** – $[(\mathbf{L})_{0\text{-}1}$ – **PA**$]_n$. In the formula, **BA** is a binding agent, for instance, an anti-MSR1, antibody, or an MSR1 antigen-binding fragment thereof. **L** is a linker, described in detail below. **PA** is a payload. Suitable payloads include any small molecule that can provide a therapeutic benefit through its delivery to MSR1. In certain embodiments, a payload may be, for instance, a steroid, an LXR modulator, or a rifamycin analog. Useful payloads are described in detail below. In the formula, **n** is an integer from 1 to 30, for instance from 1 to 4, *e.g.*, 2 or 4. Each **L** – **PA** is covalently bonded to a functional group

of **PA**. In some particular embodiments, each **L – PA** is covalently bonded to a lysine side chain, a cysteine side chain, a glutamine side chain, or an amino terminus of **BA**.

[0172] Techniques and linkers for conjugating to residues of an antibody or antigen binding fragment are known in the art. Exemplary amino acid attachments that can be used in the context of this aspect, *e.g.*, lysine (*see*, *e.g.*, US 5,208,020; US 2010/0129314; Hollander *et al.*, *Bioconjugate Chem.*, 2008, 19:358-361; WO 2005/089808; US 5,714,586; US 2013/0101546; and US 2012/0585592), cysteine (*see*, *e.g.*, US 2007/0258987; WO 2013/055993; WO 2013/055990; WO 2013/053873; WO 2013/053872; WO 2011/130598; US 2013/0101546; and US 7,750,116), selenocysteine (*see*, *e.g.*, WO 2008/122039; and Hofer *et al.*, *Proc. Natl. Acad. Sci., USA, 2008, 105*:12451-12456), formyl glycine (*see*, *e.g.*, Carrico *et al.*, *Nat. Chem. Biol.*, 2007, 3:321-322; Agarwal *et al.*, *Proc. Natl. Acad. Sci., USA, 2013, 110*:46-51, and Rabuka *et al.*, *Nat. Protocols*, 2012, *10*:1052-1067), non-natural amino acids (*see*, *e.g.*, WO 2013/068874, and WO 2012/166559), and acidic amino acids (*see*, *e.g.*, WO 2012/05982). Linkers can also be conjugated to an antigen-binding protein via attachment to carbohydrates (*see*, *e.g.*, US 2008/0305497, WO 2014/065661, and Ryan *et al.*, *Food & Agriculture Immunol.*, 2001, *13*:127-130) and disulfide linkers (*see*, *e.g.*, WO 2013/085925, WO 2010/010324, WO 2011/018611, and Shaunak *et al.*, *Nat. Chem. Biol.*, 2006, *2*:312-313). Site specific conjugation techniques can also be employed to direct conjugation to particular residues of the antibody or antigen binding protein (*see, e.g.*, Schumacher *et al. J Clin Immunol* (2016) 36(Suppl 1): 100). Site specific conjugation techniques, include, but are not limited to glutamine conjugation via transglutaminase (*see e.g.*, Schibli, Angew Chemie Inter Ed. 2010, 49 ,9995).

[0173] Linkers can be conjugated to one or more glutamine residues via transglutaminase-based chemo-enzymatic conjugation (*see, e.g.,* Dennler *et al., Bioconjugate Chem*. 2014, 25, 569-578, and WO 2017/147542). For example, in the presence of transglutaminase, one or more glutamine residues of an antibody can be coupled to a primary amine compound. Briefly, in some embodiments, an antibody having a glutamine residue (*e.g.*, a Gln295 residue) is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. Primary amine compounds include payloads or linker-payloads, which directly provide antibody drug conjugates via transglutaminase-mediated coupling. Primary amine compounds also include linkers and spacers that are functionalized with reactive groups that can be subsequently reacted with further compounds towards the synthesis of antibody drug conjugates. Antibodies comprising glutamine residues can be isolated from natural sources or engineered to comprise one or more glutamine residues. Techniques for engineering glutamine residues into an antibody polypeptide chain (glutaminyl-modified

antibodies or antigen binding molecules) are within the skill of the practitioners in the art. In certain embodiments, the antibody is aglycosylated.

[0174] In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises at least one glutamine residue in at least one polypeptide chain sequence. In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue. In further embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises one or more glutamine residues at a site other than a heavy chain 295. In some embodiments, an antibody can be prepared by site-directed mutagenesis to insert a glutamine residue at a site without resulting in disabled antibody function or binding. For example, included herein are antibodies bearing Asn297Gln (N297Q) mutation(s) as described herein. In some embodiments, an antibody having a Gln295 residue and/or an N297Q mutation contains one or more additional naturally occurring glutamine residues in their variable regions, which can be accessible to transglutaminase and therefore capable of conjugation to a linker or a linker-payload. An exemplary naturally occurring glutamine residue can be found, *e.g.*, at Q55 of the light chain. In such instances, the antibody conjugated via transglutaminase can have a higher than expected DAR value (*e.g.*, a DAR higher than 4). Any such antibodies can be isolated from natural or artificial sources.

[0175] The primary amine compound useful for the transglutaminase mediated coupling of an antibody (or antigen binding compound) comprising a glutamine can be any primary amine compound deemed useful by the practitioner of ordinary skill. Generally, the primary amine compound has the formula H_2N-R, where R can be any group compatible with the antibody and reaction conditions. In certain embodiments, R is alkyl, substituted alkyl, heteroalkyl, or substituted heteroalkyl.

[0176] In some embodiments, the primary amine compound comprises a reactive group or protected reactive group. Useful reactive groups include azides, alkynes, cycloalkynes, thiols, alcohols, ketones, aldehydes, acids, esters, hydrozides, analines, and amines. In certain embodiments, the reactive group is selected from the group consisting of azide, alkyne, sulfhydryl, cycloalkyne, aldehyde, and carboxyl.

[0177] In certain embodiments, the primary amine compound is according to the formula H_2N-**LL**-**X**, where **LL** is a divalent spacer and **X** is a reactive group or protected reactive group. In particular embodiments, **LL** is a divalent polyethylene glycol (PEG) group. In certain embodiments, **X** is selected from the group consisting of –SH, $–N_3$, alkyne, aldehyde, and tetrazole. In particular embodiments, **X** is $–N_3$.

[0178] In certain embodiments, the primary amine compound is according to one of the following formulas:

H_2N-$(CH_2)_n$-X;

H_2N-$(CH_2CH_2O)_n$-$(CH_2)_p$-X;

H_2N-$(CH_2)_n$-N(H)C(O)-$(CH_2)_m$-X;

H_2N-$(CH_2CH_2O)_n$-N(H)C(O)-$(CH_2CH_2O)_m$-$(CH_2)_p$-X;

H_2N-$(CH_2)_n$-C(O)N(H)-$(CH_2)_m$-X;

H_2N-$(CH_2CH_2O)_n$-C(O)N(H)-$(CH_2CH_2O)_m$-$(CH_2)_p$-X;

H_2N-$(CH_2)_n$-N(H)C(O)-$(CH_2CH_2O)_m$-$(CH_2)_p$-X;

H_2N-$(CH_2CH_2O)_n$-N(H)C(O)-$(CH_2)_m$-X;

H_2N-$(CH_2)_n$-C(O)N(H)-$(CH_2CH_2O)_m$-$(CH_2)_p$-X; and

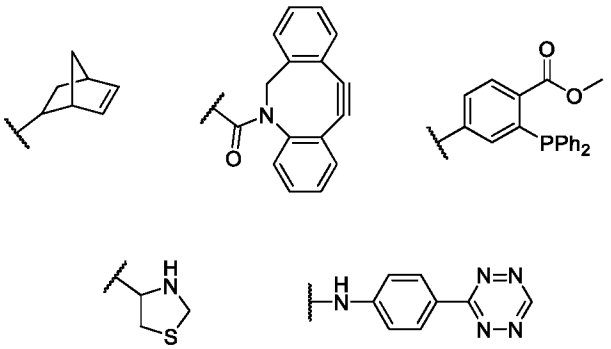
H_2N-$(CH_2CH_2O)_n$-C(O)N(H)-$(CH_2)_m$-X;

where **n** is an integer selected from 1 to 12;

m is an integer selected from 0 to 12;

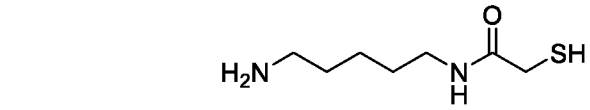
p is an integer selected from 0 to 2;

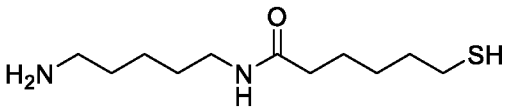
and X is selected from the group consisting of –SH, $-N_3$, –C≡CH, –C(O)H, tetrazole, and any of

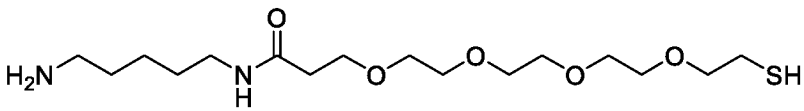


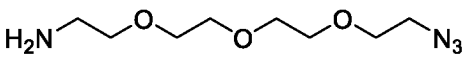
[0179] In the above, any of the alkyl or alkylene (*i.e.*, $-CH_2-$) groups can optionally be substituted, for example with C_{1-8}alkyl, methylformyl, or $-SO_3H$. In certain embodiments, the alkyl groups are unsubstituted.

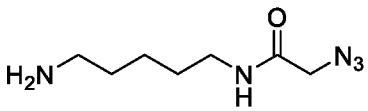
[0180] In certain embodiments, the primary amine compound is selected from the group consisting of:



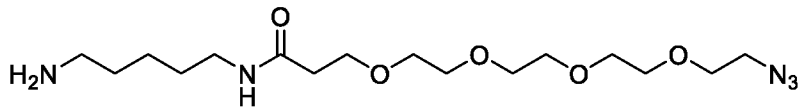




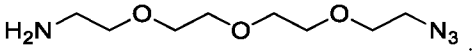




and

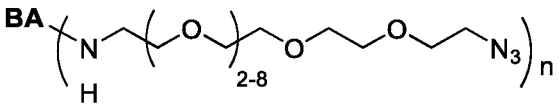


[0181] In particular embodiments, the primary amine compound is



[0182] Exemplary conditions for the above reactions are provided in the Examples below:

[0183] Accordingly, provided herein are modified anti-MSR1 antibodies, and antigen-binding fragments thereof, linked to one or more primary amine compounds. In particular embodiments, provided herein are modified anti-MSR1 antibodies, and antigen-binding fragments thereof, according to the formula:



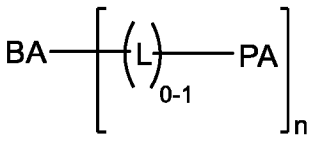
[0184] In the formula, **BA** is an anti-MSR1 antibody, or an antigen binding fragment thereof. The variable **n** is an integer from 1 to 30. In certain embodiments, **n** is from 1 to the number of glutamine residues in **BA**. In certain embodiments, **n** is from 1 to 4. In certain embodiments, **n** is 1, 2, 3, or 4. In some embodiments, n is 2. In some embodiments, n is 4. The modified anti-MSR1 antibodies, and antigen-binding fragments thereof, are useful, for example, for linking to one or more **L – PA** molecules to form an ADC.

[0185] In certain embodiments, **BA** comprises two or four glutamine residues. In certain embodiments, **BA** comprises a Q295 residue. In certain embodiments, **BA** comprises an N297Q mutation. In certain embodiments, **BA** comprises Q295 and N297Q. In such embodiments, because **BA** can be dimeric, **BA** has four glutamine residues for conjugation to **L – PA** moieties.

Compounds

[0186] In one aspect, provided herein is a compound and/or an antibody-drug conjugate comprising any antibody, or antigen-binding fragment thereof, described herein conjugated to a payload residue optionally through a linker or through a linker-spacer,

[0187] In a group of embodiments, the compound and/or the antibody-drug conjugate has the structure of Formula (**I**):



Formula (**I**)

wherein:

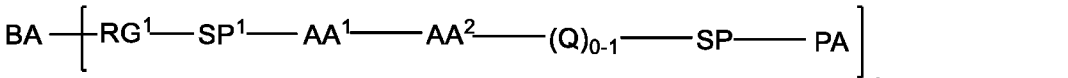
BA is a binding agent;

L is a linker;

PA is a payload moiety selected from the group consisting of a steroid residue, a LXR modulator residue, or a rifamycin analog residue; and

subscript **n** is an integer from 1 to 30.

[0188] In a group of embodiments, a compound and/or antibody-drug conjugate described above and herein has the structure of Formula (**IA**):



Formula (**IA**)

wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

AA^1 is absent, or a divalent or trivalent linker comprising an amino acid residue which is optionally bonded directly or indirectly to a group **HG**;

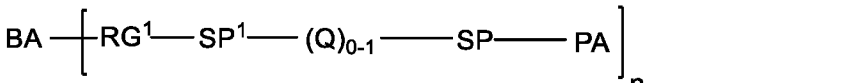
AA^2 is absent, or a dipeptide, tripeptide, or tetrapeptide residue;

Q, when present, is a connector group residue;

SP is absent, or a spacer; and

HG, when present, is a hydrophilic group.

[0189] In a group of embodiments, a compound and/or antibody-drug conjugate described above and herein has the structure of Formula (**IB-1**):



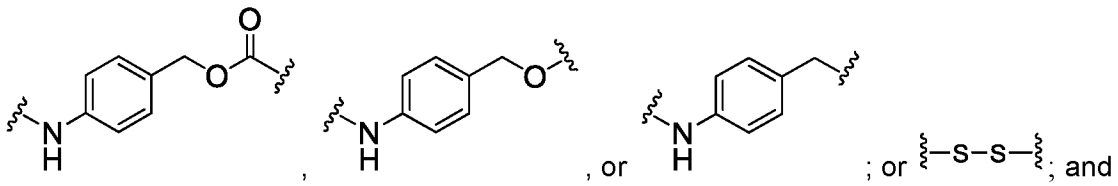
Formula (**IB-1**)

wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

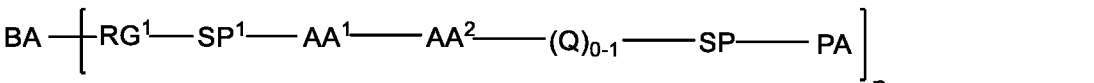
Q, when present, is



SP is absent, or a spacer;

wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula.

[0190] In a group of embodiments, a compound and/or antibody-drug conjugate described above and herein has the structure of Formula (**IB-2**):



Formula (**IB-2**)

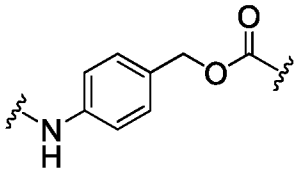
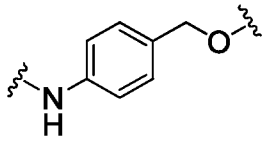
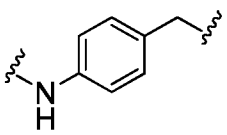
wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

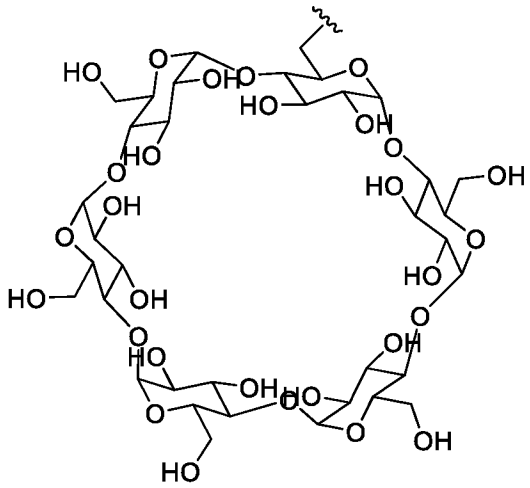
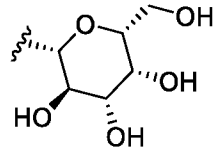
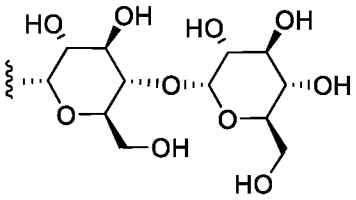
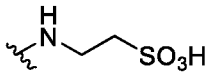
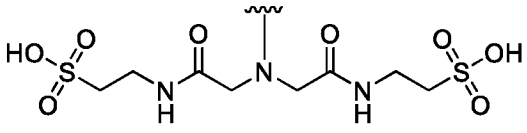
AA^1 is absent, or a divalent or trivalent linker comprising an amino acid residue which is optionally bonded directly or indirectly to a group **HG**;

AA^2 is absent, or a dipeptide, tripeptide, or tetrapeptide residue;

Q, when present, is  ,  , or  ;

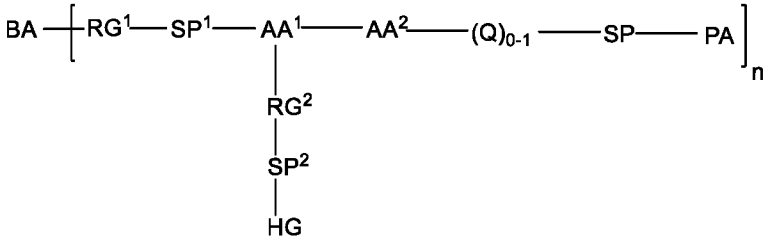
SP is absent, or a spacer; and

HG, when present, is

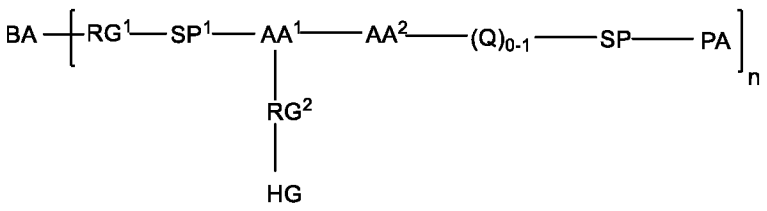
 ,  ,  ,  , or  ,

wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula.

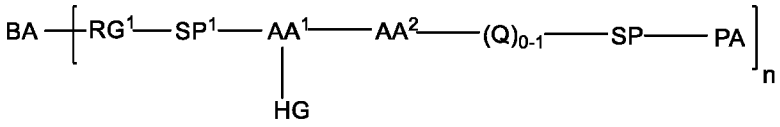
[0191] In a group of embodiments, a compound and/or antibody-drug conjugate described above and herein has the structure of Formula (**IC**), Formula (**ID**), or Formula (**IE**):



Formula (**IC**)



Formula (**ID**)



Formula (**IE**)

wherein

SP^1 is absent, or a spacer;

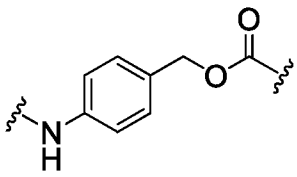
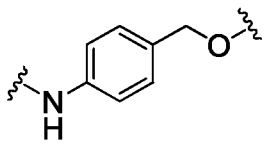
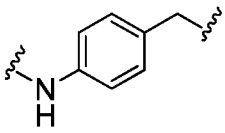
RG^1 is a reactive group residue;

SP^2 is absent, or a spacer;

RG^2 is a reactive group residue;

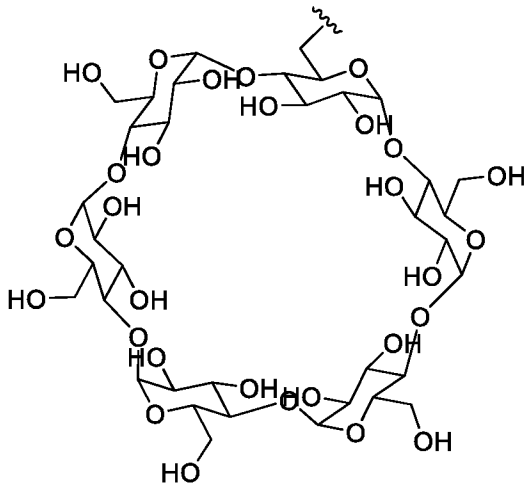
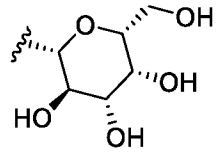
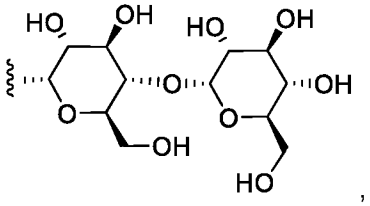
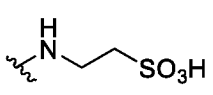
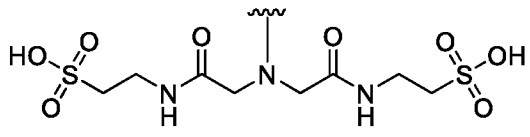
AA^1 is a divalent or trivalent linker comprising an amino acid residue;

AA^2 is a dipeptide, tripeptide, or tetrapeptide residue;

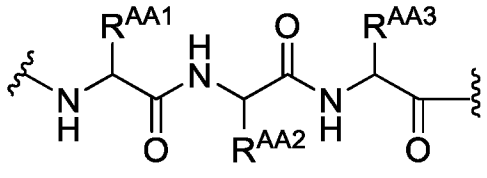
Q, when present, is  ,  , or  ;

SP is absent, or a spacer; and

HG is

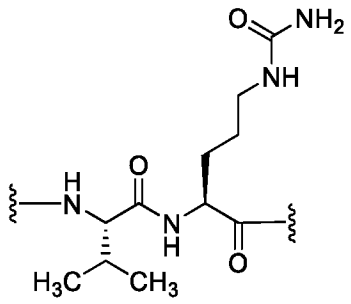
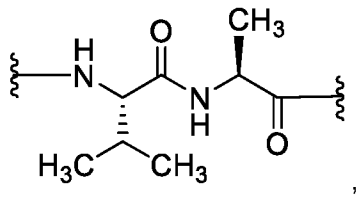
 ,  ,  ,  , or  ,

[0192] wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula. In some or any instances, for any compound and/or antibody-drug conjugate described above and herein, **AA**^{1}-**AA**^{2} is according to Formula (**LL1**):

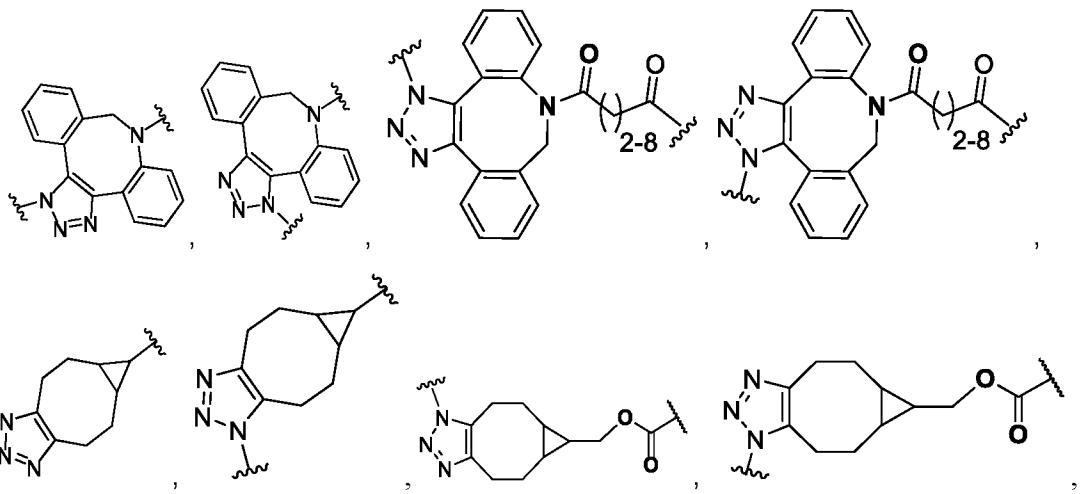


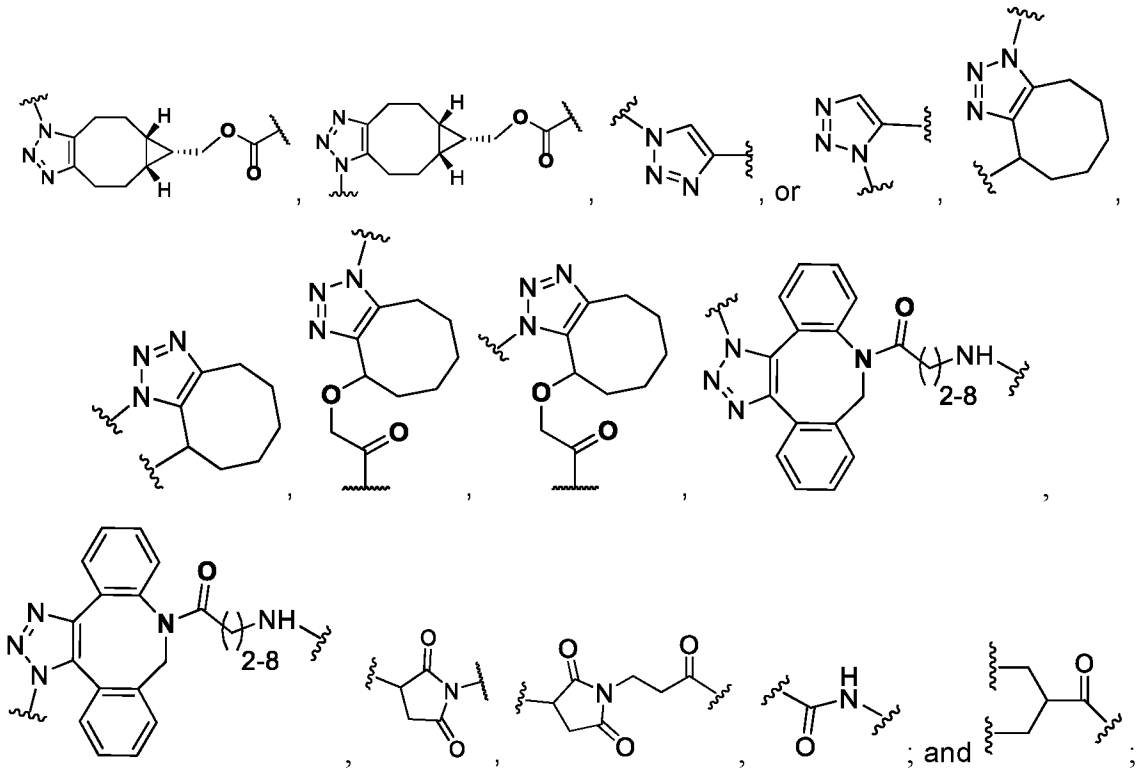
(LL1)

wherein R^{AA1}, R^{AA2}, and R^{AA3} are each, independently, amino acid side chains, at least one of which is bonded to $-(RG^2)$-SP^2-HG, $-(RG^2)$-HG or HG; wherein the indicates the atoms through which AA^1-AA^2 is bonded to the adjacent groups in the formula. In some of such embodiments, R^{AA1} is a lysine, glutamine, glutamic acid or aspartic acid side chain bonded directly or indirectly to HG, and R^{AA2} and R^{AA3} are either valine and alanine or valine and citrulline sidechains respectively.

[0193] In some or any instances, for any compound and/or antibody-drug conjugate described above and herein, AA^1-AA^2 is  or  , wherein the indicates the atoms through which AA^1-AA^2 is bonded to the adjacent groups in the formula.

[0194] In some or any instances, for any compound and/or antibody-drug conjugate described above and herein, the RG^1 and RG^2 residues are independently, in each instance, selected from the group consisting of:

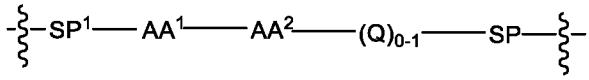


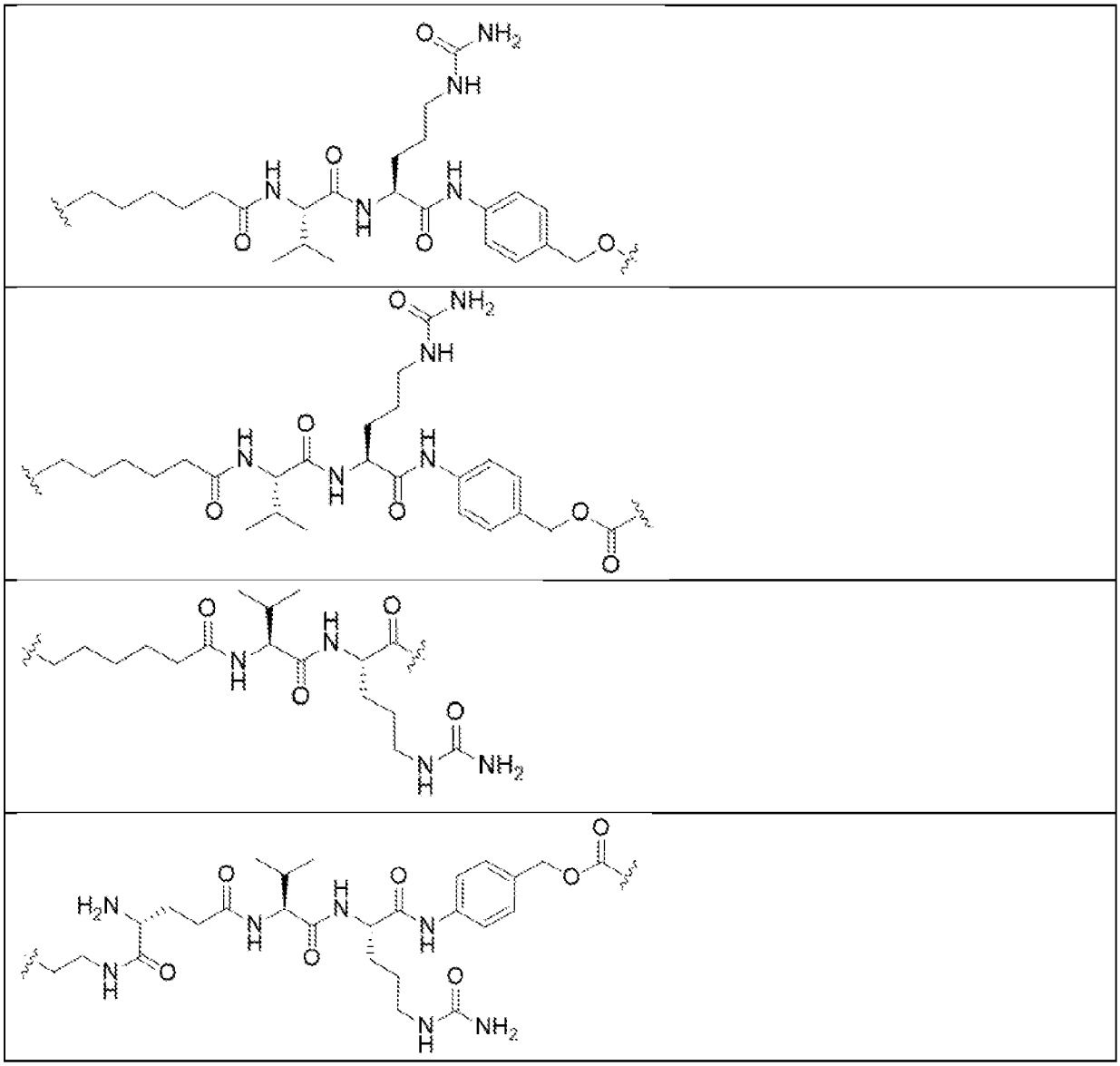


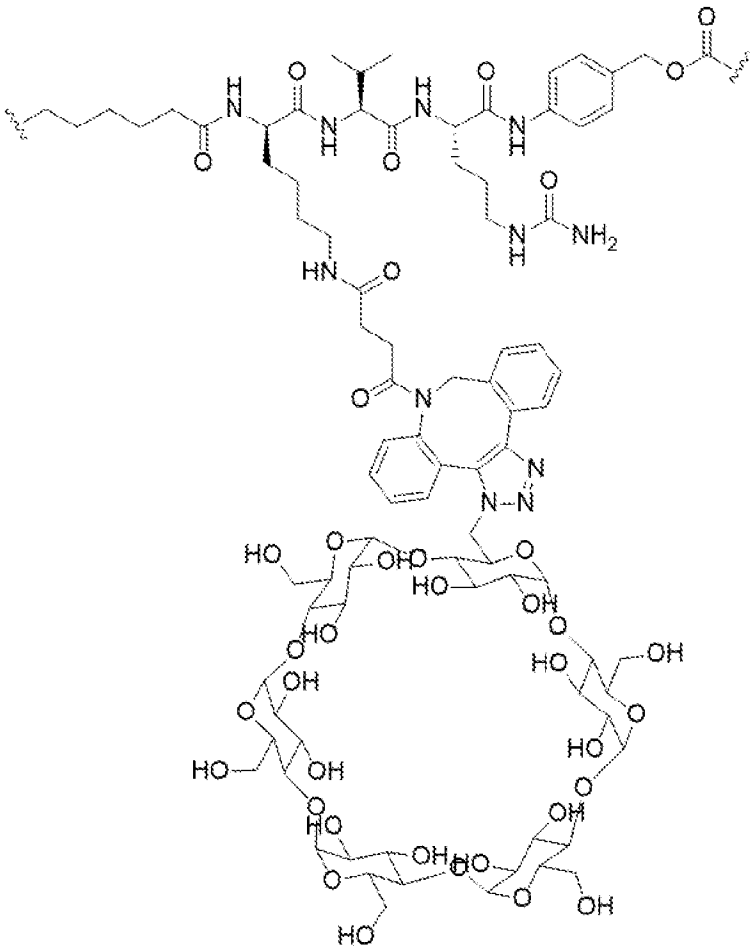
wherein the indicates the atom through which the **RG**^{1} or **RG**^{2} residue is bonded to the adjacent groups in the formula.

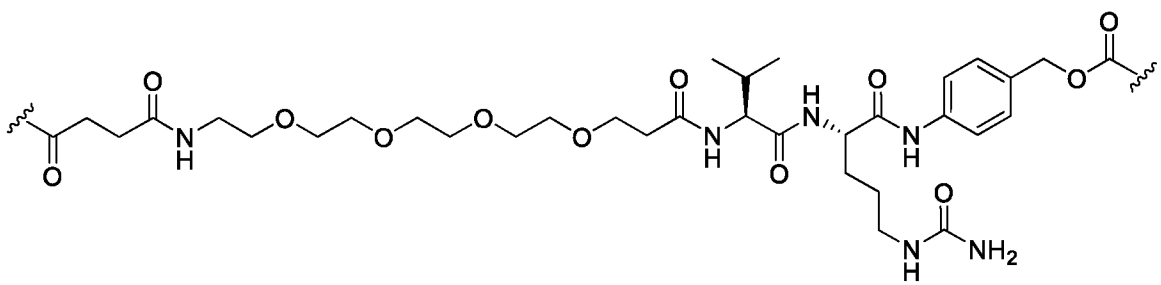
[0195] In some or any instances, for any compound and/or antibody-drug conjugate described above and herein, **SP**, **SP**^{1} and **SP**^{2} are independently, in each instance, absent, or selected from the group consisting of C_{1-6} alkylene, -NH-, -S-, -O-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-CH_2-CH_2-$(-O-CH_2-CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, $(glycine)_4$-serine, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8.

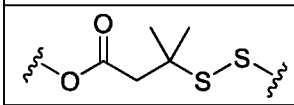
[0196] In some or any instances, for any compound and/or antibody-drug conjugate described above and herein, n is an integer from 1 to 10, from 1 to 8, from 1 to 6, from 1 to 4, or n is 1, 2, 3, or 4.

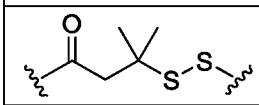
[0197] In some or any instances, for any compound, linker-payload, and/or antibody-drug conjugate described above and herein,  is

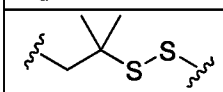


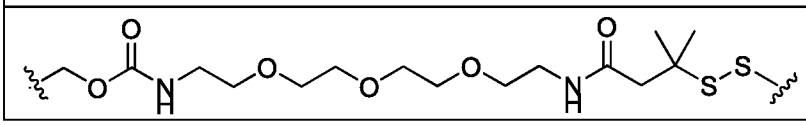


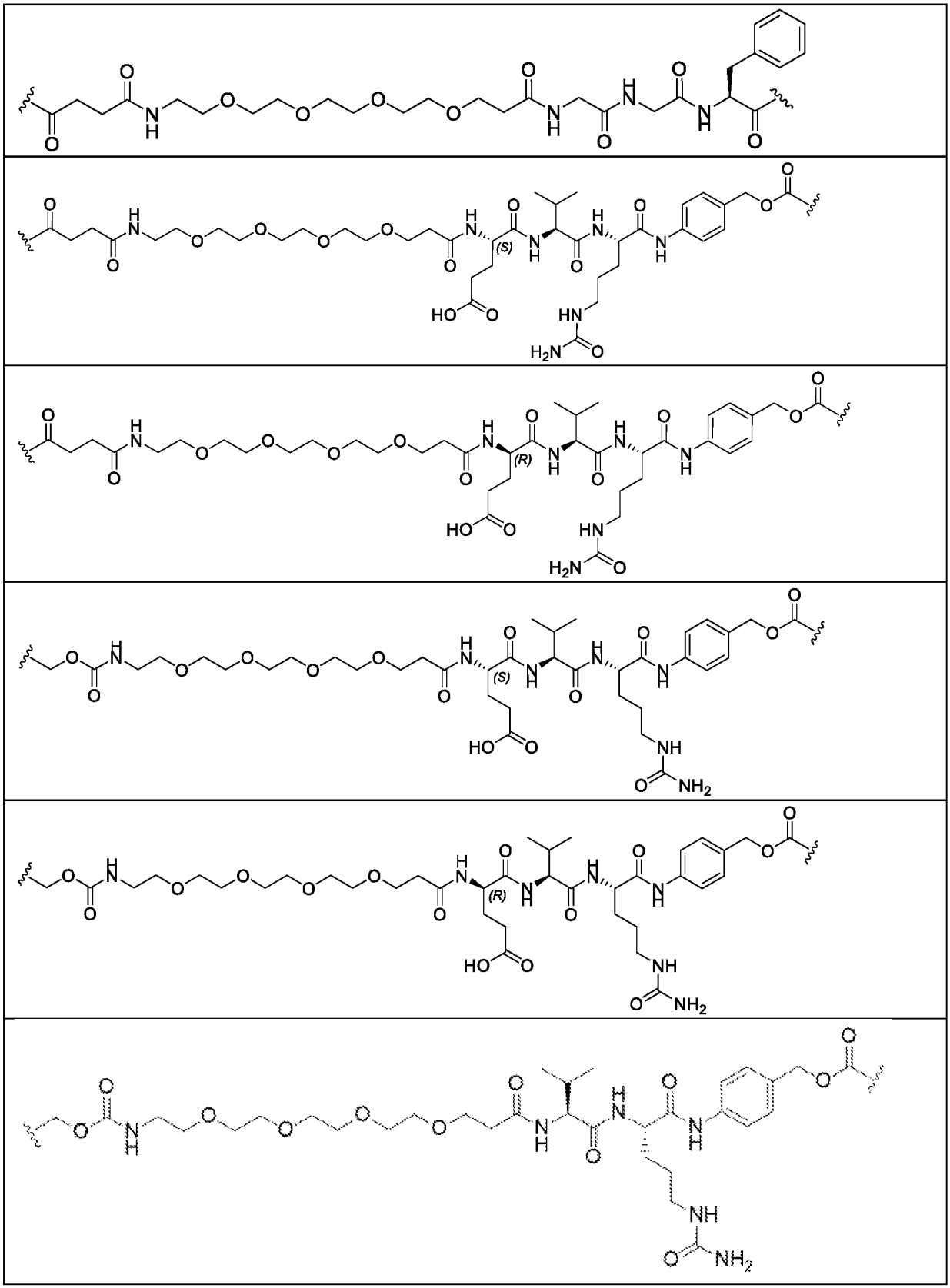


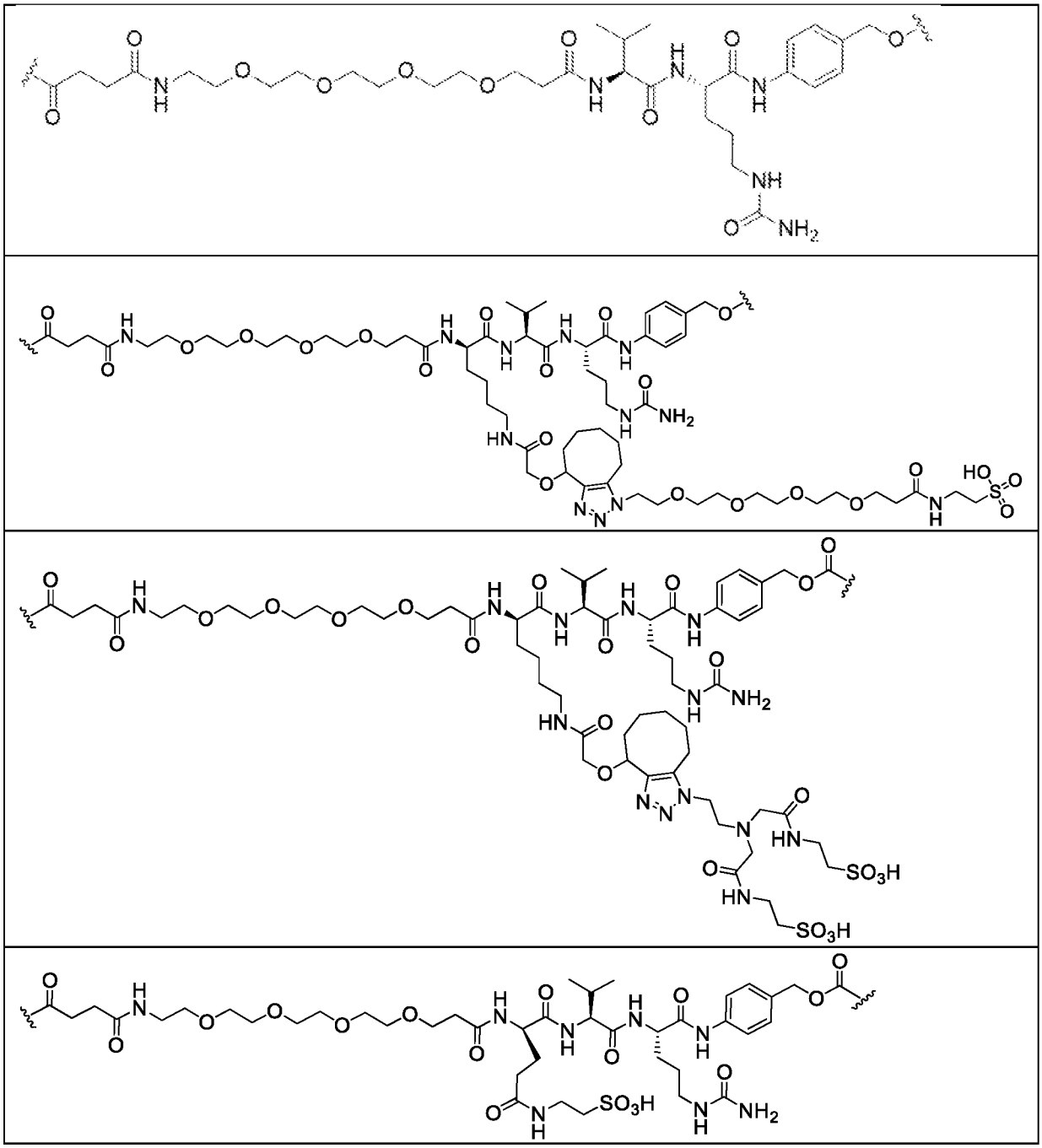


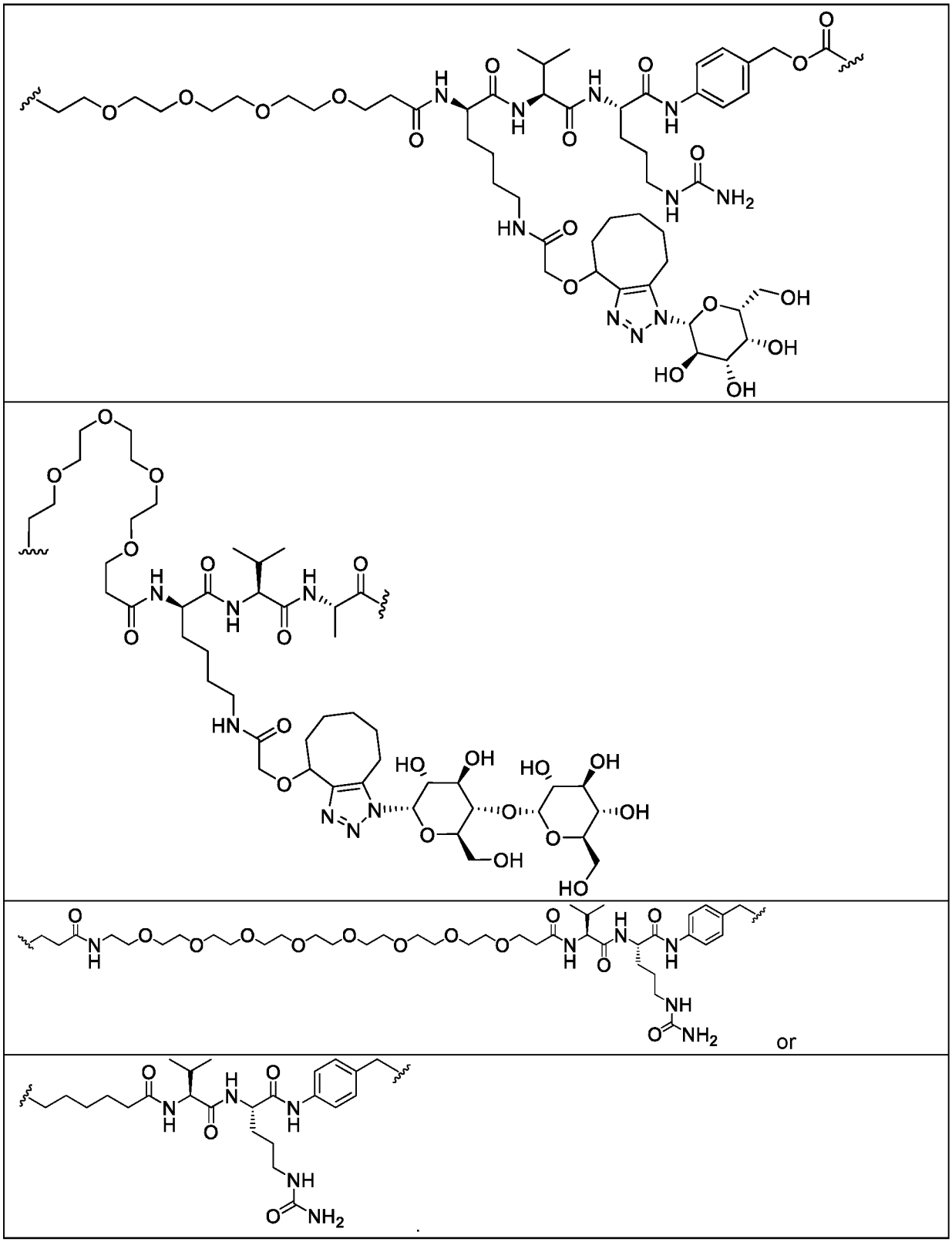












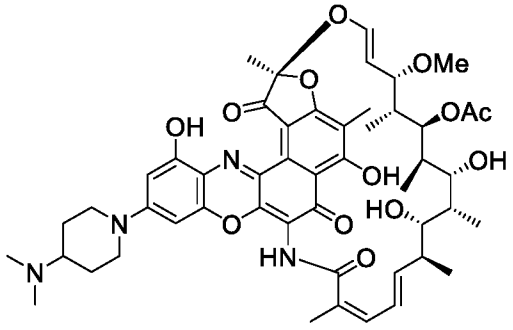
[0198] In some or any embodiments, for any compound and/or antibody-drug conjugate described above and herein, the antibody, or antigen-binding fragment thereof, is conjugated to a steroid payload through a linker or a linker-spacer.

[0199] In some or any embodiments, for any compound and/or antibody-drug conjugate described above and herein, the antibody, or antigen-binding fragment thereof, is conjugated to a LXR modulator payload through a linker.

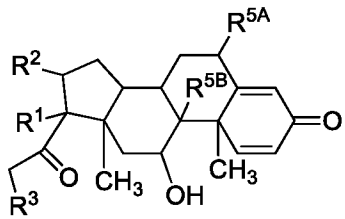
[0200] In some or any embodiments, for any compound and/or antibody-drug conjugate described above and herein, the antibody, or antigen-binding fragment thereof, is conjugated to a rifamycin analog payload through a linker.

Payloads

[0201] In the Formula (**I**) **BA** – [**L** – **PA**]$_n$, **PA** can be any payload deemed useful. Such payloads include small molecules that provide a therapeutic benefit through their delivery via MSR1. In certain embodiments, **PA** is the residue of a molecule selected from the group consisting of a steroid,an LXR modulator, or a rifamycin analog. In some cases, **PA** is a steroid. In some cases, **PA** is an LXR modulator. In some cases, **PA** is an LXR agonist. In some embodiments, **PA** is an LXR antagonist. Exemplary LXR modulator payloads are described, *e.g.*, in U.S. Application No. 62/508,327, filed May 18, 2017, entitled "BIS-OCTAHYDROPHENANTHRENE CARBOXAMIDES AND PROTEIN CONJUGATES THEREOF," published as US 2018/0334426, which is incorporated herein by reference in its entirety. In some instances **PA** is a rifamycin analog, including any rifamycin analog described herein. In some instances, **PA** is rifalogue, having the following structure:



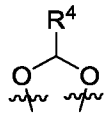
[0202] In certain embodiments, the payloads in compounds of Formula (**I**) are glucocorticoids according to Formula (**A**):



Formula (**A**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein

R^1 and R^2 are, independently, –H, alkyl, alkyl-C(O)-O–, –OH, or halo; or R^1 and R^2 together form  ,

wherein R^4 is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl,

wherein the alkyl, aryl, arylalkyl, and N-containing heterocycloalkyl are, independently in each instance, optionally substituted with -$NR^{Aa}R^{Ab}$;

R^3 is –OH, R^Z-C(O)-**X**–, heteroalkyl, piperidinyl, –$NR^{Aa}R^{Ab}$, -oxyaryl–$NR^{Aa}R^{Ab}$ or -**Z**-**A′**$(R^P)_t$;

R^Z is alkyl;

X is O or NR^{Aa};

Z is S, S(O), $S(O)_2$, SO_2NR^{Aa}, O, C(O)NR^{Aa}, C(O), or NR^{Aa};

A′ is aryl, arylalkyl, or heteroaryl;

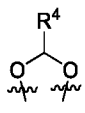
R^P is, independently in each instance, halo, optionally substituted alkyl, –OH, or -$NR^{Aa}R^{Ab}$;

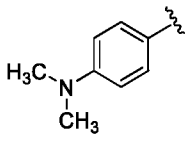
R^{Aa} and R^{Ab} are, independently in each instance, –H, optionally substituted alkyl, or optionally subtitued aryl;

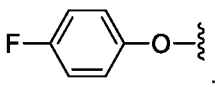
subscript **a** is an integer from 0-19; and

t is an integer from 1-3;

with the proviso that:

(1) R^3 is not –OH (a) when R^1 is –OH or (b) when R^1 and R^2 together form  ,

wherein R^4 is $C_{1\text{-}9}$alkyl or  and

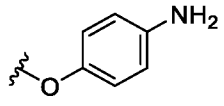
(2) R^3 is not  .

and

R^{5A} and R^{5B} are each, independently, halo or a hydrogen atom;

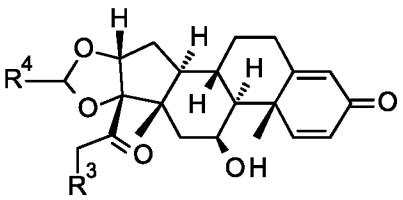
wherein the group R^3 or R^4 is bonded to the linker.

[0203] In some of such embodiments, R^3 is NH_2. In some other of such embodiments, R^3 is

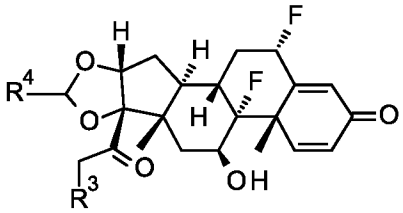


wherein ~ indicates the atom through which R^3 is attached to the adjacent groups in Formula (I).

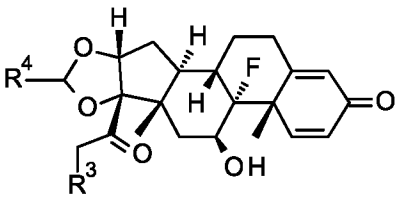
[0204] In certain embodiments, **PA** is a steroid. Exemplary steroid payloads are described, *e.g.*, in U.S. Application No. 62/614,905, filed January 8, 2018, entitled "STEROIDS AND ANTIBODY CONJUGATES THEREOF," and U.S. Application No. 15/806,197, filed November 7, 2017, entitled "STEROIDS AND PROTEIN-CONJUGATES THEREOF," published as US 2018/0155389, each of which is incorporated herein by reference in its entirety. In certain embodiments, PA is selected from



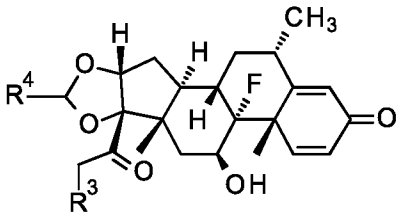
1110



1120

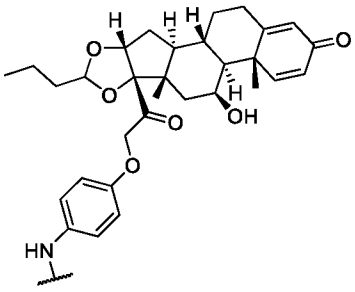


1130



1140

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments according to any of Formulas 1110-1140, R^3 is -O-aryl, -$NR^{Aa}R^{Ab}$, -alkylene-$NR^{Aa}R^{Ab}$, -X-arylene-Y-$NR^{Aa}R^{Ab}$, -X-heteroarylene-Y-$NR^{Aa}R^{Ab}$, or N-containing heterocycloalkyl; wherein X is absent, -N-, -CH_2-, or -O-; wherein Y is absent or -CH_2-; and R^4 is alkyl, aryl, alkylaryl, or arylalkyl. In certain embodiments, R^3 is -O-arylene-$NR^{Aa}R^{Ab}$, -O-heteroarylene-$NR^{Aa}R^{Ab}$; wherein aryl or heteroaryl is optionally substituted with halogen, deuterium, hydroxyl, or methoxyl. In certain embodiments, R^3 is -O-phenyl-$NR^{Aa}R^{Ab}$, -O-heteroarylene-$NR^{Aa}R^{Ab}$; wherein phenyl or heteroaryl is optionally substituted with halogen or deuterium. In certain embodiments, R^4 is n-propyl. In certain embodiments, R^{Aa} and R^{Ab} are each independently hydrogen or alkyl. In particular embodiments, one of R^{Aa} and R^{Ab} is substituted with a bond to the linker (e.g., **L** or **LL**). In certain embodiments, **PA** is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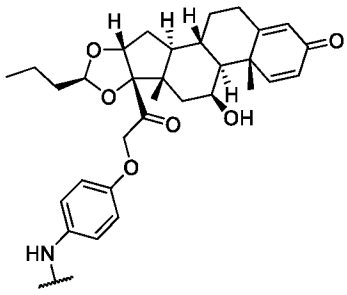
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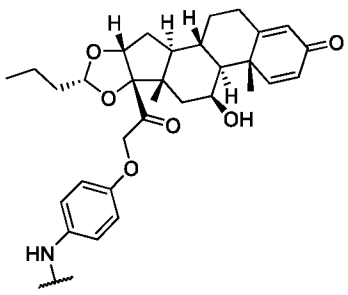
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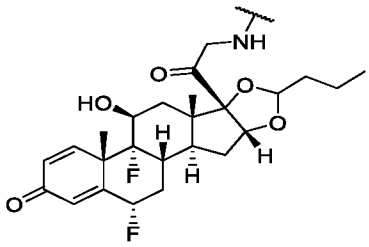
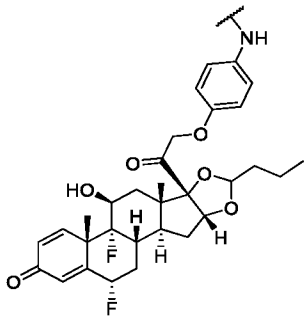
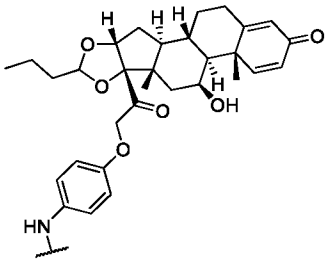
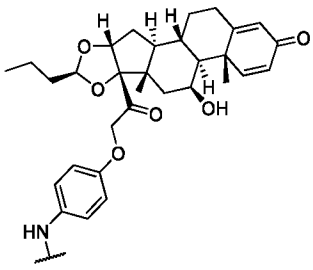
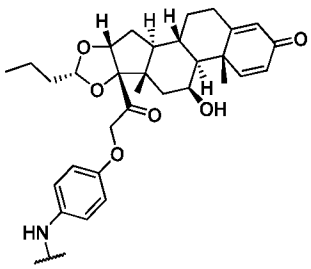
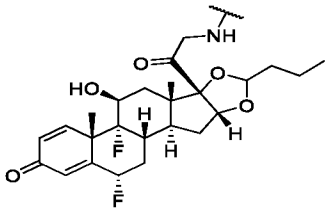
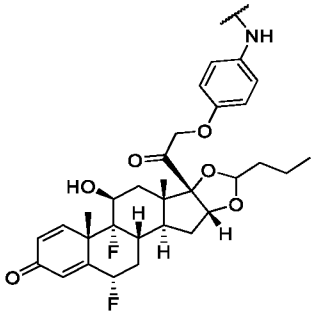

, or

,

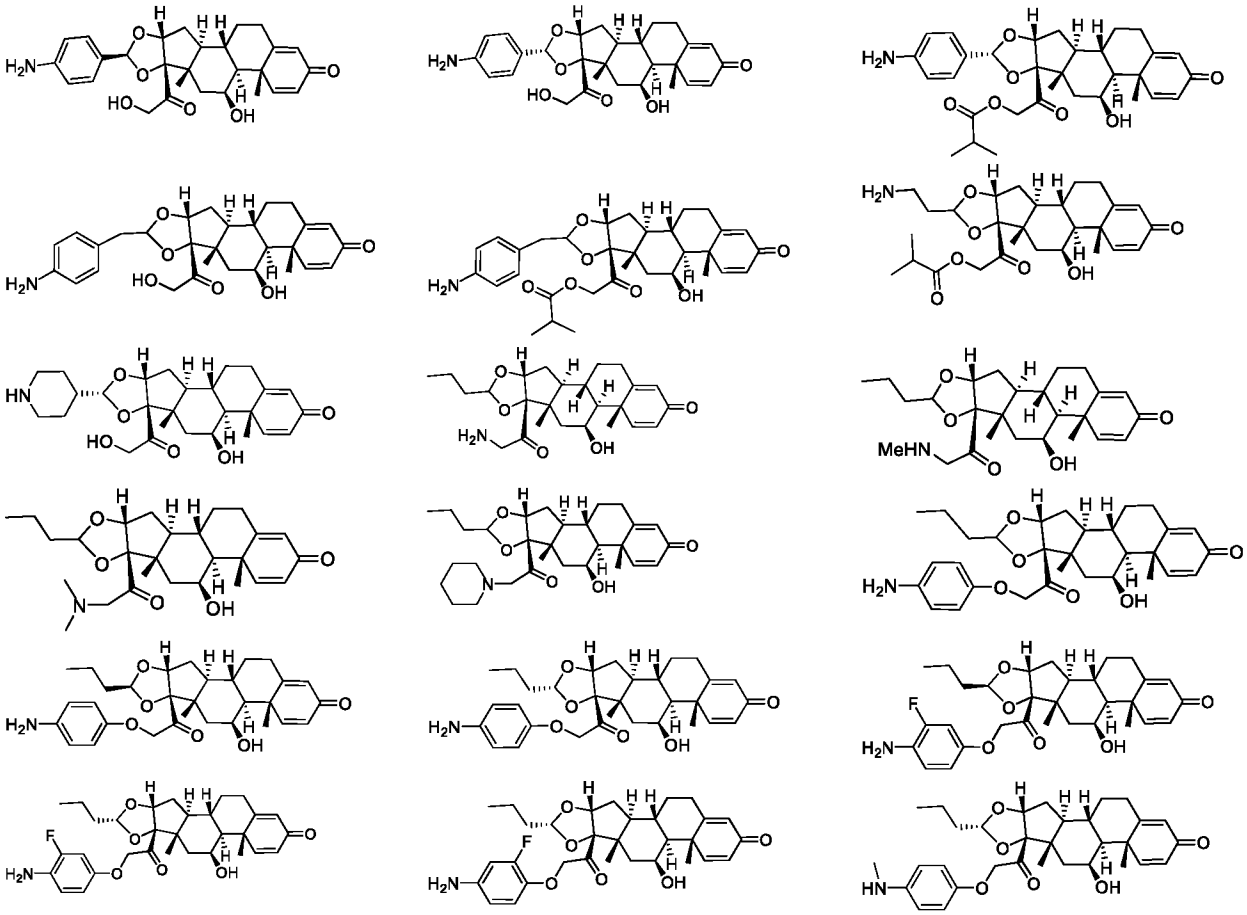
or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments,

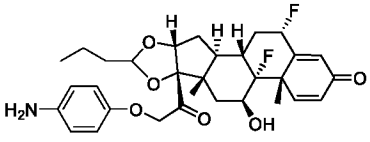
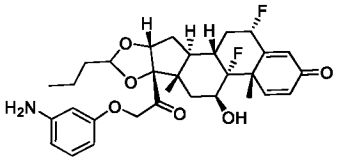
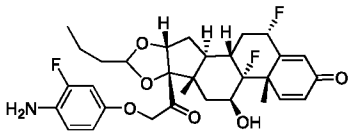
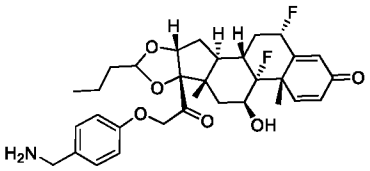
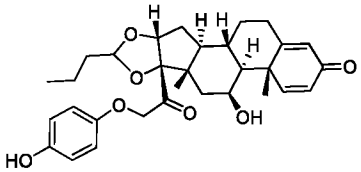
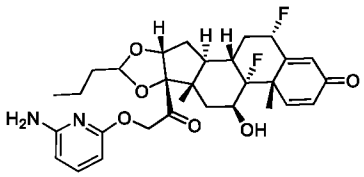
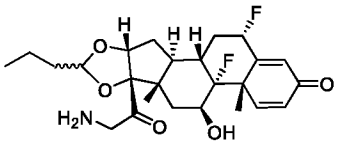
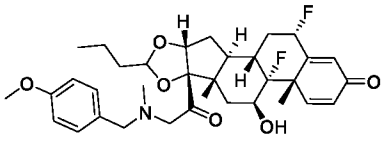
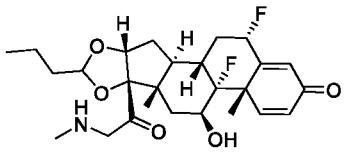
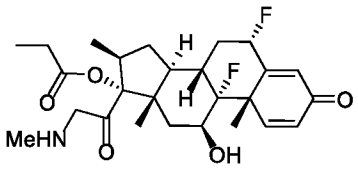
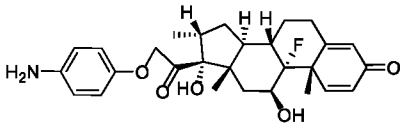
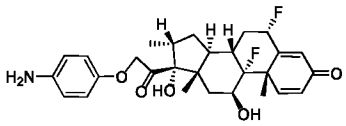
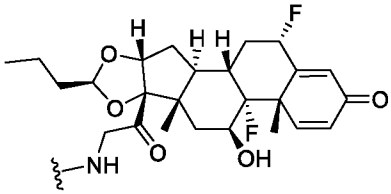
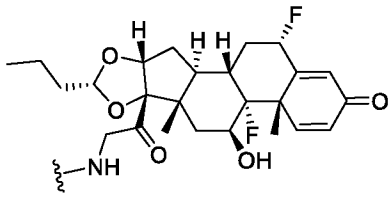
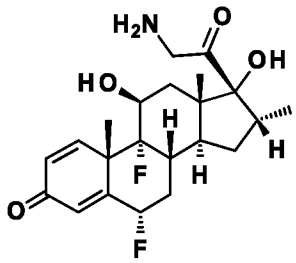
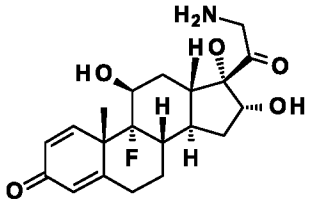
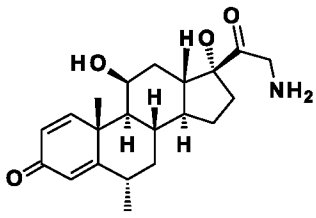
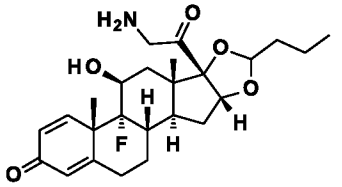
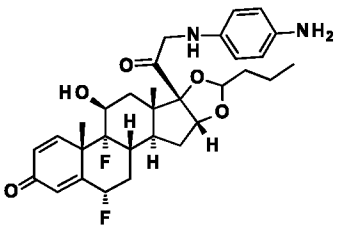
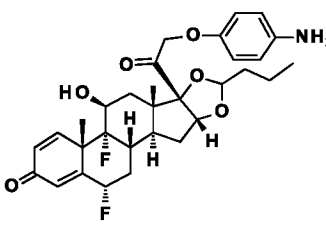
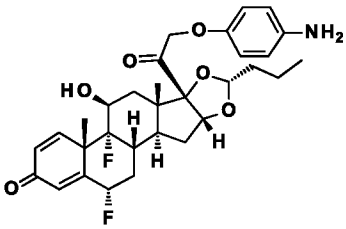
PA is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof In certain embodiments, **PA** is

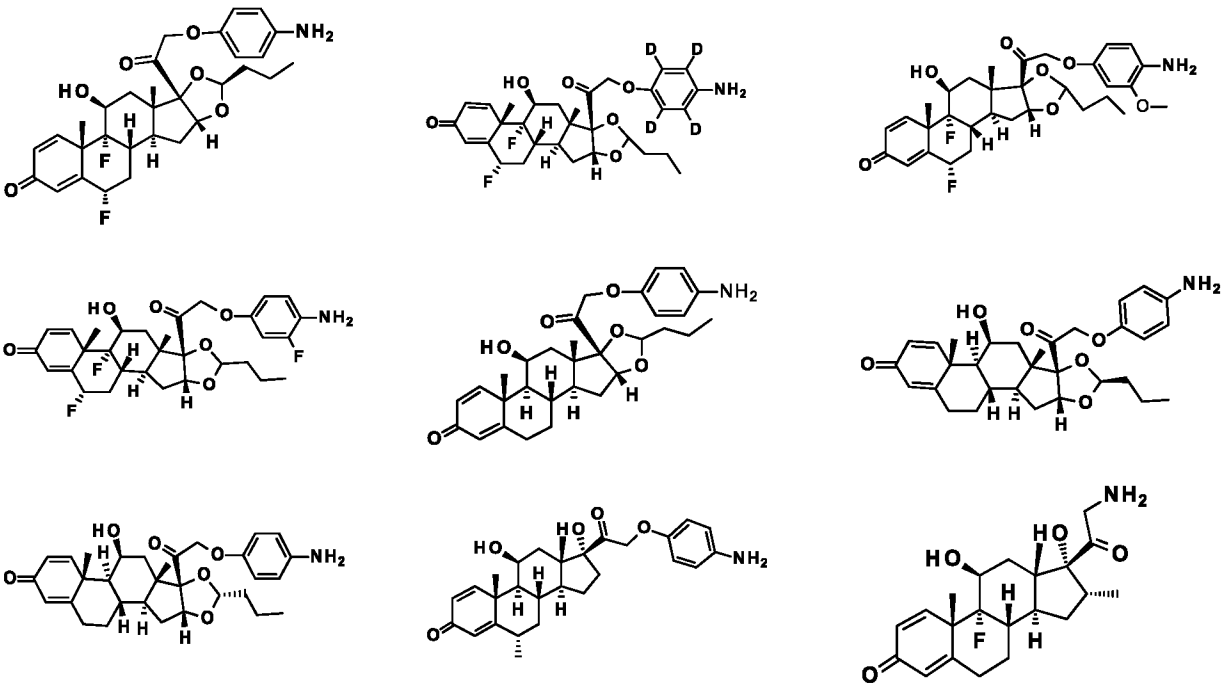

, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof.

In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In such embodiments, the wavy line indicates a bond to the linker (e.g., **L** or **LL**).

[0205] In certain embodiments, **PA** is selected from residues of the following:

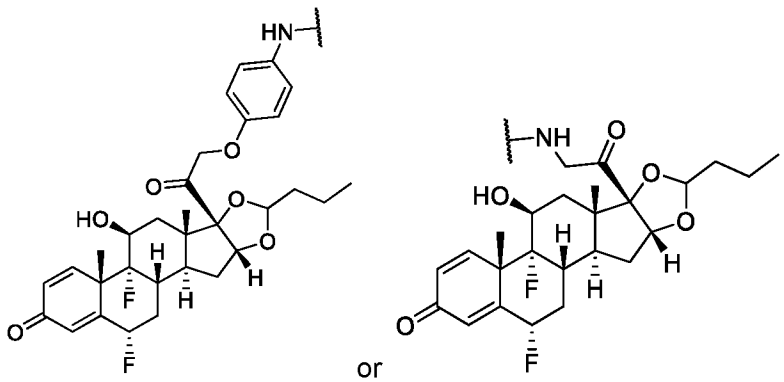


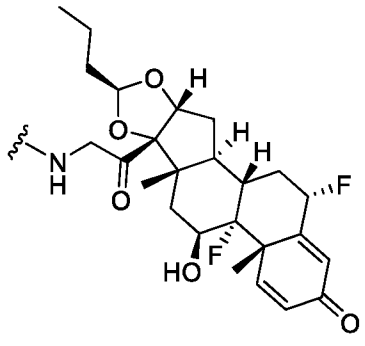
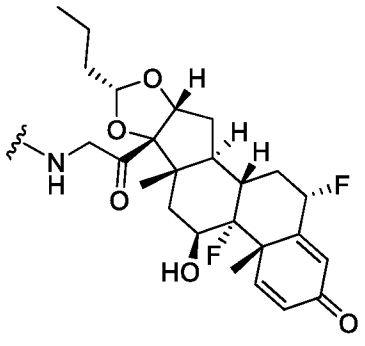


or a pharmaceutically acceptable salt or stereoisomer thereof. In particular embodiments, a primary or secondary amine is linked to a linker **L** to form a linker payload, which is linked to **BA** to form a conjugate. Those of skill will recognize that the above compounds can be linked to L with a bond to a primary or secondary amine group, substituting for an H.

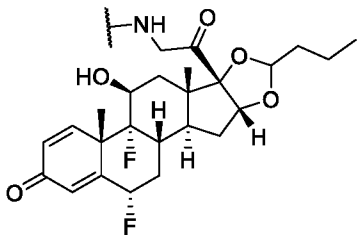
[0206] In some embodiments, the anti-MSR1 antibodies described herein are conjugated to:

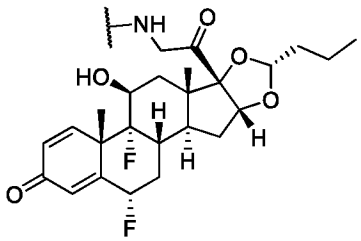


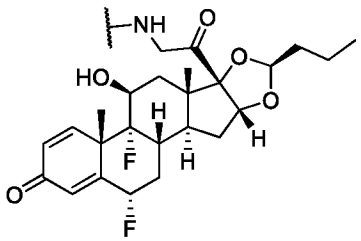
[0207] In some embodiments, the antibody-drug conjugates described herein comprise a steroid payload, wherein the steroid payload is selected from the group consisting of:

 and  , or mixtures thereof.

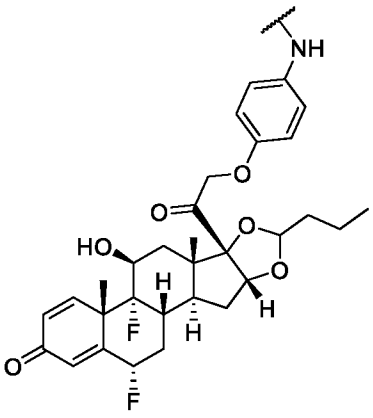
[0208] In certain embodiments, **PA** is:

 , or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:

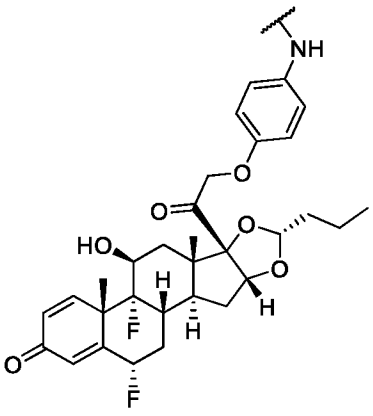
 , or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:

 , or a pharmaceutically acceptable salt or stereoisomer thereof.

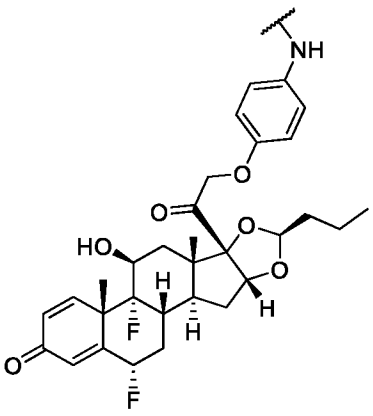
[0209] In certain embodiments, **PA** is:



, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:

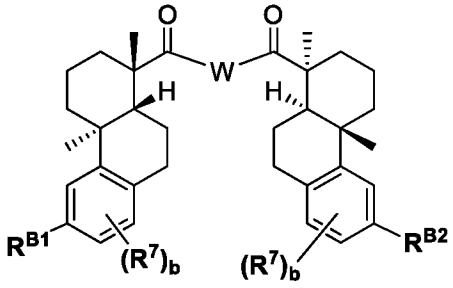


, or a pharmaceutically acceptable salt or stereoisomer thereof In certain embodiments, **PA** is:



, or a pharmaceutically acceptable salt or stereoisomer thereof.

[0210] In certain embodiments, **PA** is a liver X receptor (LXR) modulator. In certain embodiments, **PA** is according to Formula (**B**):



Formula (B)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, wherein

W is $–CH_2–$, –N(H)–, or –O–;

$\mathbf{R^{B1}}$ is –H, –OH, $–NH_2$, alkyl, or$–OP(O)(OR^6)_2$;

$\mathbf{R^{B2}}$ is –H, –OH, $–CH_2NH_2$, $\mathbf{R^{B3}}$, $\mathbf{R^{B4}}$, $\mathbf{R^{B5}}$, or $–O–\mathbf{R^{B5}}$, wherein $\mathbf{R^{B1}}$ and $\mathbf{R^{B2}}$ are not simultaneously –H;

$\mathbf{R^{B3}}$ is $–N(\mathbf{R^6})_2$;

$\mathbf{R^{B4}}$ is **–X–Y–Z**;

X is selected from the group consisting of –O– and –N(H)–;

Y is selected from the group consisting of alkylene, substituted alkylene (including, without limitation, oxo substitution, i.e., =O)), heteroalkylene, and substituted heteroalkylene (including, without limitation, oxo substitution (i.e., =O));

Z is selected from the group consisting of –OH and $–NH_2$;

$\mathbf{R^{B5}}$ is alkyl, heterocycloalkyl, or substituted heterocycloalkyl, wherein each heterocycloalkyl or substituted heterocycloalkyl includes one, two, or three heteroatoms selected from nitrogen and oxygen, and includes at least one –OH and $–CH_2OH$ substituent, or at least one primary or secondary nitrogen, for instance, O-glucose;

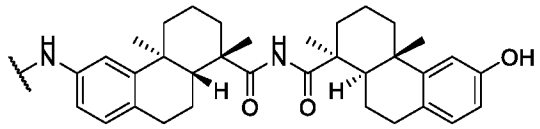
each $\mathbf{R^6}$ is in each instance, –H, an amino acid residue, an *N*-alkyl amino acid residue, a peptide, or alkyl; and

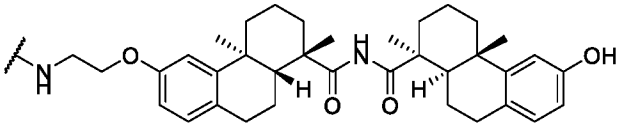
each $\mathbf{R^7}$ is, independently, halo, $C_{1\text{-}6}$ alkyl, $C_{1\text{-}6}$ alkoxy, –CN, O-glucose, O-amino acid residue, and $O\text{-}PEG_b$, wherein each subscript **b** is an integer from 0-3;

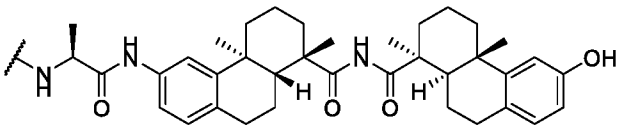
wherein the group $\mathbf{R^{B1}}$, $\mathbf{R^{B2}}$ or $\mathbf{R^7}$ is bonded to the linker.

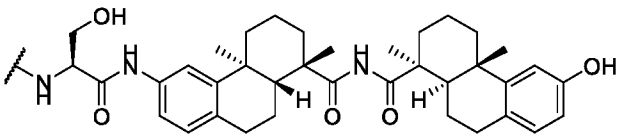
[0211] In particular embodiments, $\mathbf{R^{B1}}$ or $\mathbf{R^{B2}}$ is substituted with a bond to the linker (e.g., **L** or **LL**).

[0212] In certain embodiments, **PA** is selected from:



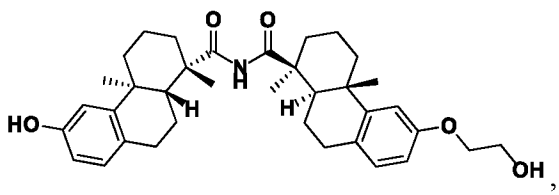


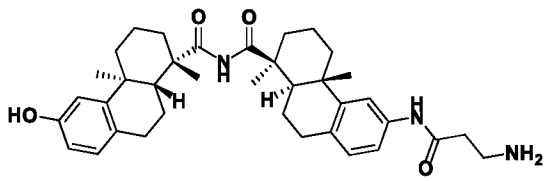


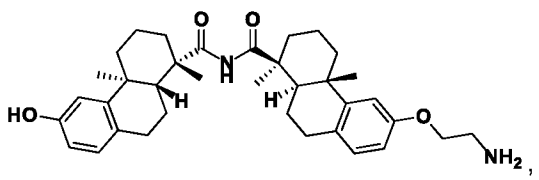


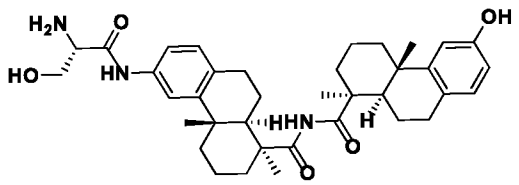
[0213] In particular embodiments, the wavy line in indicates a bond to the linker (e.g., **L** or **LL**).

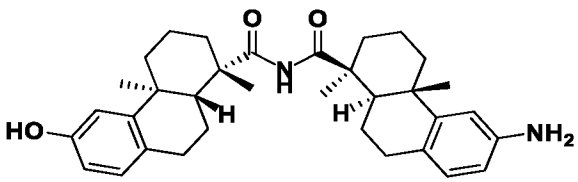
[0214] In certain embodiments, the payload of Formula (**B**) is selected from the group consisting of:

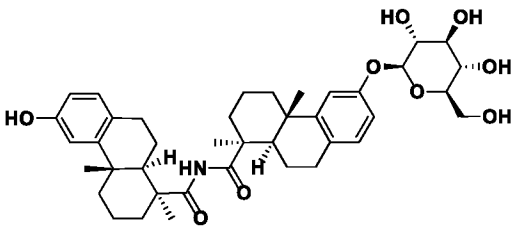


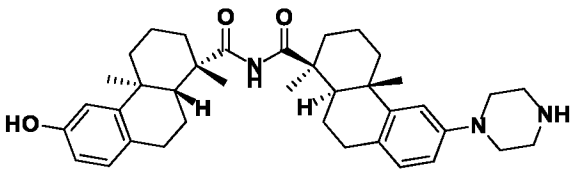







,


,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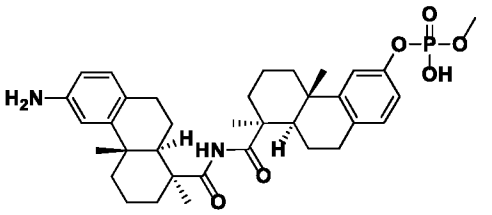
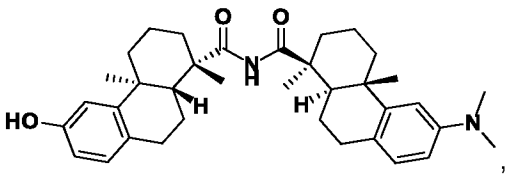
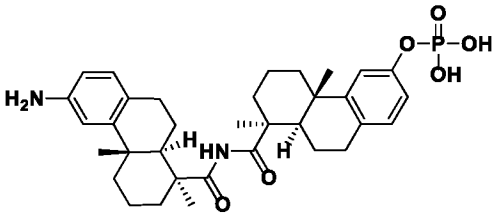
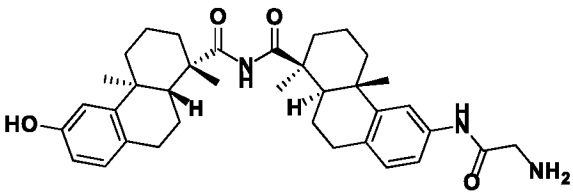
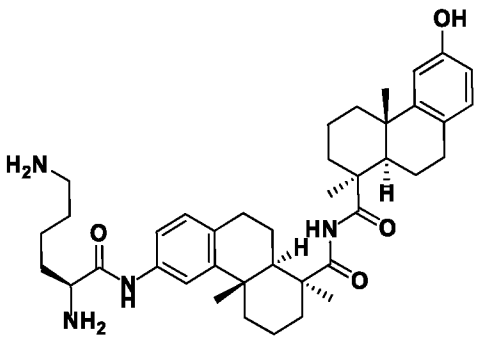
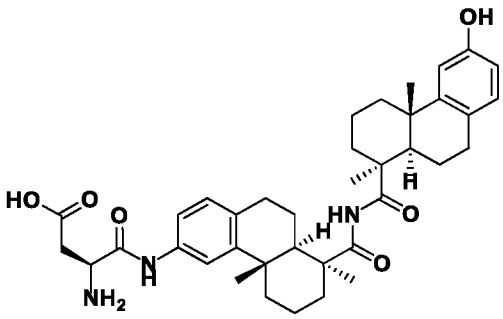

,

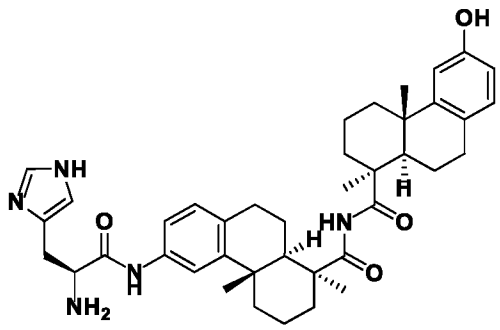

,


,


,

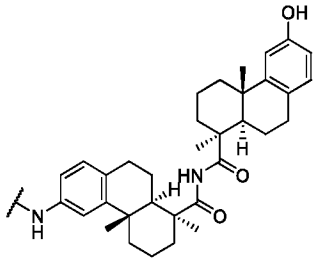

,


, and


,

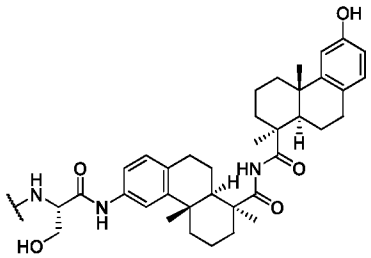
or a pharmaceutically acceptable stereoisomeric form thereof.

[0215] In certain embodiments, **PA** is:



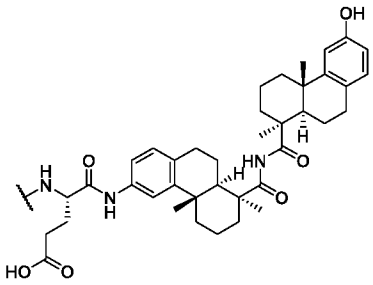
, or a pharmaceutically acceptable salt or stereoisomer thereof.

[0216] In certain embodiments, **PA** is:



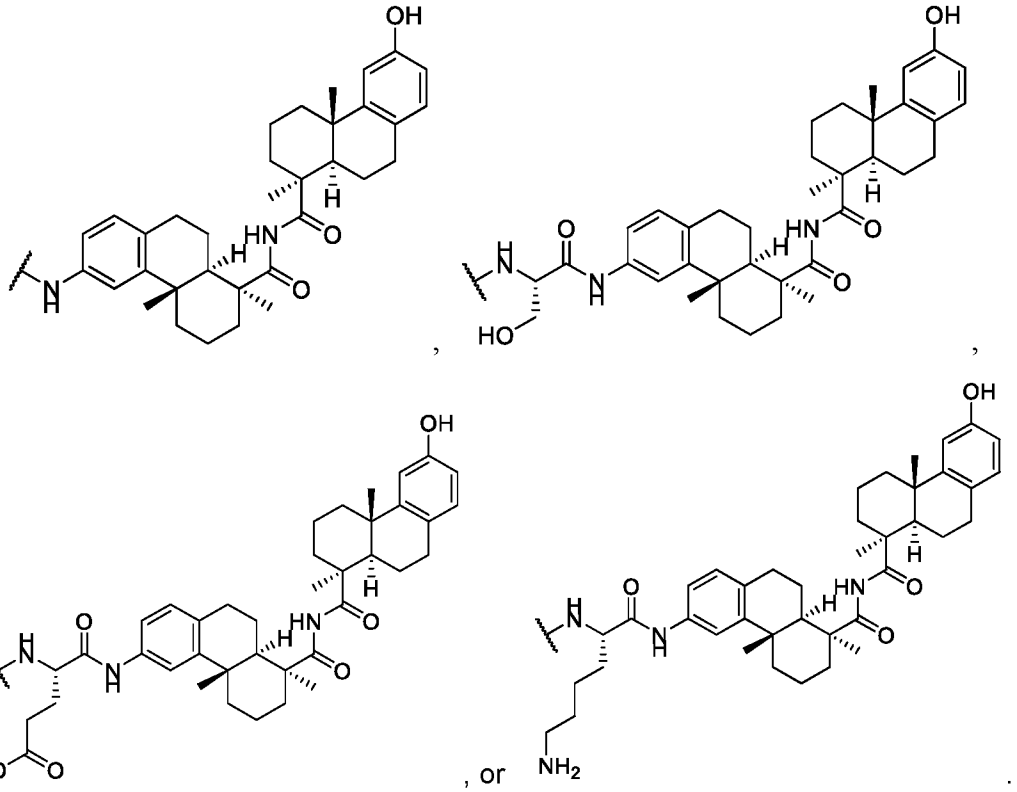
, or a pharmaceutically acceptable salt or stereoisomer thereof.

[0217] In certain embodiments, **PA** is:

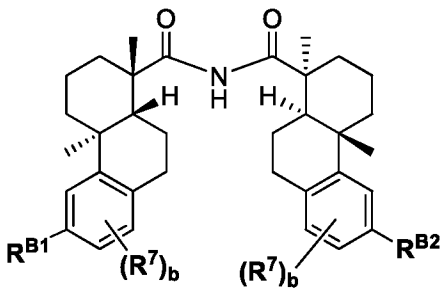


, or a pharmaceutically acceptable salt or stereoisomer thereof.

[0218] In some embodiments, the anti-MSR1 antibodies described herein are conjugated to:



[0219] In some embodiments, **PA** is a liver X receptor (LXR) modulator according to Formula (**B-1**):



Formula **(B-1)**

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof,

wherein

R^{B1} is –N(H)R^8 or –N(R^9)$_2$;

R^{B2} is –N(H)R^8;

each R^8 is, independently in each instance, hydrogen, an amino acid residue, an *N*-alkyl amino acid residue, a peptide residue, a biodegradable moiety, or alkyl;

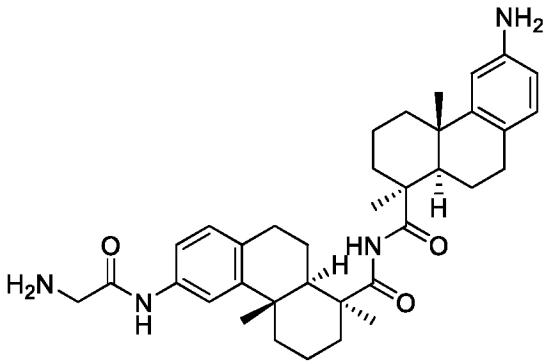
R^9 is alkyl, aryl, arylalkyl, heterocycloalkyl, or substituted heterocycloalkyl, wherein each heterocycloalkyl or substituted heterocycloalkyl comprises one, two, or three

heteroatoms selected from nitrogen and oxygen, and when substituted includes at least one –OH and -CH_2OH, or at least one primary or secondary nitrogen;

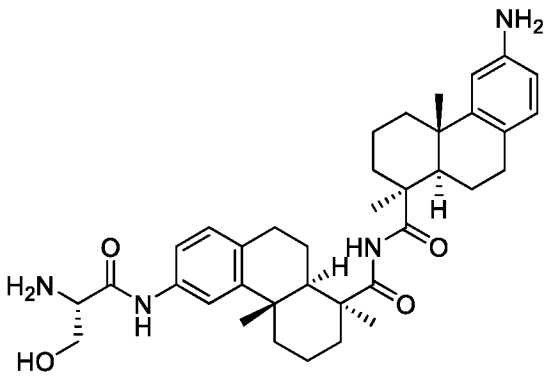
each $\mathbf{R^7}$ is independently halo, $C_{1\text{-}6}$ alkyl, $C_{1\text{-}6}$ alkoxy, –CN, O-glucose, O-amino acid residue, or O-PEG_b, wherein each subscript **b** is an integer from 0-3;

wherein the group $\mathbf{R^{B1}}$, $\mathbf{R^{B2}}$ or $\mathbf{R^7}$ is bonded to the linker.

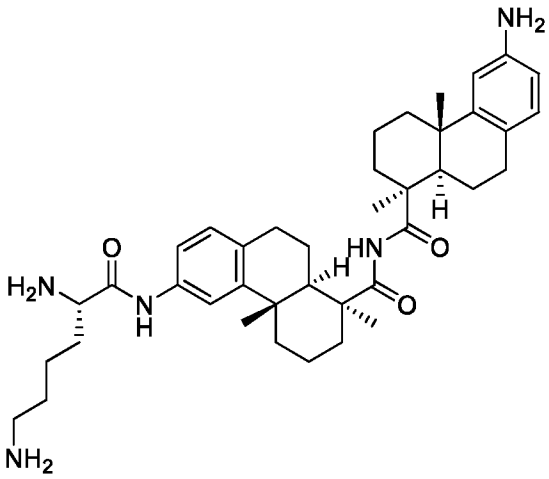
[0220] In some instances, a compound of Formula (**B-1**) is selected from the group consisting of:



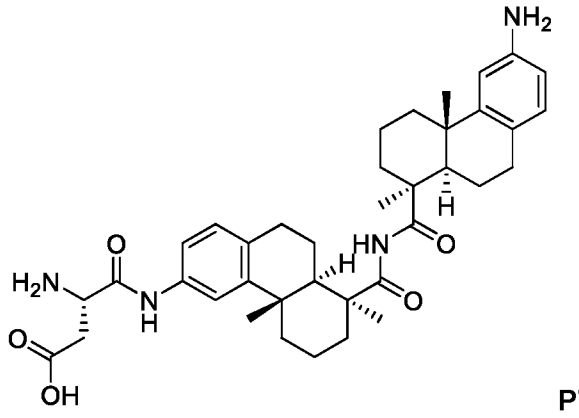
P4B;



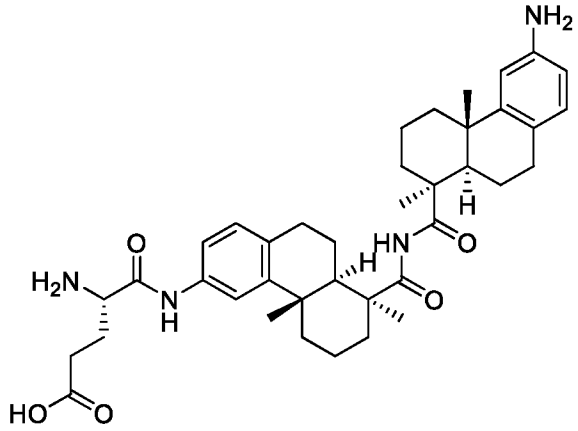
P5B;



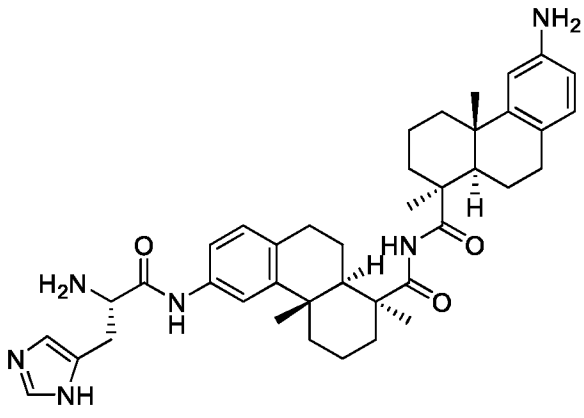
P6B;



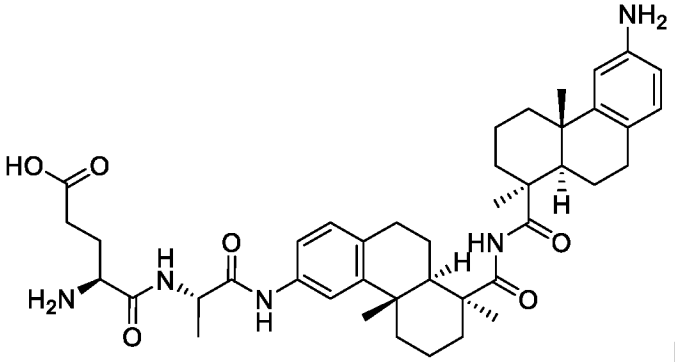
P7B;



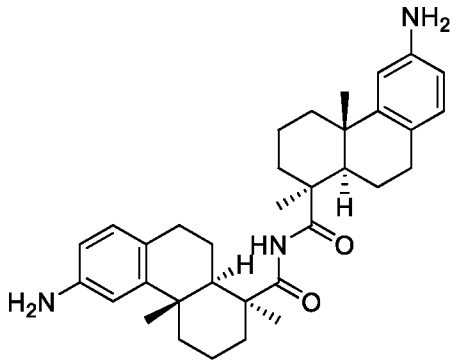
P8B;



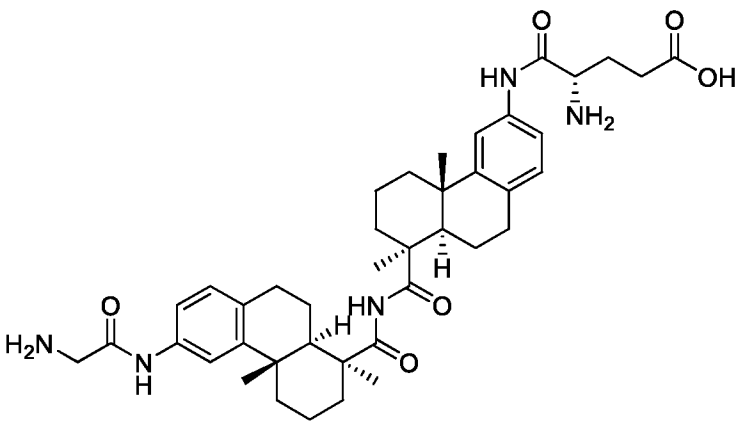
P9B;



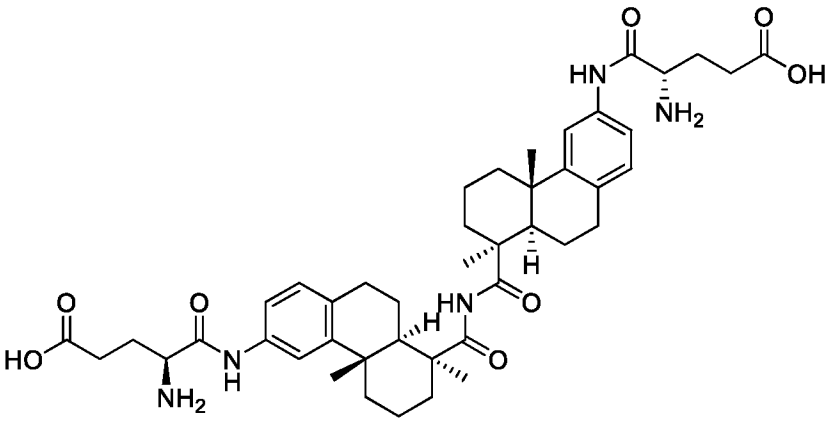
P12B;



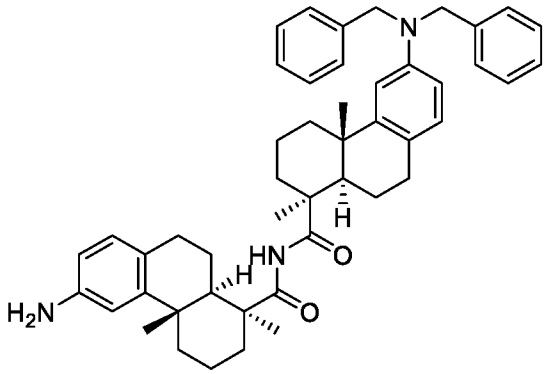
P2B;



P10B;



P11B; and

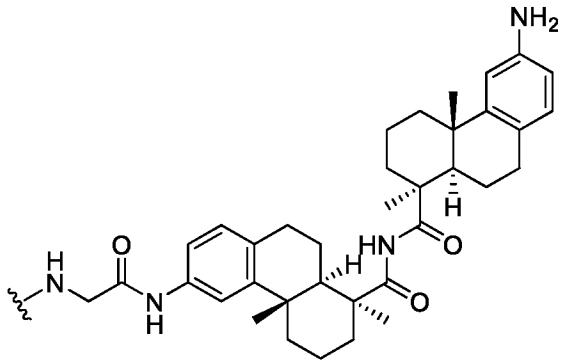


P3B;

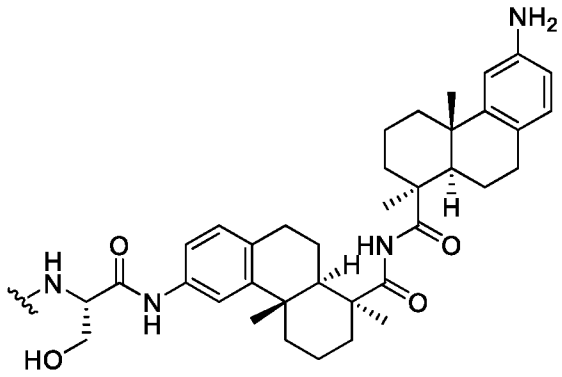
or a pharmaceutically acceptable salt or solvate thereof.

[0221] In particular embodiments, R^{B1} or R^{B2} is substituted with a bond to the linker (e.g., **L** or **LL**).

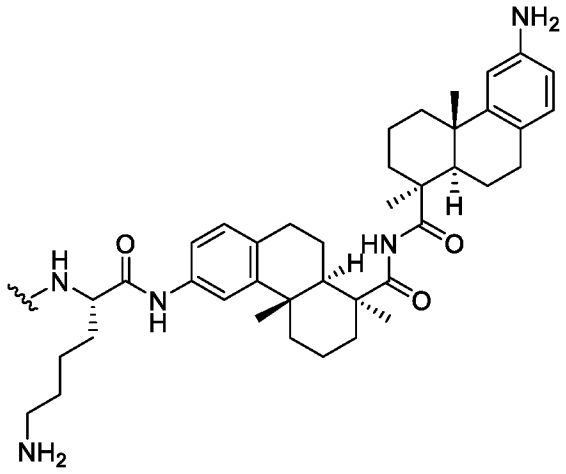
[0222] In certain embodiments, **PA** is



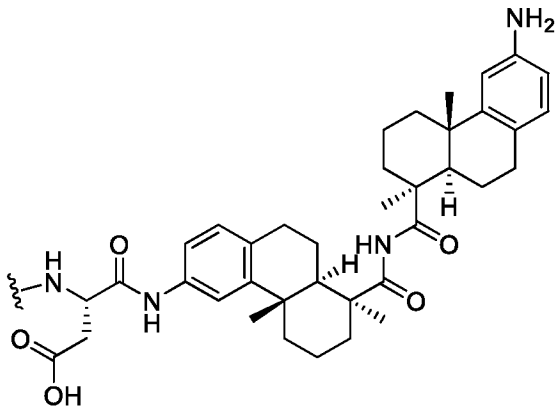
P4B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:



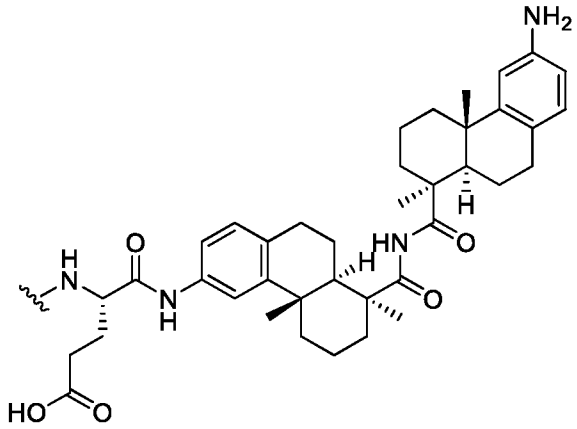
P5B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:



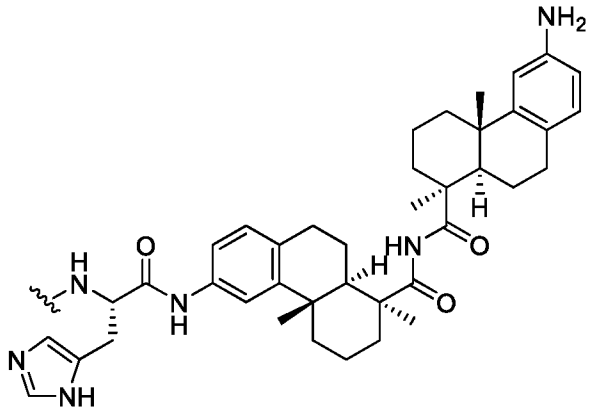
P6B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is:



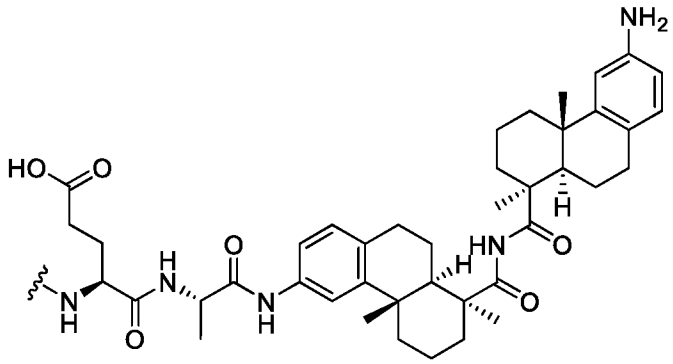
P7B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is



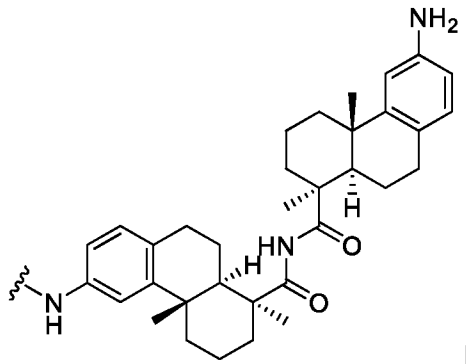
P8B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is



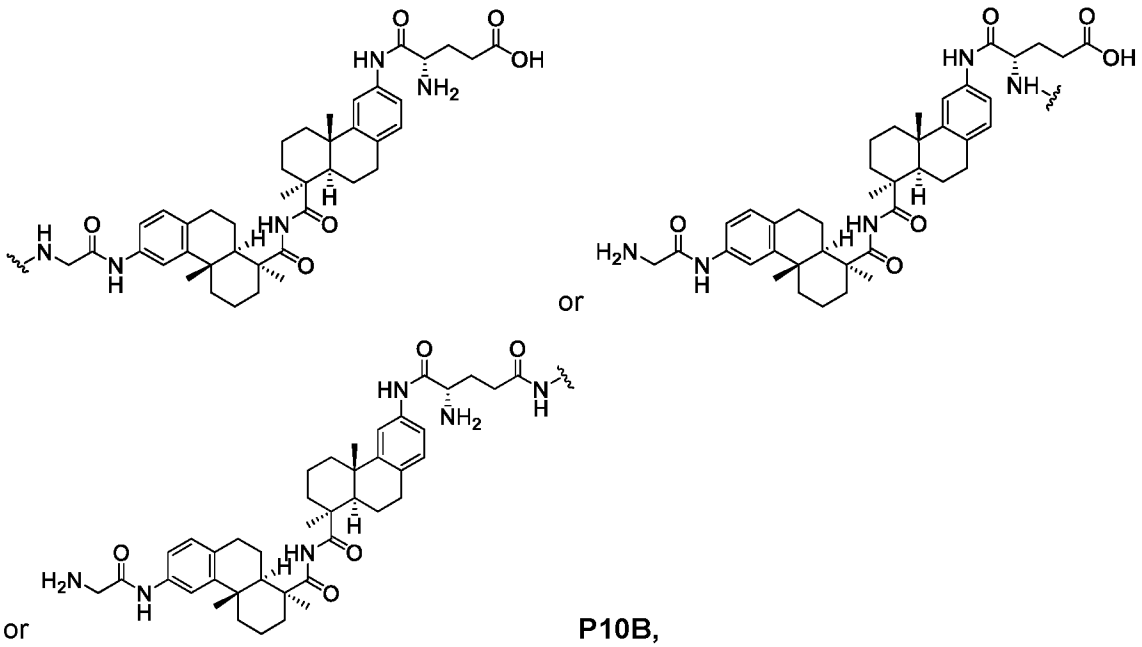
P9B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is



P12B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is

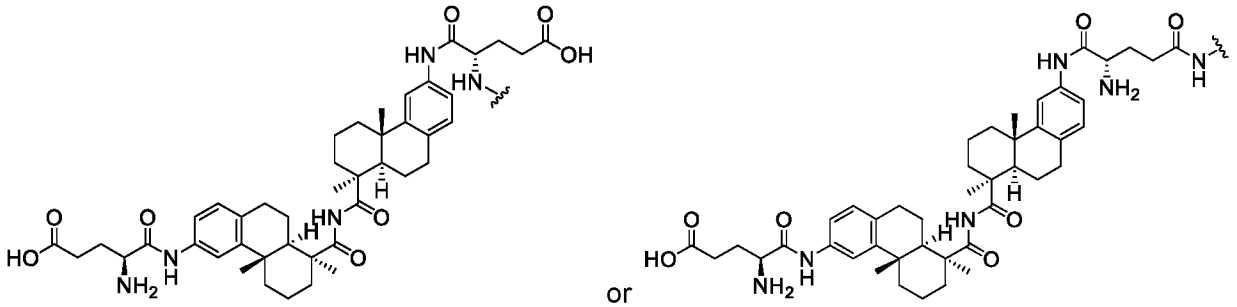


P2B, or a pharmaceutically acceptable salt or stereoisomer thereof. In certain embodiments, **PA** is



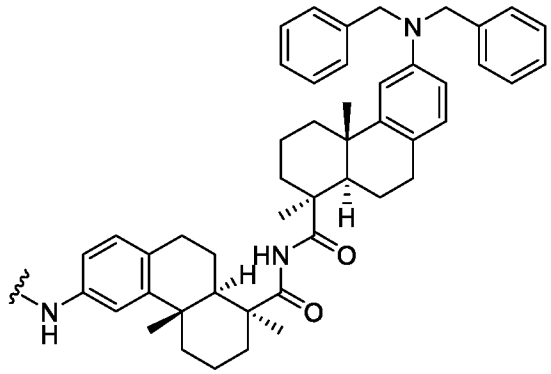
P10B,

or a pharmaceutically acceptable salt or stereoisomer thereof. In some of such embodiments, the carboxylic acid group of **P10B** is bonded to the linker through an amide linkage as shown above. In certain embodiments, **PA** is



P11B,

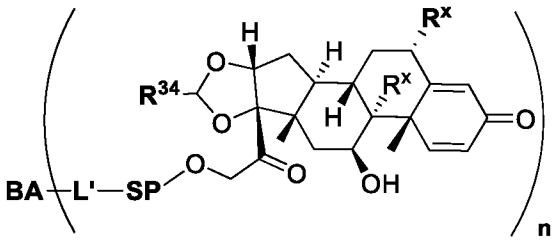
or a pharmaceutically acceptable salt or stereoisomer thereof. In some of such embodiments, the carboxylic acid group of **P11B** is bonded to the linker through an amide linkage as shown above. In certain embodiments, **PA** is



P3B, or a pharmaceutically acceptable salt or stereoisomer thereof.

For any embodiments described in this paragraph, the wavy line in indicates a bond to the linker (e.g., **L** or **LL**) as described herein.

[0223] In a group of embodiments, provided herein are compounds of Formula (**III**):

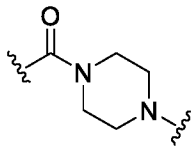
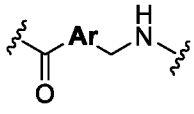


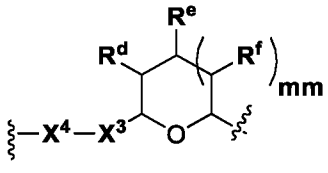
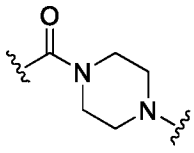
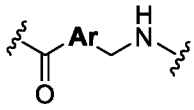
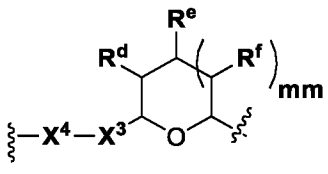
Formula (**III**);

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof,

wherein:

R^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl;

both R^x are hydrogen; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^1- where X^1 is attached to **L′** in Formula (**III**), -C(O)-N(H)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (**III**), -C(O)-N(C_{1-6}alkyl)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (**III**),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (**III**), -CH_2-NH- where the N is attached to **L′** in Formula (III),  where the N is attached to **L′** in Formula (**III**) and where **Ar** is optionally substituted arylene or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more

hydroxy, -C(O)-N(**R**^{35})-C_1-C_{10}-alkylene-C(O)NH-**X**^{2}- where **X**^{2} is attached to **L′** in Formula (**III**), or  where **X**^{4} is attached to **L′** in Formula (**III**); or both **R**^{x} are fluoro; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-**X**^{1b}- where **X**^{1b} is attached to **L** in Formula (**III**), -C(O)-N(H)-(C_1-C_{10}-alkylene)-**X**^{1b}- where **X**^{1b} is attached to **L′** in Formula (**III**),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (**III**), -CH_2-NH- where the N is attached to **L′** in Formula (III),  where the N is attached to **L′** in Formula (**III**) and where **Ar** is optionally substituted arylene or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (**III**), -C(O)-(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (**III**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(**R**^{35})-(C_1-C_{10}-alkylene)-C(O)NH-**X**^{2}- where **X**^{2} is attached to **L′** in Formula (**III**), or  where **X**^{4} is attached to **L′** in Formula (**III**); and

X^1 is -N(C_{1-6}alkyl)-;

X^{1b} is -S-, -NH-, or -N(C_{1-6}alkyl)-;

X^2 is -NH-;

X^3 is -CH_2-, **X**^{3} is -CH_2-O-(C_1-C_{10}-alkylene)-C(O)- where the C(O) is attached to **X**^{4}, or **X**^{3} is -C(O)-;

X^4 is -O-;

R^{35} is H, -OH, -OCH_3, or C_{1-6}alkyl;

R^{50} and **R**^{50a} are independently hydrogen or C_1-C_6-alkyl;**R**^{d}, **R**^{e}, and **R**^{f} are independently -H, -OH, hydroxyalkyl, alkoxycarbonyl, -C(O)OH, or -CH_2O**R**^{g}, where each **R**^{g} is independently -CH_2C(O)OH or -CH_2C(O)O(alkyl); and

mm is 0 or 1;

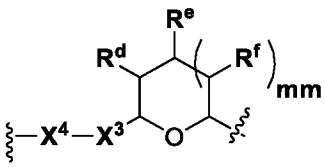
n is an integer selected from 1-30, inclusive;

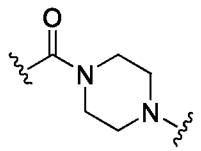
L′ is a linker; and

BA is a binding agent.

[0224] In some embodiments, in a compound of Formula (**III**), both R^x are hydrogen, and **SP**, **BA**, **L′**, R^{34} , and **n** are as described herein in some or any embodiments. In some embodiments, in a compound of Formula (**III**), both R^x are fluoro, and **SP**, **BA**, **L′**, R^{34} , and **n** are as described herein in some or any embodiments. In some embodiments, R^{34} is in the R-configuration. In some embodiments, R^{34} is in the S-configuration. In some embodiments, R^{34} is a mixture of the R- and S-configurations. In some embodiments, R^{34} is a mixture of the R- and S-configurations, wherein the R:S mixture is about 1:1, about 2:1, about 3;1, about 4:1, about 5:1, about 6;1, about 7:1, about 8:1, about 9:1, or about 10:1.

[0225] In some embodiments are compounds of Formula (**III**) where

both R^x are hydrogen; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^1- where X^1 is attached to **L′** in Formula (**III**), -C(O)-N(H)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (**III**), -C(O)-N(C_{1-6}alkyl)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (**III**), -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(R^5)-C_1-C_{10}-alkylene-C(O)NH-X^2- where X^2 is attached to **L′** in Formula (**III**), or  where X^4 is attached to **L′** in Formula (**III**); or

both R^x are fluoro; and **SP** is -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^{1b}- where X^{1b} is attached to **L′** in Formula (**III**), -C(O)-N(H)-(C_1-C_{10}-alkylene)-X^{1b}- where X^{1b} is attached to **L′** in Formula (**III**),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (**III**), -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (**III**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, or -C(O)-N(R^5)-(C_1-C_{10}-alkylene)-C(O)NH-X^2- where X^2 is attached to **L′** in Formula (**III**).

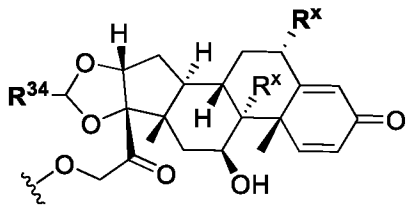
[0226] In some embodiments, a compound of Formula (**I**) is a compound of Formula (**3000**):

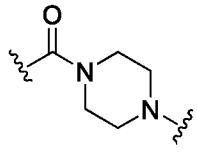
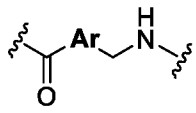
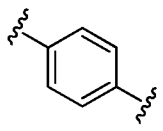
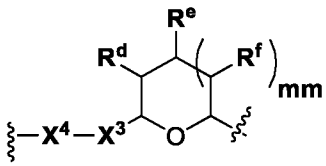
BA-(**L′-SP-D**)$_n$ Formula (**3000**)

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof,

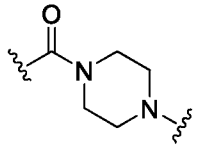
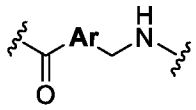
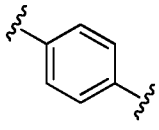
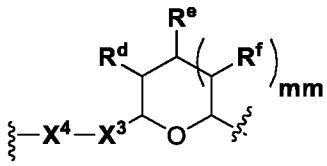
wherein

D is selected from

a)  Formula (a),

where both R^x in Formula (a) are hydrogen; R^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^1- where X^1 is attached to **L′** in Formula (3000), -C(O)-N(H)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (3000), -C(O)-N(C_{1-6}alkyl)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (3000),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (3000), -CH_2-NH- where the N is attached to **L′** in Formula (3000),  where the N is attached to **L′** in Formula (3000) and where **Ar** is optionally substituted arylene (in some embodiments ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (3000), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to **L′** in Formula (3000) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(R^{35})-C_1-C_{10}-alkylene-C(O)NH-X^2- where X^2 is attached to **L′** in Formula (3000), or  where X^4 is attached to **L′** in Formula (3000); or

where both R^x in Formula (a) are fluoro; R^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^{1b}- where X^{1b} is attached to **L′** in Formula (3000), -C(O)-N(H)-(C_1-C_{10}-

alkylene)-**X**^{1b}- where **X**^{1b} is attached to **L′** in Formula (3000),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (3000), -CH_2-NH- where the N is attached to **L′** in Formula (3000),  where the N is attached to **L** in Formula (3000) and where **Ar** is optionally substituted arylene (in some embodiments ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (3000), -C(O)-(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (3000) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(**R**^{35})-(C_1-C_{10}-alkylene)-C(O)NH-**X**^{2}- where **X**^{2} is attached to **L′** in Formula (3000), or  where **X**^{4} is attached to **L′** in Formula (3000); and/or

b) the compounds in Table A below, where the compounds in Table A are linked to **BA** of the Compound of Formula (**III**) through the hydroxy of the -C(O)CH_2OH group, *i.e.* by -C(O)CH_2-O-**SP**-**L′**-, or through the hydroxy of Mapracorat, *i.e.* by -O-**SP**-**L′**-;

X^1 is -N(C_{1-6}alkyl)-;

X^{1b} is -S-, -NH-, or -N(C_{1-6}alkyl)-;

X^2 is -NH-;

X^3 is -CH_2-, **X**^{3} is -CH_2-O-(C_1-C_{10}-alkylene)-C(O)- where the C(O) is attached to **X**^{4}, or **X**^{3} is -C(O)-;

X^4 is -O-;

R^{35} is H, -OH, -OCH_3, or C_{1-6}alkyl;

R^{50} and **R**^{50a} are independently hydrogen or C_1-C_6-alkyl;

R^d, **R**^{e}, and **R**^{f} are independently -H, -OH, hydroxyalkyl, alkoxycarbonyl, -C(O)OH, or -CH_2O**R**^{g}, where each **R**^{g} is independently -CH_2C(O)OH or -CH_2C(O)O(alkyl); and

mm is 0 or 1;

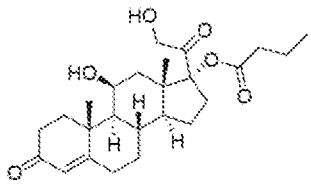
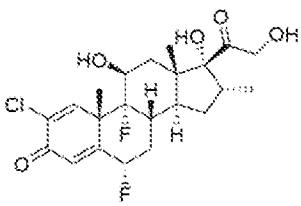
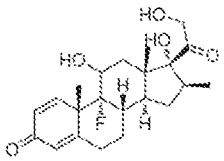
n is an integer selected from 1-30, inclusive;

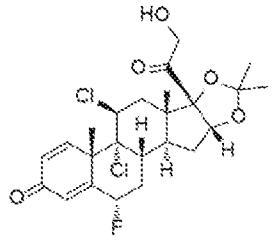
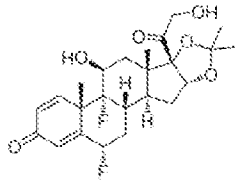
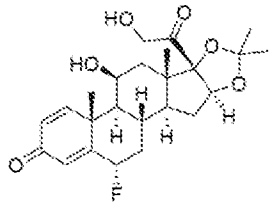
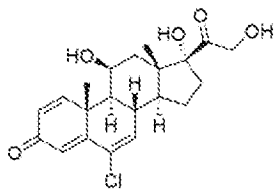
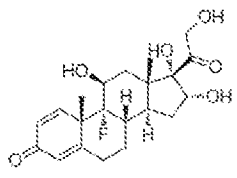
L′ is a linker; and

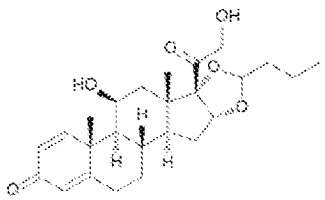
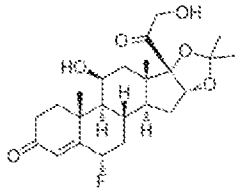
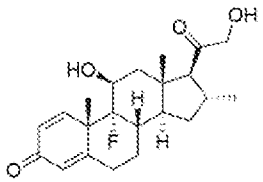
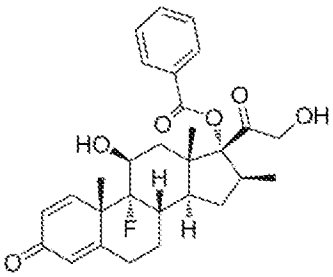
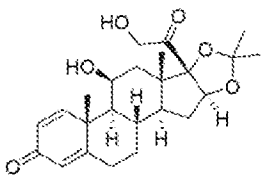
BA is a binding agent.

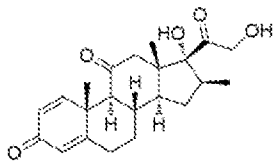
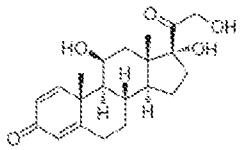
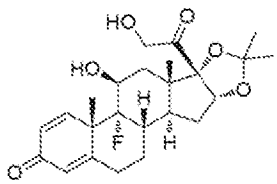
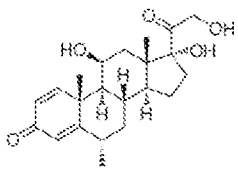
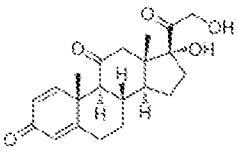
[0227] In some instances of Formula (3000), **D** is budesonide or any other steroid shown in Table A. In some instances of Formula (3000), **D** is any budesonide analog described herein. The compounds in Table A are linked to **BA** of the Compound of Formula (**III**) through the hydroxy of the -C(O)CH_2OH group, *i.e.* by -C(O)CH_2-O-**SP**-**L′**-, or through the hydroxy of Mapracorat, *i.e.* by -O-**SP**-**L′**-. In some instances of Formula (3000), the moiety **SP**-**D** (or **H-SP-D**) is refererred to herein as a "budesonide-spacer" and includes, e.g., budesonide-spacers shown in Table B, in the examples section and in Scheme 1 in **FIG. 22**.

Table A.

Trade name	**Structure**
Hydrocortisone butyrate Locoid®	
Halometasone Sicorten®/ (C-48401-Ba)	
Betamethasone Celestone®/Rinderon®/Diprosone® (NSC-39470; Sch-4831)	

Trade name	Structure
Fluclorolone Acetonide Cutanit®/Topicon® (RS-2252)	
Fluocinolone Acetonide Flucort®/Fluonid®/Iluvien®/Retisert®/Synalar®/Synalar-HP®/Synemol® (NSC-92339; DF-277)	
Flunisolide (RS-3999; RS-1320)	
Cloprednol (RS-4691)	
Triamcinolone Aristocort®/Kenacort®	

Trade name	Structure
Budesonide	
Flurandrenolide Cordran®	
Desoximetasone Topicort® (DSXS)	
Betamethasone benzoate Uticort® (W-5975)	
Desonide Desonate® (D-2083)	

Trade name	Structure
Meprednisone (NSC-527579; Sch-4358)	
Prednisolone Delta-Cortef® (NCS-9120)	
Triamcinolone Acetonide	
Methylprednisolone Depo-Medrol; Medrol; Urbason® (NSC-19987)	
Prednisone Decortin®/Deltasone®/Lodotra®/Meticorten®/Rayos® (NSC-10023)	

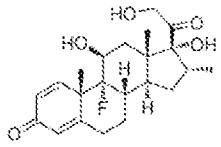
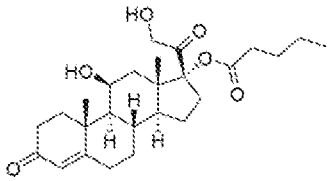
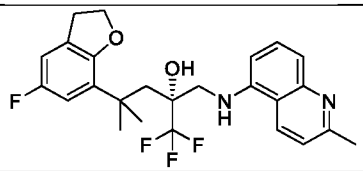
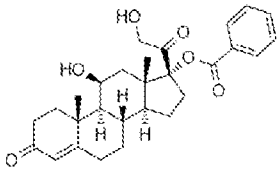
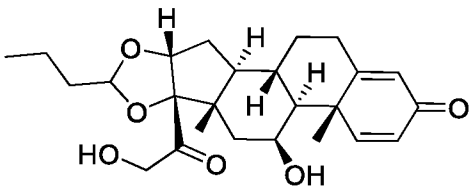
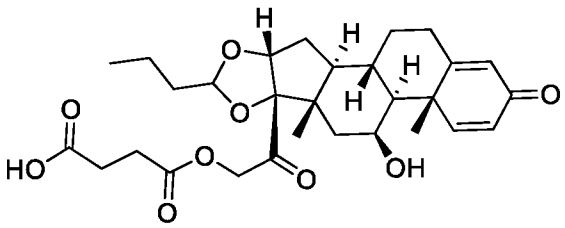
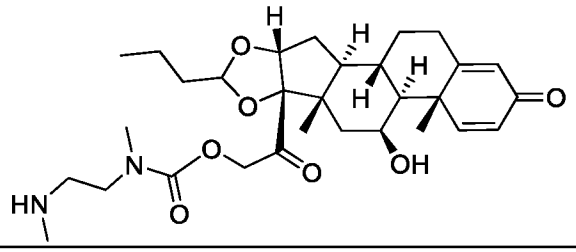
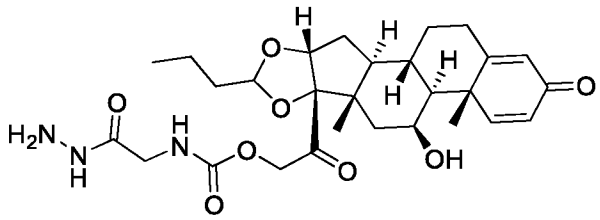
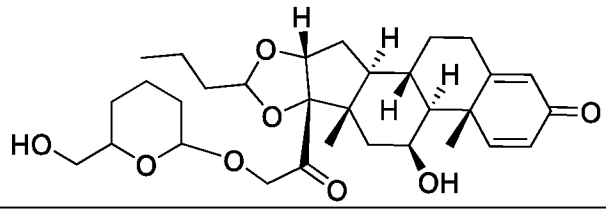
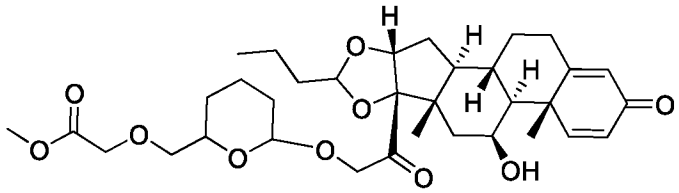
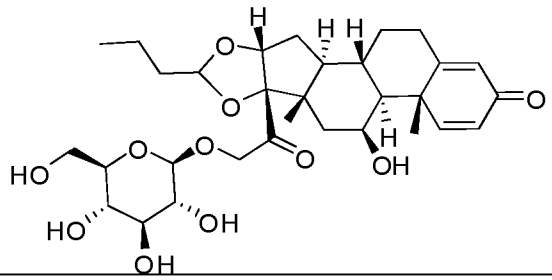
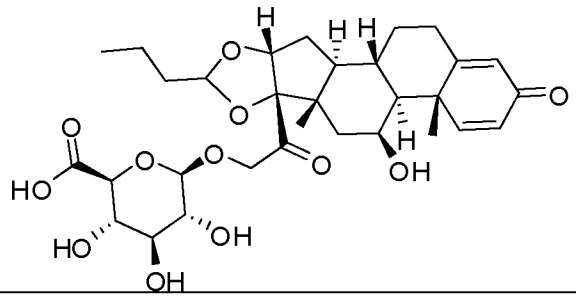
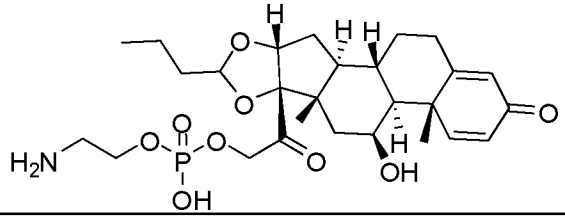
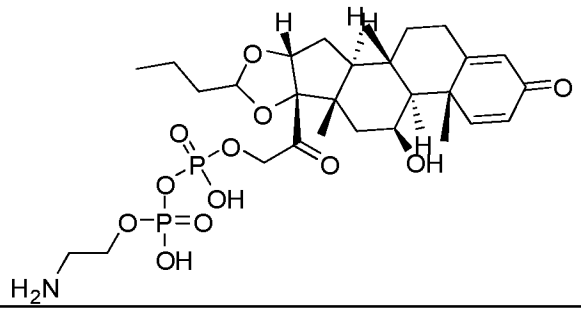
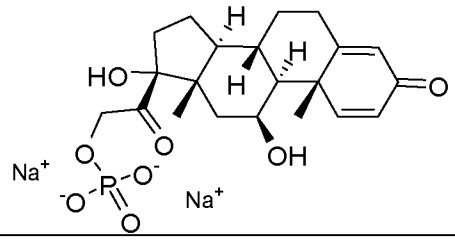
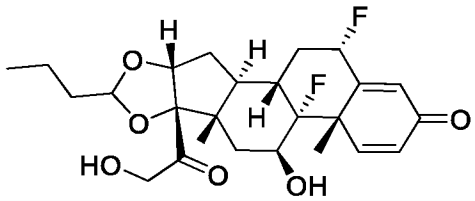
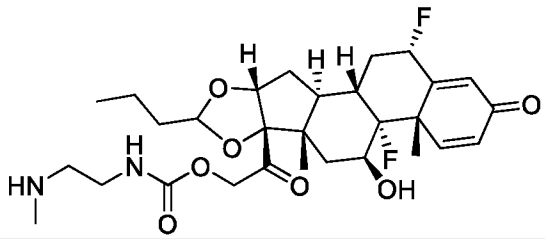
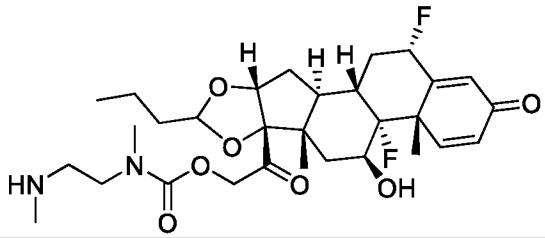
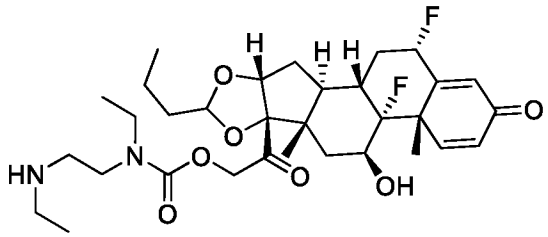
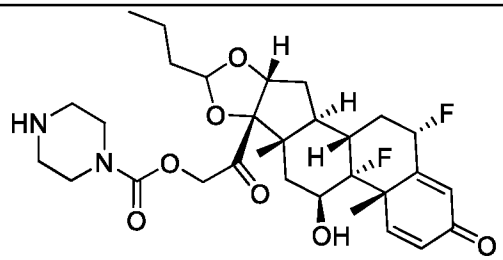
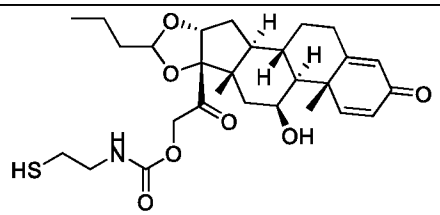
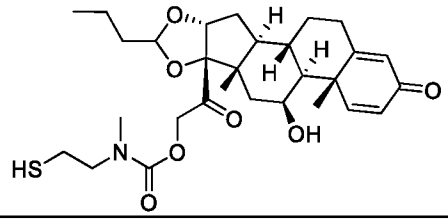
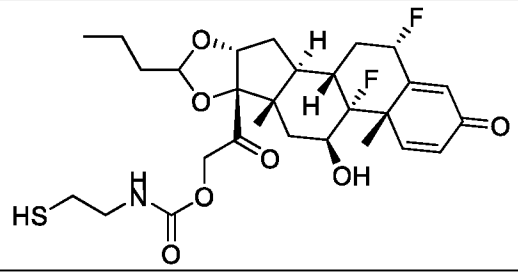
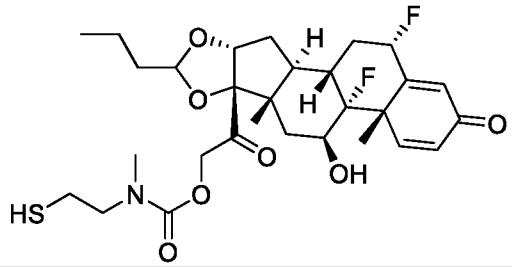
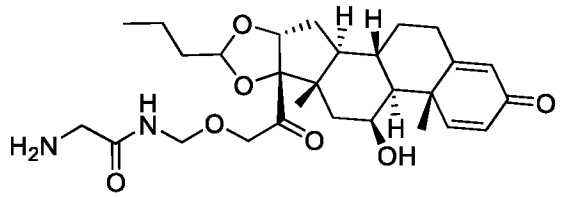
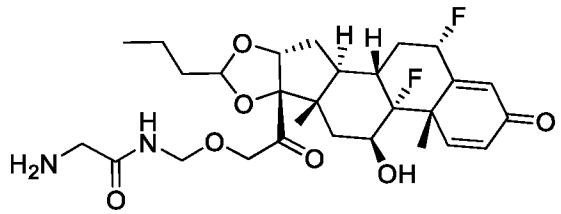
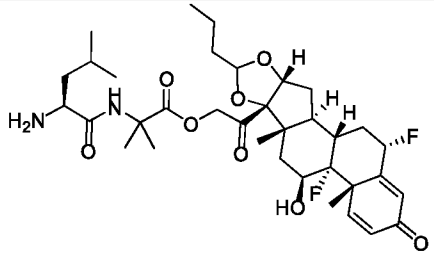
Trade name	Structure
Dexamethasone Decadron® (FT-4145; ENV-1105; IBI-10090; ISV-305; OTO-104)	
Hydrocortisone valerate Westcort®	
Mapracorat (BOL-242X; BOL-303242-X; ZK-245186; BAY-865319; BOL-303242-X)	
Benzodrocortisone	

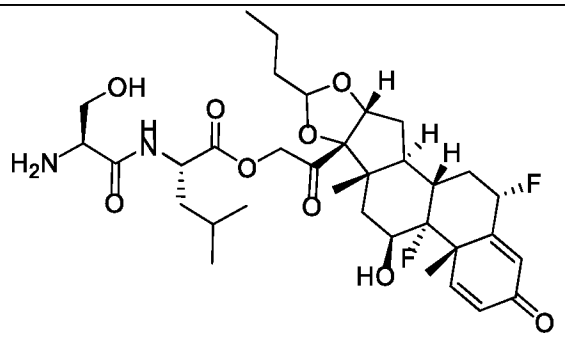
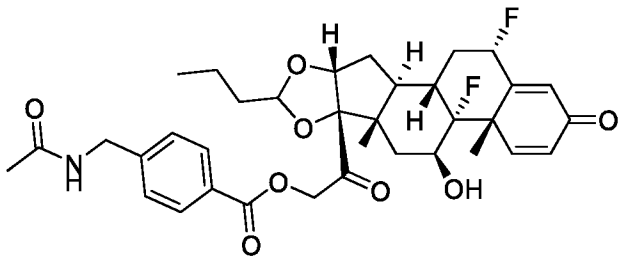
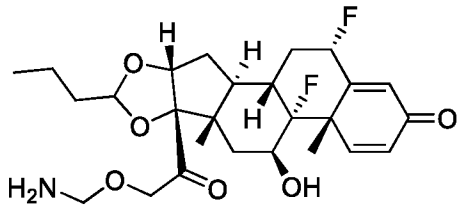
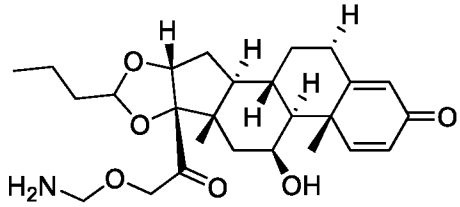
Table B. Budesonide-spacer (Budesonide-SP)

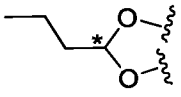
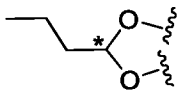
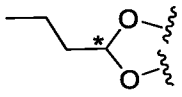
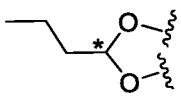
Compound No.	Structure
1a (**Budesonide**)	
1c	

Compound No.	Structure
1d	
1e	
1g	
1h	
1i	
1j	
1k	

Compound No.	Structure
1l	
1m	
100	
101a	
101b	
101c	
101d	

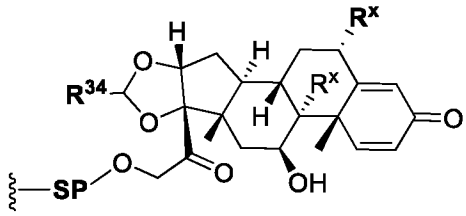
Compound No.	Structure
102c	
102d	
102e	
102f	
103a	
103b	
104a	

Compound No.	Structure
104b	
105aa	
106a	
107a	

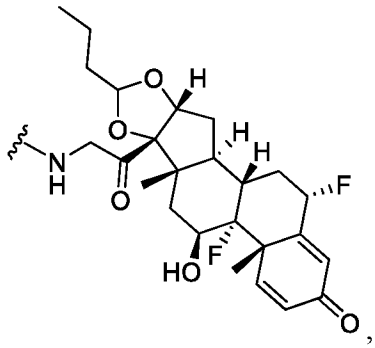
or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In some instances, the n-propyl of  in each of the above structures is in the R-configuration, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is in the S-configuration, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is a mixture of the R- and S-configurations, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is a mixture of the R- and S-configurations, i.e. at the carbon indicated by the asterisk, wherein the R:S mixture is about 1:1,

about 2:1, about 3;1, about 4:1, about 5:1, about 6;1, about 7:1, about 8:1, about 9:1, or about 10:1.

[0228] In various embodiments, **-SP-D** of **Ab-L′-SP-D** or



of Formula (**3000**) is selected from



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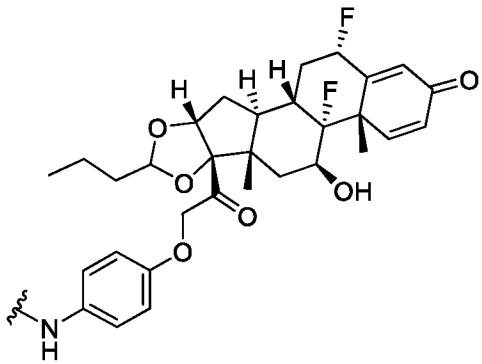

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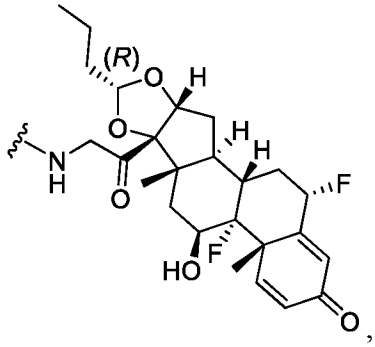

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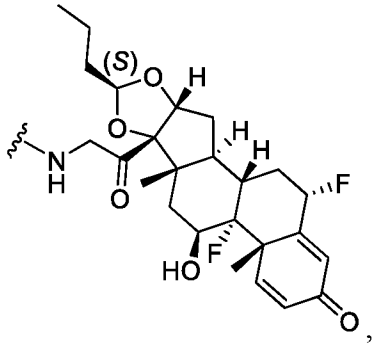

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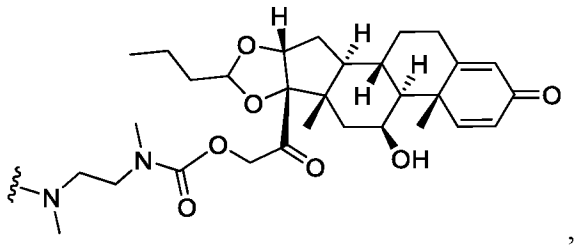

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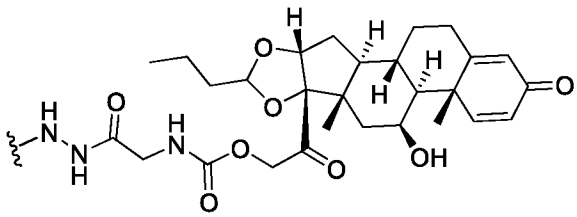

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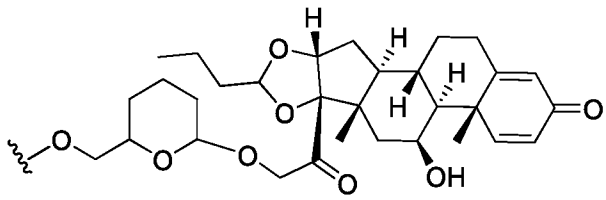

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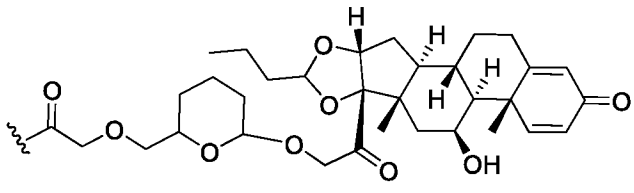

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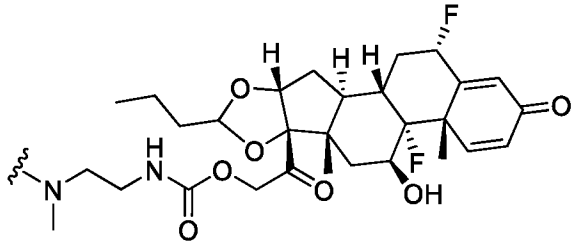

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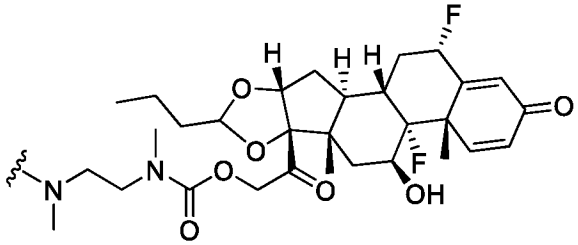

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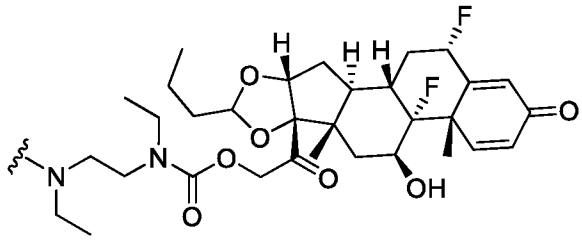

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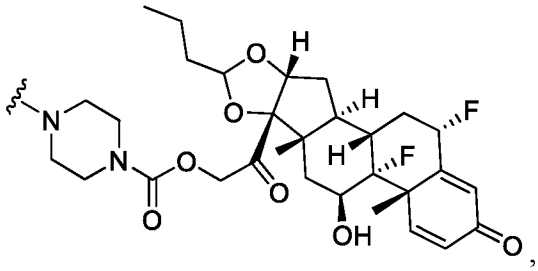

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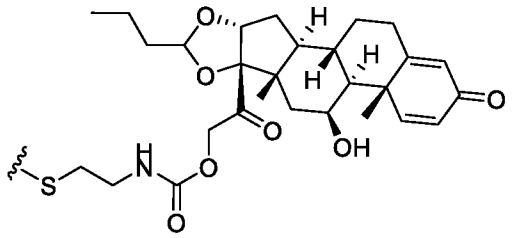

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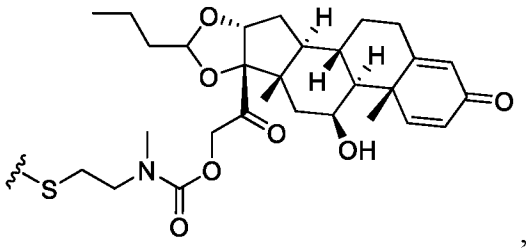

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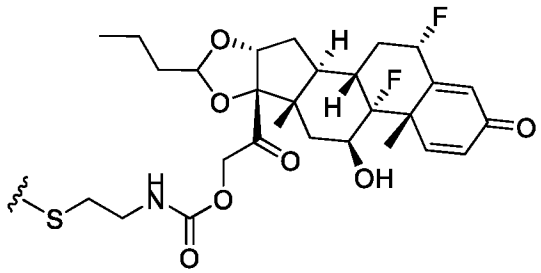

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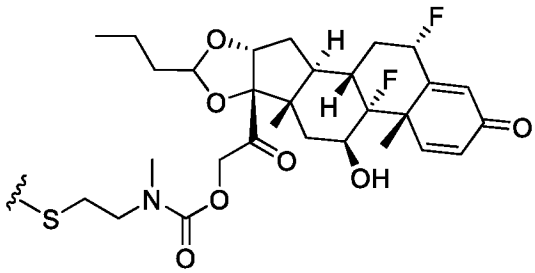

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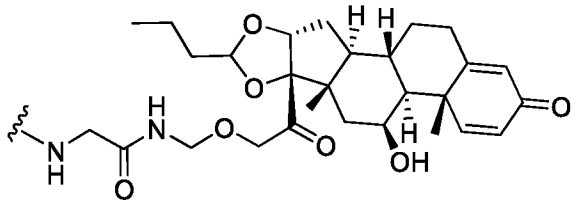

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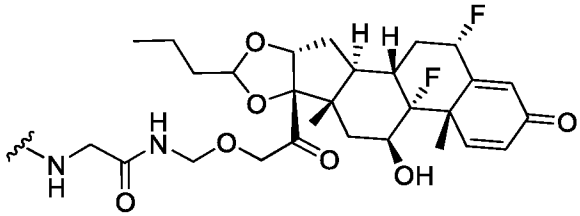

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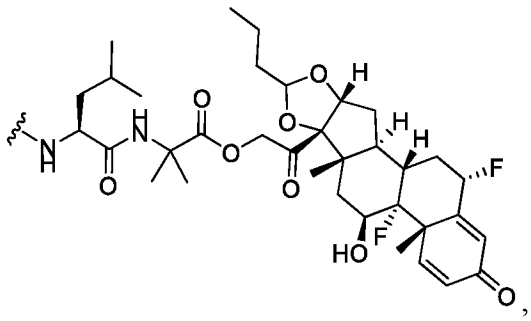

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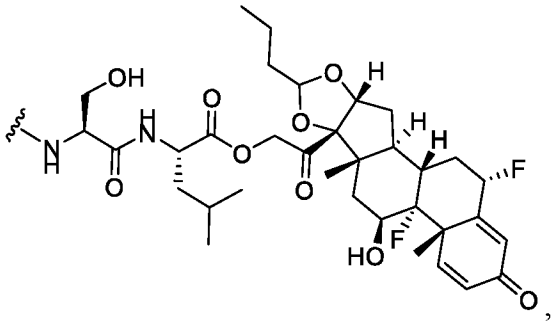

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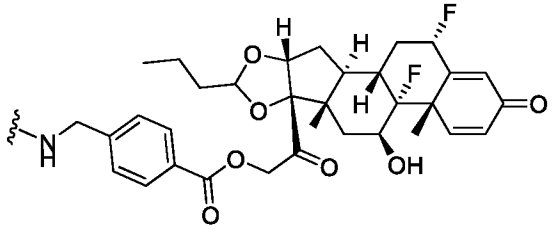
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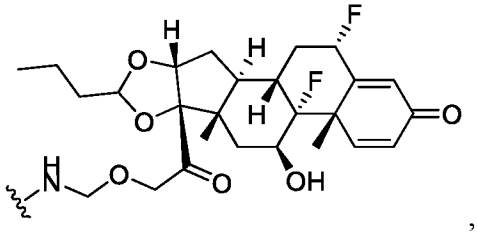
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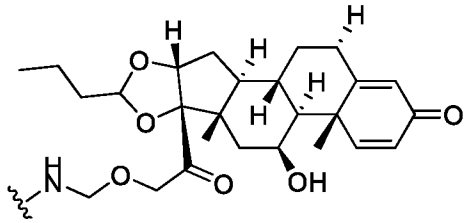
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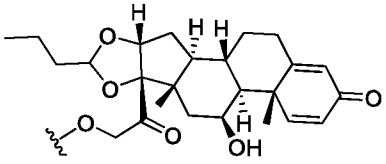
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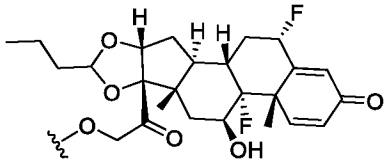
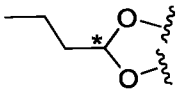
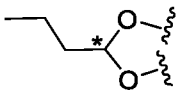
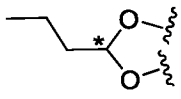
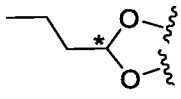
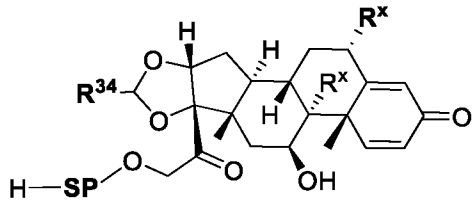
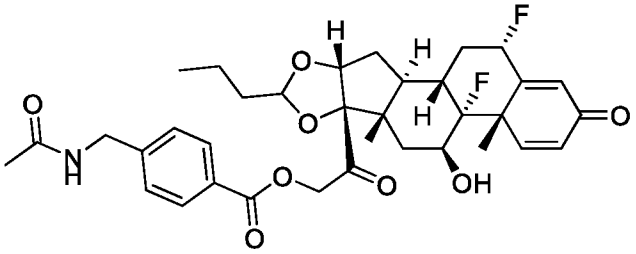


wherein the is the bond to the linker.

[0229] In some instances, the n-propyl of  in each of the above structures is in the R-configuration, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is in the S-configuration, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is a mixture of the R- and S-configurations, i.e. at the carbon indicated by the asterisk. In some instances, the n-propyl of  in each of the above structures is a mixture of the R- and S-configurations, i.e. at the carbon indicated by the asterisk, wherein the R:S mixture is about 1:1, about 2:1, about 3;1, about 4:1, about 5:1, about 6;1, about 7:1, about 8:1, about 9:1, or about 10:1.

[0230] In various embodiments,  is selected from Table B, or is selected from



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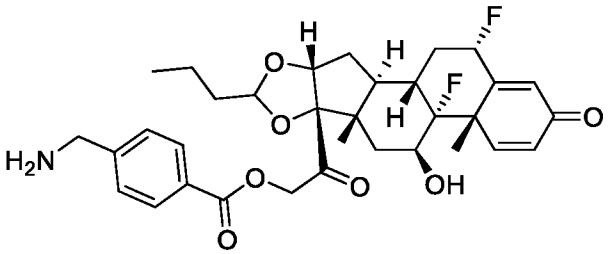

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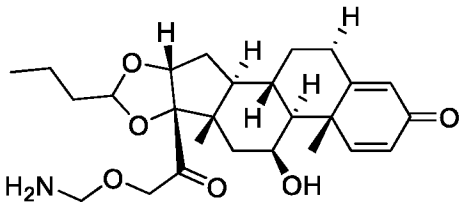
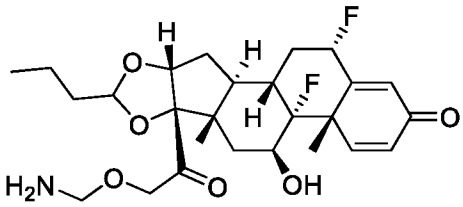
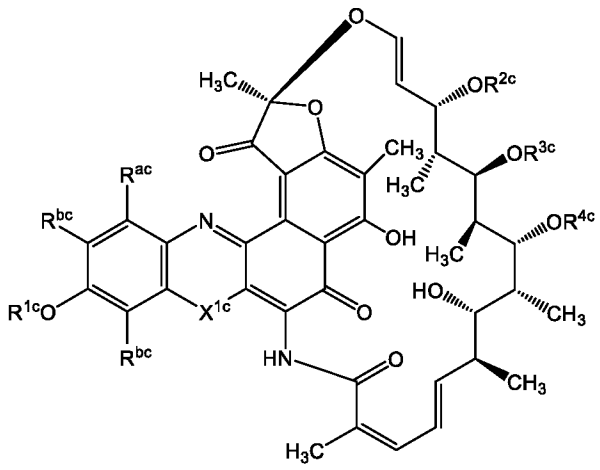


, or



.

[0231] In some embodiments, the payload is a rifamycin analog having the structure of Formula (**C**):



Formula (**C**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{X^{1c}}$ is selected from –O–, –S—, and –N$\mathbf{R^{5c}}$;

$\mathbf{R^{1c}}$ is hydrogen; amino-$C_{1\text{-}6}$alkyl; $C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; di-$C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; hydroxy-$C_{1\text{-}6}$alkyl; HS-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-N($\mathbf{R^{5c}}$)-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-O-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-S-$C_{1\text{-}6}$alkyl; heterocycloalkyl or heterocycloalkyl-$C_{1\text{-}6}$alkyl; wherein heterocycloalkyl includes one, two, or three

heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, C_{1-6}alkyl, -OH, =O, or $-N(R^{5c})_2$;

R^{2c}, R^{3c}, and R^{4c} are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-R^{5c};

R^{ac} is selected from -F; -Cl; -Br; -I; -OH; $-NH_2$; and C_{1-6}alkoxy;

R^{bc} is hydrogen at each occurrence; and

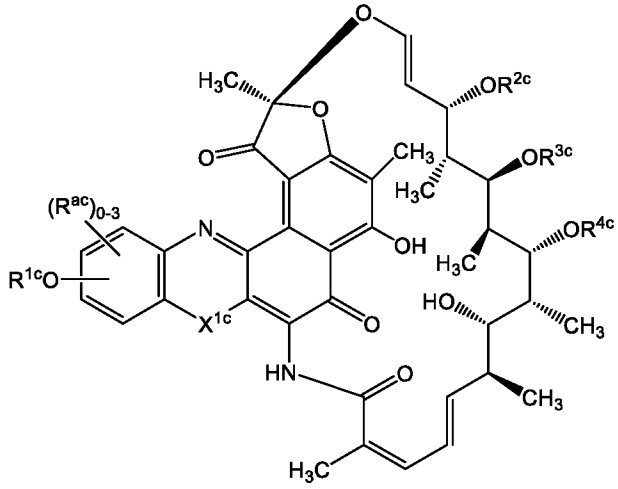
R^{5c} is independently, at each occurrence, selected from hydrogen; and C_{1-6}alkyl;

with a proviso that R^{1c} is not an n-butyl group; and

a further proviso that when X^{1c} is -O-, R^{1c} is not hydrogen;

wherein the group R^{1c} is bonded to the linker.

[0232] In some embodiments, the payload is a rifamycin analog having the structure of Formula (**C-1**):



Formula (**C-1**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

X^{1c} is selected from –S-; -O- and $-NR^{5c}$;

R^{1c} is amino-C_{1-6}alkyl; C_{1-6}alkylaminoC_{1-6}alkyl; di-C_{1-6}alkylaminoC_{1-6}alkyl; hydroxy-C_{1-6}alkyl; HS-C_{1-6}alkyl; $(R^{5c})_2$N-C_{1-6}alkylene-N(R^{5c})-C_{1-6}alkyl; $(R^{5c})_2$N-C_{1-6}alkylene-O-C_{1-6}alkyl; $(R^{5c})_2$N-C_{1-6}alkylene-S-C_{1-6}alkyl; heterocycloalkyl or heterocycloalkyl-C_{1-6}alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, C_{1-6}alkyl, -OH, =O, or $-N(R^{5c})_2$;

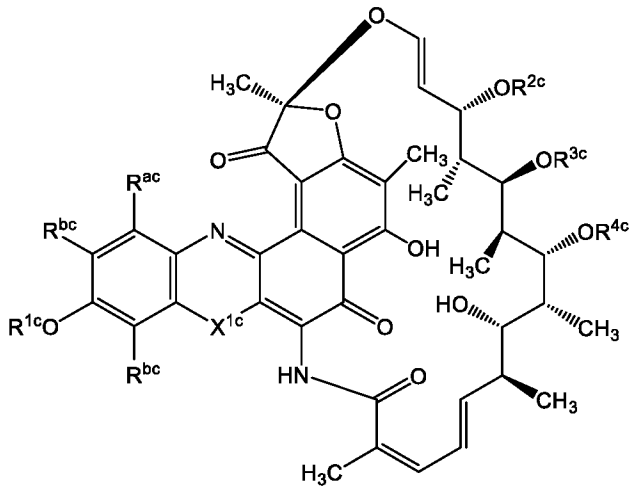
R^{2c}, R^{3c}, and R^{4c} are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-R^{5c};

each R^{ac}, when present, is independently selected from -F; -Cl; -Br; -I; -OH; $-NH_2$; and C_{1-6}alkoxy; and

R^{5c} is independently, at each occurrence, selected from hydrogen; and C_{1-6}alkyl;

wherein the group R^{1c} is bonded to the linker.

[0233] In some embodiments, the payload is a rifamycin analog having the structure of Formula (**C-2**):



Formula (**C-2**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{X^{1c}}$ is selected from –S–; –O– and –N$\mathbf{R^{5c}}$;

$\mathbf{R^{1c}}$ is amino-$C_{1\text{-}6}$alkyl; $C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; di-$C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; hydroxy-$C_{1\text{-}6}$alkyl; HS-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-N($\mathbf{R^{5c}}$)-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-O-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-S-$C_{1\text{-}6}$alkyl; heterocycloalkyl or heterocycloalkyl-$C_{1\text{-}6}$alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, $C_{1\text{-}6}$alkyl, -OH, =O, or –N$(\mathbf{R^{5c}})_2$;

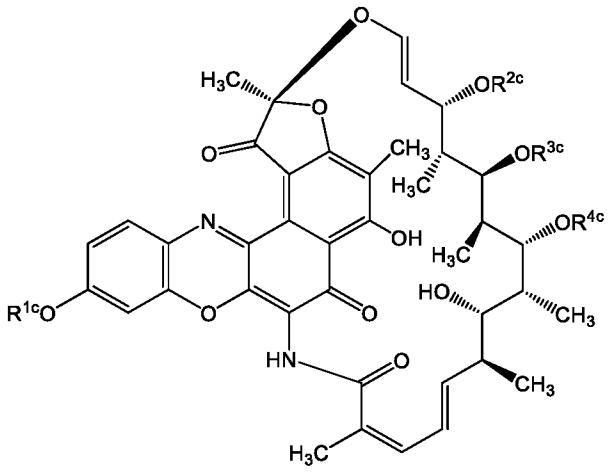
$\mathbf{R^{2c}}$, $\mathbf{R^{3c}}$, and $\mathbf{R^{4c}}$ are independently selected from hydrogen, $C_{1\text{-}6}$alkyl, and -(C=O)-$\mathbf{R^{5c}}$;

$\mathbf{R^{ac}}$and $\mathbf{R^{bc}}$ are independently selected from -F; -Cl; -Br; -I; -OH; -NH_2; and $C_{1\text{-}6}$alkoxy; and

$\mathbf{R^{5c}}$ is independently, at each occurrence, selected from hydrogen; and $C_{1\text{-}6}$alkyl;

wherein the group $\mathbf{R^{1c}}$ is bonded to the linker.

[0234] In some embodiments, the payload is a rifamycin analog having the structure of Formula (**C-3**):



Formula (**C-3**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

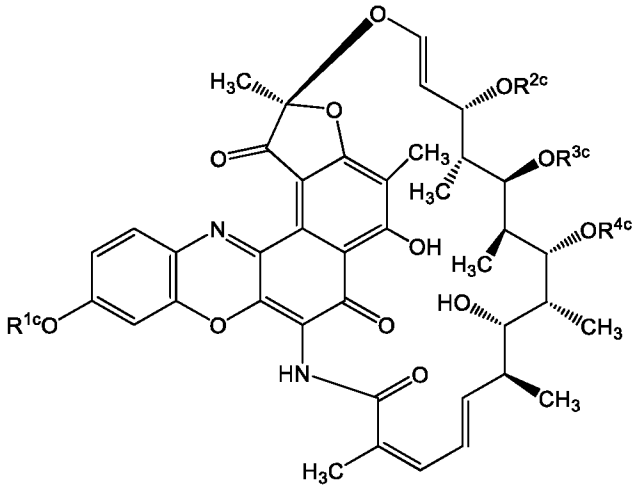
R^{1c} is di-C_{1-6}alkylaminoC_{1-6}alkyl; $(R^{5c})_2N$-C_{1-6}alkylene-N(R^{5c})-C_{1-6}alkyl; heterocycloalkyl or heterocycloalkyl-C_{1-6}alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, C_{1-6}alkyl, -OH, =O, or –N(R^{5c})$_2$; and

R^{2c}, R^{3c}, and R^{4c} are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-R^{5c}; and

R^{5c} is selected from hydrogen and C_{1-6}alkyl;

wherein the group R^{1c} is bonded to the linker.

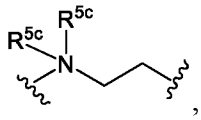
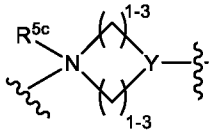
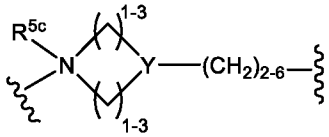
[0235] In some embodiments, the payload is a rifamycin analog having the structure of Formula (**C-4**):



Formula (**C-4**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

R^{1c} is  ,  ; or  ;

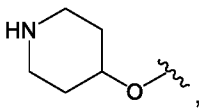
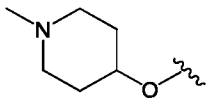
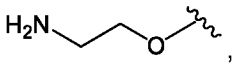
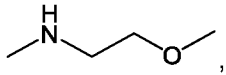
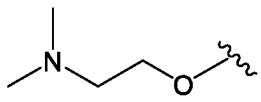
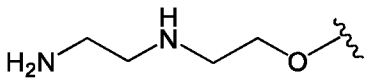
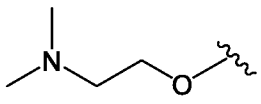
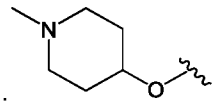
wherein **Y** is C or N;

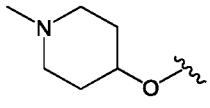
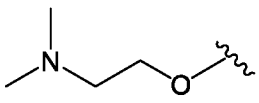
R^{2c}, R^{3c}, and R^{4c} are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-R^{5c}; and

R^{5c} is selected from hydrogen and C_{1-6}alkyl;

wherein the group R^{1c} is bonded to the linker via the nitrogen atom as indicated by the wavy line .

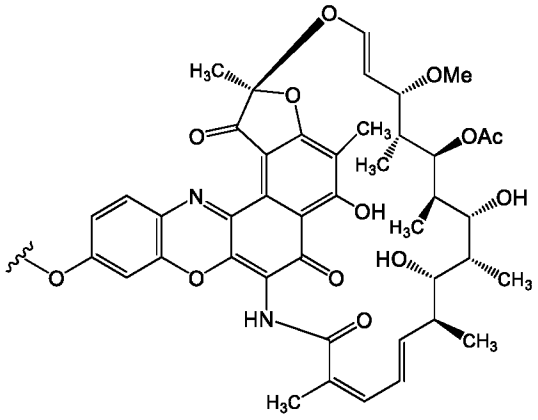
[0236] In some or any embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), R^{bc} is hydrogen and/or R^{ac} is hydrogen. In some or any embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), R^{2c} is methyl, ethyl, propyl or isopropyl, preferably methyl. In some or any embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), R^{3c} is CH_3-(C=O)- (acetyl) group, CH_3CH_2-(C=O)-. $CH_3CH_2CH_2$-(C=O)-, or $(CH_3)_2CH$-(C=O)-; preferably acetyl. In some or any embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), R^{4c} is hydrogen.

[0237] In some embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), -OR^{1c} is one or more of  ,  ,  ,  ,  or  . In some embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), -OR^{1c} is  and/or  .

[0238] In some or any embodiments of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**), X^{1c} is O, and -OR^{1c} comprises a tertiary amine, said tertiary amine, after bonding to a linker, changes to a quarternary amine. In some of such embodiments, -OR^{1c} is  or  . In such embodiments, where a quarternary amine is formed, a suitable counter ion is present, i.e., the rifamycin analog is present as a pharmaceutically acceptable salt

comprising a quarternary amine bearing a positive charge, and, as a salt, for instance, a charge-balancing negatively charged counter ion (e.g., I^-, Br^-, Cl^- or any other suitable counter ion).

[0239] In some embodiments. a compound of Formula (**C-1**) and/or (**C-2**) an/or (**C-3**) and/or (**C-4**) is selected from the group consisting of:



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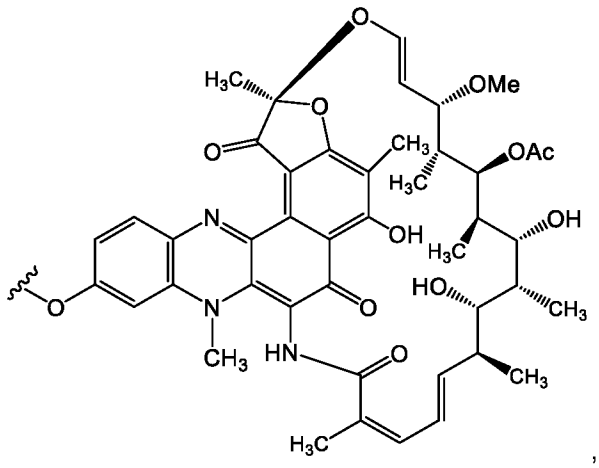

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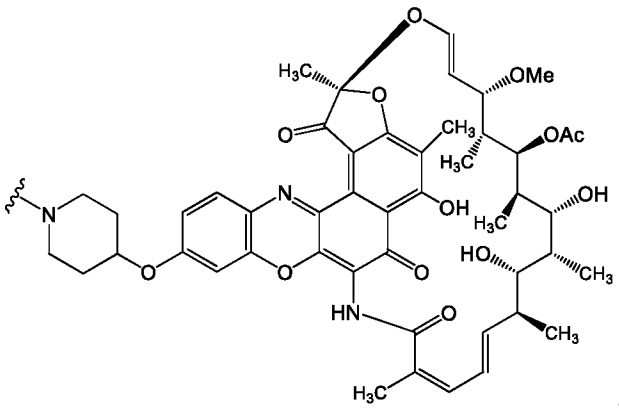

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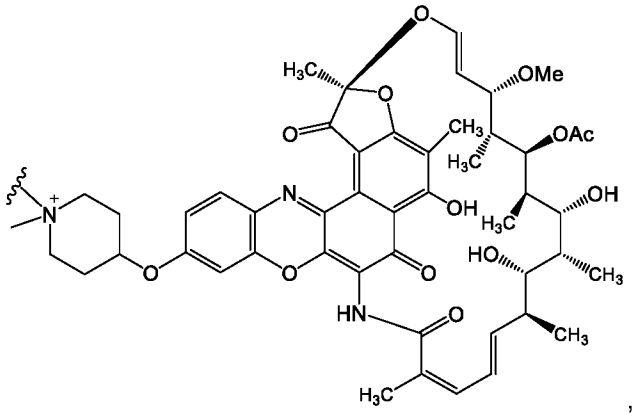

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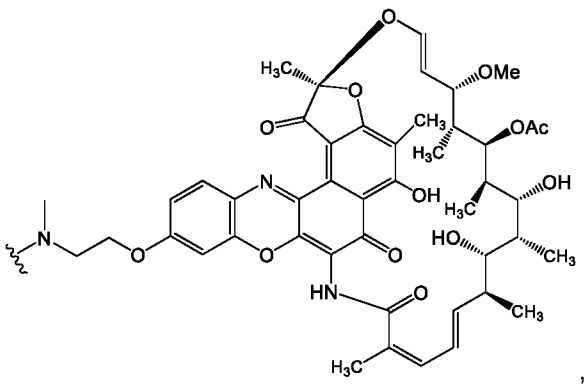

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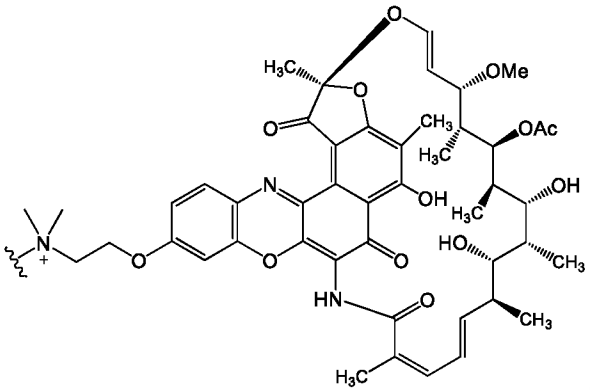
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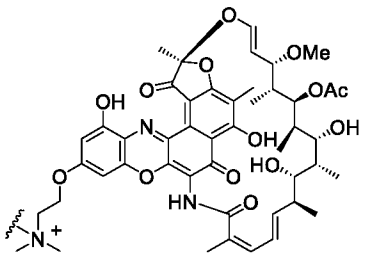
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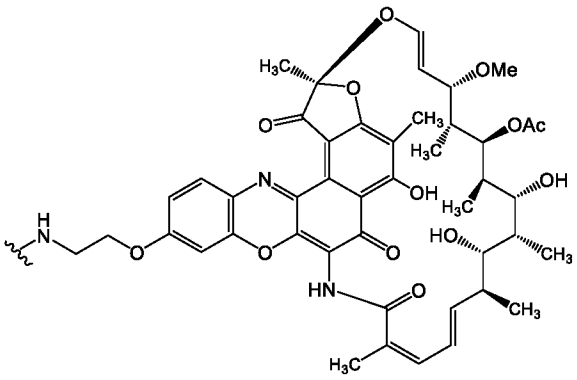
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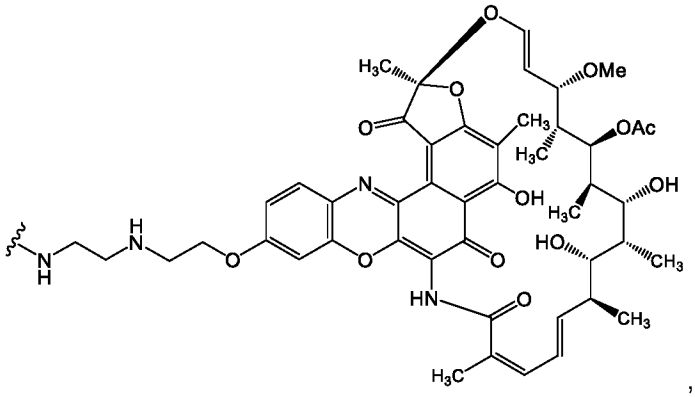
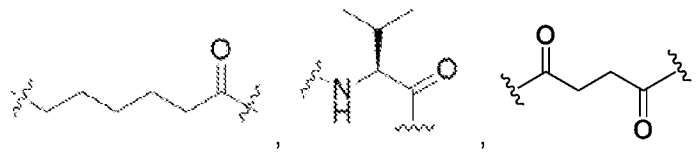

,

wherein the is the bond to the linker.

Linkers

[0240] In some embodiments of Formula (**I**), (**III**), or (**3000**), **L** comprises -L^1-L^2-$(L^3)_{0-1}$- and L^2 comprises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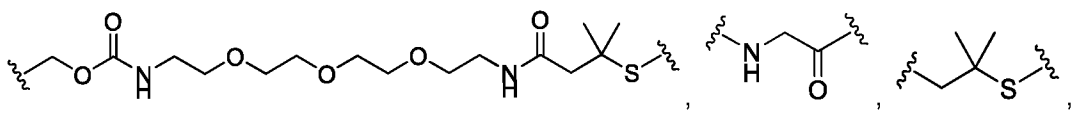
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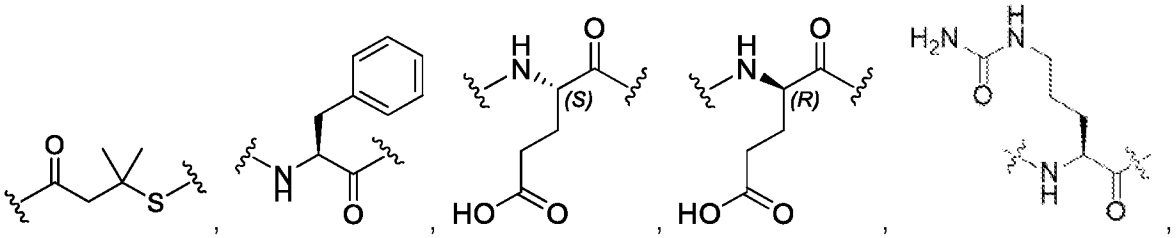
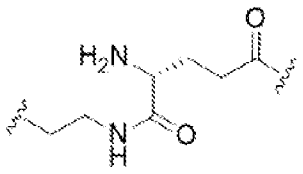
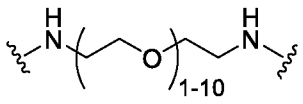
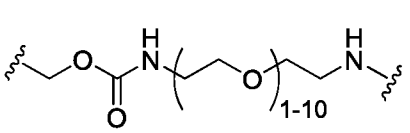
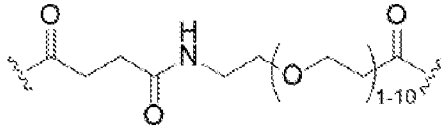
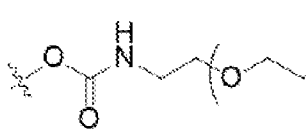
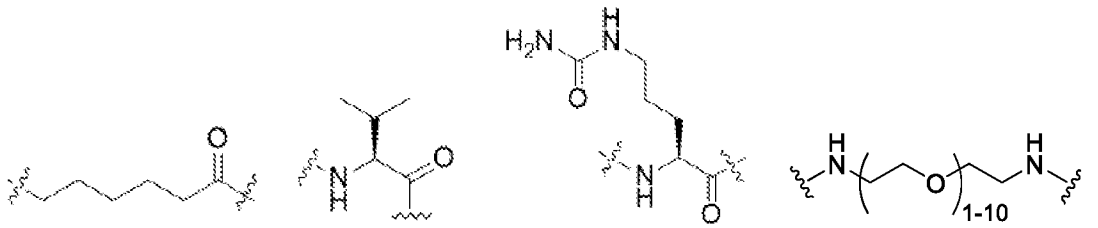
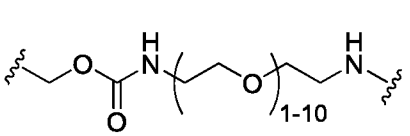
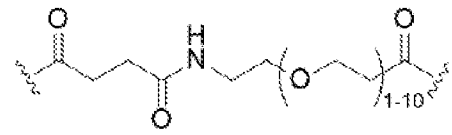
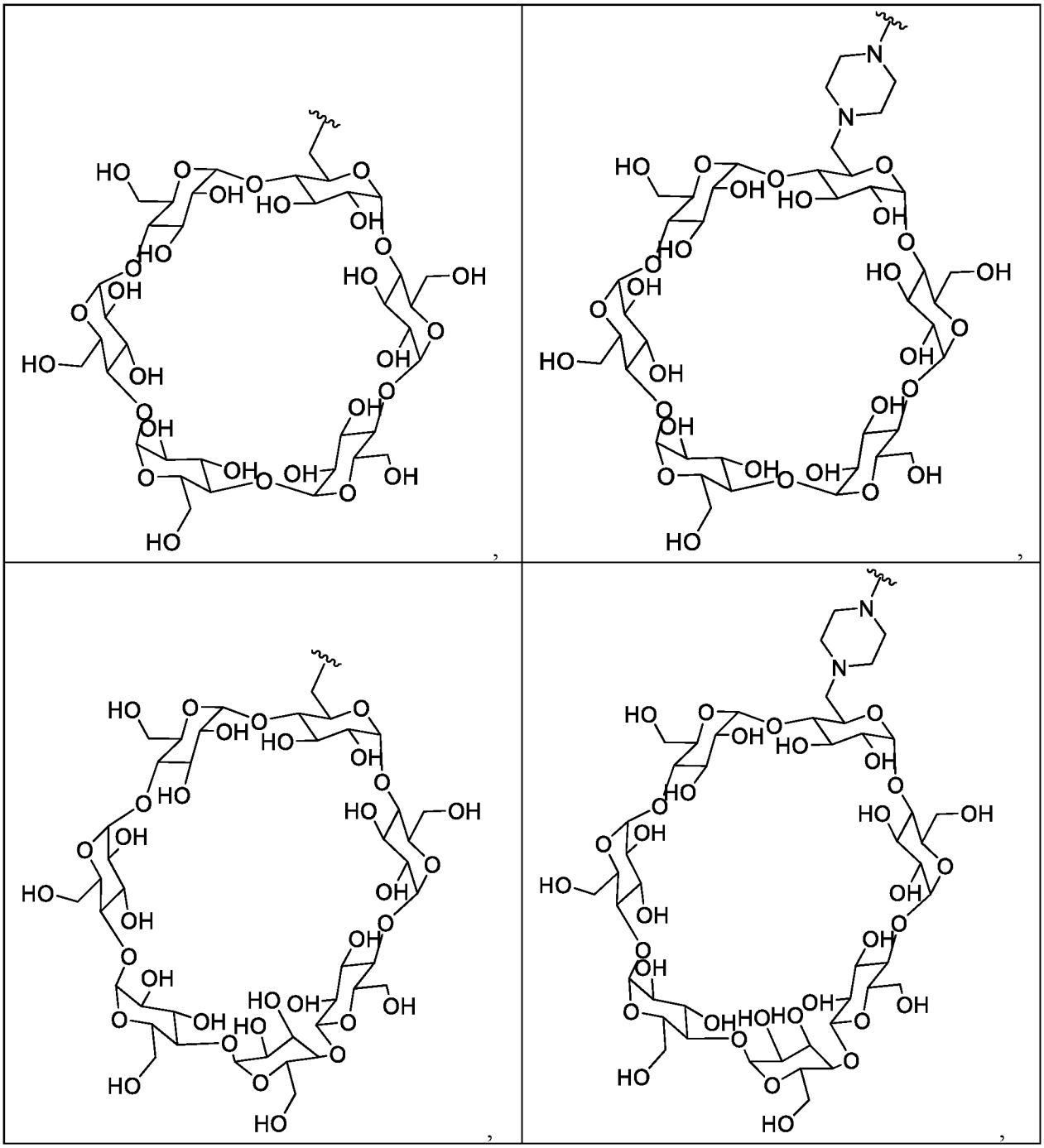
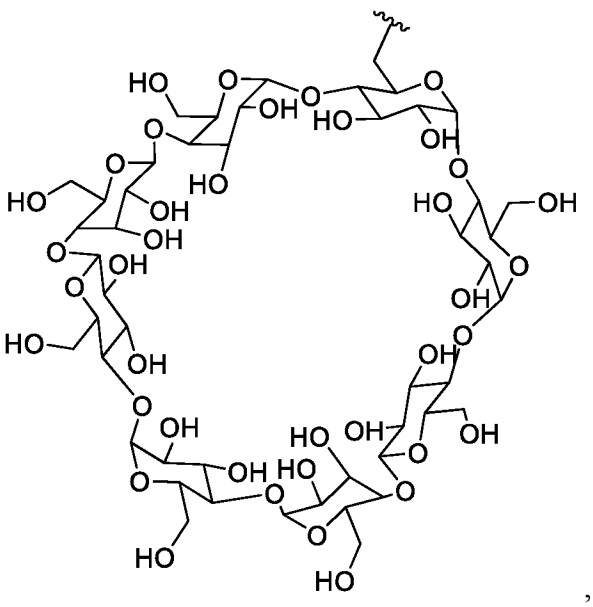







, -C(O)CH$_2$CH$_2$C(O)NH-, , , , -OCH$_2$C(O)-, or cyclodextrin residue (**CD**); or combinations thereof. In some embodiments, **L** comprises -**L**^{1}-**L**^{2}-(**L**^{3})$_{0\text{-}1}$- and **L**^{2} comprises

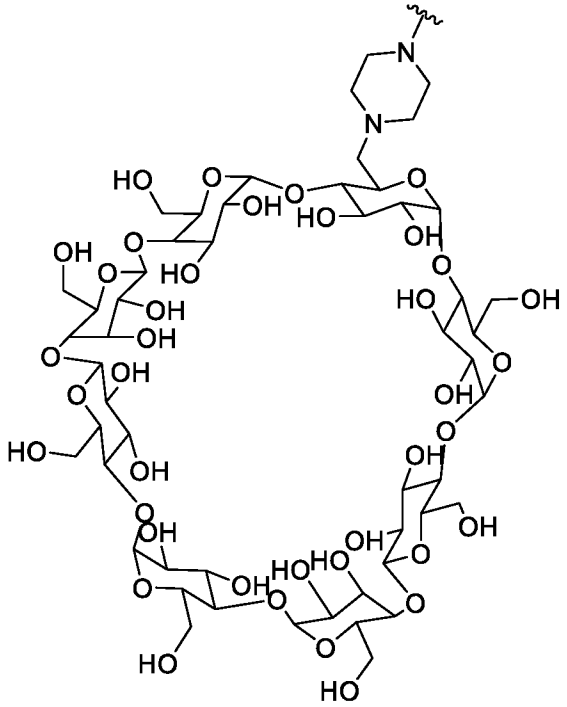




, or **CD**, or combinations thereof. In some embodiments, **L** comprises -**L**^{1}-**L**^{2}-(**L**^{3})$_{0\text{-}1}$- and **L**^{2} comprises **CD**. In some embodiments, where **L** and/or **L**^{2} comprises **CD**, **CD** is selected from the group consisting of



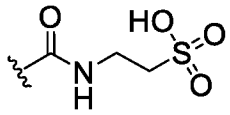


and

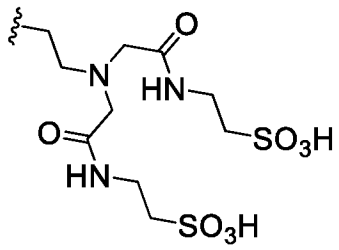


.

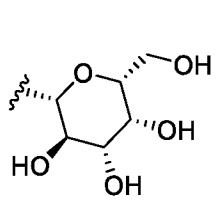
[0241] In some embodiments of Formula (**I**), (**III**), or (3000), **L** comprises -L^1-L^2-$(L^3)_{0\text{-}1}$- and L^2 comprises **HG** as described herein. In some embodiments, **L** and/or L^2 comprises



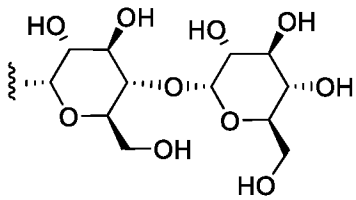
or



. In some embodiments, **L** and/or L^2 comprises



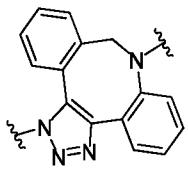
, or



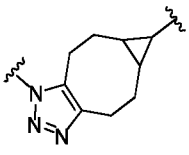
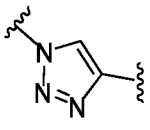
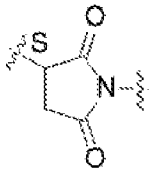
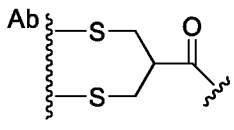
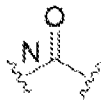
.

[0242] In some embodiments of Formula (**I**), (**III**), or (**3000**), **L** comprises -L^1-L^2-$(L^3)_{0\text{-}1}$- and

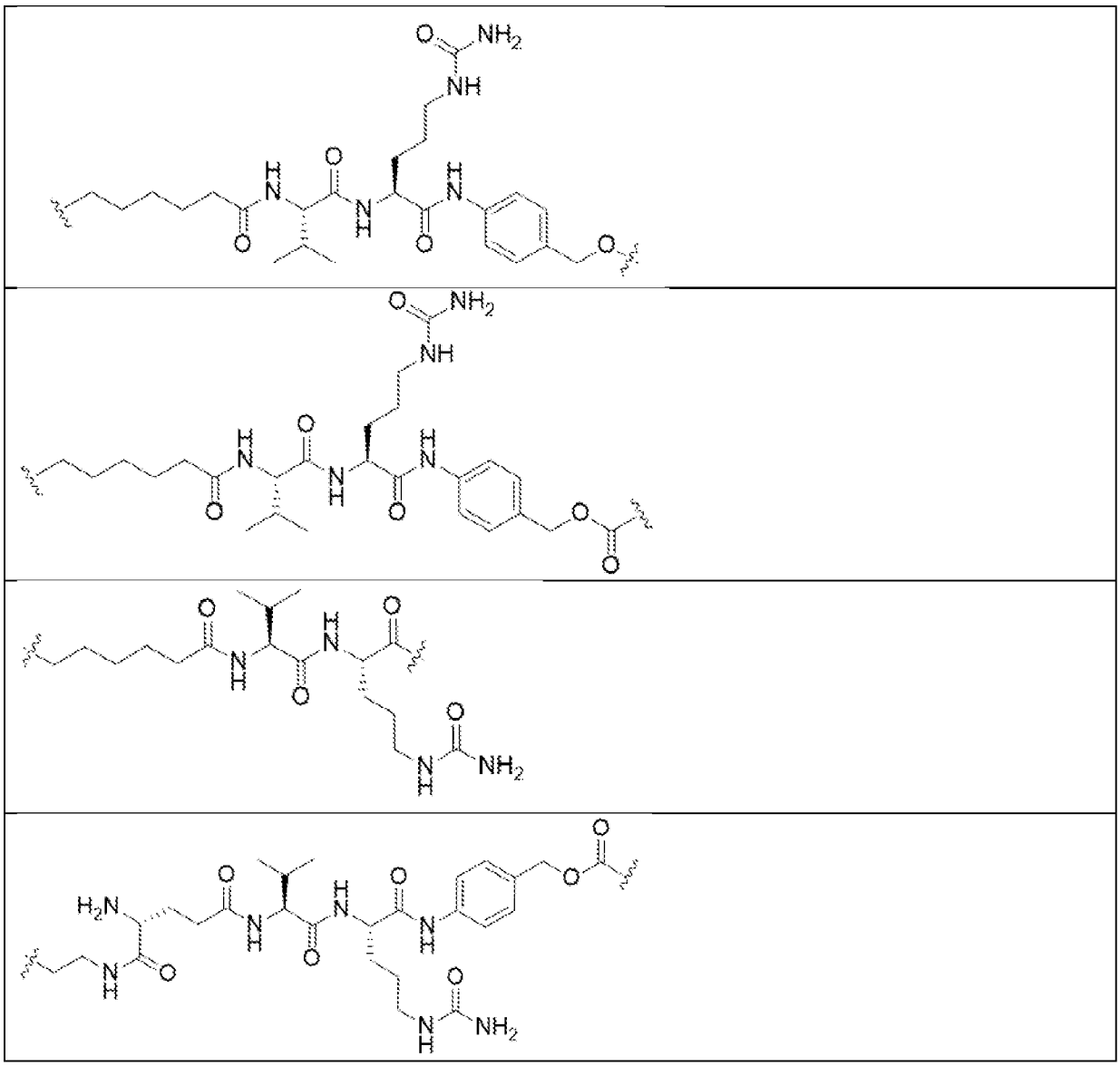
L^1 is selected from

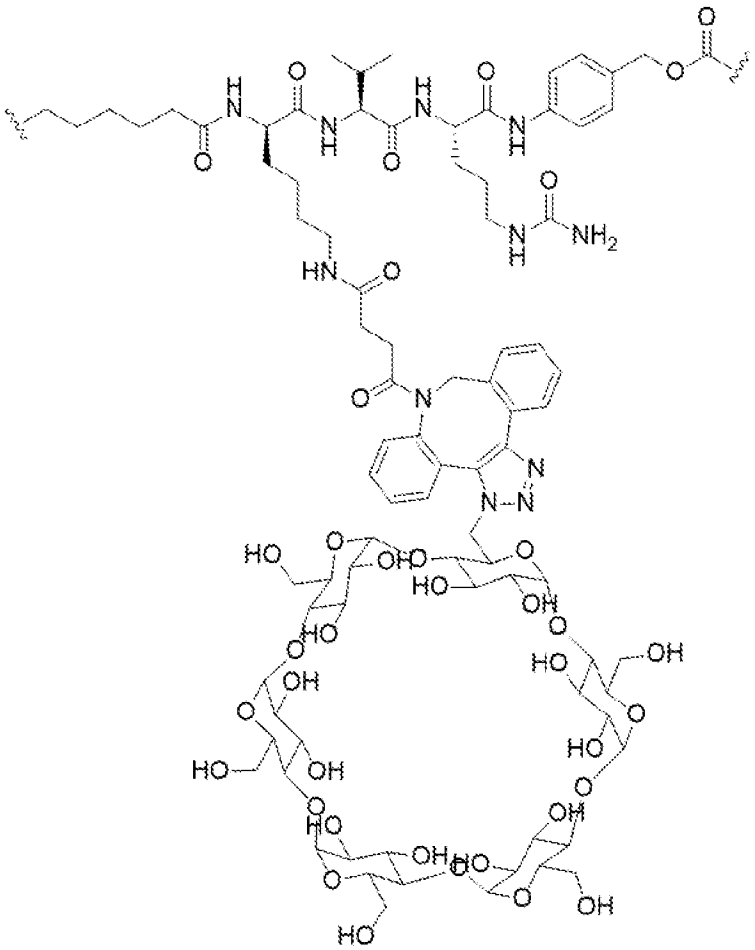


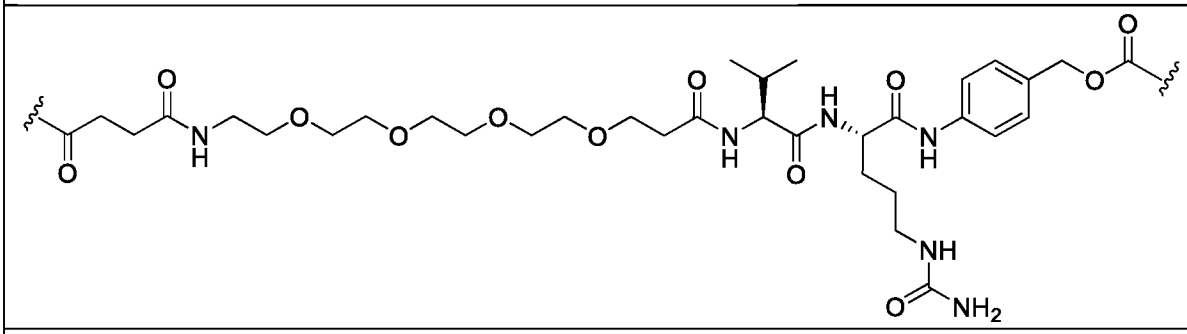
or a regioisomer or mixture of isomers thereof;

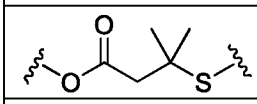

; 
; 
; 
; or a steroisomer or mixture of stereoisomers thereof, where S refers to the S atom on a cysteine residue through which the reactive group residue is attached to **BA**; and  where N refers to the N atom on a lysine residue through which the reactive group residue is attached to **BA**.

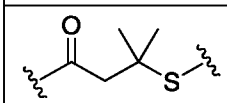
[0243] In some embodiments, **L** comprises -L^1-L^2-$(L^3)_{0-1}$- and -L^2-$(L^3)_{0-1}$- as described herein. In some embodiments, **L** is **L′-SP** as described herein for Formula (III) and Formula (3000). In some embodiments **L** comprises:

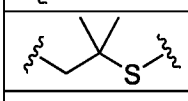


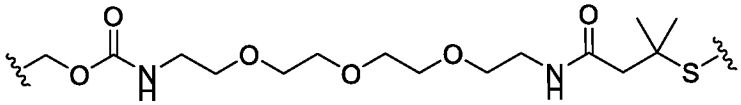


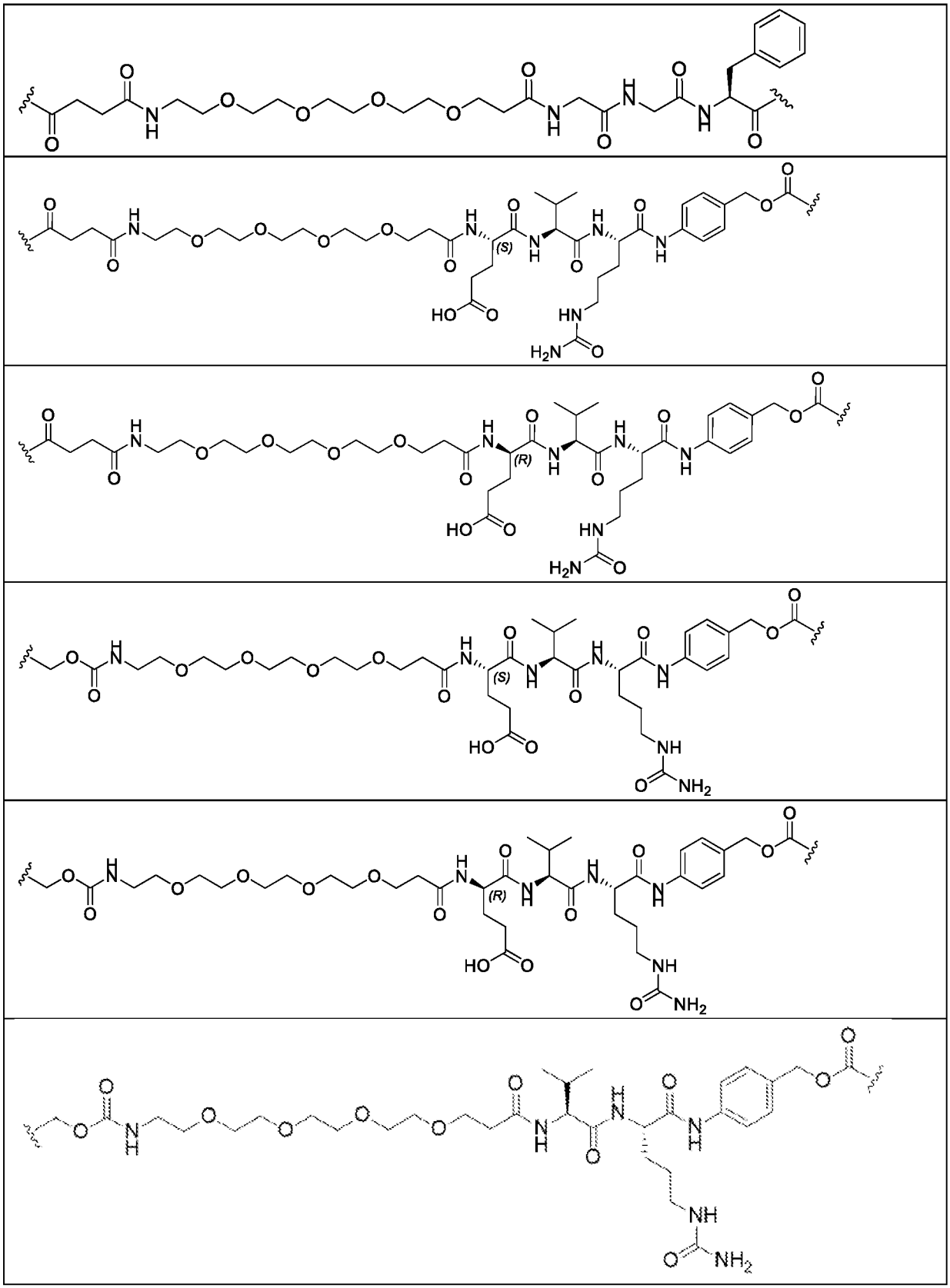


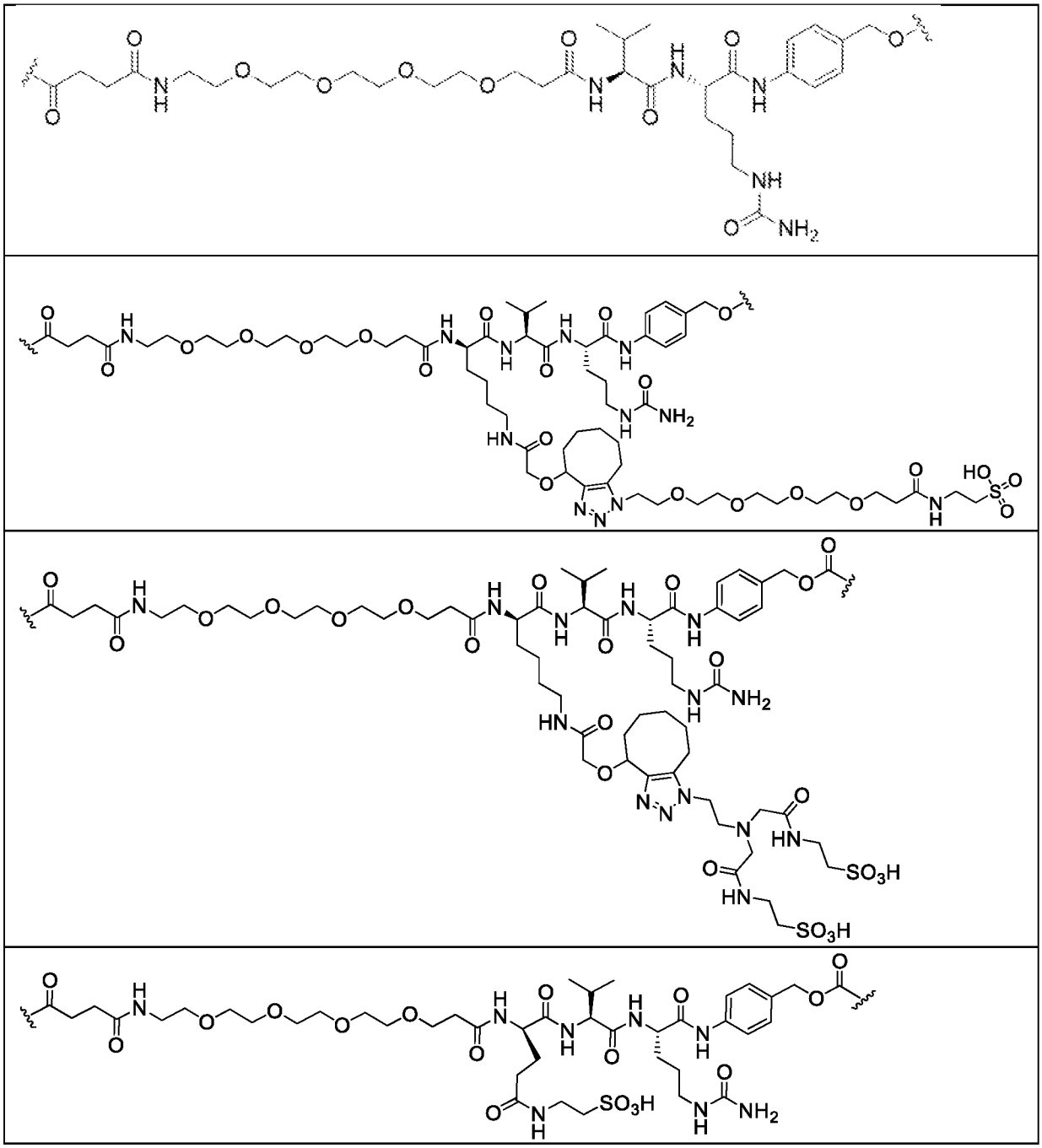


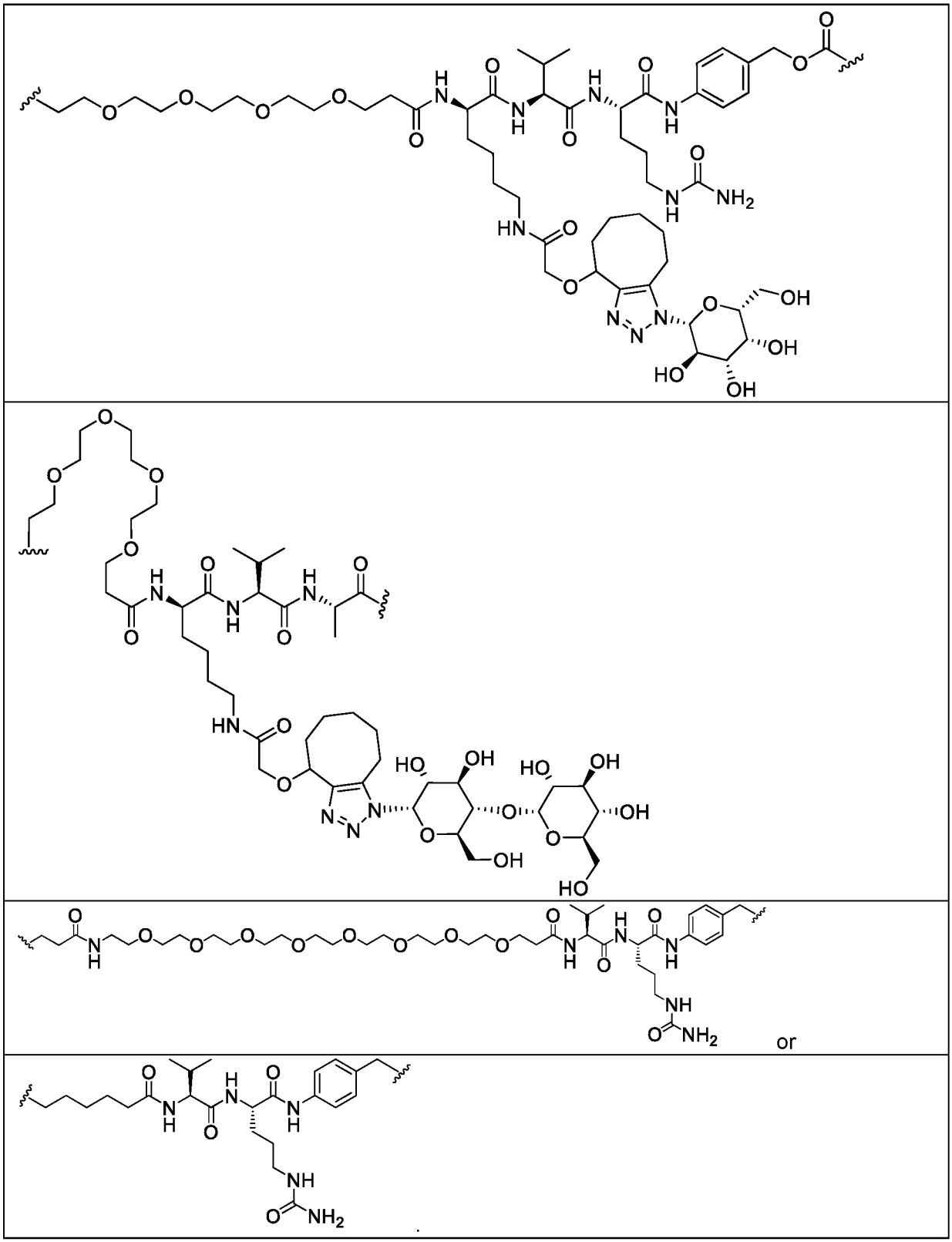




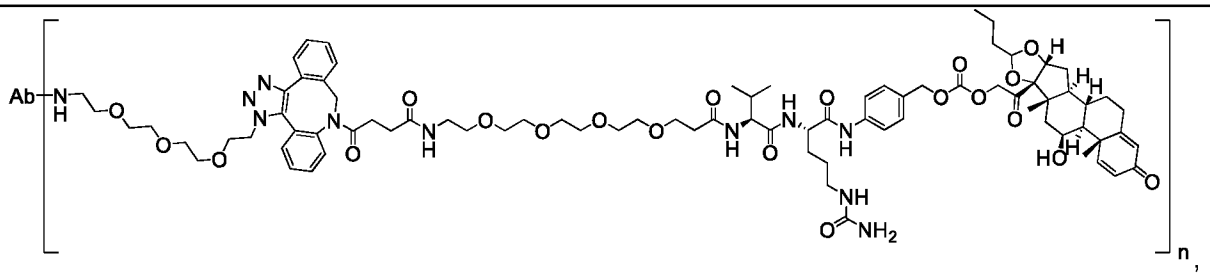




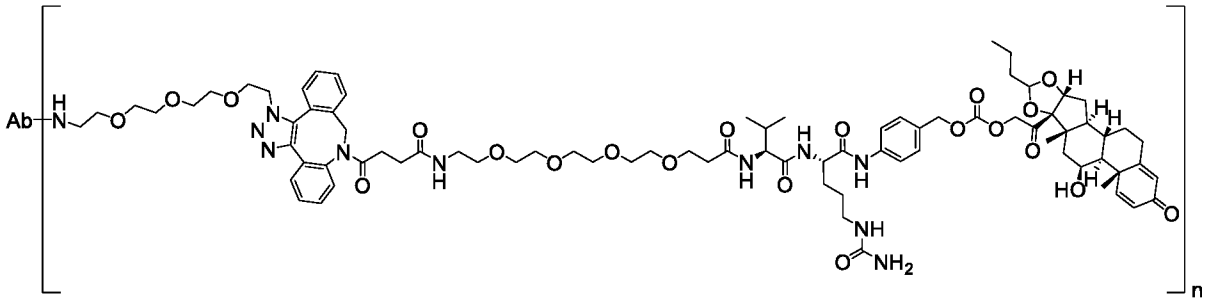




[0244] In some instances, a compound of Formula (I) or (III) or (3000), is selected from



and



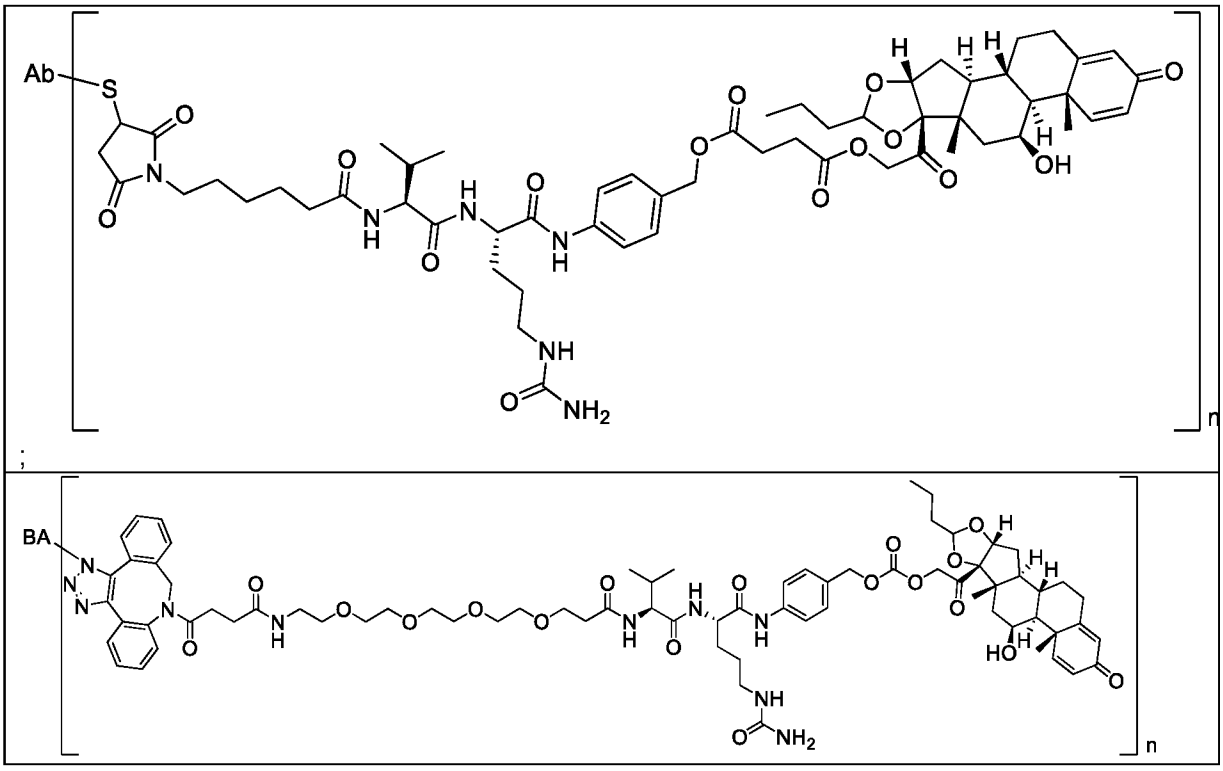
or a mixture thereof;

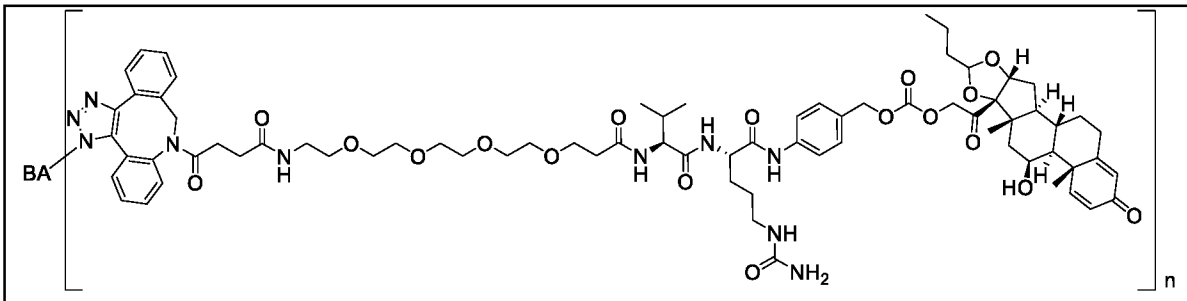
or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

each Ab is an anti-MSR1 antibody, or an antigen binding fragment thereof; and

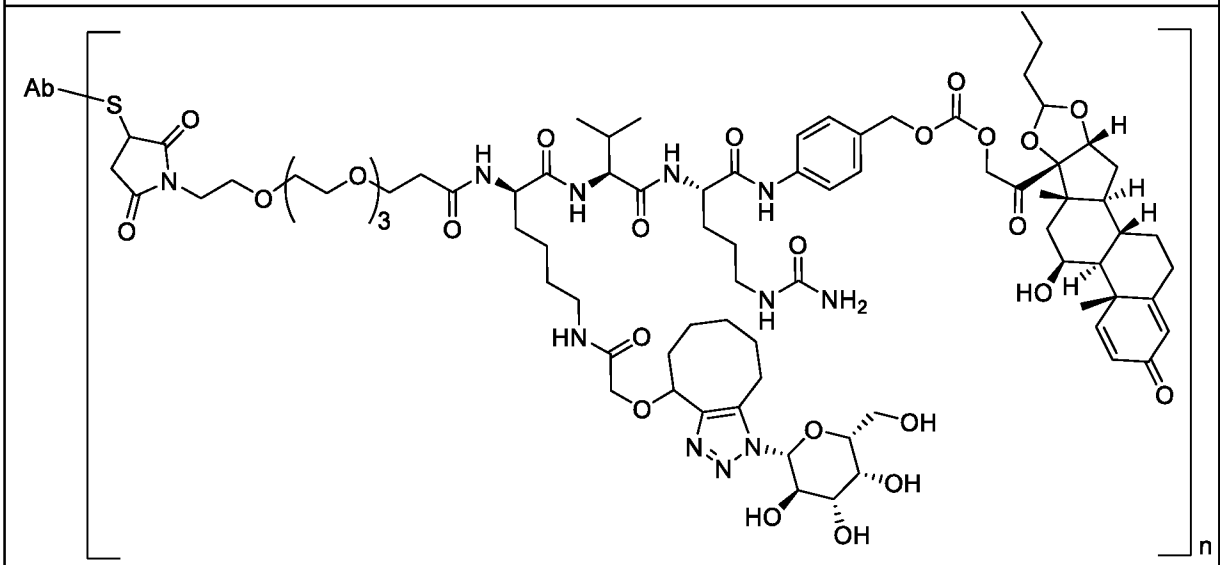
each n is an integer from 1 to 4.

[0245] In some instances, a compound of Formula (**I**) or (**III**) or (3000), is selected from

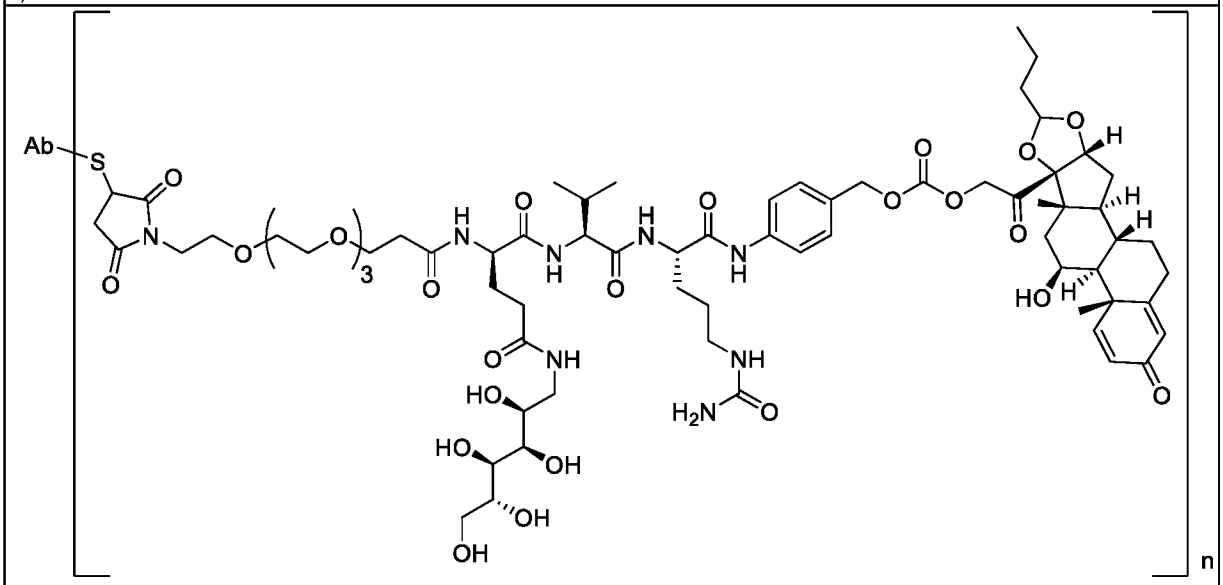




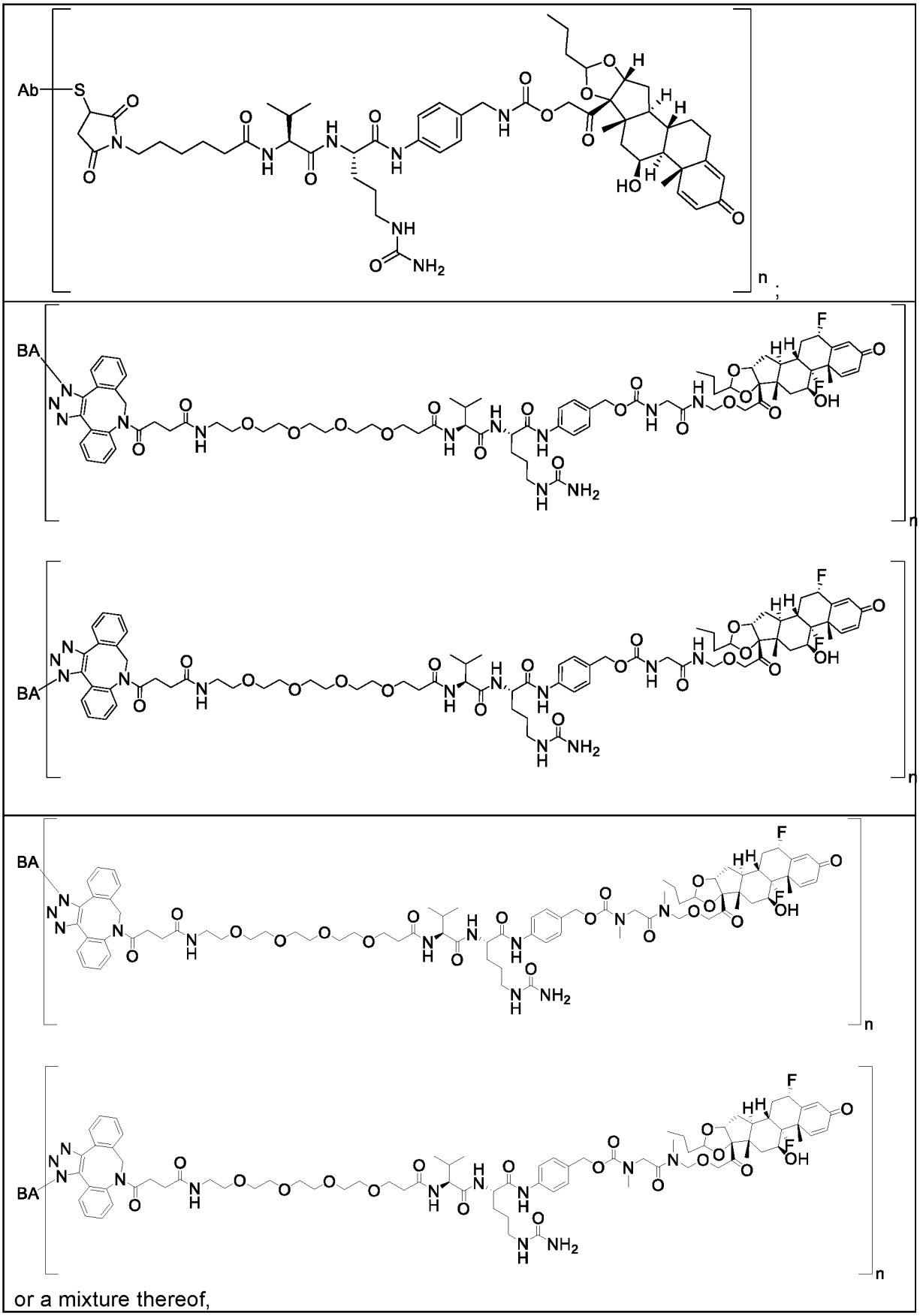
or a mixture thereof;

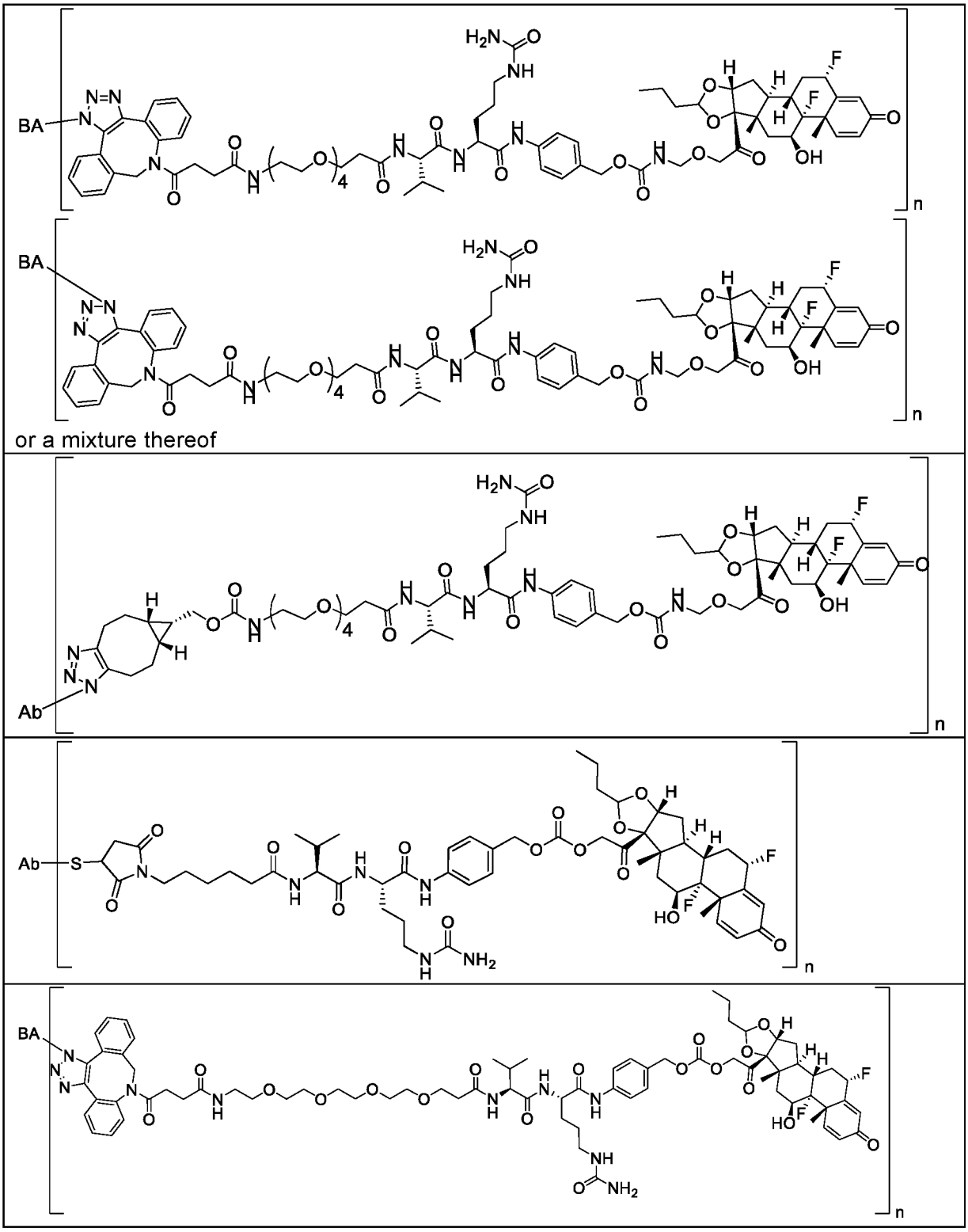


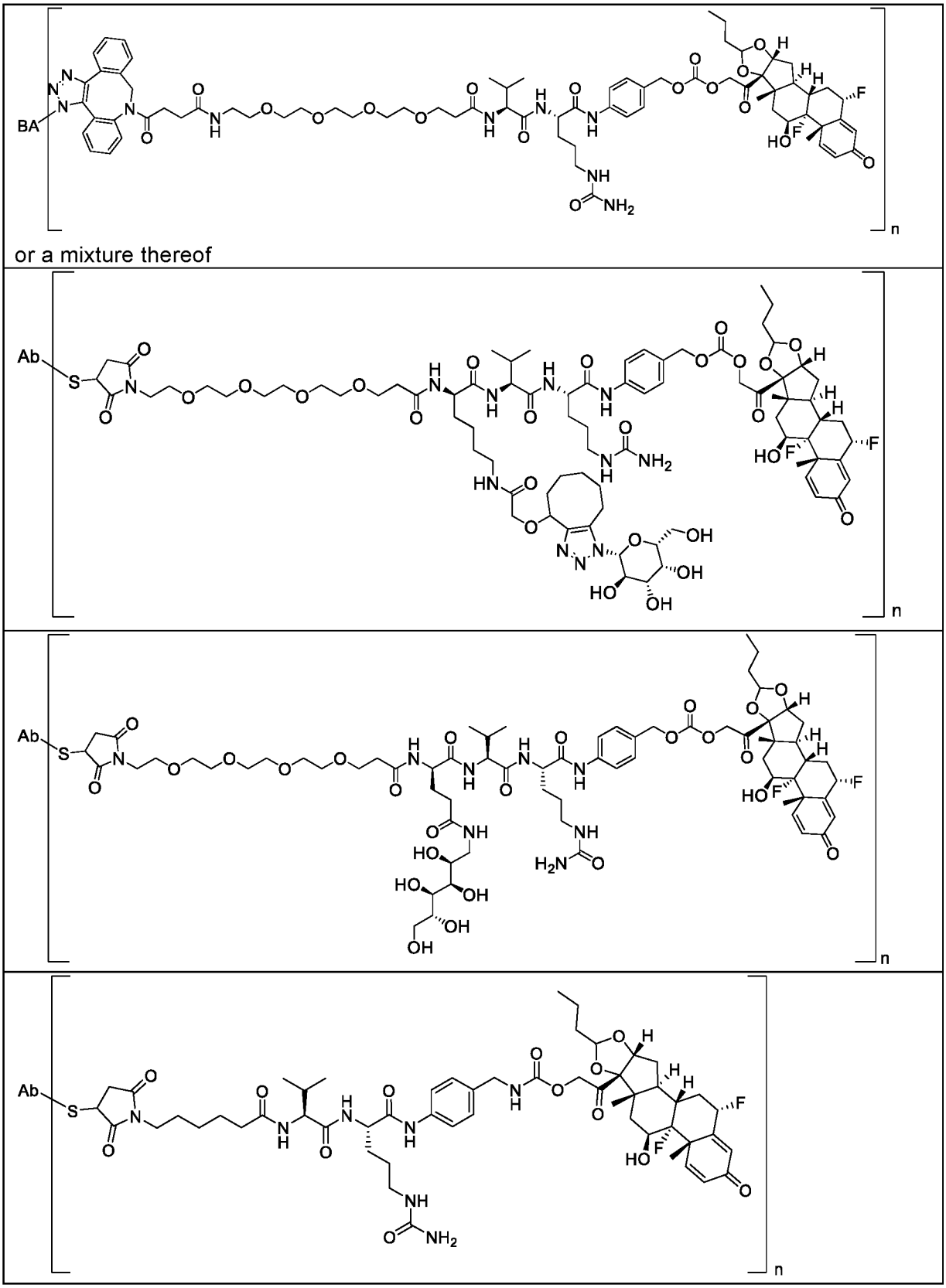
;

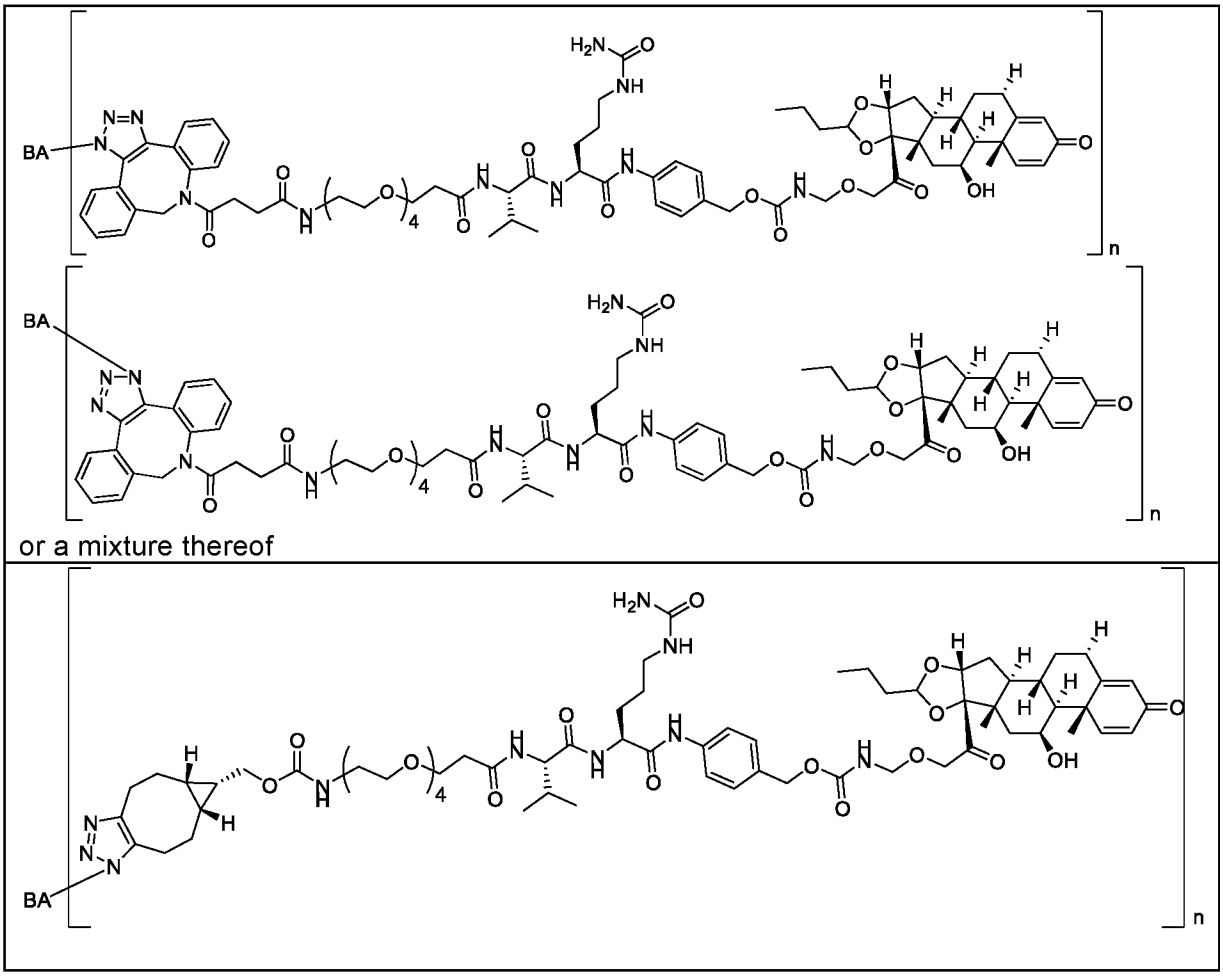


;


Ab
S
BA
HO
F
OH
NH2
n
or a mixture thereof,

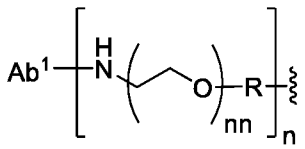

BA
Ab
n
or a mixture thereof


BA
n
or a mixture thereof
Ab
S
n
Ab
S
n
Ab
S
n



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

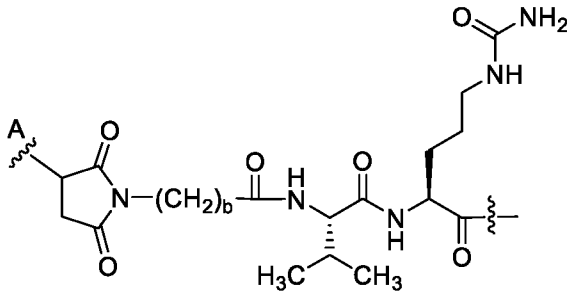
each Ab is an anti-MSR1 antibody, or an antigen binding fragment thereof;

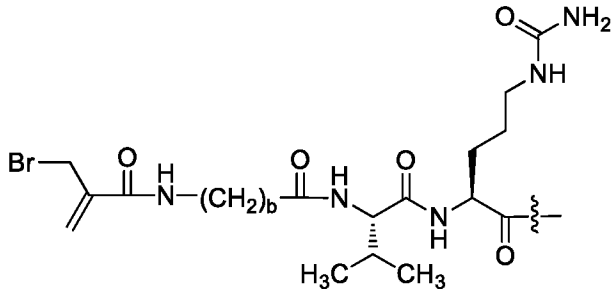
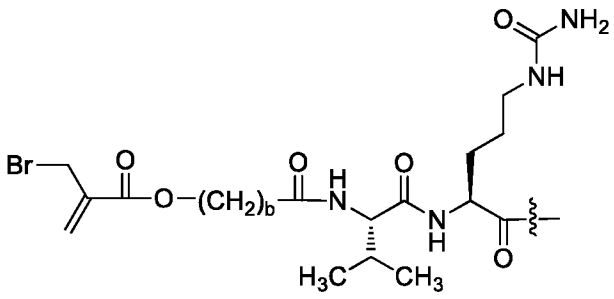
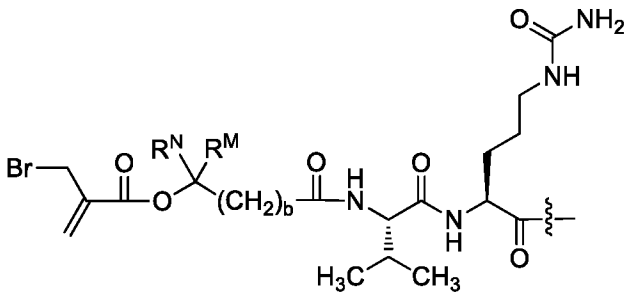
each BA is  where **Ab**^{1} is an anti-MSR1 antibody, or an antigen-binding fragnment thereof; **R** is C_{2-4}-alkylene; and **nn** is an integer selected from 2 to 4, inclusive.

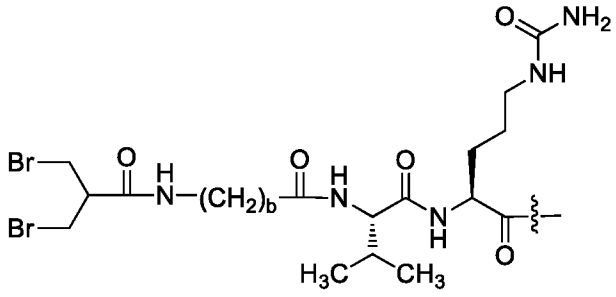
and

each n is an integer from 1 to 4.

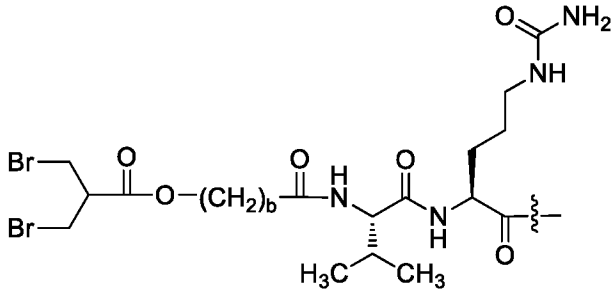
[0246] In some additional embodiments, the ADCs described herein may comprise linkers described in US 9,951,141 B2, filed November 29, 2016, and issued on April 24. 2018, which linkers are incorporated herein by reference. In some instances, the linker comprises:



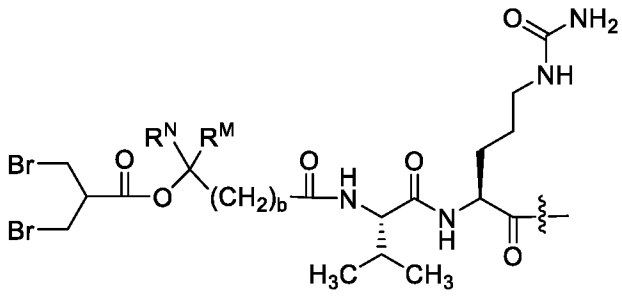
, wherein b is an integer from 2 to 8 and is a bond to the binding agent. In some embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:  ; wherein b is an integer from 2 to 8. In further embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:  ; wherein b is an integer from 2 to 8. In some other embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:  ; wherein b is an integer from 2 to 8, R^N is a hydrogen atom or alkyl, and R^M is alkyl. In additional embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:



; wherein b is an integer form 2 to 8. In certain embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:



; wherein b is an integer from 2 to 8. In some embodiments, the linker comprises two reactive groups and can form bonds with, for example, thiols on two different chains of an antibody, or antigen binding fragment thereof:



; wherein b is an integer from 2 to 8; R^N is a hydrogen atom or alkyl; and R^M is alkyl.

[0247] In the Formulae (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**III**), (**3000**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), (**6005**), (**7001**), (**7002**), (**7003**), (**7004**), and/or (**7005**) described herein, and/or in **BA** – $[(\mathbf{L})_{0\text{-}1}$ – **PA**$]_n$, **PA** can be linked to BA with any linker **L** deemed suitable. Linkers are any group or moiety that links, connects, or bonds the antibody or antigen-binding proteins described herein with a therapeutic moiety, *e.g.* a steroid or an LXR modulator. Suitable linkers may be found, for example, in *Antibody-Drug Conjugates and Immunotoxins*; Phillips, G. L., Ed.; Springer Verlag: New York, 2013; *Antibody-Drug Conjugates*; Ducry, L., Ed.; Humana Press, 2013; *Antibody-Drug Conjugates*; Wang, J., Shen, W.-C., and Zaro, J. L., Eds.; Springer International Publishing, 2015, the contents of each incorporated herein in their entirety by reference. Generally, suitable binding agent linkers for the antibody conjugates described herein are those that are sufficiently stable to exploit the circulating half-life of the antibody and, at the same time, capable of releasing its payload after antigen-

mediated internalization of the conjugate. Linkers can be cleavable or non-cleavable. Cleavable linkers include linkers that are cleaved by intracellular metabolism following internalization, *e.g.*, cleavage via hydrolysis, reduction, or enzymatic reaction. Non-cleavable linkers include linkers that release an attached payload via lysosomal degradation of the antibody following internalization. Suitable linkers include, but are not limited to, acid-labile linkers, hydrolysis-labile linkers, enzymatically cleavable linkers, reduction labile linkers, self-immolative linkers/groups, and non-cleavable linkers. Suitable linkers also include, but are not limited to, those that are or comprise peptides, glucuronides, succinimide-thioethers, polyethylene glycol (PEG) units, hydrazones, mal-caproyl units, dipeptide units, valine-citruline units, and para-aminobenzyl (PAB) units.

[0248] Any linker molecule or linker technology known in the art can be used to create or construct an ADC of the present disclosure. In certain embodiments, the linker is a cleavable linker. According to other embodiments, the linker is a non-cleavable linker. Exemplary linkers that can be used in the context of the present disclosure include, linkers that comprise or consist of *e.g.*, MC (6-maleimidocaproyl), MP (maleimidopropanoyl), val-cit (valine-citrulline), val-ala (valine-alanine), dipeptide site in protease-cleavable linker, ala-phe (alanine-phenylalanine), dipeptide site in protease-cleavable linker, PAB (p-aminobenzyloxycarbonyl), SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate), and variants and combinations thereof. Additional examples of linkers that can be used in the context of the present disclosure are provided, *e.g.*, in US 7,754,681 and in Ducry, Bioconjugate Chem., 2010, *21*:5-13, and the references cited therein, the contents of which are incorporated by reference herein in their entireties.

[0249] In certain embodiments, the linkers are stable in physiological conditions. In certain embodiments, the linkers are cleavable, for instance, able to release at least the payload portion in the presence of an enzyme or at a particular pH range or value. In some embodiments, a linker comprises an enzyme-cleavable moiety. Illustrative enzyme-cleavable moieties include, but are not limited to, peptide bonds, ester linkages, hydrazones, and disulfide linkages. In some embodiments, the linker comprises a cathepsin-cleavable linker.

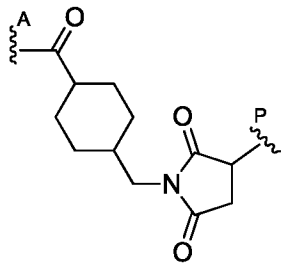
[0250] In some embodiments, the linker comprises a non-cleavable moiety.

[0251] Suitable linkers also include, but are not limited to, those that are chemically bonded to two cysteine residues of a single binding agent, *e.g.*, antibody. Such linkers can serve to mimic the antibody's disulfide bonds that are disrupted as a result of the conjugation process.

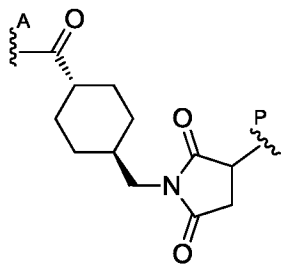
[0252] In some embodiments, the linker comprises one or more amino acids. Suitable amino acids include natural, non-natural, standard, non-standard, proteinogenic, non-proteinogenic, and L- or D- α-amino acids. In some embodiments, the linker comprises alanine, valine, glycine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or combination thereof. In certain embodiments, one or more side chains of the amino acids is linked to a side chain group, described below. In some embodiments, the linker comprises valine and citrulline. In some embodiments, the linker comprises lysine, valine, and citrulline. In some embodiments, the linker comprises lysine, valine, and alanine. In some embodiments, the linker comprises valine and alanine.

[0253] In some embodiments, the linker comprises a self-immolative group. The self-immolative group can be any such group known to those of skill. In particular embodiments, the self-immolative group is *p*-aminobenzyl (PAB), or a derivative thereof. In some embodiments, the self-immolative group is *p*-aminobenzyloxy. In some embodiments the self-immolative group comprises a cleavable di-sulfide group. Useful derivatives include *p*-aminobenzyloxycarbonyl (PABC). Those of skill will recognize that a self-immolative group is capable of carrying out a chemical reaction which releases the remaining atoms of a linker from a payload.

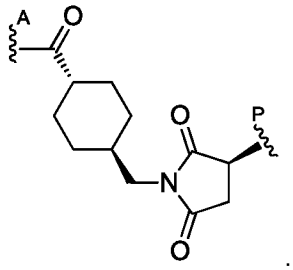
[0254] In some embodiments, the linker is:



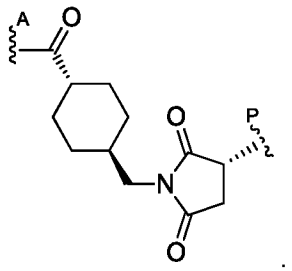
wherein is a bond to the antibody or antigen-binding protein (*e.g.*, via lysine residue) and is a bond to the payload. In some embodiments, the linker is:



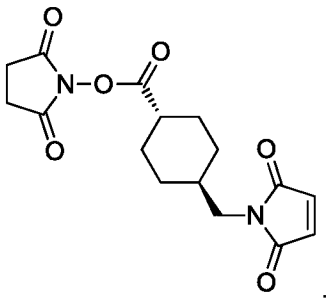
wherein is a bond to the antibody or antigen-binding protein (*e.g.*, via lysine residue) and is a bond to the payload. In certain embodiments, the linker is:



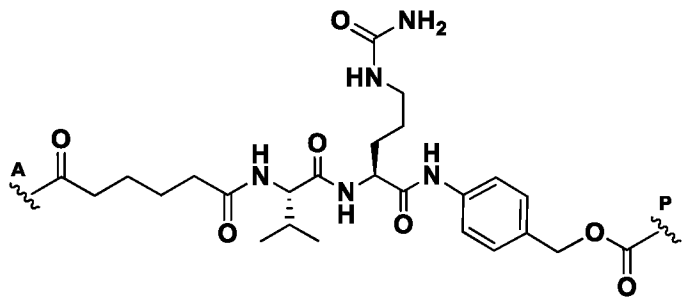
[0255] In certain embodiments, the linker is:



[0256] In some embodiments, the linker is derived from maleimidylmethyl-4-trans-cyclohexanecarboxysuccinate:



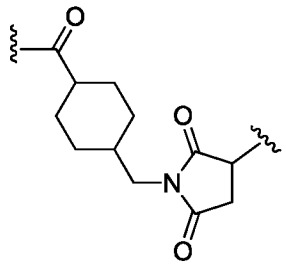
[0257] In some embodiments, the linker is:



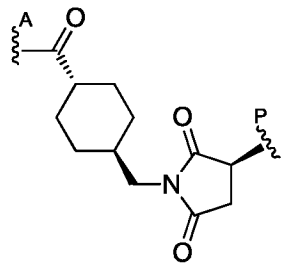
wherein is a bond to the antibody or antigen-binding protein (*e.g.*, via lysine residue) and is a bond to the payload.

[0258] In some embodiments, **L** is a cleavable linker. In some embodiments, **L** is a non-cleavable linker. In some embodiments, **L** comprises a dipeptide. In some embodiments, **L** comprises a **PAB** moiety. In some embodiments, **L** comprises a disulfide moiety.

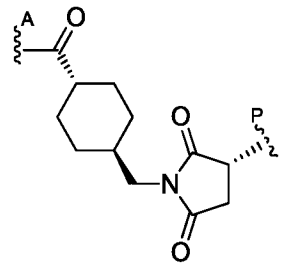
[0259] In some embodiments, **L** comprises a moiety having the following structure:



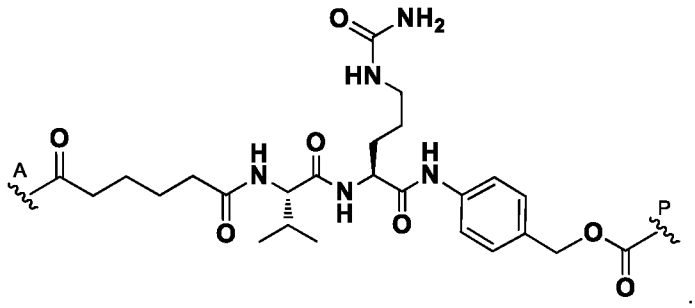
[0260] In some embodiments, L comprises a moiety having the following structure:



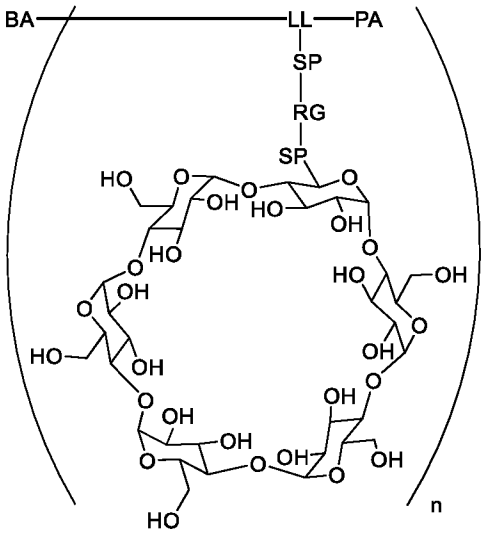
[0261] In some embodiments, L comprises a moiety having the following structure:



[0262] In some embodiments, L comprises a moiety having the following structure:



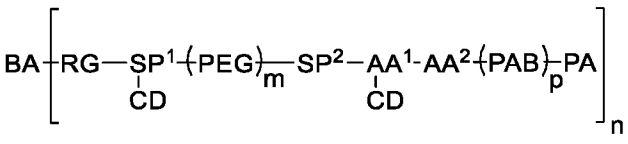
[0263] In certain embodiments, the linker comprises a cyclodextrin group. In certain embodiments, the linker provides an ADC according to Formula (Ia):



(**Ia**)

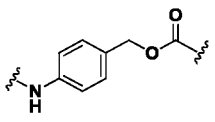
[0264] In Formula (**Ia**), **BA** is an anti-MSR1 antibody, or an antigen-binding fragment thereof, **LL** is a trivalent linker, **RG** is a reactive linker residue, **SP** is, independently in each instance, absent or a spacer group, subscript **n** is an integer from 1 to 30; and **PA** is a payload. In certain embodiments, **n** is from 1 to 4. In certain embodiments, **n** is 4. In certain embodiments, **n** is 2. In certain embodiments, **n** is 1. In certain embodiments, **n** is 3.

[0265] In certain embodiments, the linker comprises a cyclodextrin group. In certain embodiments, the linker provides an ADC according to Formula (Id):

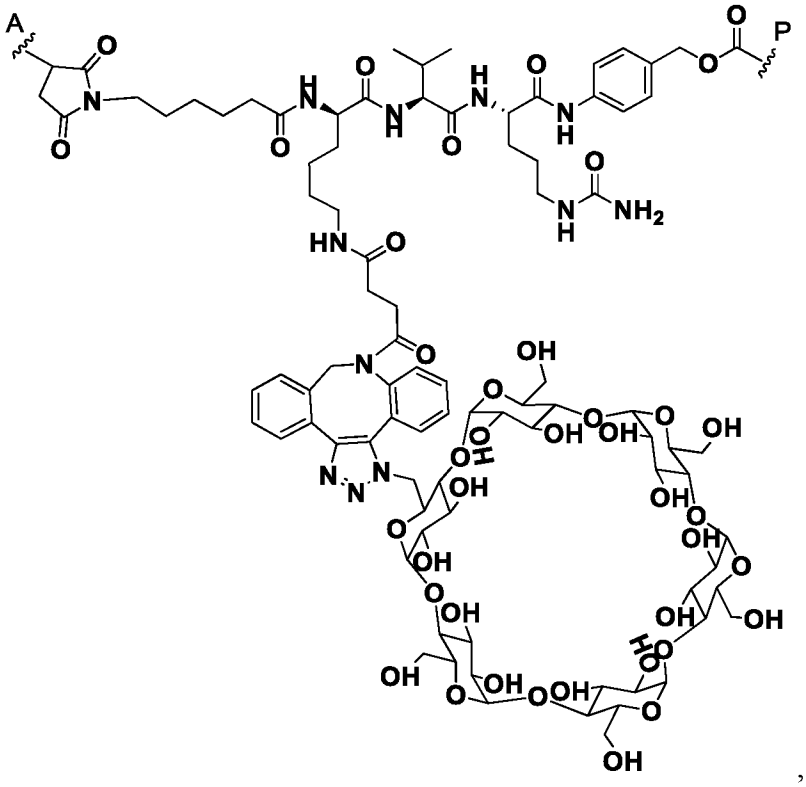


(**Id**)

[0266] In Formula (**Id**), **BA** is an anti-MSR1 antibody, or an antigen-binding fragment thereof; **RG** is a reactive group residue; SP^1 and SP^2 are each, independently in each instance, absent

or a spacer group residue, and wherein **SP**^{1} comprises a trivalent linker; **AA**^{1} is a trivalent linker comprising an amino acid residue; **AA**^{2} is a di-peptide residue; **PEG** is a polyethylene glycol residue; **PAB** is  , wherein the indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula, **CD** is, independently in each instance, absent or a cyclodextrin residue, wherein at least one **CD** is present, subscript **n** is an integer from 1 to 30; subscript **m** is an integer from 0 to 5; subscript **p** is 0 or 1; and **PA** is a payload moiety. In these examples, subscript **m** is 0, 1, 2, 3, 4, or 5. In some examples, subscript **m** is 0. In some examples, subscript **m** is 1. In some examples, subscript **m** is 2. In some examples, subscript **m** is 3. In some examples, subscript **m** is 4. In some examples, subscript **m** is 5. In some examples, subscript **p** is 0. In some examples, subscript **p** is 1. In some examples, any one of **AA**^{1} or **AA**^{2} comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, **AA**^{1} is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, **AA**^{1} is lysine. In certain embodiments, **AA**^{1} is lysine or a derivative of lysine. In certain embodiments, the **AA**^{2} is valine-citrulline. In some embodiments, the **AA**^{2} is citrulline-valine. In some embodiments, the **AA**^{2} is valine-alanine. In some embodiments, the **AA**^{2} is alanine-valine. In some embodiments, the **AA**^{2} is valine-glycine. In some embodiments, the **AA**^{2} is glycine-valine. In some embodiments, the **AA**^{1}-**AA**^{2} glutamine-valine-citrulline. In some embodiments, the **AA**^{1}-**AA**^{2} is glutamine-valine-citrulline. In some embodiments, the **AA**^{1}-**AA**^{2} is lysine-valine-alanine. In some embodiments, the **AA**^{1}-**AA**^{2} is lysine-valine-citrulline. In some embodiments, the **AA**^{1}-**AA**^{2} is glutamine-valine-citrulline. In certain embodiments, the lysine is L-lysine. In certain embodiments, the lysine is D-lysine. In some examples, **SP**^{1} is independently in each instance, selected from the group consisting of C_{1-6} alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-CH_2-CH_2-$(-O-CH_2-CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, **SP**^{2} is independently in each instance, selected from the group consisting of C_{1-6} alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-CH_2-CH_2-$(-O-CH_2-CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8.

[0267] In certain embodiments, for Formulas (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**III**), (**3000**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), (**6005**), (**7001**), (**7002**), (**7003**), (**7004**), and/or (**7005**), the linker is selected from:



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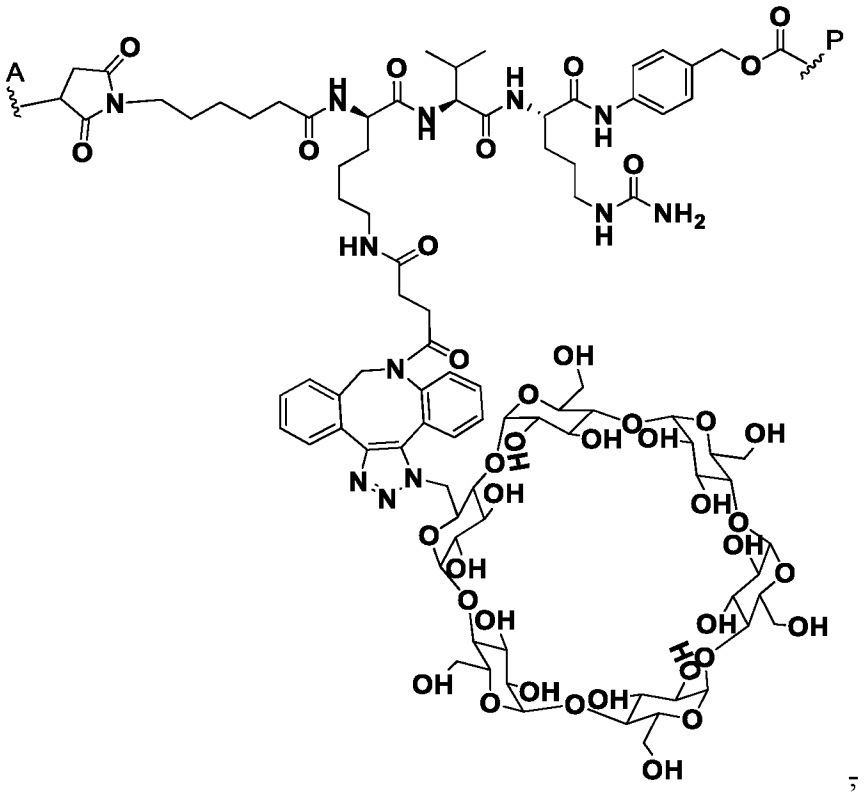

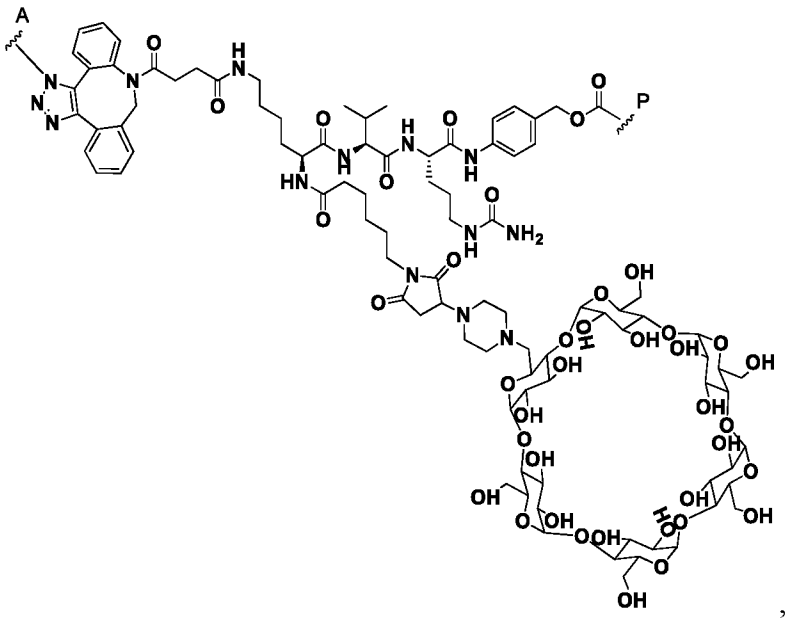

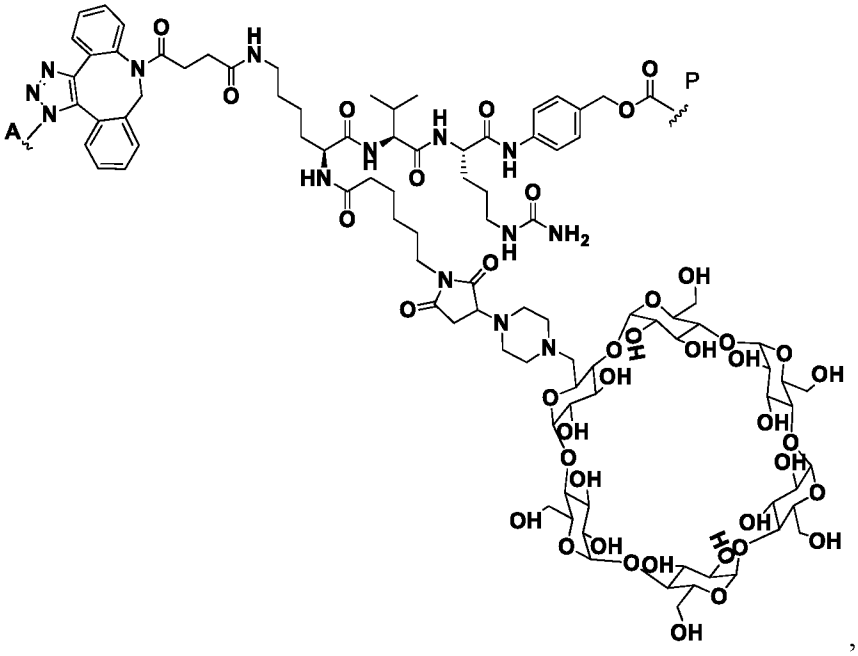

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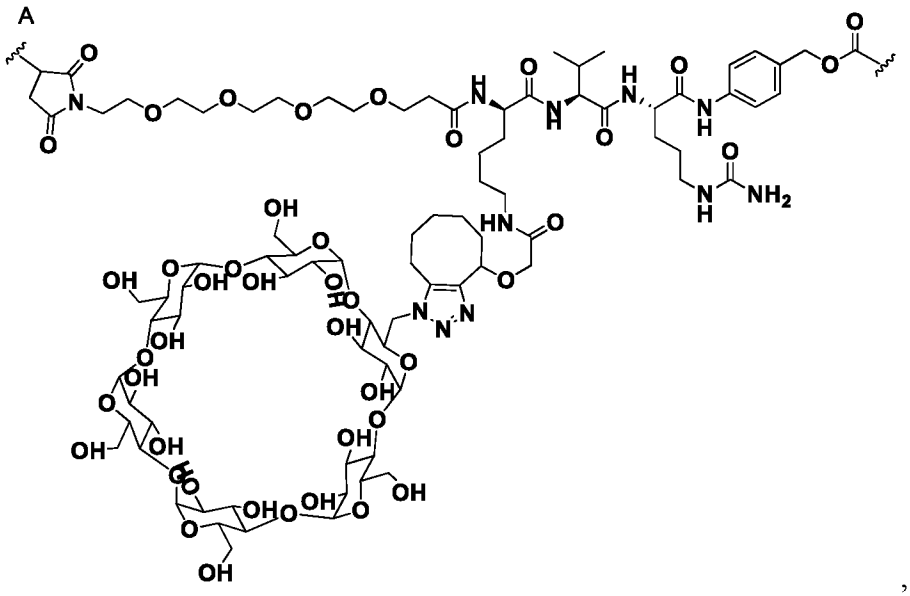

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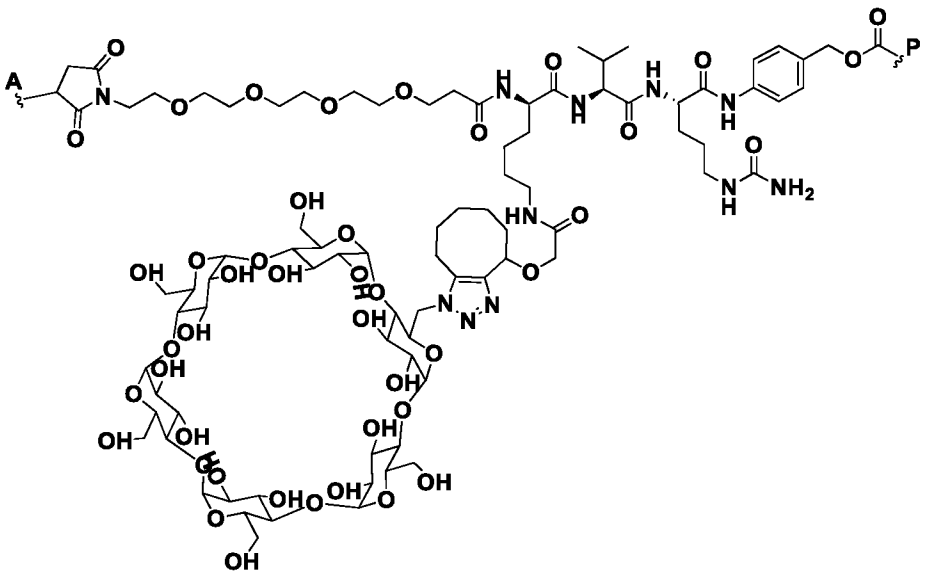

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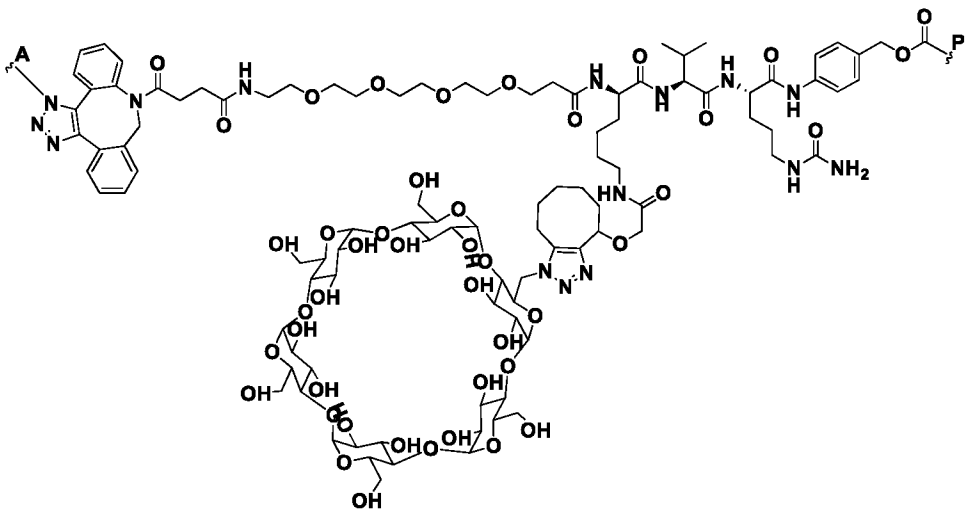

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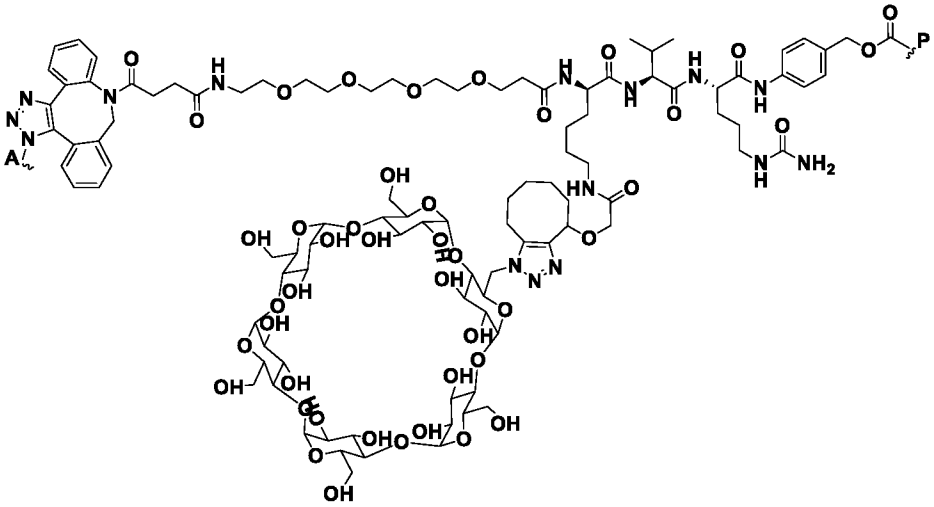

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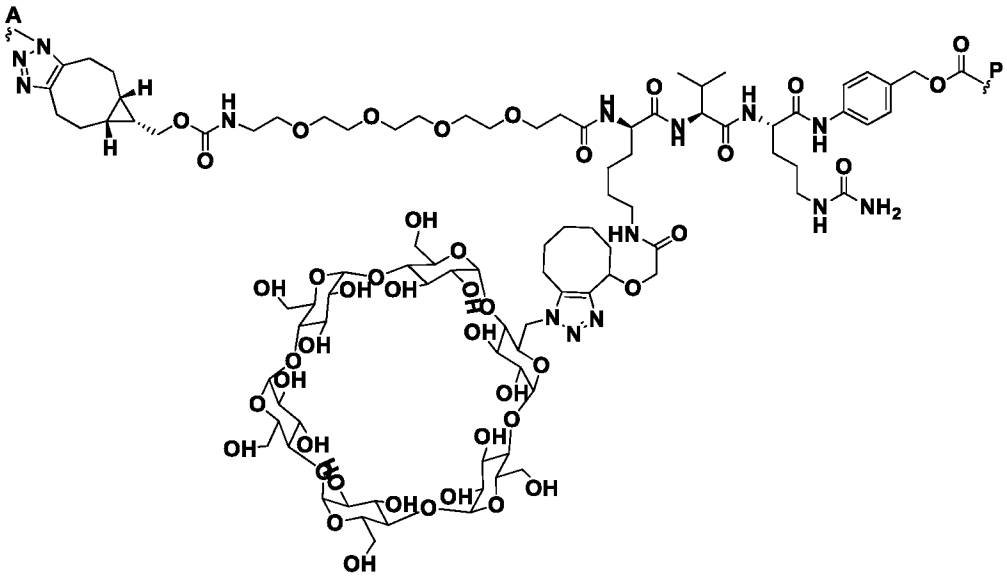

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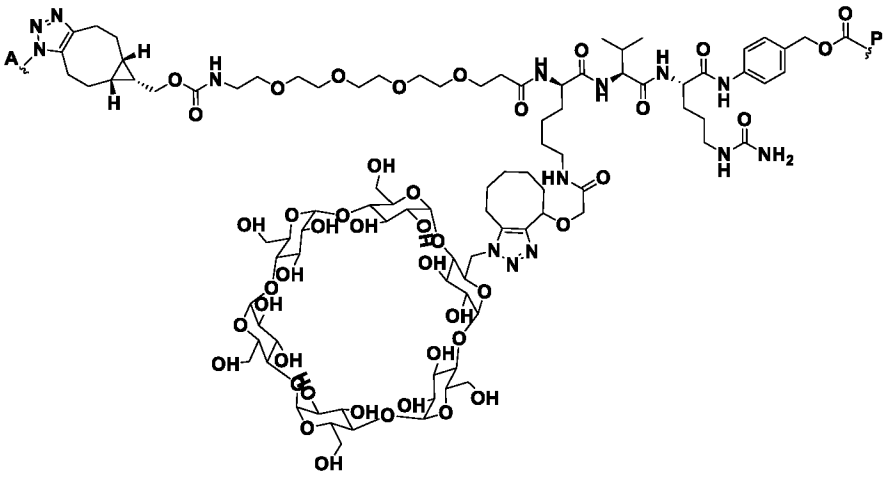

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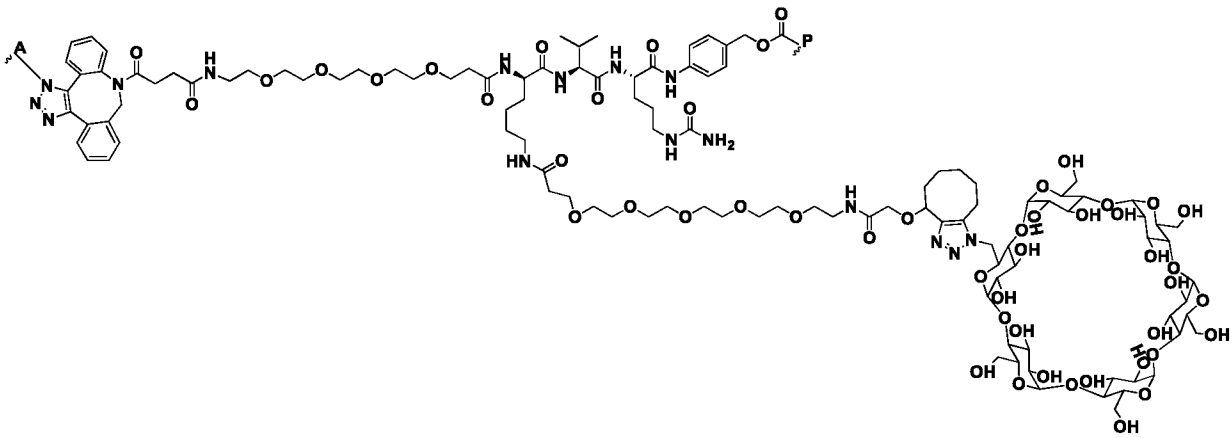

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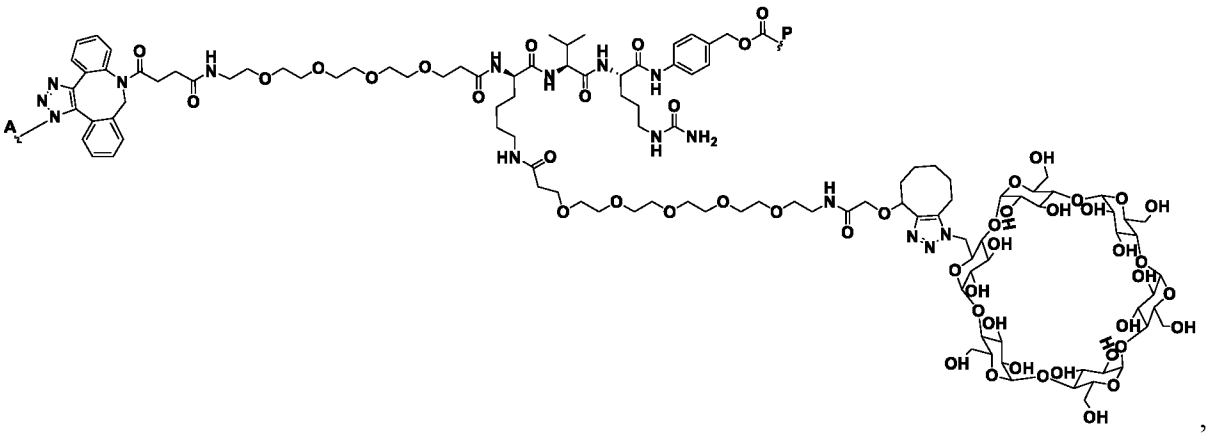

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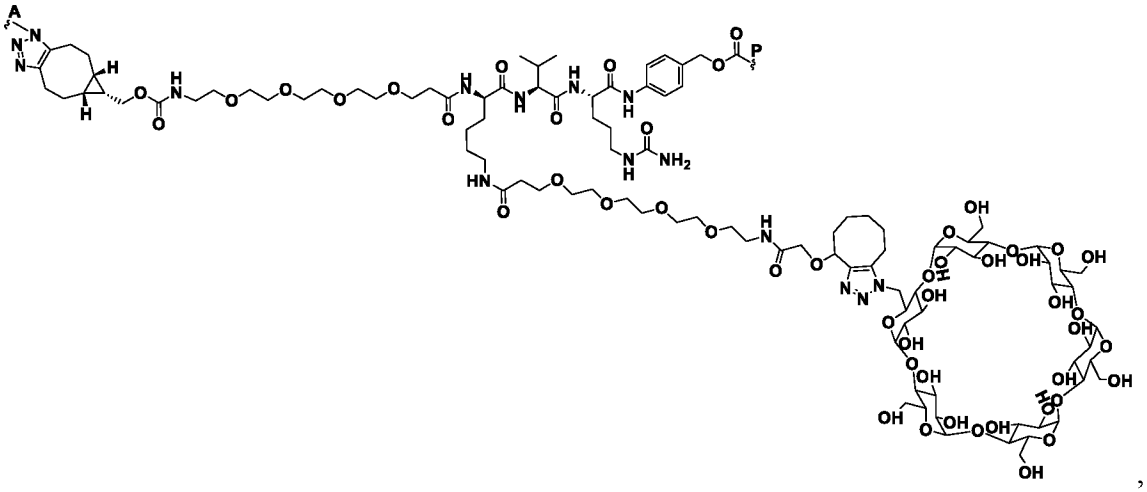

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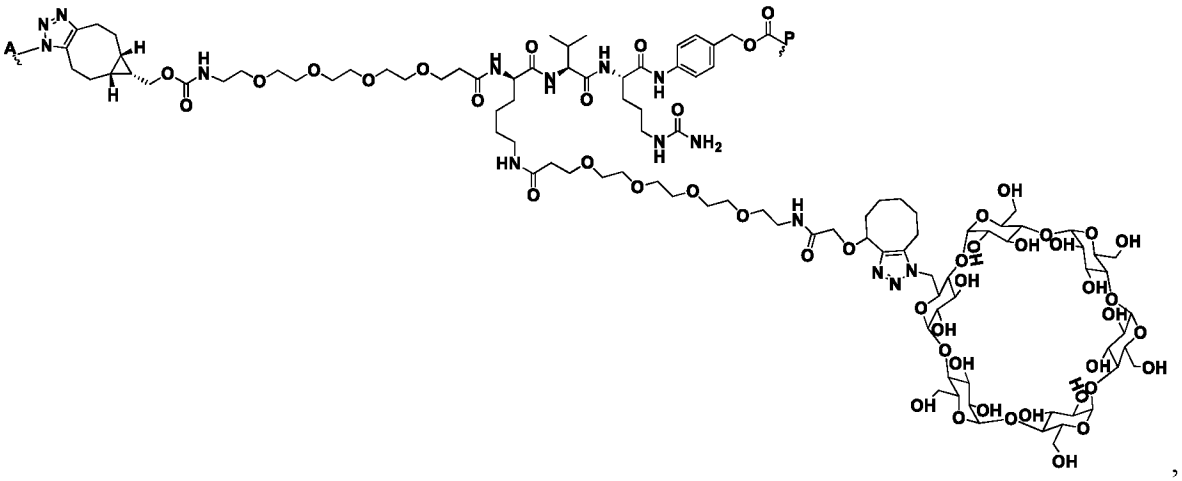

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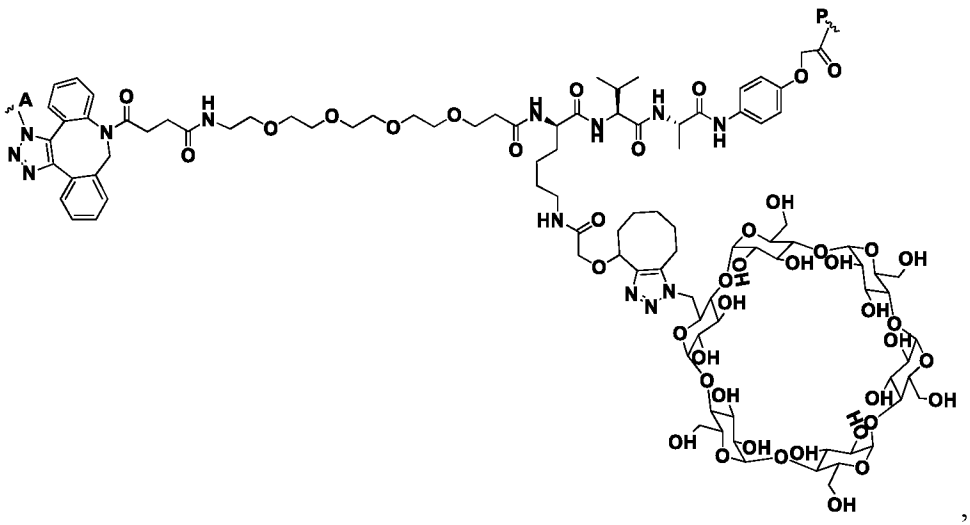

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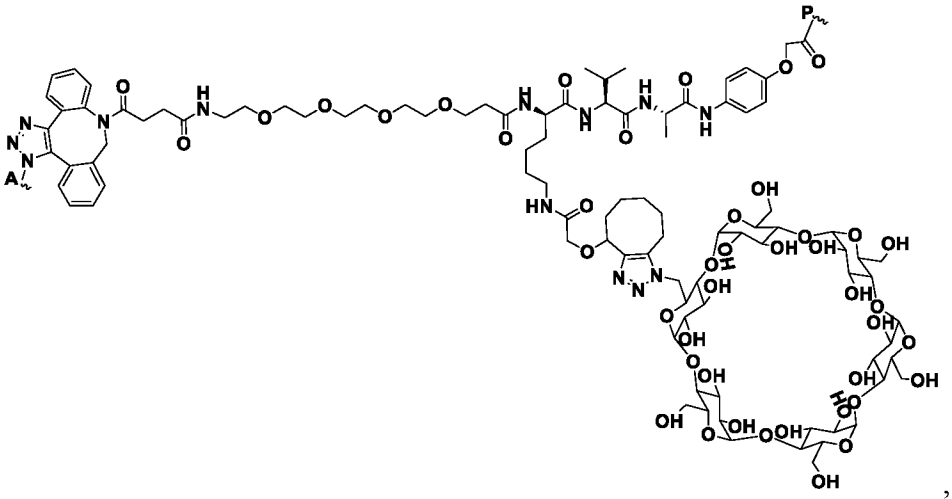

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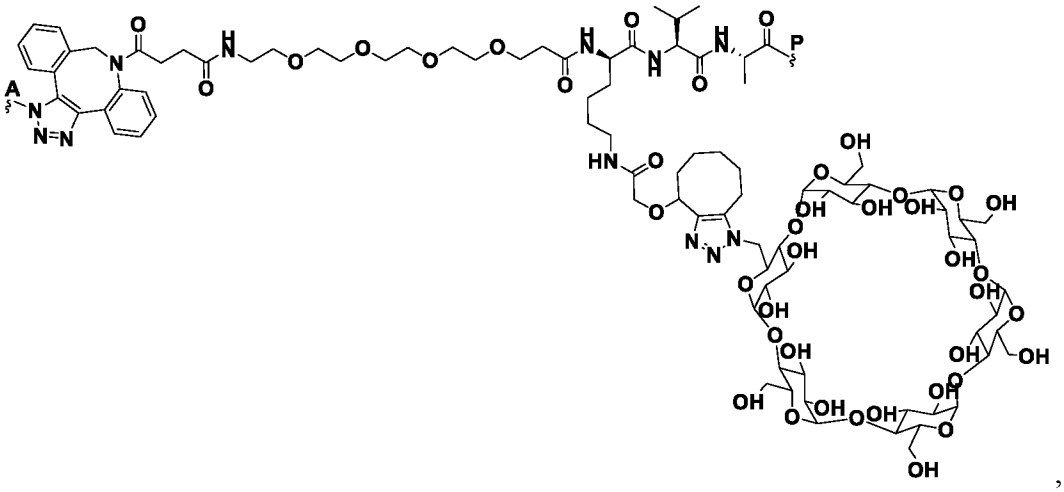

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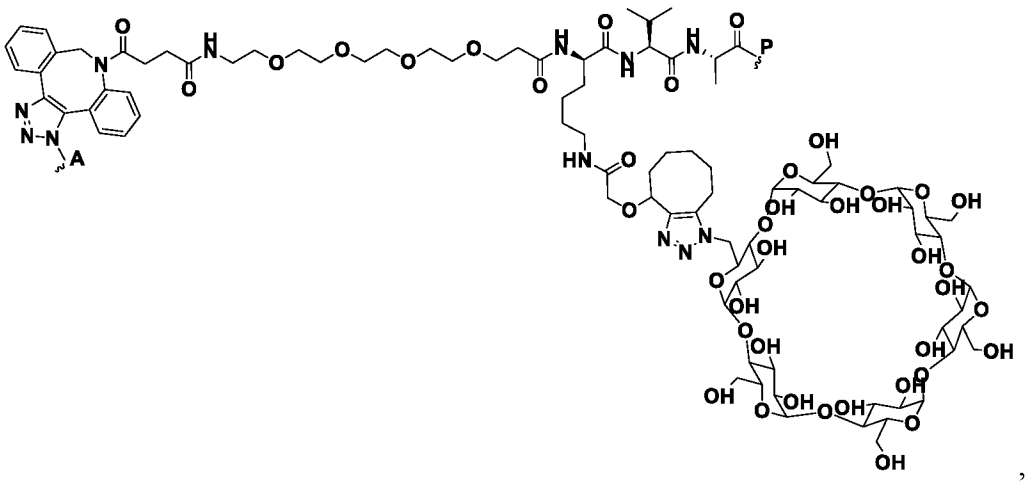

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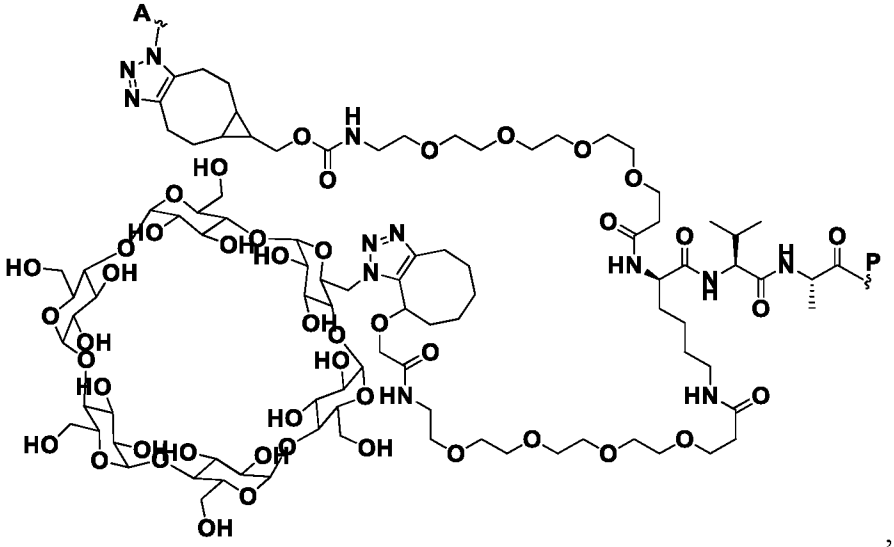

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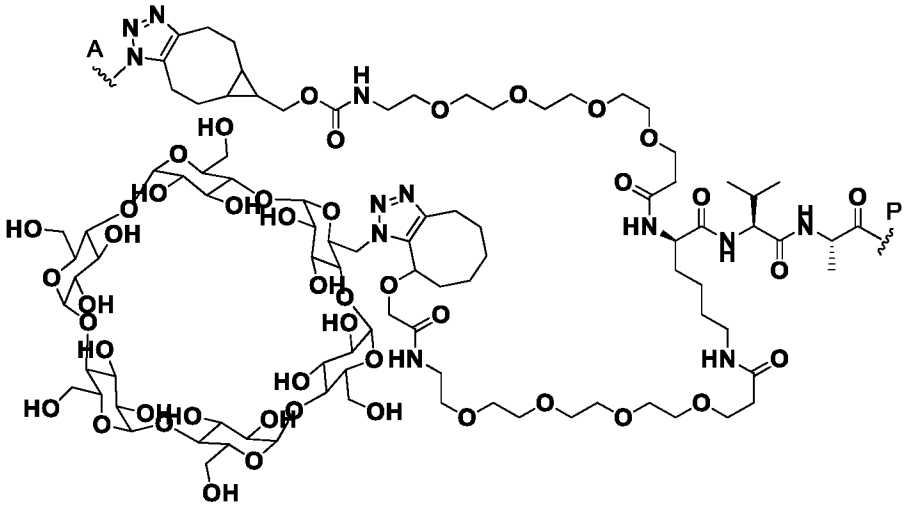

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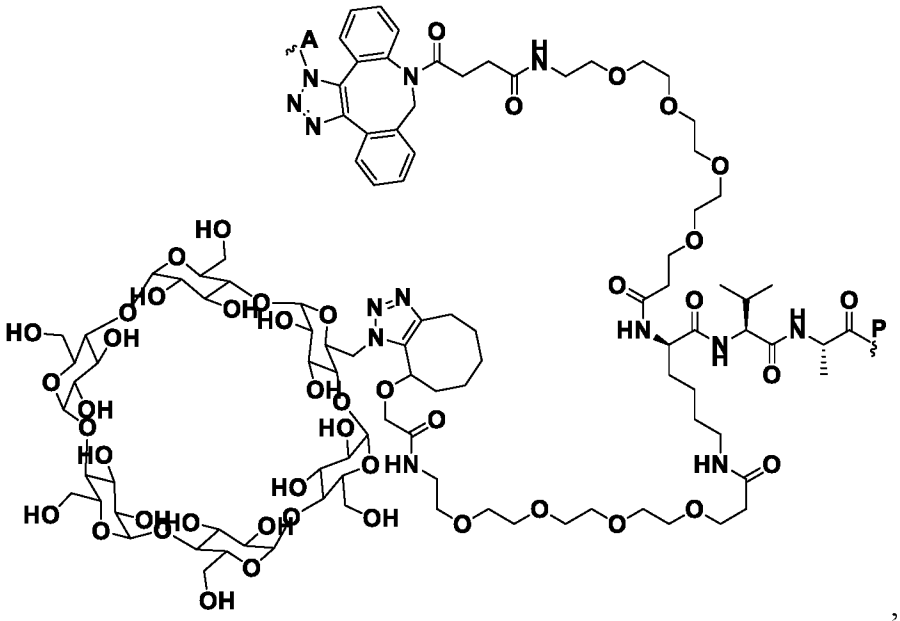

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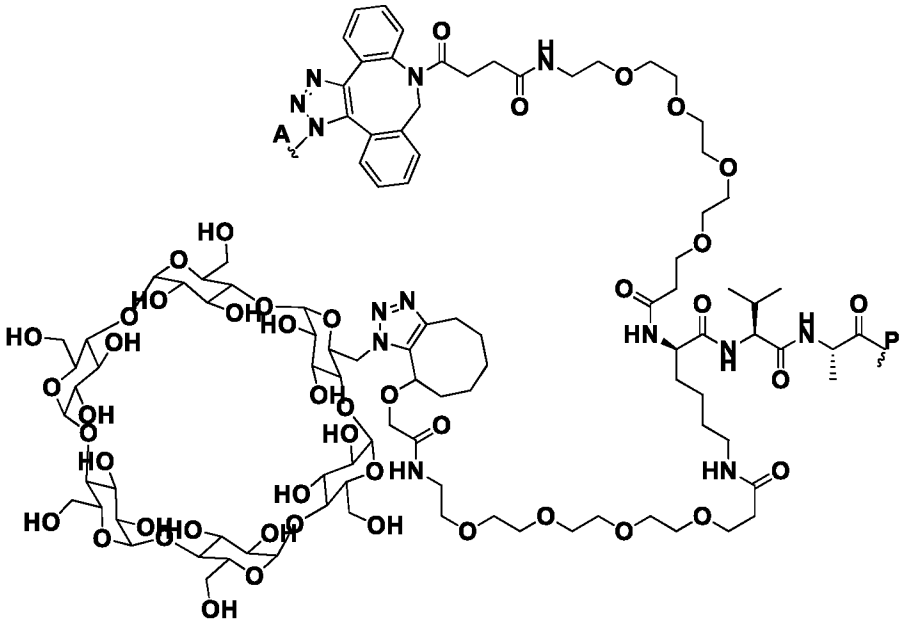

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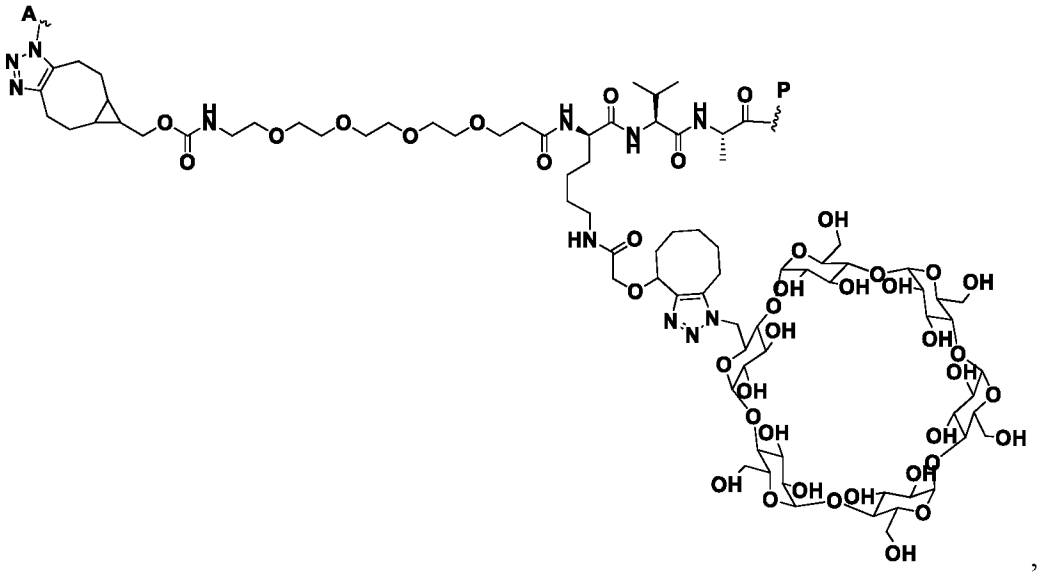

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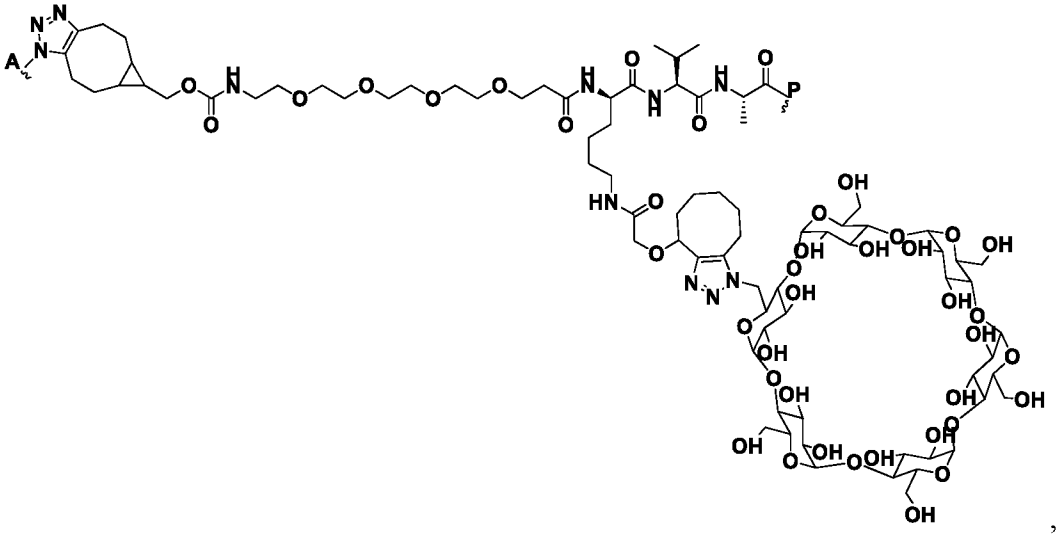

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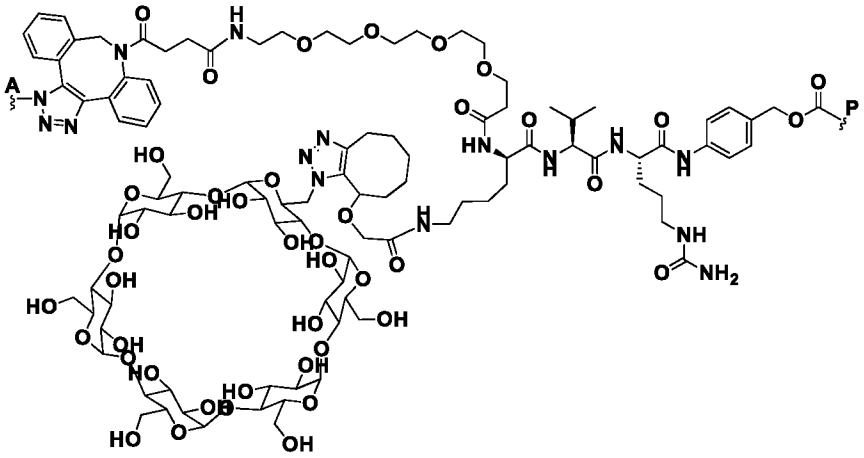

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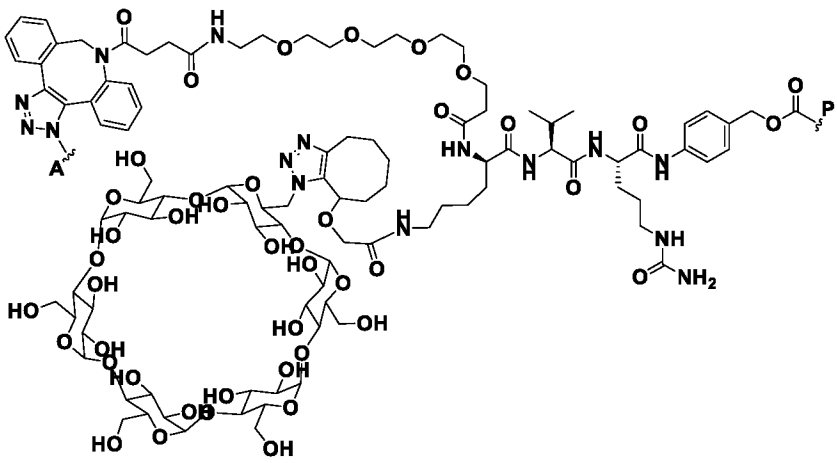

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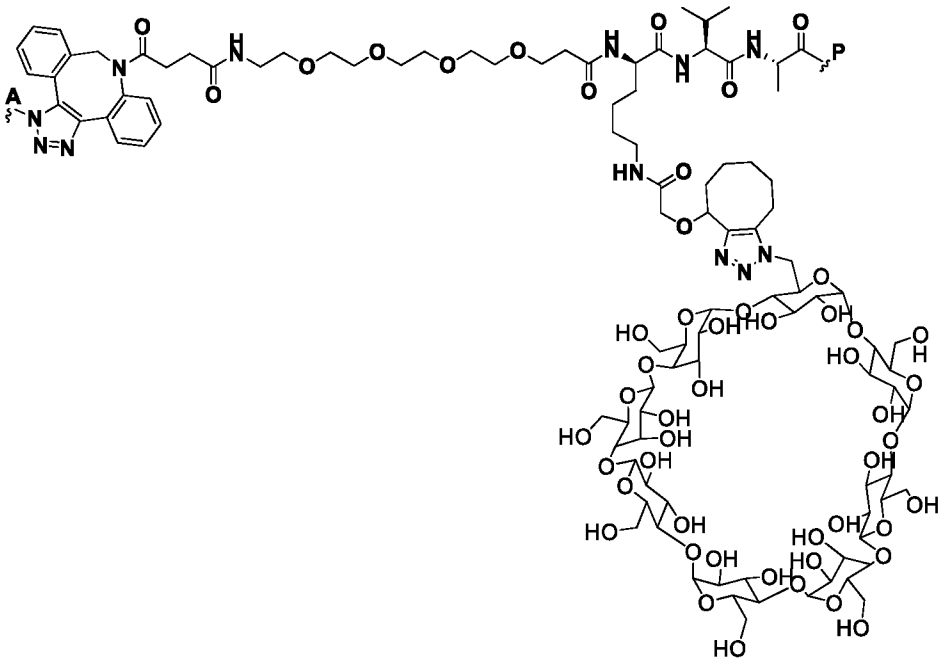

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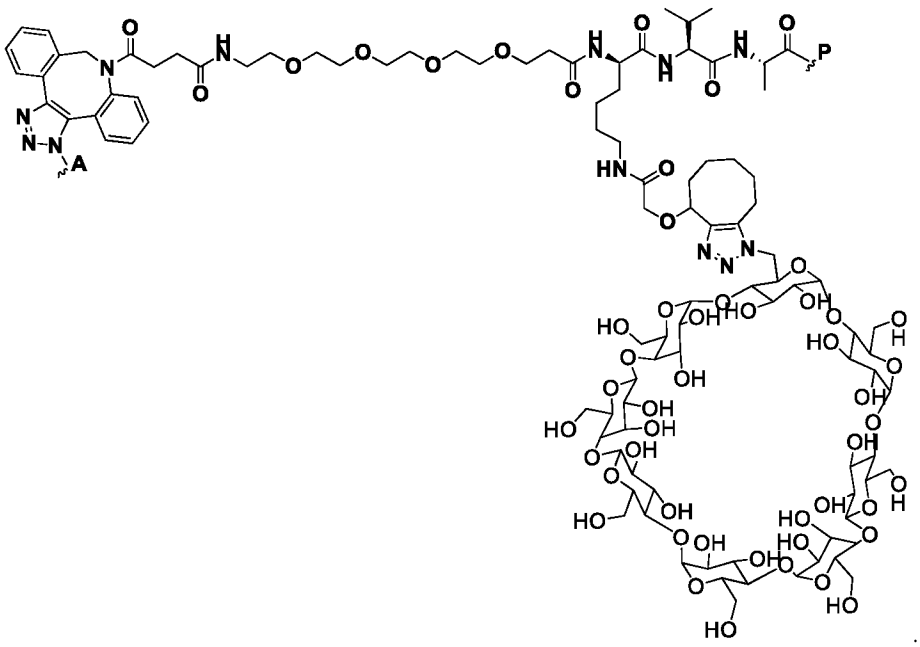

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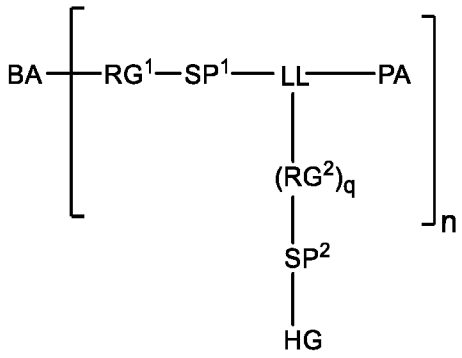
Also included in these examples, is a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, a regioisomer thereof, or mixture of regioisomers thereof, wherein each is a bond to the binding agent; and each is a bond to the payload.

[0268] In certain embodiments, the linker comprises a terminal hydrophilic group (**HG**). In certain embodiments, the linker comprises a taurine group. In certain embodiments, the linker

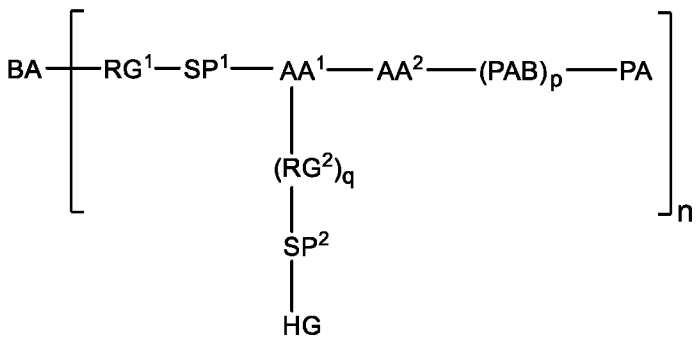
comprises a terminal sulfonic acid group. In certain embodiments, the linker provides an ADC according to Formula (**II**):



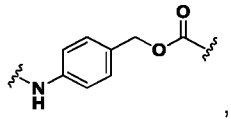
(**II**)

wherein, in Formula (**II**), **BA** is a binding agent; **LL** is a trivalent linker; **RG^1** and **RG^2** are reactive group residues; **SP^1** and **SP^2** are independently, in each instance, absent, or a spacer group residue; **HG** is a hydrophilic residue; **PA** is a payload residue; subscript **n** is an integer from 1 to 30; and subscript **q** is 0 or 1. In some instances more than one trivalent linker **LL** may be present. In some instances, **n** is an integer from 1 to 4. In some instances **n** is 1. In some instances **n** is 2. In some instances **n** is 3. In some instances **n** is 4. In some instances, **HG** is a terminal hydrophilic group. In some instances, **HG** comprises one terminal sulfonic acid group or a salt thereof. In other instances, **HG** comprises more than one terminal sulfonic acid groups or salts thereof. In some instances, **HG** comprises one terminal phosphonic acid group or a salt thereof. In other instances, **HG** comprises more than one terminal phosphonic acid groups or salts thereof. In some instances, **HG** comprises one terminal tertiary amine group or a salt thereof. In other instances, **HG** comprises more than one terminal tertiary amine groups or salts thereof. In some instances, **HG** comprises one terminal polyol (e.g., glucose, maltose) or a derivative thereof. In other instances, **HG** comprises more than one terminal polyol (e.g., glucose, maltose) or derivatives thereof.

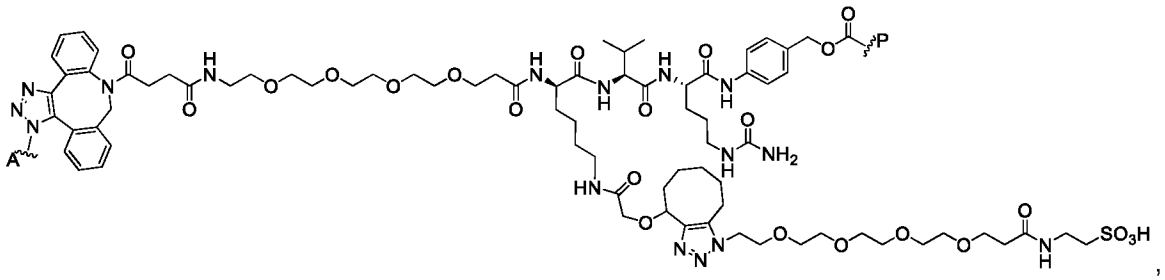
[0269] In another example, the compound of Formula (**II**) is according to Formula (**IV**):



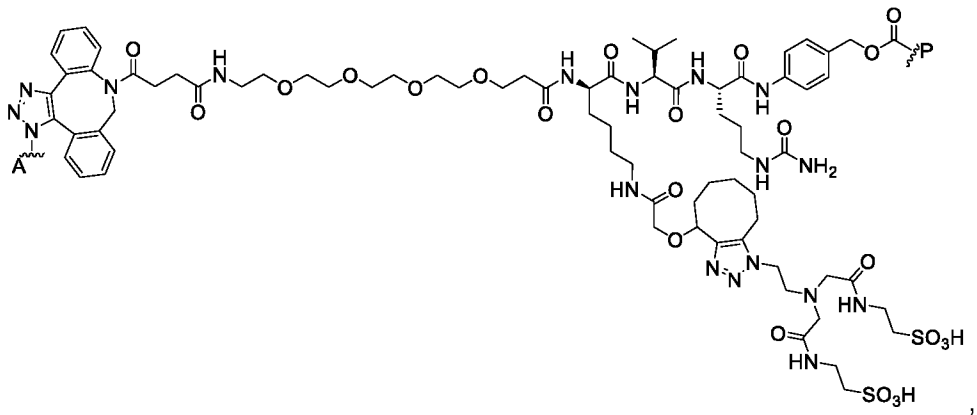
(**IV**).

[0270] In Formula (**IV**), **BA**, **RG**^{1}, **SP**^{1}, **RG**^{2}, **SP**^{2} and **HG** are as defined above, **AA**^{1} is a trivalent linker comprising an amino acid residue; **AA**^{2} is a dipeptide residue; and **PAB** is  , wherein the indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula; subscript **p** is 0 or 1; and subscript **q** is 0 or 1. In some instanes, subscript **p** is 0 and subscript **q** is 0. In some instances, subscript **p** is 1; and subscript **q** is 0. In some instances, subscript **p** is 0; and subscript **q** is 1. In some instances, subscript **p** is 1; and subscript **q** is 1. In some instances **SP**^{1} comprises from 0-5 polyethylene glycol (**PEG**) residues. In some instances **SP**^{2} comprises from 0-5 **PEG** residues. In some examples, **SP**^{1} is independently in each instance, selected from the group consisting of C_{1-6} alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-CH_2-CH_2-(-O-CH_2-CH_2)$_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, **SP**^{2} is independently in each instance, selected from the group consisting of C_{1-6} alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-CH_2-CH_2-(-O-CH_2-CH_2)$_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, any one of **AA**^{1} or **AA**^{2} comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, **AA**^{1} is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, **AA**^{1} is lysine. In certain embodiments, **AA**^{1} is lysine or a derivative of lysine. In certain embodiments, **AA**^{1} is glutamic acid. In certain embodiments, the **AA**^{2} is valine-citrulline. In some embodiments, the **AA**^{2} is citrulline-valine. In some embodiments, the **AA**^{2} is valine-alanine. In some embodiments, the **AA**^{2} is alanine-valine. In some embodiments, the **AA**^{2} is valine-glycine. In some embodiments, the **AA**^{2} is glycine-valine. In some embodiments, the **AA**^{1}-**AA**^{2} is glutamine-valine-citrulline. In some embodiments, the **AA**^{1}-**AA**^{2} is lysine-valine-citrulline. In some embodiments, the **AA**^{1}-**AA**^{2} is lysine-valine-alanine. In some embodiments, the **AA**^{1}-**AA**^{2} is glutamine-valine-alanine. In certain embodiments, the lysine is L-lysine. In certain embodiments, the lysine is D-lysine.

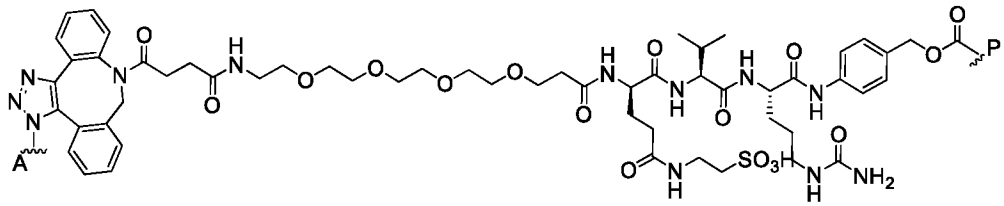
[0271] In certain embodiments, for Formulas (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**III**), (**3000**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), (**6005**), (**7001**), (**7002**), (**7003**), (**7004**), and/or (**7005**), the linker is selected from:



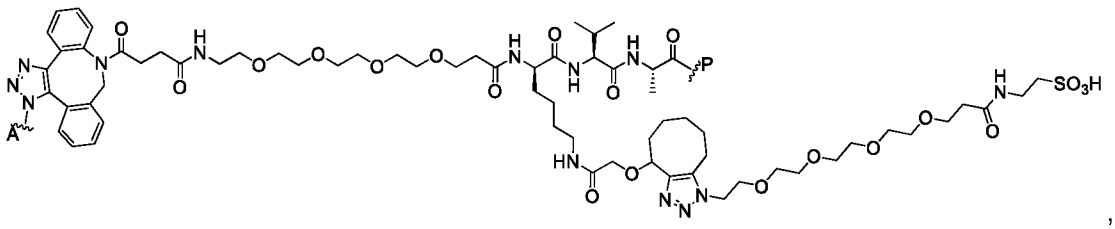
,



,

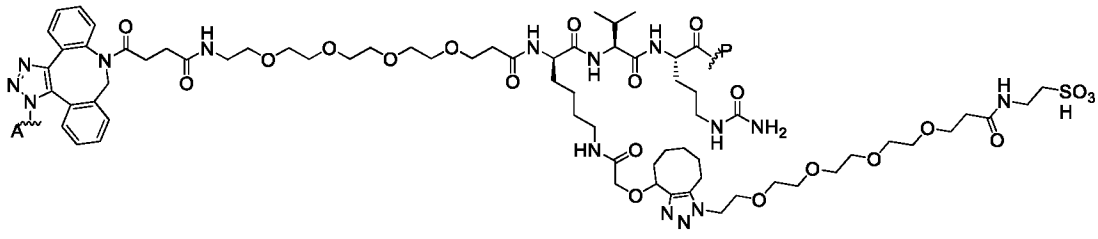


,



,

and



,

or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

each is a bond to the binding agent; and

each is a bond to the payload residue.

[0272] In some instances, the moiety **L-PA** is attached to a reactive group (**RG**) to form **RG-L-PA** (e.g., linker payloads shown in Table 3). In some instances, **BA** (or a modified from of **BA**, e.g., PEG-modified **Ab**, as shown in Table 2, or **Ab**^{1}) reacts with linker payloads to form the ADCs described in Table 1 and Table 2. Also contemplated within the scope of embodiments presented herein are ADCs prepared from any linker payloads described in Table 5A and Table 5B.

Linker payloads

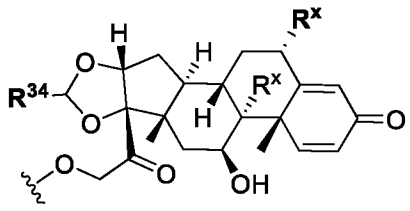
[0273] Provided herein are linker-steroids according to Formula (**2000**),

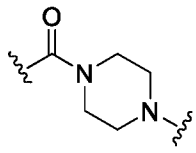
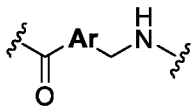
RG-L^{2}-(L^{3})$_{0-1}$-SP-D

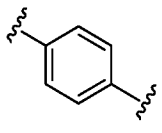
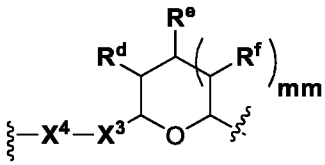
or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof; which are useful in the preparation of antibody-drug conjugates,

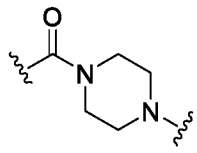
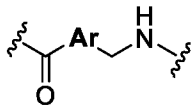
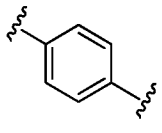
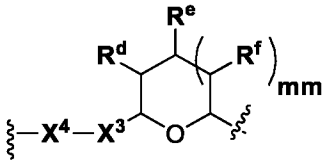
wherein

D is selected from

a)  Formula (a),

where both **R^{x}** in formula (a) are hydrogen; **R^{34}** is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C$_{1}$-C$_{10}$-alkylene-C(O)-, -C(O)-N(C$_{1-6}$alkyl)-C$_{1}$-C$_{10}$-alkylene-**X^{1}**- where **X^{1}** is attached to (**L^{3}**)$_{0-1}$ in Formula (**2000**), -C(O)-N(H)-(C$_{1}$-C$_{10}$-alkylene)-S- where S is attached to (**L^{3}**)$_{0-1}$ in Formula (**2000**), -C(O)-N(C$_{1-6}$alkyl)-(C$_{1}$-C$_{10}$-alkylene)-S- where S is attached to (**L^{3}**)$_{0-1}$ in Formula (**2000**),  where the point of attachment on the right hand side (*i.e.* at N) is to (**L^{3}**)$_{0-1}$ in Formula (**2000**), -CH$_{2}$-NH- where N is attached to (**L^{3}**)$_{0-1}$ in Formula (2000),  where the N is

attached to $(L^3)_{0-1}$ in Formula (**2000**) and where **Ar** is optionally substituted arylene (in some embodiments, ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to $(L^3)_{0-1}$ in Formula (**2000**), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to $(L^3)_{0-1}$ in Formula (**2000**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(R^5)-C_1-C_{10}-alkylene-C(O)NH-X^2- where X^2 is attached to $(L^3)_{0-1}$ in Formula (**2000**), or  where X^4 is attached to $(L^3)_{0-1}$ in Formula (**2000**); or

where both R^x in formula (a) are fluoro; R^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^{1b}- where X^{1b} is attached to $(L^3)_{0-1}$ in Formula (**2000**), -C(O)-N(H)-(C_1-C_{10}-alkylene)-X^{1b}- where X^{1b} is attached to $(L^3)_{0-1}$ in Formula (**2000**),  where the point of attachment on the right hand side (*i.e.* at N) is to $(L^3)_{0-1}$ in Formula (**2000**), -CH_2-NH- where N is attached to $(L^3)_{0-1}$ in Formula (2000),  where the N is attached to $(L^3)_{0-1}$ in Formula (**2000**) and where **Ar** is optionally substituted arylene (in some embodiments, ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to $(L^3)_{0-1}$ in Formula (**2000**), -C(O)-(C_1-C_{10}-alkylene)-NR^{50}C(O)-(C_1-C_{10}-alkylene)-NR^{50a}- where NR^{50a} is attached to $(L^3)_{0-1}$ in Formula (**2000**) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(R^5)-(C_1-C_{10}-alkylene)-C(O)NH-X^2- where X^2 is attached to $(L^3)_{0-1}$ in Formula (**2000**), or  where X^4 is attached to $(L^3)_{0-1}$ in Formula (**2000**); and/or

b) the compounds in Table A above, where the compounds in Table A are linked to **RG** of the Compound of Formula (**2000**) through the hydroxy of the -C(O)CH_2OH group, *i.e.* by -C(O)CH_2-O-**SP**-($\mathbf{L^3}$)$_{0-1}$ -, or through the hydroxy of Mapracorat, *i.e.* by -O-**SP**-($\mathbf{L^3}$)$_{0-1}$ -:

$\mathbf{X^1}$ is -N(C_{1-6}alkyl)-;

$\mathbf{X^{1b}}$ is -S-, -NH-, or -N(C_{1-6}alkyl)-;

$\mathbf{X^2}$ is -NH-;

$\mathbf{X^3}$ is -CH_2-, $\mathbf{X^3}$ is -CH_2-O-(C_1-C_{10}-alkylene)-C(O)- where the C(O) is attached to $\mathbf{X^4}$, or $\mathbf{X^3}$ is -C(O)-;

$\mathbf{X^{34}}$ is -O-;

$\mathbf{R^{35}}$ is H, -OH, -OCH_3, or C_{1-6}alkyl;

$\mathbf{R^{50}}$ and $\mathbf{R^{50a}}$ are independently hydrogen or C_1-C_6-alkyl;

$\mathbf{R^d}$, $\mathbf{R^e}$, and $\mathbf{R^f}$ are independently -H, -OH, hydroxyalkyl, alkoxycarbonyl, -C(O)OH, or -CH_2O$\mathbf{R^g}$, where each $\mathbf{R^g}$ is independently -CH_2C(O)OH or -CH_2C(O)O(alkyl); and

mm is 0 or 1;

RG is a reactive group residue;

$\mathbf{L^2}$ is a connecting linker; and

$\mathbf{L^3}$, when present, is a self-immolative group.

[0274] Provided herein are linker-LXR-modulators according to Formula (**4000**),

RG-L-E Formula **(4000)**

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof;

which are useful in the preparation of antibody-drug conjugates,

wherein

E is selected from

a) compounds of Formula (**B**) described above and herein; and/or

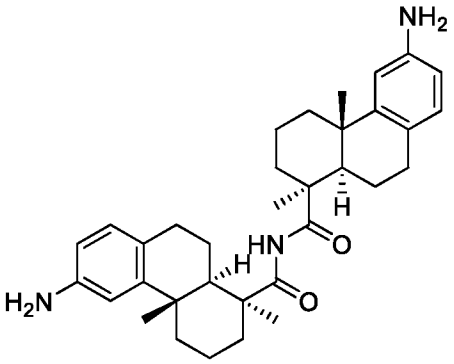
b) compounds of Formula (**B-1**) described above and herein; and/or

c) payloads described in **Table C** herein;

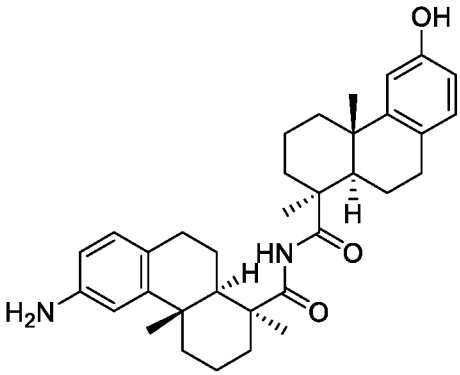
L is a linker described herein; and

RG is any reactive group residue described herein.

[0275] In some embodiments, the LXR payload is

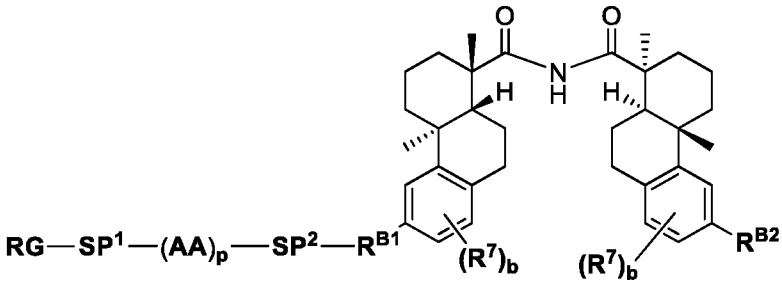


. In some embodiments, the LXR payload is

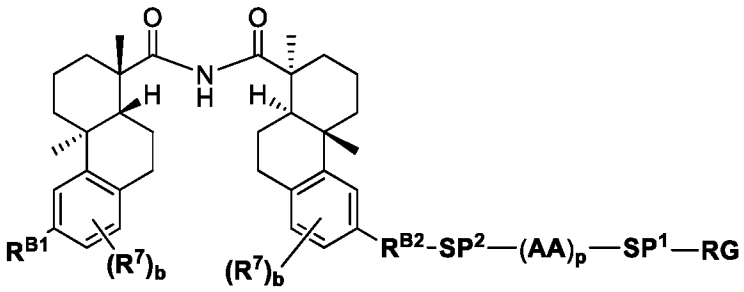


.

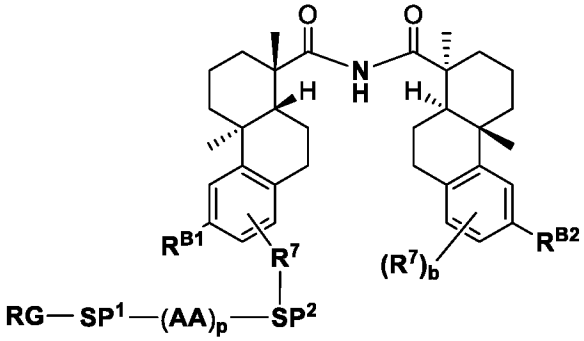
[0276] In some embodiments, linker-payloads of Formula (**4000**) have the structures of Formula (**4001**), (**4002**), (**4003**), or (**4004**):



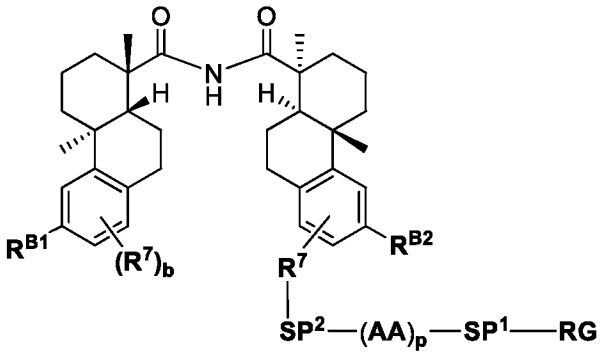
Formula (**4001**)



Formula (**4002**)



Formula (**4003**) or



Formula (**4004**)

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof;

wherein SP^1 and SP^2, when present, are spacer groups as defined herein in some and any embodiments;

each **AA** is an amino acid residue;

p is an integer from 1 to 10;

RG is a reactive group residue; and

R^{B1}, R^{B2}, R^7 and **b** are as defined herein in some or any embodiments.

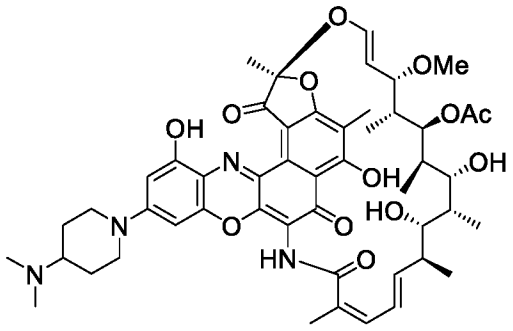
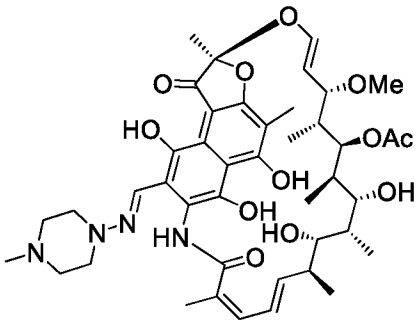
[0277] In some embodiments, linker-payloads of Formula (**4000**), (**4001**), (**4002**), (**4003**), or (**4004**) are selected from linker-payloads in Table 3.

[0278] Provided herein are linker-rifamycin analogs according to Formula (**7000**),

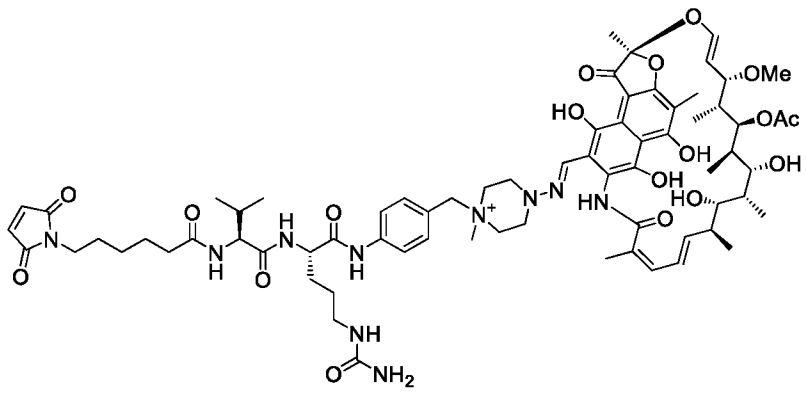
RG-L-F Formula **(7000)**

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof;

wherein **F** is a rifamycin analog;
L is a linker described herein; and
RG is any reactive group residue described herein.

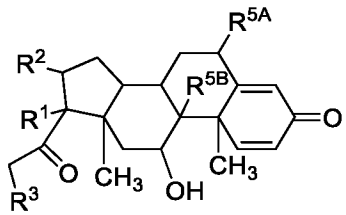
[0279] In some embodiments, **F** is a rifamycin analog described herein. In some embodiments, **F** is rifalogue:  . In some embodiments, **F** is rifampicin:  .

[0280] Also provided herein is a linker payload according to Formula (**D**):



Formula (**D**).

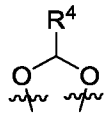
[0281] Provided herein are linker-steroids wherein the steroid conjugated to the antibody, or antigen-binding fragment thereof, through a linker or a linker-spacer is a compound of Formula (**A-1**)



Formula (**A-1**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein

R^1 and R^2 are, independently, –H, alkyl, alkyl-C(O)-O–, –OH, or halo; or R^1 and R^2 together form  ,

wherein R^4 is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl,

wherein the alkyl, aryl, arylalkyl, and N-containing heterocycloalkyl are, independently in each instance, optionally substituted with -$NR^{Aa}R^{Ab}$;

R^3 is –O, R^Z-C(O)-**X**–, –heteroalkyl, –piperidinyl, –$NR^{Aa}R^{Ab}$, –oxyaryl–$NR^{Aa}R^{Ab}$ or –**Z**-**A′**$(R^P)_t$;

R^Z is alkyl;

X is O or NR^{Aa};

Z is S, S(O), $S(O)_2$, SO_2NR^{Aa}, O, C(O)NR^{Aa}, C(O), or NR^{Aa};

A′ is aryl, arylalkyl, or heteroaryl;

R^P is, independently in each instance, halo, optionally substituted alkyl, –OH, or -$NR^{Aa}R^{Ab}$;

R^{Aa} and R^{Ab} are, independently in each instance, –H, optionally substituted alkyl, or optionally subtitued aryl;

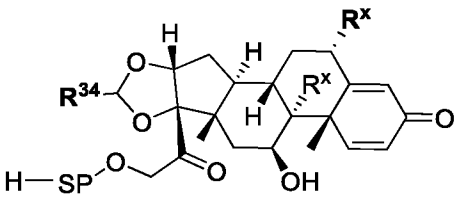
subscript **a** is an integer from 0-19; and

t is an integer from 1-3;

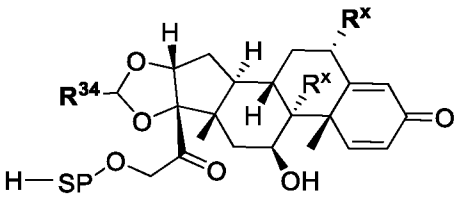
and

R^{5A} and R^{5B} are each, independently, halo or a hydrogen atom;

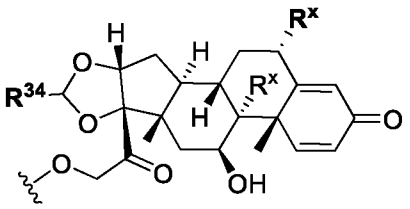
wherein the group R^3 or R^4 is bonded to the linker.

[0282] Provided herein are antibody-drug conjugates of formula **Ab-L′-SP-D or BA-L′-SP-D**, where **D** is a budesonide prodrug or a prodrug of a budesonide analog or derivative (including fluorinated analogs and derivatives), and where **Ab**, **BA**, **L′**, and **SP** are as defined in any embodiment described herein. In some embodiments, **SP** is a moiety capable of releasing **D** or  . In some embodiments, under physiological conditions the

bond between **L** and **SP** is cleaved to release a steroid prodrug, i.e. **H-SP-D** or

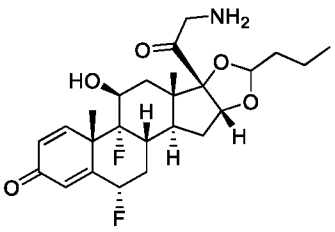


, and the bond between **SP** and **D,** or between **SP** and

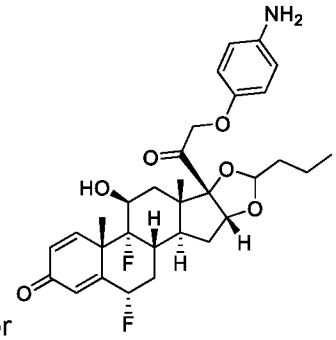


, is subsequently cleaved to release a biologically active steroid.

[0283] In some instances, ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**III**), or (**3000**), are ADCs described in Table 1 and/or Table 2 and the Examples section. In some embodiments, the steroid payload in any antibody drug conjugate described herein is

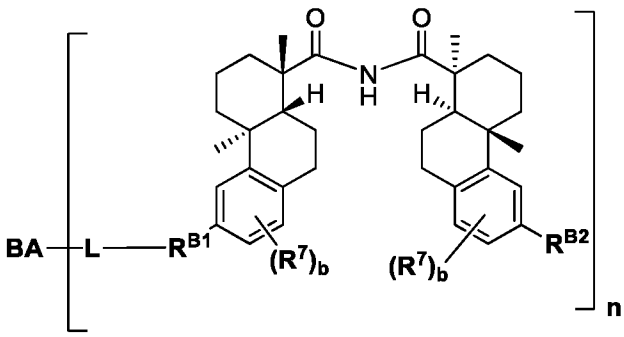


, or

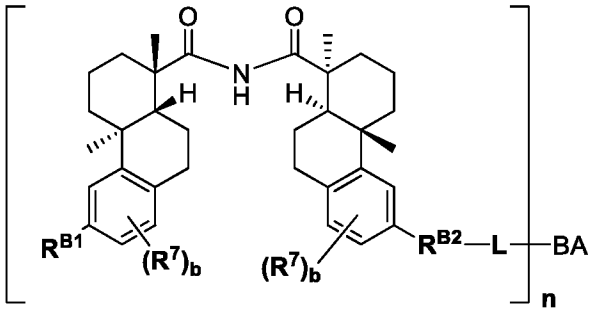


, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof.

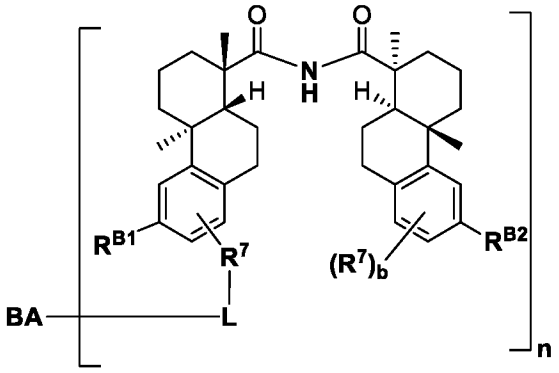
[0284] Provided herein are antibody-drug conjugates of any LXR-modulator compounds described herein of Formula (**5001**), (**5002**), (**5003**), or (**5004**):



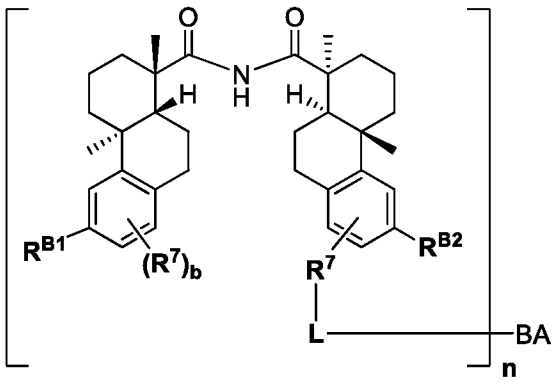
Formula (**5001**)



Formula (**5002**)



Formula (**5003**) or



Formula (**5004**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

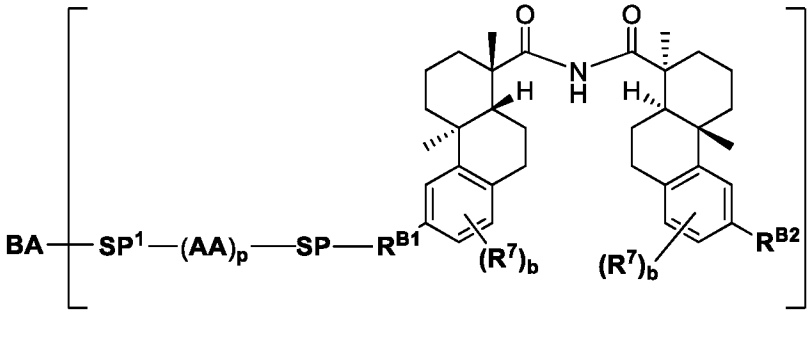
wherein **L** is any linker described herein in some or any embodiments;

n is an integer from 1 to 30;

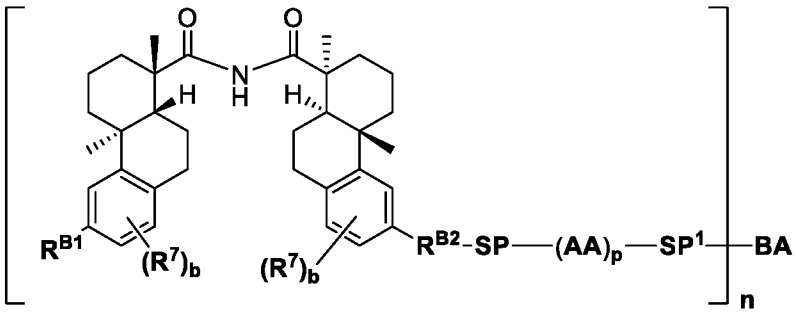
BA is a binding agent or a PEG-modified binding agent; and

R^{B1}, R^{B2}, R^7 and **b** are as defined herein in some or any embodiments.

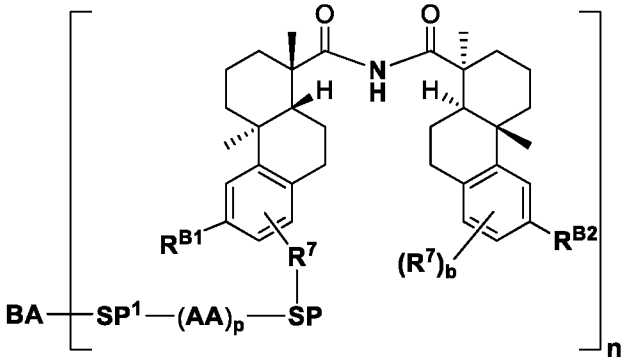
[0285] Provided herein are antibody-drug conjugates of any LXR-modulator compounds described herein of Formula (**6001**), (**6002**), (**6003**), or (**6004**):



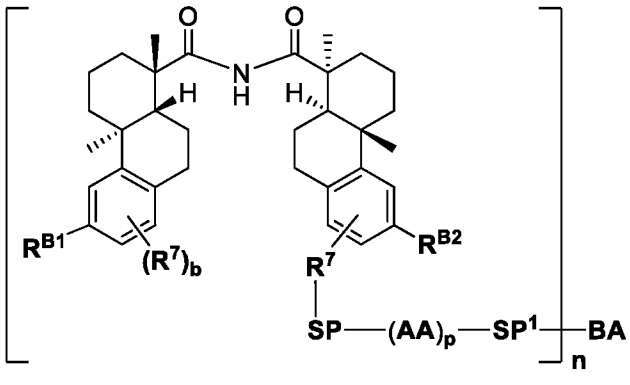
Formula (**6001**)



Formula (**6002**)



Formula (**6003**) or



Formula (**6004**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

wherein **SP**^{1} and **SP**, when present, are spacer groups as defined herein in some and any embodiments;

each **AA** is an amino acid residue;

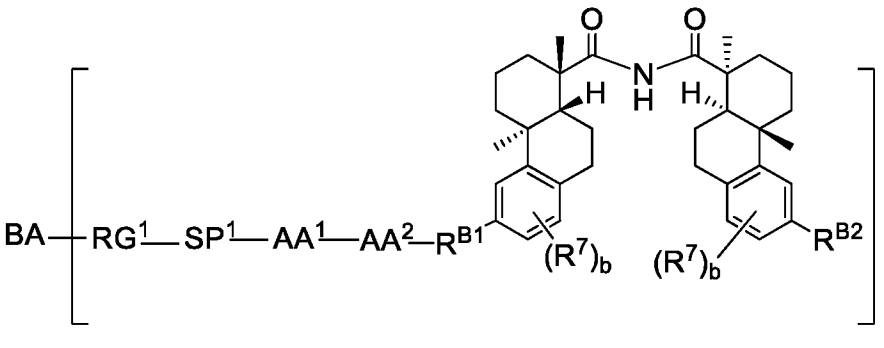
p is an integer from 1 to 10;

n is an integer from 1 to 30;

BA is a binding agent or a PEG-modified binding agent; and

R^{B1}, R^{B2}, R^7 and **b** are as defined herein in some or any embodiments.

[0286] Provided herein are antibody-drug conjugates of any LXR-modulator compounds described herein of Formula (**6005**):

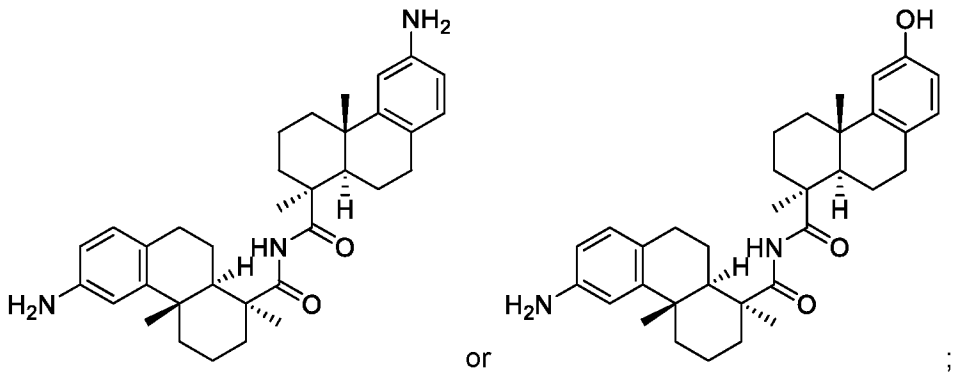


Formula (**6005**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

wherein R^{B1}, R^{B2}, R^7, **b**, **BA**, RG^1, SP^1, AA^1 and AA^2 are as defined herein in some or any embodiments.

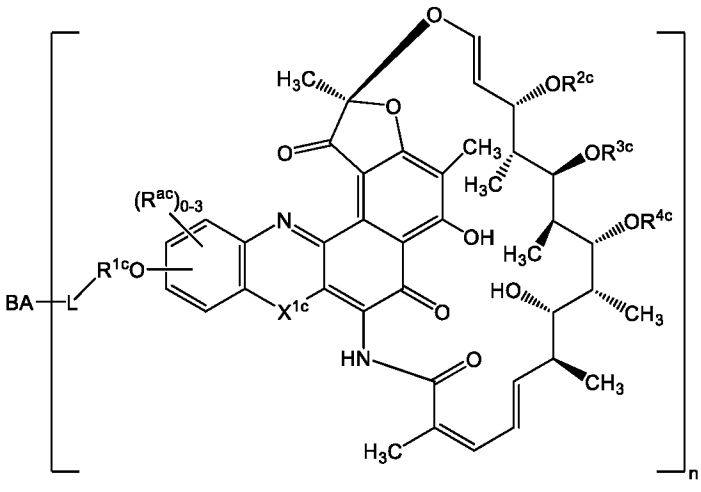
[0287] In some instances, ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), or (**6005**), are ADCs described in Table 1 and/or Table 2 and the Examples section. In some embodiments, the LXR modulator payload in any antibody drug conjugate described herein is



or ;

or a pharmaceutically acceptable salt or solvate thereof.

[0288] Provided herein are antibody-drug conjugates of any rifamycin analogs described herein and having the structure of Formula (**7001**)



Formula (**7001**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

wherein:

X^{1c} is selected from –S-; -O- and –NR^{5c};

R^{1c} is amino-C_{1-6}alkyl; C_{1-6}alkylaminoC_{1-6}alkyl; di-C_{1-6}alkylaminoC_{1-6}alkyl; hydroxy-C_{1-6}alkyl; HS-C_{1-6}alkyl; $(R^{5c})_2N$-C_{1-6}alkylene-$N(R^{5c})$-C_{1-6}alkyl; $(R^{5c})_2N$-C_{1-6}alkylene-O-C_{1-6}alkyl; $(R^{5c})_2N$-C_{1-6}alkylene-S-C_{1-6}alkyl; heterocycloalkyl or heterocycloalkyl-C_{1-6}alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, C_{1-6}alkyl, -OH, or =O;

R^{2c}, **R^{3c}**, and **R^{4c}** are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-**R^{5c}**;

each **R^{ac}**, when present, is independently selected from -F; -Cl; -Br; -I; -OH; $-NH_2$; and C_{1-6}alkoxy; and

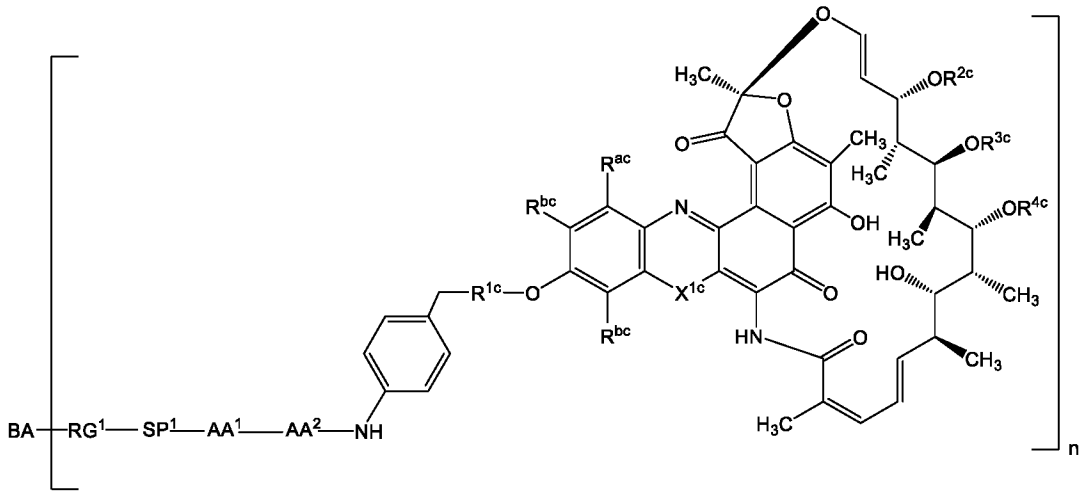
R^{5c} is independently, at each occurrence, selected from hydrogen; and C_{1-6}alkyl;

L is a linker;

BA is a binding agent; and

subscript **n** is an integer from 1 to 30.

[0289] Provided herein are antibody-drug conjugates of any rifamycin analogs described herein and having the structure of Formula (**7002**):

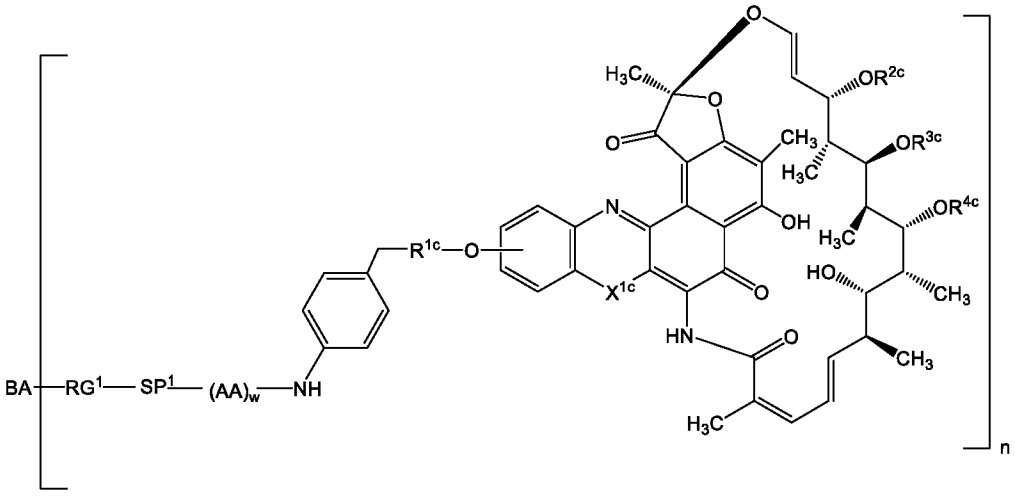


Formula (**7002**);

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

wherein **BA**, **RG**^{1}, **SP**^{1}, **AA**^{1}, **AA**^{2}, **R**^{1c}, **R**^{bc}, **R**^{ac}, **X**^{1c}, **R**^{2c}, **R**^{3c}, **R**^{4c} and **n** are as defined herein in some or any embodiments.

[0290] Provided herein are antibody-rifamycin analog conjugates having the structure of Formula (**7003**):

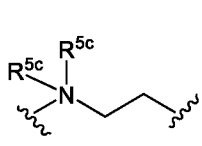
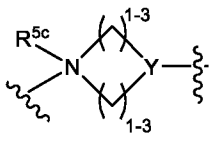
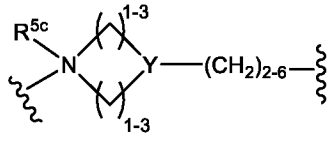


Formula (**7003**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, or regioisomer thereof, or mixtures thereof;

wherein:

X^{1c} is selected from –S-; -O- and c;

R^{1c} is  ,  ; or  ; wherein **Y** is C or N;

R^{2c}, **R**^{3c}, and **R**^{4c} are independently selected from hydrogen, $C_{1\text{-}6}$alkyl, and -(C=O)-**R**^{5c};

R^{5c} is independently, at each occurrence, absent, or selected from hydrogen; and C_{1-6}alkyl;

each **AA** is an independently selected amino acid;

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

BA is an anti-MSR1 antibody or antigen binding fragment thereof;

subscript **n** is an integer from 1 to 30;

subscript **w** is 2, 3, or 4;

wherein R^{1c} is bonded to the linker via a nitrogen atom as indicated by the wavy line .

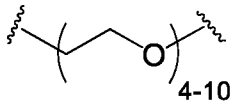
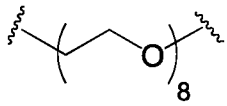
[0291] In various embodiments of Formula (**7003**), SP^1, RG^1 and **AA** are as defined herein in some and/or any particular embodiments.

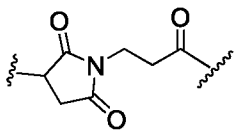
[0292] In some embodiments of Formula (**7003**), X^{1c} is O. In some embodiments of Formula (**7003**), X^{1c} is S. In some embodiments of Formula (**7003**), X^{1c} is In some embodiments of Formula (**7003**), X^{1c} is NR^{5c}.

[0293] In some embodiments of Formula (**7003**), **BA** is an anti-MSR1 antibody or antigen binding fragment thereof; comprising an N297Q mutation.

[0294] In some embodiments of Formula (**7003**), subscript **w** is 2.

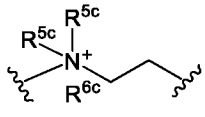
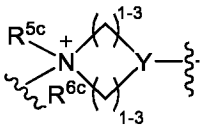
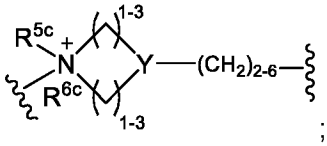
[0295] In some embodiments of Formula (**7003**), $(AA)_2$ is valine-citrulline.

[0296] In some embodiments of Formula (**7003**), SP^1 comprises  . In some embodiments of Formula (**7003**), SP^1 comprises  .

[0297] In some embodiments of Formula (**7003**), RG^1 comprises  .

[0298] In some embodiments of Formula (**7003**),

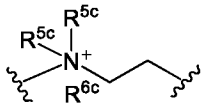
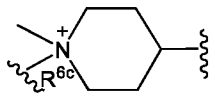
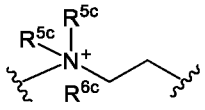
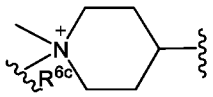
X^{1c} is O;

R^{1c} is  ,  ; or  ; wherein **Y** is C or N;

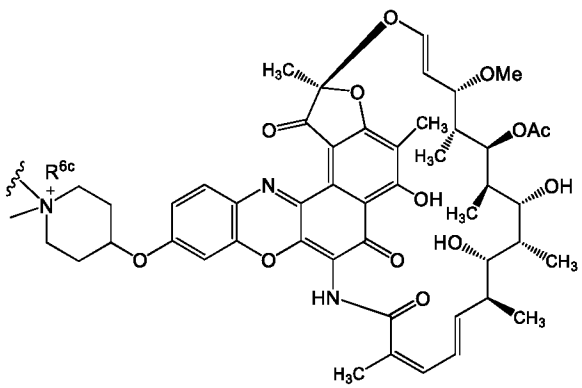
wherein R^{1c} is bonded to the linker via the quarternary nitrogen atom of R^{1c};

R^{5c} is C_{1-6}alkyl; and

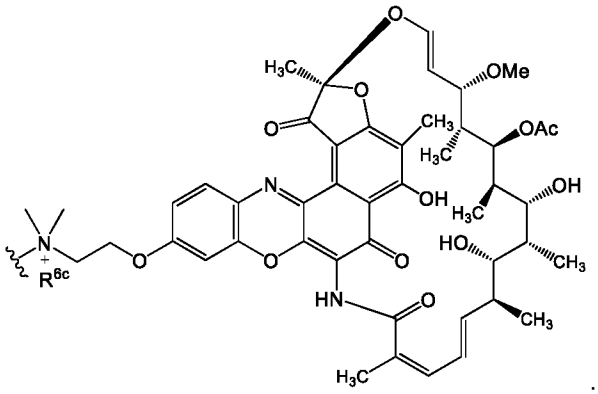
R^{6c} is a counter ion.

[0299] In some of such embodiments, R^{1c} is  , or  . In some of such embodiments, R^{1c} is  . In some of such embodiments, R^{1c} is  . In some of such embodiments, R^{6c} is I^-, Cl^- or Br^-, and in some instances, R^{6c} is I^-.

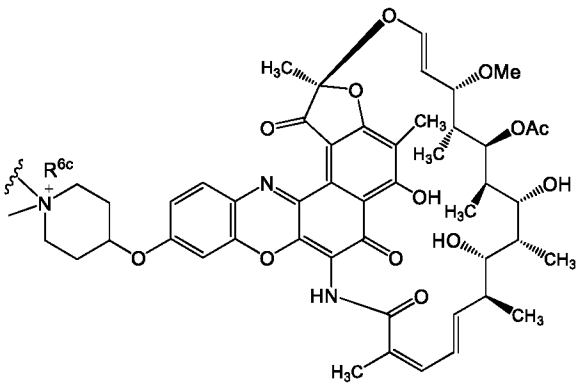
[0300] In some embodiments of Formula (**7003**), the rifamycin analog is:



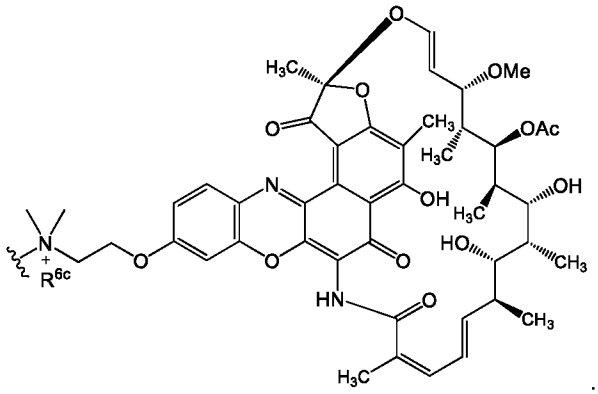
or

 .

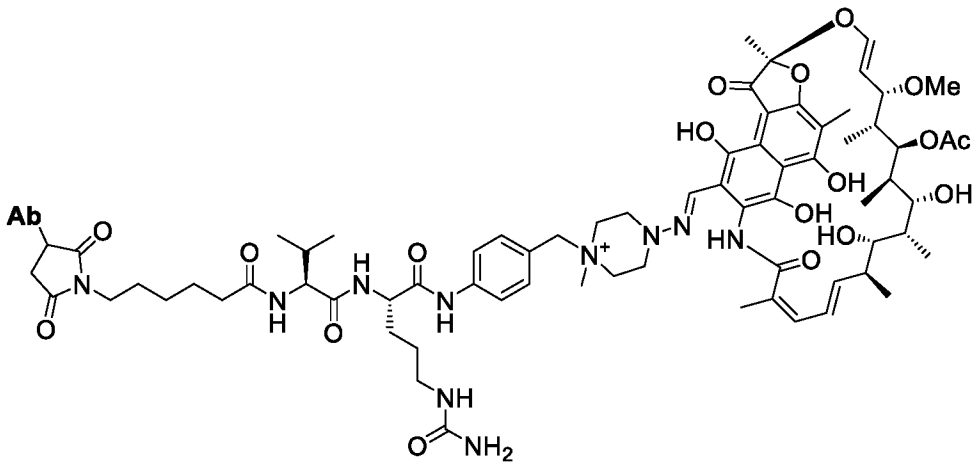
In some embodiments of Formula (**7003**), the rifamycin analog is:



[0301] . In some embodiments of Formula (**7003**), the rifamycin analog is:

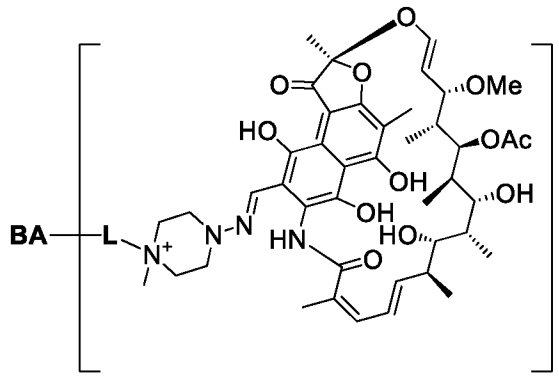


[0302] Further provided herein is an antibody-drug conjugate comprising a rifamycin compound (e.g., rifampicin), according to Formula (**7004**):



Formula (**7004**).

[0303] Further provided herein is an antibody-drug conjugate comprising a rifamycin compound (e.g., rifampicin), according to Formula (**7005**):



Formula (**7005**)

wherein **L** and **BA** are as defined herein in some or any embodiments.

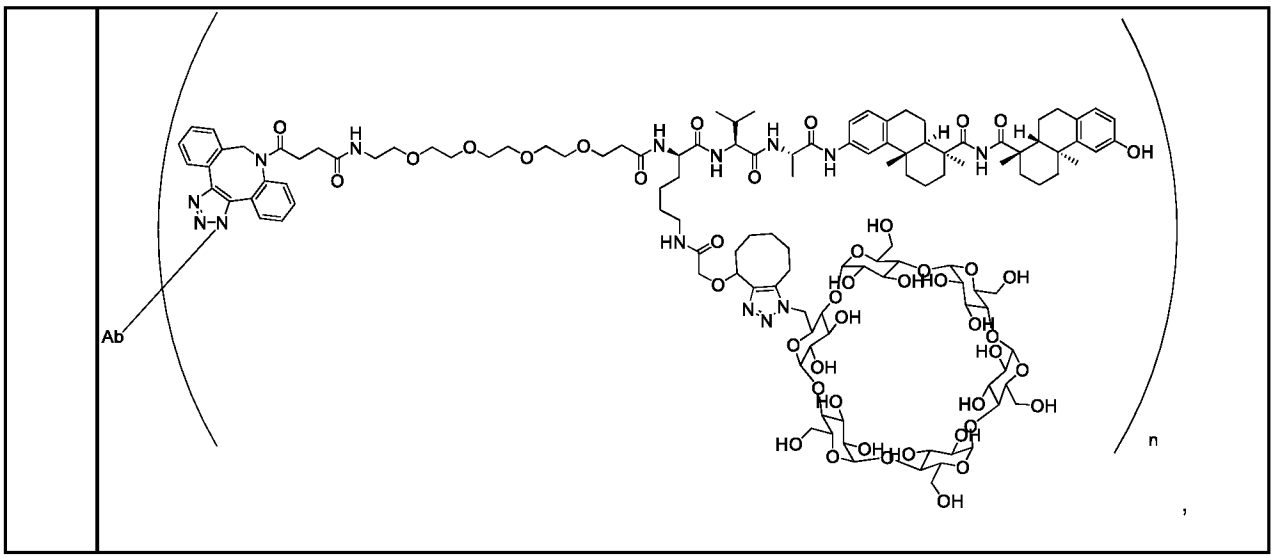
[0304] In some instances, ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**7001**) (**7002**), (**7003**), (**7004**), and/or (**7005**) are ADCs described in Table 2 and/or the Examples section.

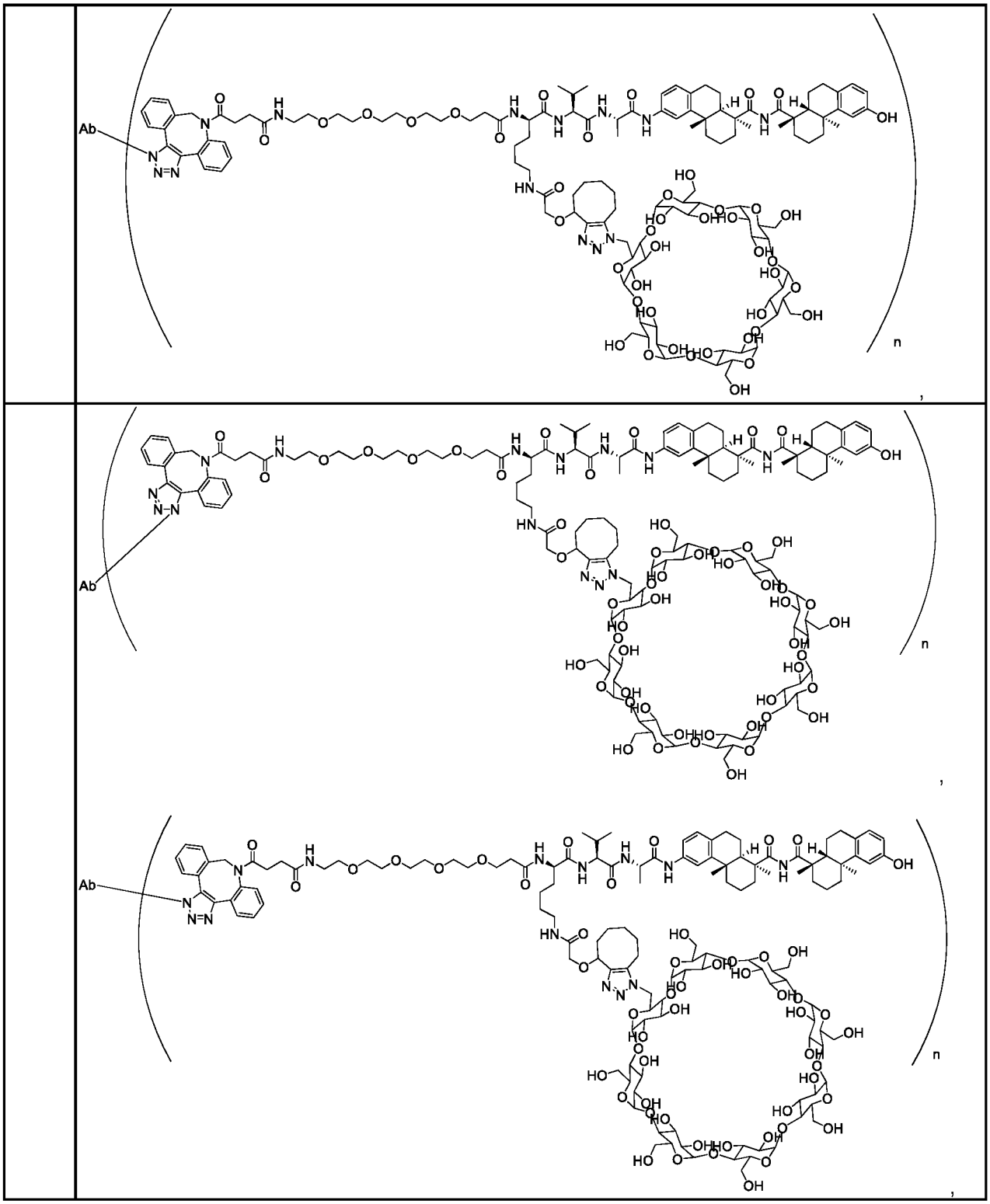
[0305] Also included in these examples of ADCs, is a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, a regioisomer thereof, or mixture of regioisomers thereof, wherein each is a bond to the binding agent; and each is a bond to the payload.

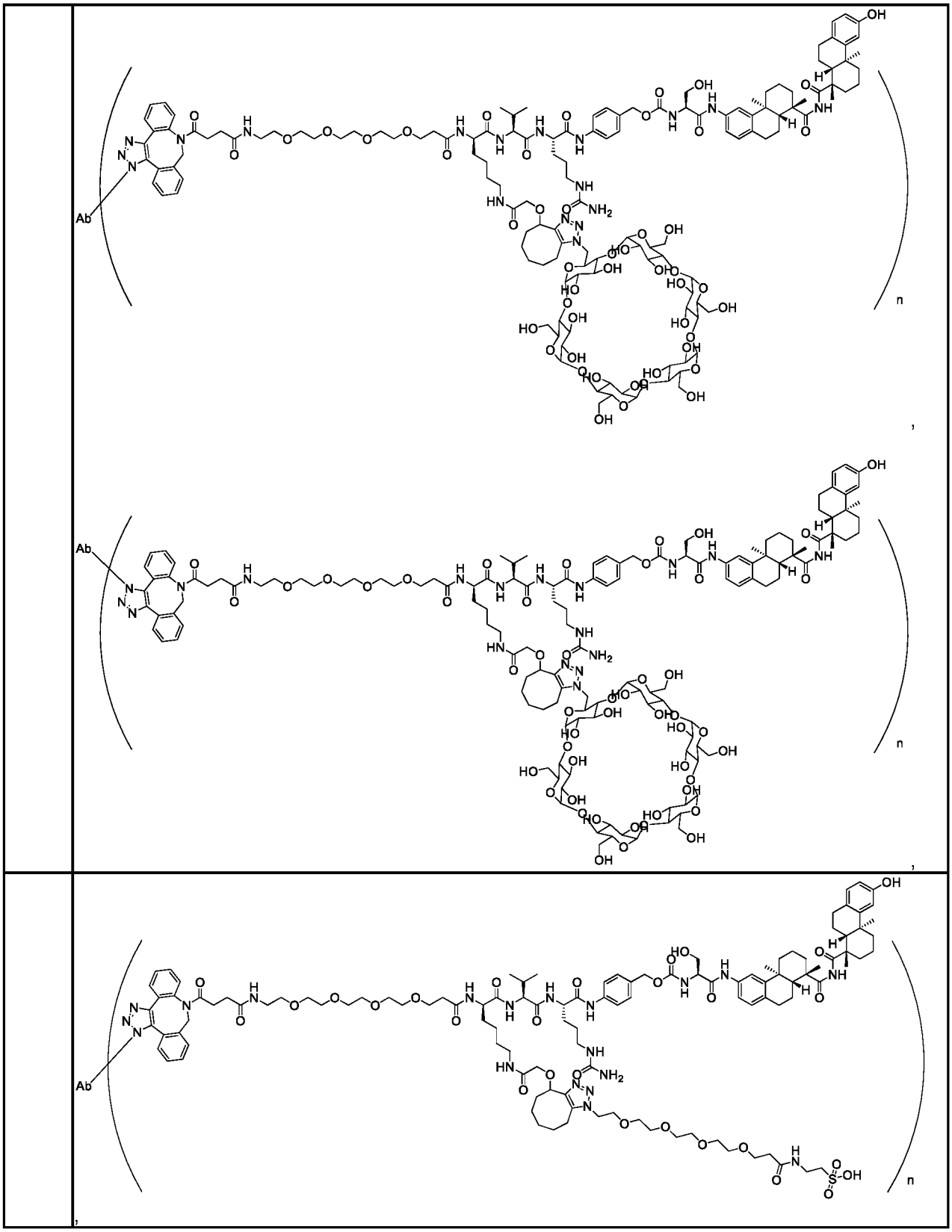
[0306] In certain embodiments, provided herein is an ADC comprising an anti-MSR1 antibody or an antigen binding fragment thereof, or a PEG-modified anti-MSR1 antibody or an antigen binding fragment thereof, disclosed herein and a linker-payload (**LP**) selected from the group consisting of the linker-payloads in Table 3, or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof.

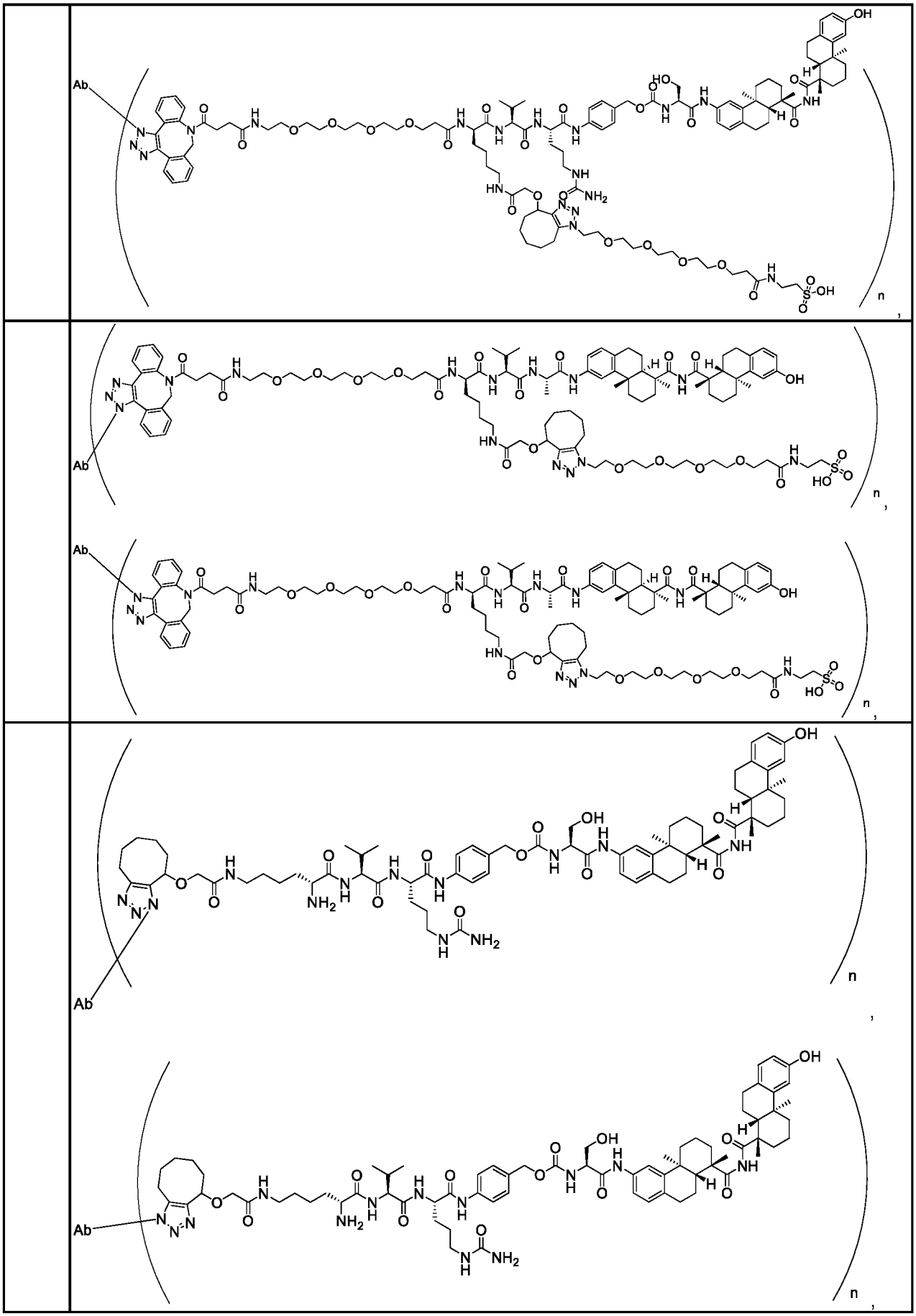
[0307] Also provided herein are antibody-radionuclide conjugates (ARCs) comprising Anti-MSR1 antibodies conjugated to one or more radionuclides. Exemplary radionuclides that can be used in the context of this aspect of the disclosure include, but are not limited to, *e.g.*, ^{225}Ac, ^{212}Bi, ^{213}Bi, ^{131}I, ^{186}Re, ^{227}Th, ^{222}Rn, ^{223}Ra, ^{224}Ra, and ^{90}Y.

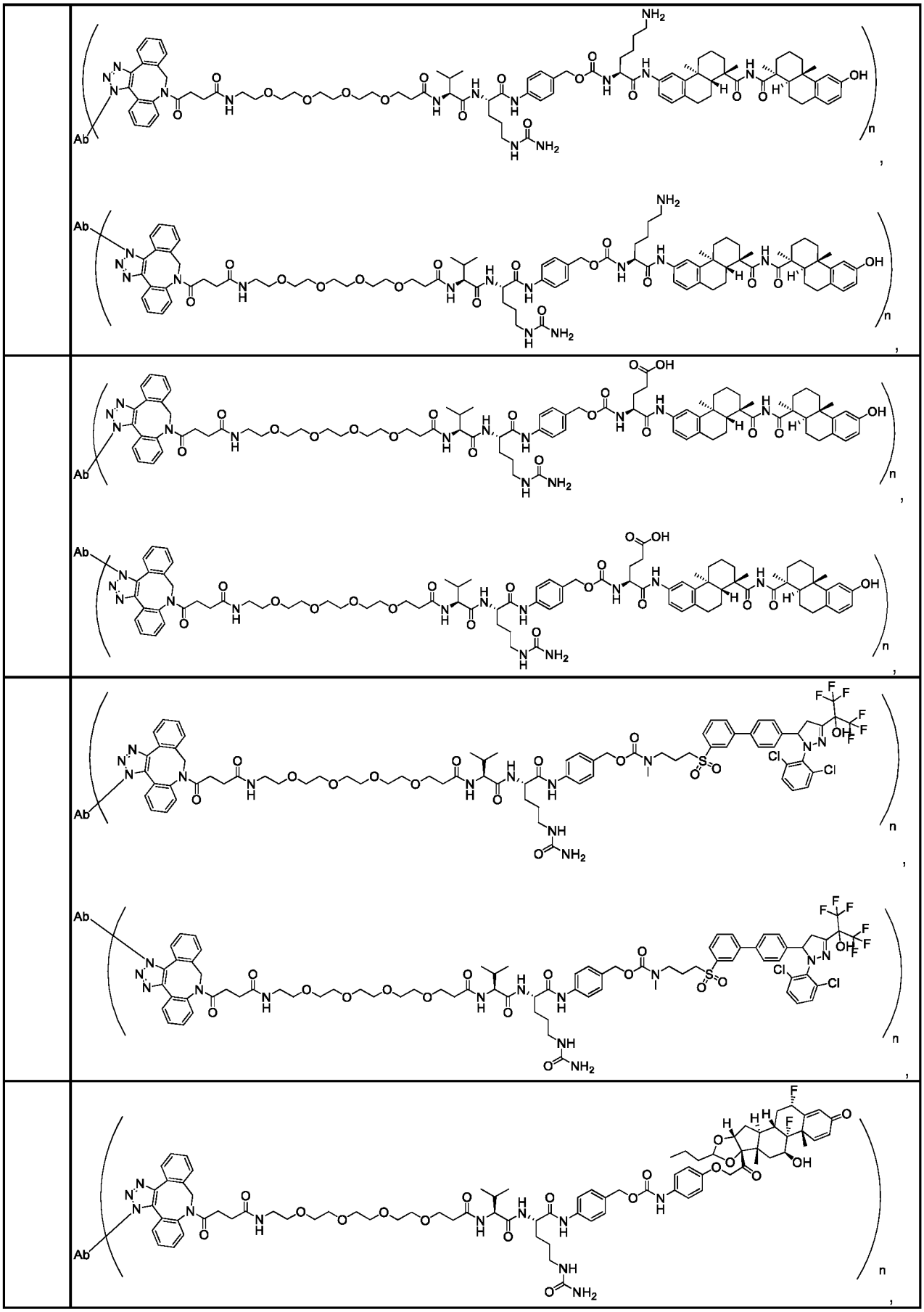
Table 1: List of ADCs and their structures

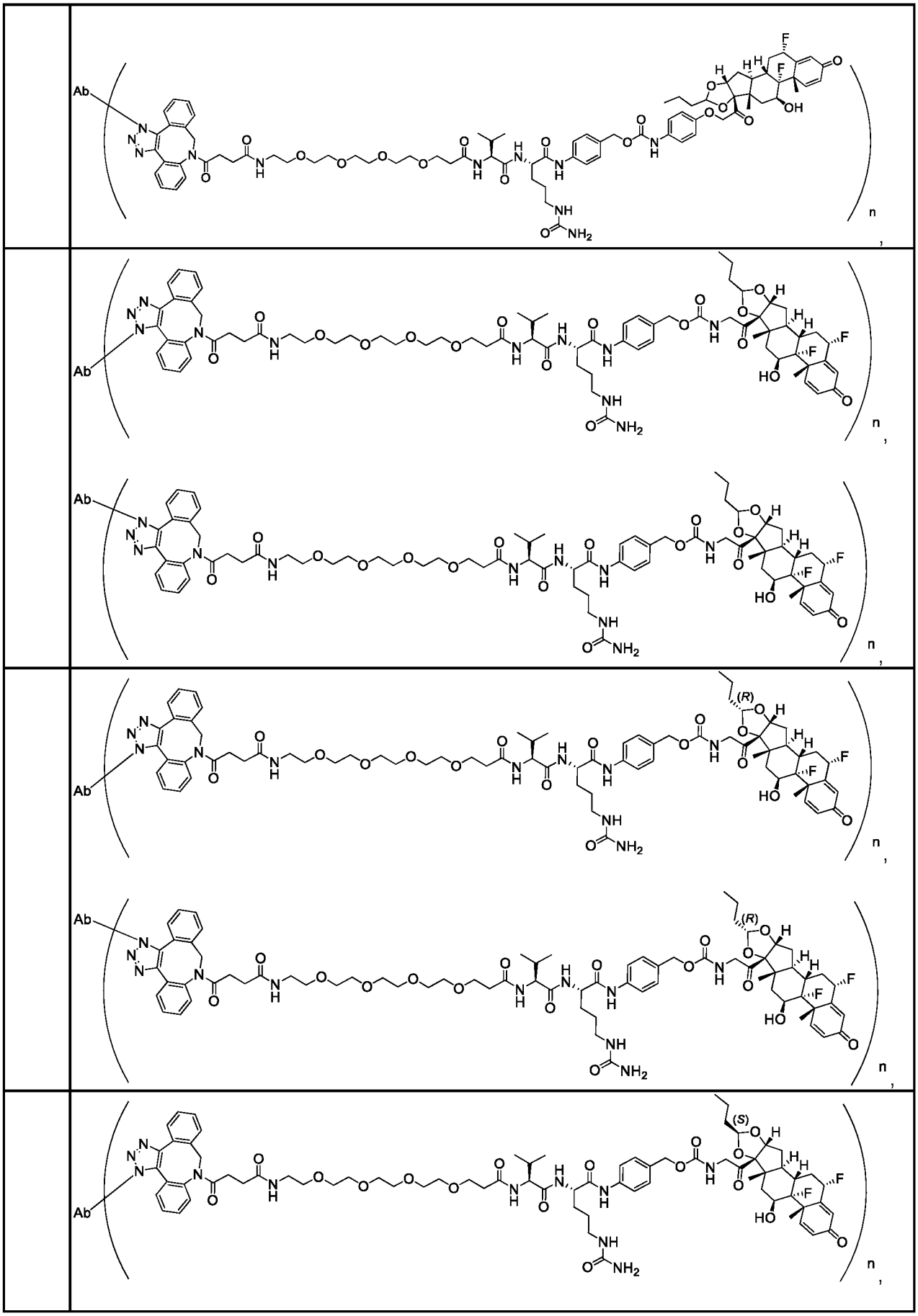


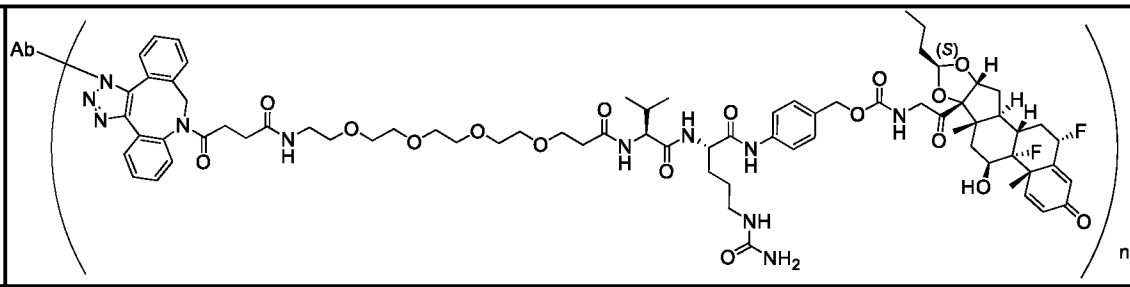


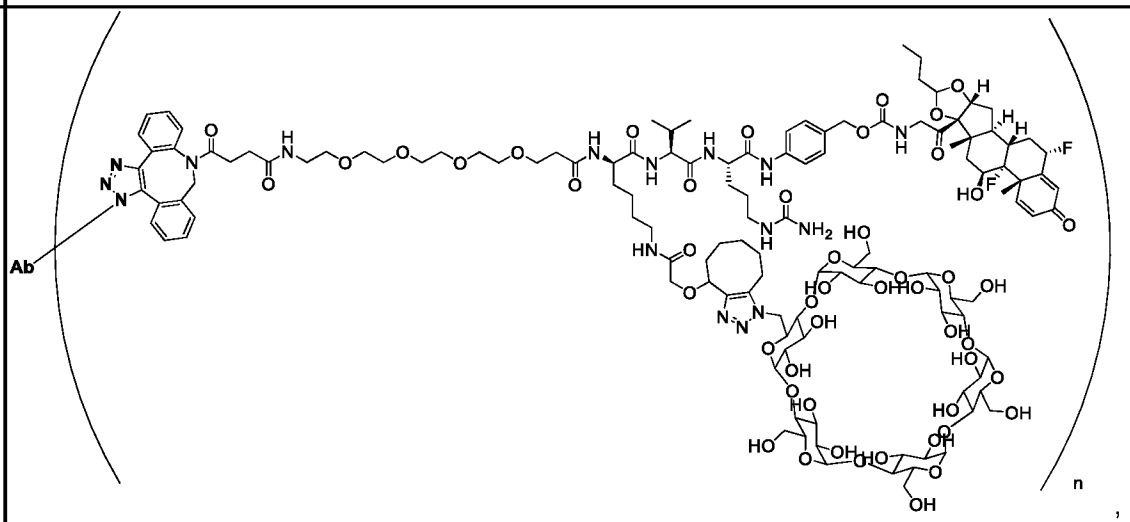


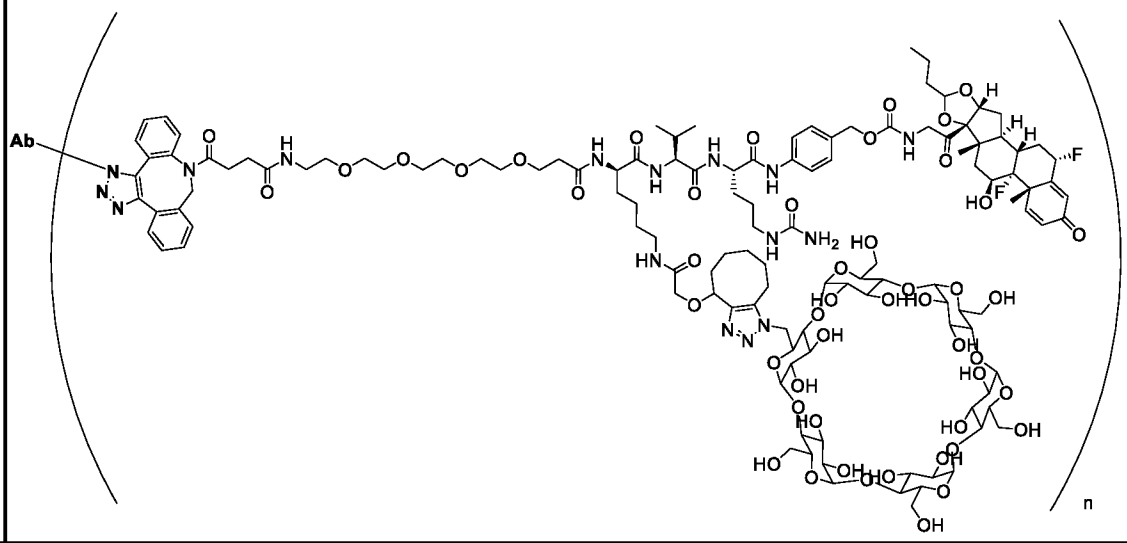


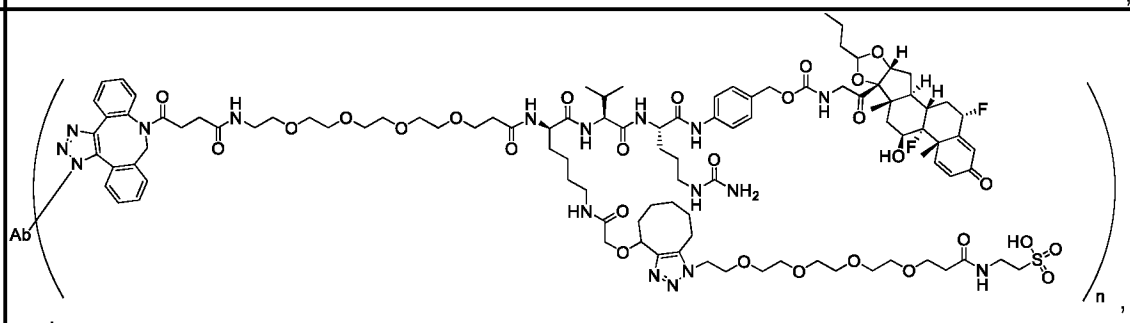




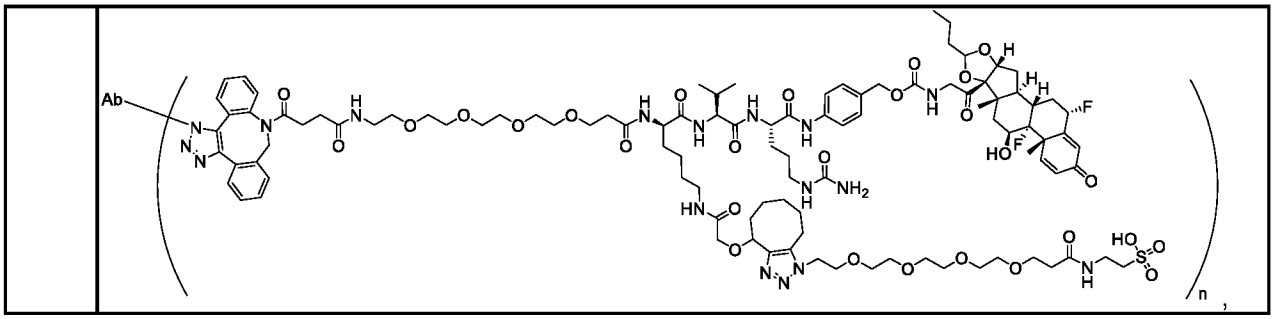


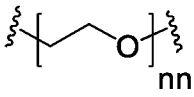
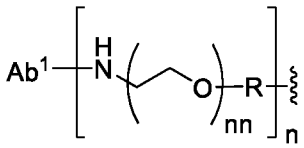






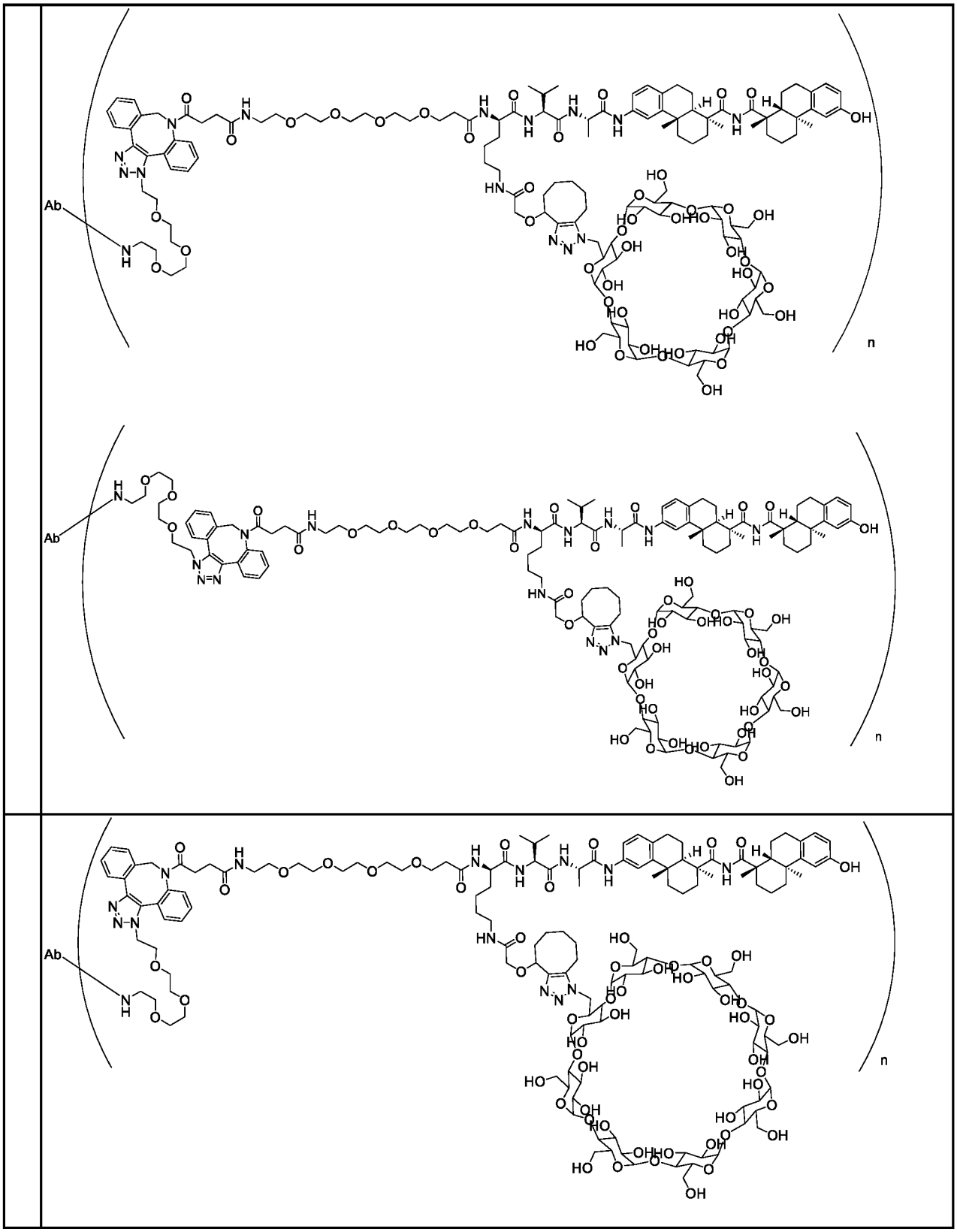
and

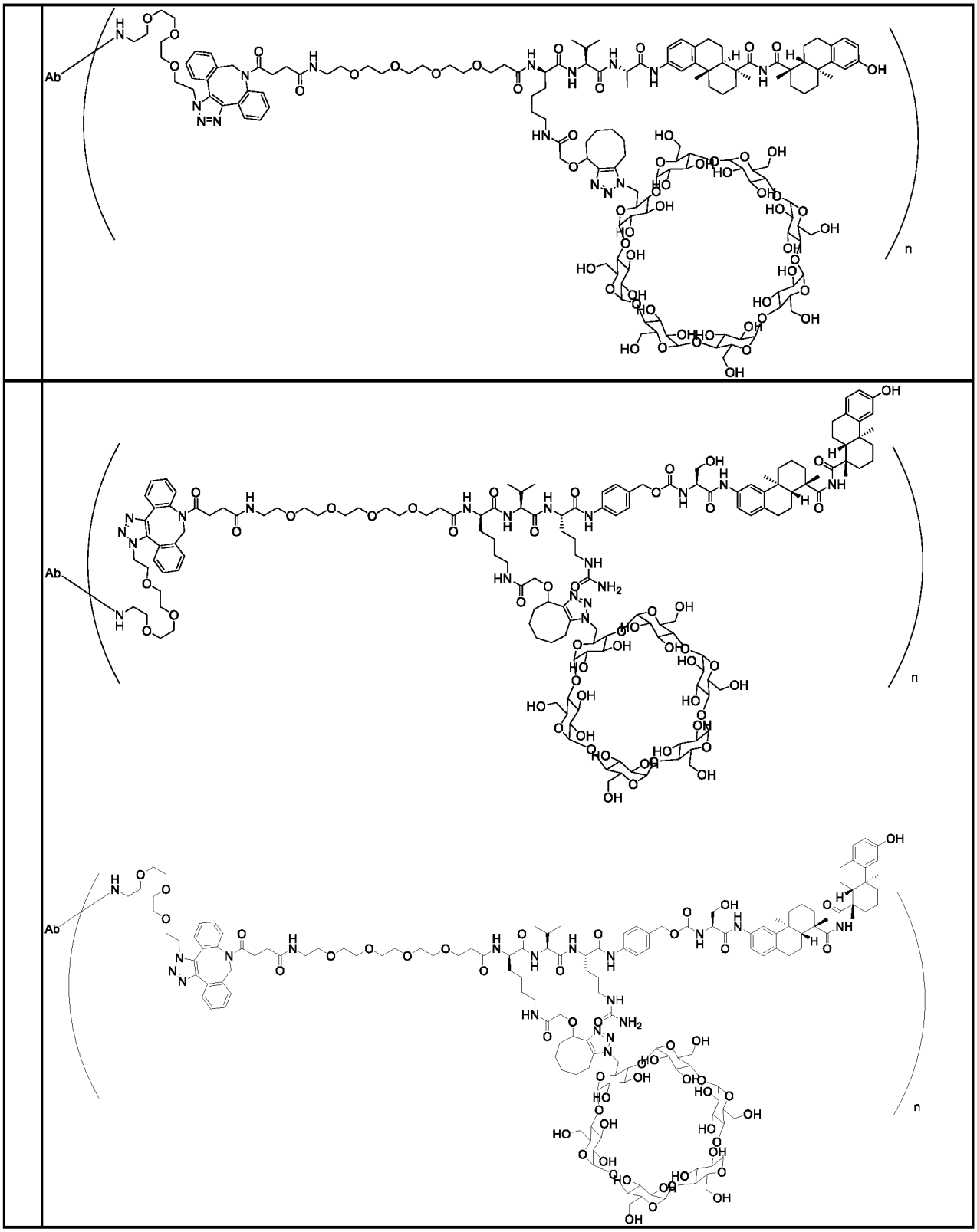


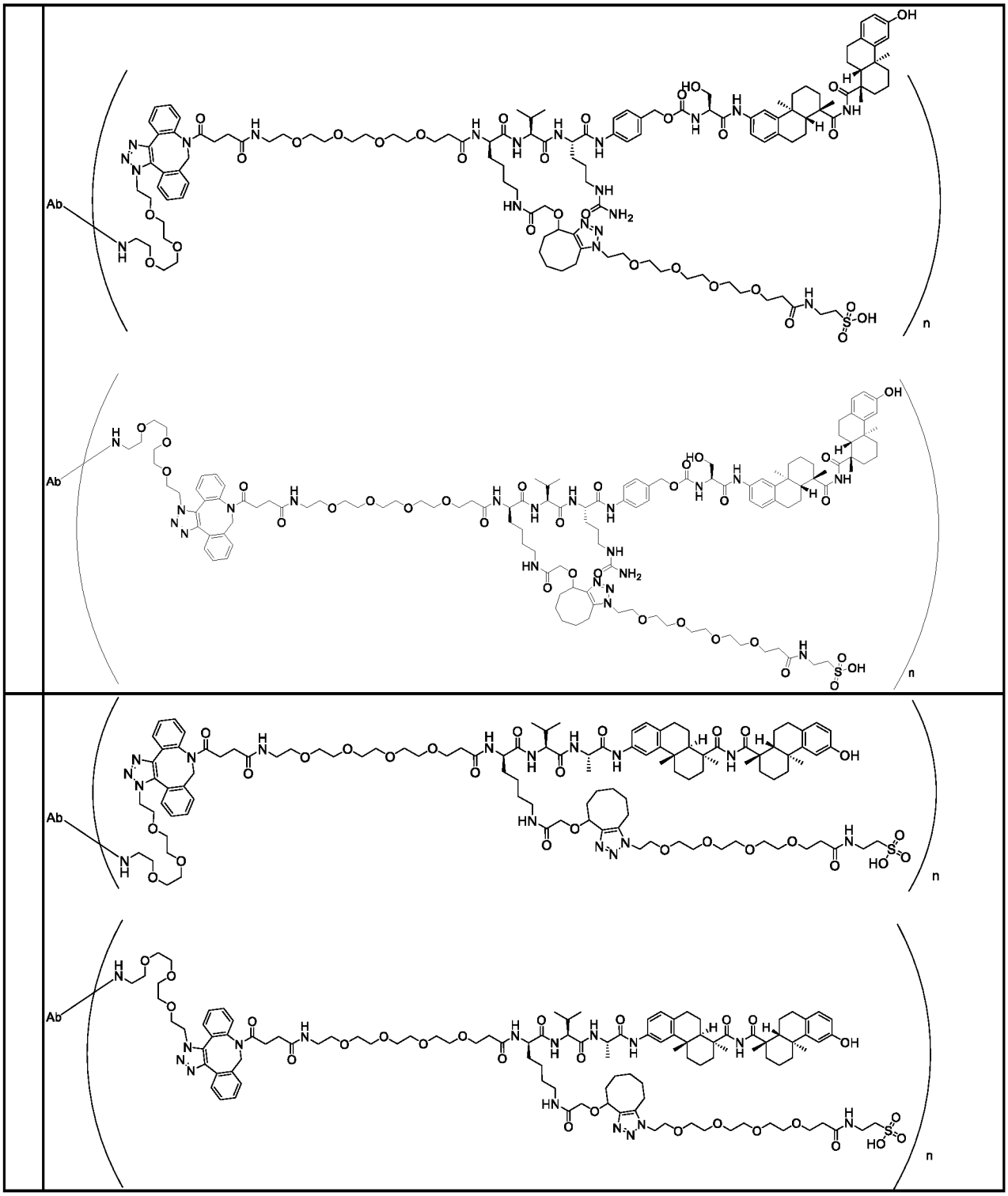
[0308] In the ADCs of Table 1, **Ab** is an anti-MSR1 antibody provided herein, or an antigen-binding fragment thereof. In particular embodiments, **Ab** is modified with a PEG group  on a glutamine side chain described herein (referred to herein as a PEG-modified antibody). In certain embodiments, the PEG group terminates with an azido group, facilitating reaction with linker-payloads described here (e.g., linker payloads described in Table 3 and in the examples). In certain embodiments, the PEG group is linked to a linker-payload through a triazole or triazole derivative as described herein. In the ADCs, **n** is an integer from 1 to 10, for instance, 1, 2, 3, or 4. In the PEG group, **nn** is an integer from 1-10, for instance, 1, 2, 3, 4, or 5. In other embodiments, each **BA** in the ADCs in Table 1 is  where Ab^1 is an anti-MSR1 antibody, or an antigen-binding fragnment thereof; **R** is C_{2-4}-alkylene; and **nn** is an integer selected from 2 to 4, inclusive, and each **n** is an integer from 1 to 10, for instance, 1, 2, 3, or 4.

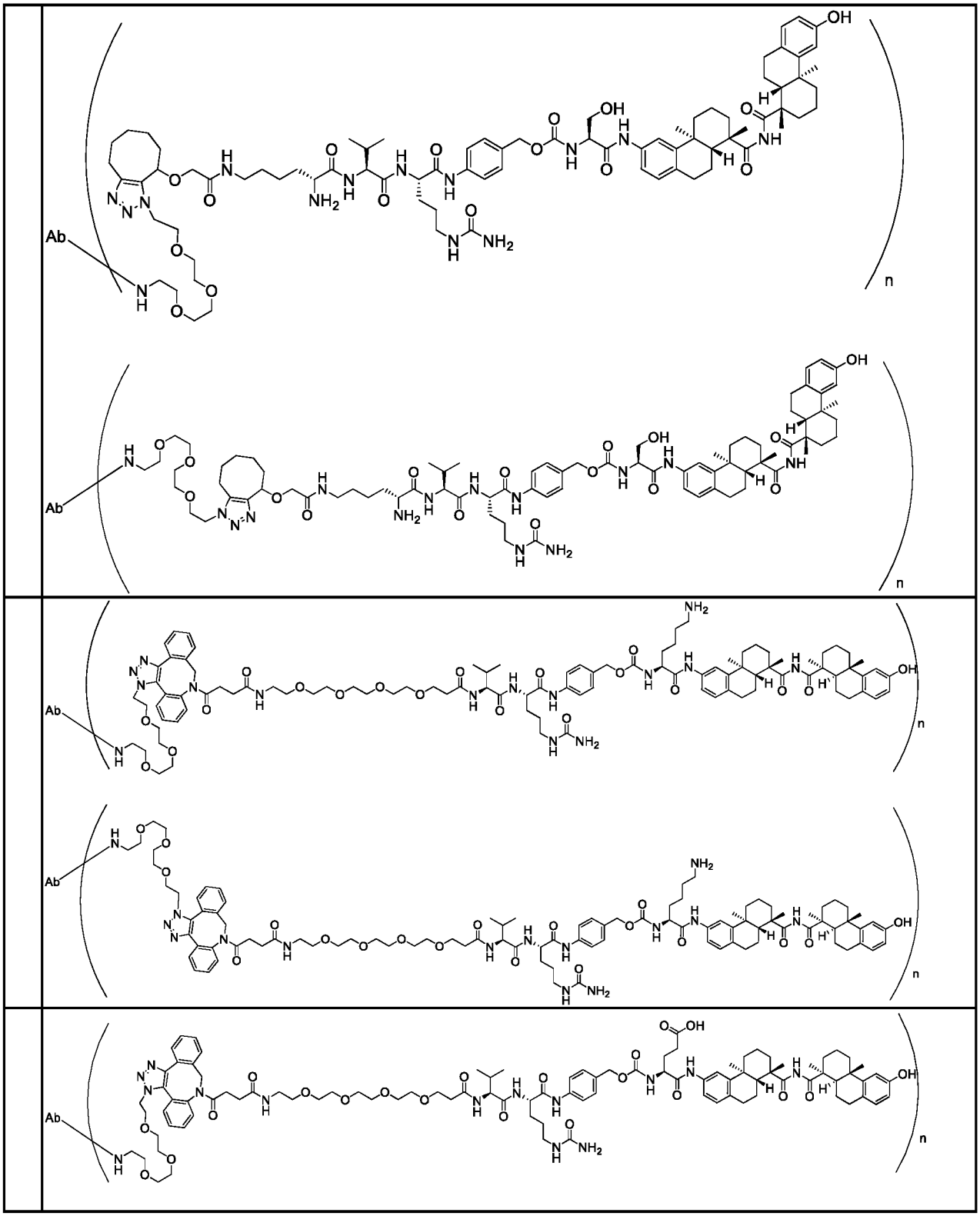
[0309] In certain embodiments, provided herein is an ADC comprising an antibody disclosed herein and a linker-payload (**LP**) selected from the group consisting of the linker-payloads in Table 2, or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof.

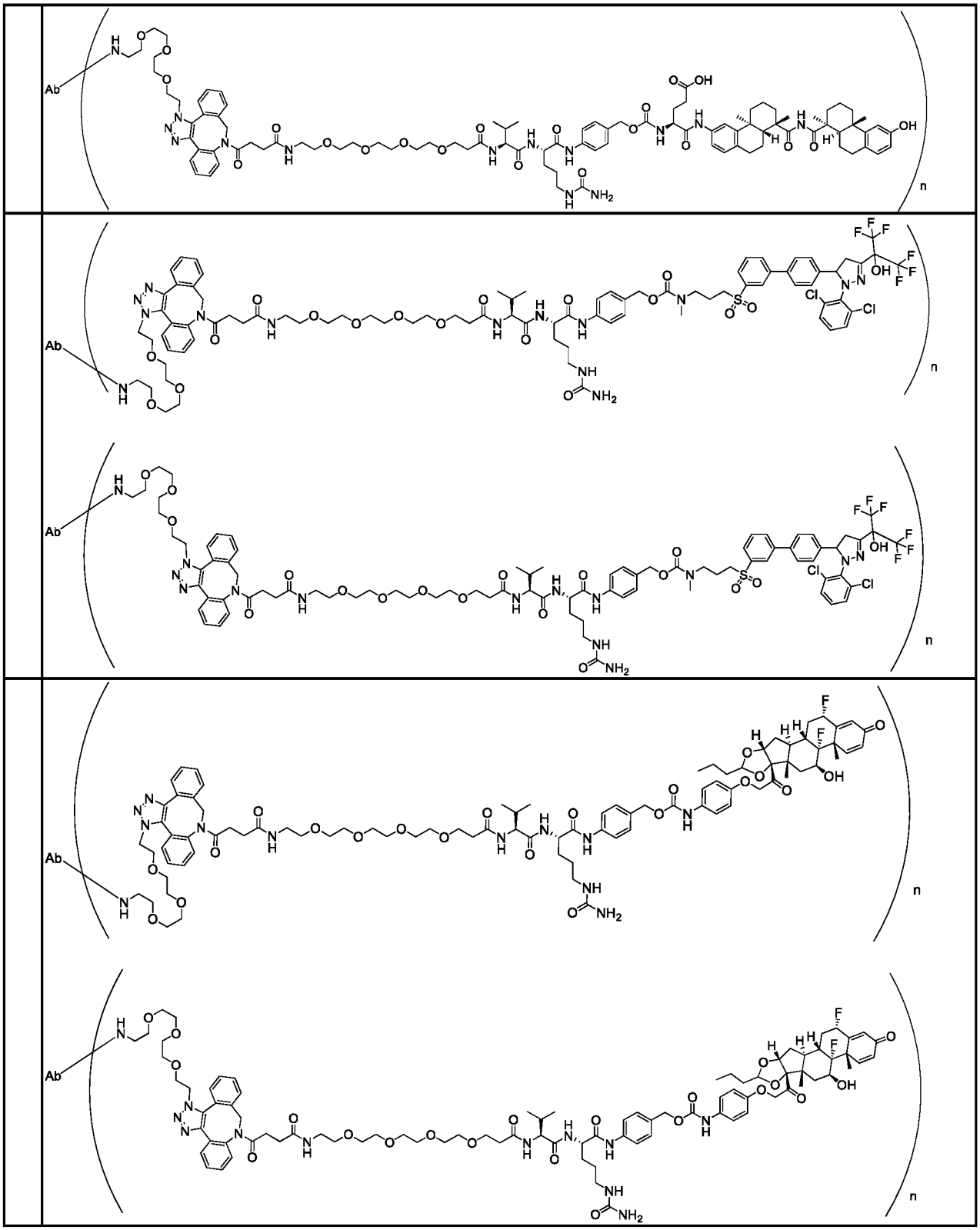
Table 2: List of ADCs and their structures

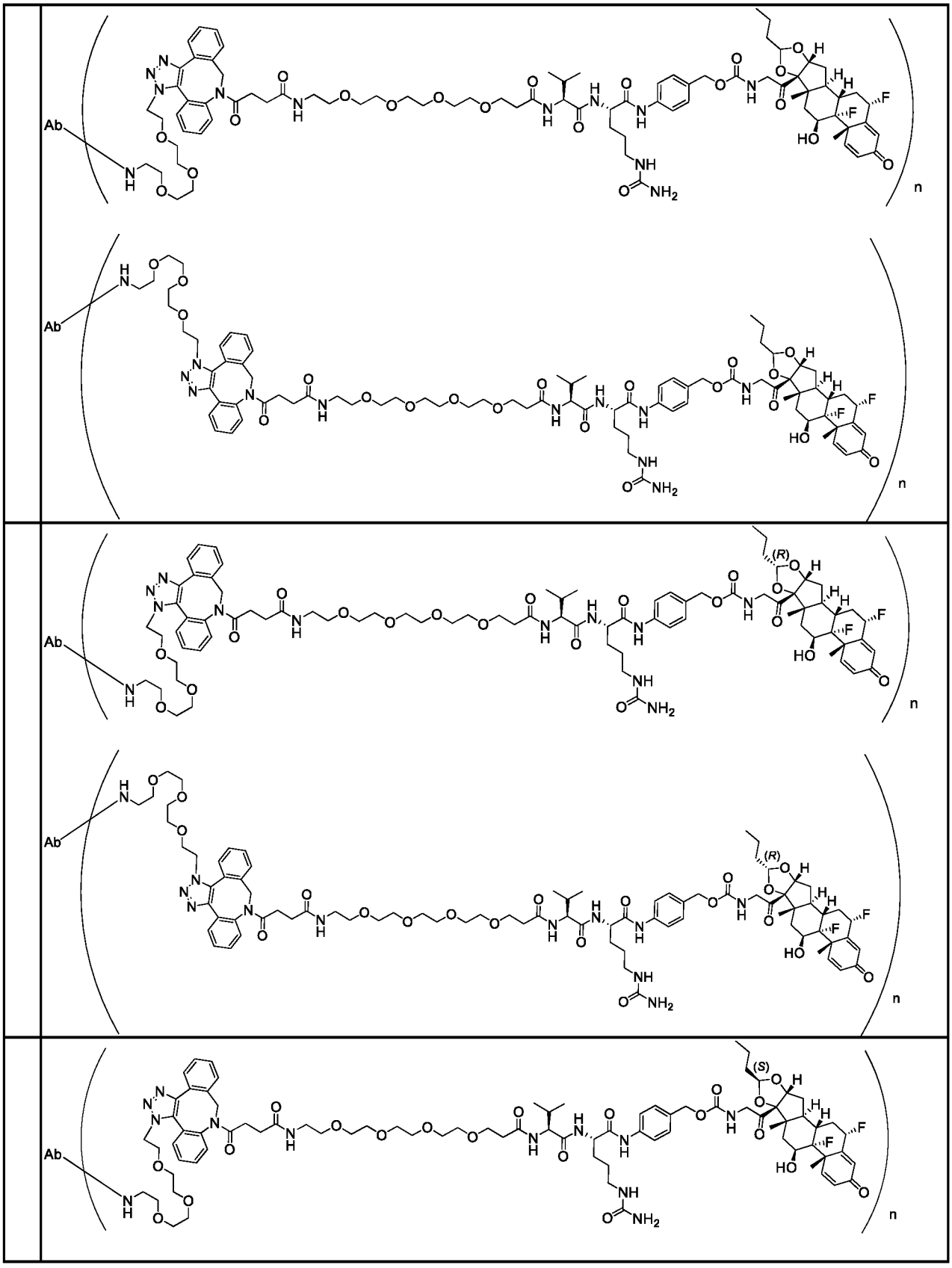


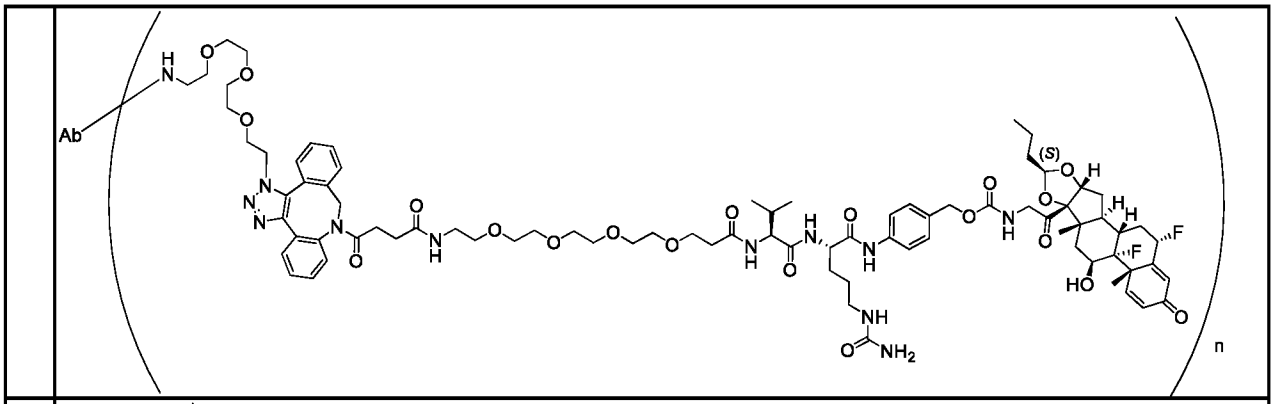

Ab
n
Ab
n
Ab
n

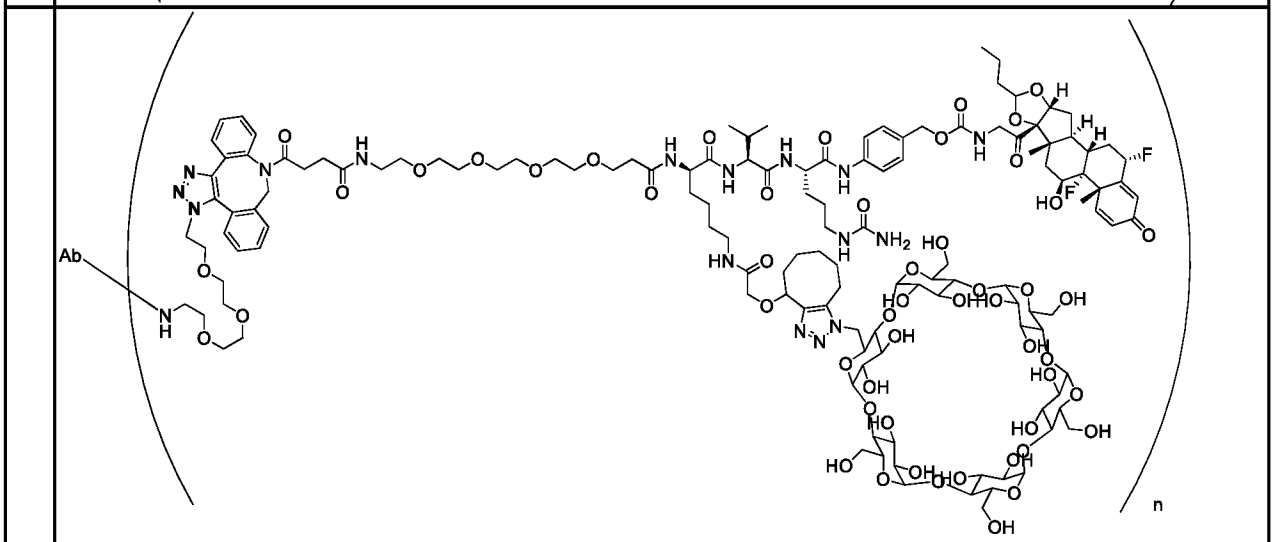


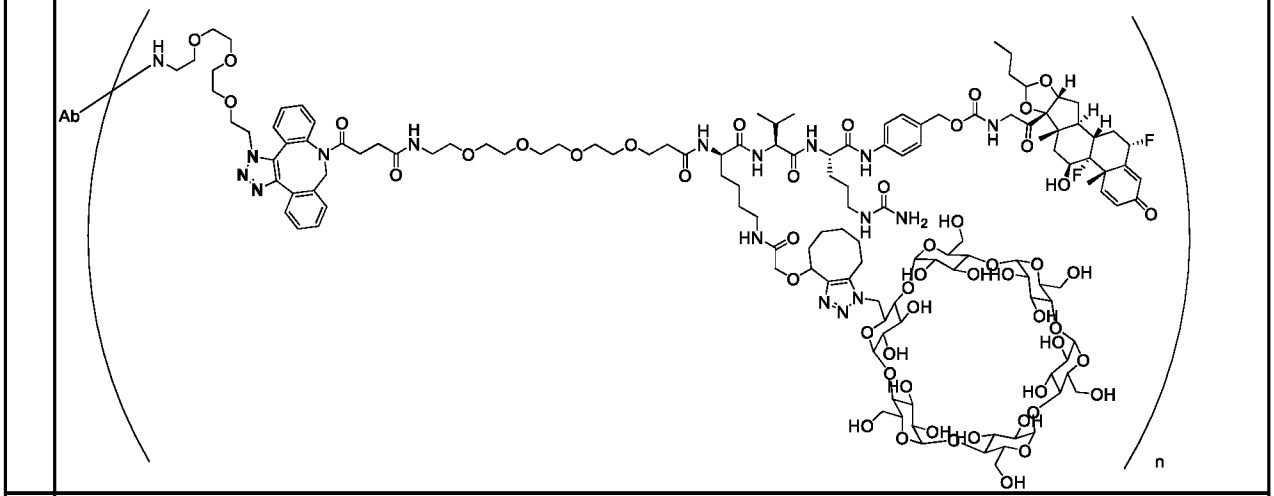


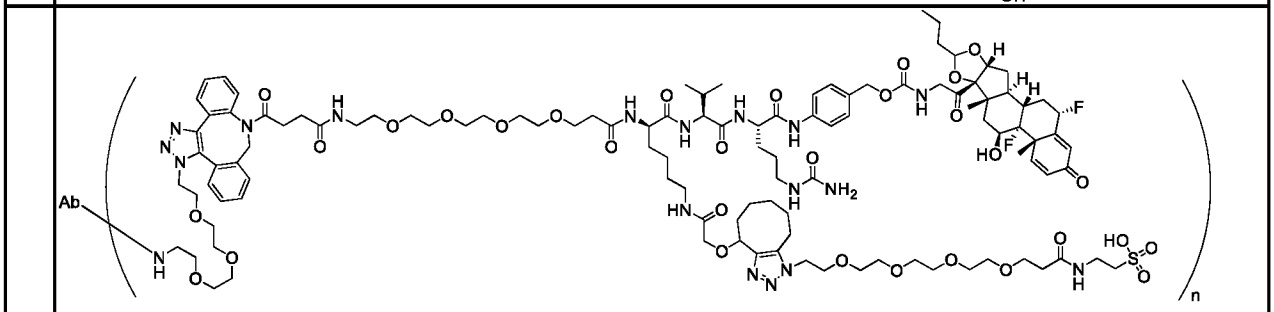




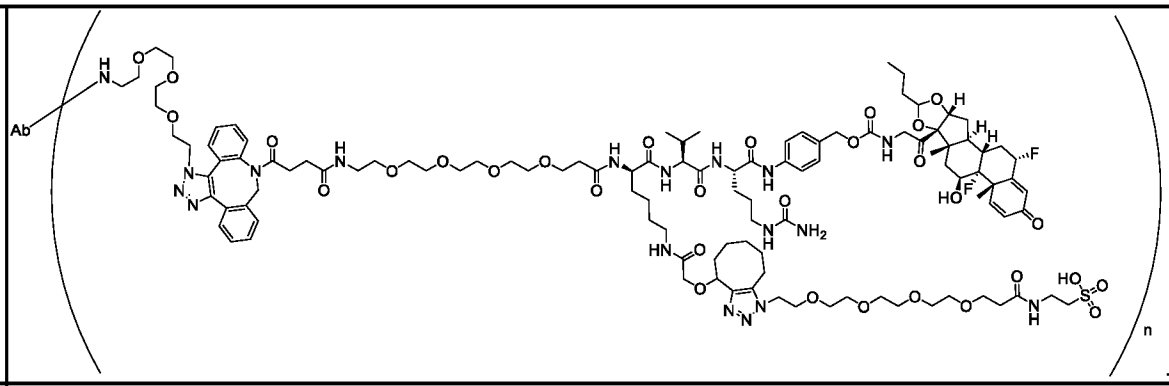


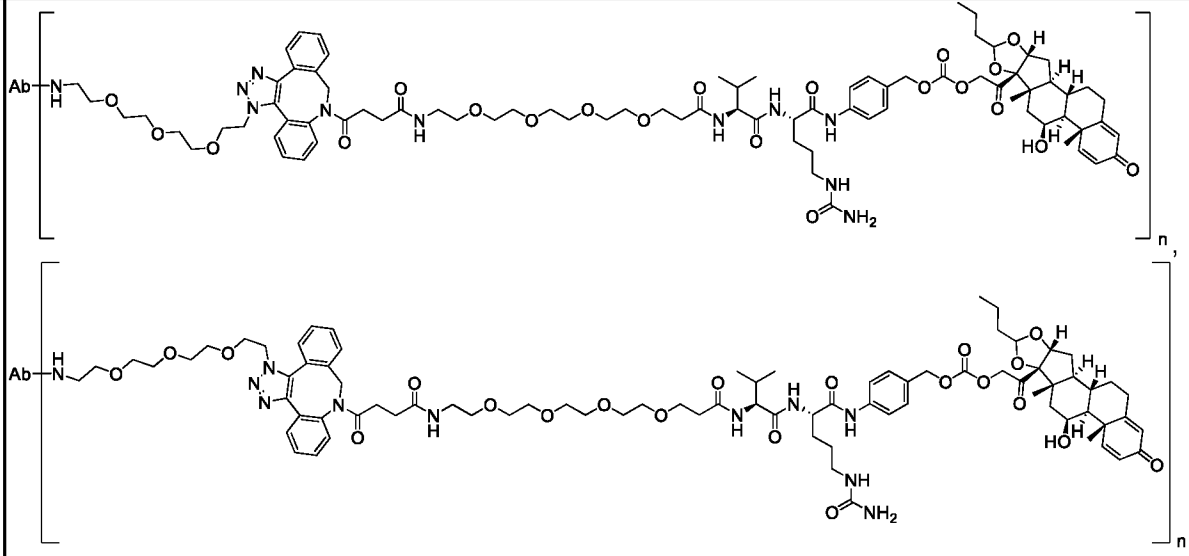






and





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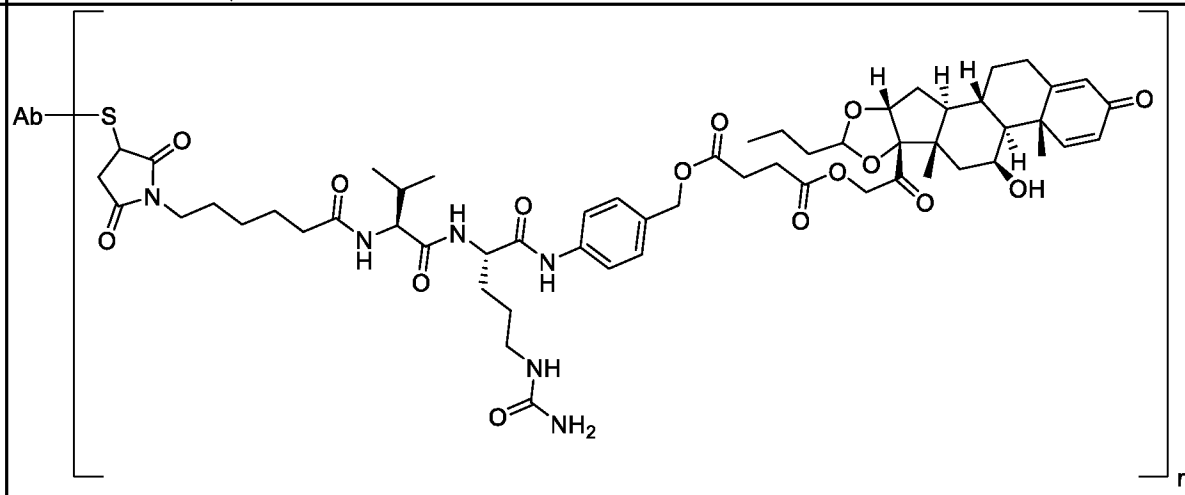
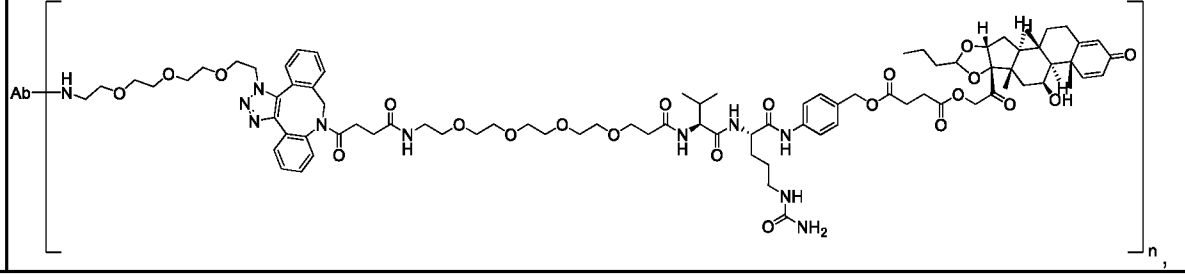
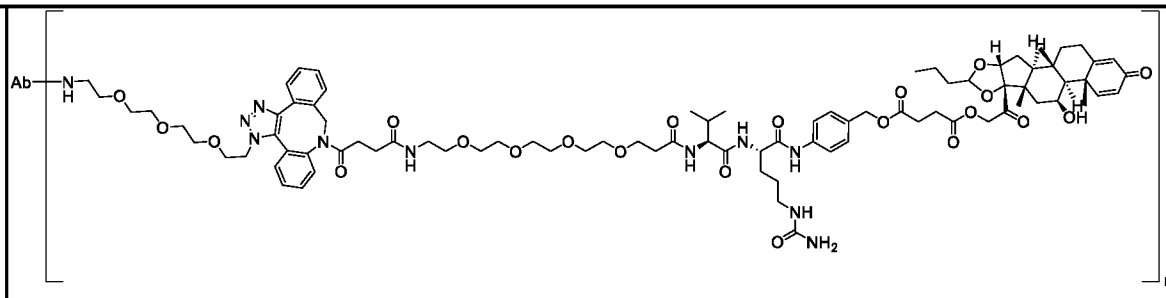


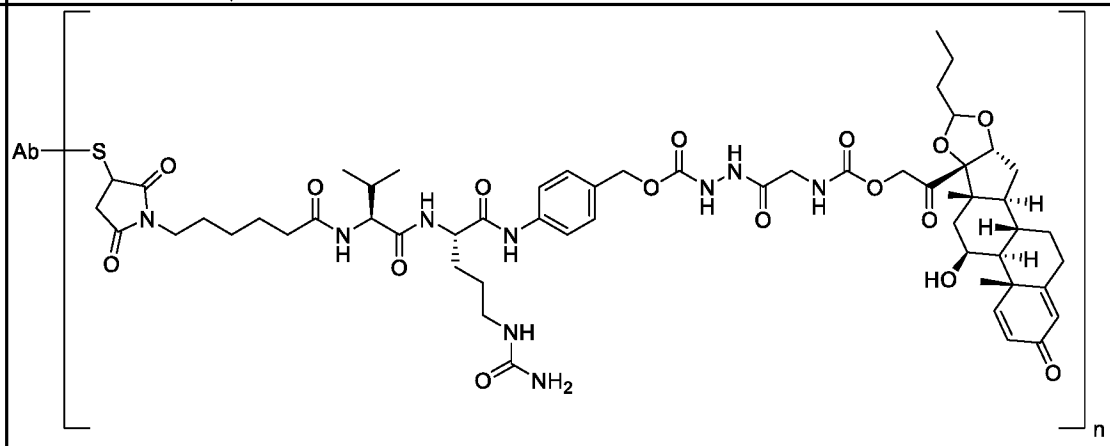
or a stereoisomer or mixture of stereoisomers thereof;



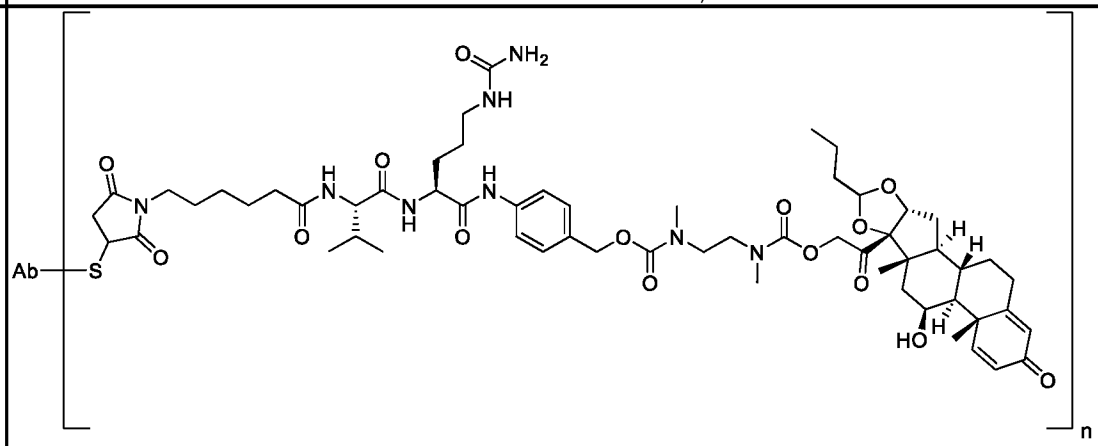


or

a mixture thereof;



or a stereoisomer or mixture of stereoisomers thereof;



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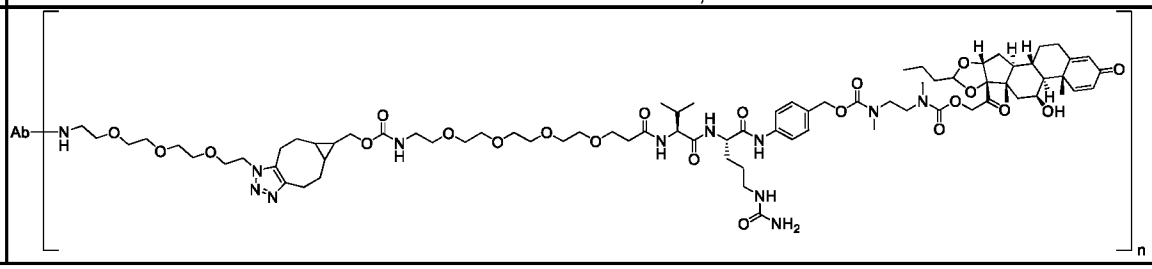
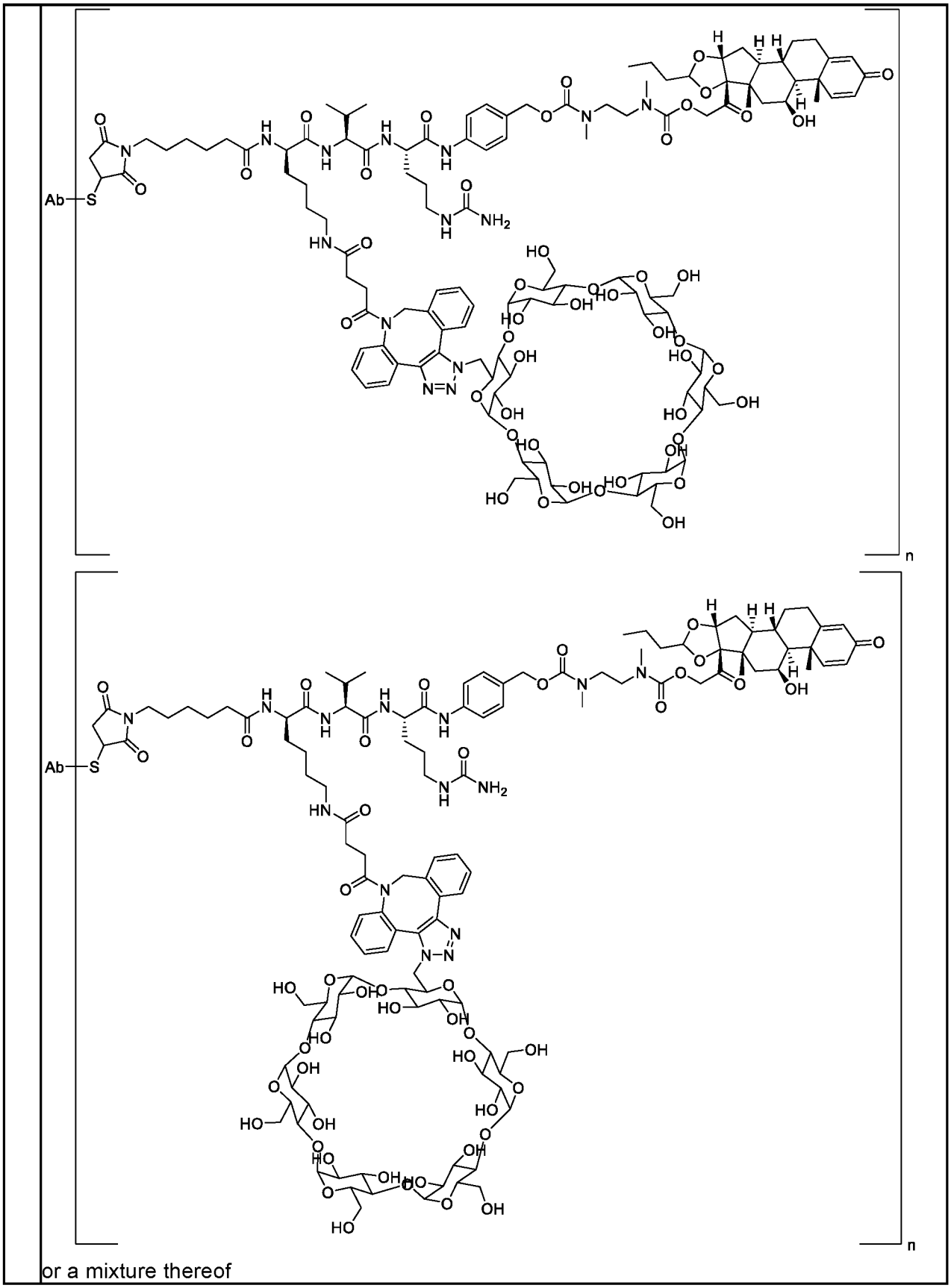
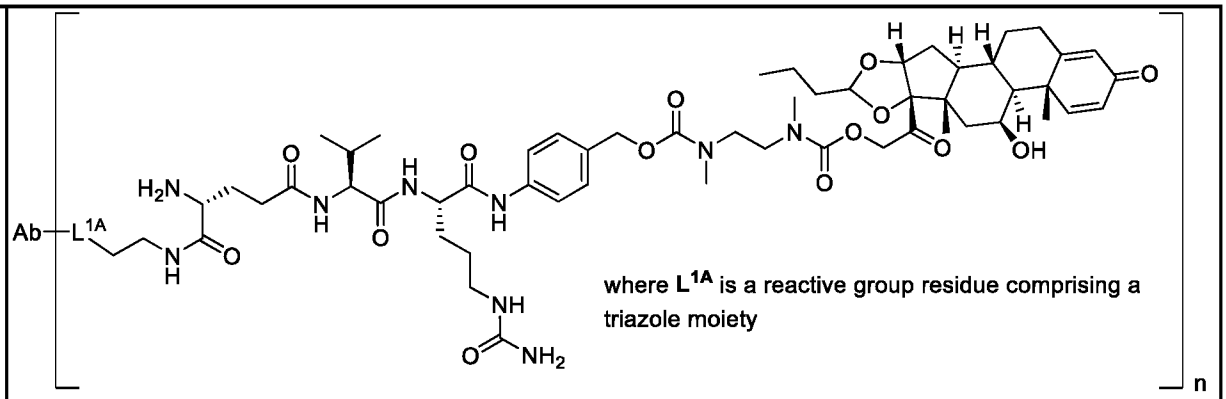
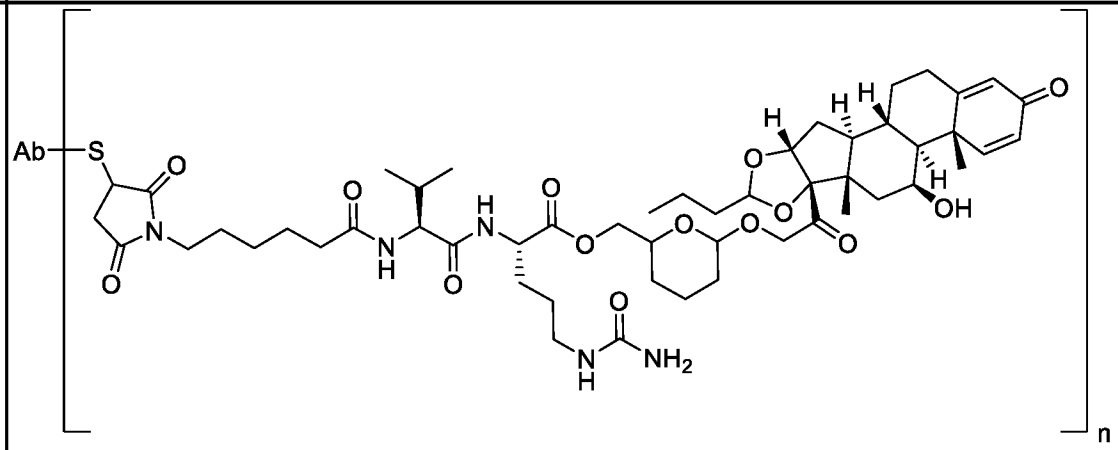




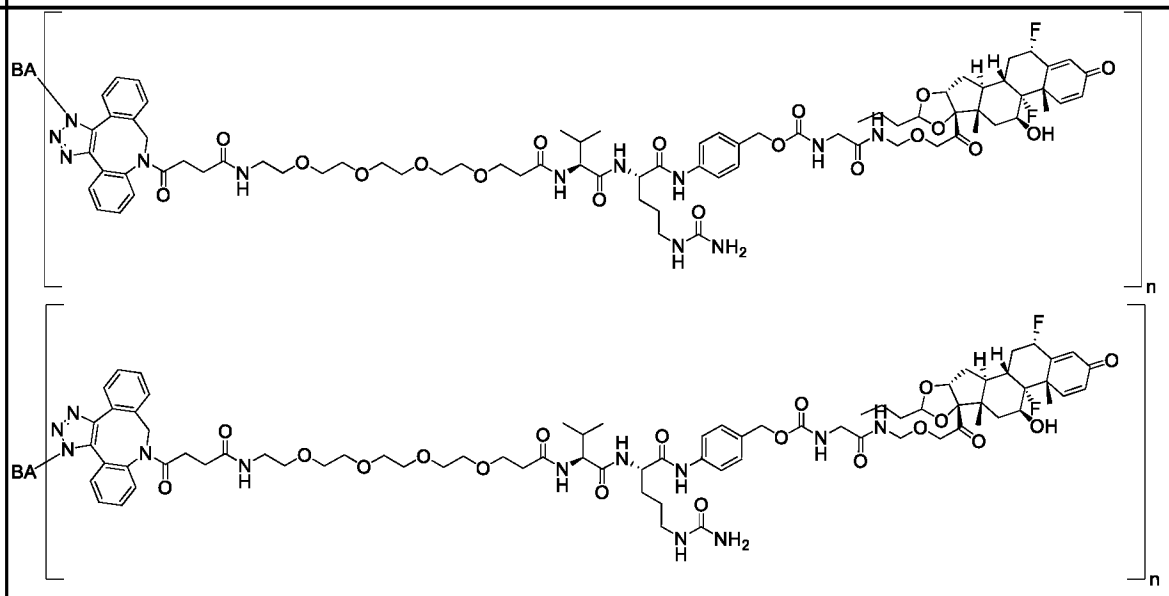
or a mixture thereof



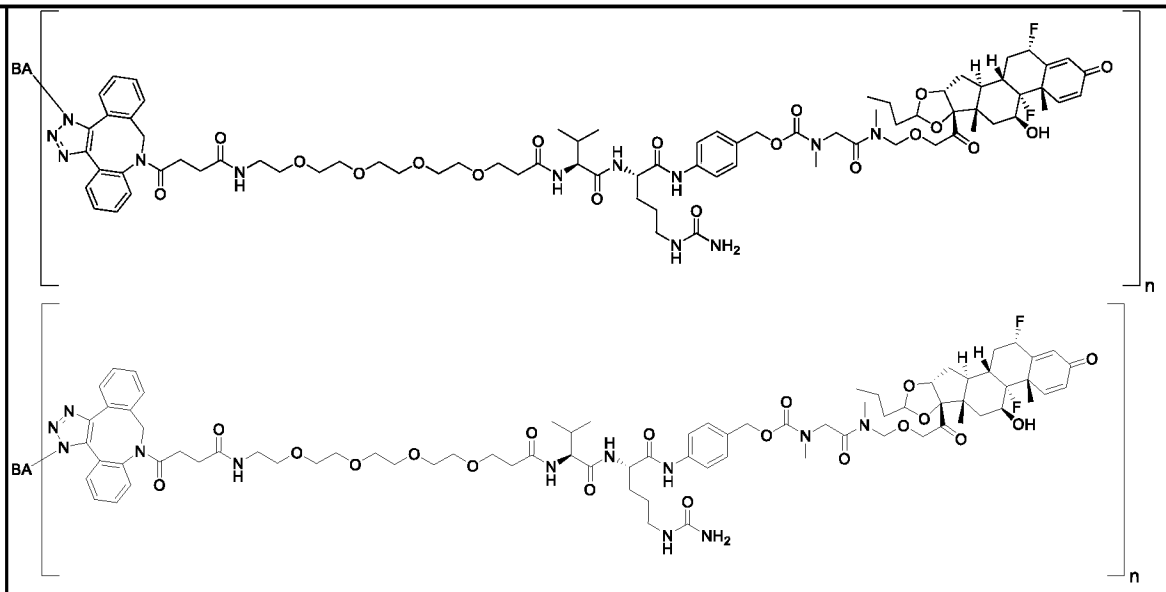
or a mixture thereof



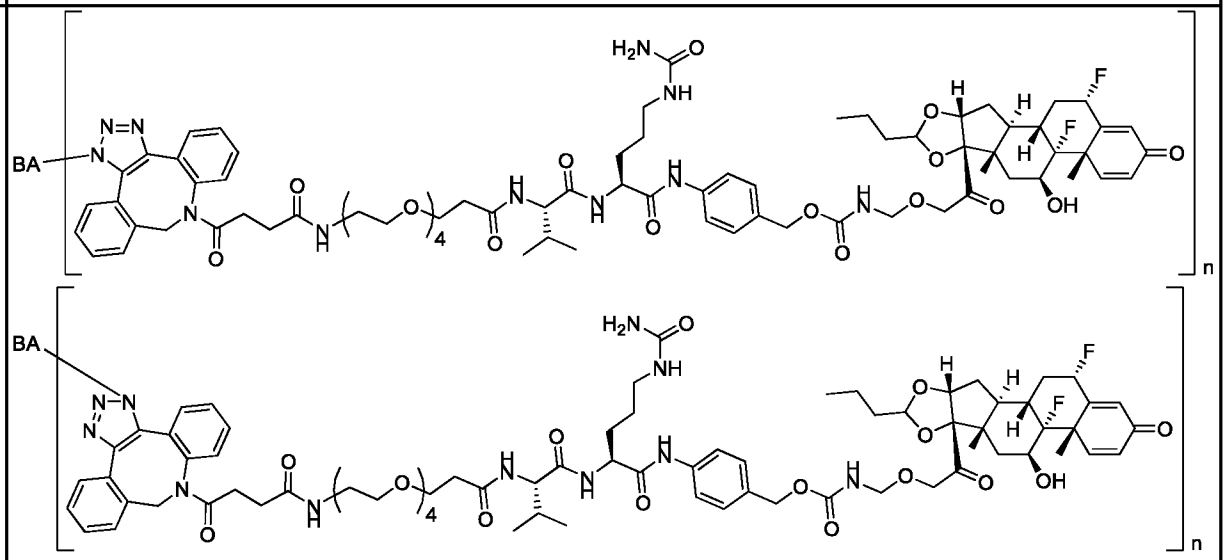
or a stereoisomer or mixture of stereoisomers thereof



or a mixture thereof



or a mixture thereof



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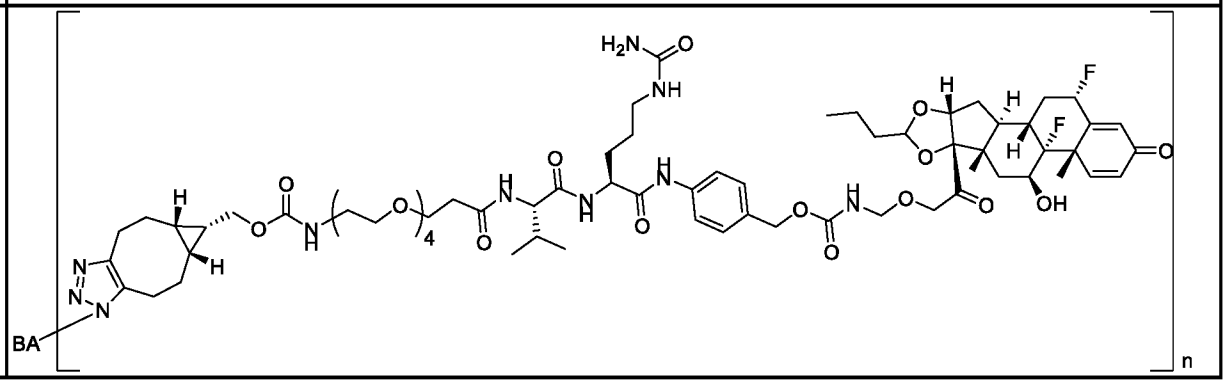
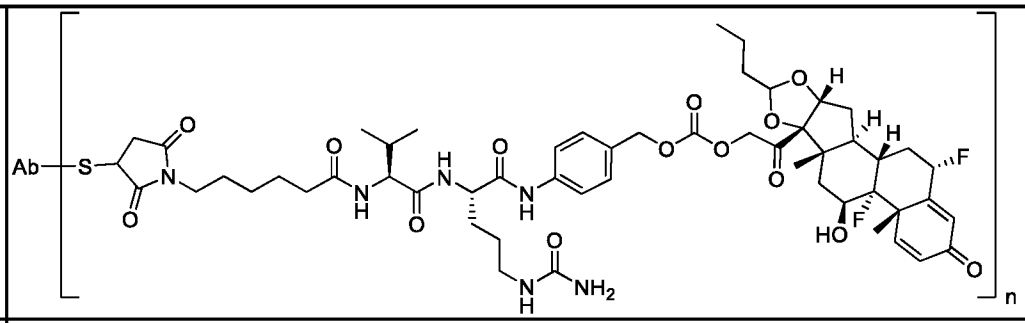
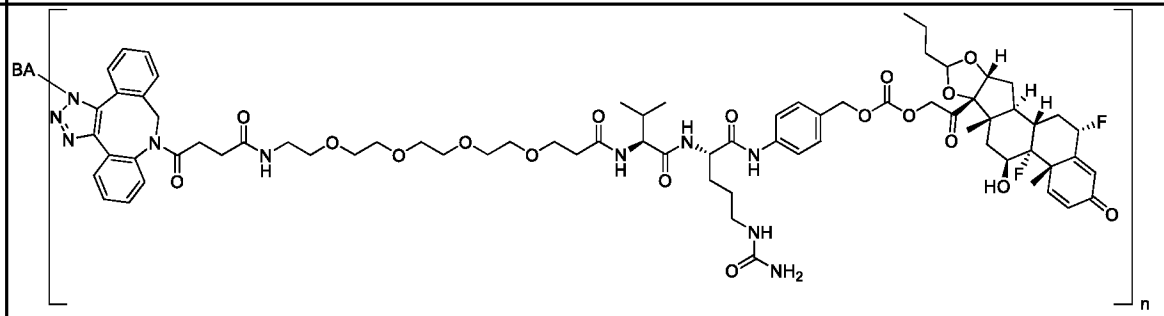
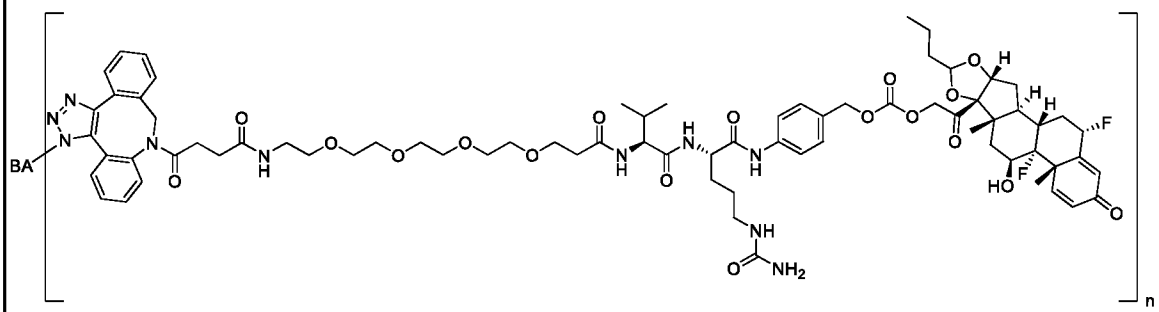
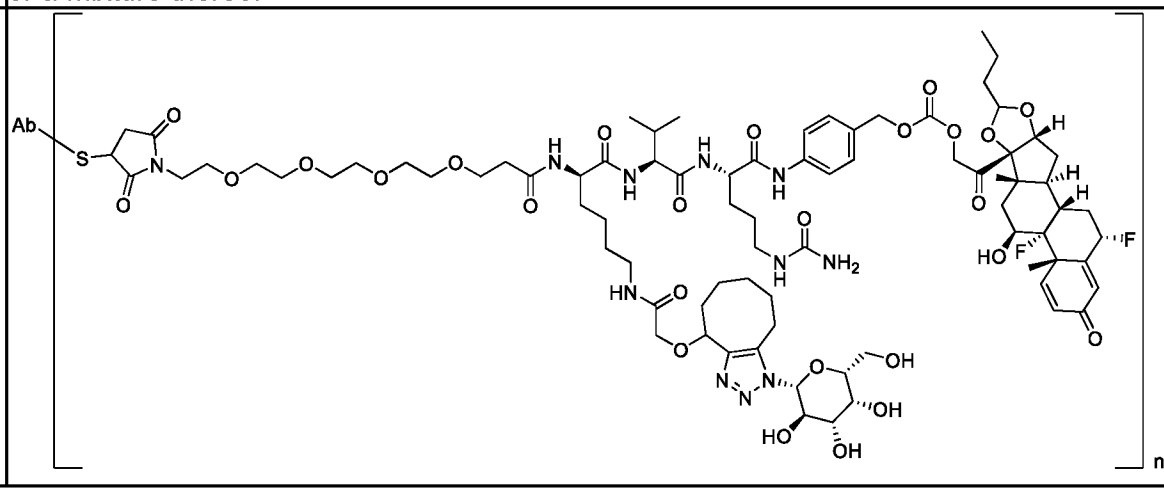


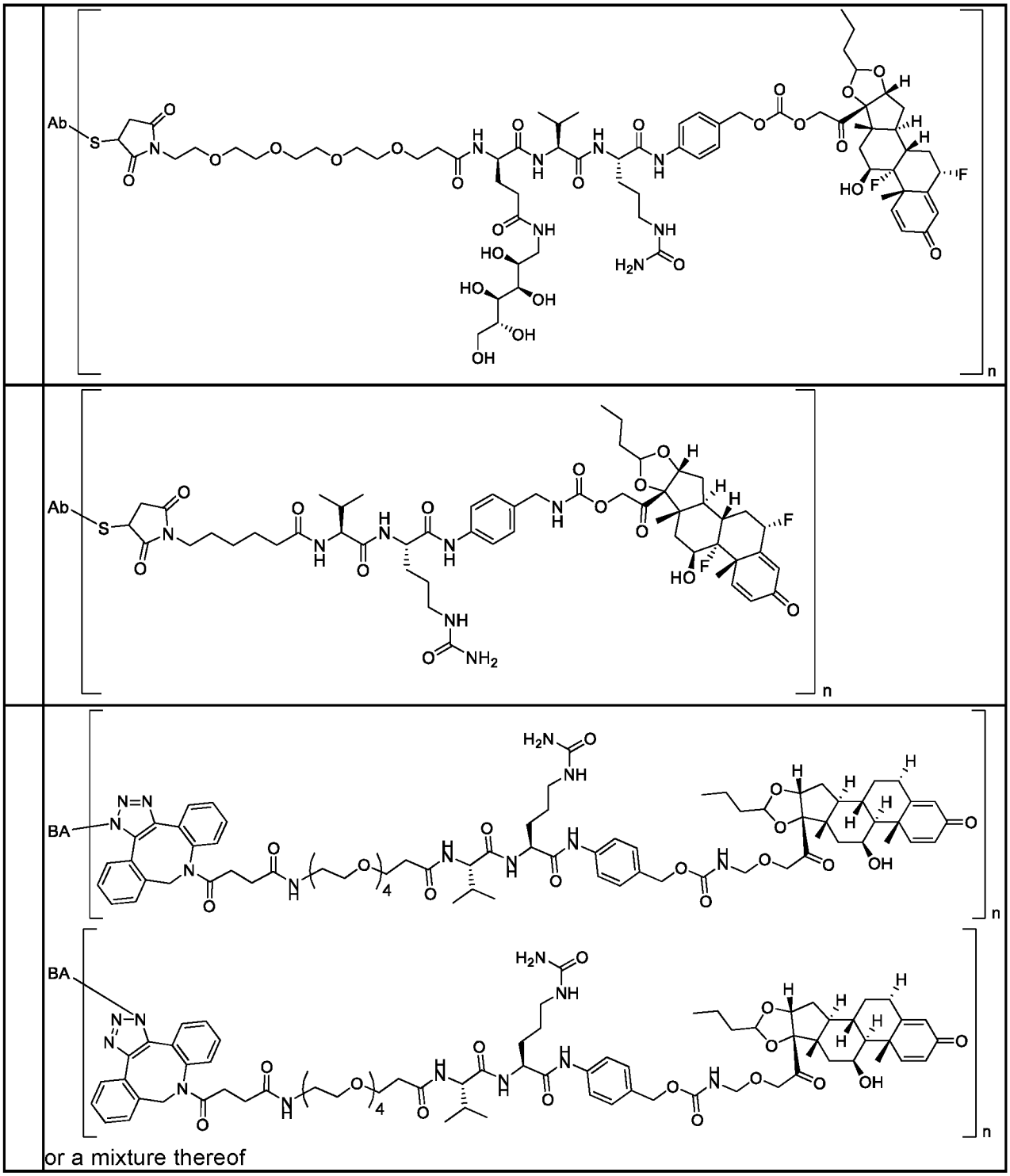






or a mixture thereof





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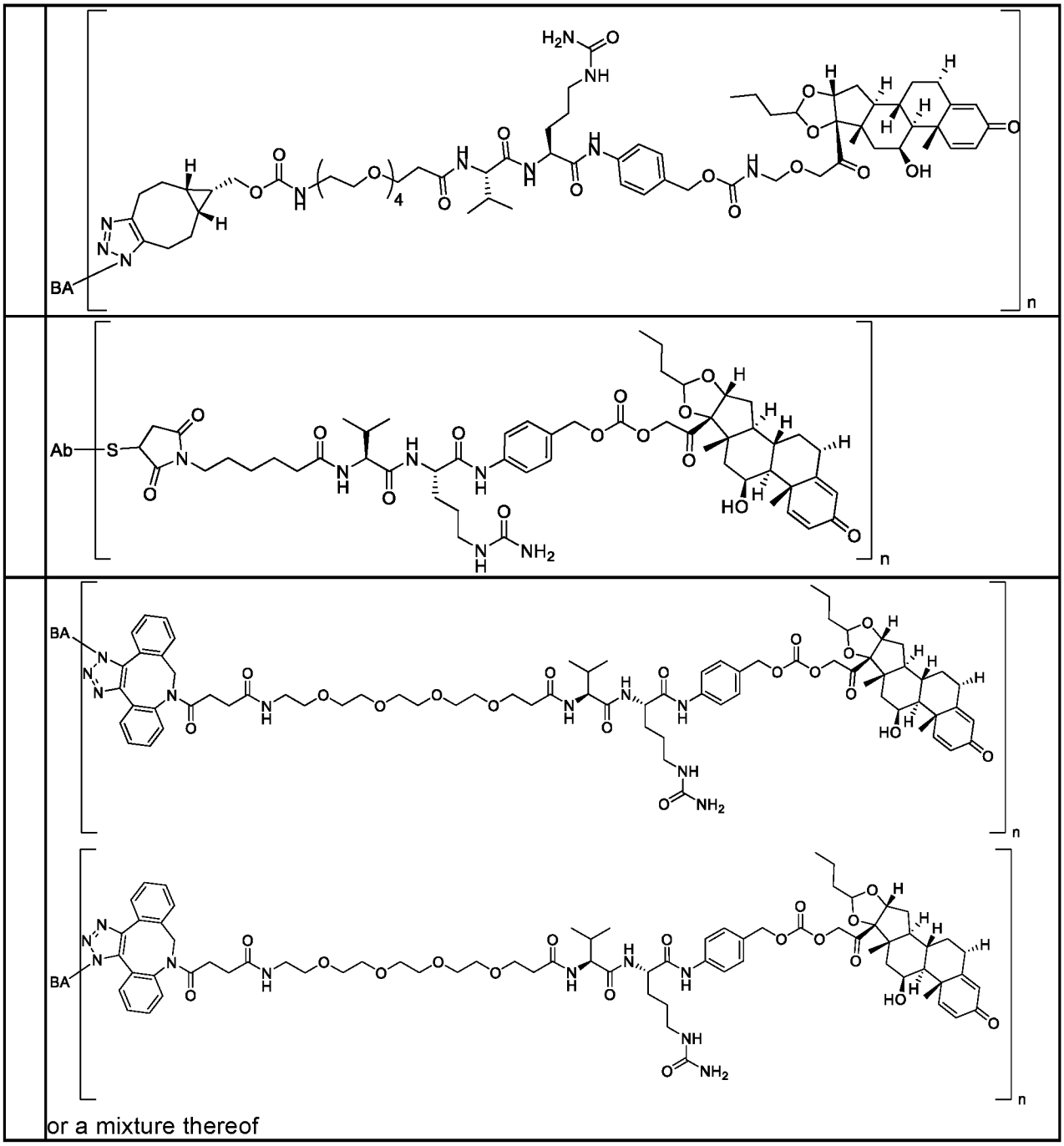

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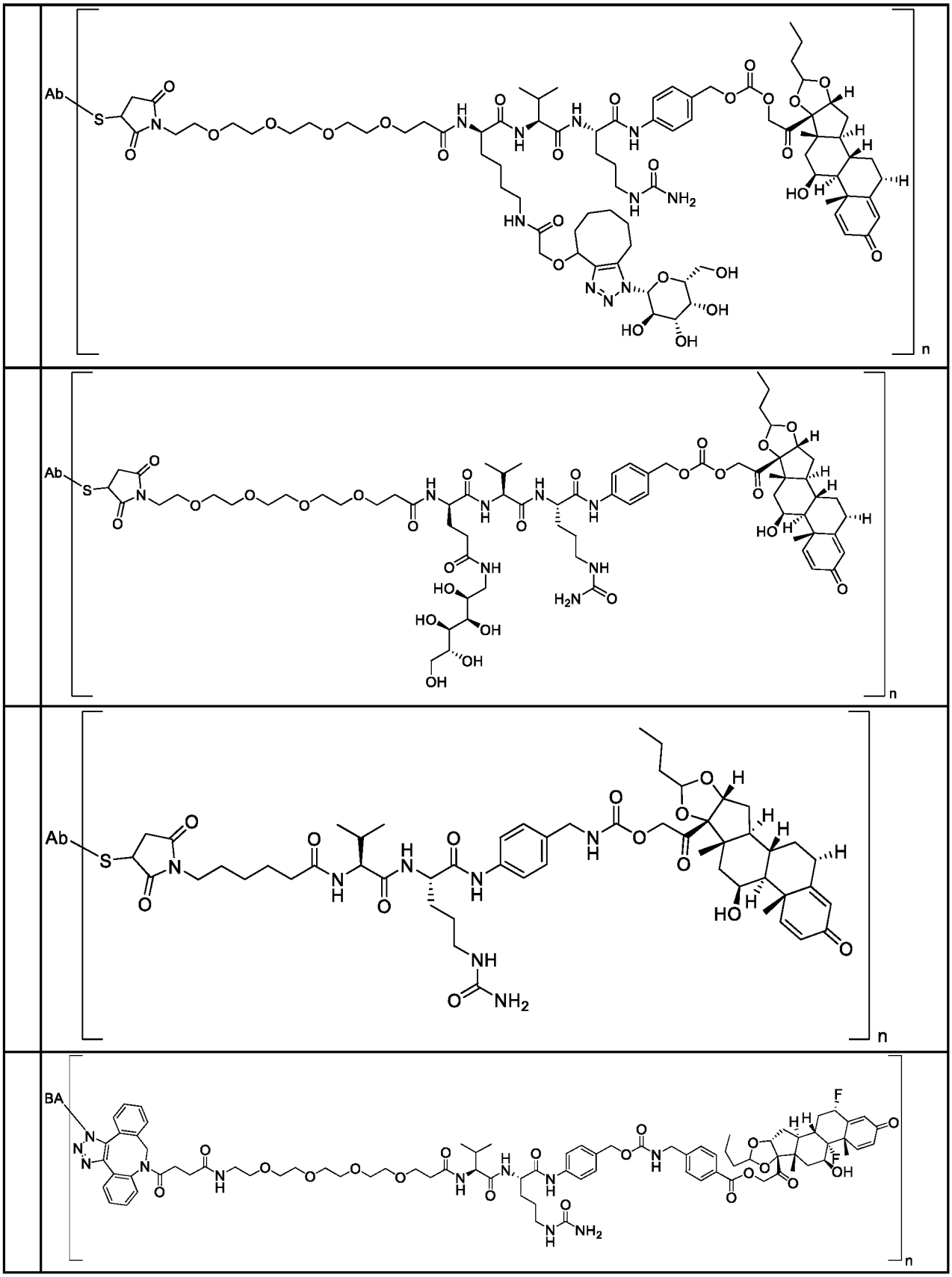

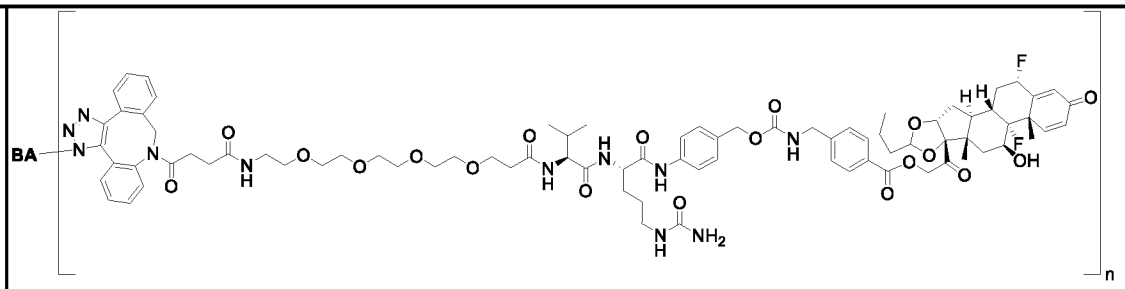

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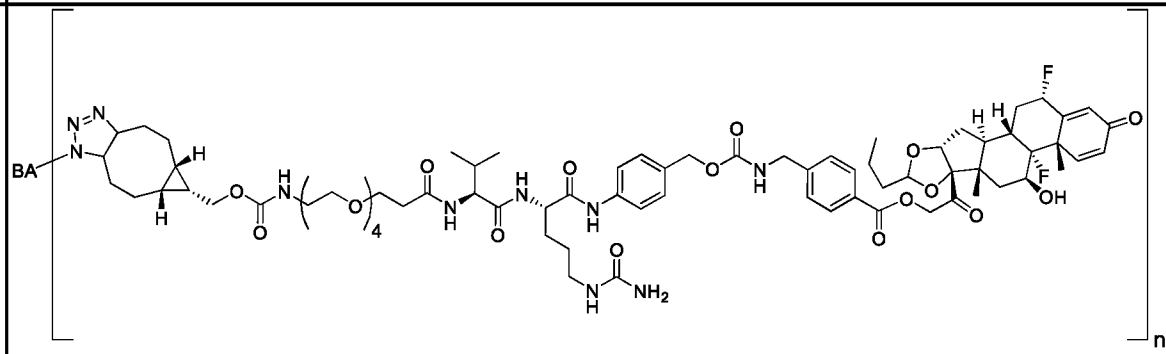

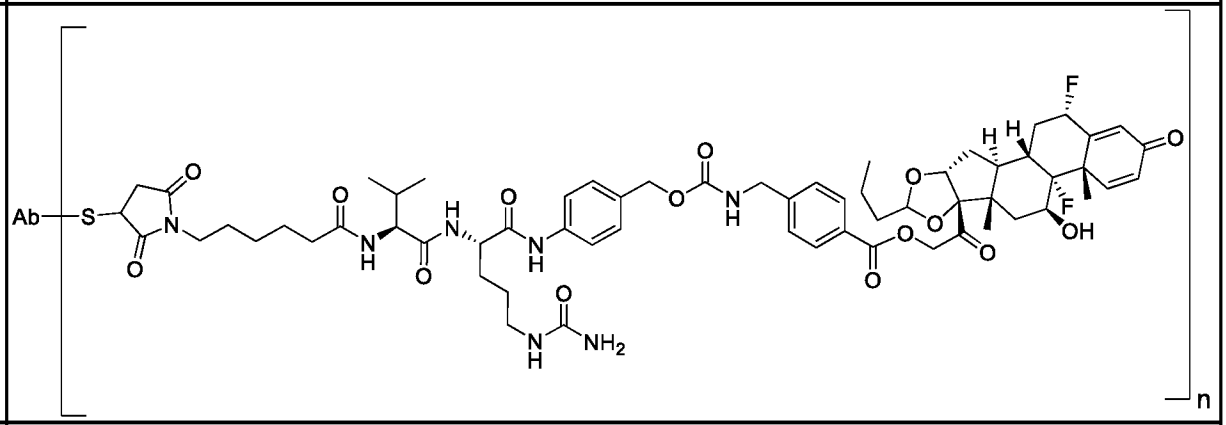

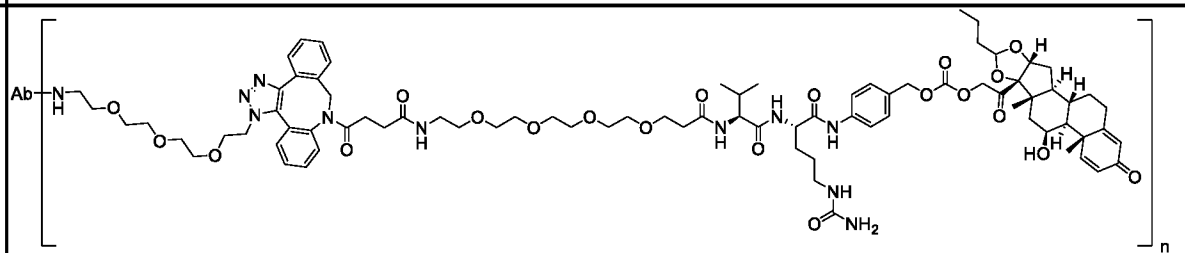

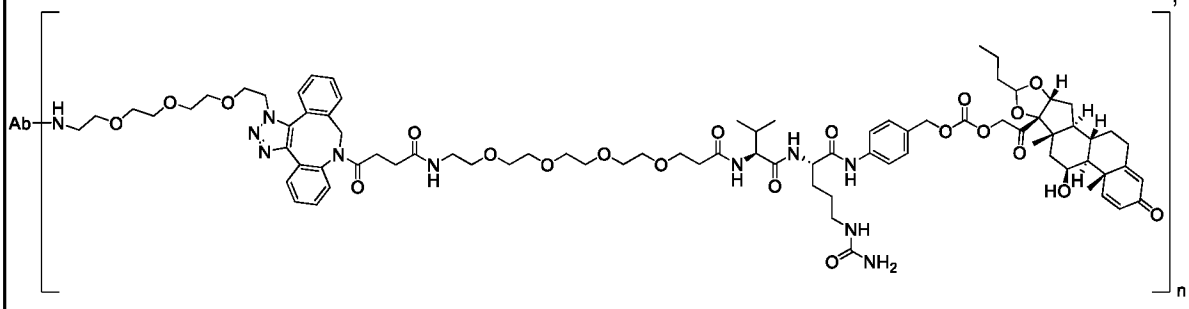

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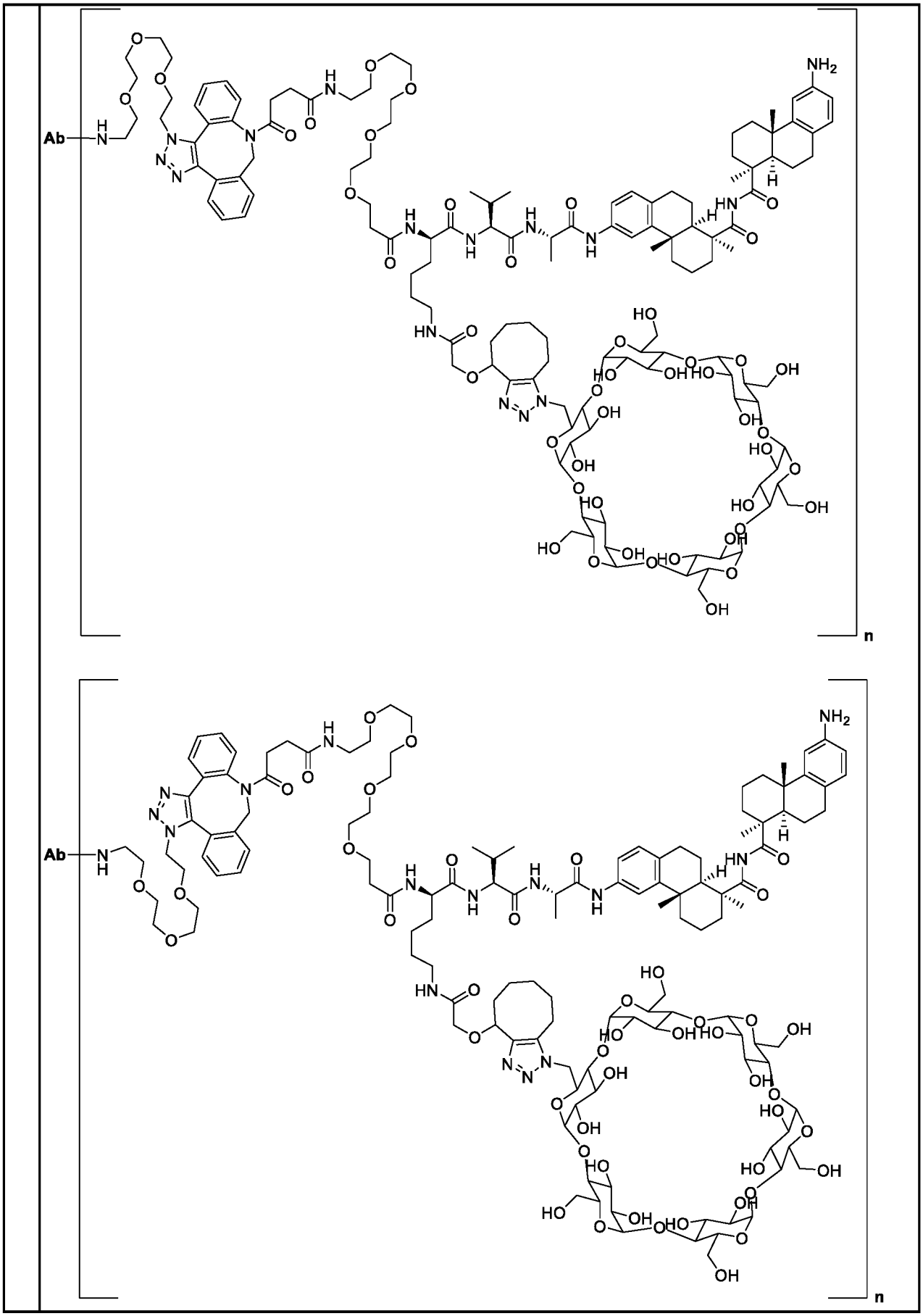
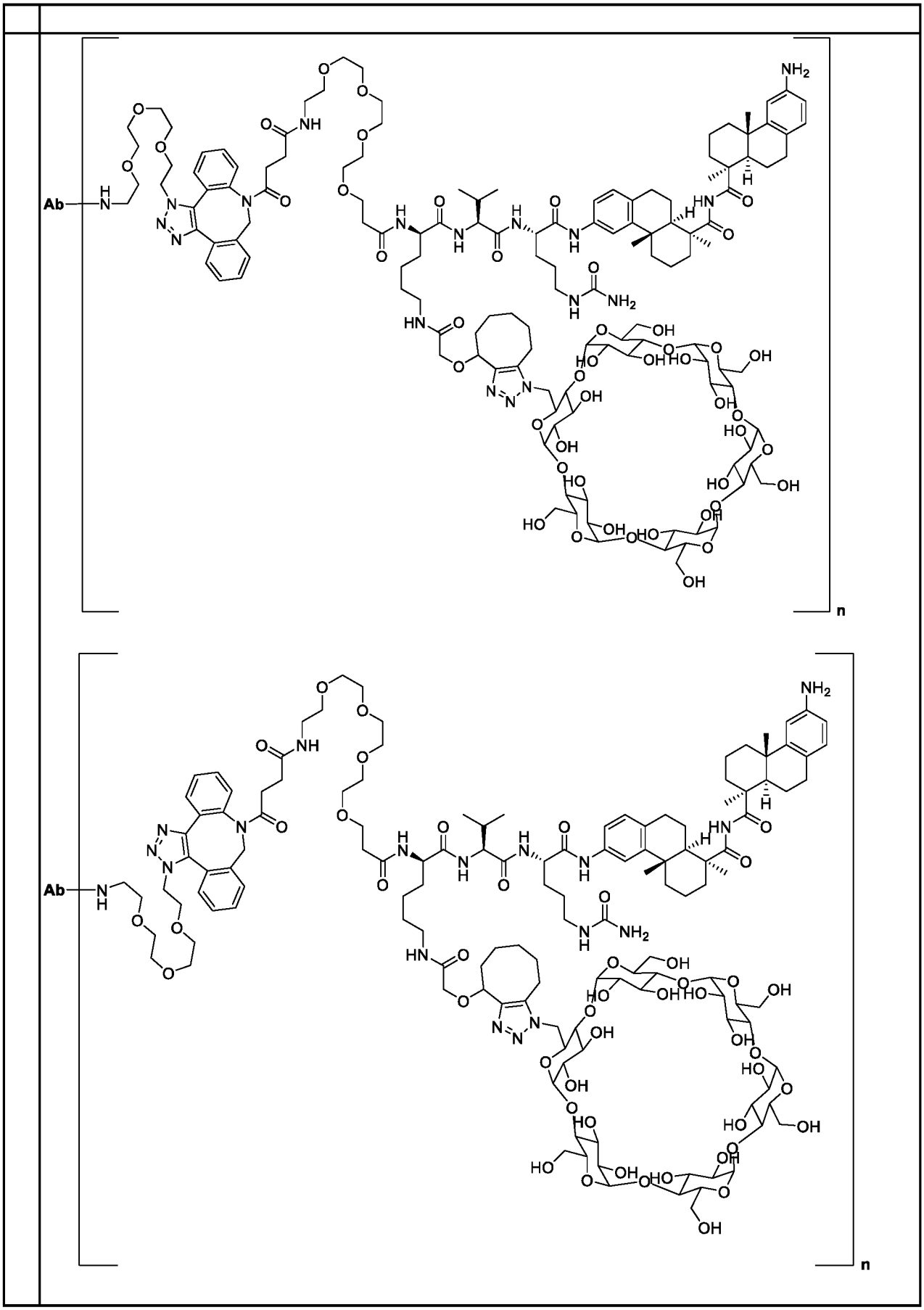
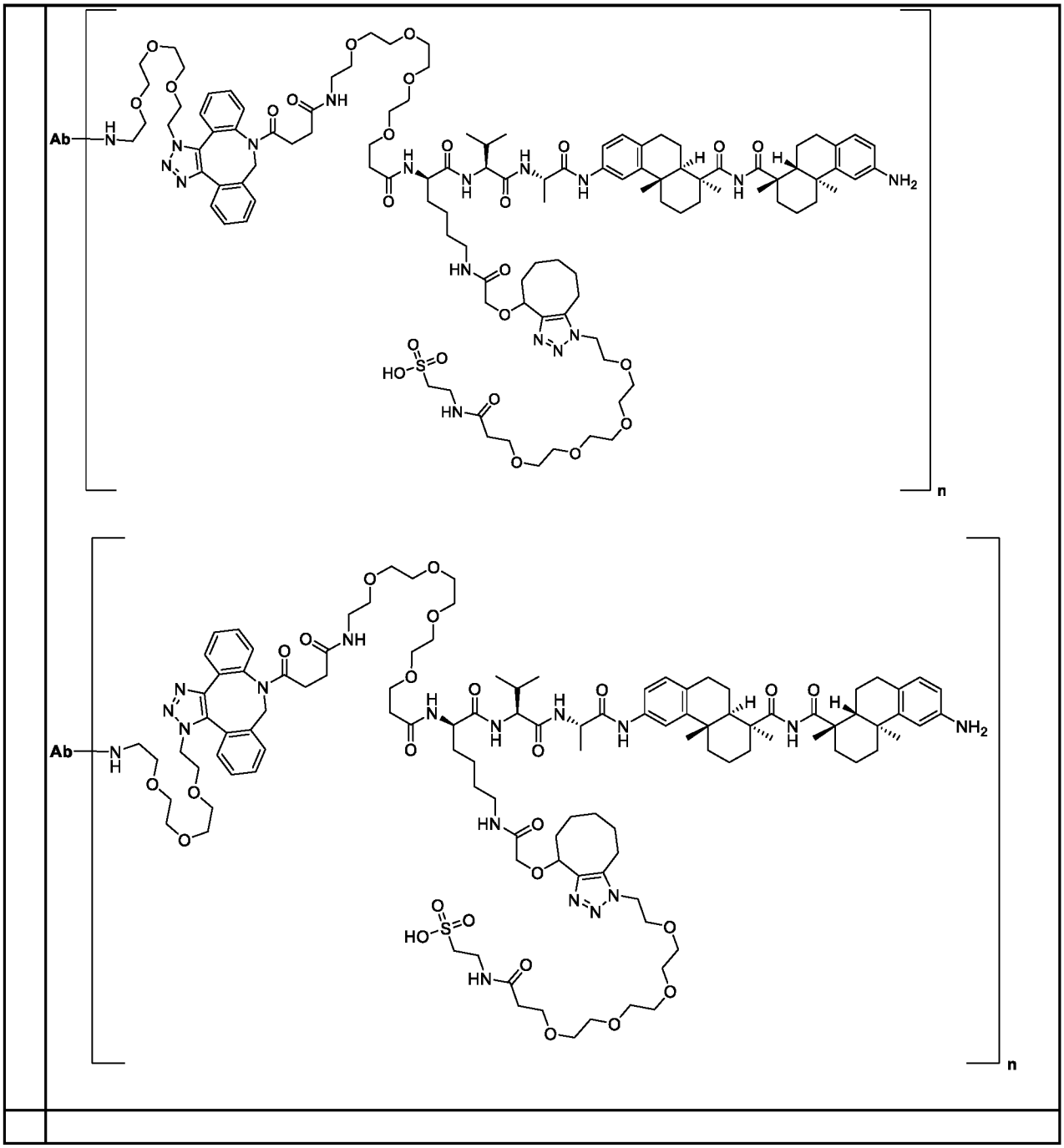
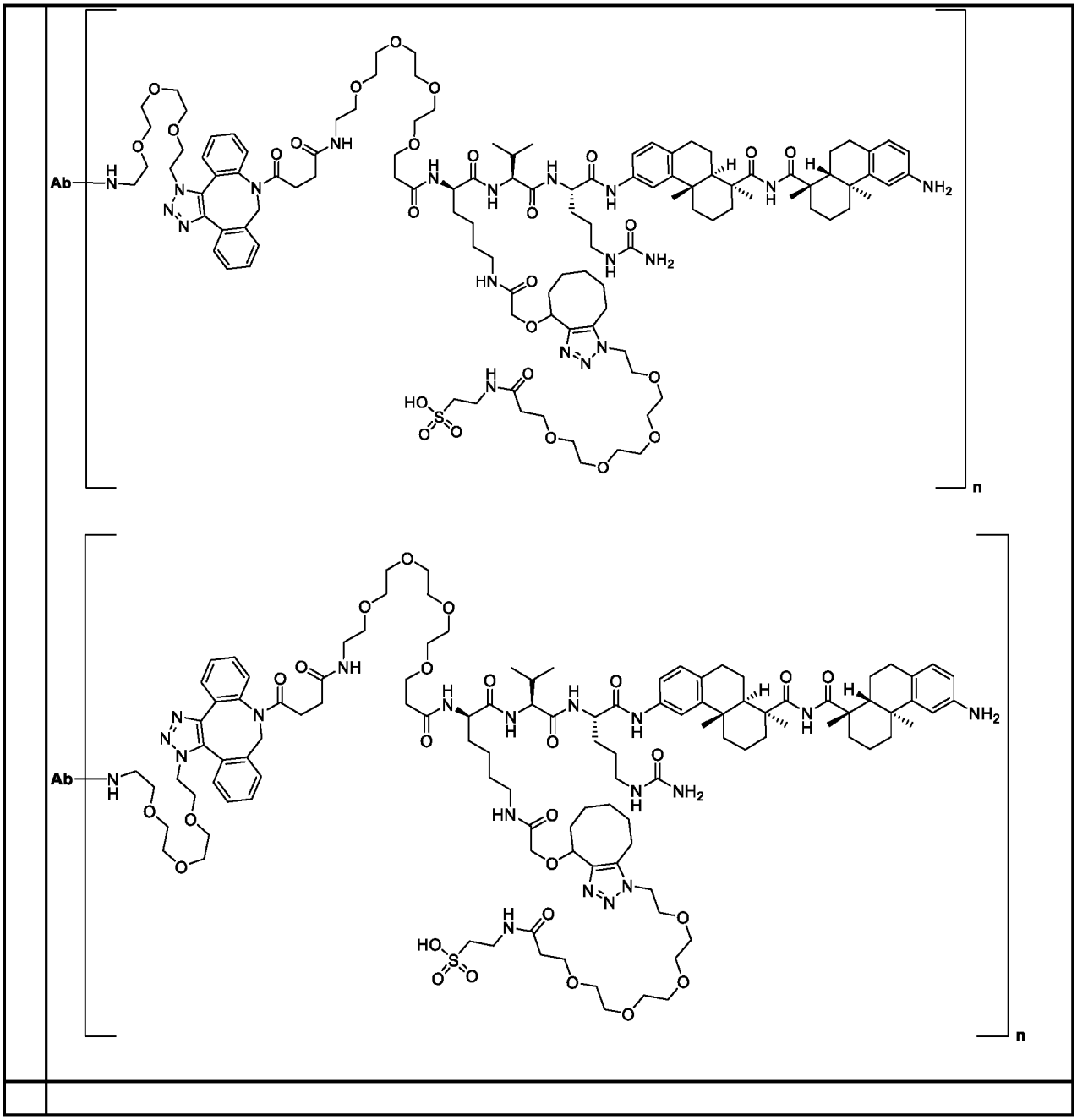
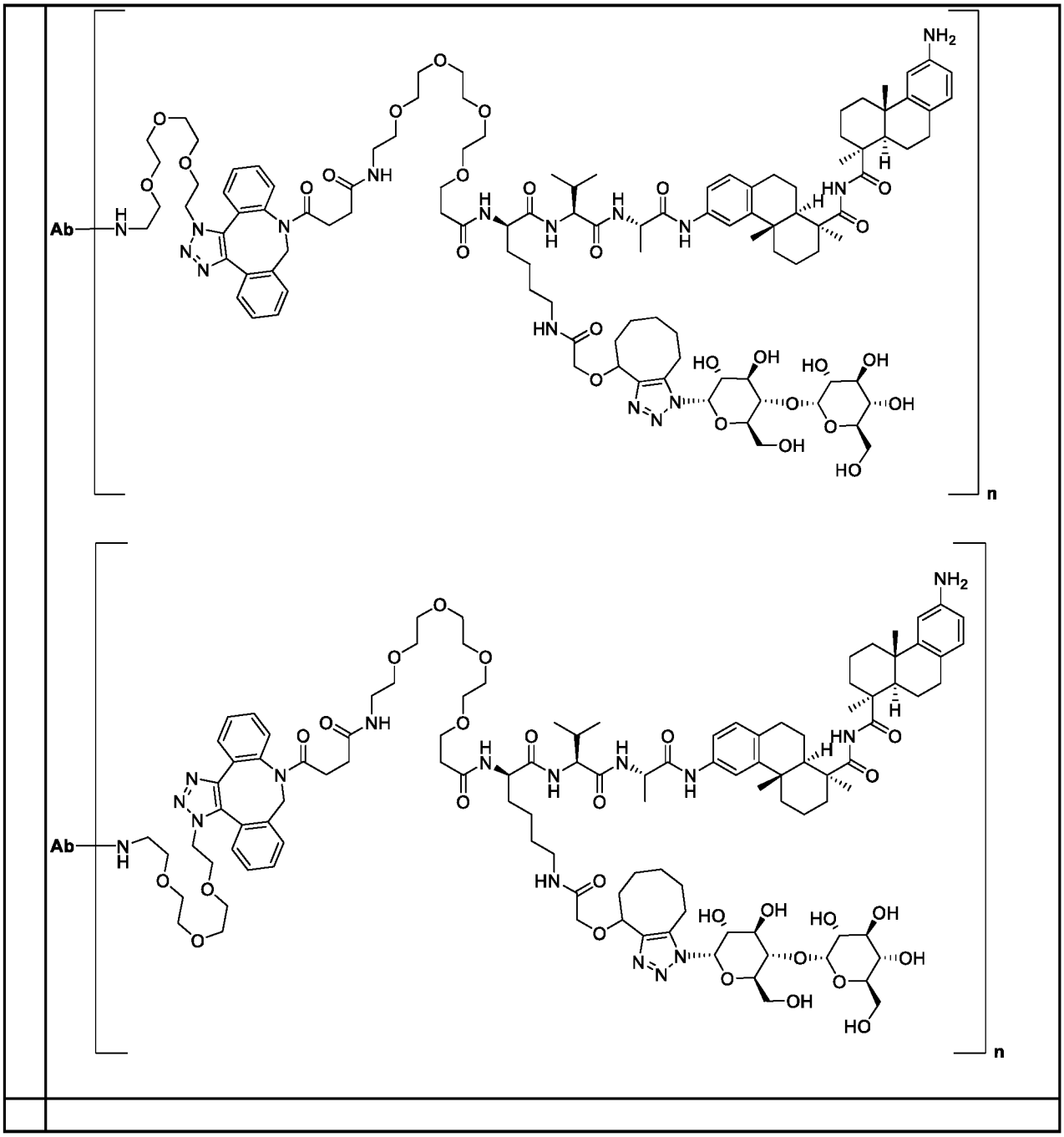
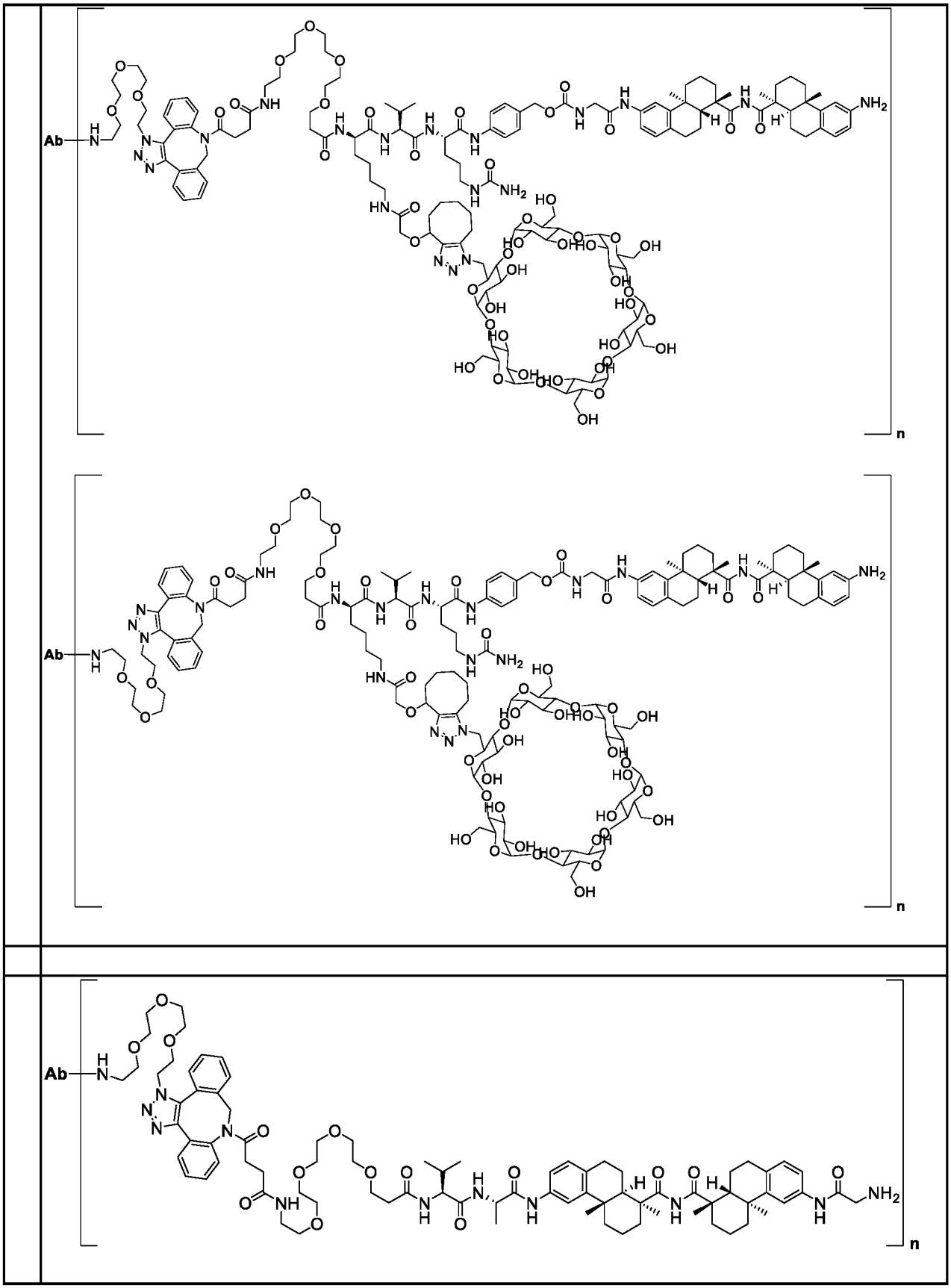

Ab
NH2
HO
OH
HN
O
N
H
n

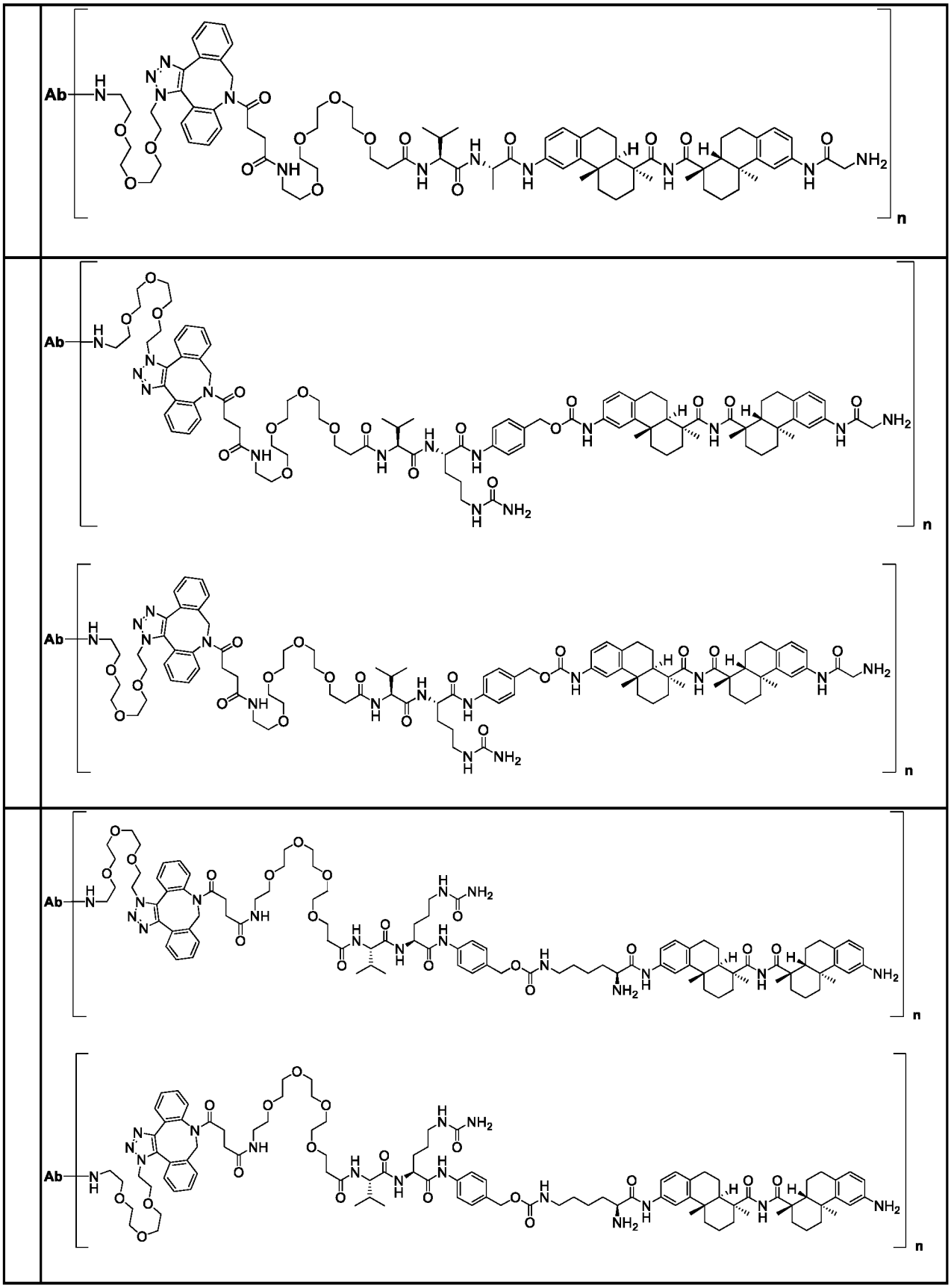

Ab
NH2
OH
HO
n
Ab
NH2
OH
HO
n

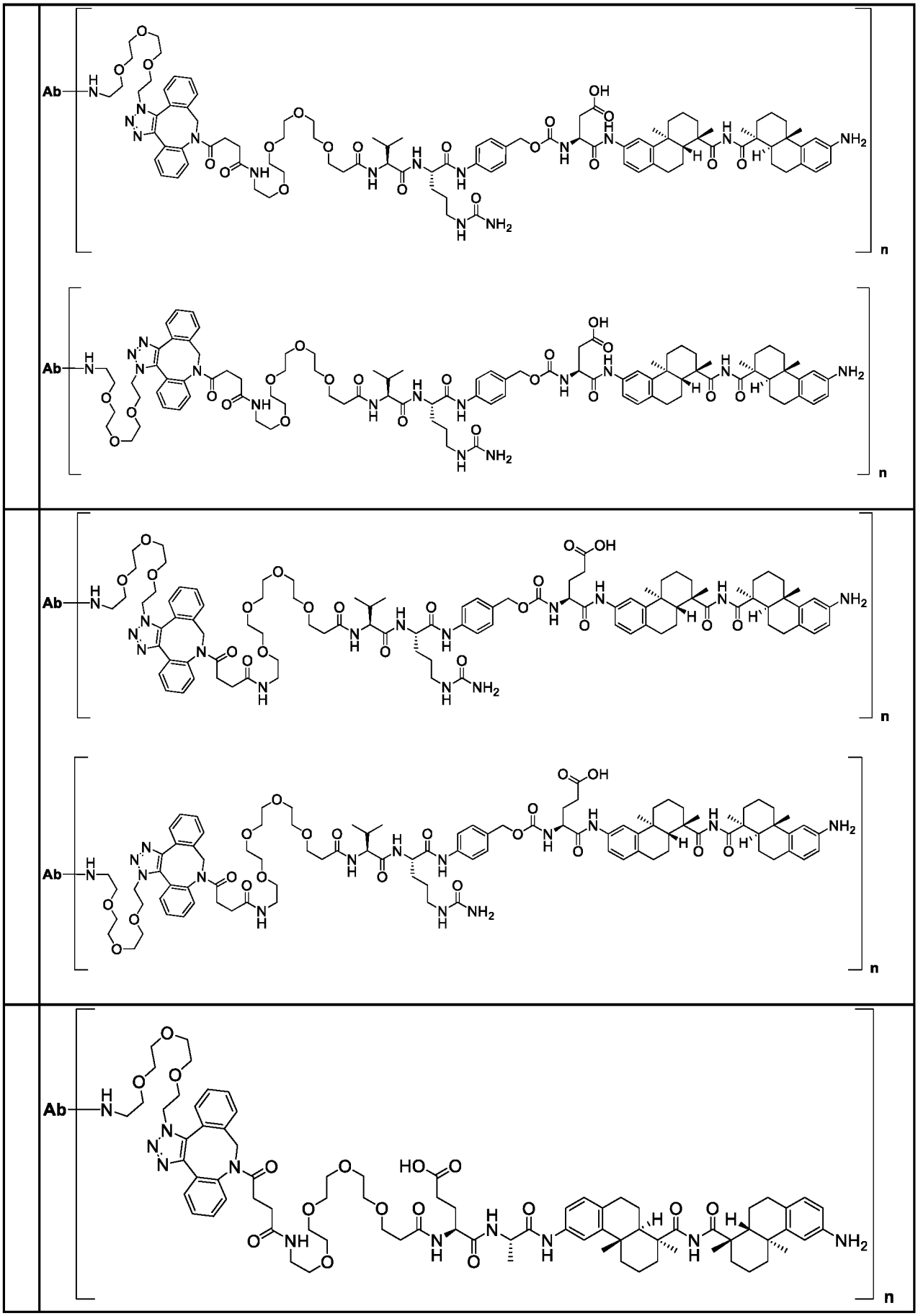


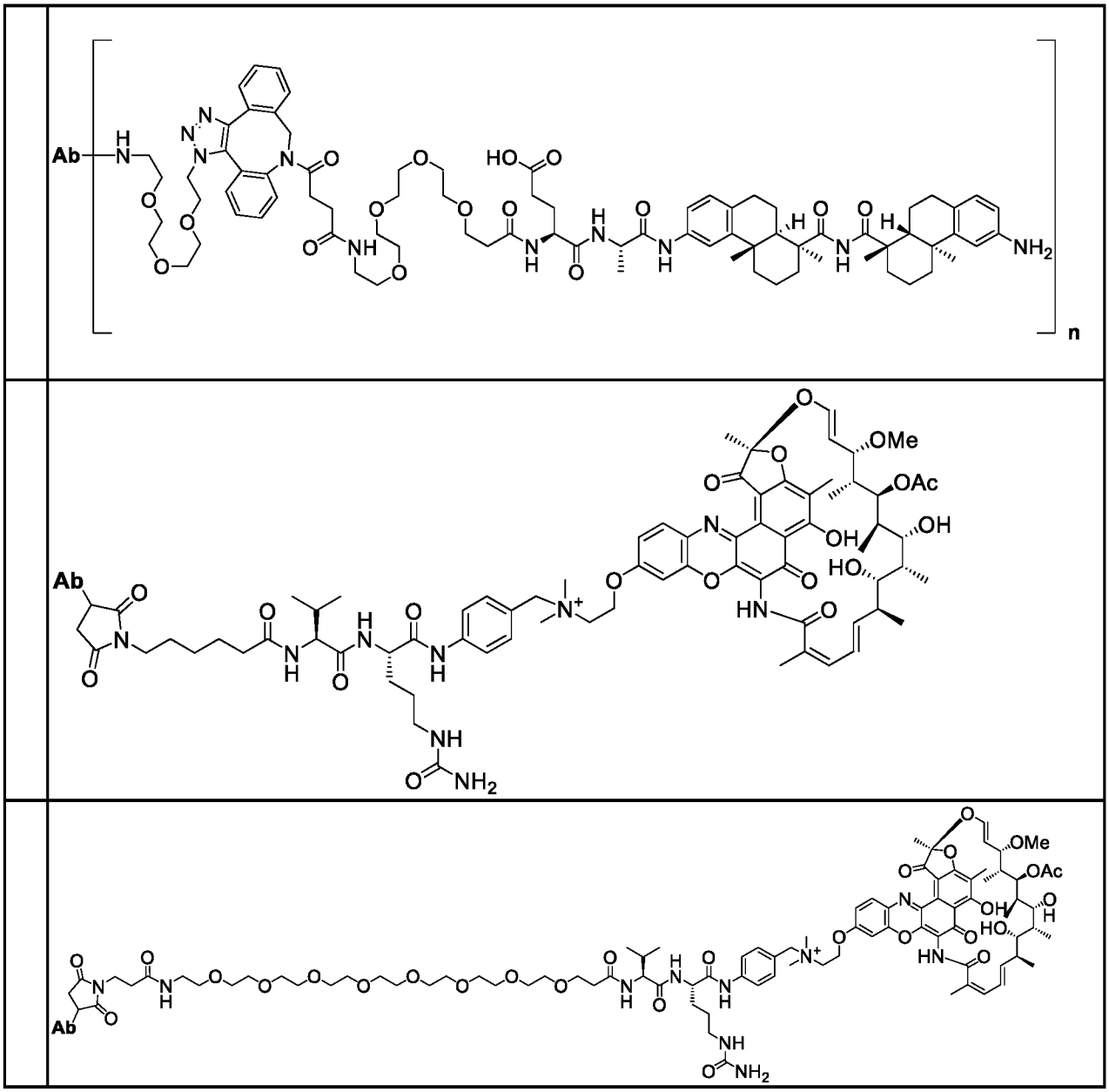



Ab
NH2
HO
OH
n


Ab
n
Ab
n
Ab
n

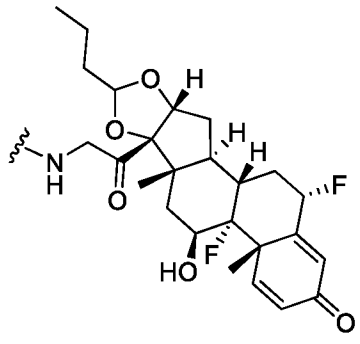






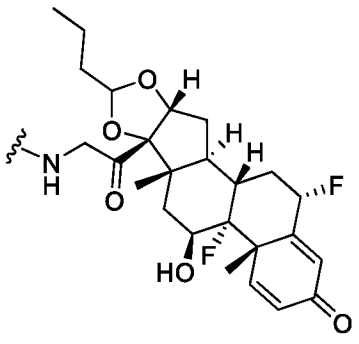
[0310] In the ADCs in Table 2, Ab is an anti-MSR1 antibody provided herein, or an antigen-binding fragment thereof. In particular embodiments, Ab is modified with a PEG group on a glutamine side chain as described herein. In the ADCs, n is an integer from 1 to 10, for instance, 1, 2, 3, or 4.

[0311] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof,



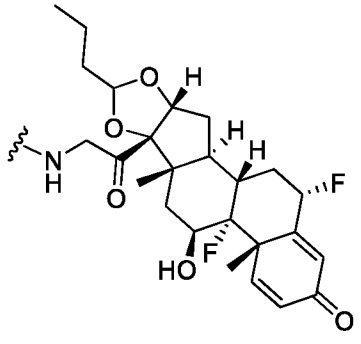
conjugated to the payload , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0312] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4 and conjugated to the payload



, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

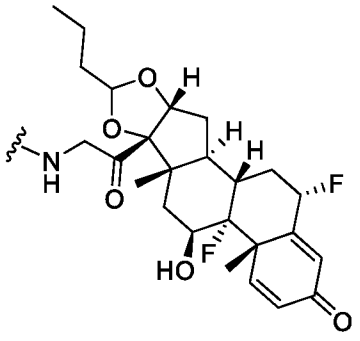
[0313] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64 and conjugated to the



payload , or pharmaceutically acceptable salt, solvate or

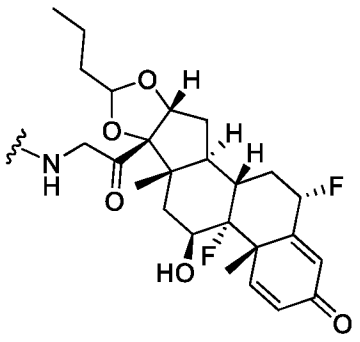
stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0314] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4 and conjugated to the payload



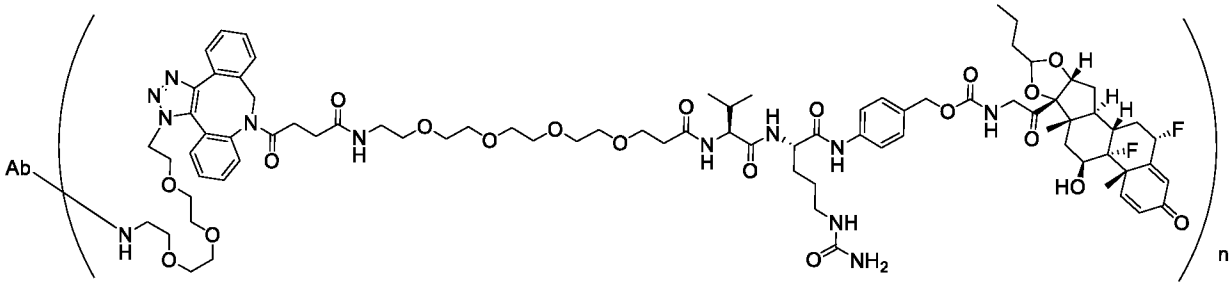
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

[0315] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58 and conjugated to the payload

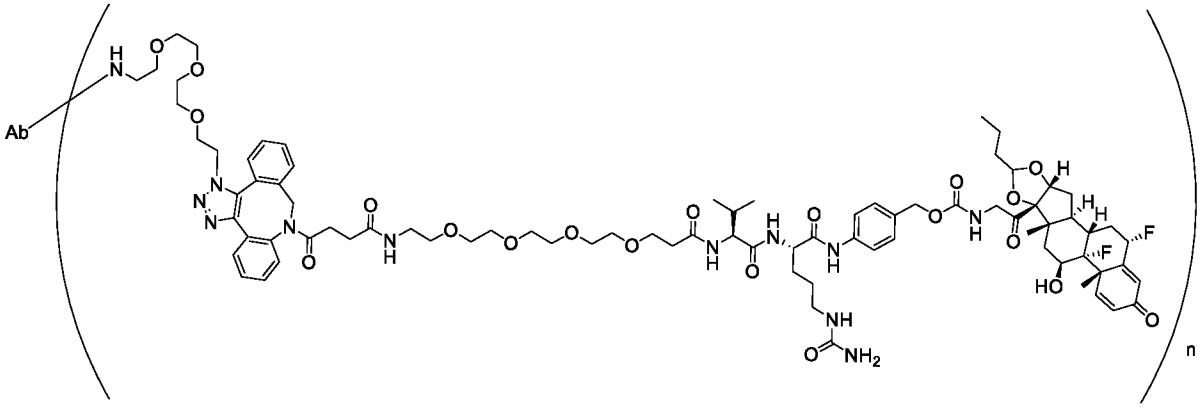


, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

[0316] An antibody-drug conjugate (ADC) according to the formulas:

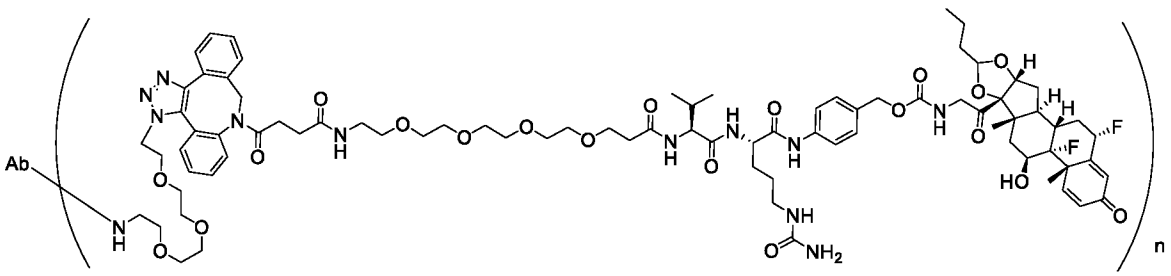


and/or

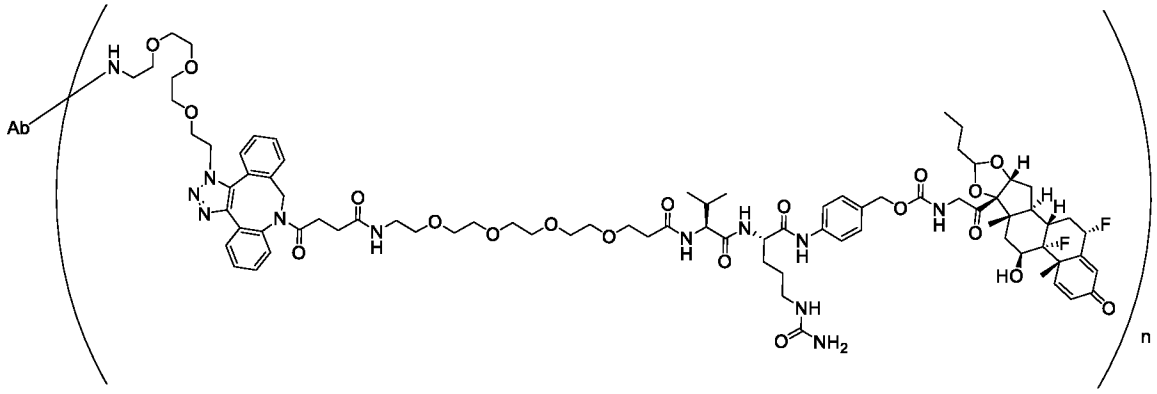


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0317] An antibody-drug conjugate (ADC) according to the formulas:

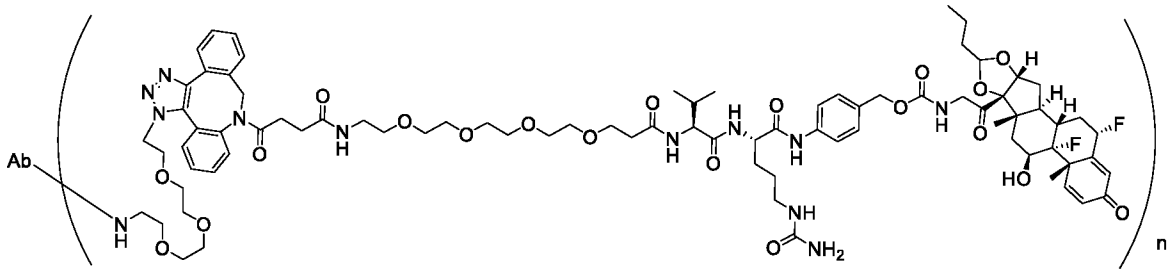


and/or

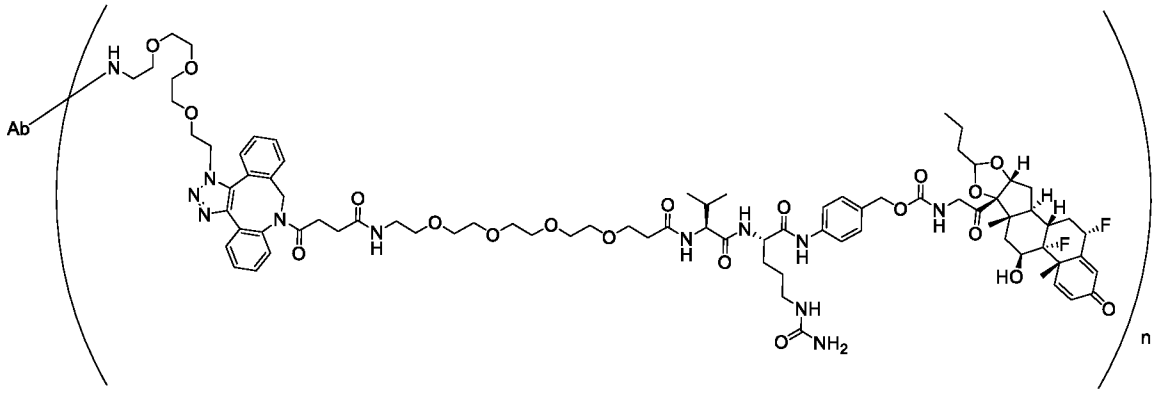


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0318] An antibody-drug conjugate (ADC) according to the formulas:

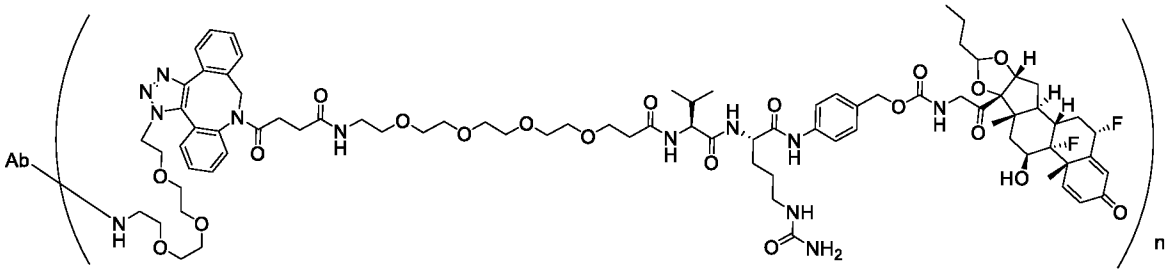


and/or

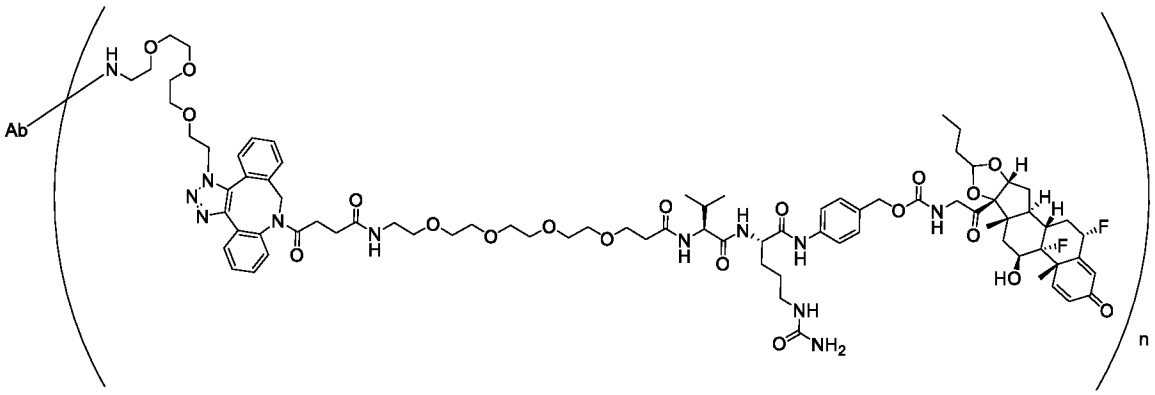


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0319] An antibody-drug conjugate (ADC) according to the formulas:



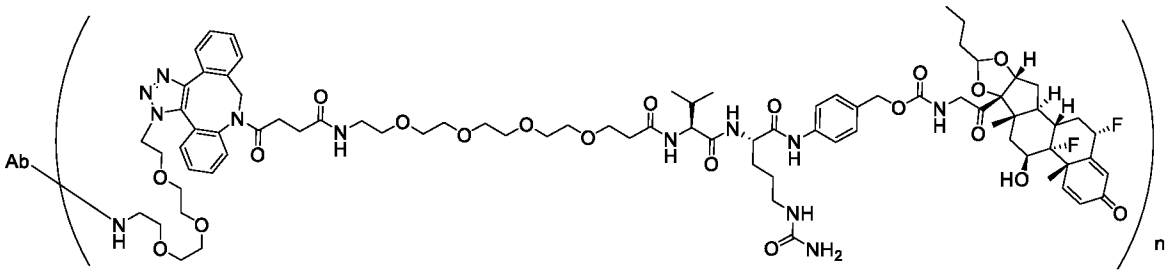
and/or



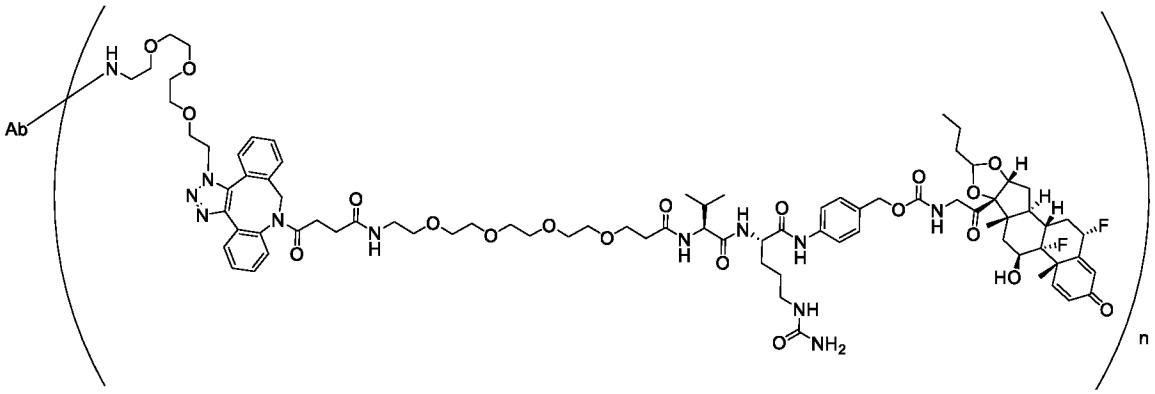
or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments,

the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodimets, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

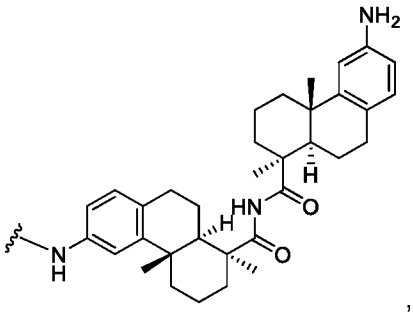
[0320] An antibody-drug conjugate (ADC) according to the formulas:

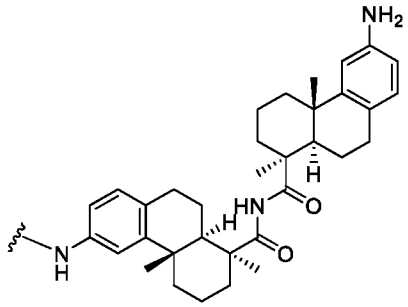


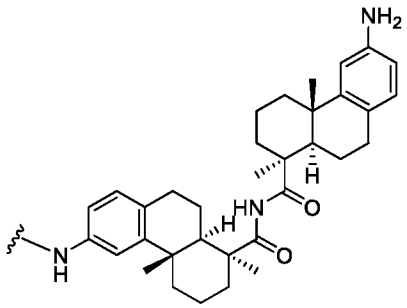
and/or



or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

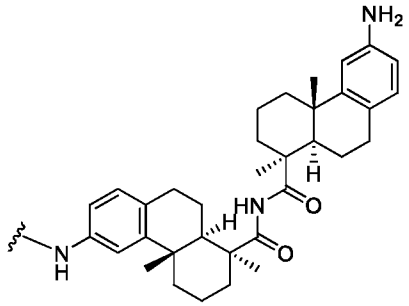
[0321] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, conjugated to the payload  , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0322] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4 and conjugated to the payload  , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0323] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64 and conjugated to the payload  , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1

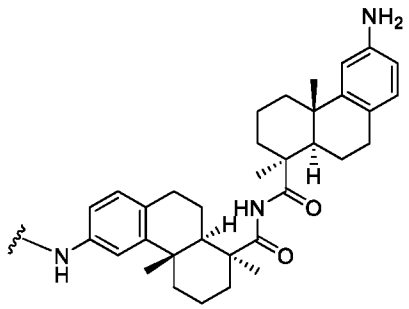
comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0324] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4 and conjugated to the payload



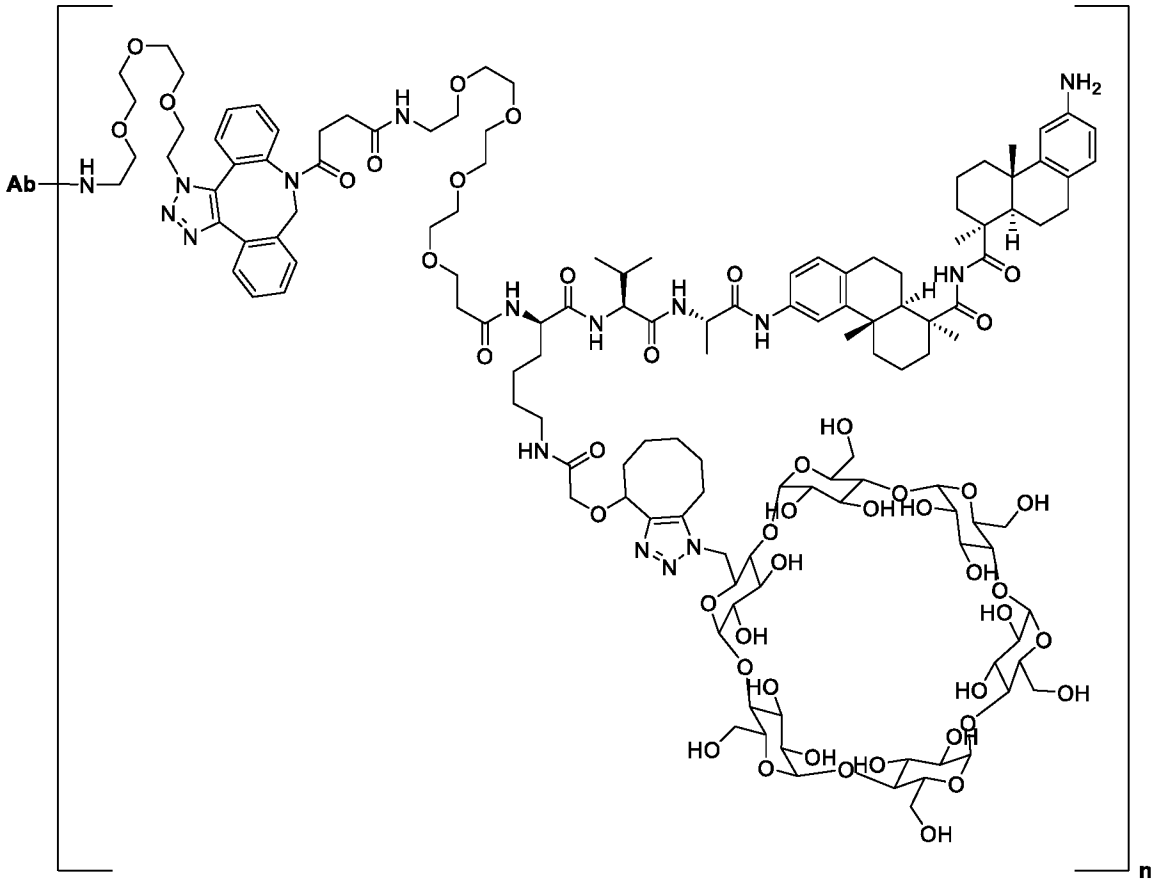
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

[0325] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58 and conjugated to the payload

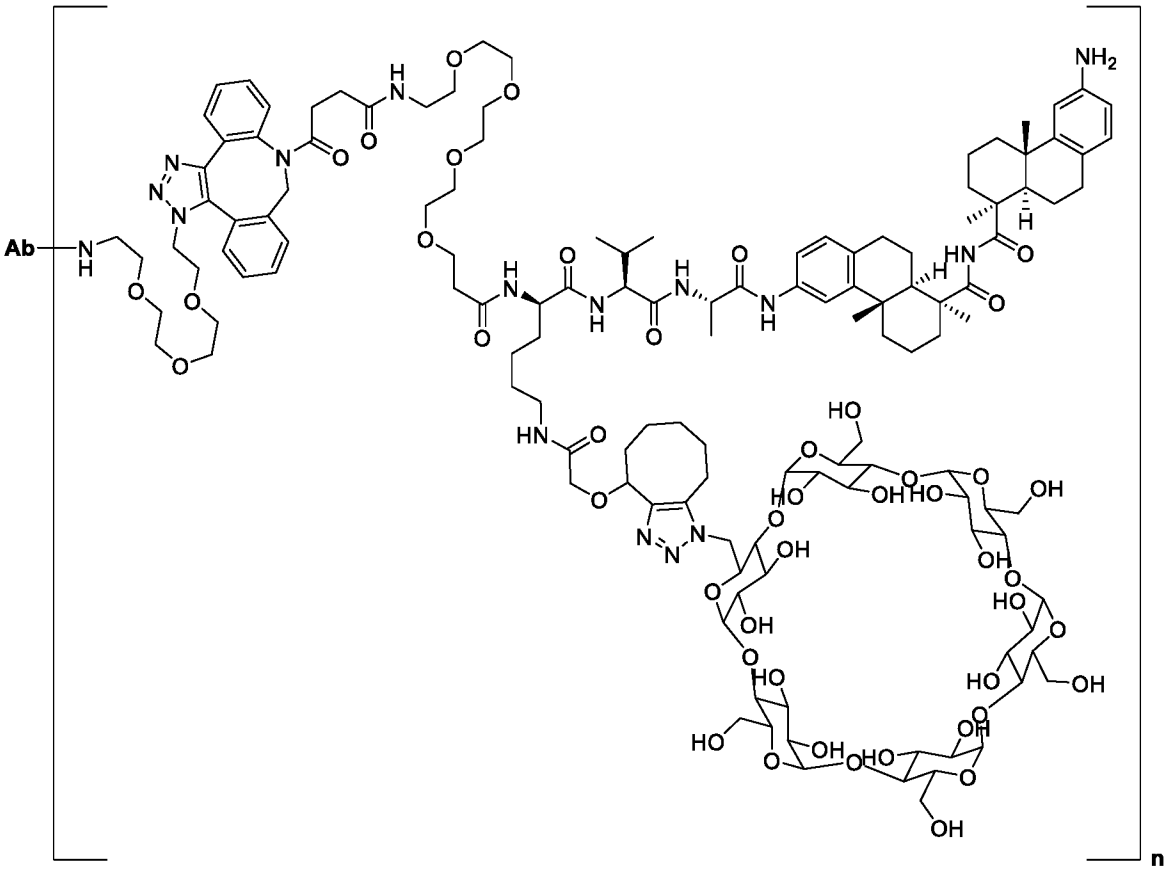


, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

[0326] An antibody-drug conjugate (ADC) according to the formulas:

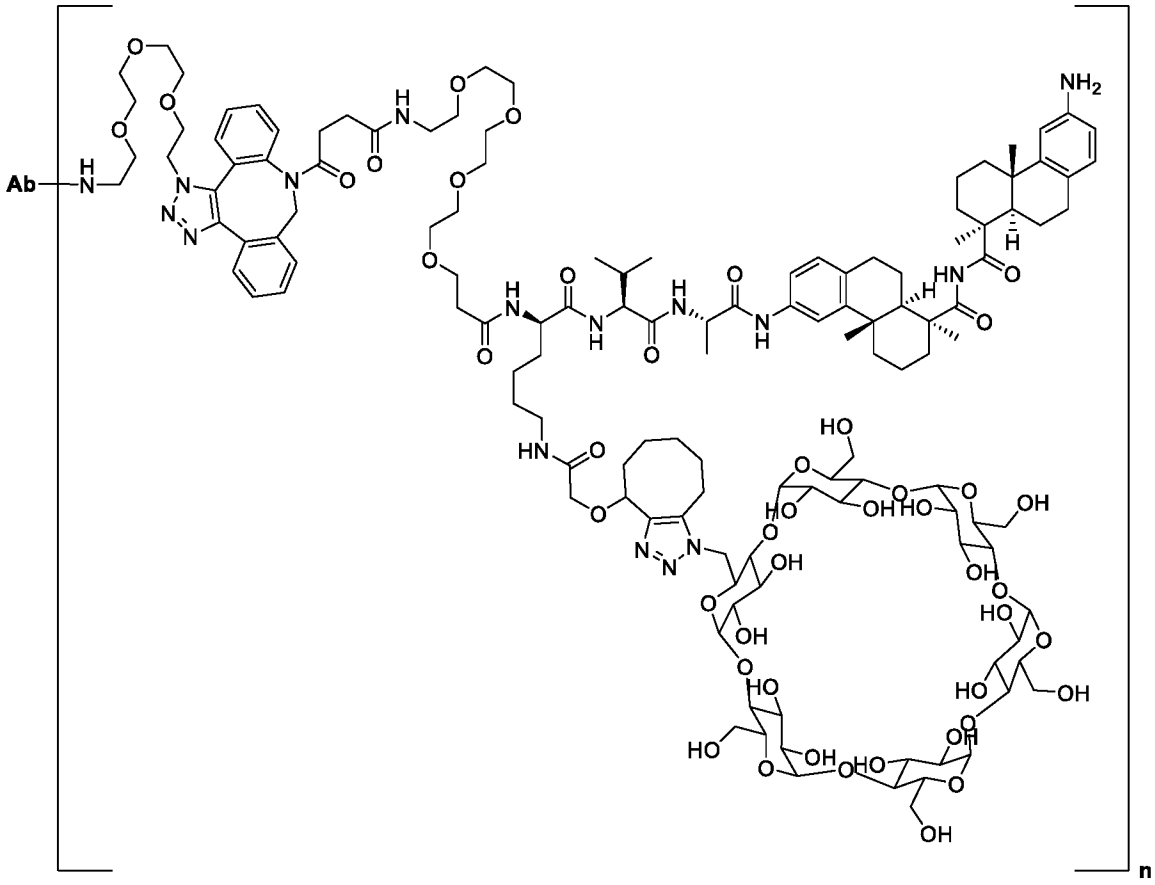


and/or

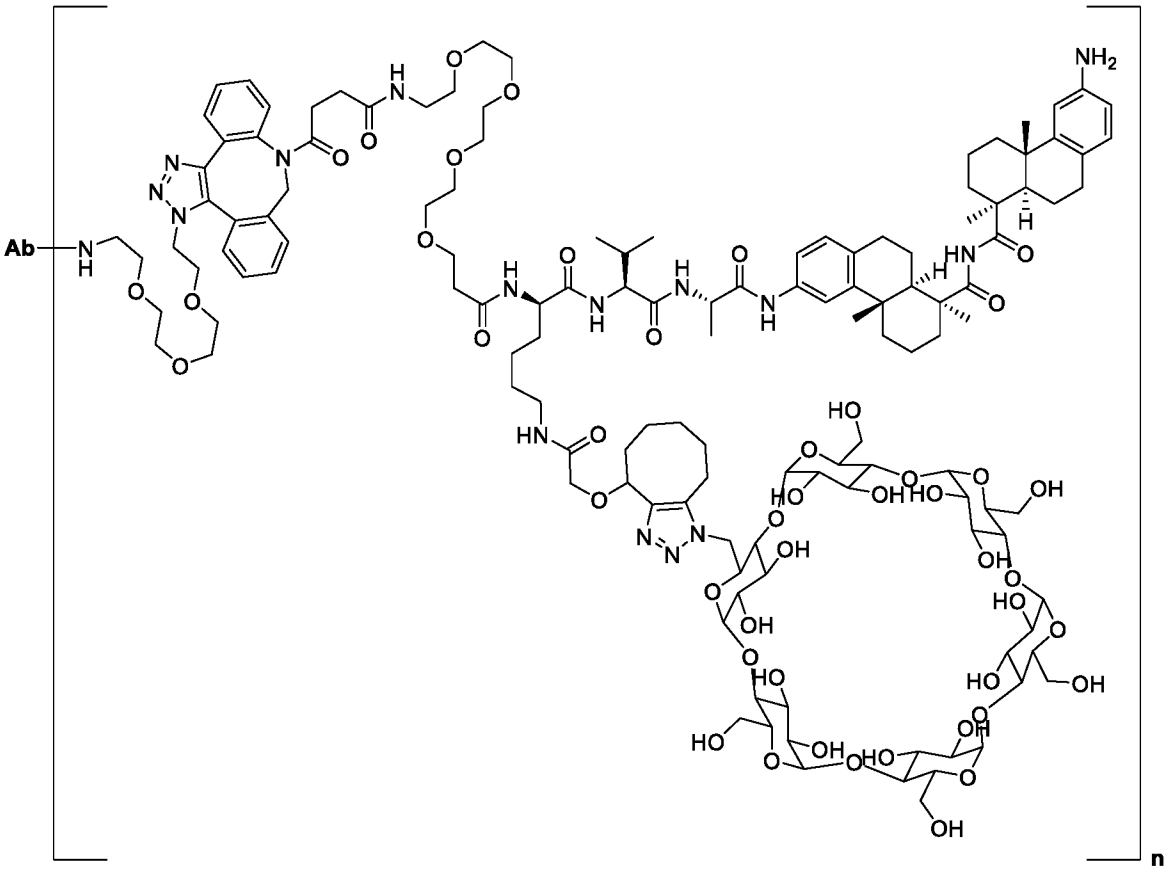


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0327] An antibody-drug conjugate (ADC) according to the formulas:

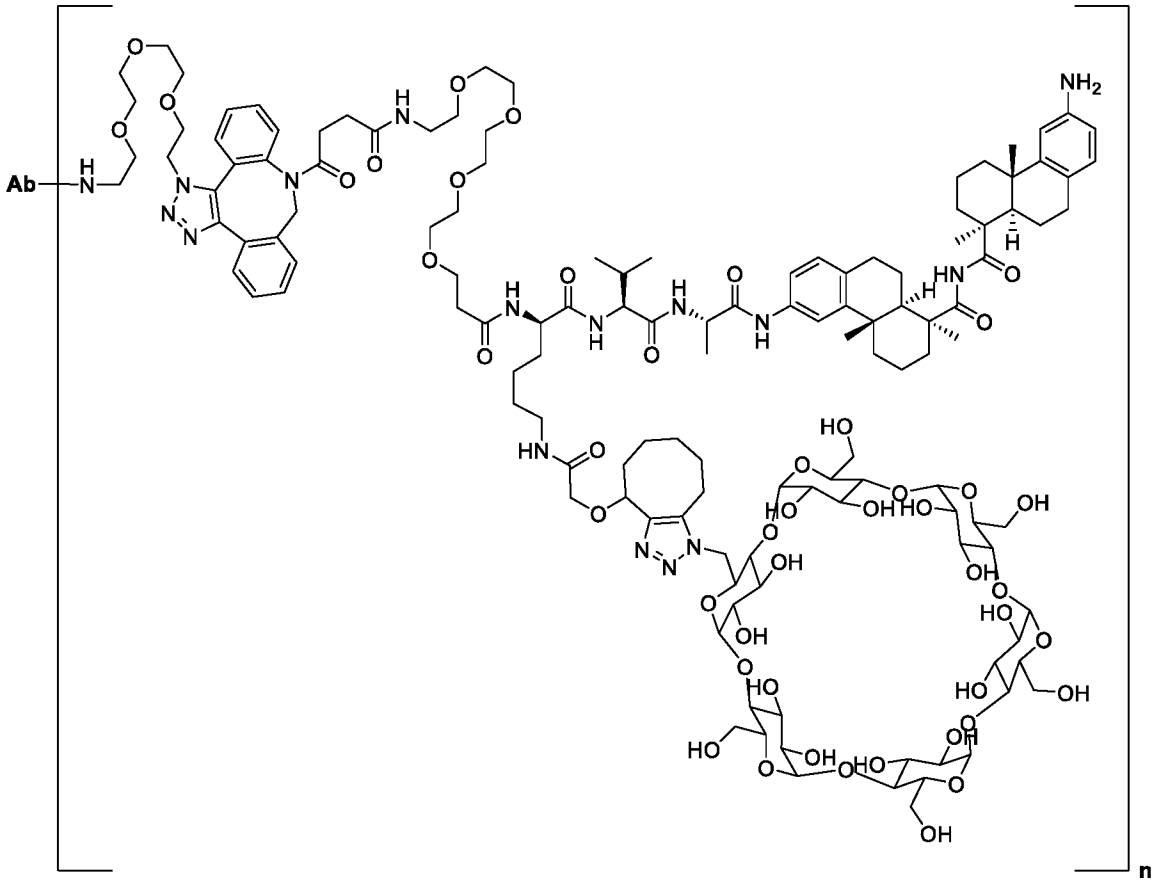


and/or

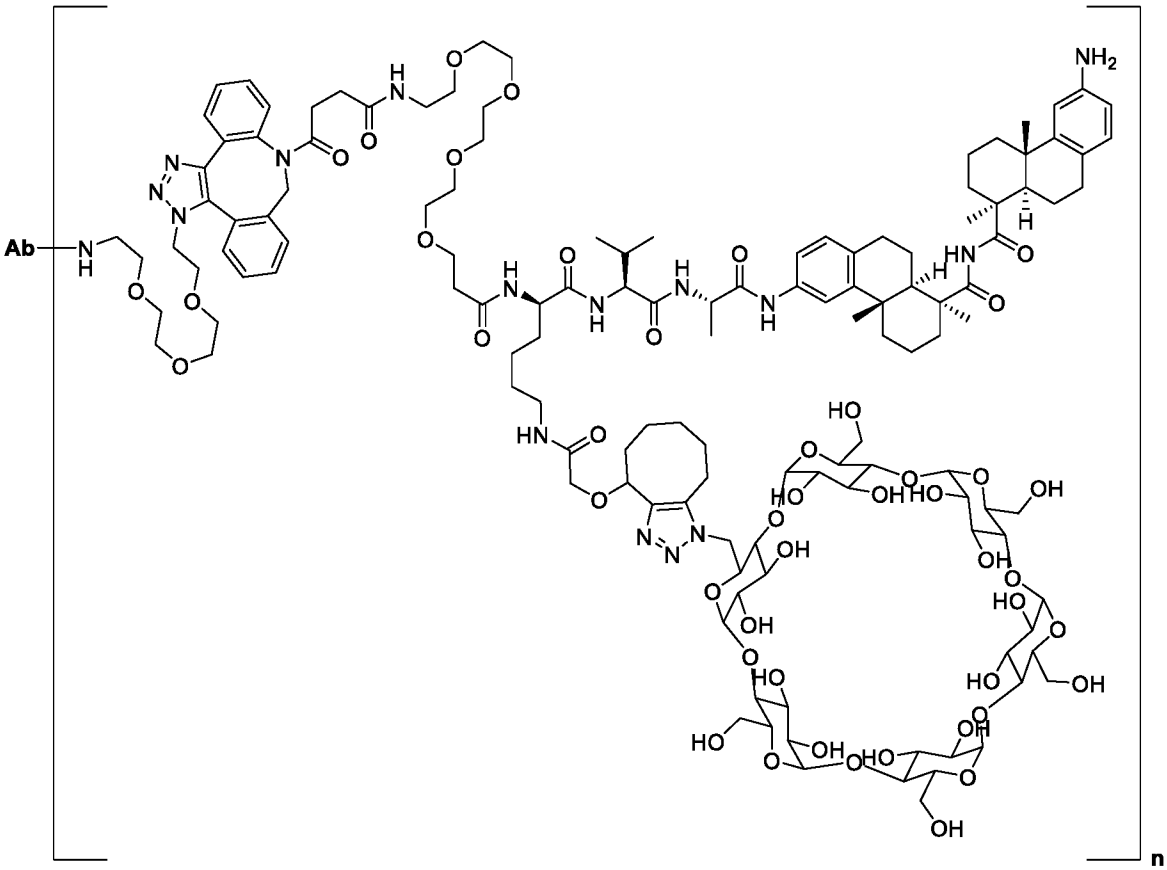


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0328] An antibody-drug conjugate (ADC) according to the formulas:

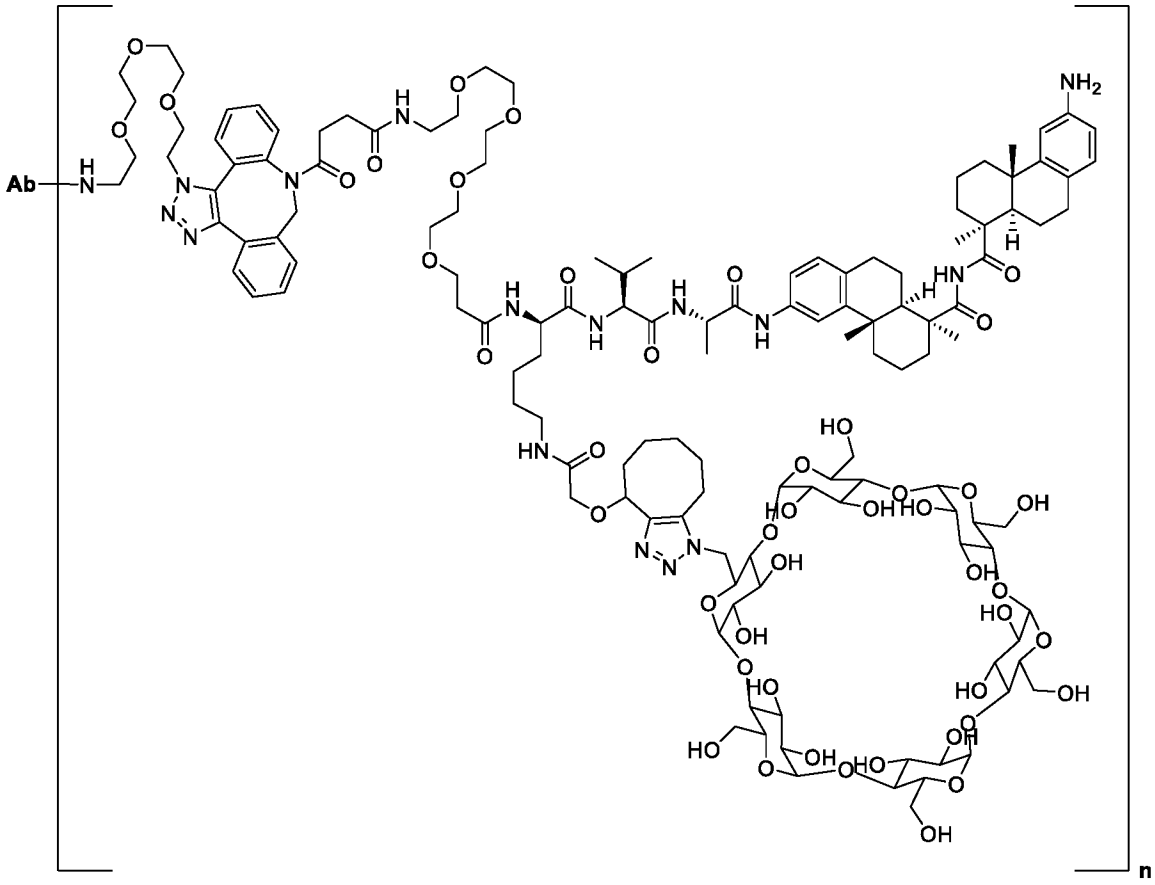


and/or

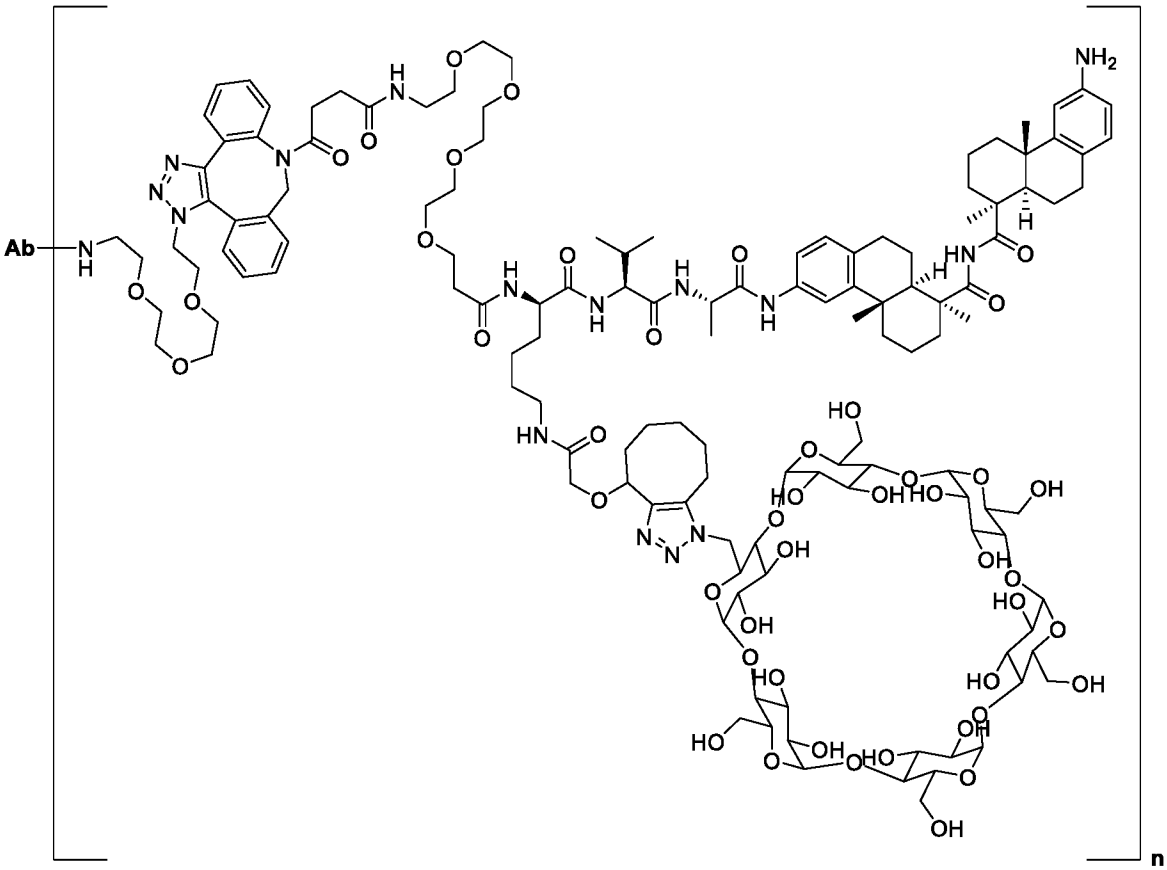


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0329] An antibody-drug conjugate (ADC) according to the formulas:

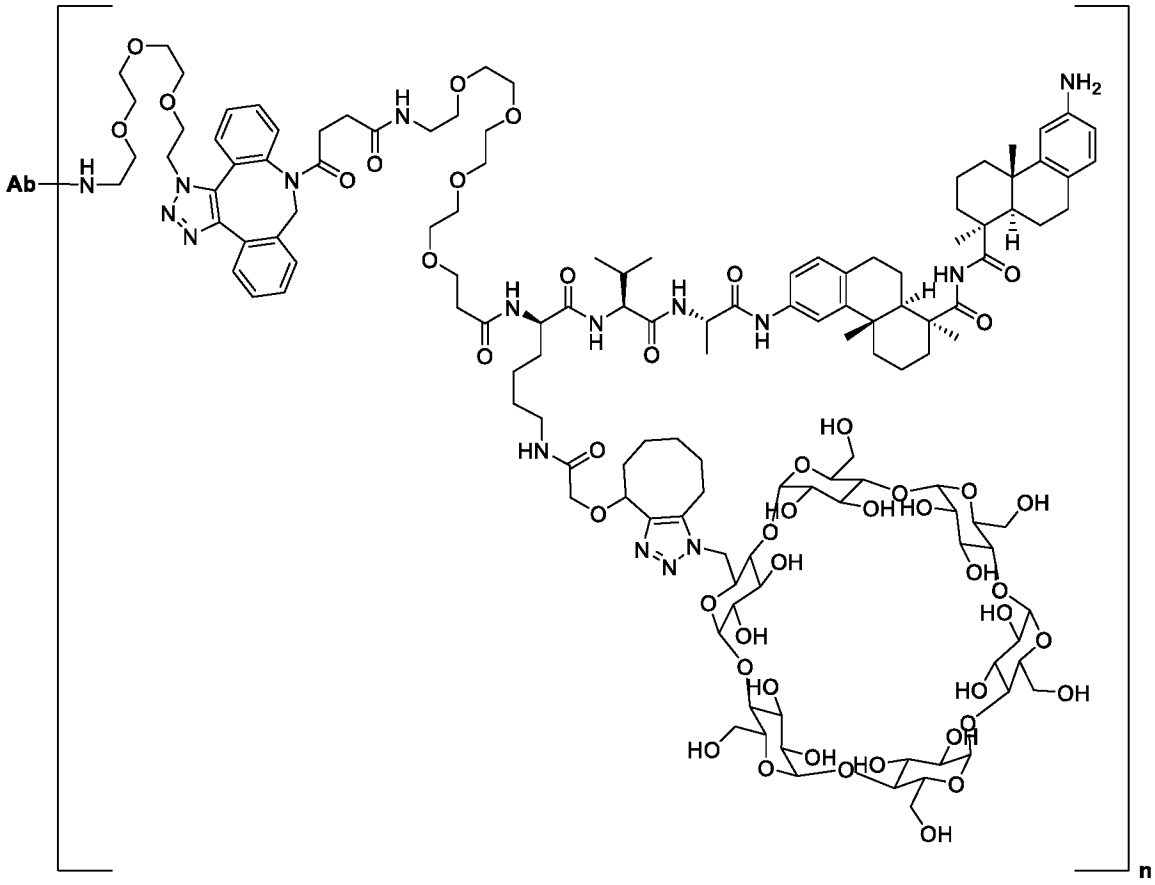


and/or

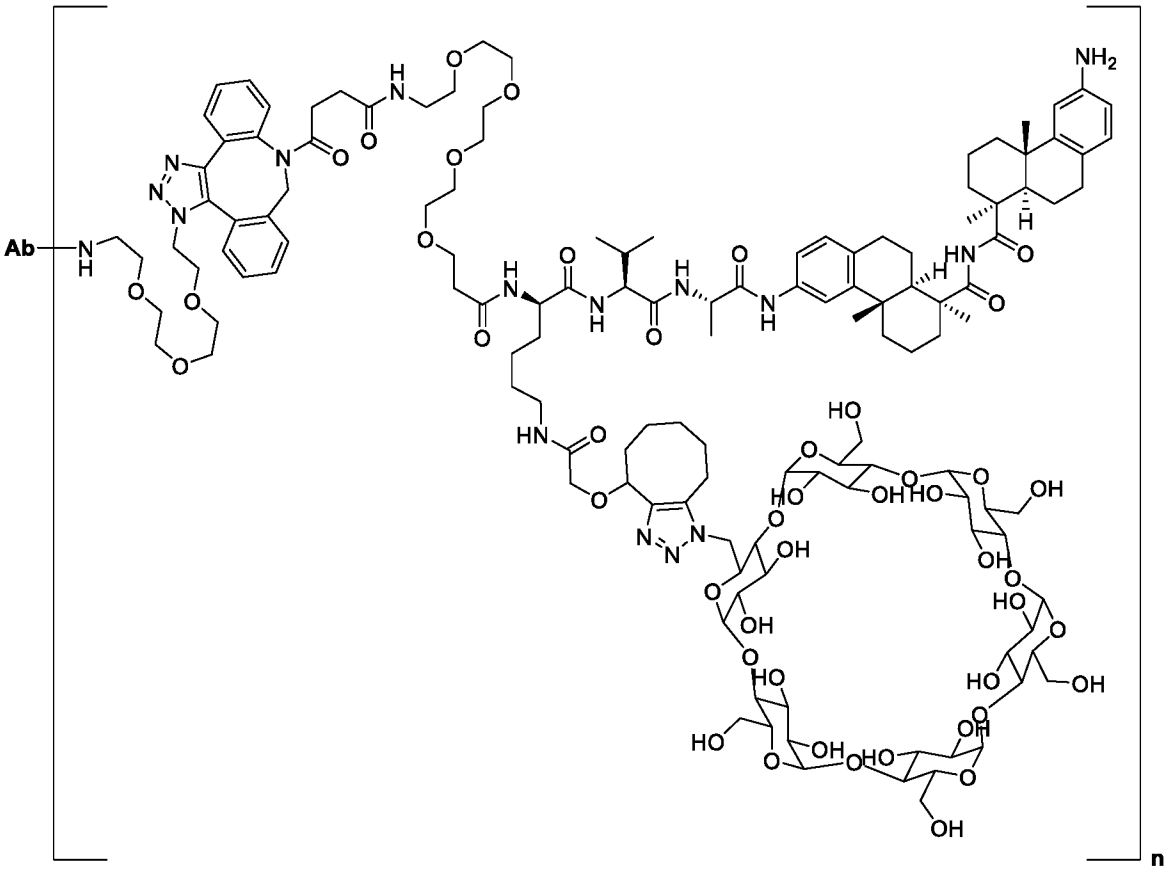


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

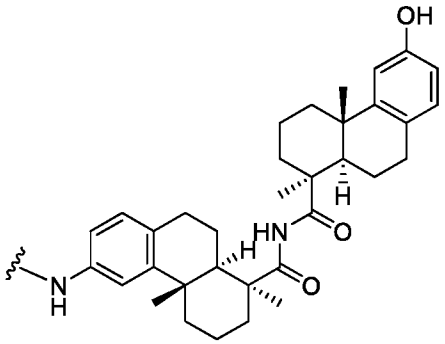
[0330] An antibody-drug conjugate (ADC) according to the formulas:



and/or

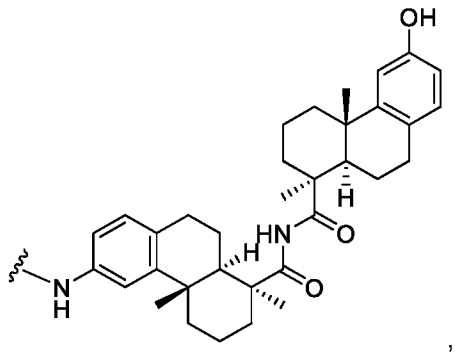


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

[0331] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, conjugated to the payload  , or pharmaceutically acceptable

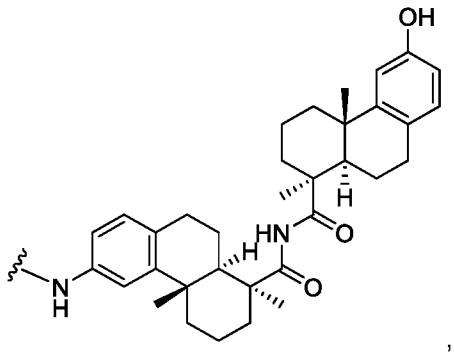
salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0332] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4 and conjugated to the payload



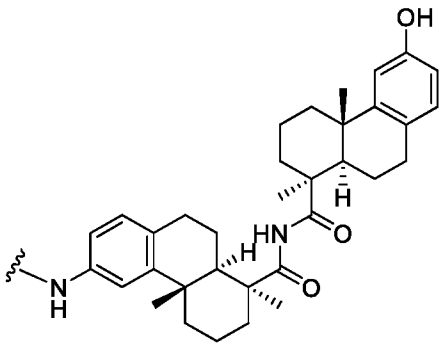
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0333] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64 and conjugated to the payload



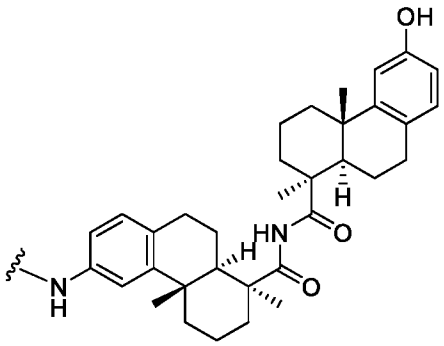
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0334] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4 and conjugated to the payload



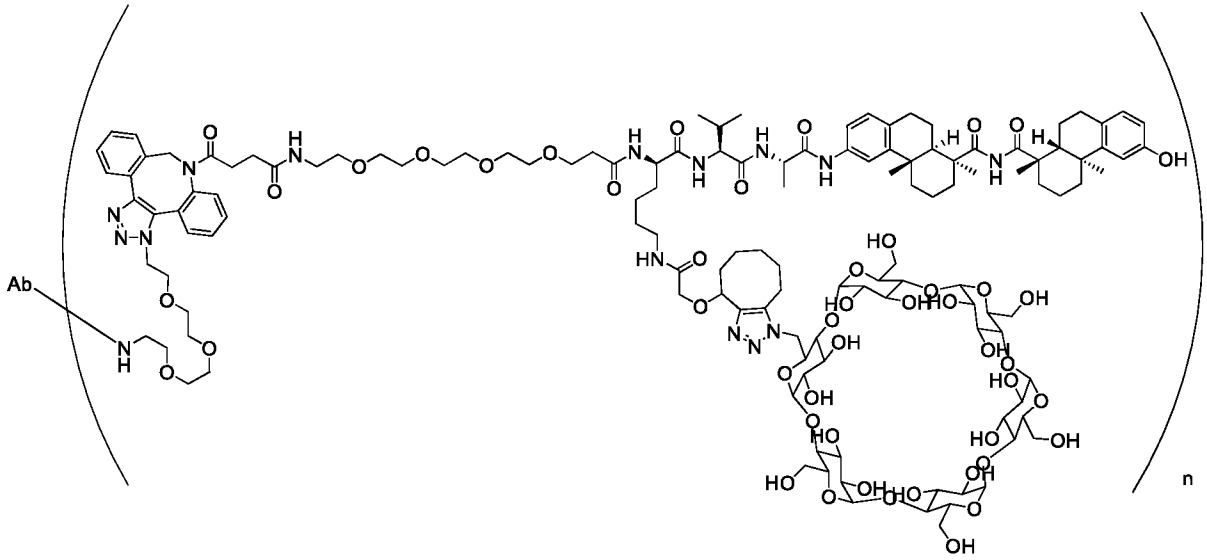
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

[0335] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58 and conjugated to the payload

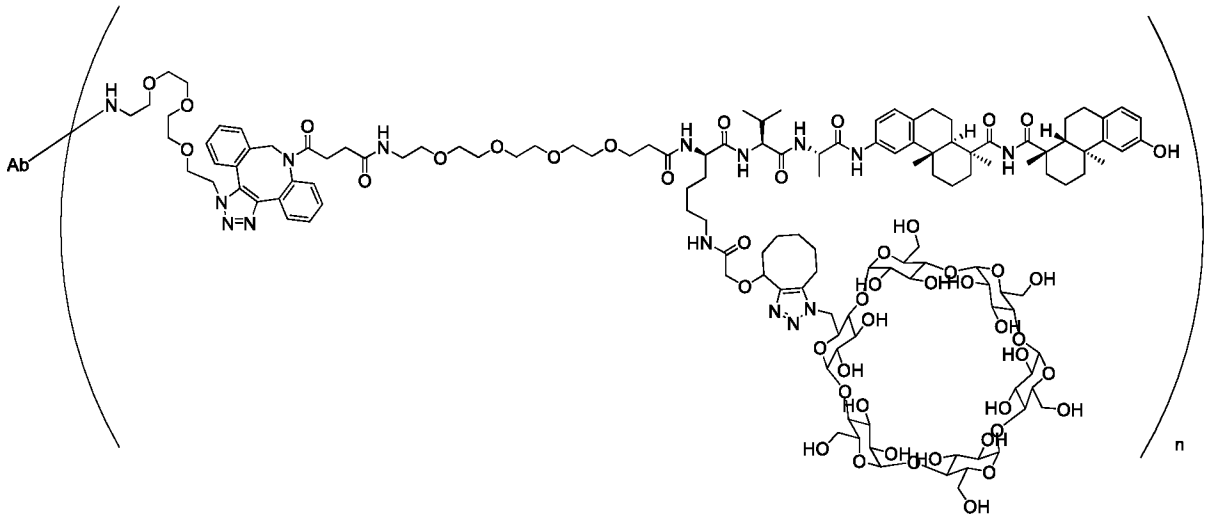


, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

[0336] An antibody-drug conjugate (ADC) according to the formulas:

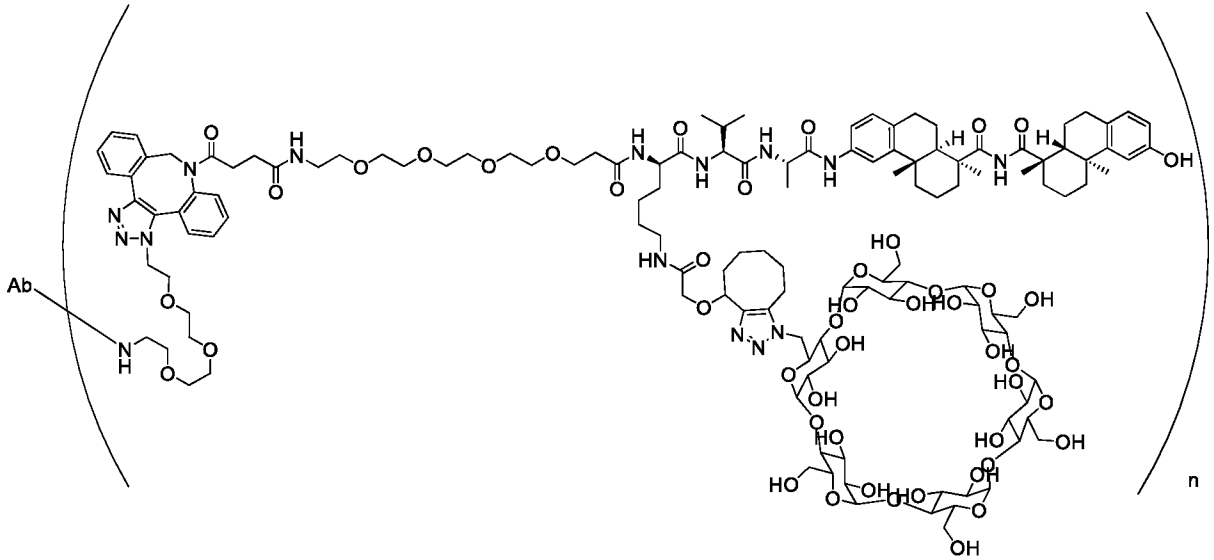


and/or

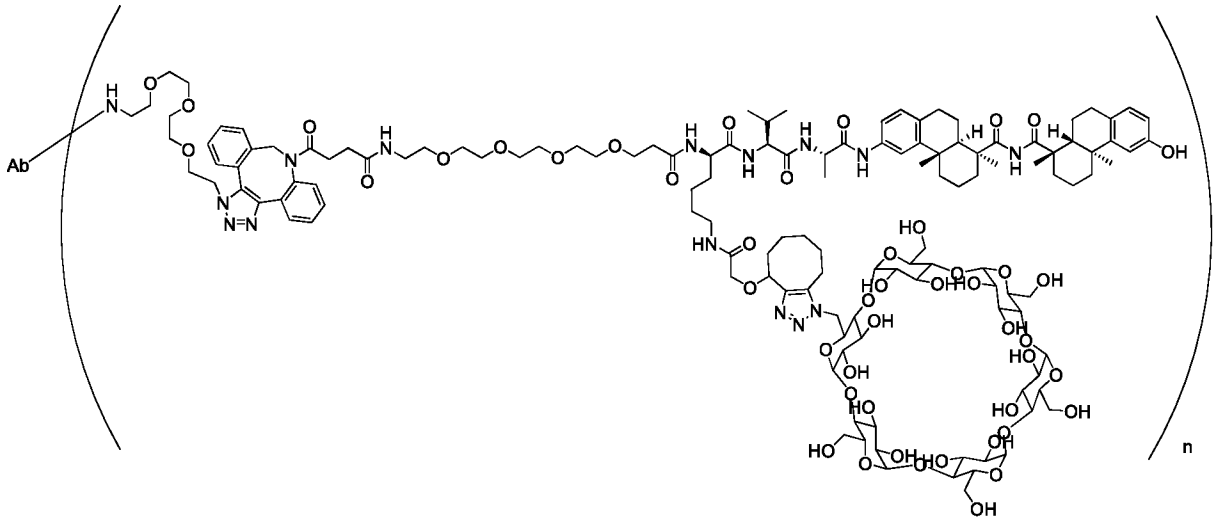


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0337] An antibody-drug conjugate (ADC) according to the formulas:

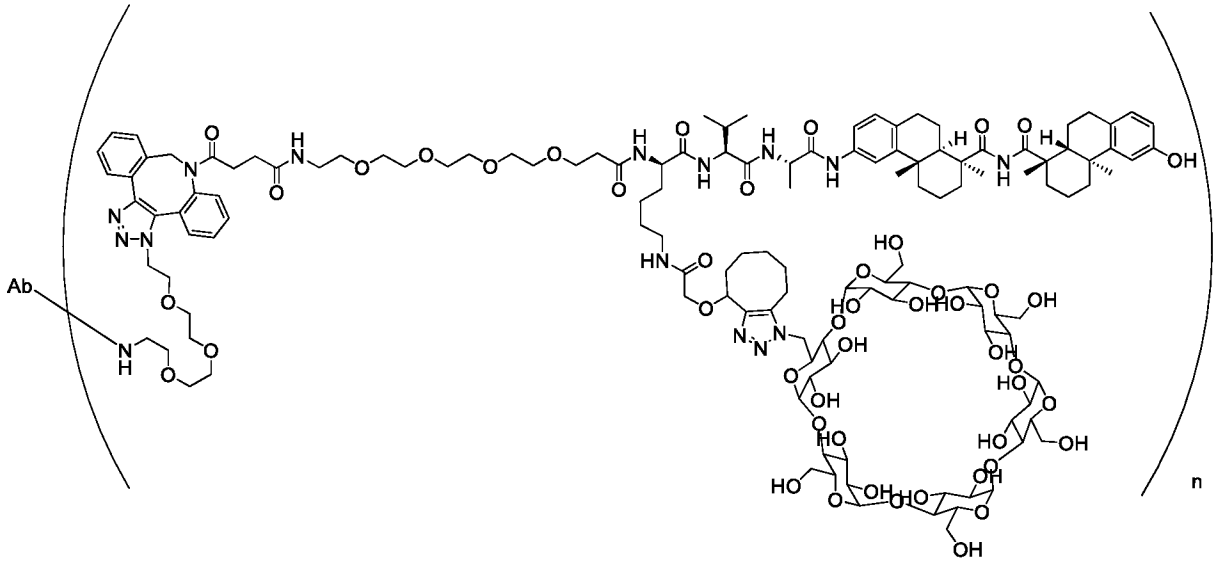


and/or

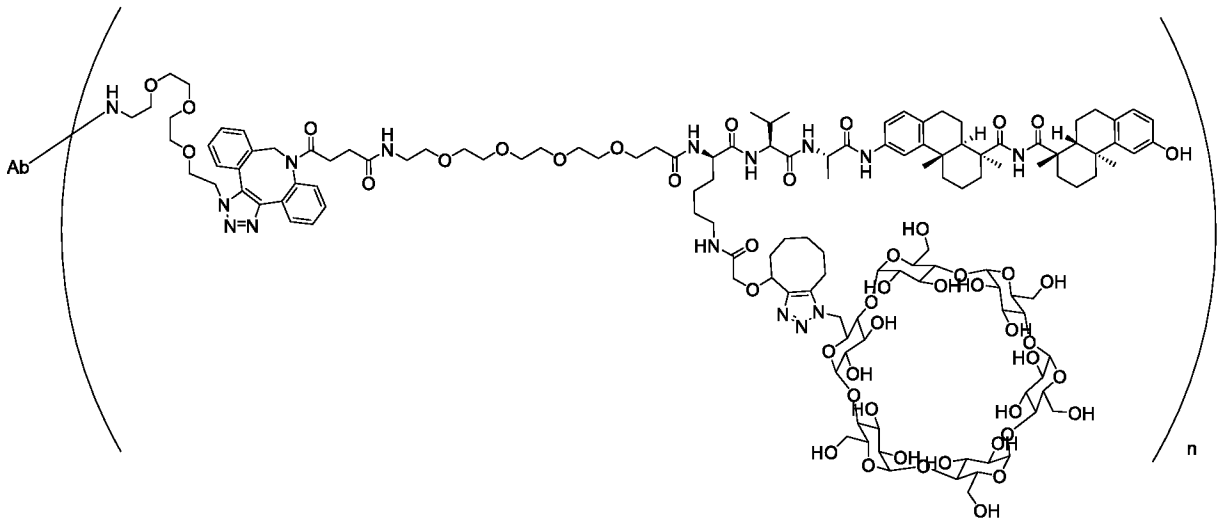


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0338] An antibody-drug conjugate (ADC) according to the formulas:



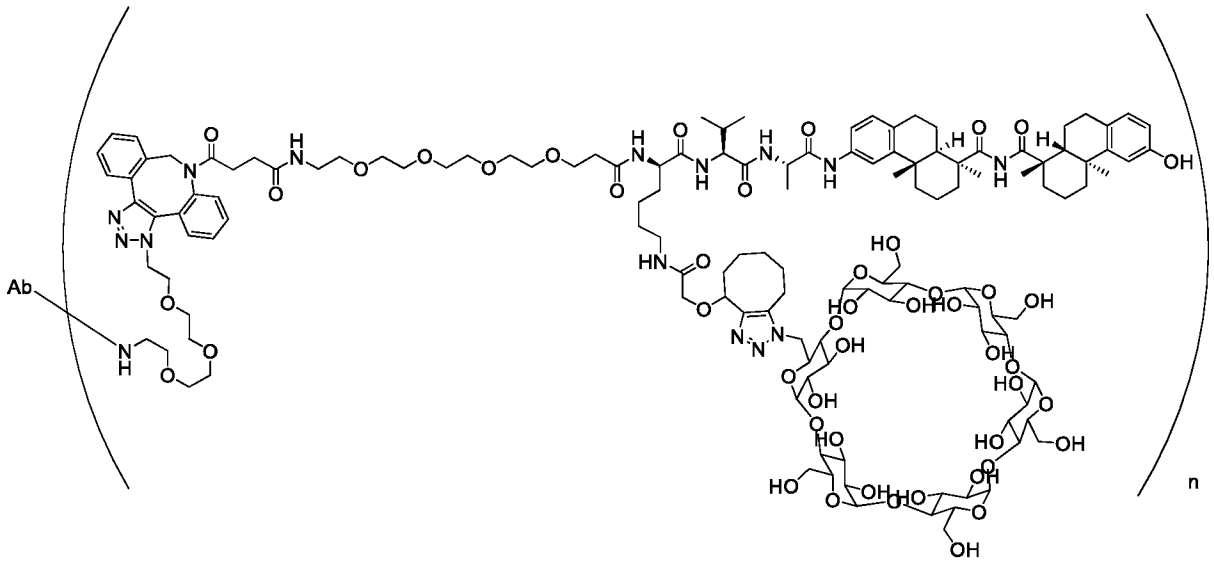
and/or



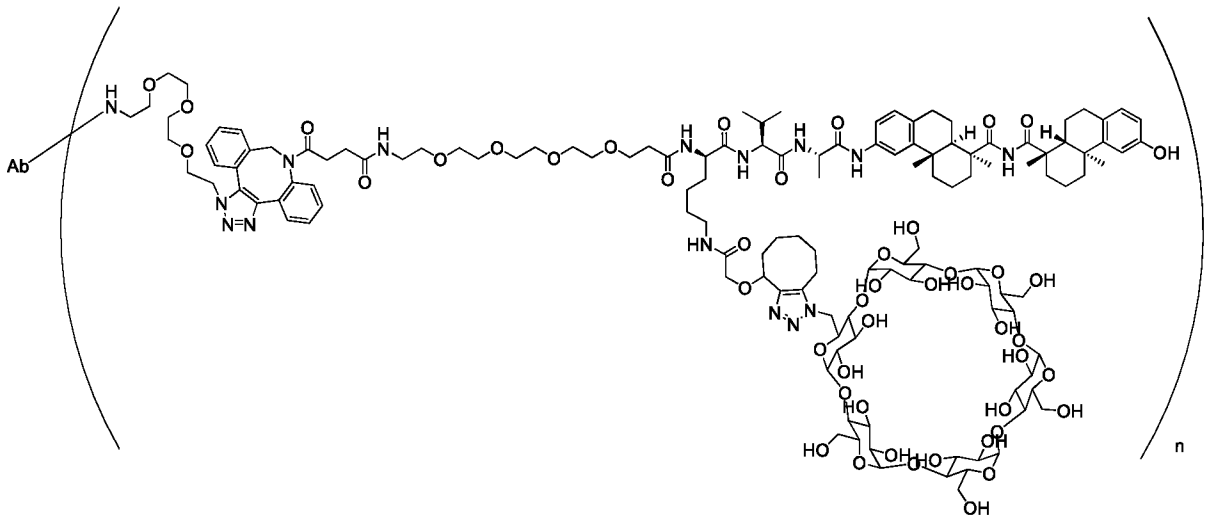
or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO:

60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0339] An antibody-drug conjugate (ADC) according to the formulas:

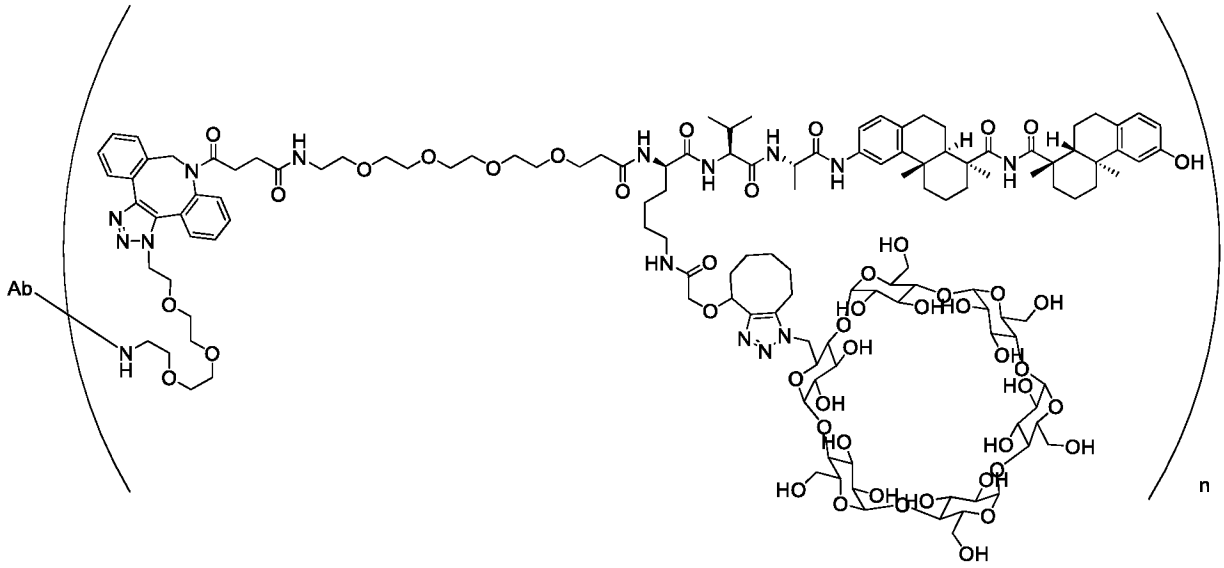


and/or

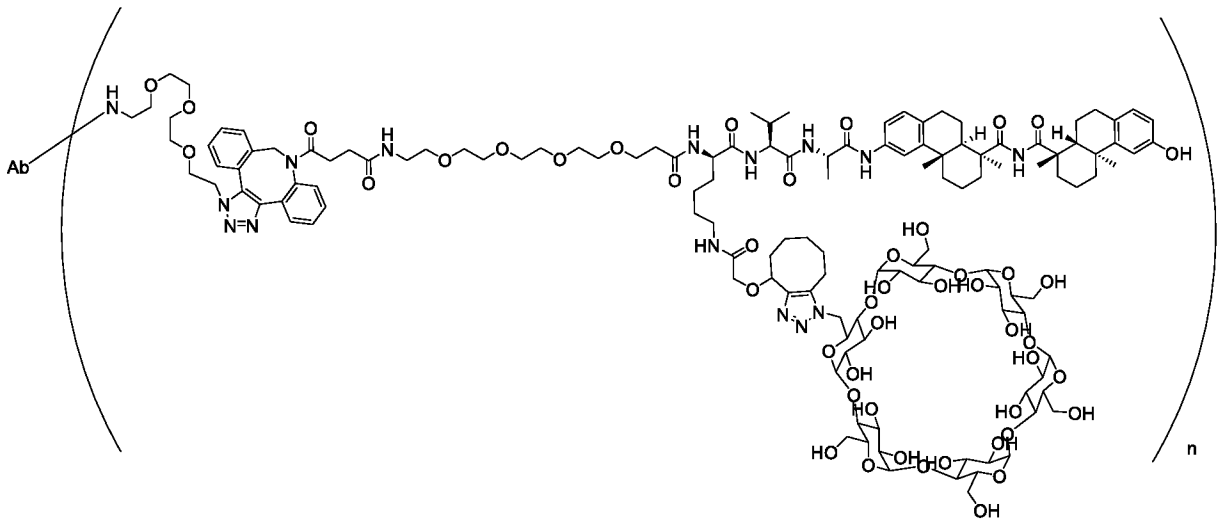


or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

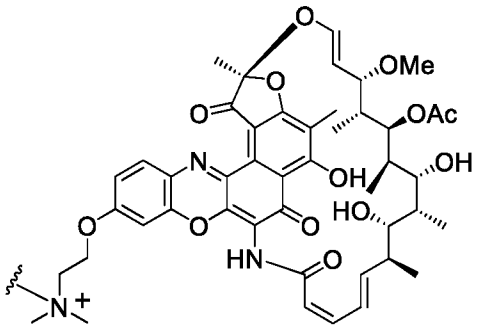
[0340] An antibody-drug conjugate (ADC) according to the formulas:

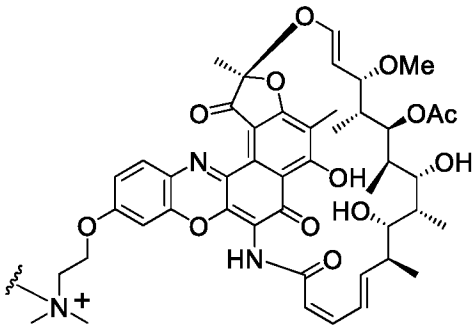


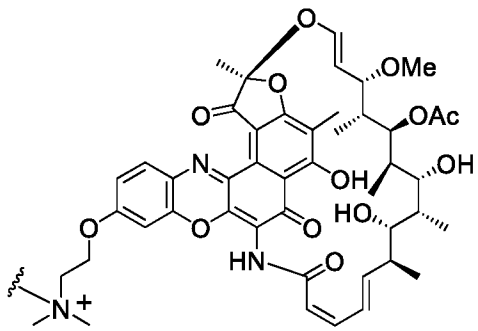
and/or



or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In som embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

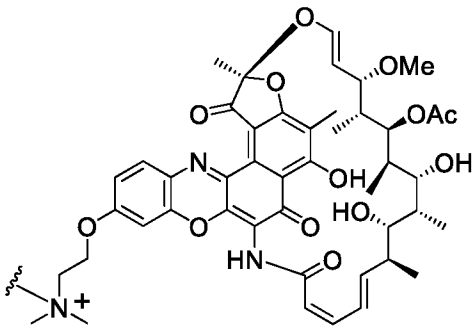
[0341] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, conjugated to the payload  , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0342] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4 and conjugated to the payload  , or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0343] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64 and conjugated to the payload  , or pharmaceutically acceptable salt, solvate or

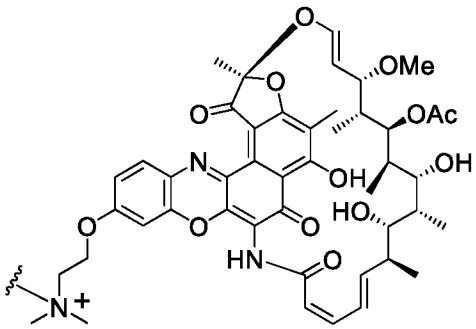
stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

[0344] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4 and conjugated to the payload



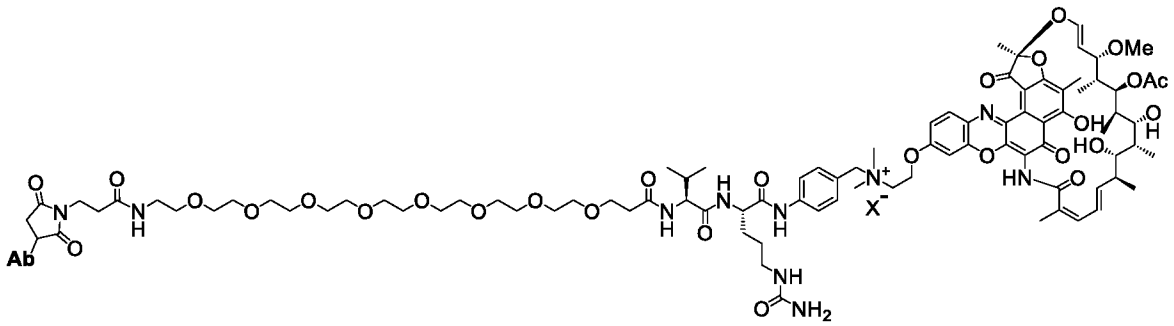
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

[0345] Provided herein is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58 and conjugated to the payload



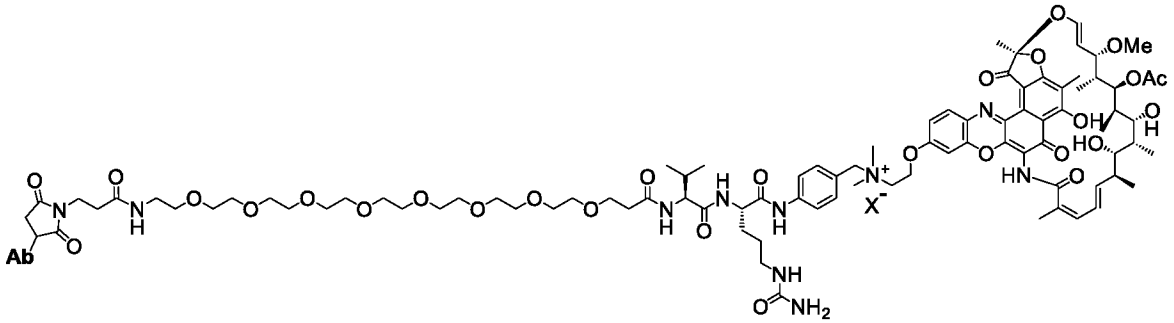
, or pharmaceutically acceptable salt, solvate or stereoisomeric form thereof. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58, and an N297Q mutation.

[0346] An antibody-drug conjugate (ADC) according to the formula:



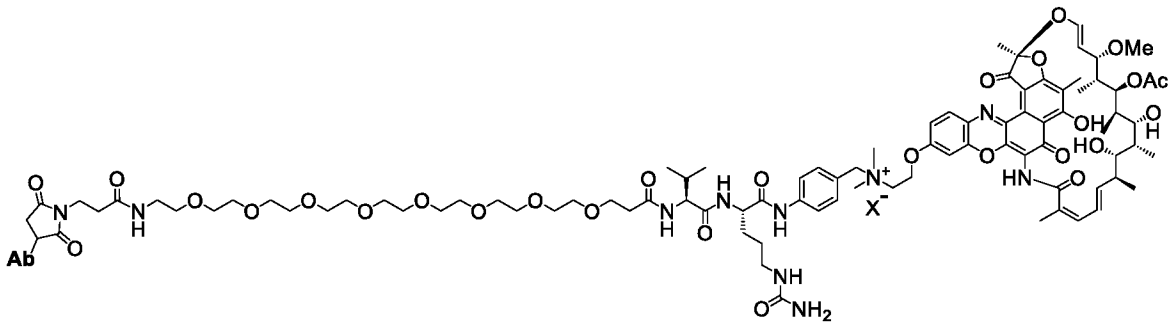
or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein X^- is a pharmaceutically acceptable counter ion and wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises an N297Q mutation.

[0347] An antibody-drug conjugate (ADC) according to the formula:



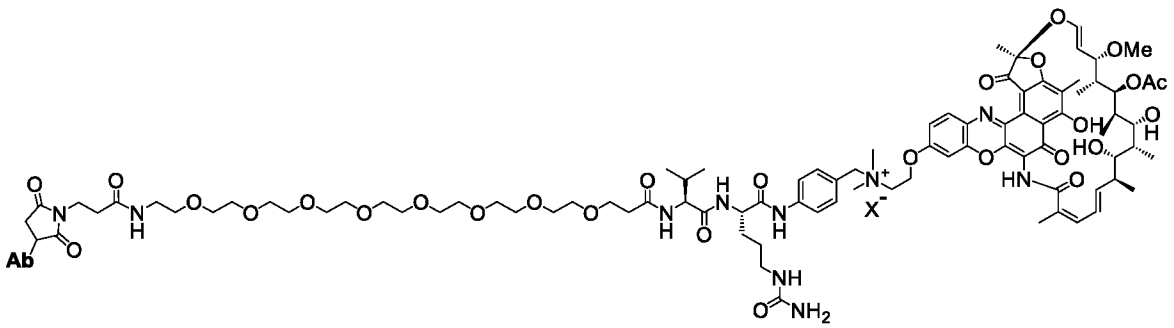
or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein X^- is a pharmaceutically acceptable counter ion and wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of six complementarity determining regions (CDRs) (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3) from Table 4, and an N297Q mutation.

[0348] An antibody-drug conjugate (ADC) according to the formula:



or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein X^- is a pharmaceutically acceptable counter ion and wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64, and an N297Q mutation.

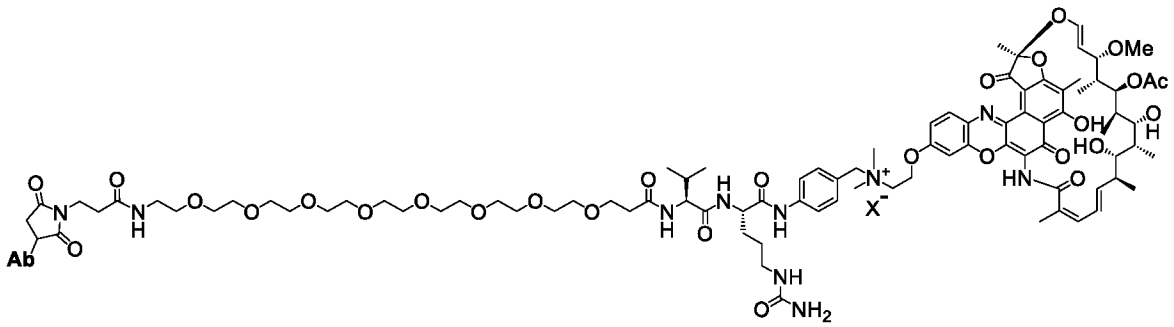
[0349] An antibody-drug conjugate (ADC) according to the formula:



or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein X^- is a pharmaceutically acceptable counter ion and wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4.

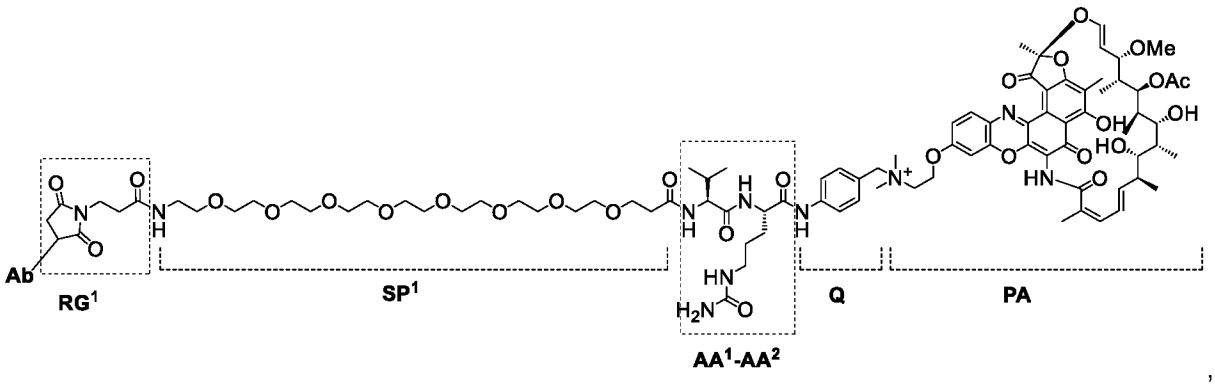
In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a set of variable regions heavy chain variable regions (HCVR) and a set of light chain variable regions (LCVR) from Table 4, and an N297Q mutation.

[0350] An antibody-drug conjugate (ADC) according to the formula:



or pharmaceutically acceptable salt, solvate, stereoisomeric form, or regioisomer thereof, or mixtures thereof; wherein $\mathbf{X}^-$ is a pharmaceutically acceptable counter ion and wherein the antibody is an anti-MSR1 antibody, or antigen binding fragment thereof, comprising a heavy chain variable region (HCVR) comprising SEQ ID NO: 50 and a light chain variable region (LCVR) SEQ ID NO: 58. In some of such embodiments, the drug antibody ratio (DAR) is from 1-4. In some embodiments, the DAR is 1. In some embodiments, the DAR is 2. In some embodiments, the DAR is 3. In some embodiments, the DAR is 4. In some embodiments, the anti-MSR1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 50; a light chain variable region (LCVR) SEQ ID NO: 58; and an N297Q mutation.

[0351] As used herein, in certain embodiments, where an amino acid is bonded to the spacer $\mathbf{SP^1}$, e.g., in the moiety $-\mathbf{SP^1}-\mathbf{AA^1}-\mathbf{AA^2}-$, the amino acid $\mathbf{AA^1}$ is bonded via its amino group to the spacer $\mathbf{SP^1}$. The spacer residue includes portions of a functional group that formed the bond with the amino group in the amino acid. By way of example only, in the following structure:



,

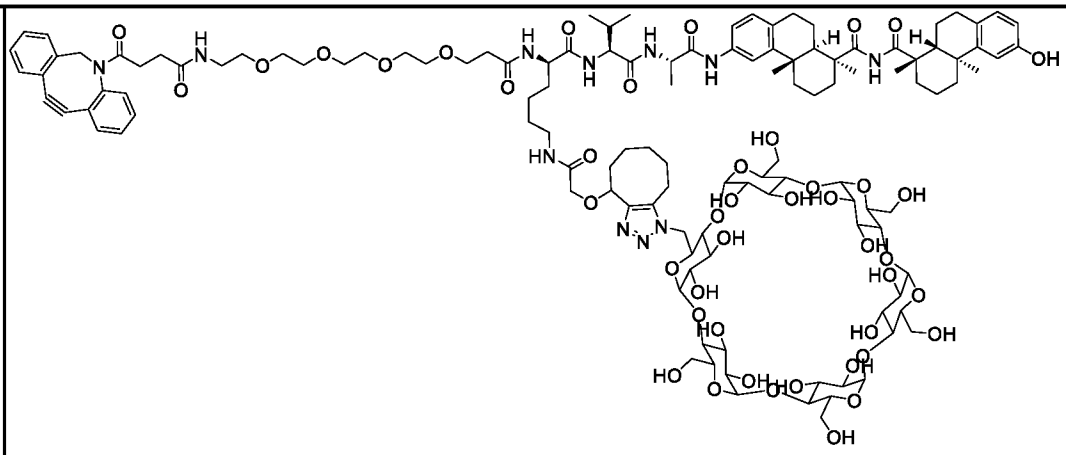
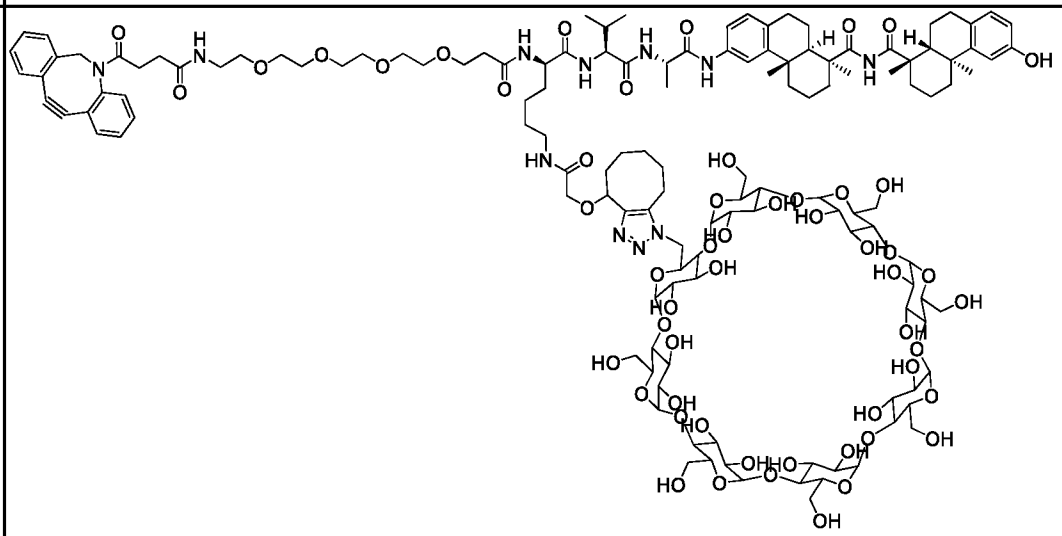
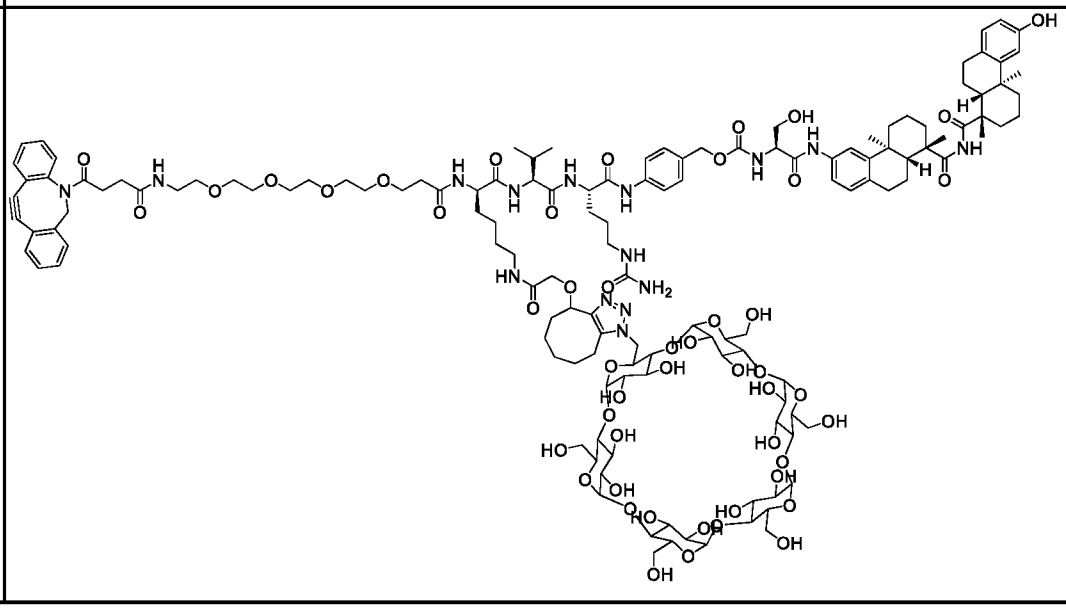
the valine residue is bonded to **SP**[1] as shown, and the **SP**[1] residue, in this example, comprises a (C=O) moiety. Also, in this example, the **SP**[1] residue comprises a functional group suitable for forming a bond with the reactive group and the **SP**[1] residue comprises a –NH– moiety. Those of skill in the art will recognize that similar groupings can apply to all other formulae described herein.

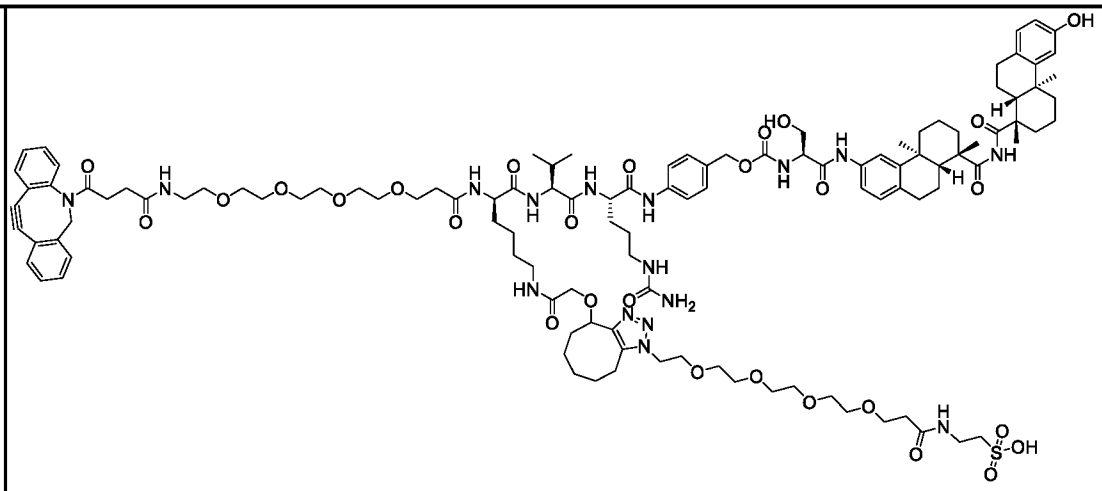
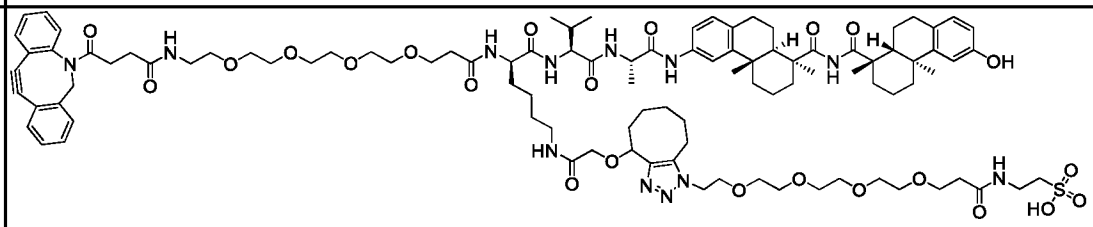
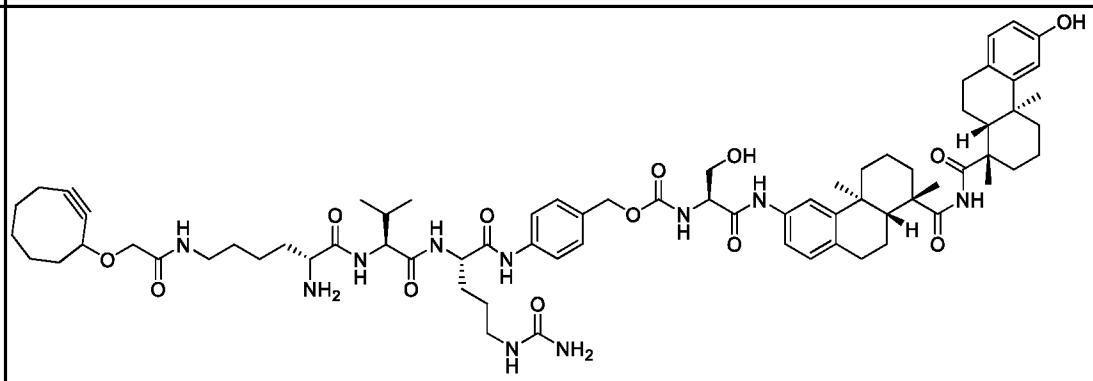
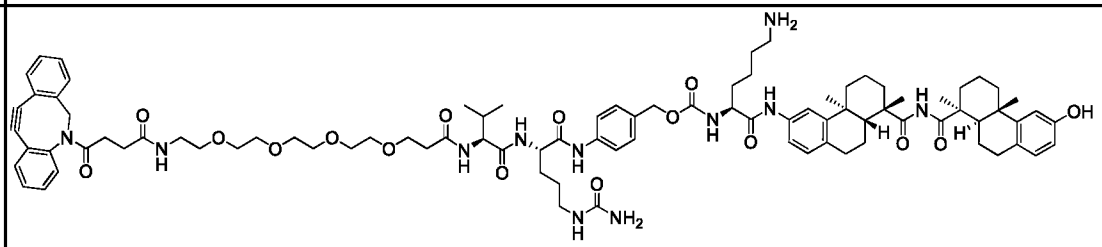
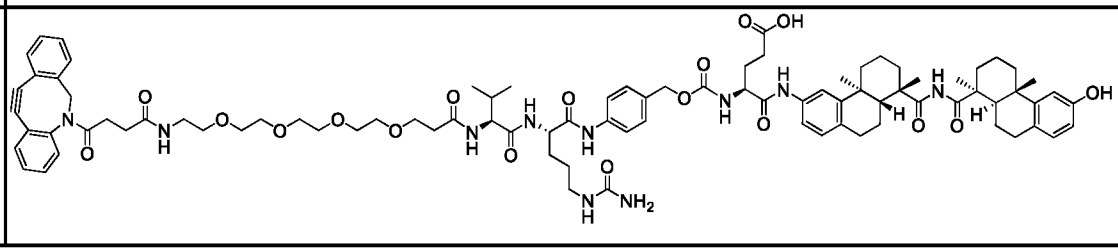
[0352] The antibody drug conjugates described herein can be prepared using conjugation conditions known to those of ordinary skill in the art, (see, *e.g.*, Doronina *et al. Nature Biotechnology* 2003, 21, 7, 778, which is incorporated herein by reference in its entirety). In some embodiments an ADC is prepared by contacting an anti-MSR1 antibody or an antigen-binding fragment thereof with a compound comprising the desired linker and payload, wherein said linker possesses a moiety that is reactive with the antibody or antigen-binding protein, *e.g.*, at the desired residue of the antibody or antigen-binding protein. Exemplary conditions are described in the Examples below.

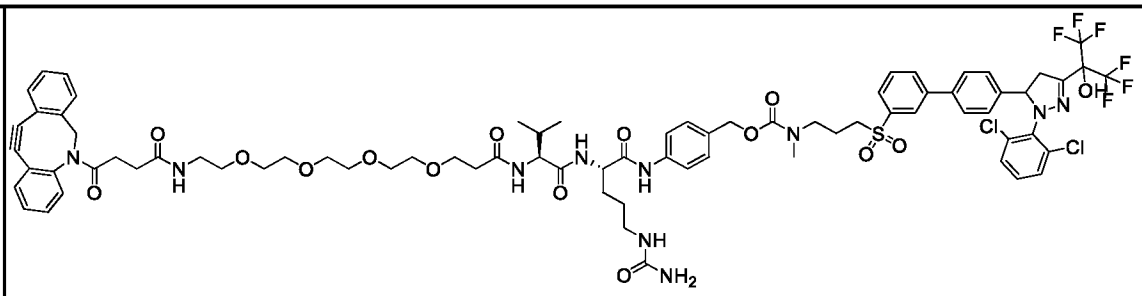
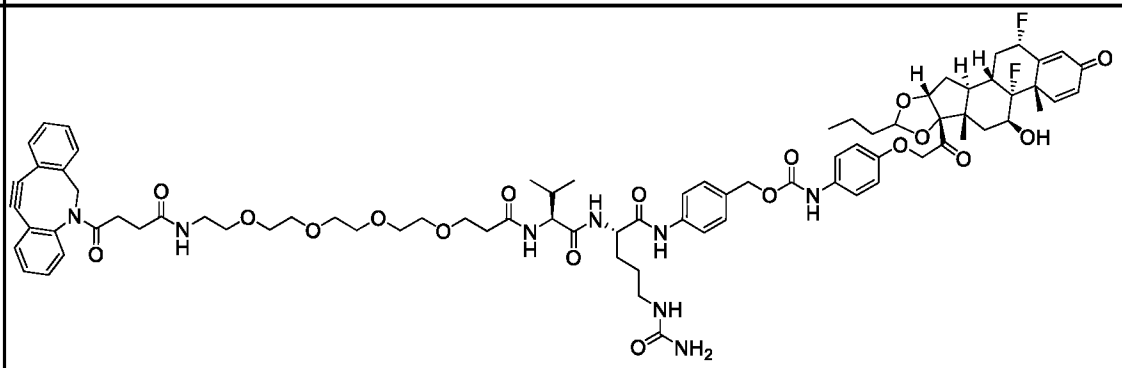
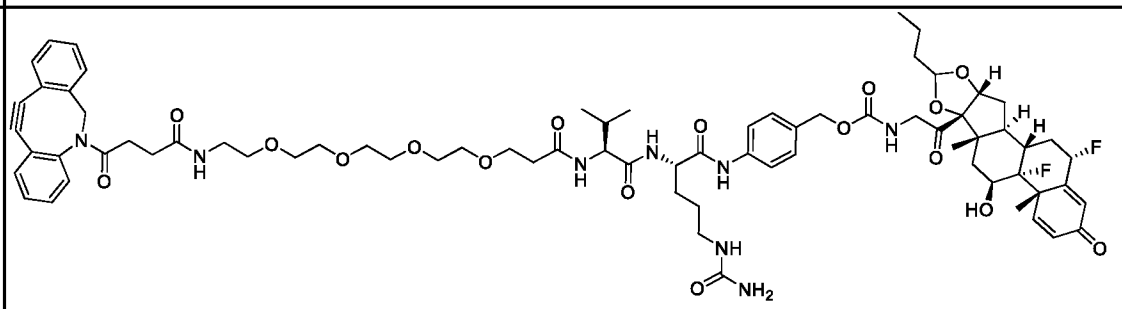
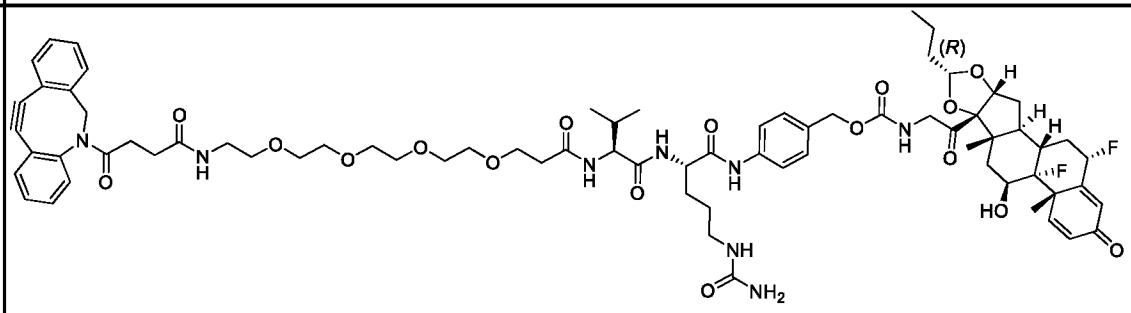
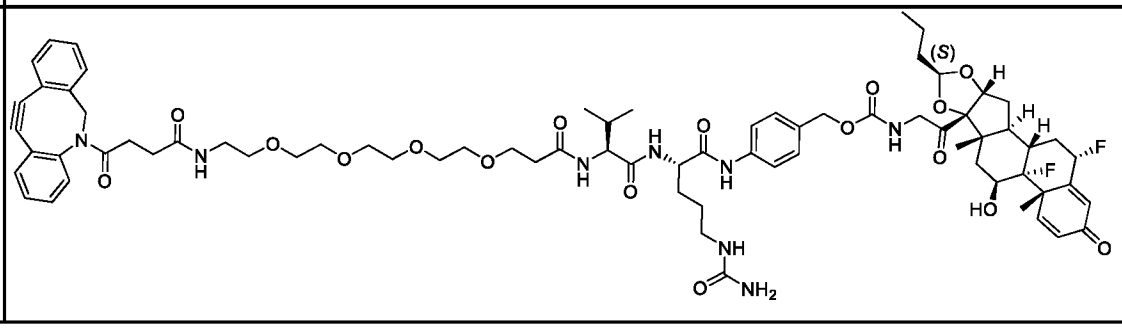
Preparation of antibody-drug conjugates

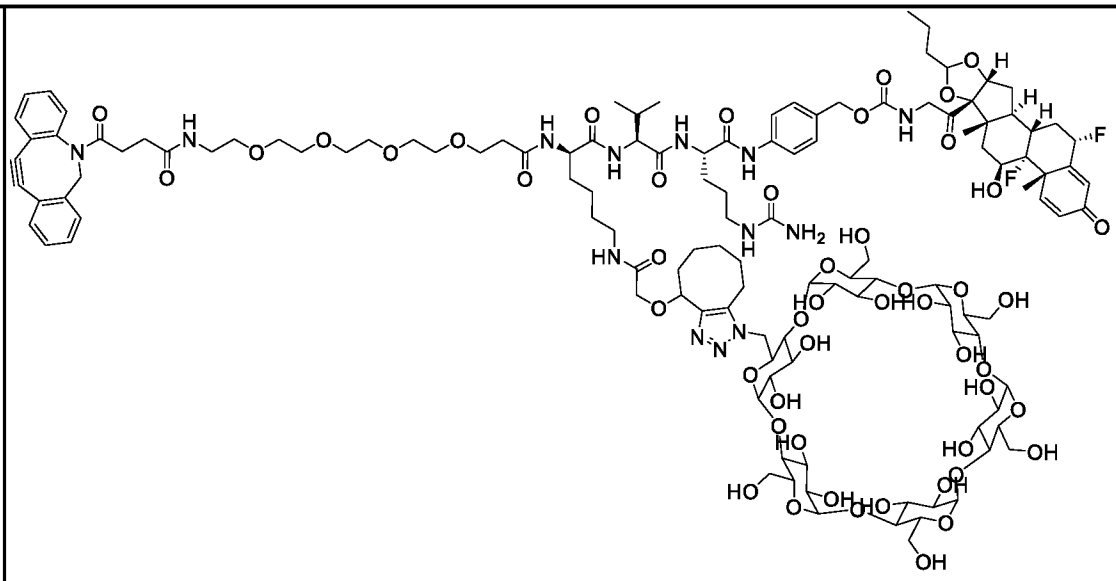
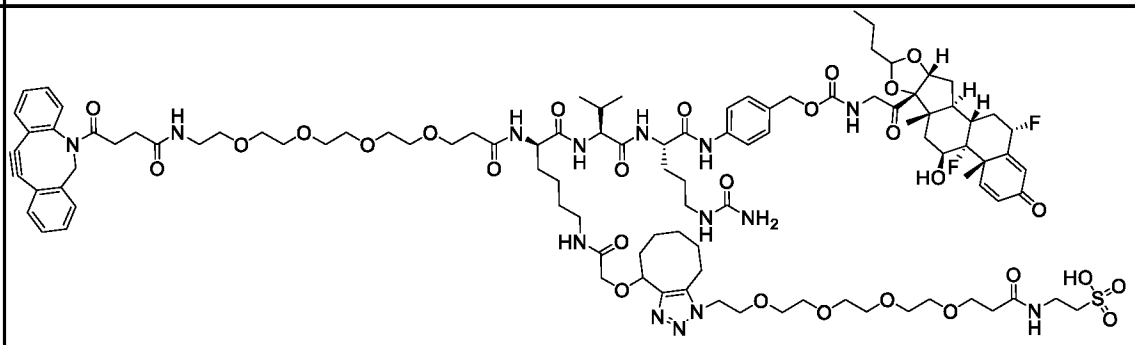
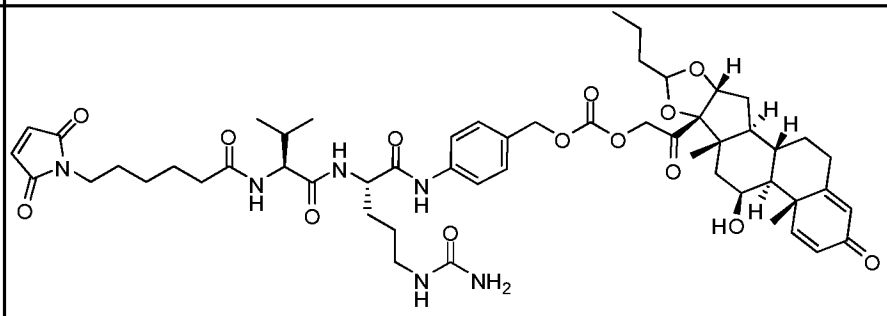
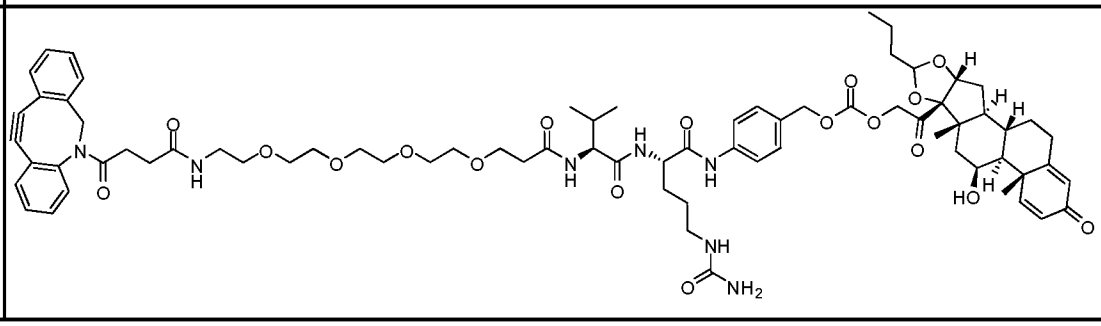
[0353] In some embodiments, provided herein are processes for preparing an antibody-drug conjugate comprising contacting an anti-MSR1 antibody, or a PEG-modified anti-MSR1 antibody, or an antigen binding fragment thereof with a linker-payload or a linker-spacer-payload selected from Table 3. Also provided herein is an antibody drug conjugate prepared by conjugating an anti-MSR1 antibody or a PEG-modified anti-MSR1 antibody, or an antigen binding fragment thereof, with a linker payload, or a linker-spacer-payload. or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, selected from Table 3.

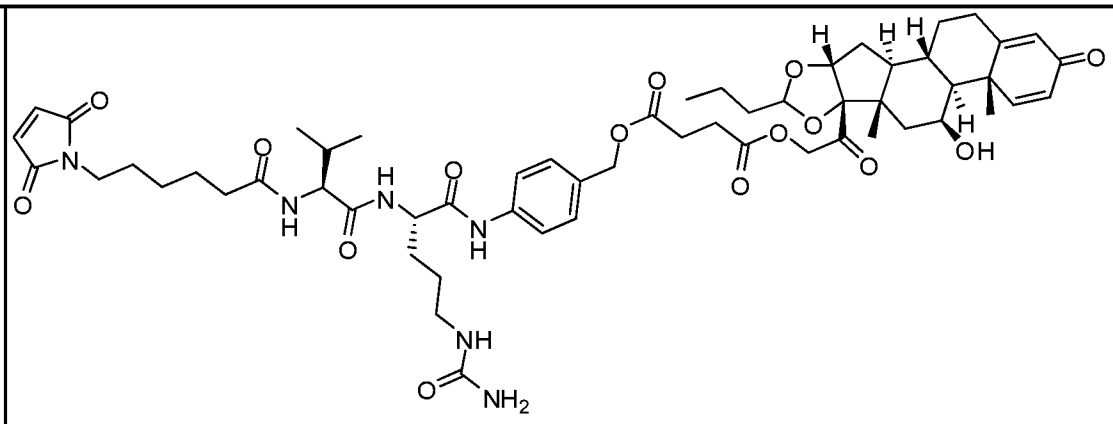
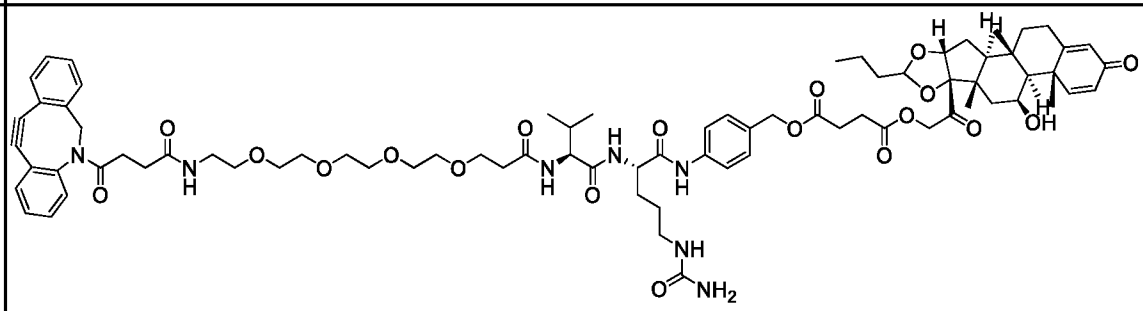
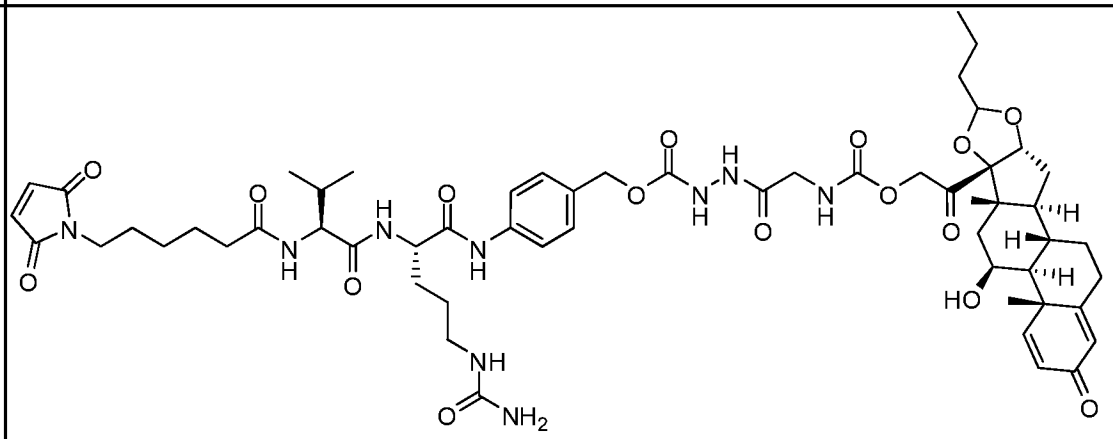
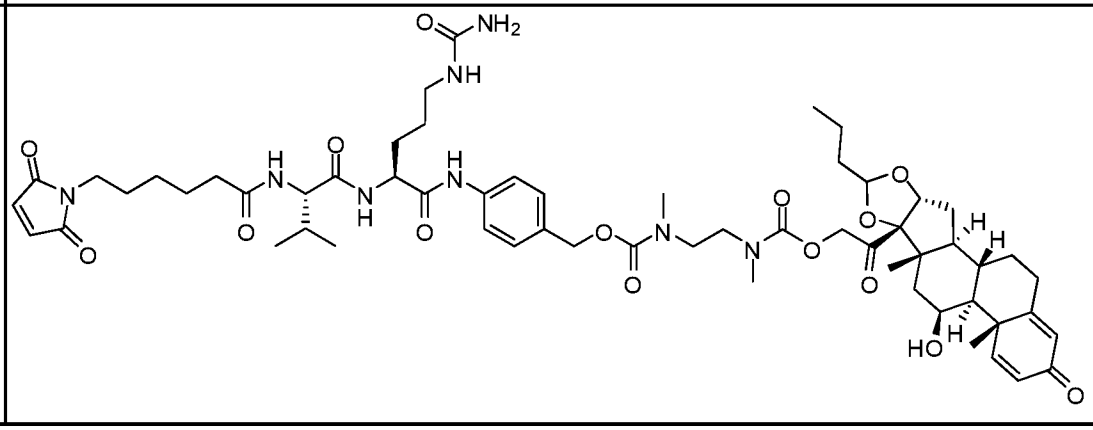
Table 3: List of linker-payloads (LPs) and their structures

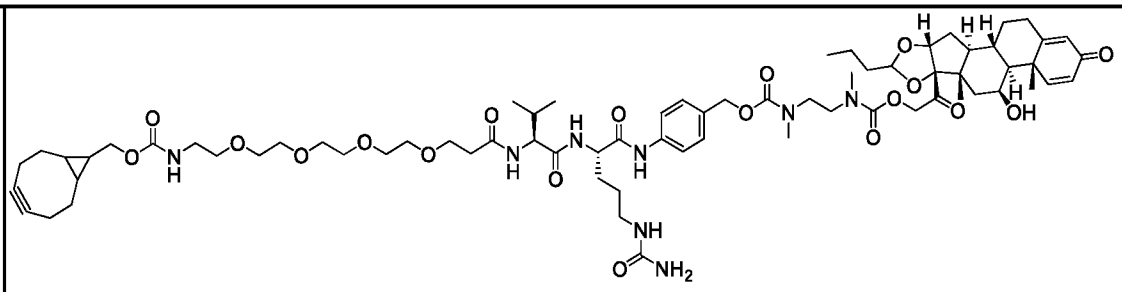
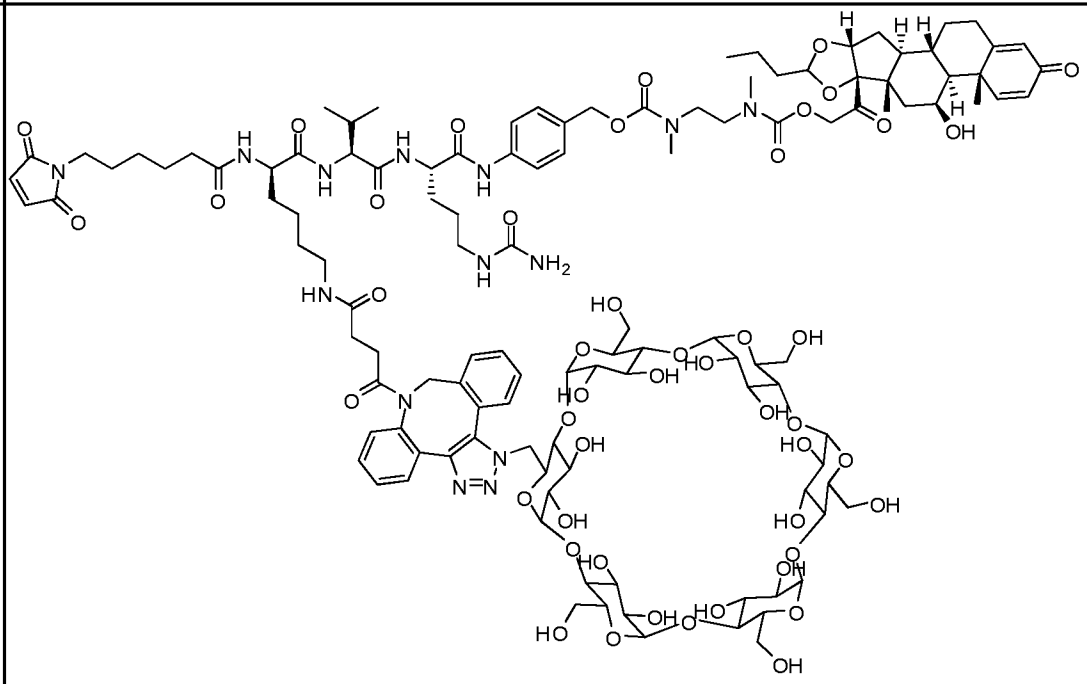
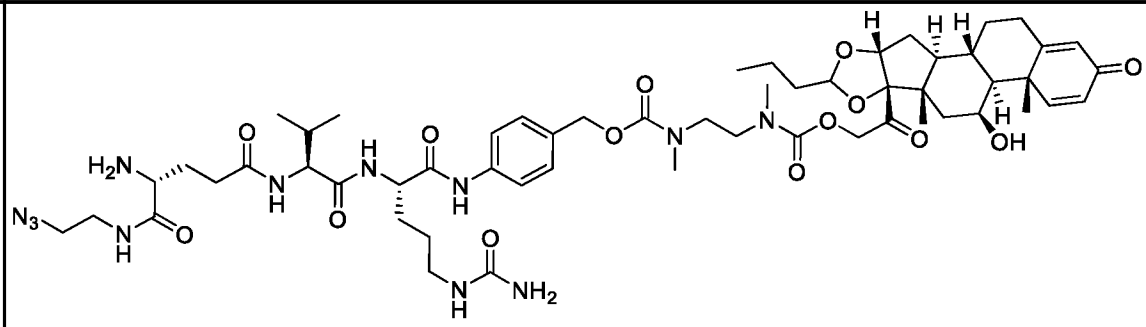
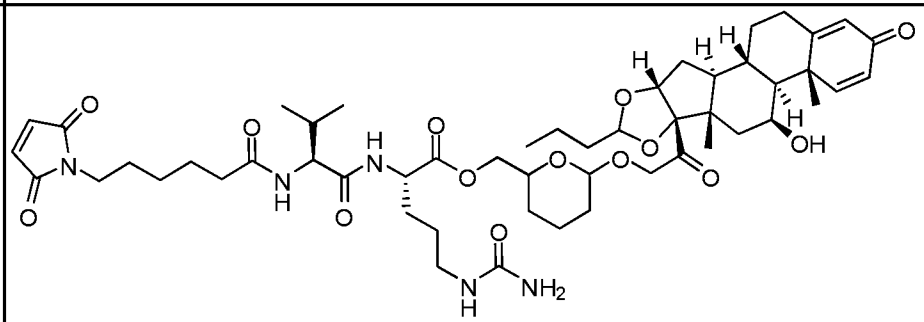
LP1	
LP2	
LP5	

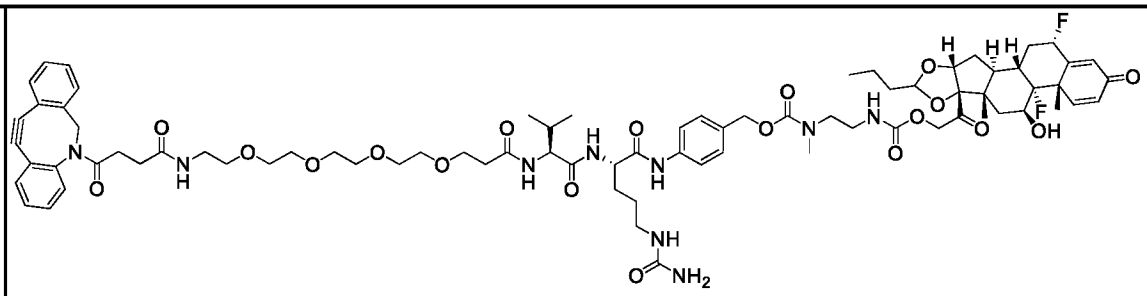
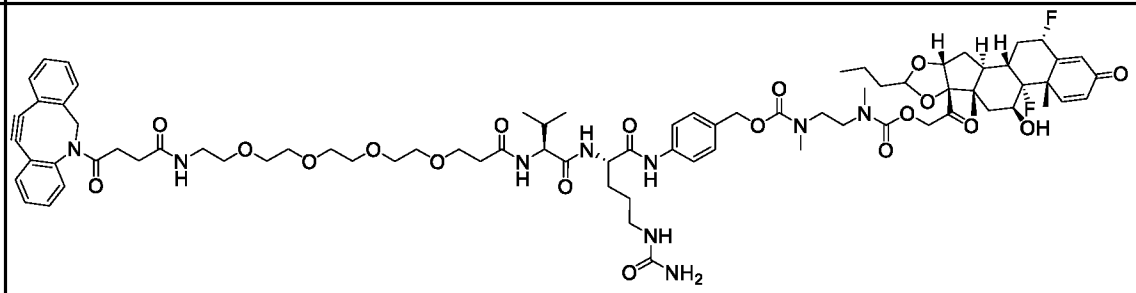
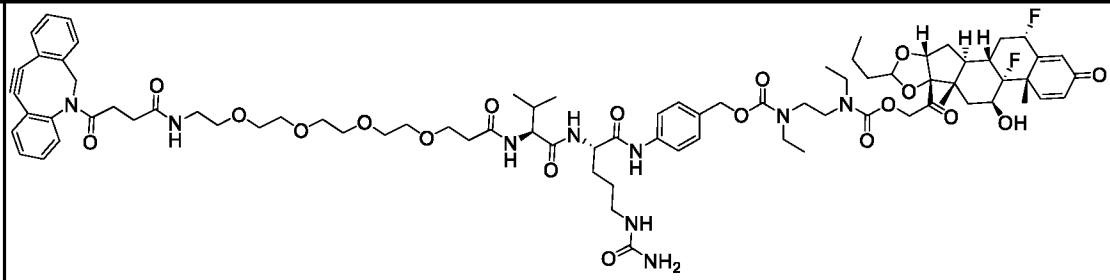
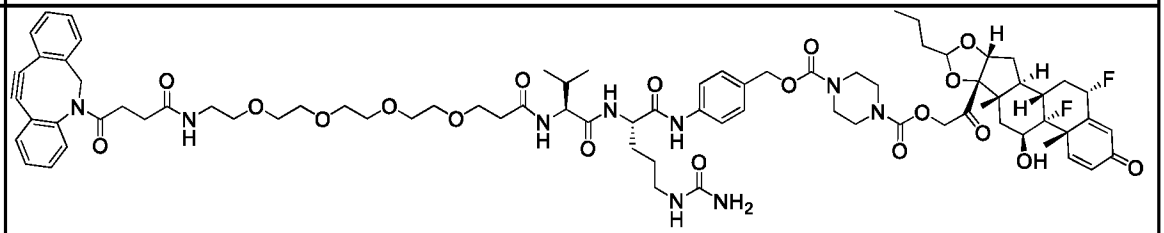
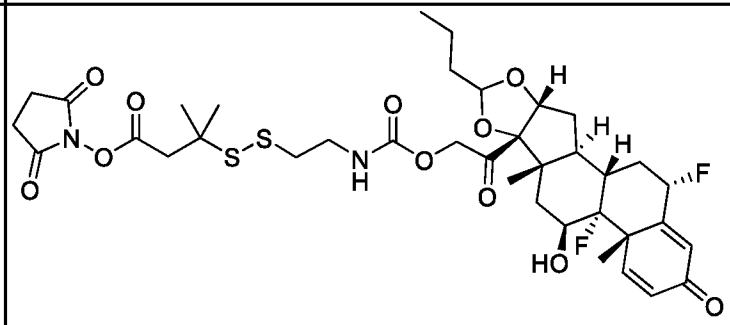
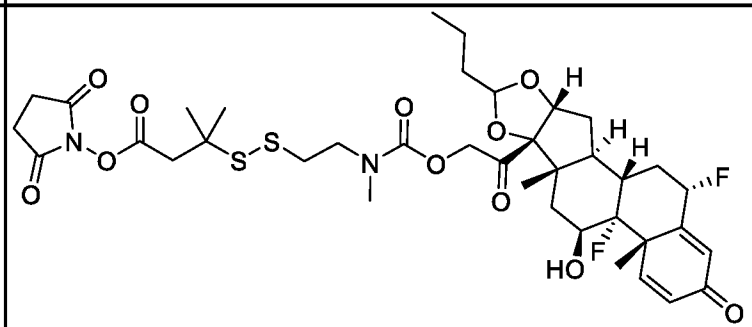
LP6	
LP18	
LP4	
LP11	
LP9	

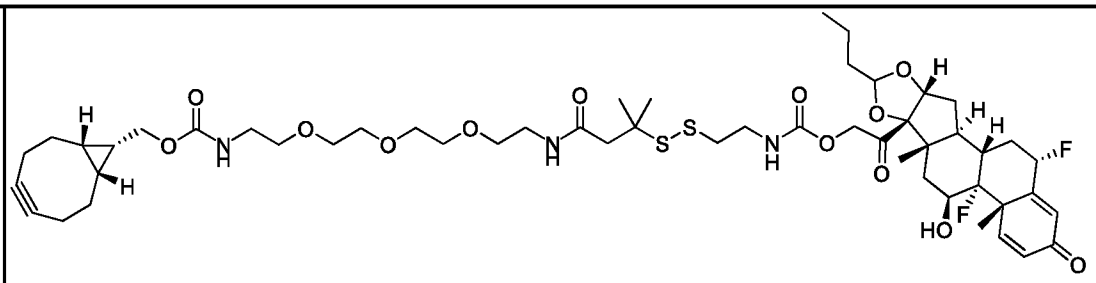
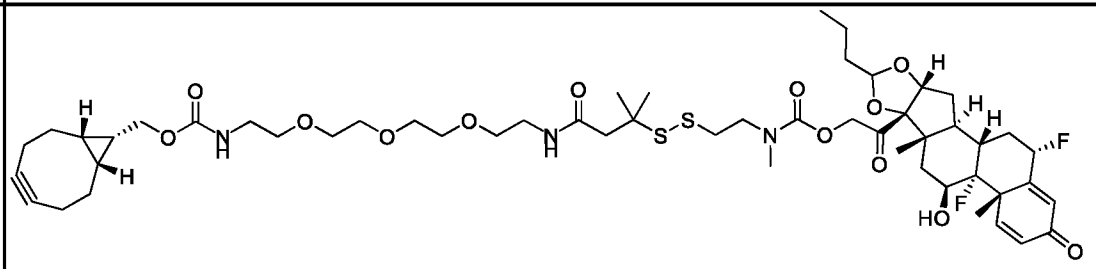
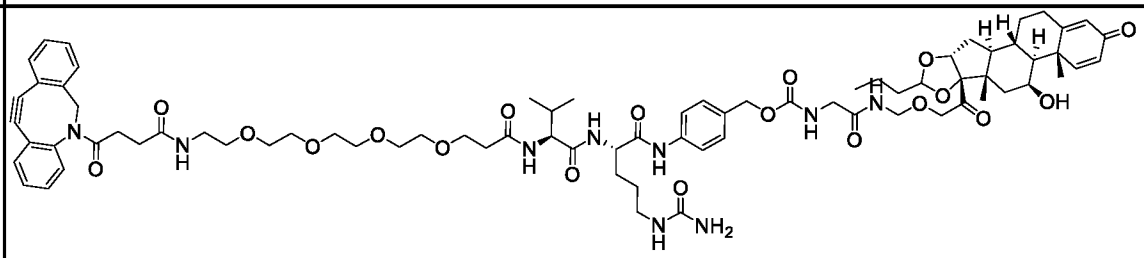
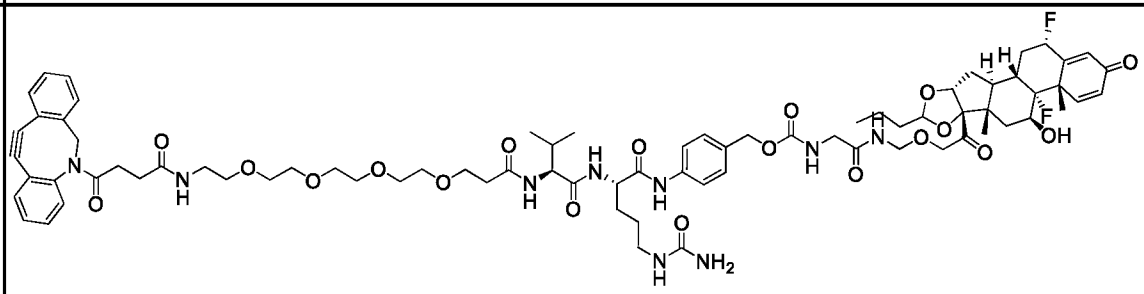
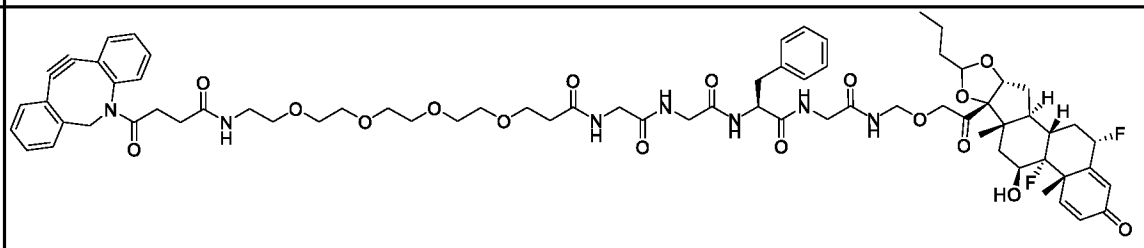
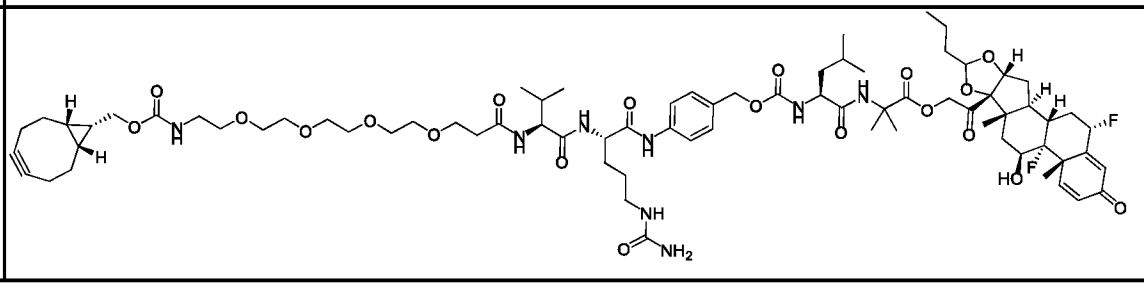
LP12	
LP3	
LP13	
	
	

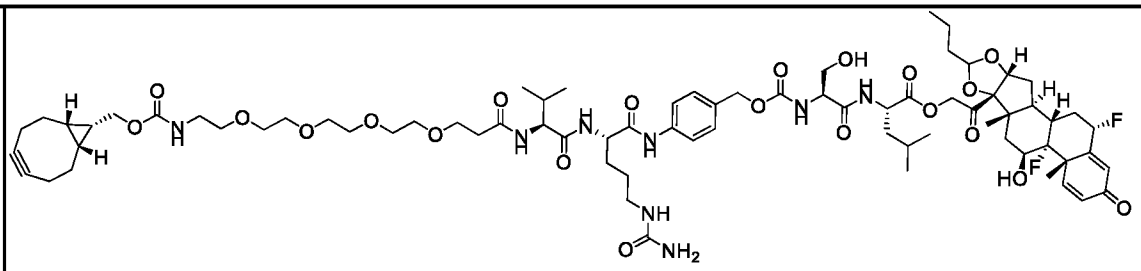
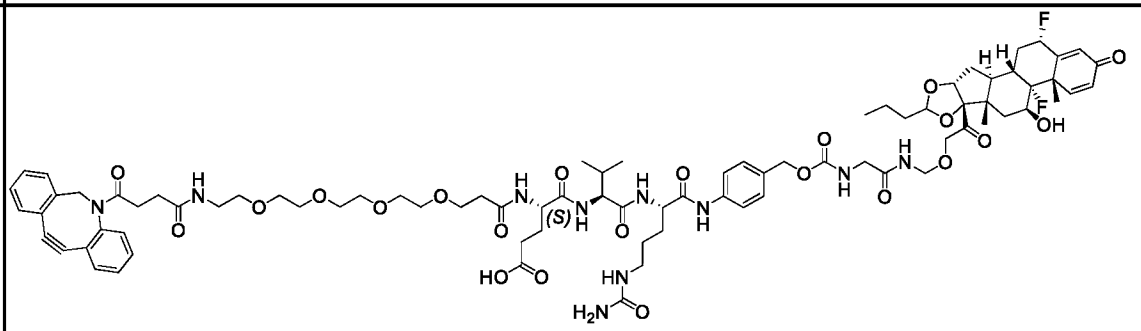
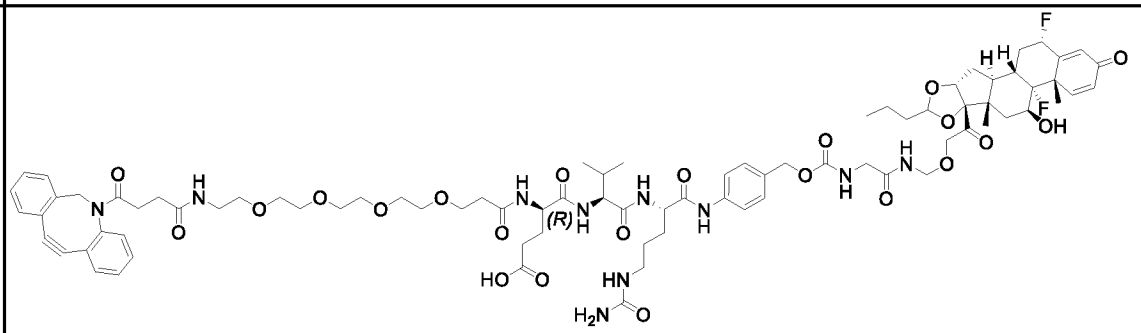
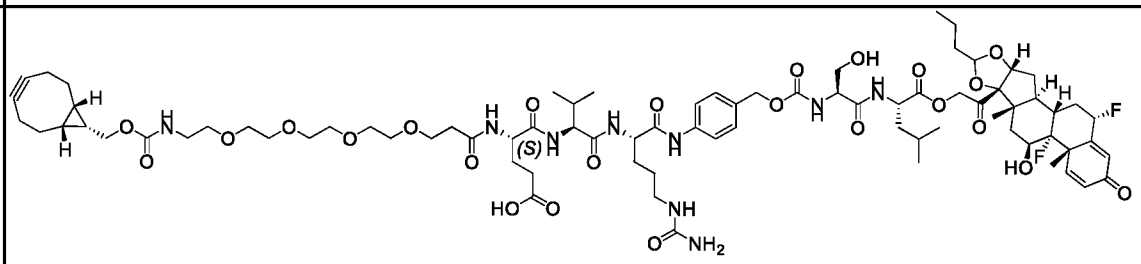
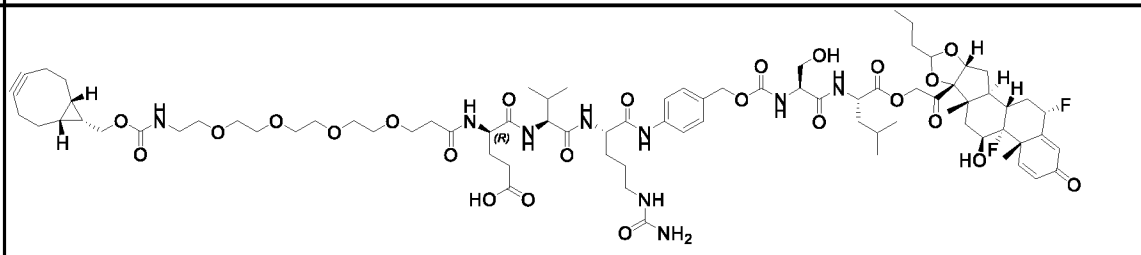
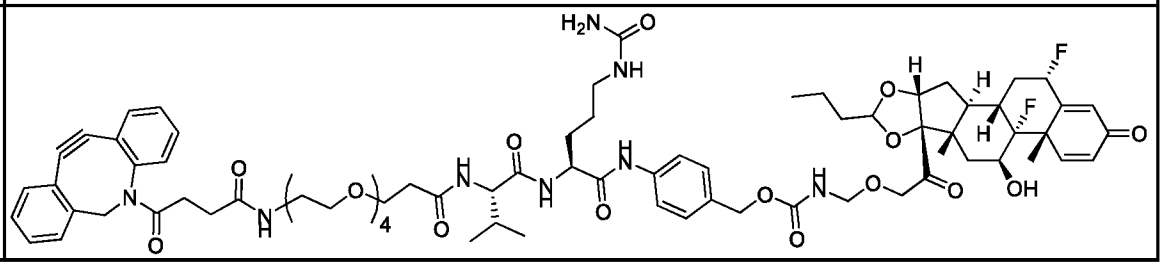
LP14	
LP15	
2a	
2b	

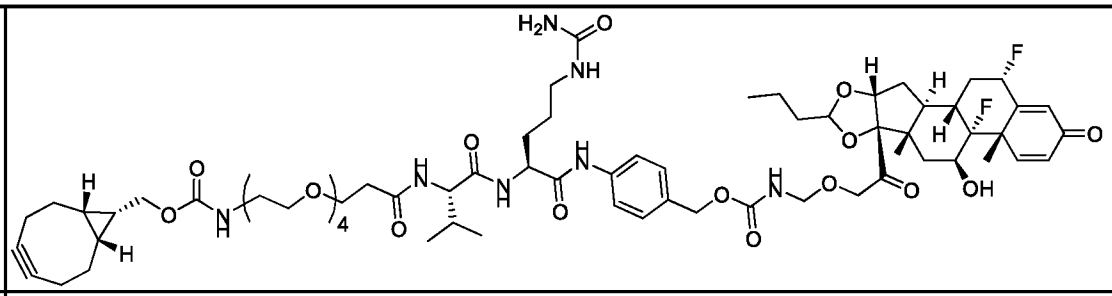
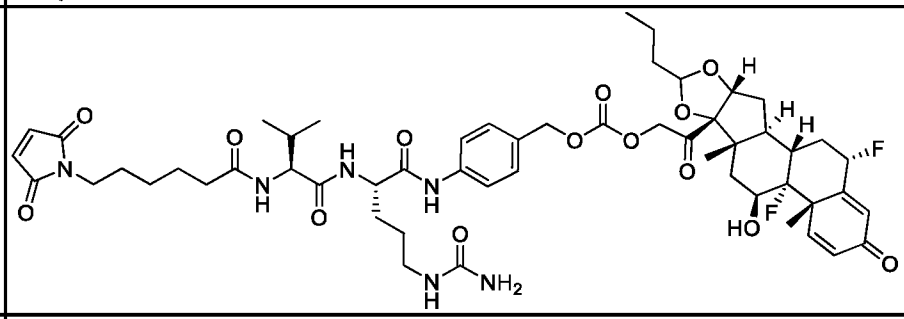
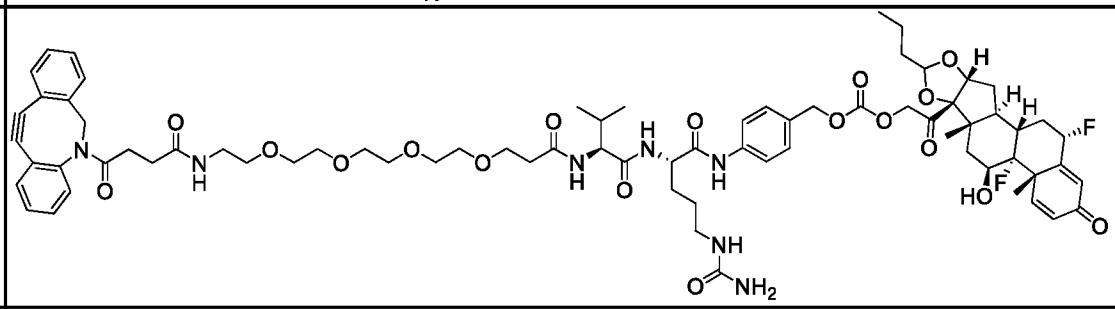
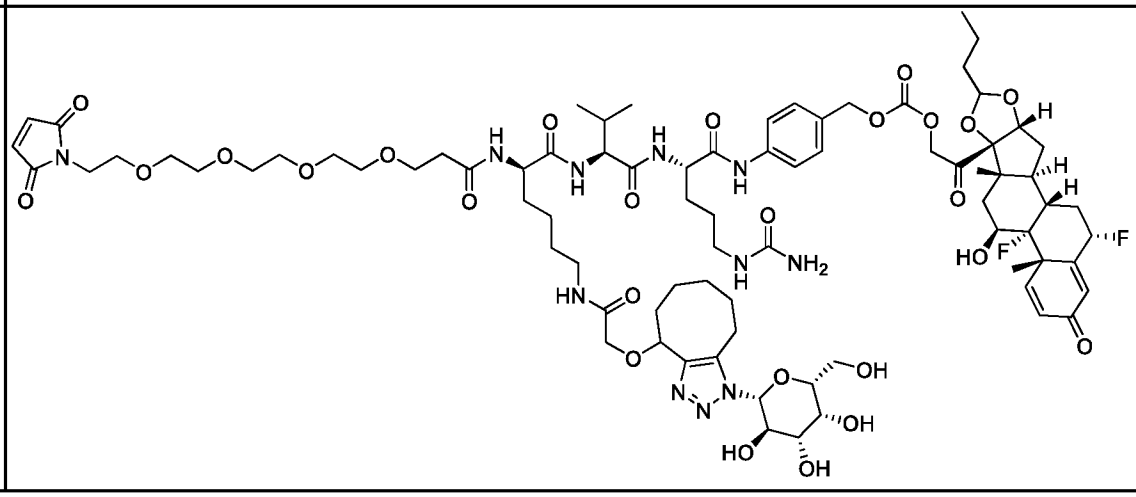
2f	
2g	
2j	
2k	

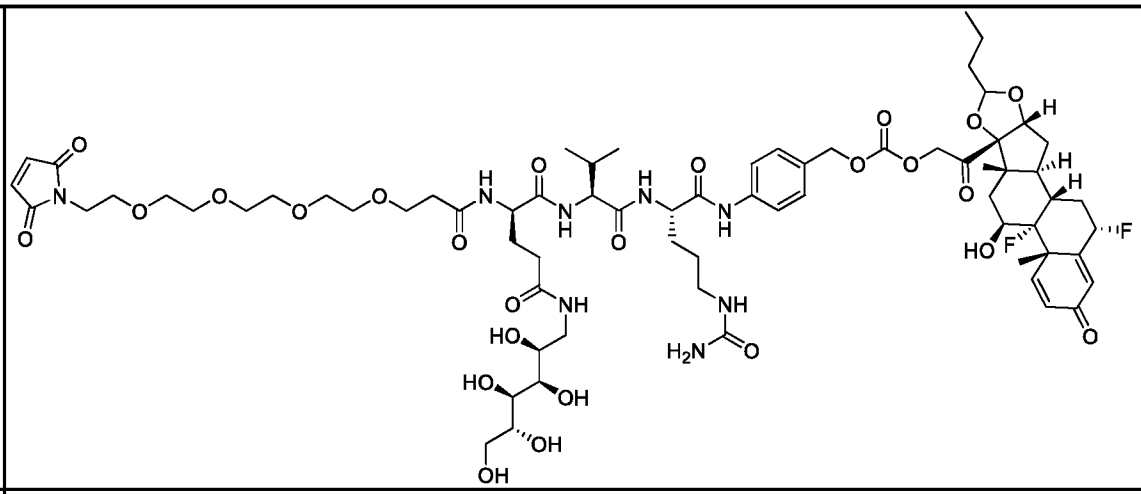
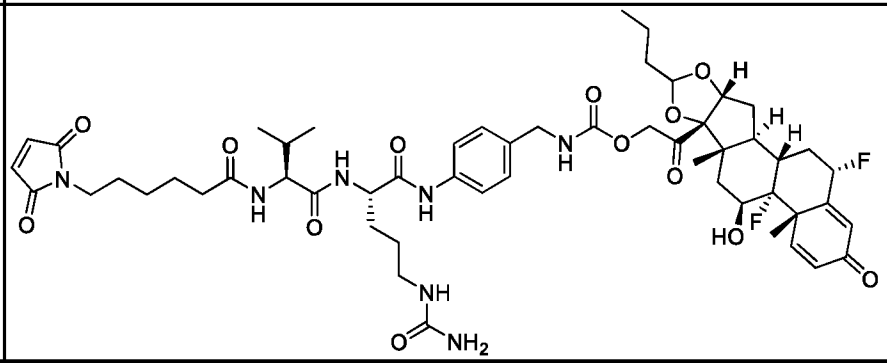
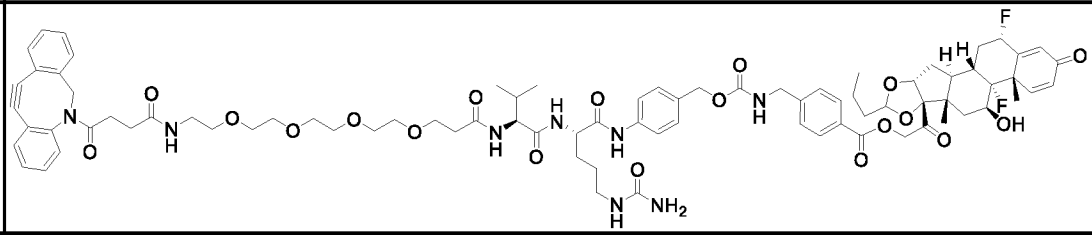
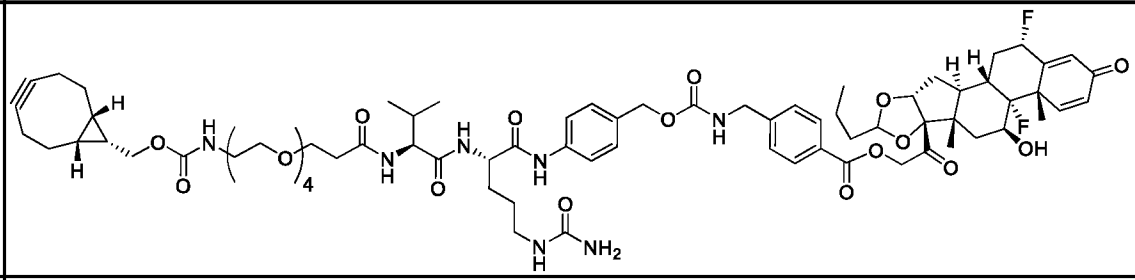
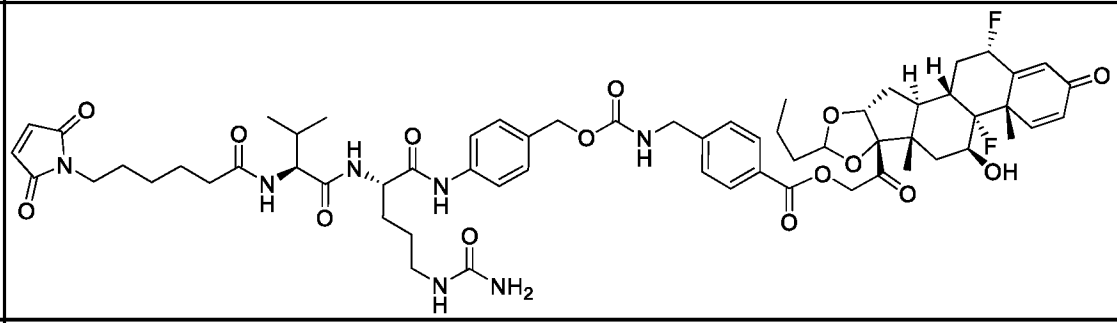
2l	
2m	
2n	 or a mixture thereof
2q	

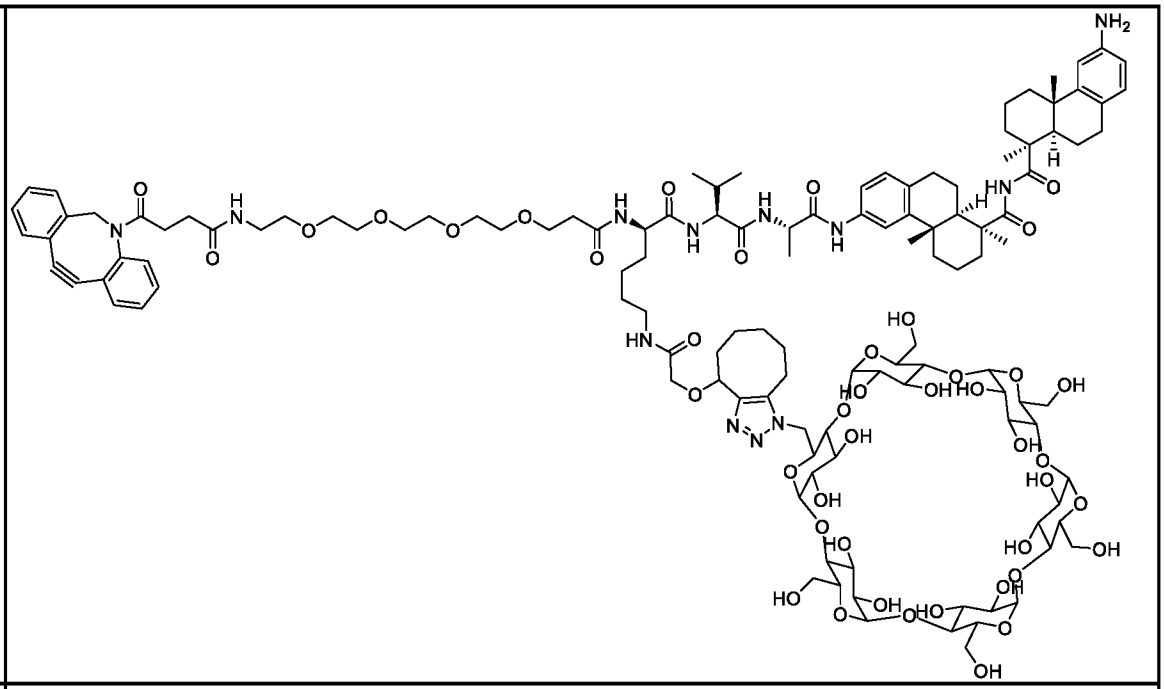
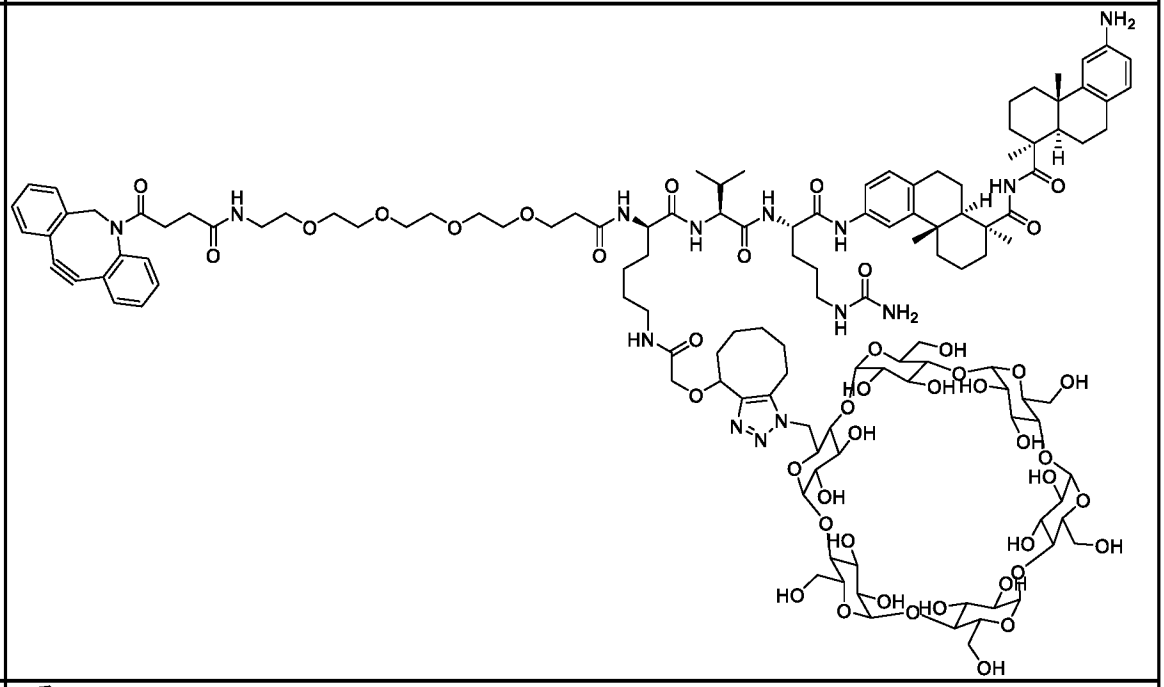
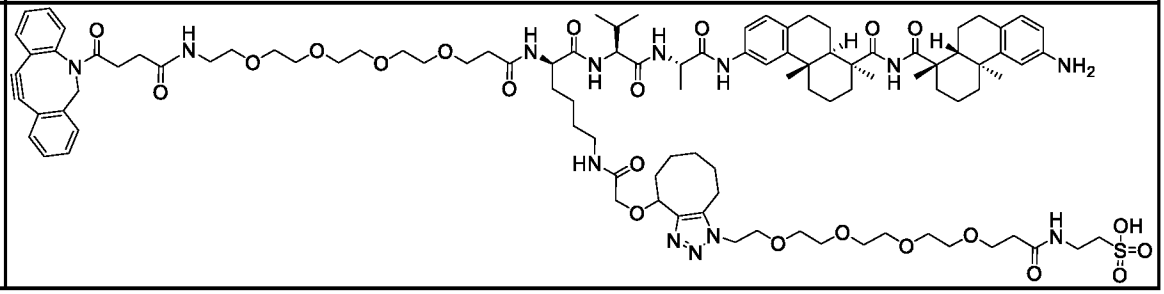
LP1A	
LP2A	
LP3A	
LP4A	
LP5A	
LP6A	

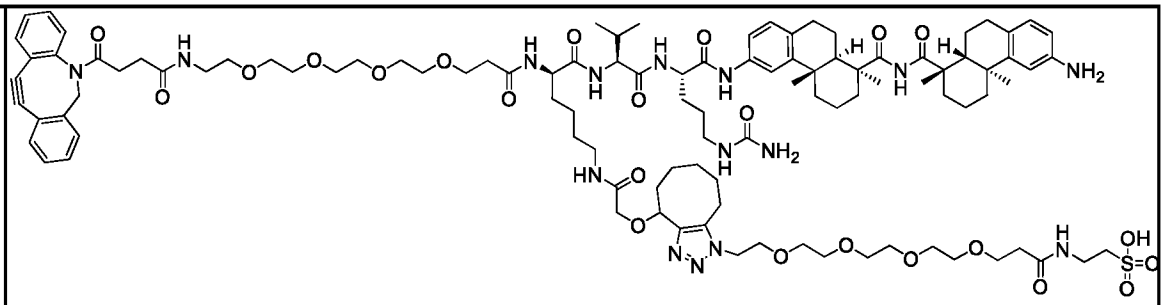
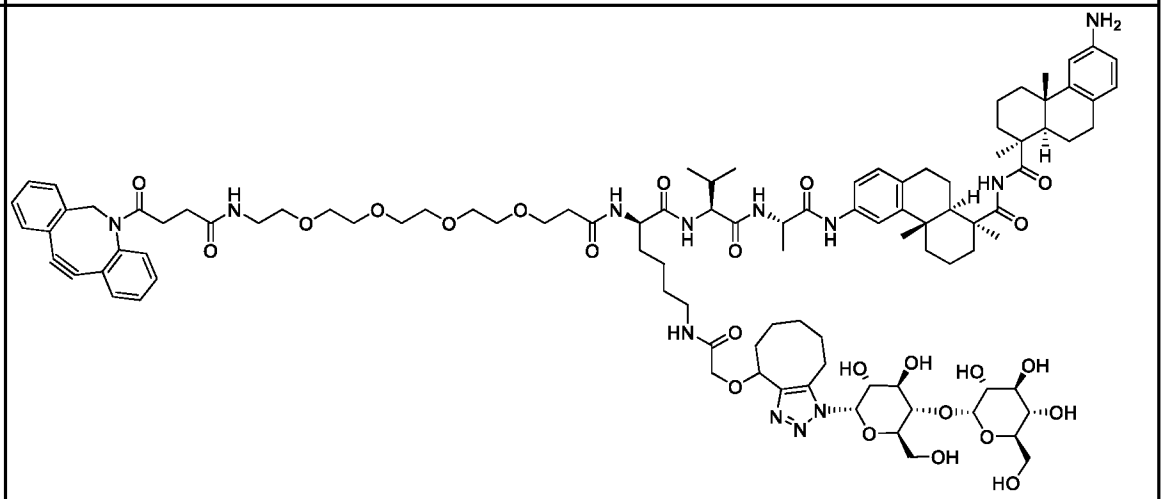
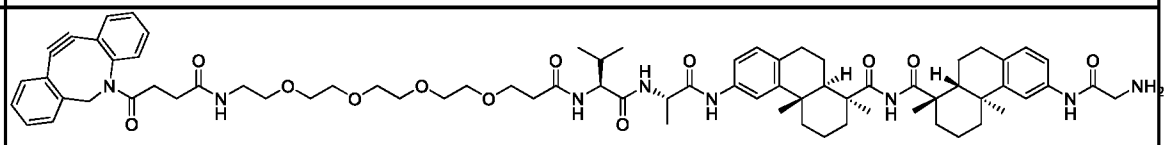
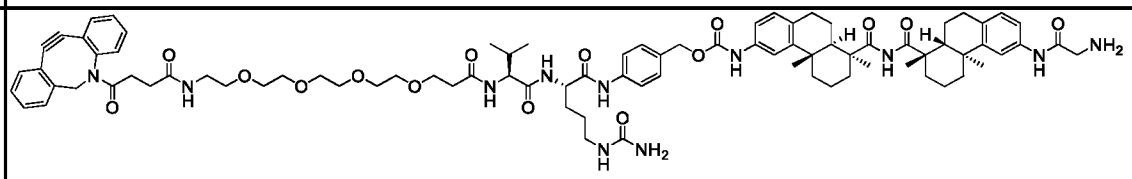
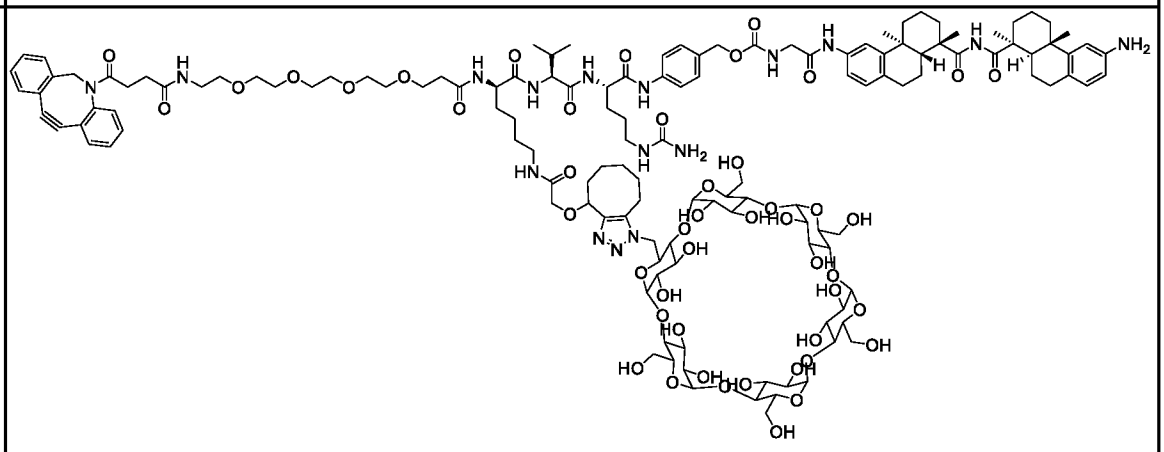
LP7A	
LP8A	
LP9A	
LP10 A	
LP11 A	
LP12 A	

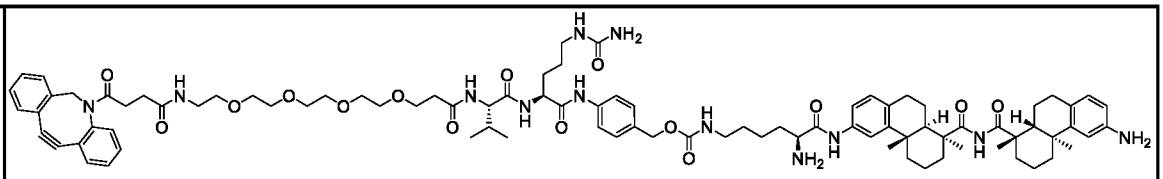
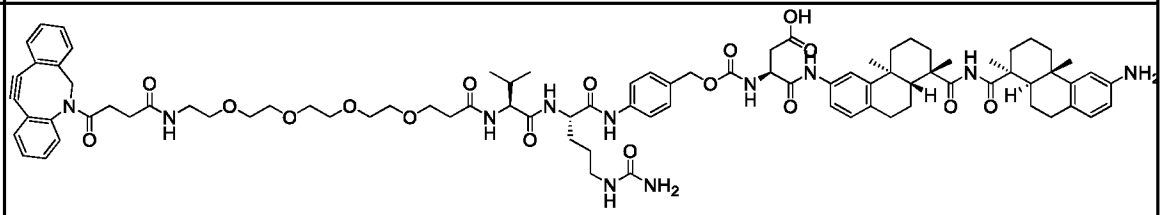
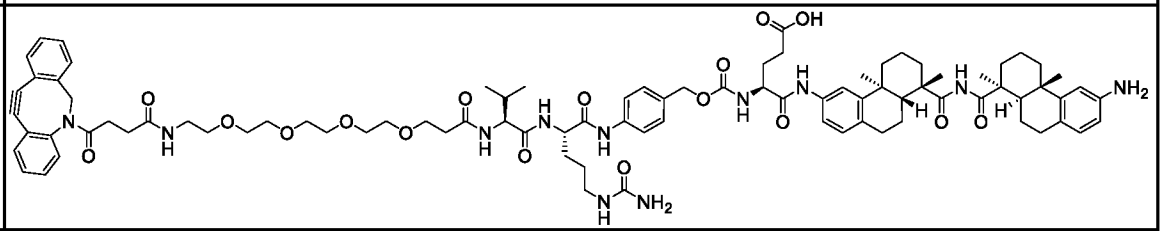
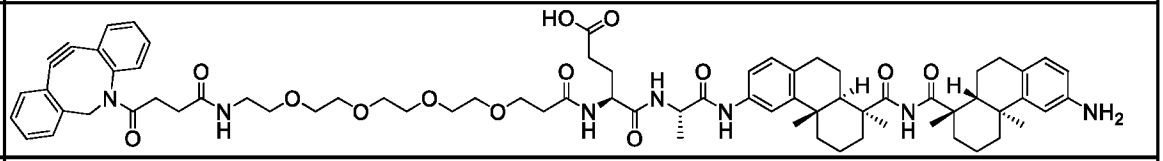
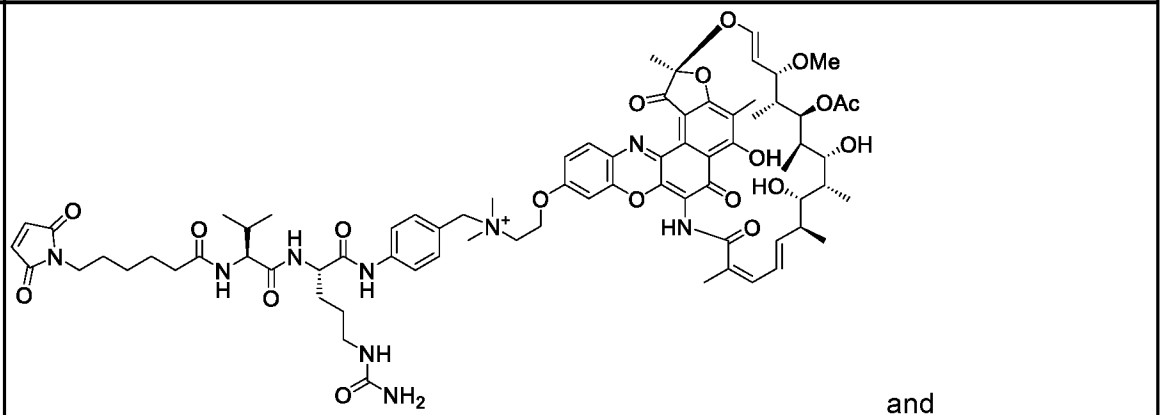
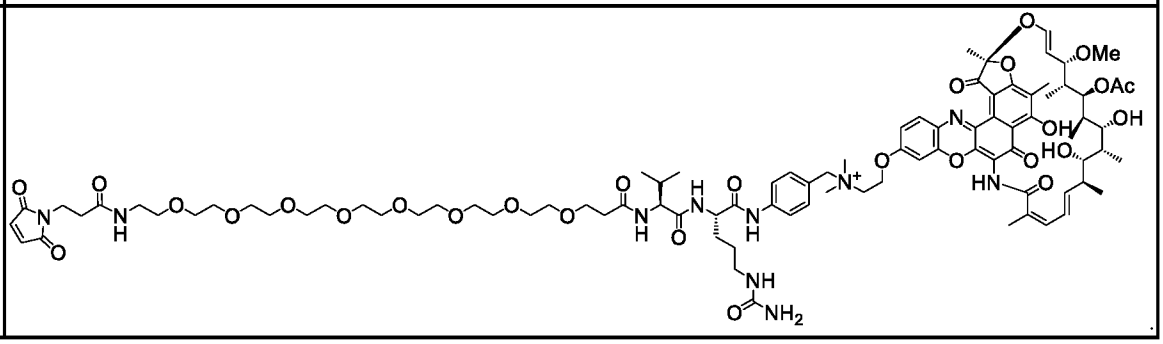
LP13 A	
LP18 A	
LP19 A	
LP20 A	
LP21 A	
LP22 A	

LP23A	
LP24A	
LP25A	
LP26A	

LP27A	
LP28A	
LP29A	
LP30A	
LP31A	

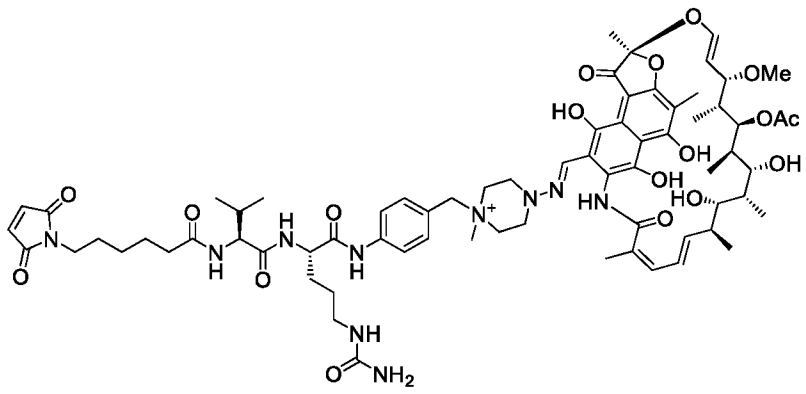
LP1B	
LP2B	
LP3B	

LP4B	
LP5B	
LP6B	
LP7B	
LP8B	

LP9B	
LP10 B	
LP11 B	
LP12 B	
R20	 and
R25	

[0354] The compounds in the tables above can be prepared as described in the Examples herein.

[0355] Also provided herein is a method of preparing an antibody-drug conjugate of a rifamycin analog (e.g., rifampicin), comprising the step of contacting an anti-MSR1 antibody, or antigen-binding fragment thereof, with a linker payload according to Formula (**D**):



Formula (**D**).

under conditions suitable for forming a bond between anti-MSR1 antibody, or antigen-binding fragment thereof.

Epitope Mapping and Related Technologies

[0356] The epitope to which the antibodies of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of an MSR1 protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of MSR1. In some embodiments, the epitope is located on or near the modified LDL-binding domain of MSR1. In other embodiments, the epitope is located outside of the modified LDL-binding domain of MSR1, *e.g.*, at a location on the surface of MSR1 at which an antibody, when bound to such an epitope, does not interfere with modified-LDL binding to MSR1.

[0357] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, Methods Mol Biol 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, Protein Science 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-

deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A.

[0358] Embodiments include anti-MSR1 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 4 herein). Likewise, embodiments also include anti-MSR1 antibodies that compete for binding to MSR1 with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 4 herein).

[0359] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-MSR1 antibody by using routine methods known in the art and exemplified herein at*, e.g.*, Example 7. For example, to determine if a test antibody binds to the same epitope as a reference anti-MSR1 antibody disclosed herein, the reference antibody is allowed to bind to a MSR1 protein. Next, the ability of a test antibody to bind to the MSR1 molecule is assessed. If the test antibody is able to bind to MSR1 following saturation binding with the reference anti-MSR1 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-MSR1 antibody. On the other hand, if the test antibody is not able to bind to the MSR1 molecule following saturation binding with the reference anti-MSR1 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-MSR1 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset

of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0360] To determine if an antibody competes for binding (or cross-competes for binding) with a reference anti-MSR1 antibody, the above-described binding methodology is performed in two orientations. In a first orientation, the reference antibody is allowed to bind to a MSR1 protein under saturating conditions followed by assessment of binding of the test antibody to the MSR1 molecule. In a second orientation, the test antibody is allowed to bind to a MSR1 molecule under saturating conditions followed by assessment of binding of the reference antibody to the MSR1 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the MSR1 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to MSR1. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Human Antibodies

[0361] The anti-MSR1 antibodies disclosed herein can be fully human antibodies. Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human MSR1.

[0362] Using VELOCIMMUNE™ technology, for example, or any other similar known method for generating fully human monoclonal antibodies, high affinity chimeric antibodies to MSR1 are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, ligand blocking activity, selectivity, epitope, *etc.* If necessary, mouse constant regions are replaced with a desired human constant region, for example wild-type or modified IgG1 or IgG4, to generate a fully human anti-MSR1 antibody. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region. In certain instances, fully human anti-MSR1 antibodies are isolated directly from antigen-positive B cells.

Bioequivalents

[0363] The anti-MSR1 antibodies and antibody fragments disclosed herein encompass proteins having amino acid sequences that vary from those of the described antibodies but that retain the ability to bind human MSR1. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to

parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti- MSR1 antibody-encoding DNA sequences disclosed herein encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-MSR1 antibody or antibody fragment that is essentially bioequivalent to an anti-MSR1 antibody or antibody fragment disclosed herein. Examples of such variant amino acid and DNA sequences are discussed above.

[0364] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0365] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0366] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0367] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0368] Bioequivalence may be demonstrated by *in vivo* and *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is

measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0369] Bioequivalent variants of anti-MSR1 antibodies disclosed herein may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-MSR1 antibody variants comprising amino acid changes which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Species Selectivity and Species Cross-Reactivity

[0370] According to certain embodiments, provided herein are anti-MSR1 antibodies that bind to human MSR1 but not to MSR1 from other species. Embodiments also include anti-MSR1 antibodies that bind to human MSR1 and to MSR1 from one or more non-human species. For example, the anti-MSR1 antibodies disclosed herein may bind to human MSR1 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomologous, marmoset, rhesus or chimpanzee MSR1. According to certain exemplary embodiments, anti-MSR1 antibodies are provided which specifically bind human MSR1 and cynomolgus monkey (*e.g.*, *Macaca fascicularis*) MSR1. Other anti-MSR1 antibodies disclosed herein bind human MSR1 but do not bind, or bind only weakly, to cynomolgus monkey MSR1.

Multispecific Antibodies

[0371] The antibodies disclosed herein may be monospecific or multispecific (*e.g.*, bispecific). Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.*, Tutt *et al.*, 1991, *J. Immunol.* 147:60-69; Kufer *et al.*, 2004, *Trends Biotechnol.* 22:238-244. The anti-MSR1 antibodies disclosed herein can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bispecific or a multispecific antibody with a second binding specificity.

[0372] Embodiments include bispecific antibodies wherein one arm of an immunoglobulin binds human MSR1, and the other arm of the immunoglobulin is specific for a second antigen. The MSR1-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as

set forth in Table 4 herein. In certain embodiments, the MSR1-binding arm binds human MSR1 and blocks modified LDL binding to MSR1. In other embodiments, the MSR1-binding arm binds human MSR1 but does not block modified LDL binding to MSR1. In some embodiments, the MSR1 binding arm binds human MSR1 and activates MSR1 signaling. In other embodiments, the MSR1 binding arm blocks MSR1-mediated receptor stimulation. Embodiments also include bispecific antibodies wherein one arm of an antibody binds a first epitope of human MSR1, and the other arm of said antibody binds a second distinct epitope of human MSR1.

[0373] An exemplary bispecific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bispecific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bispecific antibody format described above are contemplated within the scope of the present invention.

[0374] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Formulation and Administration

[0375] Embodiments relate to pharmaceutical compositions comprising the anti-MSR1 antibodies or antigen-binding fragments thereof disclosed herein. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0376] The dose of antibody administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. In an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering anti-MSR1 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351).

[0377] Various delivery systems are known and can be used to administer the pharmaceutical compositions disclosed herein, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.*, 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0378] A pharmaceutical composition as disclosed herein can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0379] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition disclosed herein. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0380] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0381] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, *etc.* These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], *etc.* As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, *etc.* The injection thus prepared is preferably filled in an appropriate ampoule.

[0382] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of Antibodies

[0383] Embodiments include methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-MSR1 antibody or an antibody-drug conjugate comprising an anti- MSR1 antibody (*e.g.*, an anti- MSR1 antibody or ADC comprising any of the HCVR/LCVR or CDR sequences as set forth in Table 4 herein). The therapeutic composition can comprise any of the anti- MSR1 antibodies, antigen-binding fragments thereof, or ADCs disclosed herein (e.g., ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**III**), (**3000**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), (**6005**), (**7001**), (**7002**), (**7003**), (**7004**) and/or (**7005**)), and a pharmaceutically acceptable carrier or diluent.

[0384] The antibodies and ADCs disclosed herein are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by MSR1 expression or activity, or treatable by binding MSR1 without competing against modified LDL, or and/or promoting MSR1 receptor internalization and/or decreasing cell surface receptor number. For example, the antibodies and ADCs disclosed herein are useful for the treatment,

attenuation, or amelioration of atherosclerosis, proliferative disorders, neurodegenerative disorders, and inflammation by targeting cells that express MSR1 and/or that respond to MSR1-mediated signaling, *e.g.*, macrophages.

[0385] In the context of the methods of treatment described herein, the anti-MSR1 antibody, or an ADC thereof, may be administered as a monotherapy (*i.e.*, as the only therapeutic agent) or in combination with one or more additional therapeutic agents (examples of which are described elsewhere herein).

[0386] Provided herein is a method of treating a proliferative disease, a metabolic disease, inflammation, a neurodegenerative disease, or disease, disorder, or condition associated with glucocorticoid receptor signaling, in a subject comprising administering to the subject an effective treatment amount of a compound described herein (e.g., an anti-MSR1 ADC), or a pharmaceutical composition comprising a compound described herein.

[0387] In some embodiments, where the payload is a steroid, the disease, disorder, or condition is allergic state, including but not limited to asthma, atopic dermatitis, contact dermatitis, allergic dermatitis, drug hypersensitivity reactions, anaphylactic rhinitis, perennial or seasonal allergic rhinitis, and serum sickness; dermatologic diseases and conditions, including but not limited to skin itching, seborrheic dermatitis, neurodermatitis, eczema, bullous dermatitis herpetiformis, exfoliative erythroderma, mycosis fungoides, pemphigus, and severe erythema multiforme (Stevens-Johnson syndrome); endocrine disorders, including but not limited to primary or secondary adrenocortical insufficiency, congenital adrenal hyperplasia, hypercalcemia associated with cancer, and nonsuppurative thyroiditis; gastrointestinal diseases; hematologic disorders, including but not limited to acquired (autoimmune) hemolytic anemia, congenital (erythroid) hypoplastic anemia (Diamond-Blackfan anemia), idiopathic thrombocytopenic purpura in adults, pure red cell aplasia, and secondary thrombocytopenia; trichinosis; tuberculous meningitis with subarachnoid block or impending block; neoplastic diseases, including but not limited to leukemias and lymphomas; nervous system disorders, including but not limited to acute exacerbations of multiple sclerosis, cerebral edema associated with primary or metastatic brain tumor, craniotomy, or head injury; ophthalmic diseases, including but not limited to sympathetic ophthalmia, temporal arteritis, uveitis, xerophthalmia, and ocular inflammatory conditions unresponsive to topical corticosteroids; renal diseases, including but not limited to for inducing a diuresis or remission of proteinuria in idiopathic nephrotic syndrome or that due to lupus erythematosus; respiratory diseases, including but not limited to berylliosis, fulminating or disseminated pulmonary tuberculosis when used concurrently with appropriate antituberculous chemotherapy, idiopathic eosinophilic pneumonias, symptomatic sarcoidosis; and Rheumatic disorders, including but not limited to

use as adjunctive therapy for short-term administration (to tide the patient over an acute episode or exacerbation) in acute gouty arthritis, acute rheumatic carditis, ankylosing spondylitis, psoriaticarthritis, rheumatoid arthritis, including juvenile rheumatoid arthritis, and for use in dermatomyositis, polymyositis, stomatitis, and systemic lupus erythematosus. In certain embodiments, provided herein are methods of treating or preventing arthritis.

[0388] In some embodiments, set forth herein is a method for treating a disease, disorder, or condition selected from an autoimmune disease, an allergy, arthritis, asthma, a breathing disorder, a blood disorder, a cancer, a collagen disease, a connective tissue disorders, a dermatological disease, an eye disease, an endocrine problem, an immunological disease, an inflammatory disease, an intestinal disorders, a gastrointestinal disease, a neurological disorder, an organ transplant condition, a rheumatoid disorder, a skin disorder, a swelling condition, a wound healing condition, and a combination thereof comprising administering a steroid payload or conjugate thereof described herein.

[0389] In some embodiments, the autoimmune disorder is selected from multiple sclerosis, autoimmune hepatitis, shingles, systemic lupus erythematosus (i.e., lupus), myasthenia gravis, Duchenne muscular dystrophy, and sarcoidosis. In some embodiments, the breathing disorder is selected from asthma, chronic respiratory disease, chronic obstructive pulmonary disease, bronchial inflammation, and acute bronchitis. In some embodiments, the cancer is selected from leukemia, lymphoblastic leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, Hodgkin's lymphoma, Non–Hodgkin's lymphoma (NHL), and multiple myeloma. In some embodiments, the collagen disease is systemic lupus erythematosus. In some embodiments, the eye disease is keratitis. In some embodiments, the endocrine problem is selected from Addison's Disease, adrenal insufficiency, adrenal cortical dysfunction, adrenocortical, and congenital adrenal hyperplasia. In some embodiments, the inflammatory disease is selected from inflammation after cataract surgery, joint inflammation, immune inflammation, tendon inflammation, bursitis, epicondylitis, Crohn's disease, inflammatory bowels disease, lipid pneumonitis thyroiditis, urticaria (hives), pericarditis, nephrotic syndrome, and uveitis. In some embodiments, the intestinal disorder is selected from collagenous colitis, ulcerative colitis, Crohn's disease, and inflammatory bowels disease. In some embodiments, the rheumatoid disorder is selected from rheumatoid arthritis, polymyalgia rheumatic, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus. In some embodiments, the skin disorder is selected from psoriasis, eczema, and poison ivy. In some embodiments, the neurological disorder is chronic inflammatory demyelinating polyradiculoneuropathy.

[0390] In some embodiments, the compounds described herein are administered to a patient to treat an acute inflammatory event, including but not limited to shock, brain edema, and graft-

vs-host disease. In some embodiments, the compounds described herein are administered to treat lympholytic effects, including but not limited to those associated with hematological malignancies, e.g., leukemias, lymphomas, and myelomas.

[0391] In some embodiments, set forth herein is a method for reducing inflammation in a subject in need thereof, comprising administering to a subject in need thereof a therapeutically effective amount of a steroid or conjugate thereof described herein. In some embodiments, set forth herein is a method for modulating the immune system in a subject in need thereof, comprising administering to a subject in need thereof a therapeutically effective amount of a steroid or conjugate thereof described herein. In some embodiments, set forth herein is a method for modulating cortisol levels in a subject in need thereof, comprising administering to a subject in need thereof a therapeutically effective amount of a steroid or conjugate thereof described herein. In some embodiments, set forth herein is a method of reducing lymphocyte migration in a subject in need thereof, comprising administering to a subject in need thereof a therapeutically effective amount of a steroid or conjugate thereof described herein. In some embodiments, set forth herein is a method of treating hypercalcemia due to cancer, Meniere's disease, a migraine headache, a cluster headache, a severe aphthous ulcer, laryngitis, severe tuberculosis, a Herxheimer reaction to syphilis, a decompensated heart failure, allergic rhinitis or nasal polyps, comprising administering to a subject in need thereof a steroid payload or conjugate thereof described herein. In some embodiments, the compounds disclosed herein can be used for treating inflammatory bowel disease, Crohn's disease, or ulcerative colitis. In some embodiments, the disease, disorder, or condition is a chronic inflammatory condition, including but not limited to asthma, skin infections, and ocular infections. In some embodiments, compounds described herein are used for immunosuppression in patients undergoing organ transplantation.

[0392] In some embodiments, the steroid payloads and conjugates thereof described herein are administered to a patient to treat a nervous disorder associated with GR signaling, including but not limited to psychiatric disorders such as schizophrenia, drug addiction, post-traumatic stress disorder (PTSD), and mood disorders, substance abuse, stress, and anxiety.

[0393] In some embodiments, the steroid payloads and conjugates thereof described herein are administered to a patient to treat a visual system disorder, including but not limited to ocular inflammation (e.g., conjunctivitis, keratitis, uveitis), macular edema, and macular degeneration. In some embodiments, the steroid payloads and conjugates thereof described herein are administered to a patient to treat a cardiovascular disorder. In some embodiments, the steroid payloads and conjugates thereof described herein are administered to a patient to treat a glucose and/or liver metabolism disorder. In some embodiments, the steroid payloads and

conjugates thereof described herein are administered to a patient to treat a musculoskeletal system disorder. In some embodiments, the steroid payloads and conjugates thereof described herein are administered to a patient to treat a cutaneous inflammatory condition, such as eczema and psoriasis.

[0394] The protein conjugates described herein provide a means for targeted delivery of its steroid payload to particular cells or organ systems, thereby reducing or preventing side effects that result from administration of the free unconjugated steroid payload. Examples of such potential side effects to be reduced or prevented include those listed in the approved drug label for Decadron® (dexamethasome), which is incorporated herein by reference in its entirety. In some embodiments, the side effect to be reduced or prevented is selected from elevation of blood pressure; sodium retention; water/fluid retention (edema, angioedema, pulmonary edema); increased excretion of potassium; reversible hypothalamic-pituitary adrenal (HPA) axis suppression; potential corticosteroid insufficiency after withdrawal of treatment; susceptibility to infecctions; exacerbation of systemic fungal infections; worsening of severity of chickenpox in pediatric and adult patients; worsening of severity of measles in pediatric and adult patients; posterior subcapsular cataracts; glaucoma with possible damage to the optic nerves; enhancement of the establishment of secondary ocular infections due to bacteria, fungi, or viruses; increase in new episodes of optic neuritis; Kaposi's sarcoma; drug-induced secondary adrenocortical insufficiency; increased risk of a perforation when active or latent peptic ulcers, diverticulitis, fresh intestinal anastomoses, and nonspecific ulcerative colitis, are present; peritoneal irritation following gastrointestinal perforation; decreased bone formation; increased bone resorption; inhibition of osteoblast function; inhibition of bone growth in pediatric patients; development of osteoporosis at any age; acute myopathy (possibly involving ocular and respiratory muscles, and potentially resulting in quadriparesis); elevation of creatinine kinase; psychic derangements, ranging from euphoria, insomnia, mood swings, personality changes, and severe depression, to frank psychotic manifestations; aggravation of existing emotional instability or psychotic tendencies; elevated intraocular pressure; bradycardia; cardiac arrest; cardiac arrhythmias; cardiac enlargement; circulatory collapse; congestive heart failure; fat embolism; hypertension; hypertrophic cardiomyopathy in premature infants; myocardial rupture following recent myocardial infarction; syncope; tachycardia; thromboembolism; thrombophlebitis; vasculitis; acne; allergic dermatitis; dry scaly skin; ecchymoses and petechiae; erythema; impaired wound healing; increased sweating; rash; striae; suppression of reactions to skin tests; thin fragile skin; thinning scalp hair; urticarial; decreased carbohydrate and glucose tolerance; development of cushingoid state; hyperglycemia; glycosuria; hirsutism; hypertrichosis; increased requirements for insulin or oral hypoglycemic agents in diabetes (insulin resistance); manifestations of latent diabetes mellitus; menstrual irregularities;

secondary adrenocortical and pituitary unresponsiveness (particularly in times of stress; as in trauma; surgery; or illness); suppression of growth in pediatric patients; congestive heart failure in susceptible patients; fluid retention; hypokalemic alkalosis; potassium loss; sodium retention; abdominal distention; elevation in serum liver enzyme levels (usually reversible upon discontinuation); hepatomegaly; increased appetite; nausea; pancreatitis; peptic ulcer with possible perforation and hemorrhage; perforation of the small and large intestine (particularly in patients with inflammatory bowel disease); ulcerative esophagitis; negative nitrogen balance due to protein catabolism; aseptic necrosis of femoral and humeral heads; loss of muscle mass; muscle weakness; osteoporosis; pathologic fracture of long bones; steroid myopathy; tendon rupture; vertebral compression fractures; convulsions; depression; emotional instability; euphoria; headache; increased intracranial pressure with papilledema (pseudotumor cerebri) usually following discontinuation of treatment; insomnia; mood swings; neuritis; neuropathy; paresthesia; personality changes; psychic disorders; vertigo; exophthalmos; glaucoma; increased intraocular pressure; posterior subcapsular cataracts; abnormal fat deposits; decreased resistance to infection; hiccups; increased or decreased motility and number of spermatozoa; malaise; moon face; and weight gain; and and those side effects associated with drug-drug interactions. In some embodiments, the side effect to be reduced or prevented are those associated with drug-drug interactions. In some embodiments, the side effect to be reduced or prevented is associated with drug-drug interactions from the use of a corticosteroid with aminoglutethimide including diminishment of adrenal suppression by corticosteroids; amphotericin B injection and potassium-depleting agents, including development of hypokalemia, cardiac enlargement, and congestive heart failure; antibiotics including a significant decrease in corticosteroid clearance; anticholinesterases including producing severe weakness in patients with myasthenia gravis; oral anticoagulants including inhibition of response to warfarin; antidiabetics including increased blood glucose concentrations; antitubercular drugs including decreased serum concentrations of isoniazid; cholestyramine including increased clearance of corticosteroids; cyclosporine including increased activity of both cyclosporine and corticosteroids, and incidence of convulsions; dexamethasone suppression test (DST) interference including false-negative results in patients being treated with indomethacin; digitalis glycosides including increased risk of arrhythmias due to hypokalemia; ephedrine including enhancement of the metabolic clearance of corticosteroids, resulting in decreased blood levels and lessened physiologic activity; estrogens, including oral contraceptives, including decreased hepatic metabolism of certain corticosteroids and associated increase in their effect; hepatic enzyme inducers, inhibitors and substrates (drugs which induce cytochrome P450 3A4 (CYP 3A4) enzyme activity e.g., barbiturates, phenytoin, carbamazepine, rifampin), including enhancing of metabolism of corticosteroids; drugs which

inhibit CYP 3A4 (e.g., ketoconazole, macrolide antibiotics such as erythromycin), including the potential for increased plasma concentrations of corticosteroids; drugs that are metabolized by CYP 3A4 (e.g., indinavir, erythromycin), including increase in their clearance, resulting in decreased plasma concentration; ketoconazole including decreased metabolism of certain corticosteroids by up to 60%, leading to increased risk of corticosteroid side effects, and inhibition of adrenal corticosteroid synthesis potentially causing adrenal insufficiency during corticosteroid withdrawal; nonsteroidal anti-inflammatory agents (NSAIDS), including increased risk of gastrointestinal side effects and increased clearance of salicylates; phenytoin, including increases or decreases in phenytoin level, altered seizure control; skin tests, including suppression of reactions to skin tests; thalidomide including toxic epidermal necrolysis; and vaccines including a diminished response to toxoids and live or inactivated vaccines due to inhibition of antibody response or potentiation of the replication of some organisms contained in live attenuated vaccines).

[0395] Thus, provided herein are methods for treating a disease, disorder, or condition associated with the glucocorticoid receptor comprising administering a conjugate of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), Formula (**III**) or Formula (**3000**), to a patient having said disease, disorder, or condition, wherein the side effects associated with administration of the free steroid payload of said conjugate is reduced. Furthermore, provided herein are methods of delivering a compound of Formula (**III**), or Formula (3000), to a cell comprising contacting said cell with a protein conjugate the compound of Formula (3000), or Formula (**III**), wherein the protein conjugate comprises an antibody or antigen binding fragment thereof that binds a surface antigen of said cell.

[0396] In some examples, where the payload is an LXR modulator, set forth herein is a method of treating a disease, disorder or condition comprising administering to a patient having said disorder a therapeutically effective amount of a compound and/or an ADC (e.g., ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), or (**6005**)) or a pharmaceutical composition thereof.

[0397] In some examples, set forth herein is a method of preventing a disease, disorder or condition comprising administering to a patient having said disorder a prophylactically effective amount of a compound and/or an ADC (e.g., ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**5001**), (**5002**), (**5003**), (**5004**), (**6001**), (**6002**), (**6003**), (**6004**), or (**6005**), or a pharmaceutical composition thereof.

[0398] In some examples, set forth herein are methods for treating or preventing any disease, disorder, or condition responsive to modulation of LXR signaling. In some examples, the disease or disorder is associated with LXR function, LXR polymorphisms, LXR agonist activity,

or LXR antagonist activity. In some examples, set forth herein is a method of treating or preventing a disease, disorder, or condition selected from the group consisting of a proliferative disorder, a neurodegenerative disorder, an immunological disorder, an autoimmune disease, an inflammatory disorder, a dermatological disease, a metabolic disease, cardiovascular disease, and a gastrointestinal disease.

[0399] The proliferative disorder can be any proliferative disorder known to those of skill. In certain embodiments, proliferative disorders include, without limitation, oncology disorders, where the oncology disorder can be any cancer disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing a melanoma. In certain embodiments, provided herein are methods of treating or preventing metastatic melanoma. In certain embodiments, provided herein are methods of treating or preventing lung cancer. In certain embodiments, provided herein are methods of treating or preventing EGFR-tyrosine kinase inhibitor resistant lung cancer. In certain embodiments, provided herein are methods of treating or preventing oral cancer. In certain embodiments, provided herein are methods of treating or preventing oral squamous cell carcinoma. In certain embodiments, provided herein are methods of treating or preventing prostate cancer. In certain embodiments, provided herein are methods of treating or preventing Hodgkin's lymphoma. In certain embodiments, provided herein are methods of treating or preventing breast cancer.

[0400] The neurodegenerative disorder can be any neurodegenerative disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing Alzheimer's disease. In certain embodiments, provided herein are methods of treating or preventing Parkinson's disease. In certain embodiments, provided herein are methods of treating or preventing Huntington's disease. In certain embodiments, provided herein are methods of treating or preventing amyotrophic lateral sclerosis. In certain embodiments, provided herein are methods of treating or preventing myelin gene expression. In certain embodiments, provided herein are methods of treating or preventing myelination and remyelination conditions, diseases, or disorders.

[0401] The immunological disorder can be any immunological disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing imflammatory bowel disease. In certain embodiments, provided herein are methods of treating or preventing ulcerative colitis. In certain embodiments, provided herein are methods of treating or preventing Crohn's disease.

[0402] The inflammatory disorder can be any inflammatory disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing arthritis. In certain embodiments, provided herein are methods of treating or preventing rheumatoid arthritis.

[0403] The metabolic disease can be any metabolic disease known to those of skill. In certain embodiments, the metabolic disease is dyslipidemia. Dyslipidemia can be any dyslipidemia known to those of skill. In certain embodiments, dyslipidemia is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hyperlipoproteinemia, HDL deficiency, ApoA-I deficiency, and cardiovascular disease such as coronary artery disease (including, for example, treatment and prevention of angina, myocardial infarction, and sudden cardiac death); atherosclerosis (including, for example, treatment and prevention of atherosclerosis); and restenosis (including, for example, preventing or treating atherosclerotic plaques which develop as a consequence of medical procedures such as balloon angioplasty). In certain embodiments, provided herein are methods of treating or preventing diabetes.

[0404] The cardiovascular disease can be any cardiovascular disease known to those of skill. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis derived from abnormal macrophage processing. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis derived from the formation of oxidized low-density lipoproteins (oxLDLs), where marcrophages fail to process oxLDLs. In certain embodiments, provided herein are methods of treating or preventing ischemic heart disease. In certain embodiments, provided herein are methods of treating or preventing stroke. In certain embodiments, provided herein are methods of treating or preventing hypertensive heart disease. In certain embodiments, provided herein are methods of treating or preventing aortic aneurysm. In certain embodiments, provided herein are methods of treating or preventing endocarditis. In certain embodiments, provided herein are methods of treating or preventing peripheral artery disease. In certain embodiments, provided herein are methods of treating or preventing combinations of any of the diseases provided in this paragraph.

[0405] In some examples, set forth herein is a method for modulating the function of a nuclear receptor. By way of non-limiting example, the function may be selected from expression/secretion of inflammatory mediators (e.g. cytokines, chemokines), cholesterol regulation, cholesterol intake, cholesterol efflux, cholesterol oxidation, migration, chemotaxis, apoptosis and necrosis, an inflammatory activity, lipid regulation, apoptosis, migration, chemotaxis, gene transcription, and protein expression.

[0406] In some examples, set forth herein herein is a method of preventing a disease, disorder or condition comprising administering to a patient having said disorder a therapeutically effective amount of a compound and/or an ADC of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**7001**), (**7002**), (**7003**), (**7004**), and/or (**7005**), or a pharmaceutical composition thereof.

[0407] *S. aureus* is a facultative intracellular bacterium that can survive phagocytosis by macrophages and other cells types (Horn, J., et al., *Inside job: Staphylococcus aureus host-pathogen interactions*. Int J Med Microbiol, 2018. 308(6): p. 607-624; Jubrail, J., et al., *Inability to sustain intraphagolysosomal killing of Staphylococcus aureus predisposes to bacterial persistence in macrophages*. Cell Microbiol, 2016. 18(1): p. 80-96). Intravital imaging has demonstrated that macrophages can serve as a reservoir where *S. aureus* replicates and then seeds other organs during infection (Surewaard, B.G., et al., *Identification and treatment of the Staphylococcus aureus reservoir in vivo*. J Exp Med, 2016. 213(7): p. 1141-51). Most antibiotics do not penetrate cells, including macrophages, very well, indicating that the intracellular *S. aureus* reservoir can evade treatment with standard of care antibiotics (Lehar, S.M., et al., *Novel antibody-antibiotic conjugate eliminates intracellular S. aureus*. Nature, 2015. 527(7578): p. 323-8). However, liposomal formulation of vancomycin increased penetration of the antibiotic into macrophages and reduced *S. aureus* organ burden more effectively than standard of care vancomycin (Surewaard, B.G., et al., *Identification and treatment of the Staphylococcus aureus reservoir in vivo*. J Exp Med, 2016. 213(7): p. 1141-51). Together, these data indicate that delivering an antibiotic to macrophages may be an effective method to eliminate the intracellular *S. aureus* reservoir.

[0408] Antibiotic resistant S. aureus remains a public health problem and roughly 40% blood stream infections are caused by methicillin-resistant S. aureus (MRSA) in the USA. Few FDA approved treatment options exist for MRSA blood stream infections, with vancomycin remaining an antibiotic of choice. In spite of appropriate antibiotic treatment, mortality from S. aureus blood stream infections is ~18%, prompting investigation into combinations that can improve treatment.

[0409] The rifamycin class of antibiotics inhibit bacterial RNA polymerase (RNAP) and have potent activity against S. aureus. Monotherapy with this class of antibiotics, however, can lead to selection of a resistant population during treatment. Therefore, rifamycin antibiotics can be used in combination with first line antibiotics to improve outcomes, commonly in infections involving prostheses or foreign devices.

[0410] The ADCs described herein comprising rifamycin analogs are useful for preventing or treating growth of a bacterium and/or bacterial infection in a subject. In some instances, the bacterium is a gram positive bacterium (a gram positive bacterium is the cause of the bacterial infection). In some instances, the bacterium is a pencillin-resistant bacterium (a penicillin-resistant bacterium is the cause of the bacterial infection). In some instances, the bacterium is a *Staphylococcus aureus*, methiciliin resistant *Staphylococcus aureus* (MRSA) bacterium (a MRSA bacterium is the cause of the bacterial infection). In some instances, the bacterium is a

methicillin susceptible *Staphylococcus aureus* (MSSA) bacterium (a MSSA bacterium is the cause of the bacterial infection). In some instances, the bacterium is a vancomycin-resistant *Staphylococcus aureus* (VRSA) bacterium (a VRSA bacterium is the cause of the bacterial infection). In some instances, the bacterium is multi-drug resistant *M. tuberculosis* (a multi-drug resistant *M. tuberculosis* bacterium is the cause of the bacterial infection). In further instances, the bacterium is *Chlamydia trachomatis* resistant to, e.g., azithromycin (*Chlamydia trachomatis* resistant to, e.g., azithromycin is the cause of the bacterial infection). In more instances, the bacterium is *Clostridium difficile* resistant to, e.g., metronidazole, vancomycin, and/or fidaxomicin (*Clostridium difficile* resistant to, e.g., metronidazole, vancomycin, and/or fidaxomicin is the cause of the bacterial infection).

[0411] Provided herein is a method of preventing or treating cellulitis, bacteremia, dermonecrosis, eyelid infection, eye infection, neonatal conjunctivitis, osteomyelitis, impetigo, boils, scalded skin syndrome, food poisoning, pneumonia, surgical infection, urinary tract infection, burn infection, meningitis, endocarditis, septicemia, toxic shock syndrome, septic arthritis, mastitis, infection associated with a prosthetic joint, infection associated with a catheter, or infection associated with an implant, in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate comprising a rifamycin analog (e.g., ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**7001**), (**7002**), (**7003**), (**7004**) and/or (**7005**)). Also provided herein is a method of preventing or treating an intracellular bacterial infection in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate of an antibody-drug conjugate comprising a rifamycin analog (e.g., ADCs of Formula (**I**), (**IA**), (**IB**), (**IB-1**), (**IB-2**), (**IC**), (**ID**), (**IE**), (**7001**), (**7002**), (**7003**), (**7004**) and/or (**7005**)).

[0412] In some instances, provided herein are therapeutic methods comprising administration an anti-MSR1 antibody, an antigen-binding portion of an MSR1 antibody, or an ADC comprising an anti-MSR1 antibody of MSR1 antigen-binding fragment thereof, to a subject in need thereof are useful for the treatment, and/or prevention of bacterial infection in a subject, and/or a disease or disorder or condition associated with *Staphylococcal* infection, for example, a *S. aureus* infection infection and/or for ameliorating at least one symptom associated with such disease, disorder or condition. Such disease, disorder or condition can be cellulitis, bacteremia, dermonecrosis, eyelid infection, eye infection, neonatal conjunctivitis, osteomyelitis, impetigo, boils, scalded skin syndrome, food poisoning, pneumonia, surgical infection, urinary tract infection, burn infection, meningitis, endocarditis, septicemia, toxic shock syndrome, or septic arthritis. In some instances, the subject has a prosthetic joint and the antibodies disclosed herein are used for treating and/or preventing *S. aureus* infection of the tissue surrounding the

prosthetic joint. In some instances, the subject has a catheter and the antibodies disclosed herein are used for treating and/or preventing *S. aureus* infection of the catheter and/or the tissue surrounding the catheter. In some instances, the subject has a foreign body implanted, and the antibodies disclosed herein are used for treating and/or preventing *S. aureus* infection of the foreign body and/or the tissue surrounding the foreign body. In some instances, the subject has mastitis, and the antibodies disclosed herein are useful for treating mastitis.

[0413] In some instances, the rifamycin analogs and/or anti-MSR1 ADCs thereof are administered in combination with one or more additional antibiotics (e.g., antibiotics that may be used for MRSA infections) such as vancomycin, trimethoprim-sulfamethoxazole, tetracycline, doxycycline/minocycline, clindamycin, cephalosporins (e.g. cephalexin), naficillin, fidaxomicin, linezolid, and the like, and/or any other suitable antibiotic(s). In some instances, the ADCs described herein comprising rifamycin analogs are administered in combination with vancomycin and are useful for preventing or treating bacterial infection in a subject.

Combination Therapies and Formulations

[0414] Embodiments include compositions and therapeutic formulations comprising any of the anti-MSR1 antibodies described herein in combination with one or more additional therapeutically active components, and methods of treatment comprising administering such combinations to subjects in need thereof.

[0415] The anti-MSR1 antibodies disclosed herein may be co-formulated with and/or administered in combination with one or more additional therapeutically active component(s) selected from the group consisting of: cytokine inhibitors, including small-molecule cytokine inhibitors and antibodies that bind to cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, IL-18, or to their respective receptors.

[0416] The anti-MSR1 antibodies disclosed herein may also be administered and/or co-formulated in combination with anti-inflammatory agents, immunomodulatory agents, analgesics, corticosteroids, steroids, antioxidants, COX inhibitors, cardioprotectants, metal chelators, IFN-gamma, and/or NSAIDs. In some embodiments, the anti-MSR1 antibodies can be administered and/or co-formulated in combination with anti-PCSK9 antibodies, anti-ANGPTL3 antibodies, statins, ezetimibe and other lipid lowering therapies.

[0417] The additional therapeutically active component(s), *e.g.*, any of the agents listed above or derivatives thereof, may be administered just prior to, concurrent with, or shortly after the administration of an anti-MSR1 antibody disclosed herein; (for purposes of the present disclosure, such administration regimens are considered the administration of an anti-MSR1 antibody "in combination with" an additional therapeutically active component). Embodiments

include pharmaceutical compositions in which an anti-MSR1 antibody disclosed herein is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[0418] According to certain embodiments, multiple doses of an anti-MSR1 antibody (or an ADC or a pharmaceutical composition comprising a combination of an anti-MSR1 antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods comprise sequentially administering to a subject multiple doses of an anti-MSR1 antibody disclosed herein. As used herein, "sequentially administering" means that each dose of anti-MSR1 antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). Embodiments include methods which comprise sequentially administering to the patient a single initial dose of an anti-MSR1 antibody, followed by one or more secondary doses of the anti-MSR1 antibody, and optionally followed by one or more tertiary doses of the anti-MSR1 antibody.

[0419] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-MSR1 antibody disclosed herein. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-MSR1 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-MSR1 antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0420] In certain exemplary embodiments, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-MSR1 antibody which is

administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0421] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-MSR1 antibody. For example, in certain embodiments, a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient. The administration regimen may be carried out indefinitely over the lifetime of a particular subject, or until such treatment is no longer therapeutically needed or advantageous.

[0422] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks or 1 to 2 months after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 12 weeks after the immediately preceding dose. In certain embodiments, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[0423] Embodiments include administration regimens in which 2 to 6 loading doses are administered to a patient at a first frequency (*e.g.*, once a week, once every two weeks, once every three weeks, once a month, once every two months, etc.), followed by administration of two or more maintenance doses to the patient on a less frequent basis. For example, according to this aspect of the invention, if the loading doses are administered at a frequency of once a month, then the maintenance doses may be administered to the patient once every six weeks, once every two months, once every three months, *etc.*

Diagnostic Uses of the Antibodies

[0424] The anti-MSR1 antibodies disclosed herein may also be used to detect and/or measure MSR1, or MSR1-expressing cells in a sample, *e.g.*, for diagnostic purposes. For example, an anti-MSR1 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of MSR1. Exemplary diagnostic assays for MSR1 may comprise, *e.g.*,

contacting a sample, obtained from a patient, with an anti-MSR1 antibody disclosed herein, wherein the anti-MSR1 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-MSR1 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein, or rhodamine; or an enzyme such as alkaline phosphatase, beta-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure MSR1 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immuno-PET (*e.g.*, ^{89}Zr, ^{64}Cu, etc.), and fluorescence-activated cell sorting (FACS).

[0425] Samples that can be used in MSR1 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of MSR1 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of MSR1 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal MSR1 levels or activity) will be measured to initially establish a baseline, or standard, level of MSR1. This baseline level of MSR1 can then be compared against the levels of MSR1 measured in samples obtained from individuals suspected of having a MSR1-related disease or condition.

EXAMPLES

[0426] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions provided herein, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric. The examples should not be construed as limiting, as the examples merely provide specific understanding and practice of the embodiments and their various aspects.

[0427] As used herein, the symbols and conventions used in the processes, and Examples, herein, are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry unless specified otherwise to the contrary. Specifically, but without limitation, the following abbreviations may be used in the Examples and throughout the specification:

Abbreviation	Term
ADC	Antibody-drug conjugate

Abbreviation	Term
Aglycosylated antibody	Antibody does not have any glycan
aq	Aqueous
BARAC	Biarylazacyclooctynone
BCN	(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl
Boc	N-tert-butoxycarbonyl
BupHTM	Thermo Scientific Prod# 28372, containing 100 mM sodium phosphate and 150 mM sodium chloride, potassium free, pH was adjusted from 7.2 to 7.6-7.8 MQ, unless otherwise noted.
CD	Cyclodextrin
COT	Cyclooctynol
Da	Dalton
DAR	Drug to antibody ratio.
DCM	Dichloromethane
DIBAC	Dibenz[b,f]azocine, 11,12-didehydro-5,6-dihydro- or Dibenzocyclooctyne or Dibenz[b,f]azocine-5(6H)-butanoic acid, 11,12-didehydro
DIBAC-Suc	Dibenz[b,f]azocine-5(6H)-butanoic acid, 11,12-didehydro
DIBACT	3H-Benzo[c]-1,2,3-triazolo[4,5-e][1]benzazocine, 8,9-dihydro-
DIBO	Dibenzocyclooctyne
DIFO	Difluorinated cyclooctyne
DIPEA	Diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
ESI	Electrospray ionization
g	Gram
HATU	2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HC	Heavy chain of immunoglobulin
HEK	Human embryonic kidney (cells)
HPLC	High performance liquid chromatography
hr or hrs	Hours
LC	Light chain of immunoglobulin
LC	Liquid chromatography
MC	Maleimidocaproyl
mg	Milligrams
min	Minutes
mL	Milliliters
mM	Millimolar
MMAE	Monomethyl auristatin E
MS	Mass spectrometry

Abbreviation	Term
MSD	Mass-selective detector
MTG	Microbial transglutaminase
MW	Molecular weight
ncADC	Non-Cytotoxic antibody drug conjugation
NHS	N-hydroxy succinimide
nM	nanomolar
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
PAB	Para-aminobezyloxy(carbonyl)
PBS	10 mM sodium phosphate buffer and 150 mM sodium chloride
PBSg	10 mM phosphate, 150 mM sodium chloride, 5% glycerol
PEG	Polyethyleneglycol
ppm	Parts per million (chemical shift)
RP	Reversed phase
RT or rt	Room temperature
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
Suc	Succinic acid
TCEP	Tris(2-carboxyethyl)phosphine hydrochloride
TEA	Triethylamine
TFA	Trifluoroacetic acid
TG	Transglutaminase
THF	Tetrahydrofuran
TOF	Time-of-flight
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet
VA	Valine-Aniline
VC	Valine-citrulline
µL	Microliters
µM	micromolar

[0428] Reagents and solvents were obtained from commercial sources such as Sinopharm Chemical Reagent Co. (SCRC), Sigma-Aldrich, Alfa, or other vendors, unless explicitly stated otherwise.

[0429] ^{1}H NMR and other NMR spectra were recorded on a Bruker AVIII 400 or Bruker AVIII 500. The data were processed with Nuts software or MestReNova software, measuring proton shifts in parts per million (ppm) downfield from an internal standard tetramethylsilane (TMS).

[0430] HPLC-MS measurements were run on an Agilent 1200 HPLC/6100 SQ System using the follow conditions:

[0431] Method A for HPLC-MS measurements included, as the Mobile Phase: A: Water (0.01% trifluoroacetic acid (TFA)), B: acetonitrile (0.01% TFA); Gradient Phase: 5% of B increased to 95% of B within 15 minutes (min); Flow Rate: 1.0 mL/min; Column: SunFire C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: Analog to Digital Converter (ADC) Evaporative Light-scattering Detector (ELSD), Diode array detector (DAD) (214 nm and 254 nm), electrospray ionization-atmospheric ionization (ES-API).

[0432] Method B for HPLC-MS measurements included, as the Mobile Phase: A: Water (10 mM NH_4HCO_3), B: acetonitrile; Gradient Phase: 5% to 95% of B within 15 min; Flow Rate: 1.0 mL/min; Column: XBridge C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), mass selective detector (MSD) (ES–API).

[0433] LC-MS measurements were run on an Agilent 1200 HPLC/6100 SQ System using the following conditions:

[0434] Method A for LC-MS measurements included, as the Instrument: WATERS 2767; column: Shimadzu Shim–Pack, PRC–ODS, 20x250mm, 15 µm, two connected in series; Mobile Phase: A: Water (0.01% TFA), B: acetonitrile (0.01% TFA); Gradient Phase: 5% of B increased to 95% of B within 3 min; Flow Rate: 1.8 – 2.3 mL/min; Column: SunFire C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), ES–API.

[0435] Method B for LC-MS measurement included, as the Instrument: Gilson GX–281; column: Xbridge Prep C18 10 µm OBD, 19x250 mm; Mobile Phase: A: Water (10 mM NH_4HCO_3), B: Acetonitrile; Gradient Phase: 5% to 95% of B within 3 min; Flow Rate: 1.8 – 2.3 mL/min; Column: XBridge C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), MSD (ES–API).

[0436] Preparative high-pressure liquid chromatography (Prep-HPLC) in an acidic or basic solvent system was utilized on a Gilson GX–281 instrument. The acidic solvent system used a Waters SunFire 10 µm C18 column (100Å, 250x19 mm), and solvent A for prep-HPLC was water/0.05% TFA and solvent B was acetonitrile. The elution conditions were a linear gradient increase of solvent B from 5% to 100% over a time period of 20 min at a flow rate of 30 mL/min. The basic solvent system included a Waters Xbridge 10 µm C18 column (100 Å, 250x19 mm), and solvent A used for prep-HPLC was water/10 mM ammonium bicarbonate (NH_4HCO_3) and solvent B was acetonitrile. The elution conditions were a linear gradient increase of solvent B from 5% to 100% over a time period of 20 min at a flow rate of 30 mL/min.

[0437] Flash chromatography was performed on a Biotage instrument, with Agela Flash Column silica–CS cartridges; Reversed phase flash chromatography was performed on Biotage instrument, with Boston ODS or Agela C18 cartridges.

[0438] Analytical chiral HPLC method - SFC conditions:

a) Instrument: SFC Method Station (Thar, Waters)

b) Column: CHIRALPAK AD-H/AS-H/OJ-H/OD-H 4.6×100mm, 5 μm (Daicel)

c) Column temperature: 40 ºC

d) Mobile phase: CO_2/ IPA(0.1% DEA)= 55/45

e) Flow: 4.0 ml/min

f) Back Pressure: 120 Bar

g) Injection volume: 2 μL.

[0439] Preparative chiral HPLC method - SFC conditions:

a) Instrument: SFC-80 (Thar, Waters)

b) Column: CHIRALPAK AD-H/AS-H/OJ-H/OD-H 20×250mm, 10 μm (Daicel)

c) Column temperature: 35 ºC

d) Mobile phase: CO_2/ IPA(0.2% Methanol Ammonia)= 30/70

e) Flow rate: 80 g/min

f) Back pressure: 100 bar

g) Detection wavelength: 214 nm

h) Cycle time: 6.0 min

i) Sample solution: 1500 mg dissolved in 70 mL Methanol

j) Injection volume: 2 mL (loading: 42.86 mg/injection).

[0440] As used herein, the symbols and conventions used in these processes, schemes, and examples, regardless of whether a particular abbreviation is specifically defined, are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry.

Example 1. Generation of Anti-MSR1 Antibodies

[0441] Anti-MSR1 antibodies were obtained by immunizing a genetically engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions

with an immunogen comprising recombinant human MSR1 extracellular domain fused to an N-terminal nonahistidine tag (R&D Systems, Catalog # 2708-MS-050, Minneapolis, MN). The mice used for the immunizations were Velocimmune mice or mice which expressed a "universal light chain" ("ULC" mice). Antibodies produced ULC mouse have different heavy chain variable regions but essentially identical light chain variable domains.

[0442] The antibody immune response was monitored by a MSR1-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce MSR1-specific antibodies. Using this technique several anti-MSR1 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained. In addition, several fully human anti-MSR1 antibodies were isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1.

[0443] Certain biological properties of the exemplary anti-MSR1 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[0444] Table 4 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-MSR1 antibodies described herein. The corresponding nucleic acid sequence identifiers are set forth in Table 5.

Table 4: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H21227N	2	4	6	8	10	12	14	16
H1H21228N	18	20	22	24	26	28	30	32
H1H21231N	34	36	38	40	42	44	46	48
H1H21234N	50	52	54	56	58	60	62	64
H1H21235N	66	68	70	72	74	76	78	80
H1H25685N	82	84	86	88	90	92	94	96
H1H25690N	98	100	102	104	106	108	110	112
H1H25695N	114	116	118	120	122	124	126	128

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1H25700N	130	132	134	136	138	140	142	144
H1H27729P	146	148	150	152	154	156	158	160
H1H27731P	162	164	166	168	170	172	174	176
H1H27732P	178	180	182	184	186	188	190	192
H1H27734P	194	196	198	200	202	204	206	208
H1H27736P	210	212	214	216	218	220	222	224
H1H27739P	226	228	230	232	234	236	238	240
H1H27747P	242	244	246	248	250	252	254	256
H1H27749P	258	260	262	264	266	268	270	272
H1H27751P	274	276	278	280	282	284	286	288
H1H27754P	290	292	294	296	298	300	302	304
H1H27756P	306	308	310	312	314	316	318	320
H1H27760P2	322	324	326	328	90	92	94	96
H1H27761P2	330	332	334	336	90	92	94	96
H1H27762P2	338	340	342	344	90	92	94	96
H1H27766P2	346	348	350	352	90	92	94	96
H1H27771P2	354	356	358	360	362	364	366	368
H1xH27759P2	370	372	374	376	90	92	94	96
H1xH27773P2	378	380	382	384	362	364	366	368
H1xH27778P2	386	388	390	392	362	364	366	368
H1xH29273P2	394	396	397	400	90	92	94	96
H1xH29282P2	402	404	406	408	90	92	94	96
H1xH29283P2	410	412	414	416	90	92	94	96
H2M21229N	420	422	424	426	428	430	432	434
H2M21230N	436	438	440	442	444	446	448	450
H2M21232N	452	454	456	458	460	462	464	466

Table 5: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1H21227N	1	3	5	7	9	11	13	15
H1H21228N	17	19	21	23	25	27	29	31
H1H21231N	33	35	37	39	41	43	45	47
H1H21234N	49	51	53	55	57	59	61	63
H1H21235N	65	67	69	71	73	75	77	79
H1H25685N	81	83	85	87	89	91	93	95
H1H25690N	97	99	101	103	105	107	109	111
H1H25695N	113	115	117	119	121	123	125	127
H1H25700N	129	131	133	135	137	139	141	143
H1H27729P	145	147	149	151	153	155	157	159
H1H27731P	161	163	165	167	169	171	173	175
H1H27732P	177	179	181	183	185	187	189	191
H1H27734P	193	195	197	199	201	203	205	207
H1H27736P	209	211	213	215	217	219	221	223
H1H27739P	225	227	229	231	233	235	237	239
H1H27747P	241	243	245	247	249	251	253	255
H1H27749P	257	259	261	263	265	267	269	271
H1H27751P	273	275	277	279	281	283	285	287
H1H27754P	289	291	293	295	297	299	301	303
H1H27756P	305	307	309	311	313	315	317	319
H1H27760P2	321	323	325	327	89	91	93	95
H1H27761P2	329	331	333	335	89	91	93	95
H1H27762P2	337	339	341	343	89	91	93	95
H1H27766P2	345	347	349	351	89	91	93	95
H1H27771P2	353	355	357	359	361	363	365	367
H1xH27759P2	369	371	373	375	89	91	93	95
H1xH27773P2	377	379	381	383	361	363	365	367
H1xH27778P2	385	387	389	391	361	363	365	367

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1xH29273P2	393	395	397	399	89	91	93	95
H1xH29282P2	401	403	405	407	89	91	93	95
H1xH29283P2	409	411	413	415	89	91	93	95
H2M21229N	419	421	423	425	427	429	431	433
H2M21230N	435	437	439	441	443	445	447	449
H2M21232N	451	453	455	457	459	461	463	465

[0445] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1H," "H2aM," *etc.*), followed by a numerical identifier (*e.g.* "21227," "21228," "21231," *etc.*), followed by a "P," "N," or "P2" suffix, as shown in Tables 4 and 5. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H21227N," "H2aM21228N," "H1H27729P," "H1H27760P2," *etc.* The prefix on the antibody designations used herein indicate the particular Fc region isotype of the antibody. In particular, an "H1H" antibody has a human IgG1 Fc (all variable regions are fully human as denoted by the first 'H' in the antibody designation), while an "H2aM" antibody has a mouse IgG2a Fc. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG4 Fc can be converted to an antibody with a human IgG1, *etc.*), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 4 and 5 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

[0446] *Antibody Modifications.* Three anti-MSR1 antibodies described in Example 1 (21227N, 21231N, 21234N) were produced with the original human **Fcγ** portion, as well as a version with an N297Q single point mutation for all three anti-MSR1 antibodies. All other antibodies described herein were made with an N297Q single point mutation in human **Fcγ** portion. A third version, an N297D mutation was produced for the 21227N antibody only.

Example 3. Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal Anti-MSR1 Antibodies

[0447] Binding affinities and kinetic constants of human anti-MSR1 antibodies for different MSR1 reagents were determined by real-time surface plasmon resonance (Biacore 4000). All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C and 37°C. The Biacore CM4

sensor chip surface was first derivatized by amine coupling with the goat anti-human Fcγ specific polyclonal antibody (Jackson ImmunoResearch Laboratories, Cat# BR-1008-39) to capture anti-MSR1 monoclonal antibodies. Binding studies were performed on human MSR1 extracellular domain expressed with a N-terminal nonahistidine tag (His9-hMSR1; R&D Systems, Cat# 2708-MS), and monkey MSR1 extracellular domain expressed with a N-terminal hexahistidine-myc-myc tag (HMM-mfMSR1; SEQ ID NO: 418). Different concentrations of His9-hMSR1 and HMM-mfMSR1 (100 nM - 3.7 nM; 3-fold serial dilution) were first prepared in HBS-ET running buffer and were injected over anti-human Fcγ captured anti-MSR1 monoclonal antibody surface for 3 minutes at a flow rate of 30 µL/minute, while the dissociation of monoclonal antibody bound MSR1 reagent was monitored for 10 minutes in HBS-ET running buffer.

[0448] The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life (t½) were calculated from the kinetic rates as:

$$K_D\ (M) = \frac{kd}{ka}, \text{ and } t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[0449] Binding kinetics parameters for His9-hMSR1 or HMM-mfMSR1 binding to different anti-MSR1 monoclonal antibodies at 25°C and 37°C are shown in Tables 6 and 7, respectively.

Table 6: Biacore Binding Affinities of Anti-MSR1 mAbs at 25 °C

Binding at 25 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H1H21227N-N297Q	His9-hMSR1	1.23E+06	4.89E-05	3.97E-11	236
	HMM-mfMSR1	1.36E+06	7.51E-05	5.53E-11	154
H1H21227N-N297D	His9-hMSR1	1.14E+06	3.79E-05	3.33E-11	305
	HMM-mfMSR1	1.35E+06	4.03E-05	2.99E-11	287
H1H21231N-N297Q	His9-hMSR1	3.99E+05	5.88E-05	1.47E-10	196
	HMM-mfMSR1	2.40E+05	9.03E-05	3.76E-10	128
H1H21234N-N297Q	His9-hMSR1	4.97E+05	1.00E-05*	2.01E-11	1155
	HMM-mfMSR1	4.08E+05	1.95E-05	4.66E-11	593
H1H27729P-N297Q	His9-hMSR1	1.97E+05	1.07E-03	5.45E-09	11
	HMM-mfMSR1	2.69E+05	2.12E-03	7.90E-09	5

Binding at 25 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H1H27731P-N297Q	His9-hMSR1	1.29E+05	2.24E-05	1.74E-10	515
	HMM-mfMSR1	9.82E+04	4.69E-05	4.77E-10	247
H1H27732P-N297Q	His9-hMSR1	1.25E+05	1.00E-05*	8.01E-11	1155
	HMM-mfMSR1	1.28E+05	3.17E-05	2.48E-10	364
H1H27734P-N297Q	His9-hMSR1	4.20E+05	1.11E-03	2.64E-09	10
	HMM-mfMSR1	4.23E+05	2.91E-03	6.88E-09	4
H1H27736P-N297Q	His9-hMSR1	5.15E+05	2.31E-04	4.48E-10	50
	HMM-mfMSR1	4.64E+05	5.87E-04	1.27E-09	20
H1H27739P-N297Q	His9-hMSR1	3.75E+05	1.03E-03	2.74E-09	11
	HMM-mfMSR1	3.52E+05	1.44E-04	4.10E-10	80
H1H27747P-N297Q	His9-hMSR1	2.43E+05	6.52E-04	2.69E-09	18
	HMM-mfMSR1	2.31E+05	8.74E-04	3.78E-09	13
H1H27749P-N297Q	His9-hMSR1	3.18E+05	1.76E-05	5.54E-11	656
	HMM-mfMSR1	2.49E+05	4.27E-05	1.71E-10	271
H1H27751P-N297Q	His9-hMSR1	1.78E+06	3.05E-04	1.72E-10	38
	HMM-mfMSR1	7.44E+05	7.49E-04	1.01E-09	15
H1H27754P-N297Q	His9-hMSR1	2.90E+05	1.00E-05*	3.44E-11	1155
	HMM-mfMSR1	2.35E+05	1.76E-05	7.50E-11	657
H1H27756P-N297Q	His9-hMSR1	3.00E+05	1.22E-04	4.06E-10	94
	HMM-mfMSR1	3.58E+05	2.44E-03	6.81E-09	5
H1H27760P-N297Q	His9-hMSR1	4.54E+05	9.09E-04	2.00E-09	13
	HMM-mfMSR1	3.63E+05	7.01E-04	1.93E-09	16
H1H27759P-N297Q	His9-hMSR1	5.99E+05	1.22E-03	2.03E-09	9
	HMM-mfMSR1	4.17E+05	9.19E-04	2.20E-09	13
H1H27761P-N297Q	His9-hMSR1	3.12E+05	5.10E-04	1.63E-09	23
	HMM-mfMSR1	3.18E+05	5.97E-04	1.88E-09	19
H1H27762P-N297Q	His9-hMSR1	9.89E+05	1.83E-03	1.85E-09	6
	HMM-mfMSR1	1.25E+06	1.99E-03	1.59E-09	6
H1H27766P-N297Q	His9-hMSR1	2.34E+05	1.86E-05	7.96E-11	620
	HMM-mfMSR1	1.57E+05	7.94E-05	5.06E-10	145
H1H27771P-N297Q	His9-hMSR1	6.86E+05	9.58E-04	1.40E-09	12
	HMM-mfMSR1	5.19E+05	5.26E-03	1.01E-08	2.2
H1H27773P-N297Q	His9-hMSR1	6.58E+05	2.63E-03	3.99E-09	4
	HMM-mfMSR1	6.43E+05	1.96E-03	3.05E-09	6
H1H27778P-N297Q	His9-hMSR1	5.75E+05	3.94E-04	6.85E-10	29
	HMM-mfMSR1	4.67E+05	1.36E-03	2.91E-09	8
H1H21234N	His9-hMSR1	6.04E+05	1.00E-05*	1.66E-11	1155
	HMM-mfMSR1	3.36E+05	1.00E-05*	2.98E-11	1155
H1H21231N	His9-hMSR1	4.77E+05	1.00E-05*	2.10E-11	1155
	HMM-mfMSR1	2.74E+05	6.39E-05	2.33E-10	181
H1H21227N	His9-hMSR1	1.20E+06	1.44E-05	1.20E-11	800
	HMM-mfMSR1	1.27E+06	4.41E-05	3.48E-11	262
Non-binding Control	His9-hMSR1	NB[$]	NB[$]	NB[$]	NB[$]
	HMM-mfMSR1	NB[$]	NB[$]	NB[$]	NB[$]

* indicates that no dissociation of His9-hMSR1 or HMM-mfMSR1 was observed under the current experimental conditions and the k_d value was manually fixed at 1.00E-05 while fitting the data

$ indicates that no binding was observed under the current experimental conditions.

Table 7: Biacore Binding Affinities of Anti-MSR1 mAbs at 37 °C

Binding at 37 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H1H21227N-N297Q	His9-hMSR1	2.67E+06	1.23E-05	4.60E-12	941
	HMM-mfMSR1	2.74E+06	1.08E-05	3.95E-12	1069
H1H21227N-N297D	His9-hMSR1	2.73E+06	1.00E-05*	3.66E-12	1155
	HMM-mfMSR1	2.68E+06	2.42E-05	9.03E-12	477
H1H21231N-N297Q	His9-hMSR1	5.34E+05	1.15E-04	2.15E-10	101
	HMM-mfMSR1	5.87E+05	1.09E-04	1.86E-10	106
H1H21234N-N297Q	His9-hMSR1	7.87E+05	1.00E-05*	1.27E-11	1155
	HMM-mfMSR1	7.50E+05	1.00E-05*	1.33E-11	1155
H1H27729P-N297Q	His9-hMSR1	2.39E+05	2.04E-03	8.53E-09	6
	HMM-mfMSR1	4.07E+05	3.49E-03	8.58E-09	3.3
H1H27731P-N297Q	His9-hMSR1	2.86E+05	1.32E-04	4.62E-10	88
	HMM-mfMSR1	2.78E+05	1.87E-04	6.74E-10	62
H1H27732P-N297Q	His9-hMSR1	2.81E+05	3.12E-05	1.11E-10	370
	HMM-mfMSR1	3.34E+05	1.06E-04	3.17E-10	109
H1H27734P-N297Q	His9-hMSR1	1.09E+06	1.90E-03	1.74E-09	6
	HMM-mfMSR1	9.49E+05	3.20E-03	3.37E-09	4
H1H27736P-N297Q	His9-hMSR1	1.02E+06	7.33E-04	7.17E-10	16
	HMM-mfMSR1	2.01E+06	1.28E-03	6.37E-10	9
H1H27739P-N297Q	His9-hMSR1	7.76E+05	2.88E-03	3.72E-09	4
	HMM-mfMSR1	2.25E+06	8.42E-04	3.74E-10	14
H1H27747P-N297Q	His9-hMSR1	5.13E+05	2.76E-03	5.37E-09	4
	HMM-mfMSR1	6.57E+05	2.28E-03	3.47E-09	5
H1H27749P-N297Q	His9-hMSR1	4.97E+05	2.42E-04	4.86E-10	48
	HMM-mfMSR1	4.77E+05	1.72E-04	3.61E-10	67
H1H27751P-N297Q	His9-hMSR1	1.45E+06	9.43E-04	6.50E-10	12
	HMM-mfMSR1	1.17E+06	1.80E-03	1.55E-09	6
H1H27754P-N297Q	His9-hMSR1	6.63E+05	2.53E-05	3.81E-11	457
	HMM-mfMSR1	7.01E+05	3.53E-05	5.04E-11	327
H1H27756P-N297Q	His9-hMSR1	6.63E+05	7.71E-04	1.16E-09	15
	HMM-mfMSR1	8.02E+05	3.19E-03	3.97E-09	4
H1H27760P-N297Q	His9-hMSR1	1.08E+06	1.89E-03	1.74E-09	6
	HMM-mfMSR1	1.52E+06	1.59E-03	1.05E-09	7
H1H27759P-N297Q	His9-hMSR1	1.03E+06	2.30E-03	2.24E-09	5
	HMM-mfMSR1	1.46E+06	1.88E-03	1.28E-09	6
H1H27761P-N297Q	His9-hMSR1	6.81E+05	2.22E-03	3.26E-09	5
	HMM-mfMSR1	9.20E+05	2.14E-03	2.32E-09	5
H1H27762P-N297Q	His9-hMSR1	3.06E+06	1.96E-03	6.40E-10	6
	HMM-mfMSR1	2.82E+06	1.97E-03	6.98E-10	6
H1H27766P-N297Q	His9-hMSR1	3.40E+05	7.72E-05	2.27E-10	150
	HMM-mfMSR1	3.43E+05	5.89E-04	1.72E-09	20
H1H27771P-N297Q	His9-hMSR1	1.35E+06	2.04E-03	1.51E-09	6
	HMM-mfMSR1	4.94E+05	9.07E-03	1.84E-08	1.3

Binding at 37 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H1H27773P-N297Q	His9-hMSR1	9.19E+05	3.07E-03	3.34E-09	4
	HMM-mfMSR1	9.60E+05	5.06E-03	5.27E-09	2.3
H1H27778P-N297Q	His9-hMSR1	1.19E+06	6.55E-04	5.49E-10	18
	HMM-mfMSR1	1.19E+06	1.25E-03	1.05E-09	9
H1H21234N	His9-hMSR1	6.76E+05	1.00E-05*	1.48E-11	1155
	HMM-mfMSR1	7.26E+05	1.00E-05*	1.38E-11	1155
H1H21231N	His9-hMSR1	7.02E+05	9.09E-05	1.30E-10	127
	HMM-mfMSR1	7.12E+05	7.82E-05	1.10E-10	148
H1H21227N	His9-hMSR1	2.56E+06	1.85E-05	7.24E-12	624
	HMM-mfMSR1	2.76E+06	1.00E-05*	3.62E-12	1155
Non-binding Control	His9-hMSR1	NB$	NB$	NB$	NB$
	HMM-mfMSR1	NB$	NB$	NB$	NB$

* indicates that no dissociation of His9-hMSR1 or HMM-mfMSR1 was observed under the current experimental conditions and the k_d value was manually fixed at 1.00E-05 while fitting the data

$ indicates that no binding was observed under the current experimental conditions.

[0450] At 25°C, all of the anti-MSR1 monoclonal antibodies of the invention bound to His9-hMSR1 with K_D values ranging from 12 pM to 5.45 nM, as shown in Table 6. At 37°C, all of the anti-MSR1 monoclonal antibodies of the invention bound to His9-hMSR1 with K_D values ranging from 3.66 pM to 8.53 nM, as shown in Table 7.

[0451] At 25°C, all of the anti-MSR1 monoclonal antibodies of the invention bound to HMM-mfMSR1 with K_D values ranging from 29.9 pM to 10.1 nM, as shown in Table 6. At 37°C, all of the anti-MSR1 monoclonal antibodies of the invention bound to HMM-mfMSR1 with K_D values ranging from 3.62 pM to 18.4 nM, as shown in Table 7.

Example 4. Octet-Derived Binding Affinities and Kinetic Constants of Human Monoclonal Anti-MSR1 Antibodies

[0452] Binding affinities and kinetic constants of human anti-MSR1 antibodies for different MSR1 reagents were determined using a real time, label-free bio-layer interferometry assay on an OCTET® HTX biosensor platform (Pall FortéBio Corp., Menlo Park, CA). The experiment was performed at 25°C in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% v/v Surfactant Tween-20, and 1 mg/mL BSA, pH7.4 (HBS-EBT) buffer with the plate shaking at the speed of 1000rpm. Binding studies were performed on human MSR1 extracellular domain expressed with a N-terminal nonahistidine tag (His9-hMSR1; R&D Systems, Cat # 2708-MS), monkey MSR1 extracellular domain expressed with a N-terminal myc-myc-hexahistidine tag (HMM-mfMSR1; SEQ ID NO: 418), and mouse MSR1 extracellular domain expressed with a N-terminal nonahistidine tag (His9-mMSR1; R&D Systems, Cat# 1797-MS). The anti-MSR1 monoclonal

antibodies were captured by dipping either anti-human Fc (AHC) or anti-mouse Fc (AMC) Octet biosensors in wells containing 5 µg/mL or 10 µg/mL of anti-MSR1 monoclonal antibody for 45-90 seconds. The AHC captured anti-MSR1 monoclonal antibodies were then dipped in wells containing 50 nM of His9-mMSR1, while the AMC captured anti-MSR1 monoclonal antibodies were dipped in wells containing different concentrations of His9-hMSR1 or HMM-mfMSR1 (100 nM, 25 nM) or 100 nM His9-mMSR1. The binding of different MSR1 reagents to the captured anti-MSR1 monoclonal antibody was measured for 4 minutes and the dissociation of monoclonal antibody bound MSR1 reagent was monitored for 8-10 minutes in HBS-EBT buffer.

[0453] The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life (t½) were calculated from the kinetic rates as:

$$K_D\ (M) = \frac{kd}{ka}, \text{ and } t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[0454] Binding kinetics parameters for His9-hMSR1, HMM-mfMSR1 or His9-mMSR1binding to different anti-MSR1 monoclonal antibodies of the invention at 25°C are shown in Tables 8 and 9.

Table 8: OCTET® Binding Affinities of Anti-MSR1 mAbs at 25 °C

Binding at 25 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H2aM21228N	His9-hMSR1	1.22E+05	1.16E-04	9.54E-10	100
	HMM-mfMSR1	1.00E+05	1.46E-04	1.45E-09	79
H2aM21229N	His9-hMSR1	3.00E+05	2.10E-04	7.00E-10	55
	HMM-mfMSR1	1.35E+05	2.22E-03	1.64E-08	5
H2aM21230N	His9-hMSR1	6.47E+05	2.87E-04	4.43E-10	40
	HMM-mfMSR1	2.37E+05	3.76E-04	1.59E-09	31
H2aM21232N	His9-hMSR1	1.30E+05	2.86E-04	2.20E-09	40
	HMM-mfMSR1	9.75E+04	3.27E-04	3.35E-09	35
H2aM21235N	His9-hMSR1	1.21E+05	4.58E-05	3.78E-10	252
	HMM-mfMSR1	1.02E+05	6.27E-05	6.12E-10	184
H2aM25700N	His9-hMSR1	5.58E+05	1.14E-04	2.05E-10	101
	HMM-mfMSR1	5.53E+05	1.15E-04	2.08E-10	100
H2aM25690N	His9-hMSR1	2.29E+05	3.11E-04	1.36E-09	37
	HMM-mfMSR1	1.64E+05	5.47E-04	3.35E-09	21
H2aM25695N	His9-hMSR1	3.60E+05	5.27E-04	1.46E-09	22
	HMM-mfMSR1	3.12E+05	5.71E-04	1.83E-09	20
H2aM25685N	His9-hMSR1	2.01E+05	3.97E-04	1.98E-09	29
	HMM-mfMSR1	6.49E+04	1.28E-03	1.97E-08	9
mIgG Isotype Control	His9-hMSR1	NB[$]	NB[$]	NB[$]	NB[$]
	HMM-mfMSR1	NB[$]	NB[$]	NB[$]	NB[$]

[$] indicates that no binding was observed under the current experimental conditions.

Table 9: OCTET® Binding Affinities of Anti-MSR1 mAbs at 25 °C

Binding at 25 °C / Antibody-Capture Format					
Antibody	**Analyte**	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H2aM21228N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM21229N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM21230N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM21232N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM21235N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM25700N	His9-mMSR1	3.60E+04	1.85E-04	5.20E-09	63
H2aM25690N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM25695N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H2aM25685N	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
mIgG Isotype Control	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H21227N-N297Q	His9-mMSR1	3.74E+05	7.08E-04	1.89E-09	16
H1H21227N-N297D	His9-mMSR1	4.61E+05	7.86E-04	1.71E-09	15
H1H21231N-N297Q	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H21234N-N297Q	His9-mMSR1	3.82E+04	5.00E-05*	1.31E-09	231
H1H27729P N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27731P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27732P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27734P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27736P-N297Q	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H27739P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27747P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27749P-N297Q	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H27751P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27754P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27756P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27760P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27759P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27761P-N297Q	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H27762P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27766P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27771P-N297Q	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H27773P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H27778P-N297Q	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]
H1H21234N	His9-mMSR1	4.60E+04	5.00E-05*	1.09E-09	231
H1H21231N	His9-mMSR1	IC[#]	IC[#]	IC[#]	IC[#]
H1H21227N	His9-mMSR1	4.15E+05	8.16E-04	1.97E-09	14
Non-binding Control	His9-mMSR1	NB[$]	NB[$]	NB[$]	NB[$]

* indicates that no dissociation of His9-mMSR1 was observed under the current experimental conditions and the k_d value was manually fixed at 5.00E-05 while fitting the data

[$] indicates that no binding was observed under the current experimental conditions.

# indicates that the binding data is inconclusive (IC)

[0455] The anti-MSR1 monoclonal antibodies bound to His9-hMSR1 with K_D values ranging from 205 pM to 2.2 nM, as shown in Table 8. The anti-MSR1 monoclonal antibodies bound to HMM-mfMSR1 with K_D values ranging from 208 pM to 19.7 nM, as shown in Table 8.

[0456] As shown in Table 9, 23 out of 35 anti-MSR1 monoclonal antibodies did not bind to His9-mMSR1, while the binding data for 6 anti-MSR1 monoclonal antibodies was inconclusive. Six (6) out of 35 anti-MSR1 monoclonal antibodies bound to His9-mMSR1 with K_D values ranging from 1.09 nM to 5.2 nM, as shown in Table 9.

Example 5. Anti-MSR1 Antibodies Display Specific Binding to Cell Surface-Expressed Human and Monkey MSR1

[0457] The ability of anti-MSR1 monoclonal antibodies to bind to human or monkey MSR1 expressing cells was determined using electrochemiluminescence (ECL) based detection.

[0458] *Generation of MSR1-expressing cell lines.* Two cell lines overexpressing either human or monkey MSR1 were generated. To generate the human MSR1 overexpressing cell line, human embryonic kidney (HEK) 293 cells were engineered by transduction with hygromycin resistant lentiviral vector encoding full length human MSR1 (hMSR1, amino acids M1-L451 of accession number NP_619729.1) with a C-terminal Myc tag. The resulting cell line is referred to as HEK293.Myc.hMSR1. Similarly, to generate the monkey MSR1 overexpressing cell line, HEK293 cells were engineered by transfection with the geneticin resistant expression plasmid encoding full length monkey MSR1 (*Macaca fascicularis*, mfMSR1, amino acids M1-L451 of accession number XP_005562705.1). The resulting cell line is referred to as HEK293.mfMSR1 cells. To measure the ability of antibodies to bind to endogenously expressed human MSR1, THP-1 human monocytic cells were treated with 200 nM of phorbol 12-myristate 13-acetate (PMA; Sigma, Cat # P8139) for 72 hours to induce high MSR1 expression prior to antibody binding. Non-transfected HEK293 cells were included as non-specific binding controls as they have no detectable expression of MSR1 by next-generation sequencing of gene expression (data not shown).

[0459] *Antibody binding assay.* To perform the antibody binding assay, cells from each of the cell lines described above were rinsed once in PBS buffer without Ca^{2+}/Mg^{2+} and incubated for 5 minutes at 37°C with Enzyme Free Cell Dissociation Solution (Millipore, Cat. # S-004-C, Burlington, MA) to detach cells from a flask. All cells were washed once with 1xPBS with Ca^{2+}/Mg^{2+} and counted with a Cellometer™ Auto T4 cell counter (Nexcelom Bioscience). Approximately $1.0x10^4$ cells were seeded separately onto 96-well carbon electrode plates [MULTI- ARRAY high bind plate, Meso Scale Diagnostics] and incubated for 1 hour at 37°C.

Non-specific binding sites were then blocked by 2% BSA (w/v) in PBS with Ca^{2+}/Mg^{2+} for 1 hour at room temperature. THP-1 cells were pre-incubated for 0.5 hours at room temperature in sample dilution buffer with: 1) 1 mg/mL Fc receptor block reagents to block Fc gamma receptors on THP-1 cell surface [whole molecule human IgG (Jackson Immunoresearch, Cat #009-000-003) for wells being tested with anti-MSR1-mFc antibodies or 2) whole molecule mIgG (Jackson Immunoresearch, Cat #015-000-003) for wells being tested with anti-MSR1-hFc antibodies. Antibody binding on HEK293.Myc.hMSR1, HEK293.mfMSR1 and HEK293 cells was tested without Fc receptor block reagents. To the plate-bound HEK293.Myc.hMSR1, HEK293.mfMSR1 and HEK293 cells or THP-1+Fc block, solutions of anti-MSR1 or control antibodies in serial dilutions ranging from 1.7 pM to 100 nM, and solutions without the presence of antibodies were added in duplicate, and the plates were incubated for 1 hour at room temperature. Plates were then washed to remove unbound antibodies an AquaMax2000 plate washer with a cell washing head (MDS Analytical Technologies). The plate-bound antibodies were detected with either a SULFO-TAG™-conjugated goat polyclonal anti-human IgG antibody specific for Fcγ fragment (Jackson Immunoresearch, Cat #109-005-098) or a SULFO-TAG™-conjugated goat polyclonal anti-mouse IgG antibody specific for Fcγ fragment (Jackson Immunoresearch, Cat #115-005-164) for 1 hour at room temperature. Plates were washed and developed with Read Buffer (Meso Scale Diagnostics, Cat # R92TD-2) according to manufacturer's recommended procedure and luminescent signals were recorded with a SECTOR Imager 600 (Meso Scale Diagnostics). Luminescence intensity, measured in relative light units (RLU), was recorded to indicate the binding intensity of each antibody at the range of concentrations. The ratio of signal detected for cell-surface binding of each anti-MSR antibody compared to isotype control antibody (both at 11 nM) was reported as an indication of specificity of MSR1 binding. Antibodies with the binding ratio on MSR-1 expressing cells of greater than or equal to 2-fold compared to the ratio on parental HEK293 cells were classified as specific binders. Antibodies with a binding ratio of less than 2-fold compared to the ratio on parental HEK293 cells were classified as non-binders as shown in. (See Table 10).

Table 10: Binding of Anti-MSR1 Antibodies to MSR1-Expressing Cells

Antibody	Ratio of 11nM Antibody Binding Signal (RLU) on MSR1-expressing cells and parental HEK293 to isotype control			
	HEK293.Myc.hMSR1	PMA-treated THP-1	HEK293.mfMSR1	HEK293
Specific Human and monkey MSR1 binders				

Antibody	Ratio of 11nM Antibody Binding Signal (RLU) on MSR1-expressing cells and parental HEK293 to isotype control			
	HEK293.Myc.hMSR1	PMA-treated THP-1	HEK293.mfMSR1	HEK293
H1H21227N-N297Q	37	5	23	1
H1H21227N-N297D	34	6	25	1
H1H21227N	37	7	26	1
H1H21231N-N297Q	64	45	69	4
H1H21231N	61	42	75	<1
H1H21234N-N297Q	43	25	35	9
H1H21234N	32	18	31	3
H1H27729P-N297Q	17	8	6	3
H1H27731P-N297Q	48	38	43	7
H1H27732P-N297Q	72	56	46	6
H1H27734P-N297Q	40	21	28	14
H1H27736P-N297Q	58	48	45	12
H1H27739P-N297Q	22	9	20	1
H1H27747P-N297Q	26	13	21	5
H1H27749P-N297Q	27	33	24	3
H1H27751P-N297Q	63	54	49	15
H1H27754P-N297Q	55	66	53	18
H1H27756P-N297Q	38	21	20	6
H1H27759P2-N297Q	23	9	25	2
H1H27760P2-N297Q	29	15	25	3
H1H27761P2-N297Q	23	10	15	3
H1H27762P2-N297Q	33	11	25	3
H1H27771P2-N297Q	42	21	7	2
H1H27773P2-N297Q	5	3	10	2
H1H27778P2-N297Q	51	32	29	4
H1xH27759P2	22	6	21	<1
H1xH29283P2	26	9	24	1
H2aM25685N	75	11	25	2
H2aM25690N	166	25	77	5
H2aM25695N	35	8	45	5
H2aM25700N	46	3	39	1
H2aM21228N	66	16	60	2

Antibody	Ratio of 11nM Antibody Binding Signal (RLU) on MSR1-expressing cells and parental HEK293 to isotype control			
	HEK293.Myc.hMSR1	PMA-treated THP-1	HEK293.mfMSR1	HEK293
H2aM21230N	78	21	55	5
H2aM21232N	86	21	51	1
H2aM21235N	68	20	40	6
Specific Human MSR1 only binders				
H2aM21229N	78	56	27	36
H1xH29282P2	9	3	1	<1
H1H27766P2-N297Q	20	9	13	8
Non-specific binder				
H1xH29273P2	20	24	27	
Isotype controls				
hIgG1 Isotype Control	1	1	1	1
mIgG Isotype Control	1	1	1	1

[0460] As illustrated in Table 10, thirty-eight of thirty-nine tested anti-MSR1 antibodies bound specifically to HEK293.Myc.hMSR1 cells with binding ratios ranging from 5- to 166-fold above isotype control at 11nM anti-MSR1 antibody concentration. Thirty-three of these anti-MSR1 antibodies specifically bound to THP-1 cells endogenously expressing human MSR1 after PMA cell differentiation with cell binding ratios ranging from 3- to 66-fold above isotype control at 11 nM. Thirty-five anti-MSR1 antibodies that bound to engineered hMSR1 cells also bound specifically to mfMSR1 engineered cells with cell binding ratios ranging from 6- to 77-fold above isotype control at 11 nM. Twelve anti-MSR1 antibodies (H1H21234N-N297Q, H1H27731P-N297Q, H1H27732P-N297Q, H1H27734P-N297Q, H1H27736P-N297Q, H1H27751P-N297Q, H1H27754P-N297Q, H1H27756P-N297Q, H2aM25695N, H2aM21235N, H2aM21229N, H1H27766P2-N297Q) bound to parental HEK293 cells with ratios 5-fold or greater above isotype control. Anti-MSR1 antibodies produced with a human IgG1 containing a N297Q or a N297D single point mutation bound cells comparable to their corresponding unmodified parental antibodies.

[0461] One anti-MSR1 antibody, H1xH29273P2, was characterized as a non-specific binder, as it bound to MSR1 cells with ratios less than 2 compared to the HEK293 cells at 11 nM antibody concentration. The hIgG1 and mIgG1 isotype controls were not specific, as expected.

Example 6. Relative Binding of Anti-MSR1 Antibodies to Cell Surface-Expressed Mouse MSR1

[0462] Relative cell surface binding of the anti-MSR1 antibodies to mouse MSR1 expressing cells was determined by flow cytometry using MSR1 positive RAW264.7 cells(ATCC, Catalog #TIB-71) and MSR1 negative B16F10.9 cells (Lin *et al.* 1998. *PNAS* 95:8829-8834). For the assay, cells were plated in PBS without calcium and magnesium (VWR Cat# 45000-446) and 2% FBS (Saradigm Cat# 1500-500) (Staining Buffer) in 96 well V-bottom plates (Axygen Scientific, Cat# P-96-450-V-C-S). To block binding to Fc receptors, RAW264.7 cells were incubated for 30 minutes at 4°C with 500 µg/mL mouse IgG (Jackson ImmunoResearch,Cat# 015-000-003) diluted in staining buffer, while B16F10.9 cells remained in staining buffer. Following Fc receptor blocking, 10 µg/mL of anti-MSR1 antibodies or an isotype control antibody were added to the cells and were subsequently incubated for 30 minutes on ice. For a positive control, a commercial anti-mouse MSR1 (Sino Biological, Cat# 50129-R004) antibody was used, while a rabbit IgG antibody (Thermo Scientific, Cat# 26102) was used as a negative control. The cells were then washed once with staining buffer and were incubated with either an APC conjugated anti-human Fc secondary antibody (Jackson ImmunoResearch, Cat# 109-136-170) or an Alexa-Flour 647 conjugated anti-rabbit Fc secondary antibody [Jackson ImmunoResearch Cat# 111-606-046] at 10 µg/mL for 30 minutes at 4°C. Cells were subsequently washed and fixed using a 50% solution of Cytofix (BD Biosciences, Cat# 554655) diluted in PBS. Samples were run on the Beckman Coulter Cytoflex and results were analyzed in Flowjo 10.2 software (BD) to calculate the mean fluorescent intensity (MFI; Table 11). The signal to noise (S/N) was determined by calculating the ratio of the anti-MSR1 antibodies or the control antibodies MFI to the unstained sample MFI (Table 11).

Table 11: Binding of Anti-MSR1 Antibodies to RAW264.7 Cells (Flow Cytometry)

Antibody	RAW264.7 MFI	B16F10.9 MFI	RAW264.7 S/N	B16F10.9 S/N
Unstained	2524	3657	1	1
Anti-human IgG secondary antibody only	3095	3174	1	1
Non-binding control	3402	3829	1	1
H1H21227N-N297Q	12810	3287	5	1
H1H21231N-N297Q	3582	3374	1	1
H1H21234N-N297Q	7052	3499	3	1
Anti-mouse MSR antibody	245149	6161	97	2
Anti-rabbit IgG secondary antibody only	8720	5389	3	1
H1H27729P-N297Q	3484	3459	1	1
H1H27731P-N297Q	3510	3688	1	1

Antibody	RAW264.7 MFI	B16F10.9 MFI	RAW264.7 S/N	B16F10.9 S/N
H1H27732P-N297Q	3425	3730	1	1
H1H27734P-N297Q	4783	4505	1	1
H1H27736P-N297Q	3554	3759	1	1
H1H27739P-N297Q	3271	3580	1	1
H1H27747P-N297Q	3630	3875	1	1
H1H27749P-N297Q	3789	3693	1	1
H1H27751P-N297Q	3823	4992	1	1
H1H27754P-N297Q	5406	4091	1	1
H1H27756P-N297Q	4573	3782	1	1
H1H27759P-N297Q	3288	3425	1	1
H1H27760P-N297Q	3429	3521	1	1
H1H27761P-N297Q	3837	3734	1	1
H1H27762P-N297Q	3519	3608	1	1
H1H27766P-N297Q	3812	3793	1	1
H1H27771P-N297Q	4055	3865	1	1
H1H27773P-N297Q	3367	3735	1	1
H1H27778P-N297Q	3648	4138	1	1

[0463] As illustrated in Table 11, two anti-MSR1 antibodies (H1H21227N-N297Q and H1H21234N-N297Q) bound weakly to RAW264.7 cells with S/N values of 5 and 3, respectively. The non-binding control antibody did not bind RAW264.7 cells. None of the 22 anti-MSR1 antibodies bound to B16F10.9 cells. A reference positive control (mouse MSR1/CD204 antibody, Sino Biological, Cat. # 50129-R004) bound Raw 264.7 cells with a S/N of 97.

Example 7. Anti-MSR1 Antibodies Bind to Distinct Epitopes on MSR1 Receptor / Cross-competition Between Anti-MSR1 Antibodies

[0464] Binding competition between different anti-MSR1 antibodies was assessed using a real time, label-free bio-layer interferometry assay on an OCTET® HTX biosensor platform (Pall FortéBio Corp., Menlo Park, CA). The experiment was performed at 25°C in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% v/v Surfactant Tween-20, and 1 mg/mL BSA, pH 7.4 (HBS-EBT) buffer with the plate shaking at the speed of 1000rpm.

[0465] To assess whether different antibodies are able to compete with one another for binding to their respective epitopes on the recombinant human MSR1 extracellular domain expressed with a N-terminal nonahistidine tag (His9-hMSR1; R&D Systems, Cat# 2708-MS), around 0.59-0.79 nM of His9-hMSR1 was first captured onto anti-Penta-His antibody coated OCTET® biosensor tips (Pall FortéBio Corp., # 18-5122) by submerging the biosensor tips for 45 seconds into wells containing a 20 µg/mL solution of His9-hMSR1. The antigen-captured

biosensor tips were then saturated with a first anti-MSR1 monoclonal antibody (subsequently referred to as "mAb-1") by immersion into wells containing a 50 µg/mL solution of mAb-1 for 4 minutes. The biosensor tips were then submerged into wells containing a 50 µg/mL solution of a second anti-MSR1 monoclonal antibody (subsequently referred to as "mAb-2") for 4 minutes. All of the biosensor tips were washed in HBS-EBT buffer in between each step of the experiment. The real-time binding response was monitored during the course of the experiment and the binding response at the end of each step was recorded. The response of mAb-2 binding to His9-hMSR1 pre-complexed with mAb-1 was compared, and the competitive/non-competitive behavior of the different anti-MSR1 monoclonal antibodies was determined using a 50% inhibition threshold. Table 12 explicitly defines the relationships of antibodies competing in both directions, independent of the order of binding.

Table 12: Cross-competition of Anti-MSR1 Antibodies for Binding to His9-hMSR1

First mAb (mAb-1) Captured using Anti-Penta-His Octet Biosensors	mAb-2 antibodies which Compete with mAb-1	First mAb (mAb-1) Captured using Anti-Penta-His Octet Biosensors	mAb-2 antibodies which Compete with mAb-1
H1H27756P-N297Q	H1H21231N	H1H27751P-N297Q	H1H21234N-N297Q
	H1H27760P-N297Q		H1H27734P-N297Q
	H1H27762P-N297Q		H1H27732P-N297Q
	H1H21231N-N297Q		H1H27731P-N297Q
	H1H27747P-N297Q		H1H27754P-N297Q
	H1H27749P-N297Q		H1H27766P-N297Q
H1H21231N	H1H27756P-N297Q		H1H21227N-N297D
	H1H27760P-N297Q	H1H27732P-N297Q	H1H27734P-N297Q
	H1H27762P-N297Q		H1H27751P-N297Q
	H1H21231N-N297Q		H1H27731P-N297Q
	H1H27747P-N297Q		H1H27754P-N297Q
	H1H27749P-N297Q	H1H27731P-N297Q	H1H27734P-N297Q
H1H27760P-N297Q	H1H27756P-N297Q		H1H27751P-N297Q
	H1H21231N		H1H27732P-N297Q
	H1H27762P-N297Q		H1H27754P-N297Q
	H1H21231N-N297Q	H1H27754P-N297Q	H1H27734P-N297Q
	H1H27747P-N297Q		H1H27751P-N297Q

First mAb (mAb-1) Captured using Anti-Penta-His Octet Biosensors	mAb-2 antibodies which Compete with mAb-1
	H1H27749P-N297Q
H1H27762P-N297Q	H1H27756P-N297Q
	H1H21231N
	H1H27760P-N297Q
	H1H21231N-N297Q
	H1H27747P-N297Q
	H1H27749P-N297Q
	H1H27766P-N297Q
H1H21231N-N297Q	H1H27756P-N297Q
	H1H21231N
	H1H27760P-N297Q
	H1H27762P-N297Q
	H1H27747P-N297Q
	H1H27749P-N297Q
H1H27747P-N297Q	H1H27760P-N297Q
	H1H27762P-N297Q
	H1H21231N-N297Q
H1H27749P-N297Q	H1H27760P-N297Q
	H1H27762P-N297Q
	H1H21231N-N297Q
H1H21234N-N297Q	H1H21234N
H1H21234N	H1H21234N-N297Q
H1H27734P-N297Q	H1H21234N-N297Q
	H1H27751P-N297Q
	H1H27732P-N297Q
	H1H27731P-N297Q
	H1H27754P-N297Q
	H1H27732P-N297Q
	H1H27731P-N297Q
H1H27761P-N297Q	H1H27736P-N297Q
	H1H27771P-N297Q
	H1H27778P-N297Q
	H1H27773P-N297Q
	H1H27739P-N297Q
H1H27736P-N297Q	H1H27761P-N297Q
	H1H27771P-N297Q
	H1H27778P-N297Q
	H1H27759P-N297Q
	H1H27773P-N297Q
	H1H27773P-N297Q
H1H27771P-N297Q	H1H27761P-N297Q
	H1H27736P-N297Q
	H1H27778P-N297Q
	H1H27759P-N297Q
	H1H27773P-N297Q
	H1H27739P-N297Q
H1H27778P-N297Q	H1H27761P-N297Q
	H1H27736P-N297Q
	H1H27771P-N297Q
	H1H27759P-N297Q
	H1H27773P-N297Q
	H1H27739P-N297Q
H1H27759P-N297Q	H1H27736P-N297Q
	H1H27771P-N297Q

First mAb (mAb-1) Captured using Anti-Penta-His Octet Biosensors	mAb-2 antibodies which Compete with mAb-1	First mAb (mAb-1) Captured using Anti-Penta-His Octet Biosensors	mAb-2 antibodies which Compete with mAb-1
	H1H27766P-N297Q		H1H27778P-N297Q
	H1H21227N-N297D		H1H27773P-N297Q
H1H27766P-N297Q	No mAb*		H1H27739P-N297Q
	H1H21234N-N297Q		H1H27749P-N297Q
H1H21227N-N297D	H1H21234N	H1H27773P-N297Q	H1H27766P-N297Q
	H1H27766P-N297Q		H1H21227N-N297D
H1H27739P-N297Q	H1H27759P-N297Q	H1H27729P-N297Q	Data Inconclusive**
H1H21227N	Data Inconclusive**	H1H21227N-N297Q	Data Inconclusive**

*Does not cross compete with any other mAb for binding to MSR1 when captured as 'mAb-1'

**mAb failed to saturate MSR1 surface or did not bind to MSR1 surface

Example 8. Ligand Uptake of Anti-MSR1 Antibodies

[0466] MSR1 can binds and internalize chemically modified or altered polyanionic molecules, including modified low density lipoproteins (LDL) (Platt, N. and S. Gordon. 2001. *J Clin Invest.* 108(5):649-654). A bioassay was generated to assess the ability of the exemplary anti-MSR1 antibodies to regulate the uptake of certain MSR1 ligands.

[0467] *Generation of MSR1-expressing cell lines for assay.* Human embryonic kidney cells (HEK293) were transduced to stably express human MSR1 (amino acids 1-451 of UniProtKB accession number NP_619729.1) with a C-terminal Myc tag. The resulting cell line, referred to here as "HEK293.Myc.hMSR1", was selected and maintained in DMEM containing 10% FBS, NEAA, penicillin/streptomycin, L-glutamine, and 100 μg/mL hygromycin.

[0468] *Ligand uptake assay.* For the bioassay, HEK293.Myc.hMSR1 cells were plated onto 96-well poly-D-lysine-coated assay plates (Greiner Bio One, Cat #655946) at 20,000 cells per well in Opti-MEM containing 0.1% FBS, penicillin/streptomycin, and L-Glutamine (assay media) and incubated at 37°C in 5% CO_2 overnight. The following day, antibodies were serially diluted from 300 nM to 5.08 pM (1:3 serial dilution) and pre-incubated with the cells, along with a negative control consisting of assay media alone, for 30 minutes at 37°C in 5% CO_2. After 30 minutes, either oxidized or acetylated low-density lipoprotein (LDL) labeled with 1,1'dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (referred to as "DiI-OxLDL" or "DiI-AcLDL," respectively) was added to the cells at a constant concentration of 10 μg/mL. To

determine the dose response of ligand uptake, DiI-OxLDL or DiI-AcLDL was serially diluted from 25 μg/mL to 24.4 pg/mL (plus assay media alone without LDL) and added to cells not iwith antibodies. After an overnight incubation at 37°C in 5% CO_2, cells were fixed with BD CytoFix™ (BD Biosciences, Cat# 554655) for 2 hours at 4°C, and ligand uptake was evaluated using a Flexstation3 plate reader (Molecular Devices) with excitation at 514 nm and emission at 565 nm. Results were analyzed using nonlinear regression (4-parameter logistics) with the Prism 7 program to obtain EC_{50} and IC_{50} values. The percentage of inhibition was calculated with the Relative Fluorescent Unit (RFU) values by using the following equation:

$$Max\ \%\ Inhibition = 100 \times \frac{RLU_{Baseline} - RLU_{Inhibition}}{RLU_{Baseline} - RLU_{Background}}$$

[0469] In the equation, "$RFU_{Baseline}$" is the fluorescence value from the cells treated with 10 μg/mL ligand without antibodies, "$RFU_{Inhibition}$" is the minimum fluorescence value with for a particular antibody with 10 μg/mL ligand, and "$RFU_{Background}$" is the fluorescence value from cells without any ligand or antibodies. The results and calculated values of the ligand uptake assay are provided in Table 13.

Table 13: Antibody Inhibition of DiI-OxLDL and DiI-AcLDL Uptake in HEK293.Myc.hMSR1 Cells

		DiI-Oxidized LDL		DiI-Acetylated LDL	
Row	**Antibodies**	**% Inhibition**	**IC_{50} (M)**	**% Inhibition**	**IC_{50} (M)**
1	H2aM21227N	90	3.1E-09	62	3.7E-09
2	H2aM21228N	79	3.1E-09	10	>1.0E-07
3	H2aM21229N	26	>1.0E-07	no inhibition	no inhibition
4	H2aM21230N	70	2.3E-09	47	>1.0E-08
5	H1M21231N	79	3.7E-09	50	>2.0E-08
6	H2aM21232N	79	4.8E-09	54	>1.0E-08
7	H2bM21234N	61	4.5E-09	19	1.8E-09
8	H2aM21235N	64	3.2E-09	24	>1.0E-07
9	Mouse IgG2a Isotype control mAb 1	no inhibition	no inhibition	no inhibition	no inhibition
10	Mouse IgG1 Isotype control mAb	no inhibition	no inhibition	no inhibition	no inhibition
11	H2aM25685N	43	>1.0E-07	22	>1.0E-07
12	H2aM25690N	83	1.7E-09	53	2.1E-09
13	H2aM25695N	69	>1.6E-08	40	>1.7E-07
14	H2aM25700N	91	2.2E-09	79	2.4E-09

		Dil-Oxidized LDL		Dil-Acetylated LDL	
Row	Antibodies	% Inhibition	IC_{50} (M)	% Inhibition	IC_{50} (M)
15	Mouse IgG2a isotype control mAb 2	no inhibition	no inhibition	no inhibition	no inhibition
16	H1H21227N-N297Q	94	1.8E-09	65	2.4E-09
17	H1H21231N-N297Q	88	3.7E-09	62	3.7E-09
18	H1H21234N-N297Q	52	6.1E-09	34	1.3E-09
19	H1H21227N	89	2.0E-09	65	2.1E-09
20	H1H21231N	78	3.6E-09	63	3.5E-09
21	H1H21234N	64	4.3E-09	36	7.1E-10
22	H1H27729P-N297Q	no inhibition	no inhibition	28	1.2E-09
23	H1H27731P-N297Q	62	>1.0E-08	37	1.8E-09
24	H1H27732P-N297Q	86	1.7E-09	62	2.2E-09
25	H1H27734P-N297Q	22	5.5E-09	no inhibition	no inhibition
26	H1H27736P-N297Q	83	3.2E-09	49	3.4E-09
27	H1H27739P-N297Q	55	>1.0E-07	no inhibition	no inhibition
28	H1H27747P-N297Q	42	>1.0E-07	26	>1.0E-07
29	H1H27749P-N297Q	42	1.7E-09	35	4.2E-10
30	H1H27751P-N297Q	85	2.9E-09	59	1.6E-09
31	H1H27754P-N297Q	73	4.6E-09	38	1.8E-09
32	H1H27756P-N297Q	76	3.4E-09	42	>1.0E-07
33	H1H27759P-N297Q	74	>1.0E-08	48	>1.0E-07
34	H1H27760P-N297Q	70	4.9E-09	46	2.6E-09
35	H1H27761P-N297Q	43	9.5E-09	29	>1.0E-07
36	H1H27762P-N297Q	78	4.0E-09	38	2.9E-09
37	H1H27766P-N297Q	72	3.7E-09	38	3.5E-09
38	H1H27771P-N297Q	73	3.0E-09	49	1.9E-09
39	H1H27773P-N297Q	41	>1.0E-07	31	>1.0E-07
40	H1H27778P-N297Q	88	2.2E-09	54	1.5E-09
41	Human IgG1-N297Q, Isotype Control mAb	no inhibition	no inhibition	no inhibition	no inhibition
42	Human IgG1 Isotype Control mAb	no inhibition	no inhibition	no inhibition	no inhibition

[0470] Suitable antibody candidates illustrate relatively efficient inhibition (*e.g.*, an IC_{50} value of less than about 10 nM). In some embodiments, suitable antibody candidates also illustrate less than about 65% maximum inhibition of ligand uptake.

[0471] As shown in Table 13 (rows 1-10), eight antibodies showed inhibition of Dil-OxLDL uptake on the HEK293.Myc.hMSR1 cells with maximum inhibition ranging from 26% to 90% and

IC_{50} values ranging from 2.3 nM to >100 nM. Seven of the 8 antibodies showed inhibition of DiI-AcLDL uptake with maximum inhibition ranging from 10% to 62% and IC_{50} values ranging from 1.8 nM to >100 nM. Antibody H2aM21229N showed no inhibition of DiI-AcLDL uptake.

[0472] As shown in Table 13 (rows 11-15), four antibodies showed inhibition of MSR1-mediated DiI-OxLDL uptake with maximum inhibition ranging from 43% to 91% and IC_{50} values ranging from 1.7 nM to >100 nM. Four antibodies of the invention showed inhibition of MSR1-mediated DiI-AcLDL uptake with maximum inhibition ranging from 22% to 79% and IC_{50} values ranging from 2.1 nM to >100 nM.

[0473] As shown in Table 13 (rows 16-42), twenty-four out of 25 antibodies showed inhibition of MSR1-mediated uptake of DiI-OxLDL with maximum inhibition ranging from 22% to 94% and IC_{50} values ranging from 1.7 nM to >100 nM. Twenty-three of the 25 antibodies showed inhibition of DiI-AcLDL uptake with maximum inhibition ranging from 26% to 65% and IC_{50} values ranging from 0.42 nM to >100 nM. Antibody H1H27729P-N297Q showed no inhibition of DiI-OxLDL uptake while antibodies H1H27739P-N297Q and H1H27734P-N297Q showed no inhibition of DiI-AcLDL uptake on HEK293.Myc.hMSR1 cells.

[0474] Human and mouse Isotype control antibodies did not show inhibition of DiI-OxLDL and DiI-AcLDL uptake by HEK293.Myc.hMSR1 cells in any of the assays.

Example 9. Binding and Internalization of Cell-Surface Expressed MSR1 by Anti-MSR1 Antibodies

[0475] Exemplary anti-MSR1 antibodies were assessed for their ability to bind and internalize MSR1 on MSR1-expressing cells.

[0476] For the assay, THP-1 cells [ATCC, Cat #TIB-202] were seeded into 96 well PDL coated plates (Perkin Elmer, Cat# 6055500) in RPMI (Irvine Scientific, Cat #9160) containing 10% FBS (ATCC, Cat# 30-2020), pencillin/streptomycin/L-glutamine (Gibco, Cat #10378-016), 50 µM Beta-Mercaptoethanol (Sigma, Cat # M7522) (growth media) plus 200 nM Phorbol Myristate Acetate (PMA; Sigma, Cat# P1585). The THP-1 cells were allowed to differentiate for 4 days at 37°C in 5% CO_2. To stain, quadruplicate plates of cells were incubated with 10 µg/mL of anti-MSR1 antibodies diluted in 2% FBS in PBS, without Calcium and Magnesium (Irving, Cat # 9240) (staining buffer) for 30 minutes at 4°C. Cells were washed twice with staining buffer incubated with an Alexa-Flour 488 conjugated secondary Ab (Jackson Immunoresearch, Cat # 115-547-003 or Jackson Immunoresearch, Cat # 109-547-003) at 10 µg/mL for 30 minutes at 4°C, and subsequently washed twice more with staining buffer. Two plates were immediately fixed and stained with 4% paraformaldehyde (PFA; ThermoFisher, Cat #28908) + 5 µM DRAQ5 (ThermoFisher, Cat #62251) in PBS for 20 minutes (non-internalization plates). The remaining

two plates were incubated at 37°C for 1 hour followed by fixation and staining for 20 minutes using a solution of 4% PFA + 5 µM DRAQ5 diluted in PBS (internalization plates). After fixation, all plates were washed once with PBS. One non-internalization plate and one internalization plate were incubated with an anti-Alexa Fluor 488 antibody (Regeneron) at 50 µg/mL in PBS overnight at 4°C to quench surface Alexa Fluor 488 fluorescence. The remaining plates were incubated with PBS only. Confocal images were acquired on the Opera Phenix (Perkin Elmer) at 40X magnification. Harmony analysis software (Perkin Elmer) was utilized to identify DRAQ5-labeled cells and the total Alexa-Fluor 488 relative fluorescent units (RFU) per cell was determined. The total binding at 4°C (RFU values of 4°C unquenched wells), total binding at 37°C (RFU values of 37°C unquenched wells), the total internalized RFU, and % Internalization were determined for each antibody as shown in Table 14.

[0477] For all calculations, background fluorescence from unstained wells was subtracted from every well. Total internalized RFU was calculated as follows: Total RFU of 37°C unquenched samples - Surface RFU at 37°C. Surface RFU is defined as unquenched RFU at 37°C - quenched RFU at 37°C)/QE. QE (quenching efficiency) is defined as: 1-(Total RFU of 4°C quenched sample/Total RFU of 4°C unquenched sample). The % Internalization was determined from the following formula: (Total internalized RFU at 37°C/Total RFU at 37°C)*100.

Table 14: Internalization and Surface Binding of Anti-MSR1 Antibodies in Differentiated THP-1 Cells

Antibody	Total Binding at 4°C	Total Binding at 37°C	Total Internalized RFU	% Internalization
H1H27729P-N297Q	1930685	6607625	3763127	57.0
H1H27731P-N297Q	1215319	1802404	977543	54.2
H1H27732P-N297Q	2513511	4924734	2414132	49.0
H1H27734P-N297Q	482859	1151348	737425	ND*
H1H27736P-N297Q	9514681	12267400	14468087	117.9**
H1H27739P-N297Q	3702857	5608380	4016378	71.6
H1H27747P-N297Q	2518361	5917858	3444330	58.2
H1H27749P-N297Q	4478384	12799899	5704834	44.6
H1H27751P-N297Q	5831744	7998767	6787876	84.9
H1H27754P-N297Q	3077308	7161570	6446236	90.0
H1H27756P-N297Q	6691792	11904608	9039171	75.9
H1H27759P-N297Q	4028970	2480463	1861578	75.0
H1H27760P-N297Q	1297337	6011876	3707164	61.7
H1H27761P-N297Q	1940392	2764577	1625899	58.8
H1H27762P-N297Q	2529645	3856573	3767717	97.7
H1H27766P-N297Q	1877240	3224247	2062539	64.0
H1H27771P-N297Q	6272656	7535203	6991358	92.8
H1H27773P-N297Q	490905	962752	-67811	ND*

Antibody	Total Binding at 4°C	Total Binding at 37°C	Total Internalized RFU	% Internalization
H1H27778P-N297Q	9910952	16552831	12518725	75.6
H1H21227N-N297Q	1800012	4110990	3226161	78.5
H1H21234N-N297Q	1953248	5451125	722651	13.3
Isotype control	185971	1087469	1704047	ND*

ND*: % internalization could not be determined due to weak binding and/or inability to determine quenching efficiency

**: A % internalized value >100% is due to the total internalized values being slightly higher than total values at 37°C. An internalization of 100% was confirmed visually by the appearance of all Alex488 fluorescence into vesicular structures at 37°C.

[0478] As shown in Table 14, 19 of 21 assayed anti-MSR1 antibodies demonstrated internalization into differentiated THP-1 cells ranging from 13.3% to 117.9% internalization. For two of the 21 anti-MSR1 antibodies, internalization could not be determined due to weak binding and/or inability to determine quenching efficiency. As a control, the isotype control did not demonstrate any measurable internalization.

Example 10. Assessing Blocking Ability of Anti-MSR1 Antibodies for Human MSR1

[0479] The ability of anti-MSR1 antibodies disclosed herein to block the binding of various ligands to human MSR1 was measured using three competition sandwich ELISA assays. The ligands used in the assays were: (1) acetylated LDL (Ac-LDL), (2) oxidized LDL (Ox-LDL), and (3) advanced glycation end-products of bovine serum albumin (AGE-BSA).

[0480] For the assay, recombinant monomeric human MSR1 protein comprised of a portion of the human MSR1 extracellular domain expressed with a N-terminal 9-Histidine tag (His9-hMSR1; R&D Systems, Cat# 2708-MS) was coated at a concentration of 2 µg/mL in PBS on a 96-well microtiter plate overnight at 4°C for use in competition ELISA assays with Ac-LDL, Ox-LDL, or biotinylated-AGE-BSA ("biot-AGE-BSA"). Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Anti-MSR1 antibodies or isotype control antibodies were serially diluted as appropriate for each tested ligand and added in duplicate for each serial dilution set to microtiter plates coated with His9-hMSR1. Buffer alone was also added to wells on each coat. After 1 hour incubation at room temperature, without wash, a final constant concentration of 50 pM Ac-LDL (Life Technologies / ThermoFisher Scientific, Cat # L-35354), 5 nM or 10 nM Ox-LDL (Alfa Aesar, Cat # J65591), or 400 pM biot-AGE-BSA (R&D Systems, Cat # BT4127) were added to plates with His9-hMSR1, and the plates were incubated for an additional 1 hour at room temperature. (Concentrations of Ac-LDL, Ox-LDL and biot-AGE-BSA for antibody inhibition assays were selected from the approximate midway point within the linear portion of

individual binding curves of Ac-LDL, Ox-LDL or biot-AGE-BSA to plate-coated His9-hMSR1.) Wells were washed, and plate-bound Ac-LDL or Ox-LDL were detected with anti-LDL rabbit antibody (Alfa Aesar, Cat # J64398) in combination with anti-rabbit IgG (H+L) specific donkey polyclonal antibodies conjugated with horseradish peroxidase (HRP) (JacksonImmunoResearch, Cat #711-035-152) and biot-AGE-BSA was detected with a streptavidin-HRP (ThermoFisher Scientific, Cat # N200). Plates were developed using TMB substrate solution (BD Biosciences, Cat #51-2606KC & Cat #51-2607KC) according to manufacturer's recommendation and absorbance at 450 nm was measured on a Victor™ Multilabel Plate Reader (PerkinElmer™). This assay was conducted in four different assay runs.

[0481] Data analysis was performed using a sigmoidal dose-response model within Prism™ software (GraphPad). Percent blockade at maximum concentration of the antibody tested in each assay was calculated as an indicator of the ability of the antibodies to block the binding of Ac-LDL, Ox-LDL or biot-AGE-BSA to His9-hMSR1 on the plate relative to the baseline of the assay. In the calculation, binding signal of the same concentrations of Ac-LDL, Ox-LDL, or biot-AGE-BSA used for the assays in the absence of antibody was referenced as 100% binding or 0% blocking, while the baseline of the assay, defined as binding signal of the sample of buffer without MSR1 ligands or antibody, was referenced as 0% binding or 100% blocking. The maximum percent blockade at the highest concentration of antibody tested in each assay are reported for all antibodies. Negative percent blockade numbers reflected higher MSR1 ligands binding to plate coated His9-hMSR1 in the presence of antibodies. The blocking results are summarized in Table 15.

Table 15: Blocking Ability of Anti-MSR1 Antibodies in Competition ELISA Assays

Antibody	anti-MSR1 antibody blocking of Ox-LDL binding to His9-hMSR1			Anti-MSR1 antibody (100 nM) blocking of Ac-LDL binding to His9-hMSR1	Anti-MSR1 antibody (300 nM) blocking of biot-AGE-BSA binding to His9-hMSR1
	anti-MSR1 antibody concentration	Ox-LDL concentration	% Blocking	% Blocking	% Blocking
Blocked>50% in all assay formats					
H2aM25700N	500 nM	10 nM	98	104	101
H1H21227N-N297Q	500 nM	10 nM	97	105	96

Antibody	anti-MSR1 antibody blocking of Ox-LDL binding to His9-hMSR1			Anti-MSR1 antibody (100 nM) blocking of Ac-LDL binding to His9-hMSR1	Anti-MSR1 antibody (300 nM) blocking of biot-AGE-BSA binding to His9-hMSR1
	anti-MSR1 antibody concentration	Ox-LDL concentration	% Blocking	% Blocking	% Blocking
H1H21227N-N297D	500 nM	10 nM	99	101	97
H1H21227N	500 nM	10 nM	100	105	99
Blocked>50% in some assay formats					
H2aM25695N	500 nM	10 nM	96	65	16
H1H27766P2-N297Q	1 µM	10 nM	54	52	27
H1H21234N-N297Q	500 nM	10 nM	49	75	-27
H1H21234N	500 nM	10 nM	98	70	21
Blocked<50% in all assay formats					
H2aM21228N	500 nM	5 nM	41	-32	-1
H2aM21229N	500 nM	5 nM	38	-11	3
H2aM21230N	500 nM	5 nM	45	-14	9
H2aM21232N	500 nM	5 nM	16	25	-50
H2aM21235N	500 nM	5 nM	-4	24	-24
H2aM25685N	500 nM	10 nM	28	-33	-86
H2aM25690N	500 nM	10 nM	8	-76	-96
H1H21231N-N297Q	500 nM	10 nM	26	-60	-50
H1H21231N	500 nM	10 nM	22	-62	-44
H1H27729P-N297Q	1 µM	10 nM	36	8	15
H1H27731P-N297Q	1 µM	10 nM	-1	-41	4
H1H27732P-N297Q	1 µM	10 nM	-60	-61	-9
H1H27734P-N297Q	1 µM	10 nM	-15	-38	-4
H1H27736P-N297Q	1 µM	10 nM	3	-31	-3
H1H27739P-N297Q	1 µM	10 nM	-41	-47	-9
H1H27747P-N297Q	1 µM	10 nM	-31	-44	-2
H1H27749P-	1 µM	10 nM	-21	-51	-1

Antibody	anti-MSR1 antibody blocking of Ox-LDL binding to His9-hMSR1			Anti-MSR1 antibody (100 nM) blocking of Ac-LDL binding to His9-hMSR1	Anti-MSR1 antibody (300 nM) blocking of biot-AGE-BSA binding to His9-hMSR1
	anti-MSR1 antibody concentration	Ox-LDL concentration	% Blocking	% Blocking	% Blocking
N297Q					
H1H27751P-N297Q	1 μM	10 nM	-31	-51	-11
H1H27754P-N297Q	1 μM	10 nM	-44	-42	-9
H1H27756P-N297Q	1 μM	10 nM	-45	-54	-8
H1H27759P2-N297Q	1 μM	10 nM	-62	-45	-19
H1H27760P2-N297Q	1 μM	10 nM	-47	-43	-4
H1H27761P2-N297Q	1 μM	10 nM	-21	-36	-4
H1H27762P2-N297Q	1 μM	10 nM	-65	-58	-16
H1H27771P2-N297Q	1 μM	10 nM	-23	-21	-1
H1H27773P2-N297Q	1 μM	10 nM	2	-11	1
H1H27778P2-N297Q	1 μM	10 nM	-59	-40	-8
Isotype control antibodies					
hIgG1 isotype control	1 μM	10 nM	3	-8	4
mIgG1 isotype control	1 μM	10 nM	16	-4	7

[0482] Four of 35 assayed anti-MSR1 antibodies were identified as blocking >50% of Ac-LDL, Ox-LDL, and biot-AGE-BSA binding to hMSR1. These four anti-MSR1 antibodies blocked greater than 95% of 50 pM Ac-LDL, 10 nM Ox-LDL and 400 pM biot-AGE-BSA binding to His9-hMSR1.

[0483] At the maximum concentration of antibody tested, four of the 35 anti-MSR1 antibodies blocked >50% Ac-LDL and/or Ox-LDL binding to hMSR1 but did not block biot-AGE-BSA binding to hMSR1. Three of these antibodies blocked both 50 pM Ac-LDL and 10 nM Ox-LDL

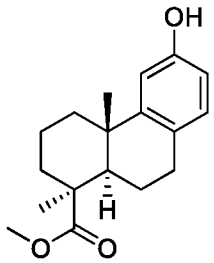
binding to hMSR1 with 52% to 98% blockade. One antibody (H1H21234N-N297Q) blocked only 50 pM Ac-LDL binding to hMSR1 with 75% blockade.

[0484] Twenty-seven (27) of the 35 anti-MSR1 antibodies and the irrelevant isotype control antibodies blocked <50% of Ac-LDL, Ox-LDL, and biot-AGE-BSA binding to hMSR1.

[0485] Three anti-MSR1 antibodies (21227N, 21231N, 21234N) were produced both with the original human **Fcγ** portion and a version with a N297Q single point mutation. The 21227N antibody was also produced as a third version with a N297D mutation. The modified versions of 21227N (H1H21227N-N297Q and H1H21227N-N297D) and 21231N (H1H21231N-N297Q) anti-MSR1 antibodies retained parental characteristics. Antibodies H1H21227N-N297Q and H1H21227N-N297D blocked >50% of Ac-LDL, Ox-LDL, and biot-AGE-BSA binding to hMSR1, while antibody H1H21231N-N297Q blocked <50% of Ac-LDL, Ox-LDL, and biot-AGE-BSA binding to hMSR1. Anti-MSR1 modified antibody H1H21234N-N297Q blocked only 50 pM Ac-LDL binding to hMSR1 in comparison to unmodified H1H21234N antibody, which blocked >50% for both Ac-LDL and Ox-LDL binding to hMSR1.

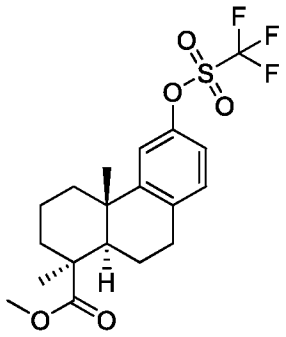
Example 11. Synthesis of Payload P1 and P2B (FIG. 1)

Methyl (1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (P1-2)



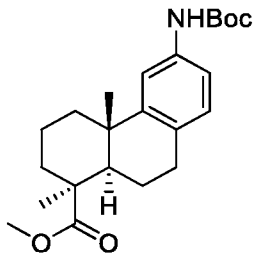
[0486] To a solution of podocarpic acid (**P1-1**, 90 g, 0.33 mol) in methanol (200 mL) and toluene (600 mL) was added with (trimethylsilyl)diazomethane (2 M in hexane, 200 mL). The reaction mixture was stirred at room temperature for 2 hours. The podocarpic acid was then totally consumed according to LCMS. The volatiles were removed *in vacuo*, and the residue was triturated from petroleum ether (2 L) to give compound **P1-2** (91 g, 96% yield) as a white solid. ESI m/z: 289 $(M + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 8.95 (s, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.48 (dd, J = 8.2, 2.4 Hz, 1H), 3.58 (s, 3H), 2.80-2.55 (m, 2H), 2.20-2.02 (m, 3H), 1.96-1.71 (m, 2H), 1.56-1.45 (m, 2H), 1.27 (t, J = 13.5 Hz, 1H), 1.21 (s, 3H), 1.09 (td, J = 13.5, 4.1 Hz, 1H), 0.91 (s, 3H) ppm.

Methyl (1*S*,4a*S*,10a*R*)-1,4a-dimethyl-6-(trifluoromethanesulfonyloxy)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (P1-3)



[0487] To a solution of compound **P1-2** (10 g, 35 mmol) in methylene chloride (200 mL) were added pyridine (3.3 g, 42 mmol) and DMAP (0.84 g, 6.9 mmol) under nitrogen atmosphere. The mixture was cooled to -78 °C and was added triflic anhydride (12 g, 42 mmol), and the resulting mixture was allowed to warm to 25 °C and stirred at 25 °C for another 4 hours. The reaction mixture was diluted with DCM (500 mL), washed with water (100 mL), *aq.* hydrochloride (1 N, 150 mL) and brine (100 mL), dried over sodium sulfate and concentrated *in vacuo* to give crude compound **P1-3** (14 g, 97% crude yield) as viscous oil, which was pure enough for the next step. The crude compound **P1-3** could be purified by flash chromatography (0-10% ethyl acetate in petroleum ether) to give pure product as viscous oil. ESI m/z: 421.2 $(M + 1)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, J = 2.5 Hz, 1H), 7.10 (d, J = 8.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.5 Hz, 1H), 3.67 (s, J = 3.4 Hz, 3H), 2.93 (dd, J = 17.2, 4.4 Hz, 1H), 2.85-2.71 (m, 1H), 2.29 (d, J = 13.5 Hz, 1H), 2.25-2.14 (m, 2H), 2.03-1.89 (m, 2H), 1.71-1.61 (m, 1H), 1.56-1.48 (m, 1H), 1.40 (td, J = 13.4, 4.2 Hz, 1H), 1.30-1.22 (m, 3H), 1.09 (td, J = 13.6, 4.2 Hz, 1H), 1.02 (s, 3H) ppm.

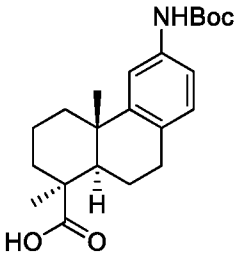
Methyl (1*S*,4a*S*,10a*R*)-6-((*tert*-butoxycarbonyl)amino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (P1-4)



[0488] To a solution of compound **P1-3** (14 g, 34 mmol) and *tert*-butyl carbamate ($BocNH_2$, 7.9 g, 68 mmol) in *tert*-butanol (100 mL) were added, successively cesium carbonate (22 g, 68 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$, 1.8 g, 2.0 mmol) and X-Phos (1.8 g, 4.0 mmol) at room temperature. The mixture was de-gassed and purged with argon three times and was then stirred at 80 °C under argon (balloon) overnight until compound **P1-3** was totally consumed, as monitored by TLC. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate and filtered through Celite. The solid was washed with ethyl

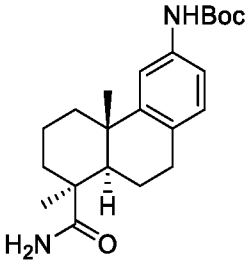
acetate for 3 times. The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (0-6.25% ethyl acetate in petroleum ether) to give compound **P1-4** (11 g, 82% yield) as a white solid. ESI m/z: 410 $(M + 23)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 9.07 (s, 1H), 7.39 (s, 1H), 7.13 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 3.59 (s, 3H), 2.76 (dd, J = 16.4, 4.5 Hz, 1H), 2.70-2.61 (m, 1H), 2.16-2.05 (m, 3H), 2.00-1.75 (m, 2H), 1.65-1.50 (m, 2H), 1.45 (s, 9H), 1.31-1.25 (m, 1H), 1.21 (s, 3H), 1.10 (td, J = 13.5, 4.1 Hz, 1H), 0.92 (s, 3H) ppm.

(1*S*,4a*S*,10a*R*)-6-{[(*tert*-Butoxy)carbonyl]amino}-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid (P1-5)



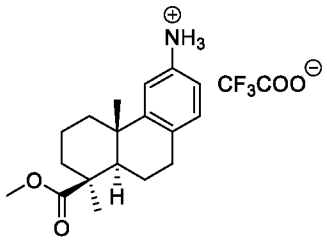
[0489] To a solution of compound **P1-4** (4.9 g, 13 mmol) in DMSO was added potassium *tert*-butoxide (15 g, 0.13 mol) in one portion at room temperature. The reaction mixture was stirred at 60 °C for 3 hours under argon until the reaction was completed according to LCMS. After cooling to room temperature, the reaction mixture was poured into ice and acidified slowly with *aq.* hydrochloride (0.5 M) to pH 5, during which the temperature did not exceed 25 °C. The precipitates were collected by filtration and washed with water several times. The crude product was further purified by silica gel column chromatography (0-20% ethyl acetate in petroleum ether) to give compound **P1-5** (4.5 g, 93% yield) as a white solid. ESI m/z: 318 $(M - 55)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 12.08 (s, 1H), 9.08 (s, 1H), 7.40 (s, 1H), 7.11 (s, 1H), 6.87 (d, J = 8.3 Hz, 1H), 2.79-2.68 (m, 1H), 2.65 (d, J = 12.6 Hz, 1H), 2.17-2.03 (m, 4H), 1.94-1.76 (m, 2H), 1.53 (d, J = 13.7 Hz, 1H), 1.46 (d, J = 7.4 Hz, 9H), 1.29-1.14 (m, 5H), 1.04 (s, 3H) ppm.

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-carbamoyl-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (P1-6)**



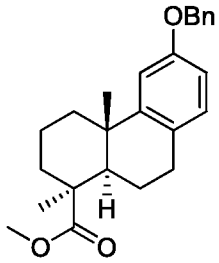
[0490] To a solution of **P1-5** (4.5 g, 12 mmol) and HATU (4.9 g, 13 mmol) in DMF (50 mL) was added diisopropylethylamine (20 mL, 0.12 mol), and the mixture was stirred at 25 °C for an hour. To the mixture was then added ammonium chloride (16 g, 0.30 mol) and the mixture was stirred at room temperature overnight. The resulting mixture was diluted with ethyl acetate, washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (0-20% ethyl acetate in petroleum ether) to give compound **P1-6** (4.2 g, 94% yield) as a white solid. ESI m/z: 373.3 $(M + 1)^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 7.20 (s, 1H), 6.97 (d, J = 7.7 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 2.77-2.68 (m, 2H), 2.66-2.55 (m, 1H), 2.20 (d, J = 12.9 Hz, 1H), 2.13 (dd, J = 13.2, 5.3 Hz, 1H), 2.08 (d, J = 14.0 Hz, 1H), 2.03-1.86 (m, 2H), 1.54 (d, J = 11.1 Hz, 1H), 1.40 (s, 9H), 1.26 (t, J = 26.7Hz, 1H), 1.18 (s, 3H), 1.14-1.03 (m, 4H) ppm.

Methyl (1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate trifluoroacetic acid salt (P1-7)



[0491] To a solution of compound **P1-4** (6.0 g, 15 mmol) in DCM (60 mL) was added TFA (12 mL) at RT. The resulting mixture was stirred at RT for 2 h until Boc was totally removed, as monitored by TLC. The reaction mixture was concentrated *in vacuo* to give crude compound **P1-7** as a TFA salt, which was used in the next step without further purification. ESI m/z: 288 $(M+1)^+$.

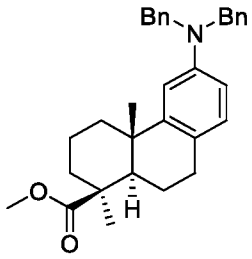
Methyl (1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (P1-8a)



[0492] A mixture of compound **P1-2** (12 g, 40 mmol) and cesium carbonate (14 g, 44 mmol) in DMF (100 mL) was stirred at 20-25 °C for 15 minutes. To the mixture was added benzyl bromide (7.1 mL, 60 mmol) at room temperature. After stirring at room temperature for 4 hours, the resulting mixture was poured into cold water and extracted with ethyl acetate. The combined

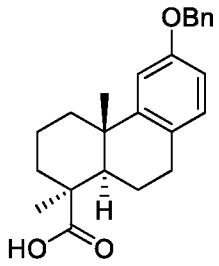
organic solution was washed with water and brine, dried over sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (0-10% ethyl acetate in petroleum ether) to give the title compound **P1-8a** (13 g, 89% yield) as a white solid. ESI m/z: 379 $(M + H)^+$. ^1H NMR (500 MHz, methanol$_{d4}$) δ 7.60-7.20 (m, 5H), 7.00-6.82 (m, 2H), 6.73 (d, J = 7.1 Hz, 1H), 5.03 (s, 2H), 3.66 (s, 3H), 2.95-2.58 (m, 2H), 2.36-2.10 (m, 3H), 2.10-1.85 (m, 2H), 1.70-1.48 (m, 2H), 1.44-1.21 (m, 4H), 1.15 (t, J = 17.2 Hz, 1H), 1.01 (s, 3H) ppm.

Methyl (1*S*,4a*S*,10a*R*)-6-(dibenzylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (8b)



[0493] To a solution of the crude compound **P1-7** obtained above (*calc.* 15 mmol) in DMF (60 mL) were added potassium carbonate (6.4 g, 46 mmol) and benzylbromide (5.8 g, 34 mmol) at RT. The reaction mixture was stirred at 80 °C overnight until the reaction was complete as monitored by TLC. After cooling to RT, the mixture was poured into cold water (300 mL) and extracted with ethyl acetate (x 3). The combined organic solution was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give crude product **8b**, which was used in the next step without further purification. ESI m/z: 468 $(M + 1)^+$.

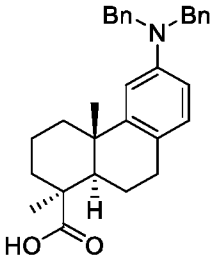
1S,4aS,10aR)-6-(Benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid (P1-9a)



[0494] A mixture of compound **P1-8a** (11 g, 29 mmol) and potassium tert-butoxide (33 g, 0.29 mol) in DMSO (0.19 L) was stirred at 100 °C for an hour until the methyl group was totally removed, as monitored by LCMS and TLC. After cooling to 25 °C, the mixture was quenched with aqueous hydrochloride (1 N) and extracted with ethyl acetate. The combined organic solution was washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (0-24% ethyl acetate in petroleum ether) to give compound **P1-9a** (7.5 g, 71% yield) as a white solid. ESI m/z: 365 $(M + H)^+$. 1H

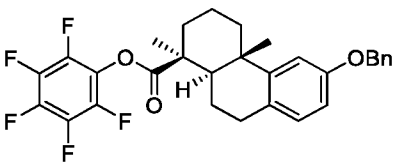
NMR (500 MHz, methanol$_{d4}$) δ 7.42 (d, J = 7.4 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.3 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 2.5 Hz, 1H), 6.72 (dd, J = 8.4, 2.5 Hz, 1H), 5.02 (s, 2H), 2.82 (dd, J = 16.3, 4.4 Hz, 1H), 2.77-2.65 (m, 1H), 2.24 (d, J = 13.2 Hz, 2H), 2.19 (dd, J = 13.8, 6.0 Hz, 1H), 2.11-1.96 (m, 2H), 1.64-1.56 (m, 1H), 1.53 (d, J = 11.0 Hz, 1H), 1.35 (td, J = 13.3, 3.7 Hz, 1H), 1.30 (s, 3H), 1.13 (s, 3H), 1.11-1.05 (m, 1H) ppm.

(1*S*,4a*S*,10a*R*)-6-(Dibenzylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid (9b)



[0495] To a solution of the crude compound **8b** obtained above (*calc.* 15 mmol) in DMSO (100 mL) was added potassium *tert*-butoxide (17 g, 0.15 mol) in one portion at RT. The reaction mixture was stirred at 100 °C for 2 h under argon until the reaction was complete according to LCMS. After cooling to RT, the reaction mixture was poured into ice and acidified slowly with *aq.* hydrochloride (4 M) to pH 5, during which the temperature was not allowed to reach higher than 25 °C. The mixture was extracted with ethyl acetate and the combined organic solution was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (0-20% ethyl acetate in petroleum ether) to give compound **9b** (6.8 g, 99% yield in 3 steps from compound **P1-4**) as a white solid. ESI m/z: 454 $(M + 1)^+$.

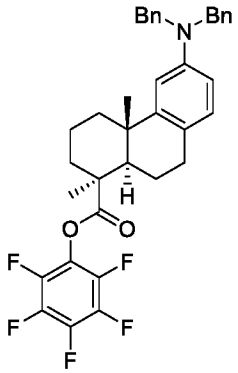
Pentafluorophenyl (1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (P1-10a)



[0496] To a solution of **P1-9a** (9.6 g, 26 mmol) in DMF (100 mL) was added DIPEA (14 mL, 79 mmol), and perfluorophenyl 2,2,2-trifluoroacetate (15 g, 53 mmol). This mixture was stirred at room temperature overnight and monitored by LCMS. The reaction mixture was then diluted with ether (200 mL) and washed with water (300 mL) and brine (200 mL). The organic solution was dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography (0-10% ethyl acetate in petroleum ether) to give compound **P1-10a** (12 g, 88% yield) as a white solid. ESI m/z: 531 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 7.43 (d, J = 7.1

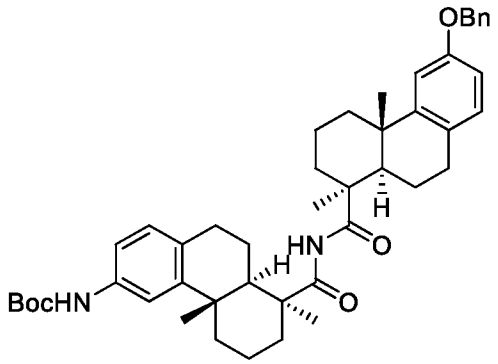
Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 1H), 6.93 (dd, *J* = 10.2, 5.5 Hz, 2H), 6.76 (dd, *J* = 8.4, 2.5 Hz, 1H), 5.05 (s, 2H), 2.81 (dd, *J* = 16.3, 4.5 Hz, 1H), 2.77-2.68 (m, 1H), 2.28-2.19 (m, 2H), 2.18 (dd, *J* = 13.4, 5.6 Hz, 1H), 2.00-1.83 (m, 2H), 1.74 (d, *J* = 11.8 Hz, 1H), 1.65 (d, *J* = 14.1 Hz, 1H), 1.47 (s, 3H), 1.38-1.27 (m, 2H), 1.08 (s, 3H) ppm.

Pentafluorophenyl (1*S*,4a*S*,10a*R*)-6-(dibenzylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (10b)



[0497] To a solution of **9b** (6.8 g, 15 mmol) in DMF (100 mL) was added DIPEA (10 mL, 0.61 mol), and perfluorophenyl 2,2,2-trifluoroacetate (10 mL, 58 mmol). This mixture was stirred at 25 °C overnight under nitrogen, and was then diluted with ether. The organics were washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0-10% ethyl acetate in petroleum ether) to give compound **10b** (7.5 g, 81% yield) as a white solid. ESI m/z: 620 $(M + 1)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.25 (m, 10H), 6.92 (d, *J* =8.4 Hz, 1H), 6.67 (d, *J* =2.8 Hz, 1H), 6.63 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.63 (m, 4H), 2.85-2.73 (m, 2H), 2.41-2.37(m, 1H), 2.22-2.20 (m, 1H), 2.15-1.91 (m, 3H), 1.71-1.65 (m, 2H), 1.51 (s, 3H), 1.37-1.19 (m, 2H), 1.10 (s, 3H) ppm.

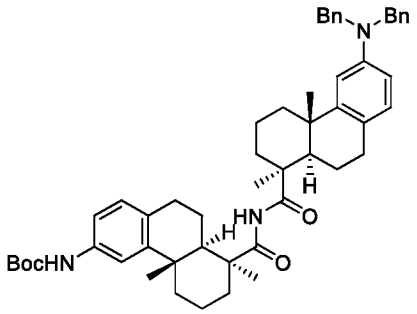
***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (P1-11a)**



[0498] To a solution of compound **P1-6** (2.3 g, 6.2 mmol) in THF (20 mL) was added dropwise *n*-BuLi (2.5 M in hexane, 5.5 mL, 14 mmol) at -78 °C. The reaction was stirred at this temperature for 1 hour. To the mixture was added a solution of **P1-10a** (3.0 g, 5.6 mmol) in THF (20 mL), and the resulting mixture was then stirred at 10-20 °C overnight until compound **P1-10a** was consumed, as monitored by LCMS. The reaction was quenched with *sat. aq.* ammonium chloride and extracted with ethyl acetate. The combined organic solution was washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (0-30% ethyl acetate in petroleum ether) to give compound **P1-11a** (1.59 g, 51% yield) as a white solid. ESI m/z: 719 $(M + 1)^+$.

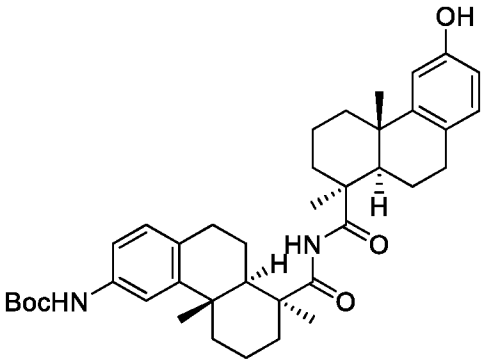
[0499] (Compared with the procedure of **11b** below, n-BuLi was used here instead of LiHMDS. Although **P1-6** was not completely consumed, this procedure led to less side-product and the yield of **P1-11a** was increased from ca. 40% to 51%. The unreacted compound **P1-6** was recovered (recovered yield: 10-20%).

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-(dibenzylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (11b)**



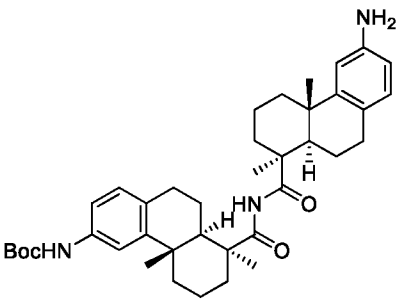
[0500] To a solution of compound **P1-6** (1 g, 2.7 mmol) in THF (15 mL) was added dropwise lithium bis(trimethylsilyl)amide (1 M in hexane, 8.0 mL) at 0 °C. The reaction was stirred at 0 °C for an hour. To the mixture was added a solution of compound **10b** (2.5 g, 4.0 mmol) in THF (10 mL), and the resulting mixture was then stirred at RT overnight. The reaction was then quenched with *sat. aq.* ammonium chloride and extracted with ethyl acetate. The combined organic solution was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography (0-35% ethyl acetate in petroleum ether) to give compound **11b** (0.95 g, 44% yield) as a white solid; and recovered compound **P1-6** (recovered yield 37%). ESI m/z: 808 $(M + 1)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.35-7.24 (m, 10H), 7.12 (d, J = 8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.62-6.59 (m, 2H), 6.42 (s, 1H), 4.62 (s, 3H), 2.98-2.75 (m, 4H), 2.31-2.22 (m, 5H), 2.10-1.97 (m, 5H), 1.69-1.63 (m, 4H), 1.56 (s, 9H), 1.32-1.27 (m, 9H), 1.43-1.27 (m, 5H), 1.05 (s, 3H) ppm.

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (P1-12a)**



[0501] To a solution of **P1-11a** (2.0 g, 2.78 mmol) in ethyl acetate (40 mL) was added wet palladium on carbon (10% Pd, 0.9 g) under nitrogen protection. The mixture was degassed and purged with hydrogen and stirred at room temperature under hydrogen balloon overnight until **P1-11a** was totally consumed, which was monitored by LCMS. The mixture was filtered through Celite and the filtration was concentrated in vacuo. The residue was purified by silica gel column chromatography (0-55% ethyl acetate in petroleum ether) to give **P1-12a** (1.06 g, 61% yield) as a white solid. ESI m/z: 629 $(M + H)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.10 (s, 1H), 8.98 (s, 1H), 8.11 (s, 1H), 7.40 (s, 1H), 7.15 (d, J = 7.5 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 2.3 Hz, 1H), 6.50 (dd, J = 8.2, 2.4 Hz, 1H), 2.84 (td, J = 16.3, 3.8 Hz, 2H), 2.77-2.64 (m, 2H), 2.30-2.22 (m, 2H), 2.14 (t, J = 10.9 Hz, 4H), 2.00-1.80 (m, 4H), 1.65-1.54 (m, 4H), 1.45 (s, 9H), 1.34-1.28 (m, 2H), 1.27 (d, J = 2.5 Hz, 6H), 1.15-1.08 (m, 2H), 0.99 (s, 6H) ppm.

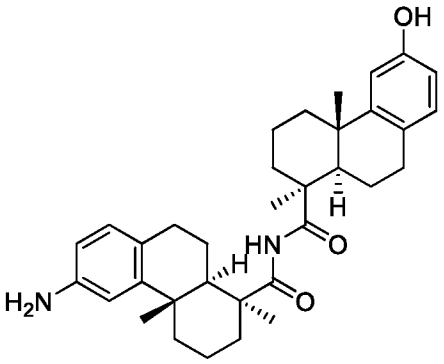
***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (12b)**



[0502] To a solution of **11b** (0.45 g, 0.56 mmol) in ethyl acetate (5 mL) was added wet palladium on carbon (10% Pd, 50 mg) under nitrogen. The mixture was degassed, purged with hydrogen 3 times, and was stirred at RT under a hydrogen balloon overnight. The mixture was

filtered through Celite and the filtrate was concentrated *in vacuo* to give compound **12b** (0.33 g, 94% yield) as a white solid. ESI m/z: 628 $(M + 1)^+$.

(1*S*,4a*S*,10a*R*)-*N*-[(1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P1)



[0503] To the solution of compound **P1-12a** (0.17 g, 0.27 mmol) in DCM (10 mL) was added dropwise TFA (3 mL) at room temperature. The reaction mixture was stirred at room temperature for an hour until Boc was removed according to LCMS. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method B) to give **P1** (0.10 g, 70% yield) as a white solid.

[0504] ESI m/z: 529.3 $(M + 1)^+$.

[0505] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 2.5 Hz, 1H), 6.65-6.57 (m, 2H), 6.50 (dd, J = 8.1, 2.3 Hz, 1H), 4.75 (s, 1H), 3.49 (s, 1H), 2.99-2.85 (m, 2H), 2.79 (tt, J = 11.6, 5.8 Hz, 2H), 2.34-2.14 (m, 6H), 2.15-1.95 (m, 4H), 1.74-1.51 (m, 5H), 1.46-1.34 (m, 2H), 1.30 (s, 6H), 1.21-1.06 (m, 8H) ppm.

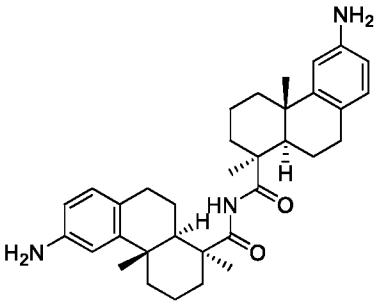
[0506] ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 8.99 (s, 1H), 8.09 (s, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.63 (d, J = 2.5 Hz, 1H), 6.50 (dd, J = 8.0, 2.5 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 6.34 (dd, J = 8.0, 2.5 Hz, 1H), 4.69 (s, 2H), 2.86-2.60 (m, 4H), 2.28-2.10 (m, 6H), 1.94-1.75 (m, 4H), 1.65-1.53 (m, 4H), 1.35-1.20 (m, 8H), 1.20-1.06 (m, 2H), 0.98 (s, 6H) ppm.

[0507] ^{13}C NMR (100 MHz, $DMSO_{d6}$) δ 174.03, 173.92, 155.34, 148.39, 147.63, 146.43, 129.56, 129.09, 124.60, 121.65, 113.23, 112.58, 111.81, 110.77, 52.32, 52.09, 45.56, 45.52, 39.20, 39.36, 38.23, 38.17, 37.18, 37.12, 31.08, 31.00, 27.65, 27.64, 23.08, 23.03, 21.43, 21.27, 19.64, 19.61 ppm.

[0508] HPLC (method B): Retention time: 8.92 min, purity: 99.4%. chiral HPLC: >99.9% (in column AD, AS, OD, and OJ).

[0509] Optical rotation (α): +2.53° (1.7 g/100 mL THF, 25 °C).

(1*S*,4a*S*,10a*R*)-6-Amino-*N*-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P2B)

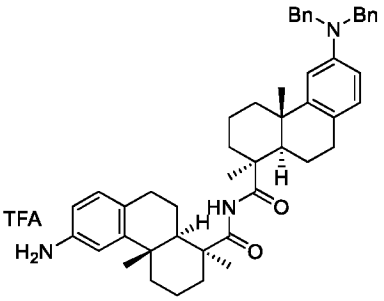


[0510] To the solution of compound **12b** (0.63 g, 1.0 mmol) in DCM (10 mL) was added dropwise TFA (3 mL) at RT. The reaction mixture was stirred at RT for 4 h until Boc was removed according to LCMS. The mixture was directly purified by prep-HPLC (method B) to give **P2B** (0.42 g, 79% yield) as a white solid. ESI m/z: 528.2 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 8.28 (s, 1H), 6.67 (d, J = 8.0 Hz, 2H), 6.47 (d, J = 2.0 Hz, 2H), 6.33 (dd, J = 8.0, 2.0 Hz, 2H), 4.69 (s, 4H), 2.80-2.75 (m, 2H), 2.70-2.60 (m, 2H), 2.26-2.20 (m, 2H), 2.19-2.05 (m, 4H), 1.95-1.75 (m, 4H), 1.62-1.50 (m ,4H), 1.33-1.20 (m, 8H), 1.12 (t, J = 8.8 Hz, 2H), 0.98 (s, 6H) ppm.

Example 11-2. Synthesis of P3B

[0511] This example demonstrates general methods for the synthesis of the podocarpic acid derivative **P3B** in Table C, below. This example refers to the compounds numbered from **11b** and **P3B** in FIG. 23.

(3*S*,8*R*,9*S*,10*R*,13*S*,14*S*)-17-imino-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-ol, trifluoroacetic acid salt (P3B)



[0512] To a solution of compound **11b** (10 mg, 12 μmol) in DCM (2 mL) was added TFA (1 mL). The reaction mixture was stirred at RT for 2 h until Boc was removed according to LCMS. The volatiles were removed *in vacuo* and the residue was dissolved in 5% acetonitrile in water.

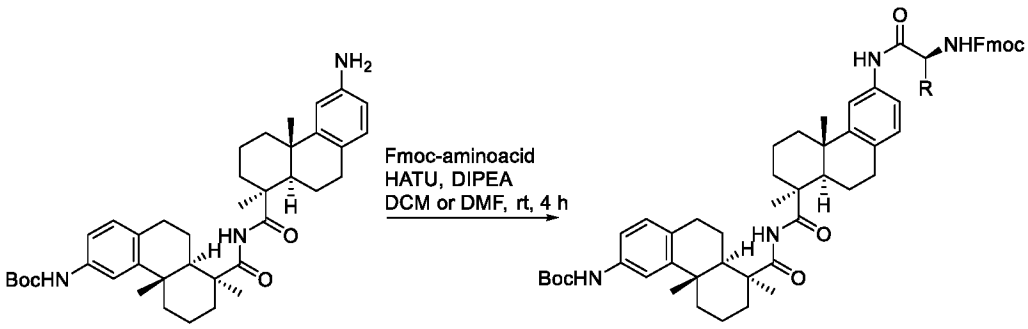
The solution was lyophilized to afford compound **P3B** (7 mg, 80% yield) as a light yellow solid. ESI m/z: 354.8 $(M/2 + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 9.38 (br s, 2H), 8.08 (s, 1H), 7.35-7.06 (m, 12H), 6.97 (d, J = 8.8 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 6.46 (d, J = 6.4 Hz, 1H), 4.68-4.55 (m, 4H), 3.00-2.50 (m, 4H), 2.40-2.00 (m, 6H), 1.94-1.73 (m, 5H), 1.70-1.50 (m, 3H), 1.50-1.00 (m, 11H), 0.98 (s, 3H), 0.83 (s, 3H) ppm. ^{19}F NMR (376 MHz, $DMSO_{d6}$) δ -74.08 ppm.

EXAMPLE 11-3

[0513] This example demonstrates general methods for the synthesis of the podocarpic acid derivatives **P4B-P8B** in Table C, below. This example refers to the compounds numbered from **12b** and **P4B-P8B** in FIG. 23.

Example 11-3a

Intermediates 13a-e

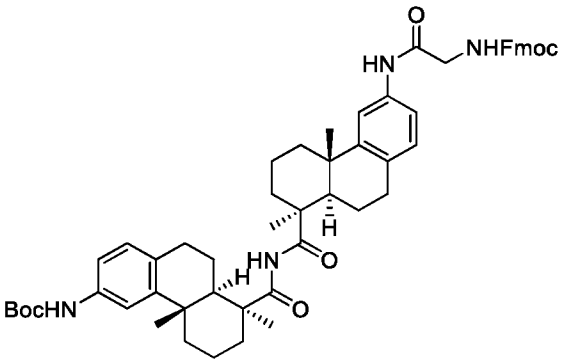


[0514] To a solution of compound **12b** (1.0 equiv.) in DMF or DCM were added Fmoc-aminoacid (1.1-1.2 equiv.), HATU (1.2-1.5 equiv.) and DIPEA (2.0-3.0 equiv.) successively. The reaction mixture was stirred at RT for 4 h, which was monitored by LCMS. The mixture was concentrated *in vacuo* (when DCM was solvent) and the residue was purified by silica gel column chromatography (0-90% ethyl acetate in petroleum ether); or the reaction mixture (when DMF was solvent) was directly purified by reverse phase flash chromatography (50-90% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **13** as white solids.

12b	Fmoc-aminoacid		HATU	DIPEA	Solvent	Time	purification	Product		
g (mmol)	R	g (mmol)	g (mmol)	g (mmol)	(mL)	(h)		#	g	Yield (%)

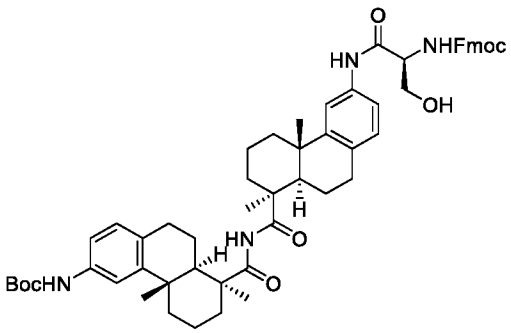
12b g (mmol)	Fmoc-aminoacid		HATU g (mmol)	DIPEA g (mmol)	Solvent (mL)	Time (h)	purification	Product		
	R	g (mmol)						#	g	Yield (%)
0.13 (0.20)	H	0.071 (0.24)	0.11 (0.29)	0.077 (0.60)	DMF (10)	4	RP-B	**13a**	0.14	78
0.31 (0.50)	CH_2OH	0.18 (0.55)	0.23 (0.60)	0.19 (1.50)	DCM (20)	4	SGC	**13b**	0.39	83
0.20 (0.32)	$(CH_2)_4NHFmoc$	0.21 (0.35)	0.15 (0.38)	0.083 (0.64)	DMF (20)	4	RP-B	**13c**	0.30	78
0.31 (0.50)	$CH_2COOtBu$	0.22 (0.55)	0.23 (0.60)	0.19 (1.50)	DCM (20)	4	SGC	**13d**	0.43	85
0.31 (0.50)	$(CH_2)_2COOtBu$	0.23 (0.55)	0.23 (0.60)	0.19 (1.50)	DCM (20)	4	SGC	**13e**	0.42	82

9*H*-Fluoren-9-ylmethyl *N*-({[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-{[(*tert*-butoxy)carbonyl]amino}-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (13a)



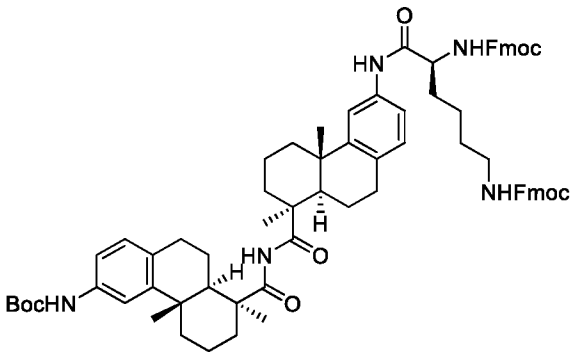
[0515] Following the general procedure for Intermediates **13a-e**, compound **13a** (0.14 g, 78% yield) was obtained as a white solid. ESI m/z: 907 $(M + H)^+$.

9*H*-Fluoren-9-ylmethyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-{[(*tert*-butoxy)carbonyl]amino}-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (13b)



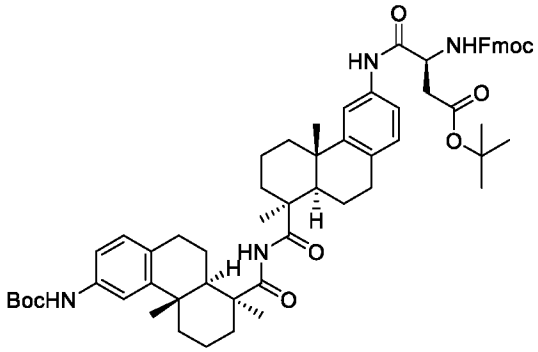
[0516] Following the general procedure for Intermediates **13a-e**, compound **13b** (0.39 g, 83% yield) was obtained as a white solid. ESI m/z: 938 $(M + H)^+$.

9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-{[(*tert*-butoxy)carbonyl]amino}-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentyl]carbamate (13c)



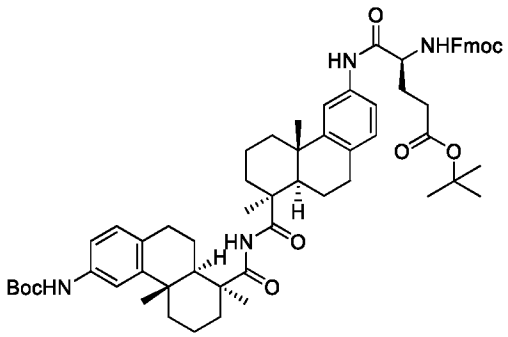
[0517] Following the general procedure for Intermediates **13a-e**, compound **13c** (0.30 g, 78% yield) was obtained as a white solid. ESI m/z: 1201 $(M + H)^+$.

(*S*)-*tert*-Butyl 3-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-4-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-(*tert*-butoxycarbonylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-4-oxobutanoate (13d)



[0518] Following the general procedure for Intermediates **13a-e**, compound **13d** (0.43 g, 85% yield) was obtained as a white solid. ESI m/z: 1021 $(M + H)^+$.

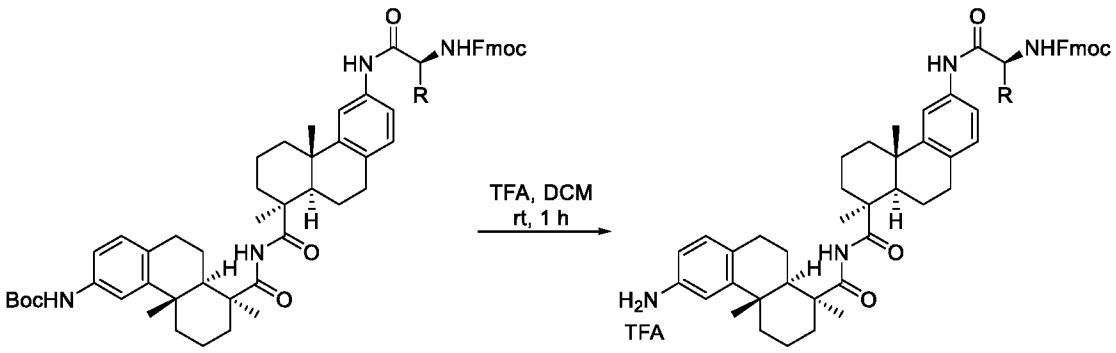
(*S*)-*tert*-Butyl 4-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-(*tert*-butoxycarbonylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-5-oxopentanoate (13e)



[0519] Following the general procedure for Intermediates **13a-e**, compound **13e** (0.42 g, 82% yield) was obtained as a white solid. ESI m/z: 1036 $(M + H)^+$.

Example 11-3b

Intermediates 14a-e



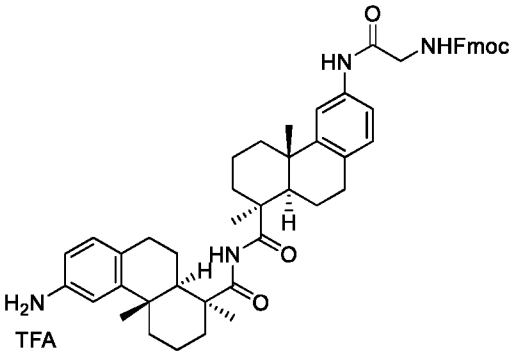
13a, R = H
13b, R = CH_2OH
13c, R = $(CH_2)_4NHFmoc$
13d, R = $CH_2COOtBu$
13e, R = $CH_2CH_2COOtBu$

14a, R = H
14b, R = CH_2OH
14c, R = $(CH_2)_4NHFmoc$
14d, R = CH_2COOH
14e, R = CH_2CH_2COOH

[0520] To a solution of compound **13** (1.0 equiv.) in DCM was added TFA at RT. The reaction mixture was stirred at RT for an hour and concentrated *in vacuo* to give crude product **14** as colorless oil, which was used for the next step without further purification.

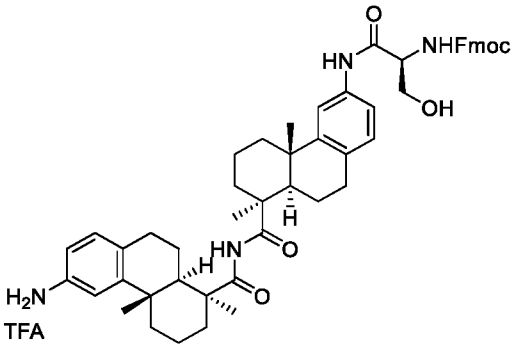
13			TFA (mL)	DCM (mL)	Crude Product **14**		
#	R	g (mmol)			#	R	g
13a	H	0.14 (0.16)	3	10	**14a**	H	0.13
13b	CH_2OH	0.10 (0.11)	3	10	**14b**	CH_2OH	0.09
13c	$(CH_2)_4NHFmoc$	0.24 (0.20)	3	10	**14c**	$(CH_2)_4NHFmoc$	0.22
13d	$CH_2COOtBu$	0.12 (0.12)	3	10	**14d**	CH_2COOH	0.10
13e	$(CH_2)_2COOtBu$	0.10 (0.10)	3	20	**14e**	$(CH_2)_2COOH$	0.08

9*H*-Fluoren-9-ylmethyl *N*-({[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate, trifluoroacetic acid salt (14a)



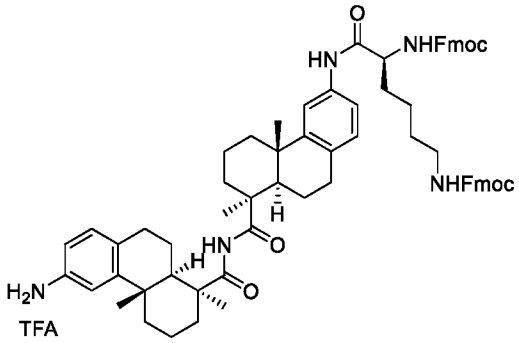
[0521] Following the general procedure for Intermediates **14a-e**, crude compound **14a** (0.14 g, 99% yield, TFA salt) was obtained as colorless oil. ESI m/z: 807 $(M + 1)^+$.

9*H*-Fluoren-9-ylmethyl (*S*)-1-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-3-hydroxy-1-oxopropan-2-ylcarbamate, trifluoroacetic acid salt (14b)



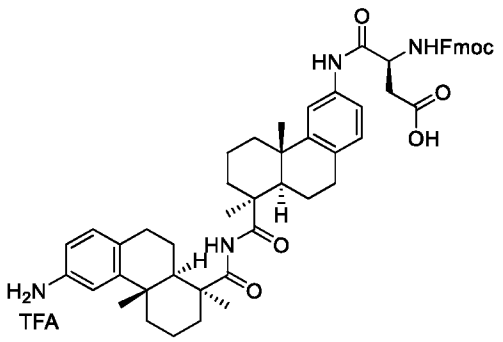
[0522] Following the general procedure for Intermediates **14a-e**, crude compound **14b** (0.14 g, 99% yield, TFA salt) was obtained as colorless oil. ESI m/z: 837 $(M + 1)^+$.

9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentyl]carbamate, trifluoroacetic acid salt (14c)



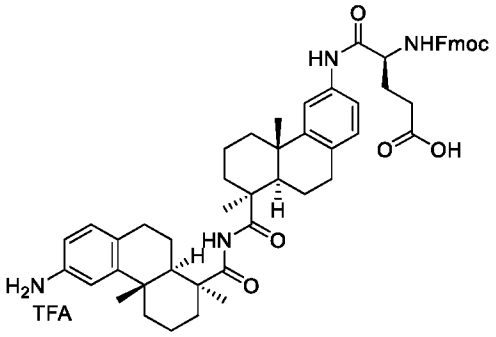
[0523] Following the general procedure for Intermediates **14a-e**, crude compound **14c** (0.22 g, 92% yield, TFA salt) was obtained as colorless oil. ESI m/z: 1101 $(M + 1)^+$.

(*S*)-3-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-4-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-4-oxobutanoic acid, trifluoroacetic acid salt (14d)



[0524] Following the general procedure for Intermediates **14a-e**, crude compound **14d** (0.10 g, 87% yield, TFA salt) was obtained as colorless oil. ESI m/z: 866 $(M + 1)^+$.

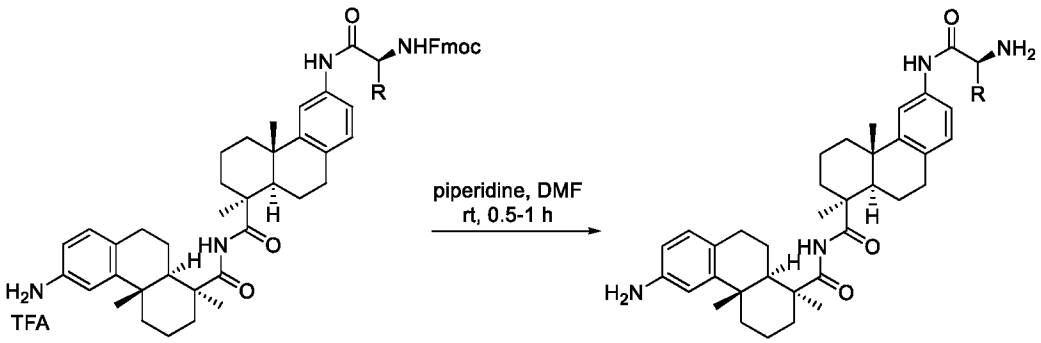
(*S*)-4-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-5-oxopentanoic acid, trifluoroacetic acid salt (14e)



[0525] Following the general procedure for Intermediates **14a-e**, crude compound **14e** (84 mg, 86% yield, TFA salt) was obtained as colorless oil. ESI m/z: 880 $(M + 1)^+$.

Example 11-3c

Payloads P4B-P8B



14a, R = H
14b, R = CH_2OH
14c, R = $(CH_2)_4NHFmoc$
14d, R = CH_2COOH
14e, R = CH_2CH_2COOH

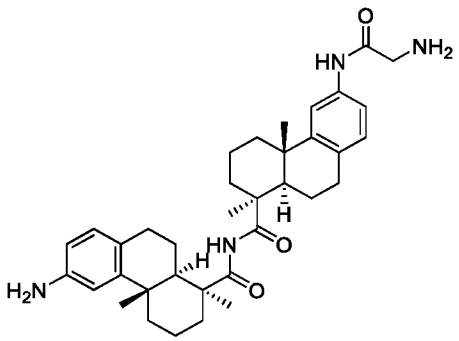
P4B, R = H
P5B, R = CH_2OH
P6B, R = $(CH_2)_4NH_2$
P7B, R = CH_2COOH
P8B, R = CH_2CH_2COOH

[0526] To a solution of crude **14,** obtained above, in DMF was added piperidine. The mixture was stirred at RT for half an hour, which was monitored by LCMS. The mixture was directly purified by prep-HPLC (method B) to give payloads **P4-8** as white solids.

Crude **14**			piperidine (mL)	DMF (mL)	Time (hour)	purification			
#	R	g (mmol)					#	R	mg (Yield)
14a	H	0.066	0.5	3	0.5	Prep-B	**P4B**	H	12

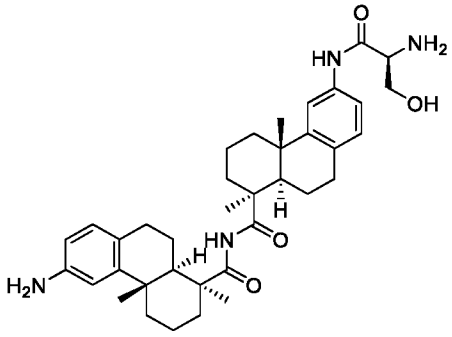
		(0.073)							(28%)
14b	CH_2OH	0.10 (0.11)	0.5	3	0.5	Prep-B	**P5B**	CH_2OH	41 (67%)
14c	$(CH_2)_4NHFmoc$	0.050 (0.045)	0.5	5	1	Prep-B	**P6B**	$(CH_2)_4NH_2$	5 (17%)
14d	CH_2COOH	0.10 (0.12)	0.5	3	0.5	Prep-B	**P7B**	CH_2COOH	39 (51%)
14e	$(CH_2)_2COOH$	0.10 (0.12)	0.5	3	0.5	Prep-B	**P8B**	$(CH_2)_2COOH$	44 (58%)

(1*S*,4a*S*,10a*R*)-*N*-[(1*S*,4a*S*,10a*R*)-6-(2-Aminoacetamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P4B)



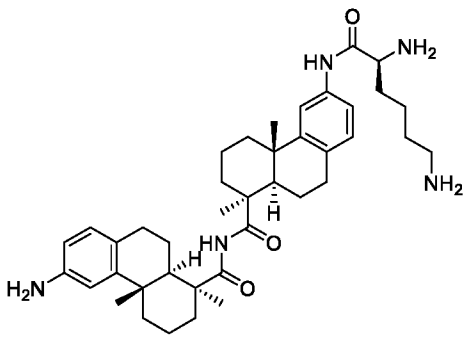
[0527] Following the general procedure for Payloads **P4B-8B**, compound **P4B** (12 mg, 28% yield) was obtained as a white solid. ESI m/z: 585 $(M + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 9.79 (s, 1H), 8.12 (s, 1H), 7.50 (s, 1H), 7.41 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 1.5 Hz, 1H), 6.34 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 4. 70 (m, 2H), 3.20 (s, 3H), 2.95-2.83 (m, 1H), 2.80-2.70 (m, 2H), 2.70-2.60 (m, 1H), 2.30-2.20 (m, 2H), 2.20-2.10 (m, 4H), 2.05-1.70 (m, 4H), 1.70-1.50 (m, 4H), 1.31-1.25 (m, 8H), 1.20-1.10 (m, 2H), 1.05 (d, J = 7.5 Hz ,6H) ppm.

(1*S*,4a*S*,10a*R*)-6-Amino-*N*-((1*S*,4a*S*,10a*R*)-6-((*S*)-2-amino-3-hydroxypropanamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P5B)



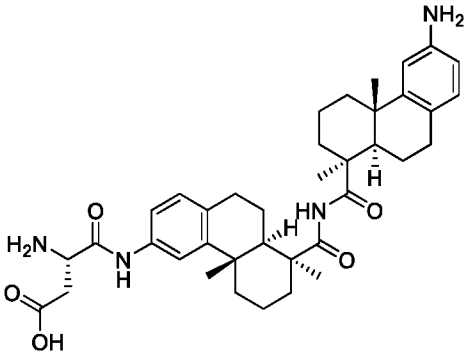
[0528] Following the general procedure for Payloads **P4B-8B**, compound **P5B** (41 mg, 67% yield) was obtained as a white solid. ESI m/z: 615 $(M + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 8.09(s, 1H), 7.52 (s, 1H), 7.41 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 6.96(d, J = 8.5 Hz, 1H), 6.68(d, J = 8.5 Hz, 1H), 6.48(d, J = 1.5 Hz, 1H), 6.34 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 4.84-4.76 (m, 1H), 4.67 (s, 2H), 3.60-3.45 (m, 2H), 2.95-2.83 (m, 1H), 2.80-2.60 (m, 4H), 2.30-2.20 (m, 3H), 2.20-2.10 (m, 4H), 1.95-1.75 (m, 5H), 1.70-1.50 (m, 4H), 1.40-1.30 (m, 2H), 1.28 (s, 3H), 1.26 (s, 3H), 1.20-1.10 (m, 3H), 1.01 (s, 3H), 0.98 (s, 3H) ppm.

(1*S*,4a*S*,10a*R*)-6-Amino-*N*-((1*S*,4a*S*,10a*R*)-6-((*S*)-2,6-diaminohexanamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P6B)



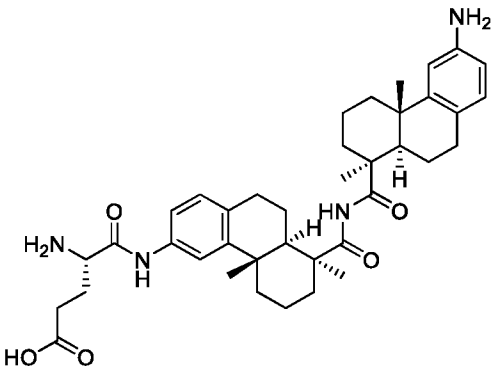
[0529] Following the general procedure for Payloads **P4B-8B**, compound **P6B** (5 mg, 17% yield) was obtained as a white solid. ESI m/z: 656 $(M + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 8.09 (s, 1H), 7.52 (s, 1H), 7.41 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 1.5 Hz, 1H), 6.34 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 4. 70 (m, 2H), 3.20 (s, 2H), 2.95-2.83 (m, 2H), 2.80-2.70 (m, 2H), 2.70-2.60 (m, 2H), 2.37-2.35 (m, 1H), 2.30-2.20 (m, 3H), 2.20-2.10 (m, 5H), 2.05-1.95 (m, 2H), 1.95-1.75 (m, 5H), 1.70-1.50 (m, 6H), 1.31-1.25 (m, 8H), 1.20-1.10 (m, 3H), 1.05 (d, J = 7.5 Hz, 6H), 0.85 (t, J = 6.0 Hz, 1H) ppm.

(*S*)-3-Amino-4-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-4-oxobutanoic acid (P7B)



[0530] Following the general procedure for Payloads **P4B-8B**, compound **P7B** (39 mg, 51% yield) was obtained as a white solid. ESI m/z: 643 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 10.5-10.0 (br, 1H), 8.09 (s, 1H), 7.52 (s, 1H), 7.41 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 1.5 Hz, 1H), 6.34 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 4.84-4.50 (m, 2H), 3.75-3.68 (m, 2H), 2.95-2.83 (m, 1H), 2.80-2.60 (m, 4H), 2.40-2.20 (m, 4H), 2.20-2.10 (m, 5H), 1.95-1.70 (m, 5H), 1.70-1.50 (m, 5H), 1.27 (d, J = 7.5 Hz, 6H), 1.20-1.10 (m, 2H), 1.01 (s, 3H), 0.98 (s, 3H) ppm.

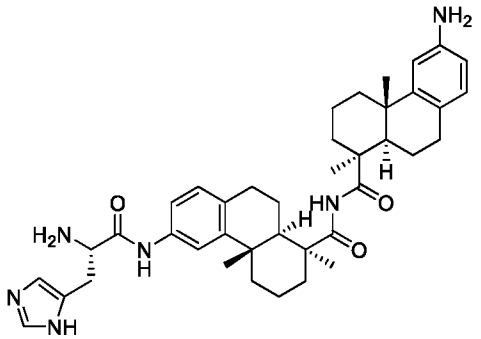
(*S*)-4-Amino-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-5-oxopentanoic acid (P8B)



[0531] Following the general procedure for Payloads **P4B-8B**, compound **P8B** (44 mg, 58% yield) was obtained as a white solid. ESI m/z: 657 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 8.09 (s, 1H), 7.52 (s, 1H), 7.41 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 6.96 (d, J = 8.5 Hz,1H), 6.68 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 1.5 Hz, 1H), 6.34 (dd, J = 7.5 Hz, 1.5 Hz, 1H), 4.70 (m, 2H), 2.95-2.83 (m, 1H), 2.80-2.60 (m, 5H), 2.30-2.20 (m, 4H), 2.20-2.10 (m, 5H), 1.95-1.75 (m, 5H), 1.70-1.50 (m,

5H), 1.50-1.40 (m, 4H), 1.27 (d, *J* =7.5 Hz, 6H), 1.20-1.10 (m, 2H), 1.01 (s, 3H), 0.98 (s, 3H) ppm.

(1S,4aS,10aR)-6-amino-N-((1S,4aS,10aR)-6-((S)-2-amino-3-(1H-imidazol-4-yl)propanamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P9B)



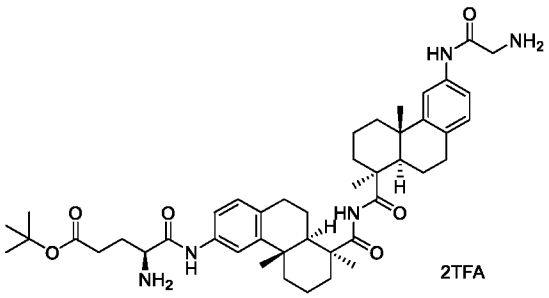
[0532] To a solution of Fmoc-His-OH (0.38 g, 1.0 mmol) in DCM (5 mL) were added Fmoc-OSu (0.37 g, 1.1 mmol) and DIPEA (0.26 g, 2.0 mmol). The reaction mixture was stirred at RT overnight. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography (5-10% methanol in DCM) to give Fmoc-His(Fmoc)-OH (0.50 g, 84% yield, ESI m/z: 600 $(M + 1)^+$) as a white solid.

[0533] To a solution of compound **12b** (0.31 g, 0.50 mmol) in DCM (20 mL) were added the Fmoc-His(Fmoc)-OH (0.33 g, 0.55 mmol), obtained above, HATU (0.23 g, 0.60 mmol) and DIPEA (0.19 g, 1.5 mmol) successively. The resulting mixture was stirred at RT for 4 h, which was monitored by LCMS. To the reaction mixture was added piperidine (0.5 mL) and the mixture was stirred at RT for an hour until Fmoc was totally removed, as monitored by LCMS. The mixture was concentrated *in vacuo* and the residue was purified by reverse phase flash chromatography (50-80% acetonitrile in water) to give **Boc-P9B** (0.15 g) as a white solid, half of which was dissolved in DCM (20 mL). To the solution was added TFA (3 mL). The mixture was stirred at RT for an hour until Boc was removed according to LCMS. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method B) to give compound **P9B** (17 mg, 12% yield) as a white solid. ESI m/z: 665 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 11.82 (s, 1H), 9.75 (s, 1H), 8.09 (s, 1H), 7.52-7.50 (m, 2H), 7.41 (dd, *J* = 8.5 Hz, 1.5 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.90-6.80 (m, 1H), 6.68 (d, *J* = 8.5 Hz,1H), 6.48 (d, *J* = 1.5 Hz, 1H), 6.34 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H), 4.84-4.50 (m, 2H), 3.60-3.45 (m, 2H), 2.95-2.83 (m, 2H), 2.80-2.70 (m, 2H), 2.70-2.60 (m, 2H), 2.40-2.10 (m, 8H), 1.95-1.70 (m, 5H), 1.70-1.50 (m, 4H), 1.27 (d, *J* = 7.5 Hz, 6H), 1.20-1.10 (m, 2H), 1.01 (s, 3H), 0.98 (s, 3H) ppm.

EXAMPLE 11-4

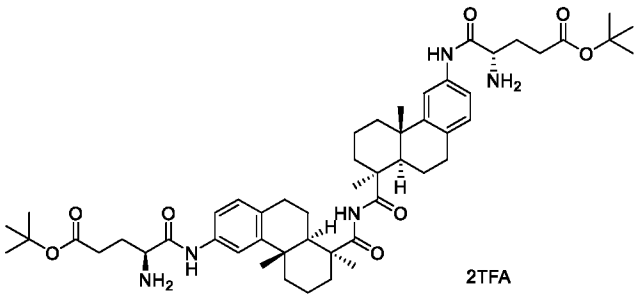
[0534] This example demonstrates general methods for the synthesis of the podocarpic acid derivatives **P10B** and **P11B** in Table C, below. This example refers to the compounds numbered from **14a**, **15a-b**, and **P10B** and **P11B** in FIG. 24.

(*S*)-*tert*-Butyl 4-amino-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-(2-aminoacetamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-5-oxopentanoate, di-trifluoroacetic acid salt (15a)



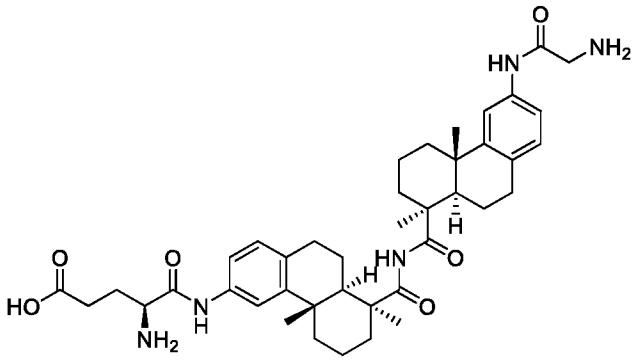
[0535] To a solution of Fmoc-Glu(OtBu)-OH (74 mg, 0.17 mmol) and DIPEA (55 µL, 0.32 mmol) in DMF (5.0 mL) was added HATU (91 mg, 0.24 mmol). The mixture was stirred at RT for 15 min before the addition of compound **14a** (0.14 g, 0.16 mmol). The reaction mixture was stirred at RT overnight, which was monitored by LCMS. To the reaction mixture was then added piperidine (1 mL) dropwise. The reaction mixture was stirred at RT for an hour until Fmoc was totally removed according to LCMS. The resulting mixture was directly separated by reverse phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.01%)) to give compound **15a** (0.13 g, 80% yield) as a yellow solid. ESI m/z: 770.5 $(M + 1)^+$.

(4*S*,4'*S*)-*tert*-Butyl 5,5'-(4b*S*,4b'*S*,8*S*,8a*R*,8'*S*,8a'*R*)-8,8'-(azanediylbis(oxomethylene))bis(4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthrene-8,3-diyl)bis(azanediyl)bis(4-amino-5-oxopentanoate) di-trifluoroacetic acid salt (15b)



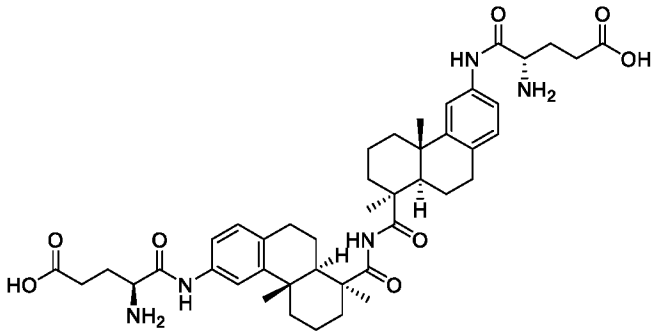
[0536] To a solution of Fmoc-Glu(OtBu)-OH (0.15 g, 0.35 mmol) and DIPEA (83 µL, 0.48 mmol) in DMF (5.0 mL) was added HATU (0.15 g, 0.40 mmol). The mixture was stirred at RT for 30 min before the addition of compound **P2B** (0.10 g, 0.16 mmol). The reaction mixture was stirred at RT overnight, which was monitored by LCMS. To the reaction mixture was then added piperidine (1 mL) dropwise. The reaction mixture was stirred at RT for 3 h until Fmoc was totally removed according to LCMS. The resulting mixture was directly separated by reverse phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.01%)) to give compound **15b** (0.14 g, 78% yield) as a yellow solid. ESI m/z: 899 $(M + 1)^+$.

(*S*)-4-Amino-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-(2-aminoacetamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-5-oxopentanoic acid (P10B)



[0537] A mixture of compound **15a** (0.13 g, 0.13 mmol) in neat TFA (2.0 mL) was stirred at RT for an hour, which was monitored by LCMS. The resulting mixture was diluted with DCM (20 mL) and concentrated *in vacuo*. The residue was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* sodium bicarbonate (10 mM)) to give **P10B** (20 mg, 22% yield) as a white solid. ESI m/z: 358 $(M/2 + 1)^+$; 714.5 $(M + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 9.85 (br s, 1H), 8.12 (s, 1H), 7.51 (s, 2H), 7.42-7.36 (m, 2H), 6.96 (dd, J = 8.0 Hz and 3.0 Hz, 2H), 3.67 (br s, 8H), 3.36-3.33 (m, 1H), 3.22 (s, 2H), 2.91-2.87 (m, 2H), 2.80-2.71 (m, 2H), 2.31-2.28 (m, 4H), 2.17-2.14 (m, 4H), 1.91-1.82 (m, 5H), 1.69-1.58 (m, 5H), 1.34-1.23 (m, 6H), 1.19-1.12 (m, 2H), 1.01 (s, 6H) ppm.

(4*S*,4'*S*)-5,5'-(4b*S*,4b'*S*,8*S*,8a*R*,8'*S*,8a'*R*)-8,8'-(Azanediylbis(oxomethylene))bis(4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthrene-8,3-diyl)bis(azanediyl)bis(4-amino-5-oxopentanoic acid) (P11B)



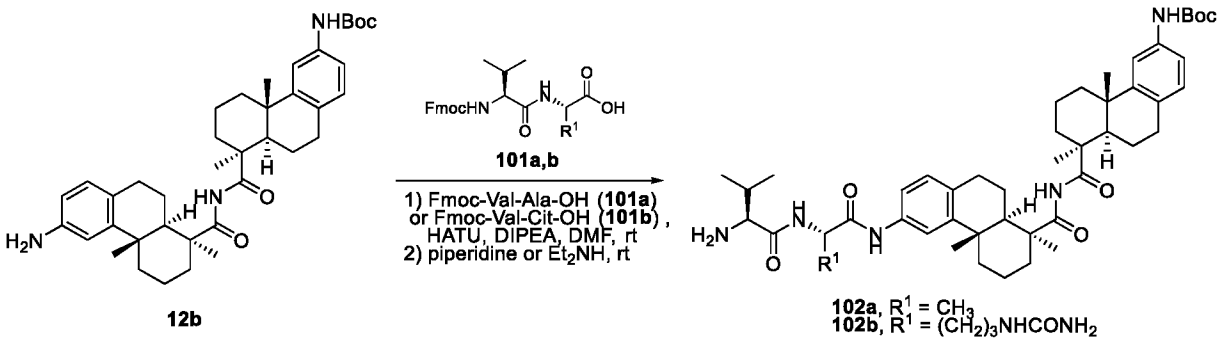
[0538] A mixture of compound **15b** (0.14 g, 0.14 mmol) in neat TFA (3.0 mL) was stirred at RT for an hour, which was monitored by LCMS. The resulting mixture was diluted with DCM (30 mL) and concentrated *in vacuo*. The residue was purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* sodium bicarbonate (10 mM)) to give **P11B** (20 mg, 18% yield) as a white solid. ESI m/z: 394 $(M/2 + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 9.85 (br s, 2H), 8.12 (s, 1H), 7.51 (s, 2H), 7.9 (dd, J = 8.4 Hz and 1.6 Hz, 2H), 6.97 (d, J = 8.0 Hz, 2H), 3.5 (br s, 6H), 3.40-3.37 (m, 2H), 3.16 (s, 1H), 2.91-2.88 (m, 2H), 2.79-2.66 (m, 2H), 2.33-2.29 (m, 7H), 2.18-2.15 (m, 4H), 1.91-1.84 (m, 6H), 1.71-1.59 (m, 6H), 1.35-1.23 (m, 6H), 1.18-1.12 (m, 2H), 1.01 (s, 6H) ppm.

EXAMPLE 11-5

[0539] This example demonstrates methods for the synthesis of the linker-payloads **LP1B-LP5B** in Table 3, above. This example refers to the compounds numbered **12b** and from **102a-b** to **106a-e** and linker-payloads **LP1B-LP5B** in FIG. 25.

Example 5a

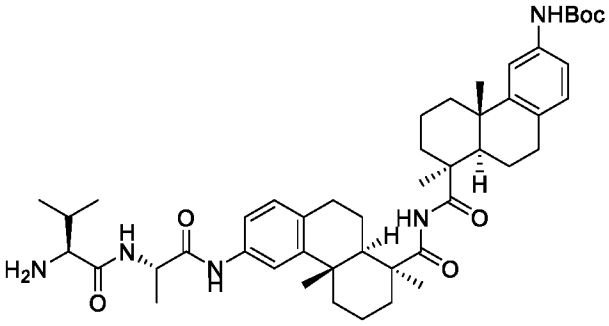
Intermediates 102a-b



[0540] To a solution of acid (Fmoc-Val-Ala-OH (**101a**, 1.2 equiv.) or Fmoc-Val-Cit-OH (**101b**, 1.2 equiv.) in DMF were added HATU (1.2 equiv.) and DIPEA (2.0-3.0 equiv.) at RT. After the mixture was stirred at RT for 5 min, compound **12b** (1.0 equiv.) was added. The resulting mixture was stirred at RT for 4-20 h until the amine was consumed according to LCMS. To the mixture was then added piperidine (excess), and the mixture was stirred at RT for 1-6 h until Fmoc was totally removed, as monitored by LCMS. The reaction mixture was filtered through a membrane and the filtrate was directly purified by prep-HPLC (method B) or reverse phase flash chromatography to give compound **102** (38-72% yield) as a white solid.

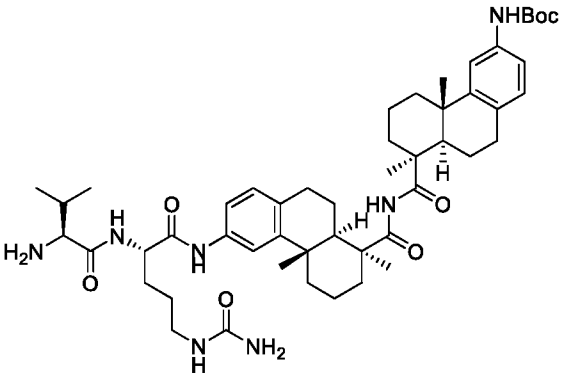
Amine g (mmoL)		Acid g (mmoL)		Step 1				Step 2		Purification	Product	
				HATU g (mmol)	DIPEA g (mmol)	DMF (mL)	Time (hr)	Piperidine (mL)	Time (hr)		#	g (yield)
12b	0.31 (0.50)	**101a**	0.25 (0.60)	0.23 (0.60)	0.19 (1.5)	20	4	0.5	1	RP	102a	0.29 (72%)
12b	0.50 (0.80)	**101b**	0.48 (0.96)	0.36 (0.96)	0.28 mL (1.6)	6	20	0.5	6	Prep-B	102b	0.27 (38%)

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (102a)**



[0541] Following the general procedure for Intermediates 102a,b, compound **102a** (0.29 g, 72% yield) was obtained as a white solid. ESI m/z: 799 $(M + 1)^+$.

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (102b)**

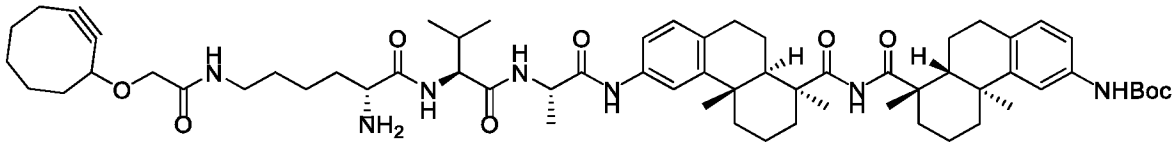


[0542] Following the general procedure for Intermediates 102a,b, compound **102b** (0.27 g, 38% yield) was obtained as a white solid. ESI m/z: 885 $(M + 1)^+$.

Example 5b

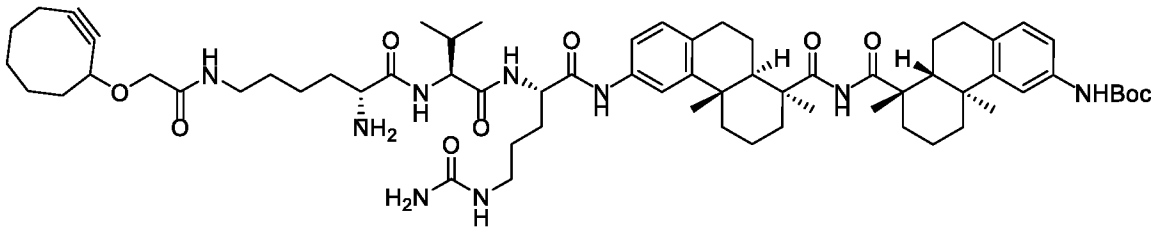
Intermediates 104a-b

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-{2-amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido}-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (104a)**



[0543] To a solution of compound **103** (70 mg, 0.13 mmol) in DMF (2 mL) were added HATU (68 mg, 0.18 mmol) and DIPEA (44 μL, 0.24 mmol) at RT. The mixture was stirred at RT for 5 min before the addition of compound **102a** (95 mg, 0.12 mmol). The reaction mixture was then stirred at RT for 3 h until compound **102a** was totally consumed, as monitored by LCMS. To the reaction mixture was then added piperidine (0.5 mL, excess). The mixture was stirred at RT for 2 h. The mixture was then filtered and the filtrate was concentrated. The residue was purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **104a** (0.13 g, 96% yield) as a white solid. ESI m/z: 518.0 $((M-55)/2)^+$.

***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-{2-amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido}-3-methylbutanamido]-5-(carbamoylamino)pentanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (104b)**



[0544] To a solution of compound **103** (0.27 g, 0.31 mmol) and DIPEA (0.11 mL, 0.61 mmol) in DMF (6 mL) was added compound **102b** (0.18 g, 0.34 mmol) followed by the addition of HATU (0.14 g, 0.37 mmol). The reaction mixture was stirred at RT for 3 h, which was monitored by LCMS. The resulting solution was directly purified by reverse phase flash chromatography to give compound **Fmoc-104b** (0.30 g, ESI m/z: 711 $((M + Na)/2 +1)^+$) as a pale yellow solid, which was dissolved in DCM (6.0 mL). To the solution was added piperidine (0.5 mL) and the reaction mixture was stirred at RT for 2 h until Fmoc was totally removed according to LCMS. The volatiles were removed *in vacuo* and the residue was triturated with petroleum ether to give compound **104b** (0.26 g, 65% yield) as a light yellow solid. ESI m/z: 1177.6 $((M + H)^+$.

Example 5c

Intermediates 105a-c

[0545] Azido intermediate α-CD-N_3 (**105a**) was synthesized according to *J. Am. Chem. Soc.*, **2012**, 134(46), 19108-19117 (FIG. 32).

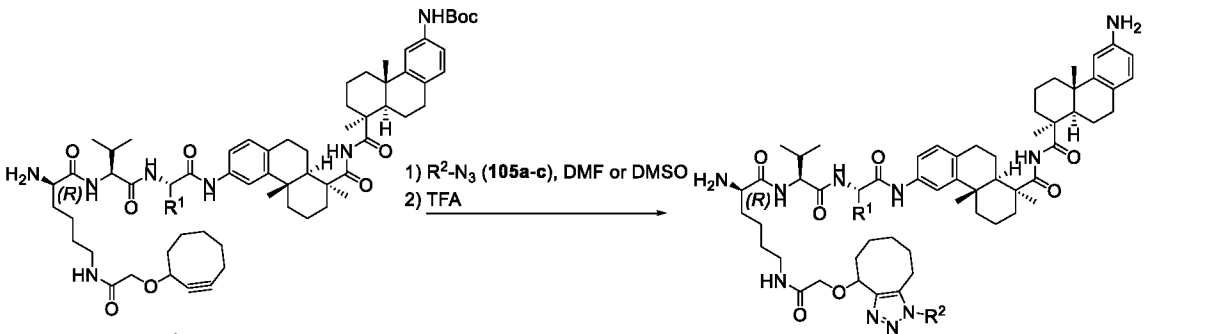
Azido-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (105b), FIG. 33

[0546] To a solution of 2,5-dioxopyrrolidin-1-yl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate (N_3-PEG_4-OSu, 0.10 g, 0.26 mmol) and taurine (39 mg, 0.31 mmol) in anhydrous DMF (4 mL) was added DIPEA (15 mg, 0.52 mmol). The mixture was stirred at RT overnight. The reaction mixture was filtered and the solution was purified by prep-HPLC (method A) to give intermediate **105b** (0.80 g, yield 78%) as colorless oil. ESI m/z: 399.1 $(M + H)^+$. ^1H NMR (500 MHz, D_2O) δ 3.69 (t, J = 6.0 Hz, 2H), 3.64-3.59 (m, 14H), 3.49 (t, J = 6.5 Hz, 2H), 3.41 (t, J = 4.5 Hz, 2H), 3.00 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 6.0 Hz, 2H) ppm.

[0547] Azido intermediate maltose-N_3 (**105c**) was synthesized according to *Tetrahedron Letters*, **2001**, 42 (7), 1325-1328 (FIG. 34).

Example 5d

Intermediates 106a-e



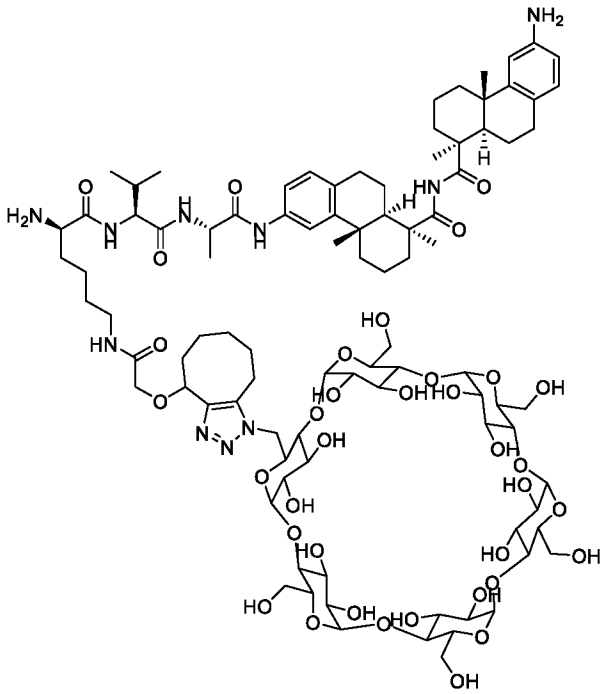
[0548] To a solution of compound **104** in DMF were added azido intermediate **105** at RT. The reaction was stirred at RT for 3-48 h until LCMS showed complete reaction. The reaction mixture was directly purified by prep-HPLC to give compound **Boc-106** as a white solid, which was dissolved in TFA solution (or neat TFA). The solution was stirred at RT for 0.5-20 h until

Boc was removed according to LCMS. The solution was concentrated to give **106** (as the TFA salt).

Alkyne g (mmoL)		Azide g (mmoL)		Step 1				Step 2			Product	
				DMF (mL)	T (°C)	Time (hr)	Purification	TFA (mL)	solvent (mL)	Time (hr)	#	g (yield)
104a	0.050 (0.045)	**105a**	0.11 (0.12)	4	RT	48	RP-A	1	DCM (3 mL)	1	**106a**	0.019* (69%)
104b	0.050 (0.042)	**105a**	0.085 (0.085)	4	RT	20	RP	2	/	0.5	**106b**	0.076 (87%)
104a	0.17 (0.16)	**105b**	0.12 (0.31)	6	RT	20	RP-A	2	/	1	**106c**	0.090 (39%)
104b	0.050 (0.042)	**105b**	0.034 (0.084)	2	RT	3	RP	1	MeOH (2 mL)	20	**106d**	0.022 (36%)
104a	0.035 (0.032)	**105c**	0.024 (0.064)	DMSO 5 mL	RT	4	RP	3	/	2	**106e**	0.034 (77%)

*not all **Boc-106a** was used for de-Boc.

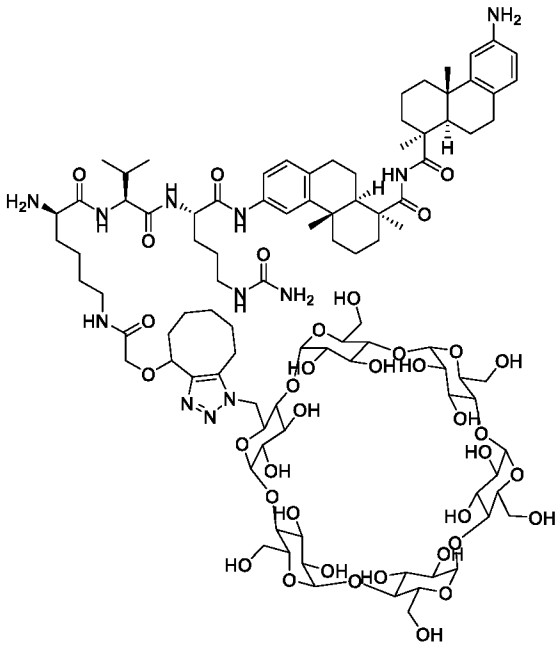
(1*S*,4a*S*,10a*R*)-*N*-[(1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carbonyl]-6-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]propanamido]-1,4a-dimethyl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carboxamide trifluoroacetic acid salt (106a)



[0549] Following the general procedure for Intermediates 106a-e, compound **Boc-106a** (72 mg, 76% yield, as a mixture of triazole regioisomers) was obtained as a white solid (ESI m/z: 1045 (M/2 + 1)$^+$). A small amount of the pure major isomer (7 mg) could be obtained after further purification by reverse phase flash chromatography (0-60% acetonitrile in *aq.* TFA (0.01%)), which was determined by ^{1}H NMR (500MHz, DMSO$_{d6}$) ((with regioisomers) δ 9.92 (s, 0.5H), 9.82 (s, 0.5H), 9.10 (s, 1H), 8.58-8.53 (m, 1H), 8.50-8.35 (m, 1H), 8.12 (s, 1H), 8.04 (s, 3H), 7.88-7.82 (m, 1H), 7.55 (s, 0.5H), 7.46 (s, 0.5H), 7.41 (s, 1H), 7.40-7.26 (m, 1H), 7.14 (d, *J* = 7.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 5.51 (br, 12H), 4.83-4.70 (m, 8H), 4.57-4.51 (m, 3H), 4.44-4.36 (m, 4H), 4.00-3.98 (m, 1H), 3.88-3.55 (m, 27H), 3.26-3.09 (m, 6H), 2.91-2.85 (m, 4H), 2.76-2.63 (m, 3H), 2.29-2.25 (m, 2H), 2.17-1.99 (m, 4H), 1.96-1.93 (m, 6H), 1.88-1.63 (m, 9H), 1.56-1.54 (m, 15H), 1.33-1.28 (m, 15H), 1.18-1.13 (m, 3H), 1.01-0.99 (m, 6H), 0.89-0.83 (m, 7H) ppm.). To a mixture of **Boc-106a** (as a mixture of regioisomers) (20 mg, 9.6 µmol) in DCM (3 mL) was added TFA (1 mL). The resulting mixture was stirred at RT for an hour until Boc was removed, as monitored by LCMS. The volatiles were removed *in vacuo* to give compound **106a** (19 mg, 69% yield from **104a**) as a pale-yellow solid, which was used for the next step without further purification. ESI m/z: 995 (M/2 + 1)$^+$.

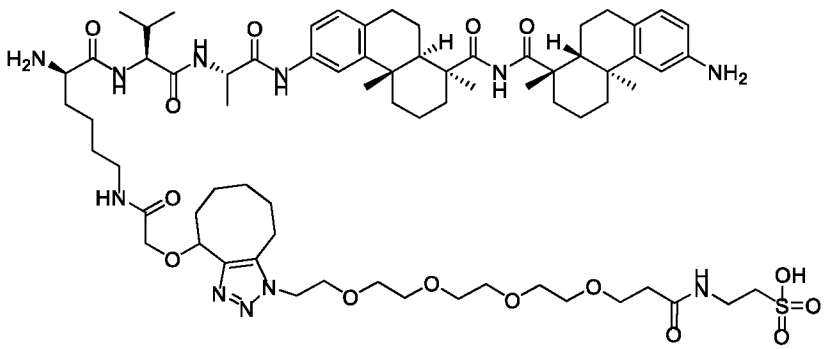
(1*S*,4a*S*,10a*R*)-*N*-{[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-

1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide trifluoroacetic acid salt (106b)



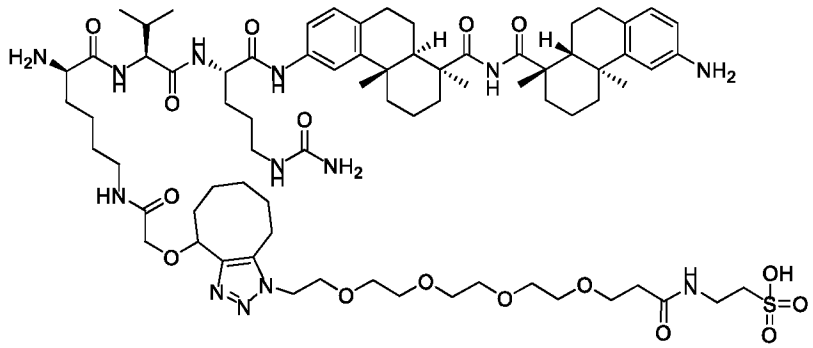
[0550] Following the general procedure for Intermediates 106a-e, compound **106b** (76 mg, 87% yield from **104b**) was obtained as a yellow solid. ESI m/z: 692 $(M/3 + 1)^+$.

1-(4-(2-(((*R*)-5-Amino-6-(((*S*)-1-(((*S*)-1-(((4b*S*,8*S*,8a*R*)-8-(((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-6-oxohexyl)amino)-2-oxoethoxy)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (106c)



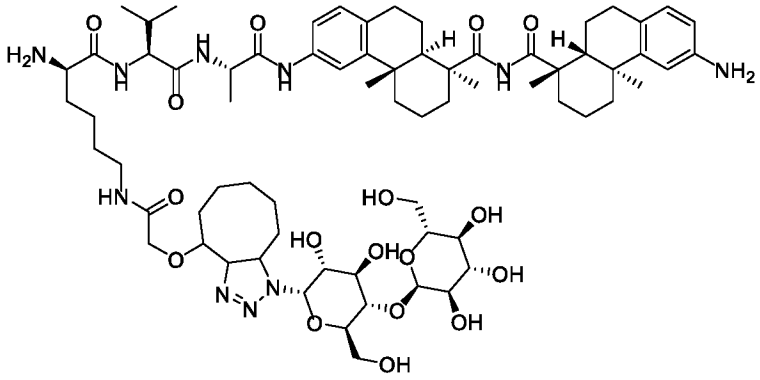
[0551] Following the general procedure for Intermediates 106a-e, compound **106c** (90 mg, 39% yield from **104c**) was obtained as a yellow solid. ESI m/z: 463.8 $(M/3 + 1)^+$.

1-(4-(((6*S*,9*S*,12*R*)-1,12-Diamino-6-(((4b*S*,8*S*,8a*R*)-8-(((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl)carbamoyl)-9-isopropyl-1,8,11,18-tetraoxo-2,7,10,17-tetraazanonadecan-19-yl)oxy)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (106d)



[0552] Following the general procedure for Intermediates 106a-e, compound **106d** as its TFA salt (22 mg, 36% yield) was obtained as a white solid after purification by reverse phase flash chromatography (0-80% acetonitrile in water during 25 minutes). ESI m/z: 788 $(M/2 + H)^+$.

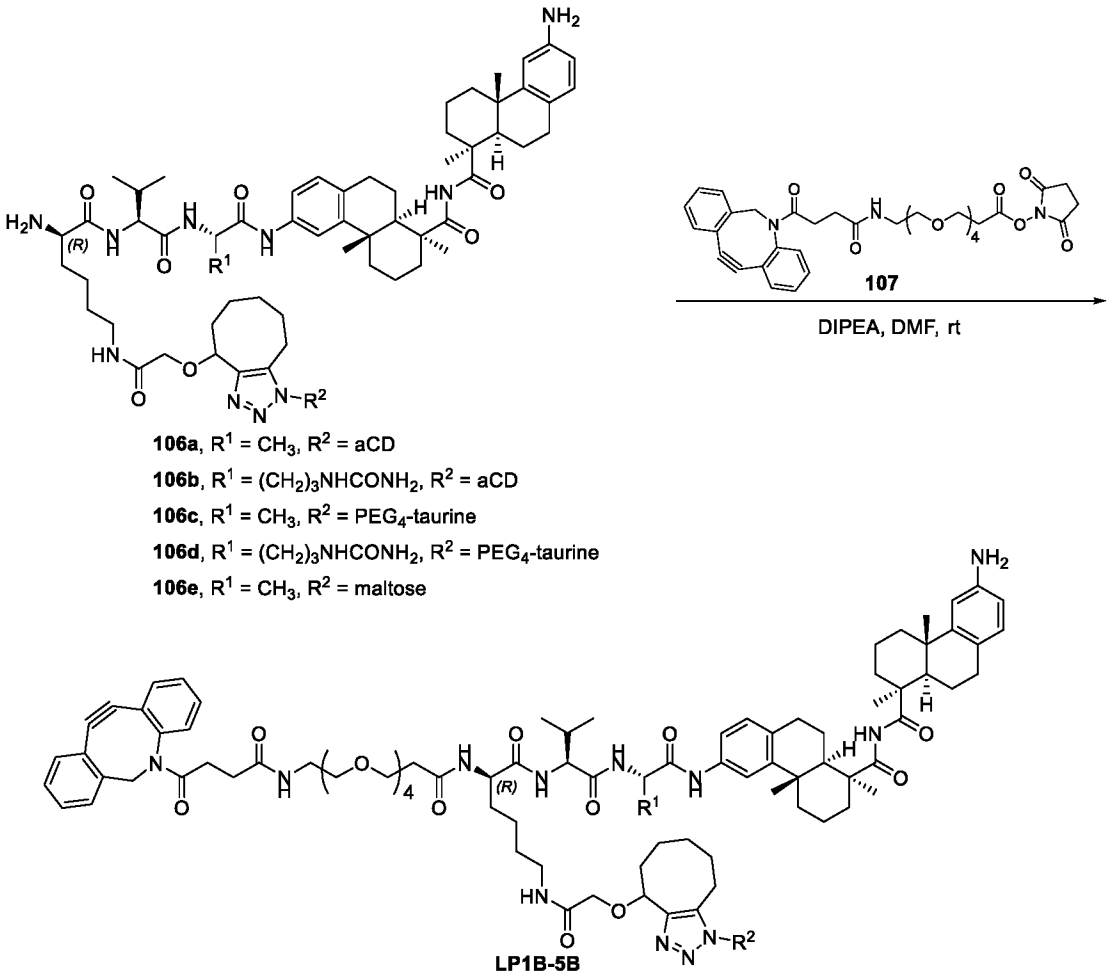
(1*S*,4a*S*,10a*R*)-6-Amino-*N*-((1*S*,4a*S*,10a*R*)-6-((2*S*)-2-((2*S*)-2-((2*R*)-2-amino-6-(2-((1-((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4-dihydroxy-6-(hydroxymethyl)-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)-3a,4,5,6,7,8,9,9a-octahydro-1*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy)acetamido)hexanamido)-3-methylbutanamido)propanamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (106e)



[0553] Following the general procedure for Intermediates 106a-e, compound **106e** as its TFA salt (34 mg, 77% yield) was obtained as colorless oil. ESI m/z: 1358 $(M + H)^+$.

Example 5e

Linker-Payloads LP1B-LP5B



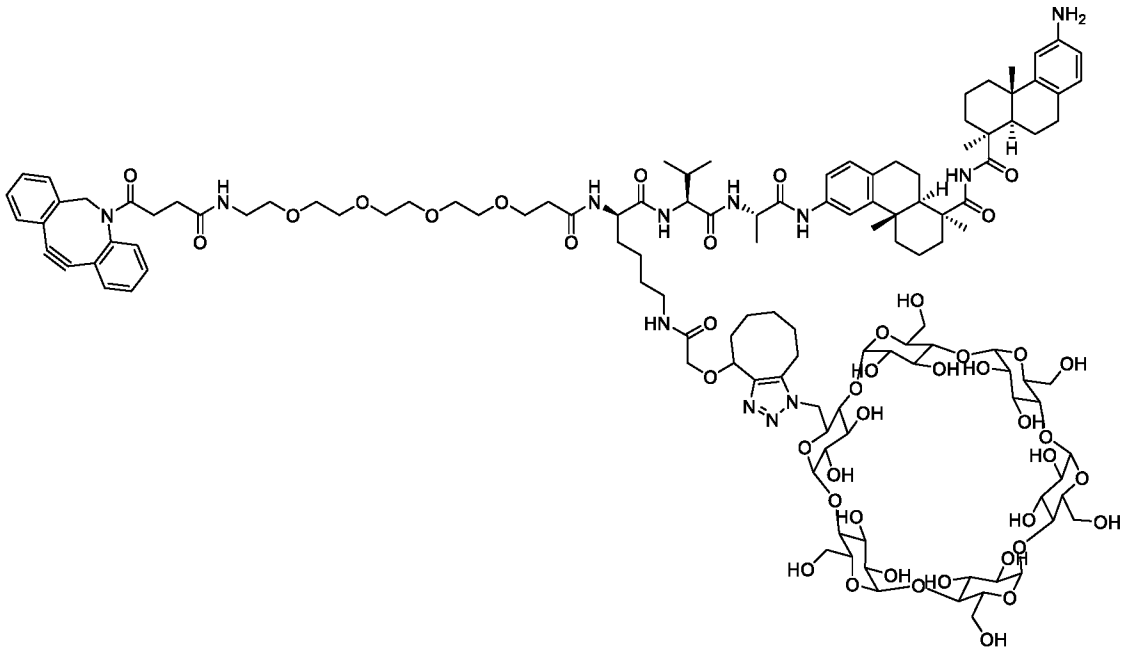
[0554] To a solution of compound **106** in DMF were added compound DIBAC-PEG$_4$-NHS **107** and DIPEA at RT. The reaction mixture was stirred at RT for 3 h. The reaction mixture was directly purified by prep-HPLC (method B) or reverse phase flash chromatography (method B) to give compound **LP1B-5B**.

Amine **106**		NHS ester **107** mg (μmol)	DIPEA (mmol)	DMF (mL)	Time (hr)	Purification		Product **LP**	
#	mg (μmol)							#	mg (yield)
106a	19 (8.7)	7.0 (11)	4.0 mg (0.031)	1.5	1.5	RP-B (twice)		**LP1B**	8 (36%)*
106b	76 (37)	24 (37)	12 μL (0.073)	5	1.5	Prep-B (twice)		**LP2B**	20 (21%)
106c	90 (65)	39 (60)	0.02 mL (0.12)	5	1.5	Prep-B		**LP3B**	60 (52%)
106d	22 (15)	9.7 (15)	5 μL (0.030)	2	1.5	Prep-B		**LP4B**	6 (20%)

106e	40 (29)	23 (35)	7.6 mg (59)	5	4	Prep-B		**LP5B**	15 (27%)

*7 mg of compound **106a** as free base was recycled.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-5,6,7,8a,9,10-hexahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}pentyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP1B)

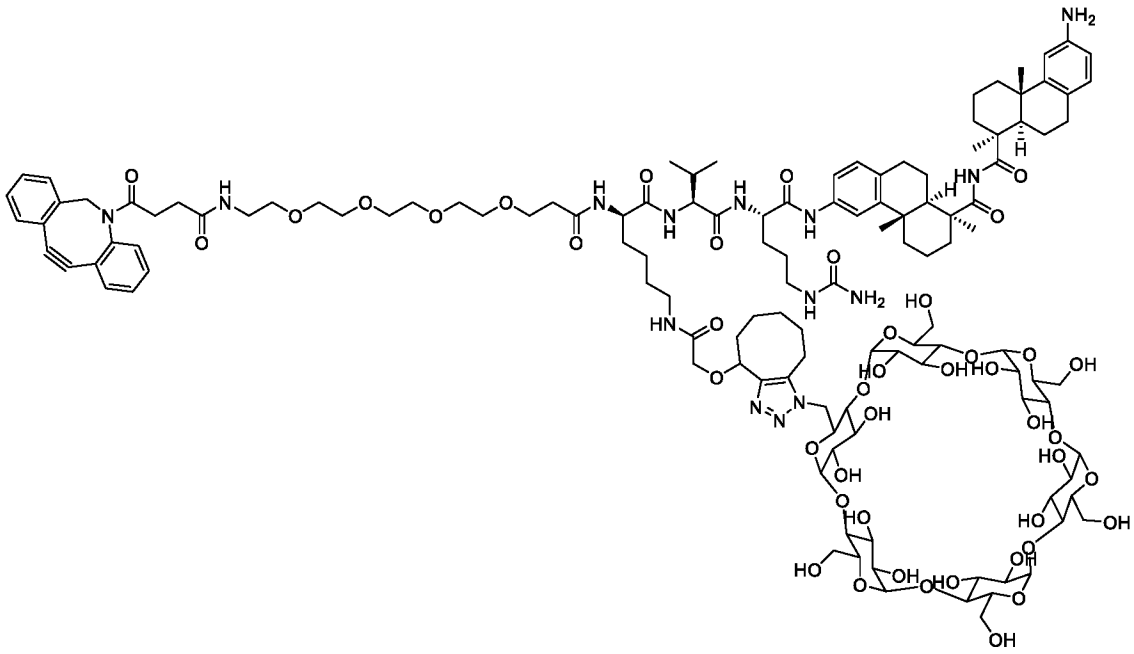


[0555] Following the general procedure for Linker-payloads LP1-5, linker-payload **LP1B** (8 mg, 36% yield) was obtained as a white solid. ESI m/z: 842 $(M/3 + 1)^+$; 1262 $(M/2 + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 9.69 (s, 0.5H), 9.28 (s, 0.5H), 8.20-8.00 (m, 5H), 7.81-7.75 (m, 2H), 7.66 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.53-7.27 (m, 9H), 6.96-6.92 (m, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.47 (s, 1H), 6.32 (d, J = 8.4 Hz, 1H), 5.57-5.47 (m, 14H), 5.13-4.99 (m, 2H), 4.81-4.68 (m, 11H), 4.59-4.52 (m, 5H), 4.36-4.29 (m, 3H), 4.16-4.08 (m, 1H), 3.99-3.98 (m, 1H), 3.84-

3.53 (m, 30H), 3.45-3.38 (m, 12H), 3.13-3.03 (m, 4H), 2.90-2.66 (m, 5H), 2.36-2.32 (m, 1H), 2.26-2.20 (m, 3H), 2.15-2.12 (m, 4H), 2.00-1.99 (m, 2H), 1.88-1.72 (m, 6H), 1.64-1.40 (m, 14H), 1.33-1.24 (m, 17H), 1.14-1.09 (m, 3H), 1.00-0.97 (m, 7H), 0.89-0.81 (m, 8H) ppm.

[0556] Anal. *HPLC*: 95%, Retention time: 7.93 min (method B).

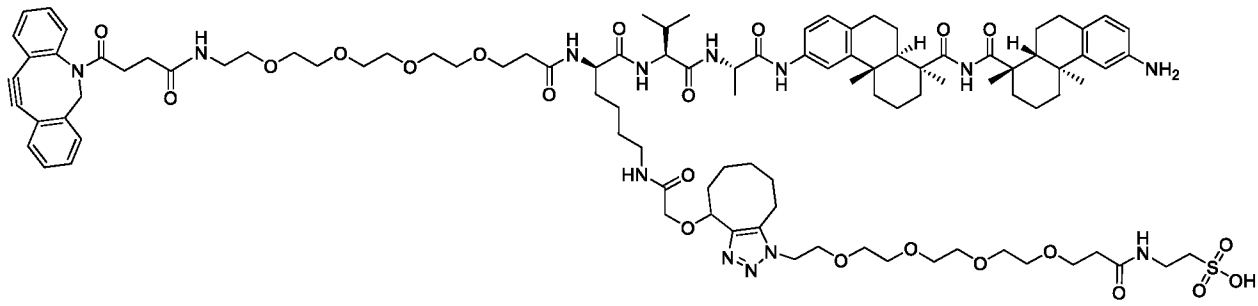
1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}pentyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP2B)



[0557] Following the general procedure for Linker-payloads LP1-5, linker-payload **LP2B** (20 mg, 21% yield) was obtained as a white solid. ESI m/z: 870 (M/3 + H)$^+$. ^{1}H NMR (400 MHz, DMSO$_{d6}$) δ 9.76 (s, 1H), 9.31 (s, 1H), 8.26-8.16 (m, 1H), 8.11-8.07 (m, 1H), 8.03-7.98 (m, 1H), 7.78-7.75 (m, 1H), 7.68-7.66 (m, 1H), 7.62-7.60 (d, *J* = 7.2 Hz, 1H), 7.55-7.52 (m, 1H), 7.48-

7.44 (m, 4H), 7.39-7.30 (m, 4H), 6.96-6.93 (m, 1H), 6.68-6.77 (d, *J* = 8.4 Hz, 1H), 6.47 (m, 1H), 6.34-6.32 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H), 5.96 (br s, 1H), 5.59-5.56 (m, 5H), 5.52-5.45 (m, 7H), 5.38 (s, 2H), 5.13 (br s, 1H), 5.04-5.00 (d, *J* = 14.0 Hz, 1H), 4.81-4.69 (m, 10H), 4.60-4.51 (m, 5H), 4.36-4.11 (m, 5H), 3.98 (br s, 2H), 3.85-3.77 (m, 11H), 3.70-3.58 (m, 10H), 3.45-3.40 (m, 20H), 3.30-3.27 (m, 4H), 3.12-3.04 (m, 5H), 2.98-2.86 (m, 4H), 2.77-2.67 (m, 4H), 2.27-2.19 (m, 4H), 2.16-2.10 (m, 4H), 2.02-1.96 (m, 2H), 1.89-1.73 (m, 8H), 1.64-1.57 (m, 11H), 1.50-1.43 (m, 7H), 1.26-1.20 (m, 12H), 1.15-1.10 (m, 4H), 1.01-0.97 (m, 6H), 0.89-0.82 (m, 6H) ppm. *Anal. HPLC*: 100%, Retention time: 7.35 min (method B). Solubility: < 0.1 mg/mL water.

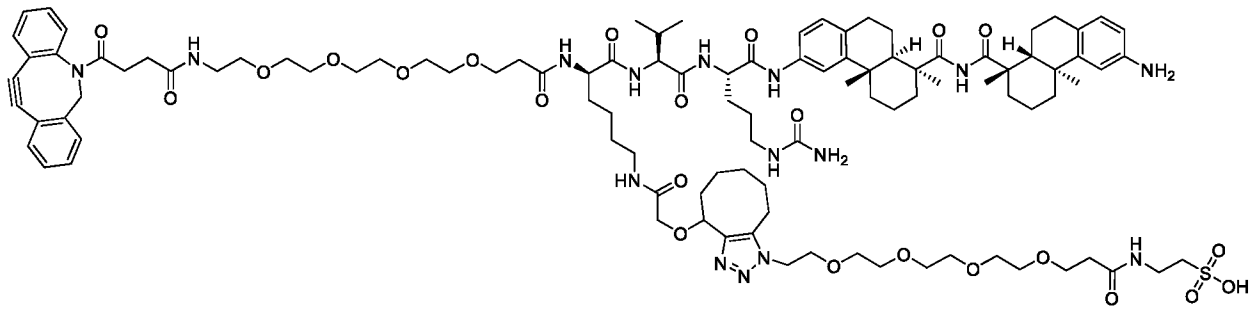
2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP3B)



[0558] Following the general procedure for Linker-payloads LP1-5, linker-payload **LP3B** (60 mg, 52% yield) was obtained as a white solid. ESI m/z: 642 (M/3 + H)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 0.6H), 9.25 (s, 0.4H), 8.22-8.07 (m, 3H), 8.02-7.86 (m, 2H), 7.77-7.72 (m, 2H), 7.68-7.60 (m, 2H), 7.54-7.52 (m, 1H), 7.50-7.42 (m, 4H), 7.39-7.27 (m, 4H), 7.22 (s, 1H), 7.08 (s, 1H), 6.97-6.94 (m, 2H), 6.74 (d, *J* = 10.5 Hz, 1H), 6.58 (s, 1H), 6.43 (dd, *J* = 10.0 Hz, 2.0 Hz, 1H), 5.62 (br s, 1H), 5.04-5.00 (m, 1H), 4.93-4.90 (m, 0.4H), 4.76-4.72 (m, 0.6H), 4.52-4.26 (m, 4H), 4.16-4.08 (m, 1H), 3.81-3.75 (m, 4H), 3.61-3.55 (m, 4H), 3.46-3.44 (m, 24H), 3.30-3.27 (m, 5H), 3.08-3.06 (m, 5H), 2.97-2.89 (m, 2H), 2.80-2.74 (m, 4H), 2.54-2.52 (m, 2H), 2.40-2.31 (m,

2H), 2.27-2.22 (m, 4H), 2.16-2.13 (m, 4H), 2.02-1.71 (m, 8H), 1.69-1.45 (m, 10H), 1.40-1.21 (m, 14H), 1.17-1.08 (m, 4H), 1.01-0.98 (m, 6H), 0.89-0.82 (m, 6H) ppm.

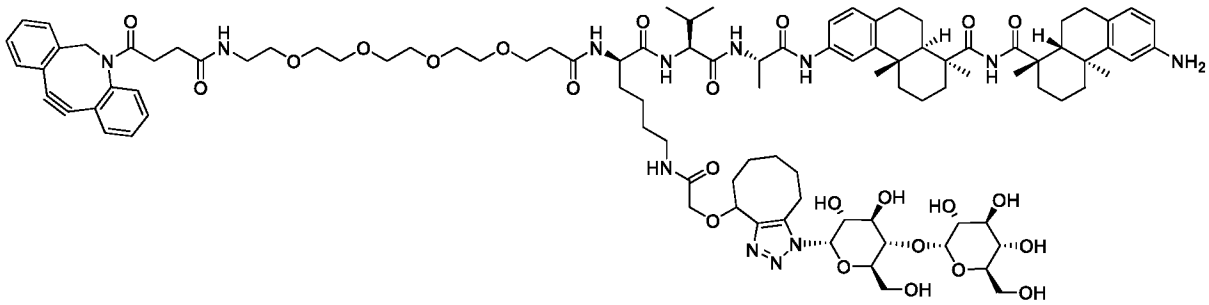
2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP4B)



[0559] Following the general procedure for Linker-payloads LP1-5, compound **LP4B** (6.0 mg, 20% yield) was obtained as a white solid. ESI m/z: 671 (M/3 + H)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1H), 9.29 (s, 1H), 8.24-8.22 (m, 1H), 8.18-8.17 (m, 2H), 8.09 (s, 2H), 8.01-7.99 (m, 1H), 7.97-7.94 (m, 2H), 7.89-7.84 (m, 1H), 7.76 (m, 5H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.55 (m, 2H), 7.51-7.43 (m, 7H), 7.39-7.28 (m, 7H), 7.08 (m, 4H), 6.96-6.93 (t, *J* = 7.0 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 2H), 6.48 (s, 2H), 6.35-6.33 (m, 2H), 5.97-5.95 (m, 2H), 5.37 (s, 4H), 5.04-5.01 (m, 2H), 4.74-4.69 (m, 5H), 4.52 (m, 1H), 4.42 (t, *J* = 5.5 Hz, 2H), 4.34-4.27 (m, 4H), 4.18 (m, 1H), 4.13-4.11 (m, 1H), 3.82-3.77 (m, 5H), 3.58-3.55 (m, 6H), 3.49-3.44 (m, 14H), 3.10-3.05 (m, 4H), 2.98-2.93 (m, 4H), 2.77-2.75 (m, 4H), 2.28-2.25 (m, 4H), 2.03-1.99 (m, 2H), 1.64-1.51 (m, 6H), 1.27-1.24 (m, 11H), 1.01-0.98 (m, 11H), 0.89-0.82 (m, 8H) ppm.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-[2-({1-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4-dihydroxy-6-(hydroxymethyl)-5-

{[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}oxan-2-yl]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]pentyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP5B)



[0560] Following the general procedure for Linker-payloads LP1-5, compound **LP5B** (15 mg, 27% yield) was obtained as a white solid. ESI m/z: 947 $(M/2 + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 9.69 (s, 0.55H), 9.27 (s, 0.45H), 8.25-8.15 (m, 2H), 8.15-8.05 (m, 2H), 8.05-7.90 (m, 1H), 7.90-7.80 (m, 1H), 7.76 (t, *J* = 6.4 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 6.8 Hz, 1H), 7.55-7.40 (m, 4H), 7.40-7.25 (m, 3H), 7.00-6.90 (m, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.47 (s, 1H), 6.32 (d, *J* = 8.0 Hz, 1H), 5.75-5.70 (m, 1H), 5.56 (d, *J* = 6.0 Hz, 1H), 5.50-5.40 (m, 2H), 5.20-4.92 (m, 4H), 4.80-4.70 (m, 1H), 4.70-4.65 (m, 2H), 4.65-4.55 (m, 2H), 4.40-4.25 (m, 2H), 4.20-3.95 (m, 2H), 3.80-3.75 (m, 2H), 3.75-3.45 (m, 23H), 3.30-3.20 (m, 3H), 3.20-2.90 (m, 5H), 2.90-2.65 (m, 3H), 2.60-2.55 (m, 1H), 2.40-1.95 (m, 12H), 1.90-1.70 (m, 5H), 1.70-1.35 (m, 14H), 1.35-1.20 (m, 15H), 1.15-1.05 (m, 3H), 1.00-0.90 (m, 7H), 0.90-0.80 (m, 7H) ppm.

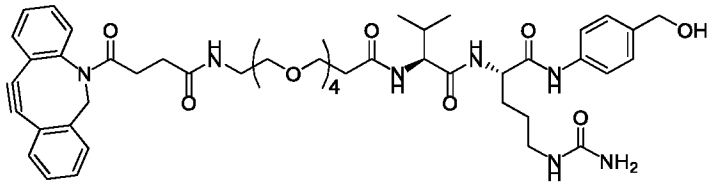
EXAMPLE 11-6

Linker-Payload LP6B

[0561] This example demonstrates methods for the synthesis of the linker-payload **LP6B** in Table 3, above. This example refers to the compounds numbered from **109** to **113** and linker-payload **LP6B** in FIG. 26.

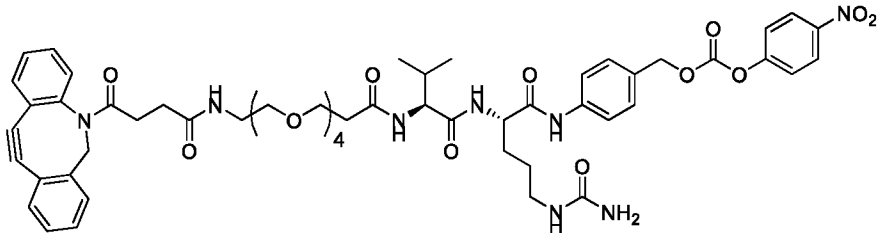
1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-

(hydroxymethyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide (110)



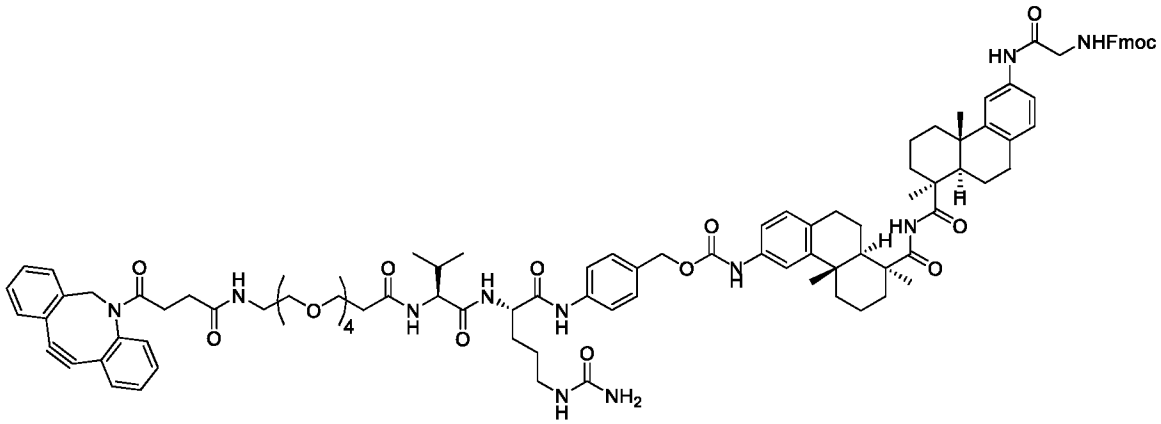
[0562] To a solution of compound **108** (0.30 g, 0.54 mmol) in DMF (10 mL) were added HATU (0.31 g, 0.81 mmol) and DIPEA (0.14 g, 1.1 mmol) successively at RT. The mixture was stirred at RT for 15 min. To the reaction solution was then added VC-PAB-OH **109** (CAS: 159857-79-1, 0.21 g, 0.54 mmol) at RT, and the resulting mixture was stirred at RT for 3 h until **108** or **109** were consumed, as monitored by LCMS. The reaction mixture was then filtered through a filtering membrane and the filtrate was concentrated and directly purified by reverse phase flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give compound **110** (0.30 g, 60% yield) as a white solid. ESI m/z: 617 $(M + H)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-nitrophenyl carbonate (112)



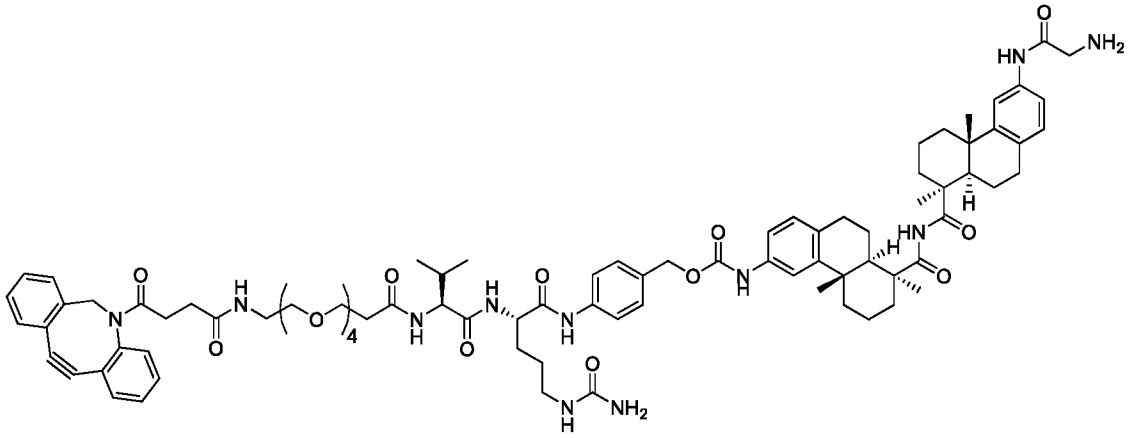
[0563] To a solution of compound **110** (0.15 g, 0.16 mmol) in DMF (10 mL) were added bis(4-nitrophenyl) carbonate **111** (0.15 g, 0.49 mmol) and DIPEA (63 mg, 0.49 mmol) successively at 0 °C. The mixture was then stirred at RT for 3 h until **110** was consumed, as monitored by LCMS. The reaction mixture was filtered through a filtering membrane and the filtrate was concentrated and directly purified by reverse phase flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give compound **112** (50 mg, 28% yield) as a white solid. ESI m/z: 1079 $(M + H)^+$.

9*H*-Fluoren-9-ylmethyl *N*-({[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (113)



[0564] To a mixture of compound **14a** (0.10 g, 0.12 mmol) and compound **112** (0.15 g, 0.14 mmol) in DMF (5 mL) were added HOBt (20 mg, 0.15 mmol) and DIPEA (48 mg, 0.37 mmol), and the mixture was stirred at RT for 4 h, which was monitored by LCMS. The reaction mixture was purified by prep-HPLC (method B) to give compound **113** (0.16 g, 72% yield) as a light yellow solid. ESI m/z: 874 $(M/2 + 1)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-(2-aminoacetamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (LP6B)



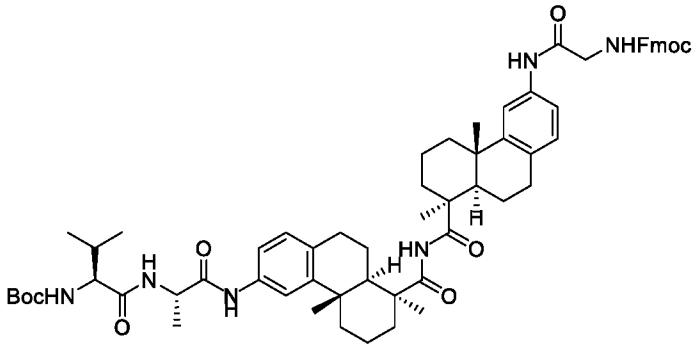
[0565] To a solution of compound **113** (0.10 g, 0.057 mmol) in DMF (5 mL) was added piperidine (1 mL) and the mixture was stirred at RT for half an hour until Fmoc was totally removed according to LCMS. The reaction mixture was directly purified by Prep-HPLC (method B) to give compound **LP6B** (35 mg, 23% yield) as a white solid. ESI m/z: 763 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 10.0 (s, 1H), 9.51 (s, 1H), 8.15-8.08 (m, 2H), 7.87 (d, J = 8.5 Hz, 1H), 7.76 (t, J = 5.0 Hz, 1H), 7.70-7.65 (m, 1H), 7.61 (t, J = 8.5 Hz, 3H), 7.51-7.42 (m, 4H), 7.41-7.30 (m, 8H), 7.20-7.10 (m, 1H), 7.00-6.90 (m, 2H), 6.00-5.95 (m, 1H), 5.40 (s, 2H), 5.35-5.30 (m, 1H), 5.10-5.00 (m, 3H), 4.40-4.35 (m, 1H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 14H), 3.25 (s, 3H), 3.10-3.00 (m, 4H), 3.00-2.85 (m, 4H), 2.80-2.70 (m, 2H), 2.63-2.61 (m, 1H), 2.60-2.55 (m, 1H),2.45-2.35 (m, 3H), 2.31-2.20 (m, 3H), 2.20-2.10 (m,4H), 2.10-1.92 (m, 5H), 1.90-1.82 (m, 4H), 1.68-1.53 (m, 6H), 1.50-1.40 (m, 2H), 1.20-1.10 (m, 2H), 1.02-0.96 (m, 6H), 0.90-0.80 (m, 8H) ppm.

EXAMPLE 11-7

Linker-Payload LP7B

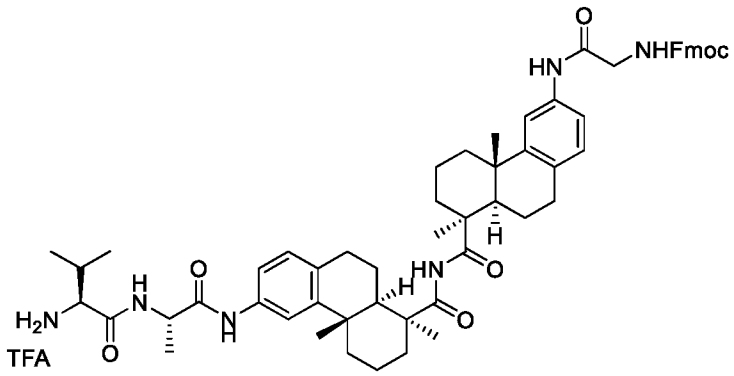
[0566] This example demonstrates methods for the synthesis of the linker-payload **LP7B** in Table 2, above. This example refers to the compounds numbered **14a**, **107**, **114** and **115** and linker-payload **LP7B** in FIG. 27.

9*H*-Fluoren-9-ylmethyl *N*-({[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (114)



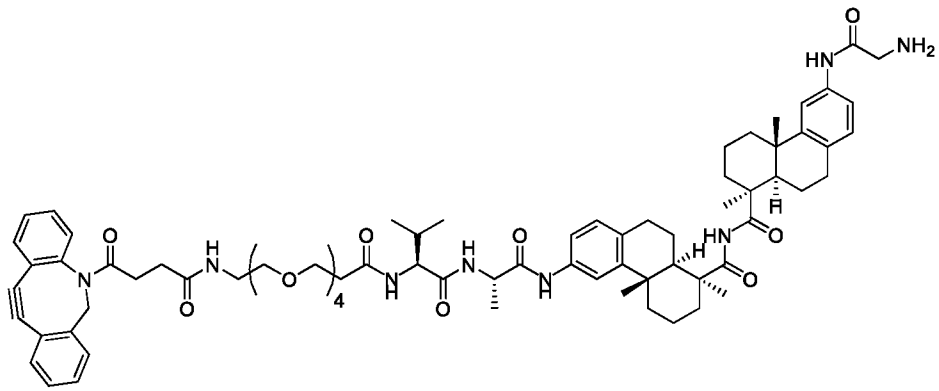
[0567] To a solution of compound **14a** (66 mg, 0.082 mmol) in DMF (10 mL) were added Boc-Val-Ala-OH **101c** (28mg, 0.098mmol), DIPEA (32 mg, 0.25 mmol) and HATU (47 mg, 0.12 mmol). The reaction mixture was stirred at RT for 4 h, and monitored by LCMS. The mixture was directly purified by reverse phase flash chromatography (50-90% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **114** (74 mg, 84% yield) as a white solid. ESI m/z: 978 $(M - Boc + 1)^+$.

9*H*-Fluoren-9-ylmethyl *N*-({[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate trifluoroacetic acid salt (115)



[0568] To a solution of compound **114** (74 mg, 0.069 mmol) in DCM (3 mL) was added TFA (1 mL). The reaction mixture was stirred at RT for an hour until Boc was totally removed according to LCMS. The volatiles were removed *in vacuo* to give crude product **115** (66 mg, 97% yield as TFA salt) as colorless oil. ESI m/z: 978 $(M + 1)^+$.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-(2-aminoacetamido)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP7B)



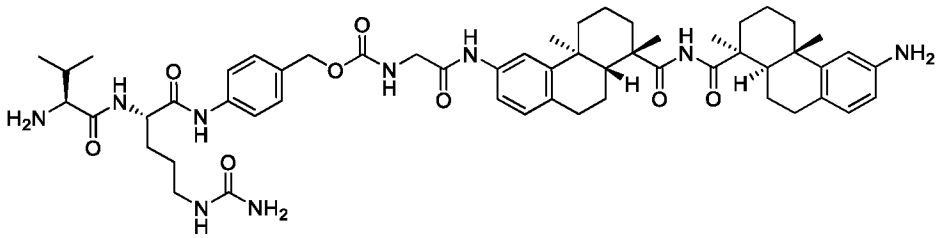
[0569] To a solution of compound **115** (60 mg, 61 μmol) in DMF (5 mL) was added DIBAC-suc-PEG4-OSu **107** (48 mg, 74 μmol) and DIPEA (24 mg, 0.18 mmol). The reaction mixture was stirred at RT for 4 h, and monitored by LCMS. To the reaction was then added piperidine (0.2 mL, excess). The reaction mixture was stirred at RT for 30 min until Fmoc was totally removed according to LCMS. The reaction mixture was directly purified by Prep-HPLC (method B) to give **LP7B** (22 mg, 28% yield) as a white solid. ESI m/z: 1292 $(M + H)^+$. ^{1}H NMR (500MHz, $DMSO_{d6}$) (with regioisomers) δ 9.66 (s, 0.5H), 9.51(s, 0.5H), 8.43 (d, J = 7.5 Hz, 0.5H), 8.15-8.10 (m, 1.5H), 8.05 (d, J = 7.5 Hz, 0.5H), 7.90 (d, J = 7.5 Hz, 0.5H), 7.77 (t, J = 5.5 Hz, 1H), 7.70-7.65 (m, 1H), 7.65-7.60 (m, 1H), 7.55-7.25 (m, 11H), 6.97 (d, J = 8.5 Hz, 2H), 5.02 (d, J = 14.5 Hz, 1H), 4.40-4.35 (m, 1H), 4.18 (t, J = 7.5 Hz, 0.5H), 4.04 (t, J = 7.5 Hz, 0.5H), 3.65-3.50 (m, 3H), 3.50-3.40 (m, 14H), 3.23 (s, 3H), 3.15-3.05 (m, 3H), 2.95-2.85 (m, 3H), 2.80-2.70 (m, 3H), 2.60-2.55 (m, 1H), 2.40-2.10 (m, 12H), 2.00-1.80 (m, 5H), 1.80-1.70 (m, 1H), 1.70-1.55 (m, 5H), 1.24 (s, 1H), 1.20-1.10 (m, 3H), 1.10-0.90 (m, 8H), 0.90-0.80 (m, 8H) ppm.

EXAMPLE 11-8

Linker-Payload LP8B

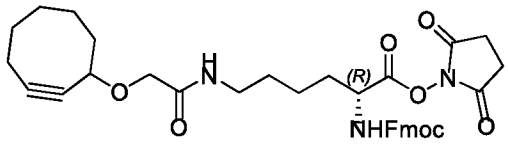
[0570] This example demonstrates methods for the synthesis of the linker-payload **LP8B** in Table 3, above. This example refers to the compounds numbered **P4B**, **117**-**120** and linker-payload **LP8B** in FIG. 28.

{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-({[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (117)



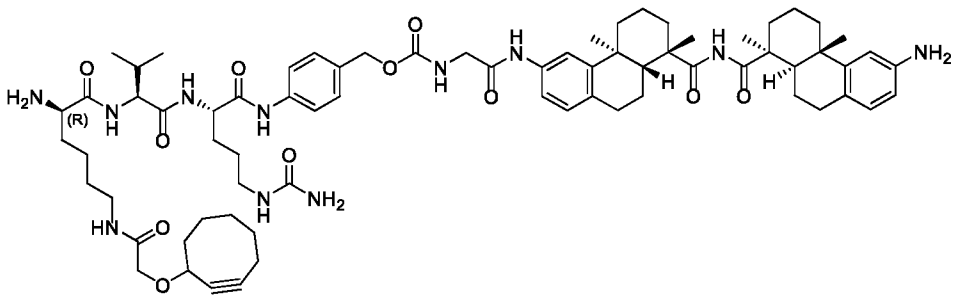
[0571] To a solution of Fmoc-vc-PAB-PNP **116** (0.14 g, 0.18 mmol) and payload **P4B** (0.11 g, 0.18 mmol) in DMF (2 mL) were added HOBt (24 mg, 0.18 mmol) and DIPEA (70 mg, 0.54 mmol) at RT by syringe. The mixture was stirred at RT for 2 h and compound **P4B** was consumed according to LCMS. To the resulting mixture was added piperidine (42 mg, 0.50 mmol) and the reaction was stirred at RT for 2 h until Fmoc was totally removed, as monitored by LCMS. After filtering through a membrane, the filtrate was concentrated and directly purified by prep-HPLC (method B) to give a compound **117** (45 mg, 27% yield) as a white solid. ESI m/z: 991 $(M + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.11 (s, 1H), 9.80 (s, 1H), 8.20-8.04 (m, 2H), 7.60 (d, J = 8.4 Hz, 2H), 7.52-7.44 (m, 2H), 7.36-7.20 (m, 3H), 6.96 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.2 Hz, 1H), 6.48 (d, J = 1.7 Hz, 1H), 6.36-6.29 (m, 1H), 5.98 (t, J = 5.6 Hz, 1H), 5.41 (s, 2H), 4.97 (s, 2H), 4.70 (s, 2H), 4.52-4.42 (m, 1H), 3.75 (d, J = 6.0 Hz, 2H), 3.07-2.85 (m, 4H), 2.82-2.61 (m, 3H), 2.31-2.06 (m, 6H), 2.00-1.78 (m, 6H), 1.72-1.52 (m, 6H), 1.44-1.09 (m, 13H), 0.99 (d, J = 8.5 Hz, 6H), 0.87 (d, J = 8.1 Hz, 3H), 0.78 (d, J = 6.8 Hz, 3H) ppm.

2,5-Dioxopyrrolidin-1-yl (2*R*)-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoate (118)



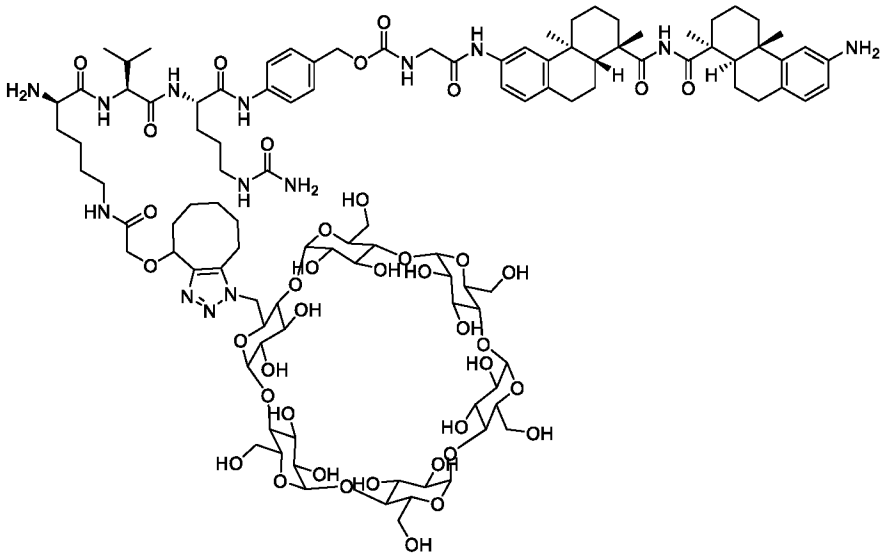
[0572] A mixture of compound **103** (0.10 g, 0.19 mmol), EDCI (72 mg, 0.38 mmol) and HOSu (43 mg, 0.38 mmol) in DCM (3 mL) was stirred at RT for 3 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (70% of ethyl acetate in petroleum ether) to give intermediate **118** (55 mg, 47% yield) as a white solid, which was used in the next step without purification. ESI m/z: 630 $(M + 1)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-({[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (119)



[0573] To a solution of compound **117** (55 mg, 56 μmol) and DIPEA (24 mg, 0.19 mmol) in DMF (1.5 mL) was added the crude intermediate **118** (40 mg, 63 μmol). The reaction mixture was stirred at RT for 2 h until **118** was consumed according to LCMS. The reaction mixture was directly purified by reverse phase flash chromatography (0-100% acetonitrile in water) to give **Fmoc-119** (60 mg, ESI m/z: 753 $(M/2 + 1)^+$) as a white solid, which was dissolved in DMF (1.5 mL). To the solution was added diethylamine (24 mg, 0.33 mmol) and the solution was stirred at RT for 2 h until Fmoc was totally removed according to LCMS. The reaction mixture was directly purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate) to give compound **119** (35 mg, 50% yield from compound **117**) as a white solid. ESI m/z: 1282 $(M + H)^+$.

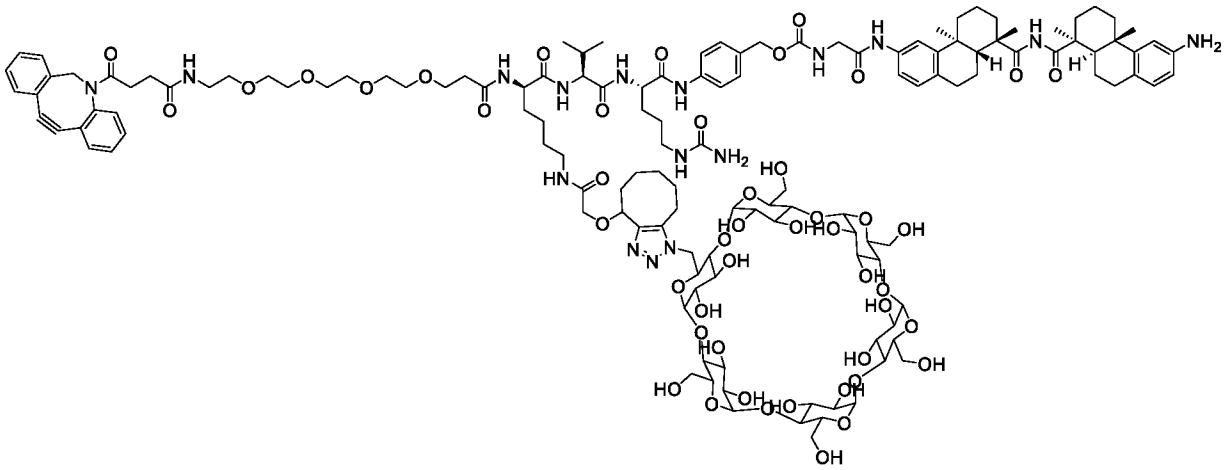
{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-({[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (120)



[0574] To a solution of compound **119** (70 mg, 54 μmol) in DMF (3 mL) was added α-CD-N_3 **105a** (0.16 g, 0.16 mmol). The reaction mixture was stirred at 50 °C for 3 days, which was monitored by LCMS. The resulting mixture was then directly purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM) to give compound **120** (20 mg, 16% yield) as a white solid. ESI m/z: 1141 $(M/2 + 1)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-({[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-

carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}methyl)carbamate (LP8B)



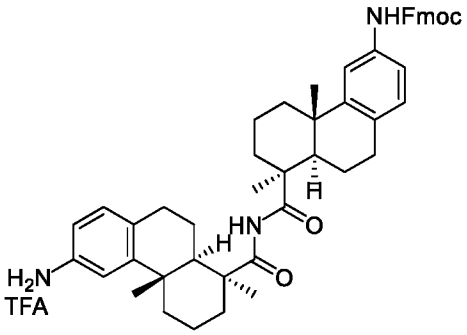
[0575] To a solution of compound **120** (10 mg, 4.4 μmol) and intermediate **107** (5 mg, 7.7 μmol) in DMF (2 mL) was added DIPEA (16 mg, 0.12 mmol) and the mixture was stirred at room temperature for 16 h. The reaction mixture was directly purified by prep-HPLC (method B) twice to give **LP8B** (1.5 mg, 12% yield) as a white solid. ESI m/z: 939 $(M/3 + H)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.87-9.66 (m, 1H), 8.22-8.08 (m, 4H), 7.91-7.76 (m, 2H), 7.71-7.61 (m, 4H), 7.56-7.45 (m, 4H), 7.40-7.28 (m, 5H), 6.96 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.48 (s, 1H), 6.34 (d, J = 8.2 Hz, 1H), 6.03-5.96 (m, 1H), 5.67-5.31 (m, 12H), 5.06-4.95 (m, 3H), 4.87-4.66 (m, 7H), 4.63-4.50 (m, 3H), 4.38-4.29 (m, 3H), 4.22-4.13 (m, 1H), 4.06-3.93 (m, 1H), 3.86-3.40 (m, 54H), 3.30-3.19 (m, 4H), 3.17-2.85 (m, 4H), 2.81-2.62 (m, 2H), 2.60-2.54 (m, 3H), 2.41-1.95 (m, 11H), 1.92-1.71 (m, 5H), 1.68-1.40 (m, 13H), 1.33-1.09 (m, 22H), 1.02-0.93 (m, 10H), 0.89-0.78 (m, 9H) ppm. *Anal. HPLC* (as a mixture of triazole regioisomers): 63%, Retention time: 6.03 min; 36%, Retention time: 6.13 min (method B).

EXAMPLE 11-9

Linker-Payload LP9B

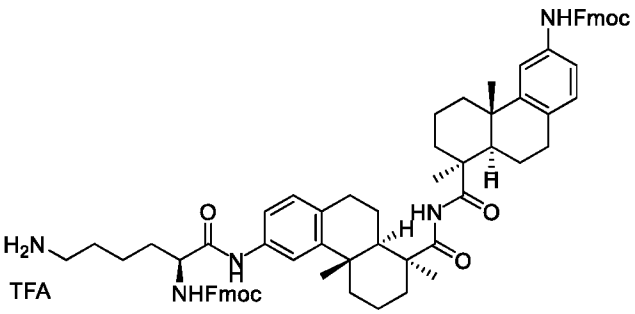
[0576] This example demonstrates methods for the synthesis of the linker-payload **LP9B** in Table 3, above. This example refers to the compounds numbered **12b**, **15**, **112**, **121**, and **122** and linker-payload **LP9B** in FIG. 29.

9*H*-Fluoren-9-ylmethyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate, trifluoroacetic acid salt (15)



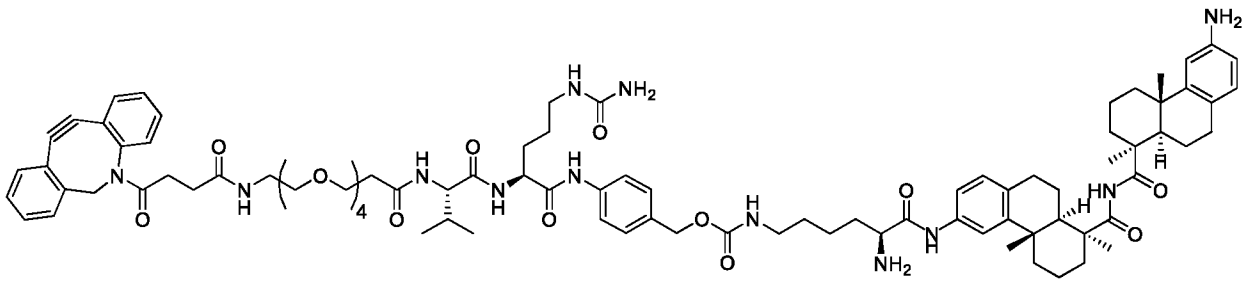
[0577] To a solution of compound **12b** (0.63 g, 1.0 mmol) in DCM (50 mL) were added Fmoc-OSu (0.40 g, 1.2 mmol) and DIPEA (0.26 g, 2.0 mmol). The mixture was stirred at RT for 16 h, which was monitored by LCMS. The mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (50-80% ethyl acetate in petroleum ether) to give **Boc-15** (0.71 g) as a white solid, which was dissolved in DCM (10 mL). To the solution was added TFA (3 mL) at RT. The reaction mixture was stirred at RT for 4 h until Boc was totally removed according to LCMS. The volatiles were removed *in vacuo* to give compound **15** as a TFA salt (0.62 g, 74% yield) and colorless oil. ESI m/z: 751 $(M + H)^+$.

9*H*-Fluoren-9-ylmethyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-6-amino-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate, trifluoroacetic acid salt (122)



[0578] To the solution of compound **15** (0.30 g, 0.40 mmol) in DMF (20 mL) were added Fmoc-Lys(Boc)-OH **121** (0.23 g, 0.48 mmol), HATU (228 mg, 0.60mmol) and DIPEA (0.16 g, 1.2 mmol) successively at RT. The reaction mixture was stirred at RT for 4 h, and monitored by LCMS. The resulting mixture was directly purified by reverse phase flash chromatography (50-90% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give **Boc-122** (0.41 g) as a white solid, 0.24 g of which was dissolved in DCM (20 mL). To the solution was added TFA (3 mL) and the reaction mixture was stirred at RT for an hour until Boc was totally removed according to LCMS. The volatiles were removed *in vacuo* to give compound **122** as a TFA salt (0.22 g, 79% yield) and colorless oil. ESI m/z: 1101 $(M + H)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(5*S*)-5-amino-5-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}pentyl]carbamate (LP9B)



[0579] To a mixture of compound **122** (14 mg, 12 μmol) and compound **112** (15 mg, 14 μmol) in DMF (5 mL) were added HOBt (2 mg, 15 μmol) and DIPEA (5 mg, 37 μmol), and the mixture was stirred at RT for 4 h, which was monitored by LCMS. To the reaction mixture was then added piperidine (0.5 mL) and the mixture was stirred at RT for 0.5 h until Fmoc was totally removed according to LCMS. The reaction mixture was then filtered through a membrane and the filtrate was concentrated and directly purified by prep-HPLC (method B) to give **LP9B** (6 mg, 31% yield) as a white solid. ESI m/z: 798 $(M/2 + H)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 10.0 (s, 1H), 8.15-8.08 (m, 2H), 7.87 (d, J = 8.5 Hz, 1H), 7.76 (t, J = 5.0 Hz, 1H), 7.67 (d, J = 7.0 Hz, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.60-7.56 (m, 2H), 7.51-7.42 (m, 4H), 7.41-7.24 (m, 6H), 7.20-7.16 (m, 1H), 6.95 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.50-6.46 (m, 1H), 6.36-6.32 (m, 1H),

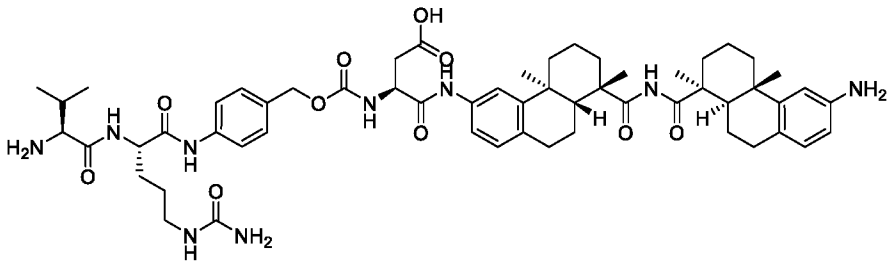
6.00-5.96 (m, 1H), 5.40 (s, 2H), 5.02 (d, *J* = 9.0 Hz, 1H), 4.91 (s, 2H), 4.69 (s, 2H), 4.40-4.35 (m, 1H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 14H), 3.24-3.20 (m, 2H), 3.10-3.05 (m, 2H), 3.00-2.92 (m, 3H), 2.92-2.85 (m, 1H), 2.80-2.70 (m, 2H), 2.65-2.55 (m, 2H), 2.45-2.35 (m, 2H), 2.31-2.20 (m, 4H), 2.20-2.10 (m,4H), 2.10-1.92 (m, 3H), 1.90-1.70 (m, 6H), 1.68-1.53 (m, 6H), 1.50-1.30 (m, 8H), 1.25-1.20 (m, 7H), 1.20-1.10 (m, 3H), 1.02-0.96 (m, 6H), 0.90-0.80 (m, 6H) ppm.

EXAMPLE 11-10

Linker-Payloads LP10B and LP11B

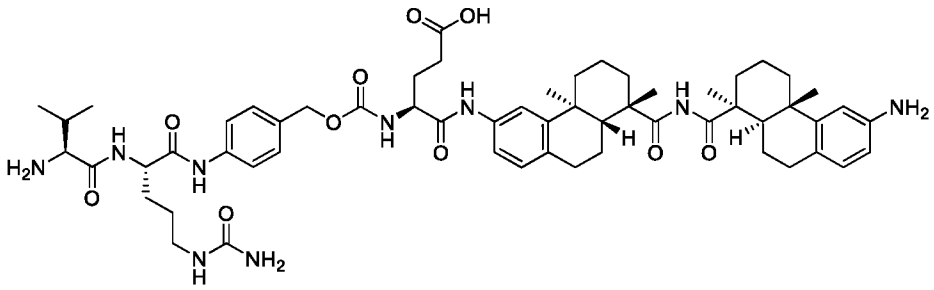
[0580] This example demonstrates methods for the synthesis of the linker-payloads **LP10B-LP11B** in Table 3, above. This example refers to the compounds numbered **P7B**, **P8B**, **116**, **123a-b**, linker-payloads **LP10B** and **LP11B** in FIG. 30.

(*S*)-4-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-3-((4-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyloxy)carbonylamino)-4-oxobutanoic acid (123a)



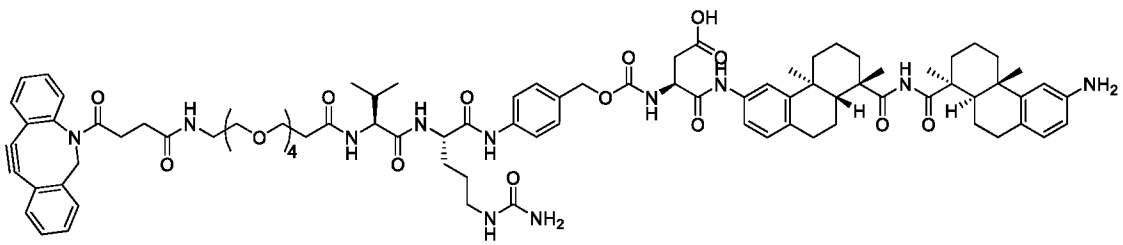
[0581] To a solution of payload **P7** (64 mg, 0.10 mmol) in DMF (5 mL) were added intermediate **116** (92 mg, 0.12 mmol) and DIPEA (26 mg, 0.20 mmol) successively at RT. The reaction mixture was stirred at RT for 4 h, and monitored by LCMS. To the mixture was then added piperidine (0.5 mL), and the reaction mixture was stirred at RT for 10 min until Fmoc was totally removed according to LCMS. The mixture was directly purified by reverse phase flash chromatography (40-70% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **123a** (41 mg, 39% yield) as a white solid. ESI m/z: 1049 $(M + H)^+$.

(*S*)-5-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-4-((4-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyloxy)carbonylamino)-5-oxopentanoic acid (123b)



[0582] Following a similar procedure for **123a** except substituting **P8** (0.53 g, 0.81 mmol) for **P7**, provides compound **123b** (0.61 g, 71% yield) as a white solid. ESI m/z: 1063 $(M + H)^+$.

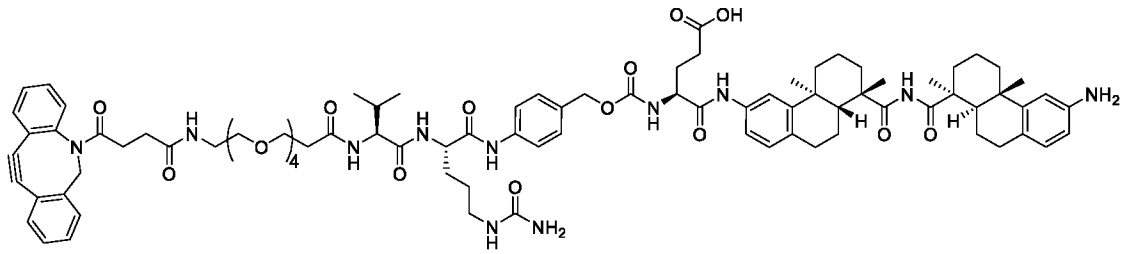
(3*S*)-3-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-3-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}propanoic acid (LP10B)



[0583] To a solution of compound **123** (21 mg, 20 μmol) in DMF (2 mL) was added intermediate **107** (16 mg, 24 μmol) and DIPEA (5 mg, 40 μmol) successively at RT. The reaction mixture was stirred at RT for 4 h, and monitored by LCMS. The resulting mixture was directly purified by Prep-HPLC (method B) to give compound **LP10B** (12 mg, 38% yield) as a white solid. ESI m/z: 792 $(M/2 + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.0 (s,1H), 8.14 (d, J = 7.2

Hz, 1H), 8.09 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.85-7.75 (m, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.65-7.55 (m, 3H), 7.55-7.40 (m, 4H), 7.40-7.25 (m, 5H), 6.95 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.05-5.95 (m, 1H), 5.43 (s, 2H), 5.05-4.90 (m, 3H), 4.80-4.60 (m, 1H), 4.40-4.35 (m, 2H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 25H), 3.20-2.85 (m, 4H), 2.80-2.55 (m, 3H), 2.40-2.30 (m, 3H), 2.30-2.20 (m, 3H), 2.20-2.05 (m, 4H), 2.0-1.50 (m, 12H), 1.50-1.30 (m, 3H), 1.30-1.20 (m, 6H), 1.20-1.05 (m, 2H), 1.05-0.90 (m, 5H), 0.90-0.80 (m, 6H) ppm.

(4*S*)-4-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-4-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}butanoic acid (LP11B)



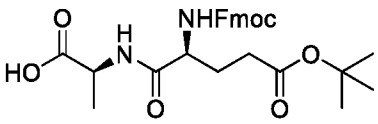
[0584] Following a similar procedure for **LP10B** except substituting **123b** (0.10 g, 94 μmol) for **123a**, provides compound **LP11B** (50 mg, 33% yield) as a white solid. ESI m/z: 799 (M/2 + H)$^+$. ^{1}H NMR (400 MHz, DMSO$_{d6}$) δ 10.0 (s, 1H), 9.91 (s, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.09 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.85-7.75 (m, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.65-7.55 (m, 3H), 7.55-7.40 (m, 4H), 7.40-7.25 (m, 6H), 6.95 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.05-5.95 (m, 1H), 5.43 (s, 2H), 5.05-4.90 (m, 3H), 4.40-4.35 (m, 2H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 4H), 3.50-3.40 (m, 25H), 3.20-2.85 (m, 4H), 2.80-2.55 (m, 3H), 2.40-2.30 (m, 3H), 2.30-2.20 (m, 3H), 2.20-2.05 (m, 4H), 2.00-1.50 (m, 12H), 1.50-1.30 (m, 3H), 1.30-1.20 (m, 6H), 1.20-1.05 (m, 2H), 1.05-0.90 (m, 5H), 0.90-0.80 (m, 6H) ppm.

EXAMPLE 11-11

Payload P12B and Linker-Payload LP12B

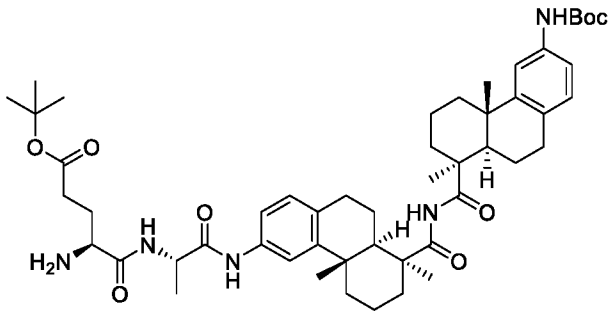
[0585] This example demonstrates methods for the synthesis of the payload **P12B** in Table C, below, and the linker-payload **LP12B** in Table C, above. This example refers to the compounds numbered **12b**, **107**, **124**, **125**, and **126** and payload **P12B** linker-payload **LP12B** in FIG. 31.

((*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(*tert*-butoxy)-5-oxopentanoyl)-*L*-alanine (125)



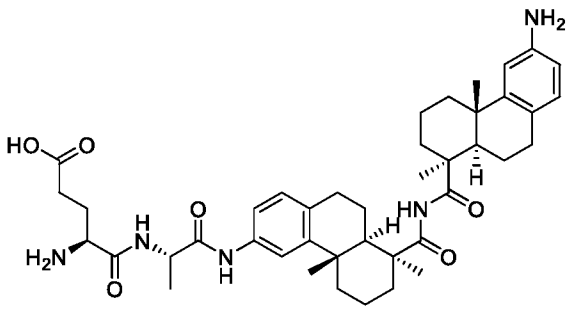
[0586] To a solution of Fmoc-Glu(OtBu)-OH (6.0 g, 14 mmol) in DCM (300 mL) were added HOSu (3.2 g, 28 mmol) and EDCI (5.4 g, 28 mmol). The reaction mixture was stirred at RT overnight, and monitored by LCMS. The resulting mixture was diluted with DCM (200 mL) and washed with water (100 mL x 2) and brine (100 mL). The organic solution was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to give crude compound **124** (8.5 g, ESI m/z: 545 $(M + 23)^+$), which was dissolved into DMF (10 mL). To the solution were added alanine (4.2 g, 48 mmol) and DIPEA (6.2 g, 48 mmol). The reaction mixture was stirred at RT overnight, and monitored by LCMS. The resulting mixture was poured into water (100 mL) and acidified with acetic acid to pH 5-6. The mixture was extracted with ethyl acetate and the combined organic solution was concentrated *in vacuo*. The crude product was purified by reverse phase flash chromatography (0-60% acetonitrile in *aq.* TFA (0.01%)) to give compound **125** (1 g, 15% yield) as a white solid. ESI m/z: 497 $(M + H)^+$.

(*S*)-*tert*-Butyl 4-amino-5-((*S*)-1-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-(*tert*-butoxycarbonylamino)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-1-oxopropan-2-ylamino)-5-oxopentanoate (126)



[0587] To a solution of compound **125** (42 mg, 84 μmol) and DIPEA (28 μl, 0.16 mmol) in DMF (3.0 mL) was added HATU (36 mg, 95 μmol). The reaction mixture was stirred at RT for 10 min before the addition of compound **12b** (50 mg, 80 μmol), and the mixture was stirred at RT overnight, which was monitored by LCMS. To the reaction mixture was then added piperidine (1.0 mL) and the mixture was stirred at RT for 3 h until Fmoc was totally removed according to LCMS. The resulting mixture was directly purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.01%)) to give compound **126** (20 mg, 28% yield) as a yellow solid. ESI m/z: 885 $(M + H)^+$.

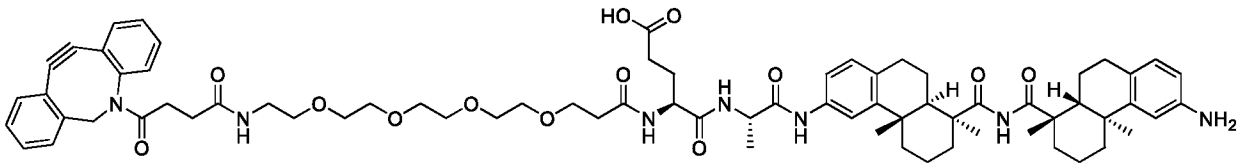
(*S*)-4-Amino-5-((*S*)-1-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-1-oxopropan-2-ylamino)-5-oxopentanoic acid (P12B)



[0588] A mixture of compound **126** (57 mg, 64 μmol) in neat TFA (2.0 mL) was stirred at RT for an hour, and monitored by LCMS. The resulting mixture was diluted with DCM (20 mL) and concentrated *in vacuo*. The residue was purified by reverse phase flash chromatography (0-100% acetonitrile in *aq.* sodium bicarbonate (10 mM)) to give **P12B** (20 mg, 43% yield) as a

white solid. ESI m/z: 365 (M/2 + H)$^+$. ^{1}H NMR (400 MHz, DMSO$_{d6}$) δ 9.90 (s, 1H), 8.30 (br s, 1H), 8.09 (s, 1H), 7.50 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.46(s, 1H), 6.33 (dd, J = 7.6 Hz and 2.0 Hz, 1H), 4.70 (br s, 1H), 4.41-4.36 (m, 1H), 3.42-3.30 (m, 6H), 2.91-2.85 (m, 1H), 2.78-2.75 (m, 2H), 2.67-2.61 (m, 1H), 2.32-2.27 (m, 4H), 2.16-2.13 (m, 4H), 1.88-1.77 (m, 4H), 1.65-1.58 (m, 4H), 1.29-1.23 (m, 12H), 1.14-1.11 (m, 2H), 1.01 (d, J = 7.6 Hz, 6H) ppm.

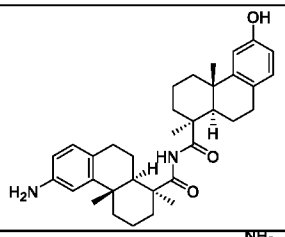
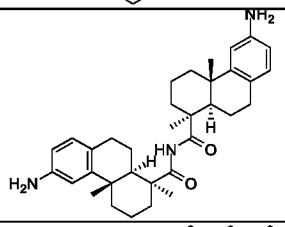
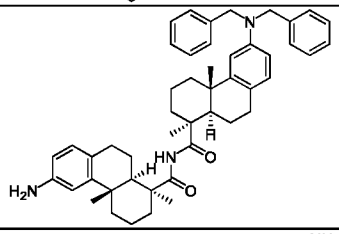
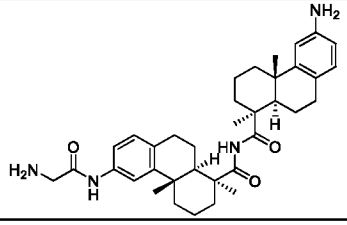
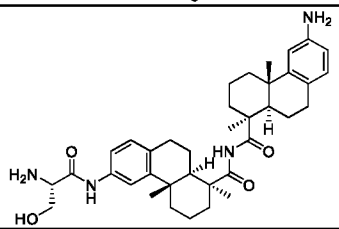
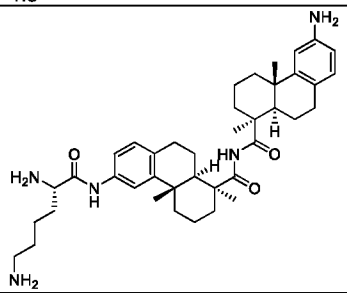
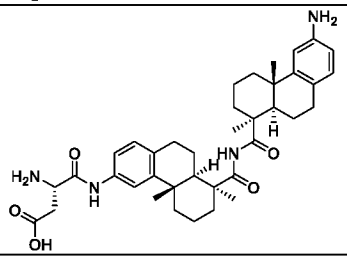
(4*S*)-4-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}butanoic acid (LP12B)

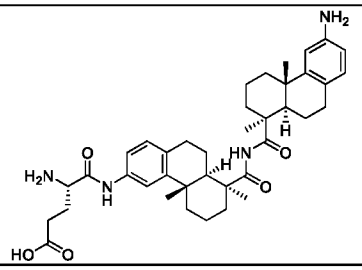
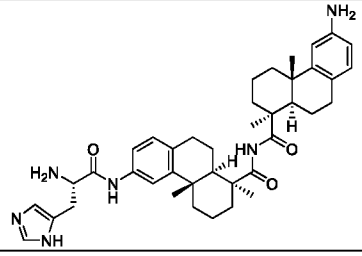
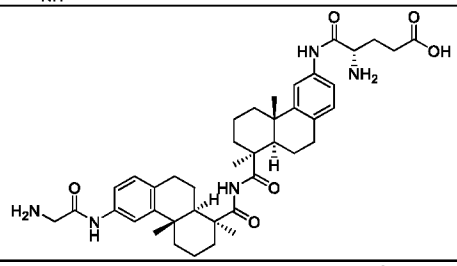
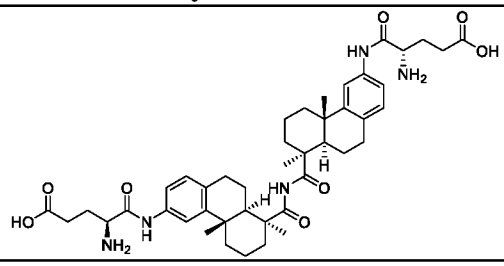
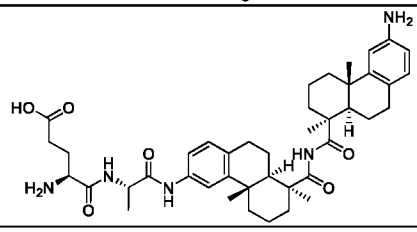


[0589] To a solution of compound **P12B** (10 mg, 14 μmol) in DMF (2.0 ml) were added DIPEA (6 μl, 21 μmol) and compound **107** (11 mg, 16 μmol). The reaction mixture was stirred at RT for 3 h, and monitored by LCMS. The resulting mixture was directly purified by Prep-HPLC (method B) to give compound **LP12B** (3.5 mg, 20% yield) as a white solid. ESI m/z: 632 (M/2 + H)$^+$. ^{1}H NMR (400 MHz, DMSO$_{d6}$) δ 9.64 (s, 1H), 9.49 (s, 1H), 8.41-8.39 (m, 1H), 8.12-8.09 (m, 1H), 7.79 (br s, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.60 (br s, 1H), 7.52-7.27 (m, 8H), 6.95 (d, J = 8.4 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 6.47 (s, 1H), 6.33 (dd, J = 8.4 Hz and 1.6 Hz, 1H), 5.02 (d, J = 14.0 Hz, 1H), 4.70-4.67 (br s, 1H), 4.36-4.30 (m, 1H), 4.21-4.14 (m, 1H), 3.61-3.58 (m, 3H), 3.46-3.45 (m, 15H), 3.08-3.06 (m, 2H), 2.90-2.55 (m, 5H), 2.42-2.09 (m, 12H), 1.98-1.73 (m, 7H), 1.65-1.54 (m, 3H), 1.29-1.25 (m, 12H), 1.17-1.11 (m, 2H), 0.99-0.97 (m, 6H) ppm.

Table C. LXR modulator payloads

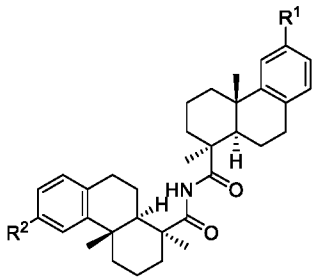
Cpd code	Structures	MF	FW	Exact Mass

Cpd code	Structures	MF	FW	Exact Mass
P1		$C_{34}H_{44}N_2O_3$	528.72	528.72
P2B		$C_{34}H_{45}N_3O_2$	527.74	527.74
P3B		$C_{48}H_{57}N_3O_2$	707.99	707.45
P4B		$C_{36}H_{48}N_4O_3$	584.79	584.37
P5B		$C_{37}H_{50}N_4O_4$	614.82	614.38
P6B		$C_{40}H_{57}N_5O_3$	655.91	655.45
P7B		$C_{38}H_{50}N_4O_5$	642.83	642.38

Cpd code	Structures	MF	FW	Exact Mass
P8B		$C_{39}H_{52}N_4O_5$	656.85	656.39
P9B		$C_{40}H_{52}N_6O_3$	664.88	664.41
P10B		$C_{41}H_{55}N_5O_6$	713.91	713.42
P11B		$C_{44}H_{59}N_5O_8$	785.97	785.44
P12B		$C_{42}H_{57}N_5O_6$	727.93	727.43

[0590] The structures, calculated LogP values, MS and HPLC results for the above payload compounds are summarized in Table D.

Table D Chemical-Physical Properties of Payloads



Cpd	R1	R2	cLogP	MS (M+H)	HPLC	
					Purity (%)	RT (min)
P1	OH	NH_2	+++	529.3	95	8.66
P2B	NH_2	NH_2	+++	528.2	95	9.11
P3B	NH_2	NBn_2	+++	354.8 (M/2+H)	95	8.87
P4B	NH_2	$NHC(O)CH_2NH_2$ (Gly)	++	585.4	98	8.20
P5B	NH_2	NHC(O)(S)-$CH(CH_2OH)NH_2$ (Ser)	++	615.4	100	7.86
P6B	NH_2	$NHC(O)CH((CH_2)_4NH_2)NH_2$ (Lys)	+++	656.5	100	8.89
P7B	NH_2	$NHC(O)CH(CH_2CO_2H)NH_2$ (Asp)	+	643.4	100	6.64
P8B	NH_2	$NHC(O)CH(CH_2CH_2COOH)NH_2$ (Glu)	+	657.4	97	6.69
P9B	NH_2	NHC(O)CH(CH_2-imidazole)NH_2 (His)	++	665.3	97	5.94
P10B	$NHC(O)CH_2NH_2$ (Gly)	$NHC(O)CH(CH_2CH_2COOH)NH_2$ (Glu)	+	714.5	100	6.12
P11B	$NHC(O)CH(CH_2CH_2COOH)NH_2$ (Glu)	$NHC(O)CH(CH_2CH_2COOH)NH_2$ (Glu)	+	393.8 (M/2+H)	100	5.45
P12B	NH_2	$NHC(O)CH(CH_3)NHC(O)CH(CH_2CH_2COOH)NH_2$ (AlaGlu)	+	728.5	100	6.61, 6.67

6 < +++ < 12; 4 < ++ < 6; 0 < + < 4

[0591] The molecular formulae, molecular weights, calculated LogP values, MS, and HPLC results for the above linker-payload compounds are summarized in Table E.

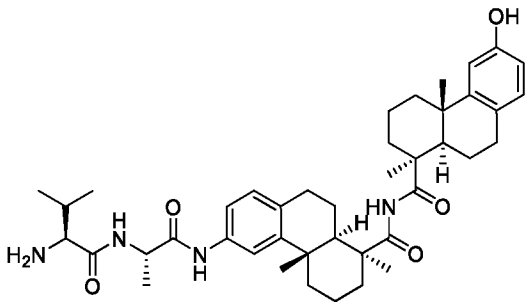
Table E Chemical-Physical Properties of Payloads

Cpd	MF	MW	cLogP	Purity (%)	MS m/z (100%)	highest MS m/z	HPLC RT (min)
LP1	$C_{124}H_{176}N_{12}O_{43}$	2522.81	+	95	841.7 [M/3+H]	1262.0 [M/2+H]	7.93 (B)
LP2B	$C_{127}H_{182}N_{14}O_{44}$	2608.87	+	100	870.3 [M/3+H]	870.3 [M/3+H]	7.35 (B)
LP3B	$C_{101}H_{143}N_{13}O_{22}S$	1923.36	+++	100	641.8 [M/3+H]	962.5 [M/2+H]	6.20 (B)
LP4B	$C_{104}H_{149}N_{15}O_{23}S$	2009.45	++	99	670.5 [M/3+H]	1004.9 [M/2+H]	5.94 (B)
LP5B	$C_{100}H_{138}N_{12}O_{24}$	1892.23	++	95	631.5 [M/3+H]	946.6 [M/2+H]	6.94 (B)
LP6B	$C_{85}H_{109}N_{11}O_{15}$	1524.84	+++	96	763.0 [M/2+H]	763.0 [M/2+H]	8.63 (B)
LP7B	$C_{74}H_{96}N_8O_{12}$	1289.60	+++	97	645.4 [M/2+H]	1290.7 [M+H] (23%)	8.67 (B)
LP8B	$C_{137}H_{192}N_{16}O_{47}$	2815.07	+	99	939.2 [M/2+H]	939.2 [M/2+H]	7.60 (B)
LP9B	$C_{89}H_{118}N_{12}O_{15}$	1595.96	+++	100	798.3 [M/2+H]	798.3 [M/2+H]	8.58 (B)
LP10B	$C_{87}H_{111}N_{11}O_{17}$	1582.88	+++	97	528.4 [M/3+H]	792.0 [M/2+H]	7.11 (B)
LP11B	$C_{88}H_{113}N_{11}O_{17}$	1596.90	+++	96	799.2 [M/2+H]	799.2 [M/2+H]	7.10 (B)
LP12B	$C_{72}H_{91}N_7O_{13}$	1262.53	+++	100	631.8 [M/2+H]	631.8 [M/2+H]	7.51 (B)

6 < +++ < 12; 4 < ++ < 6; -2 < + < 4

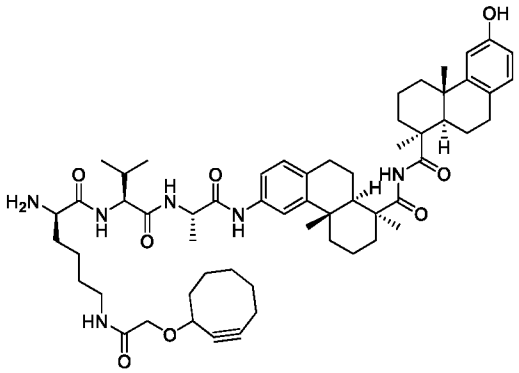
Example 12. Synthesis of LP1 (FIG. 2)

(1*S*,4a*S*,10a*R*)-6-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)propanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (LP1-2)



[0592] To a solution of **P1** (53 mg, 0.10 mmol) in DMF (1 mL) were added Fmoc-Val-Ala-OH (41 mg, 0.10 mmol), HATU (38 mg, 0.1 mmol) and diisopropylethylamine (26 mg, 0.20 mmol) successively. After stirring at 25 °C for 24 hours until **P1** was consumed according to LCMS, the mixture was added piperidine (0.1 mL) and the resulting solution was stirred at 25 °C for another 3 hours. After filtration, the filtrate was directly purified by prep-HPLC (method B) to give compound **LP1-2** (45 mg, 64% yield) as a white solid. ESI m/z: 699 $(M + 1)^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 8.40 (s, 1H), 7.47 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.56 (dd, J = 8.3, 2.4 Hz, 1H), 4.60-4.48 (m, 1H), 3.22-3.11 (m, 1H), 3.02-2.93 (m, 1H), 2.92-2.76 (m, 3H), 2.74-2.70 (m, 1H), 2.43-2.31 (m, 3H), 2.28 (d, J = 14.1 Hz, 3H), 2.16-1.96 (m, 3H), 1.81 (s, 1H), 1.78-1.65 (m, 4H), 1.53-1.42 (m, 4H), 1.38 (d, J = 5.3 Hz, 6H), 1.33-1.22 (m, 2H), 1.14 (d, J = 6.6 Hz, 6H), 1.09 (d, J = 18.6 Hz, 6H) ppm.

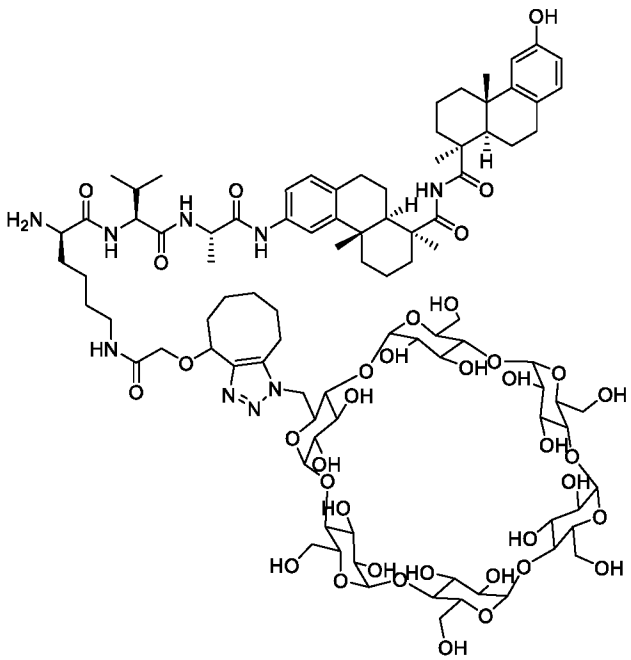
(1*S*,4a*S*,10a*R*)-6-((2*S*)-2-((2*S*)-2-((2*R*)-2-Amino-6-(2-(cyclooct-2-ynyloxy)acetamido)hexanamido)-3-methylbutanamido)propanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (LP1-4)



[0593] To a solution of compound **LP1-3** (35 mg, 0.064 mmol) in DMF (1 mL) were added HATU (24 mg, 0.064 mmol) and compound **LP1-2** (45 mg, 0.064 mmol) in succession at room temperature. The mixture was stirred for a few minutes at room temperature until the mixture was homogenous. To this mixture was added diisopropylethylamine (41 mg, 0.32 mmol) at room temperature by syringe. The resulting mixture was stirred at room temperature overnight

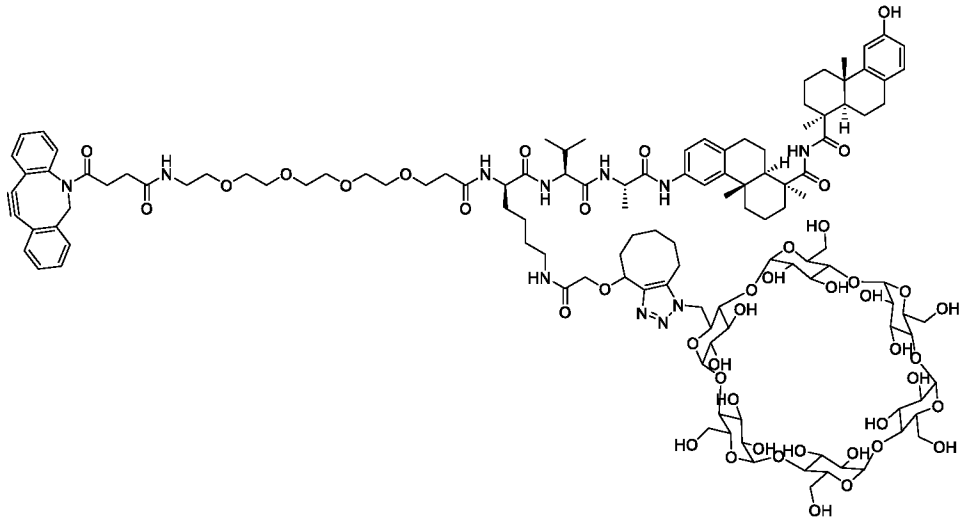
(16 hours) until **LP1-2** was mostly consumed according to LCMS. To this reaction mixture was then added piperidine (0.1 mL, excess) dropwise at room temperature and the mixture was stirred for another 3 hours until Fmoc was removed, as monitored by LCMS. The reaction mixture was directly purified by reversed phase flash chromatography or prep-HPLC (method B, basic condition) to give compound **LP1-4** (30 mg, 47% yield) as a white solid. ESI m/z: 991 (M + 1)$^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 7.51 (d, J = 1.5 Hz, 1H), 7.37 (dd, J = 8.3, 2.0 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.56 (dd, J = 8.3, 2.5 Hz, 1H), 4.64-4.57 (m, 1H), 4.48 (q, J = 7.1 Hz, 1H), 4.33-4.27 (m, 1H), 4.20 (d, J = 6.7 Hz, 1H), 3.93 (m, 2H), 3.43 (t, J = 6.6 Hz, 1H), 3.24 (t, J = 6.9 Hz, 2H), 3.02-2.93 (m, 2H), 2.92-2.76 (m, 3H), 2.40-2.32 (m, 2H), 2.33-2.23 (m, 4H), 2.22-2.12 (m, 3H), 2.12-2.00 (m, 5H), 1.99-1.91 (m, 1H), 1.91-1.81 (m, 2H), 1.78-1.66 (m, 6H), 1.66-1.58 (m, 1H), 1.58-1.49 (m, 2H), 1.45 (d, J = 7.1 Hz, 6H), 1.38 (d, J = 4.0 Hz, 6H), 1.34-1.22 (m, 4H), 1.14 (d, J = 7.0 Hz, 6H), 1.06-0.98 (m, 6H) ppm.

(1*S*,4a*S*,10a*R*)-*N*-{[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (LP1-5)



[0594] To a solution of compound **LP1-4** (30 mg, 30 μmol) in DMF (0.5 mL) was added a solution of CD-N_3 (60 mg, 60 μmol) in DMF (0.5 mL) at room temperature by syringe. The mixture was stirred at 20-25 °C for 3 days. Compound **LP1-4** was mostly consumed according to LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP1-5** (14 mg, 23% yield) as a white solid. ESI m/z: 995 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 8.40 (s, 1H), 7.56-7.52 (m, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.56 (dd, J = 8.2, 2.3 Hz, 1H), 5.24-5.16 (m, 1H), 5.01-4.95 (m, 6H), 4.65-4.59 (m, 1H), 4.52-4.44 (m, 1H), 4.31-4.22 (m, 2H), 4.13-3.73 (m, 22H), 3.63-3.43 (m, 14H), 3.14-2.72 (m, 7H), 2.45-2.32 (m, 3H), 2.28 (d, J = 13.8 Hz, 3H), 2.22-1.85 (m, 11H), 1.82-1.59 (m, 9H), 1.55-1.41 (m, 8H), 1.38 (d, J = 5.3 Hz, 6H), 1.31-1.26 (m, 3H), 1.14 (d, J = 7.2 Hz, 6H), 1.06-0.93 (m, 6H) ppm.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}pentyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP1)

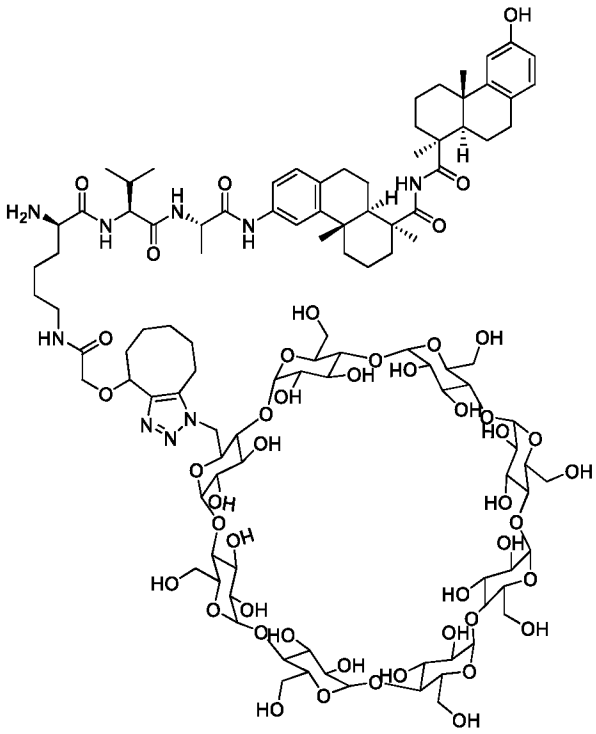


[0595] To a solution of compound **LP1-5** (14 mg, 7.4 μmol) and DIBAC-Suc-PEG$_4$-OSu (6.5 mg, 10 μmol) in DMF (1mL) was added triethylamine (2.0 mg, 20 μmol) and the mixture was stirred at 20-25 °C for 16 hours. Most of the volatiles were removed in vacuo and the residue was purified by prep-HPLC (method B) to give compound **LP1** (5.0 mg, 27% yield) as a white

solid. ESI m/z: 1261 (M/2 + 1)$^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 7.69-7.44 (m, 6H), 7.41-7.30 (m, 3H), 7.26 (d, *J* = 6.8 Hz, 1H), 7.04-6.96 (m, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 1.9 Hz, 1H), 6.56 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.25-5.18 (m, 1H), 5.17-5.08 (m, 1H), 5.01-4.94 (m, 4H), 4.61 (s, 16H), 4.53-4.13 (m, 5H), 4.03-3.80 (m, 18H), 3.74-3.64 (m, 3H), 3.63-3.41 (m, 23H), 3.28-2.76 (m, 12H), 2.76-2.65 (m, 1H), 2.56-2.44 (m, 2H), 2.42-2.31 (m, 4H), 2.30-2.23 (m,4H), 2.18-1.92 (m, 9H), 1.79-1.55 (m, 9H), 1.49-1.34 (m, 9H), 1.33-1.22 (m, 3H), 1.18-1.10 (m, 6H), 1.06-0.94 (m, 6H) ppm.

Example 13. Synthesis of LP2 (FIG. 3)

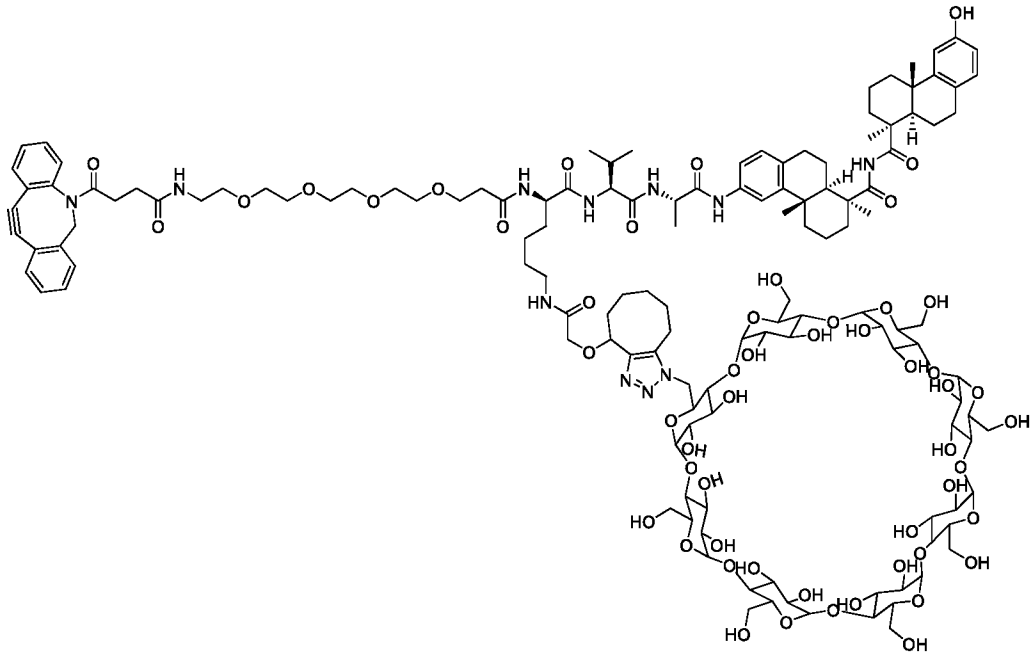
(1*S*,4a*S*,10a*R*)-*N*-{[(1*S*,4a*S*,10a*R*)-6-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56-hexadecahydroxy-10,15,20,25,30,35,40-heptakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29,32,34,37,39-hexadecaoxanonacyclo[36.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}.2^{28,31}.2^{33,36}]hexapentacontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]propanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]carbonyl}-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (LP2-5)



[0596] To a solution of compound **LP1-4** (30 mg, 0.030 mmol) in DMF (2 mL) was added γCD-N_3 (0.12 mg, 0.091 mmol). The mixture was stirred at RT for 16 hours, which was monitored by LCMS. The mixture was filtered through membrane and the filtrate was then purified by prep-

HPLC (method A) to give compound **LP2-5** (40 mg, 57% yield) as a white solid. ESI m/z: 1157.6 $(M/2 + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 9.82 (s, 1H), 9.00 (s, 1H), 8.55 (d, J = 9.0 Hz, 1H), 8.39 (d, J = 6.5 Hz, 1H), 8.11-8.04 (m, 4H), 7.93-7.88 (m, 1H), 7.45 (s, 1H), 7.35 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.63 (s, 1H), 6.50 (d, J = 11 Hz, 1H), 5.89-5.68 (m, 16H), 5.16-4.32 (m, 19H), 3.94-3.80 (m, 4H), 3.69-3.51 (m, 50H), 3.18-2.64 (m, 8H), 2.33-1.85 (m, 12H), 1.65-1.11 (m, 25H), 0.99 (d, J = 9.5 Hz, 6H), 0.89-0.83 (m, 6H) ppm.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-{2-[(1-{[41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56-hexadecahydroxy-10,15,20,25,30,35,40-heptakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29,32,34,37,39-hexadecaoxanonacyclo[36.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}.2^{28,31}.2^{33,36}]hexapentacontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}pentyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP2)

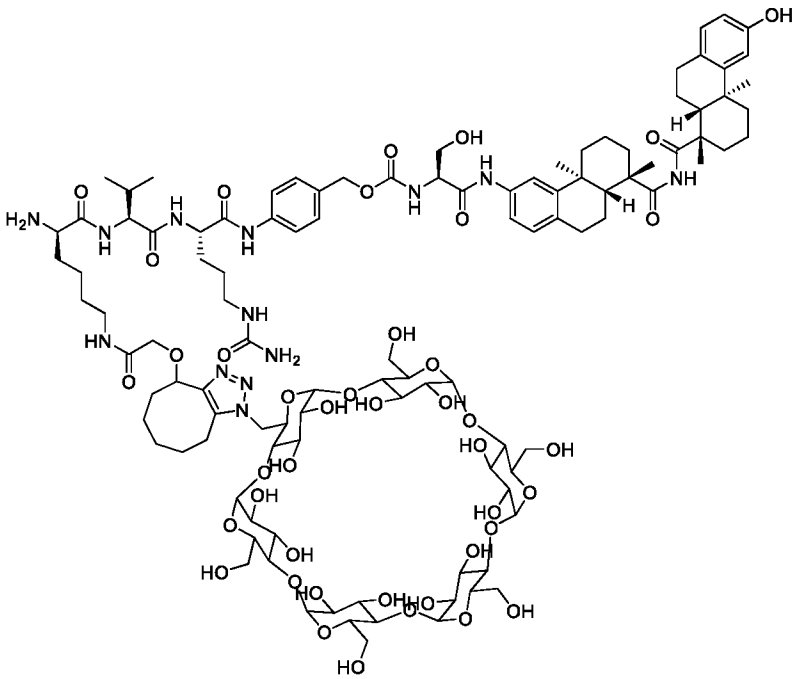


[0597] To a solution of compound **LP2-6** (4.3 mg, 7.8 μmol) in anhydrous DMF (1 mL) was added HATU (3.0 mg, 7.8 μmol). The mixture was stirred at 10 °C for 10 minutes before compound **LP2-5** (15 mg, 6.5 μmol) and DIPEA (1.7 mg, 13 μmol) was added. The mixture was stirred at RT for 2 hours until **LP2-5** was totally consumed, as monitored by LCMS. The mixture was filtered through a membrane and the filtrate was purified by prep-HPLC (method B) to give compound **LP3** (6.0 mg, 32% yield) as a white solid. ESI m/z: 1424.2 $(M/2 + 1)^+$. ^1H NMR (500

MHz, $DMSO_{d6}$) δ 9.26 (s, 1H), 8.95 (s, 1H), 8.24-7.98 (m, 4H), 7.81 (d, *J* = 5.6 Hz, 1H), 7.72 (t, *J* = 5.5 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.55 (s, 1H), 7.53-7.25 (m, 7H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 2.1 Hz, 1H), 6.50 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.94-5.58 (m, 15H), 5.39-4.43 (m, 17H), 4.37-4.24 (m, 3H), 4.13-4.08 (m, 1H), 3.98-3.33 (m, 52H), 3.26-2.52 (m, 18H), 2.40-1.18 (m, 48H), 1.18-0.63 (m, 19H) ppm. Solubility: 0.075 mg/mL H_2O.

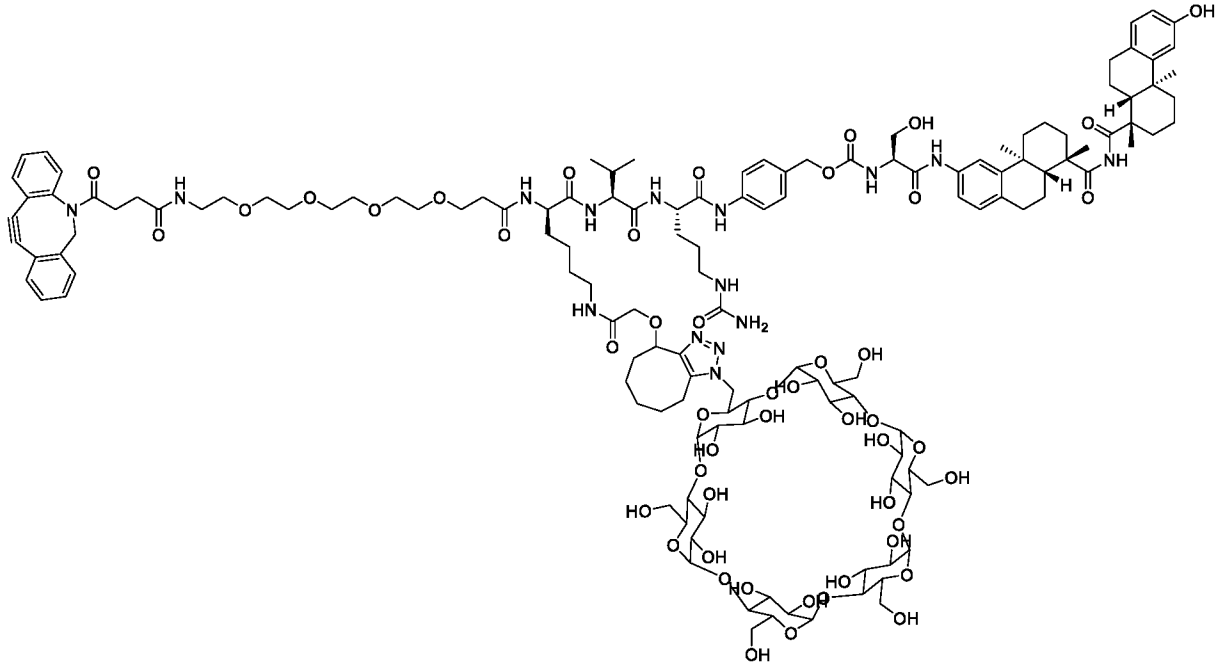
Example 14. Synthesis of LP5 (FIG. 4)

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.$2^{3,6}$.$2^{8,11}$.$2^{13,16}$.$2^{18,21}$.$2^{23,26}$]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (LP5-1)



[0598] To a solution of compound **LP4** (20 mg, 15 μmol) in DMF (1 mL) was added a solution of CD-N_3 (46 mg, 45 μmol) in acetonitrile (2 mL) and water (2 mL) at RT. The mixture was stirred at 30 °C for 16 hours. Compound **LP4** was mostly consumed according to LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP5-1** (20 mg, 57% yield) as a white solid. ESI m/z: 1156.0 $(M/2 + 1)^+$.

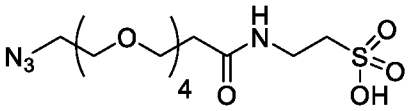
{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (LP5)



[0599] To a solution of DIBAC-PEG$_4$-acid **LP5-2** (4.3 mg, 7.8 μmol) in DMF (1 mL) were added HATU (3.0 mg, 3.6 μmol) and DIPEA (1.7 mg, 13 μmol) at RT. The resulting mixture was stirred at RT for 10 minutes. To the mixture was then added **LP5-1** (15 mg, 6.5 μmol). The reaction mixture was stirred at 30 °C for 2 hours until the reaction completed, as monitored by LCMS. The reaction mixture was filtered and purified by prep-HPLC (method B) to give **LP5** (10 mg, 42% yield) as a white solid. ESI m/z: 1424.3 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 9.81-9.67 (m, 2H), 8.99 (s, 1H), 8.20-8.06 (m, 5H), 7.85-7.22 (m, 18H), 6.97-6.49 (m, 2H), 5.98 (s, 1H), 5.65-5.33 (m, 15H), 5.14-4.92 (m, 5H), 4.82-4.72 (m, 6H), 4.60-4.54 (m, 4H), 4.36-4.28 (m, 3H), 4.18-3.96 (m, 3H), 3.85-3.55 (m, 27H), 3.49-3.39 (m, 23H), 3.28-3.08 (m, 8H), 2.94-2.57 (m, 4H), 2.42-2.07 (m, 8H), 1.99-1.45 (m, 22H), 1.28-1.11 (m, 23H), 1.05-0.95 (m, 6H), 0.89-0.79 (m, 7H) ppm.

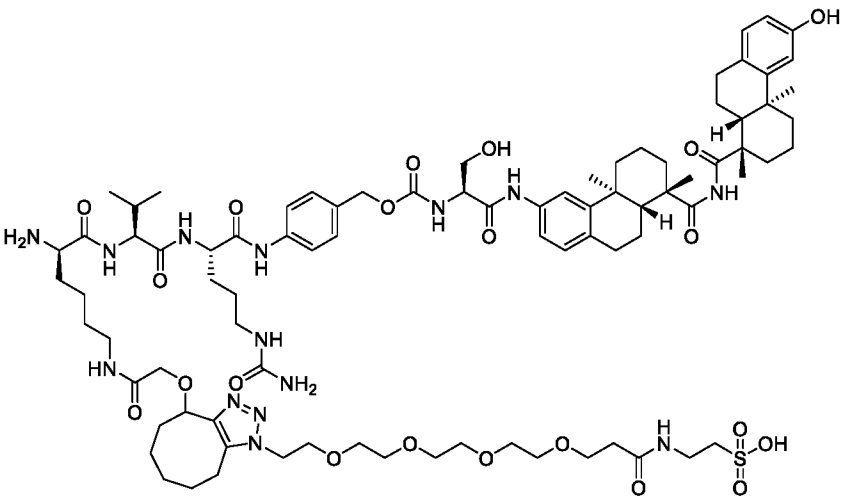
Example 15. Synthesis of LP6 (FIG. 5)

1-Azido-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (L6-2)



[0600] To a solution of azido-PEG_4-NHS (**L6-1**, 0.10 g, 0.26 mmol) in anhydrous DMF (4 mL) were added taurine (39 mg, 0.31 mmol) and DIPEA (15 mg, 0.52 mmol). The mixture was stirred at 25 °C overnight. The mixture was filtered and the filtrate was purified by prep-HPLC (method A) to give compound **LP6-2** (80 mg, 78% yield) as colorless oil. ESI m/z: 399.1 $(M + H)^+$. ^1H NMR (500 MHz, D_2O) δ 3.69 (t, J = 6.0 Hz, 2H), 3.64-3.59 (m, 14H), 3.49 (t, J = 6.5 Hz, 2H), 3.41 (t, J = 4.5 Hz, 2H), 3.00 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 6.0 Hz, 2H) ppm.

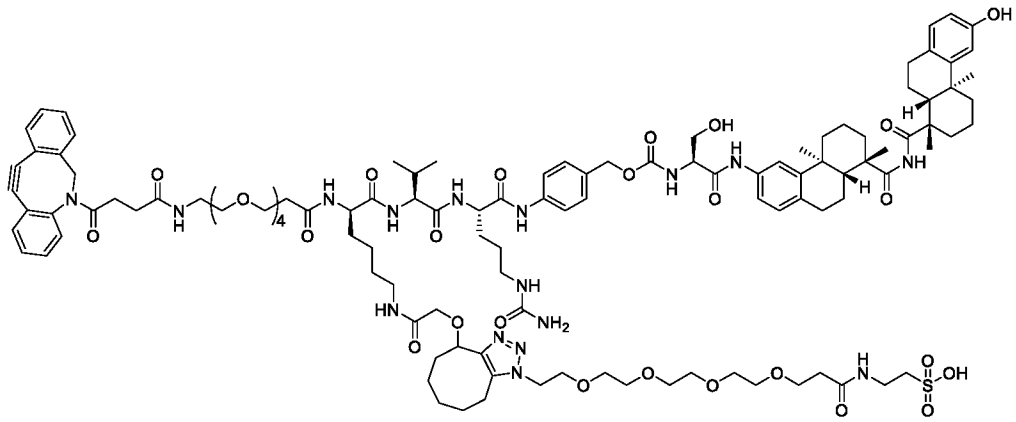
2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-1-({4-[({[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP6-3)



[0601] To a solution of compound **LP6-2** (20 mg, 50 μmol) in water (1 mL) was added dropwise *sat. aq.* sodium bicarbonate solution at 0 °C until pH ~ 7. To the stirred solution was then added a solution of compound **LP4** (28 mg, 21 μmol) in acetontrile (1 mL) by syringe. The mixture was stirred at 25 °C overnight. The reaction mixture was monitored by LCMS until compound **LP4** was totally consumed. The reaction mixture was filtered and purified by prep-

HPLC (method A) to give compound **LP6-3** (15 mg, 41% yield) as a white solid. ESI m/z: 856.5 $(M/2 + 1)^+$.

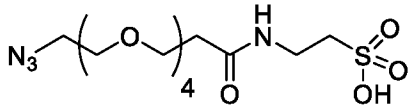
2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-({4-[({[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP6)



[0602] To a solution of compound **LP6-3** (15 mg, 8.8 μmol) and commercially available DIBAC-Suc-PEG$_4$-OSu **LP6-4** (5.7 mg, 8.8 μmol, CAS 1427004-19-0) in DMF (1 mL) was added DIPEA (2.3 mg, 18 μmol) and the mixture was stirred at RT for 2 hours. Most of the volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method B) to give **LP6** (6.0 mg, 30% yield) as a white solid. ESI m/z: 1123.8 $(M/2 + H)^+$, 749.5 $(M/3 + H)^+$. ^{1}H NMR (500 MHz, methanol$_{d4}$) δ 7.76-7.16 (m, 14H), 7.06-7.00 (m, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.72-6.71 (m, 1H), 6.56-6.55 (m, 1H), 5.39-5.33 (m, 1H), 5.14-5.09 (m, 5H), 4.61 (s, 18H), 4.50-4.43 (m, 2H), 4.33-4.30 (m, 1H), 3.99 (s, 2H), 3.89-3.85 (m, 3H), 3.73-3.42 (m, 28H), 3.25-2.72 (m, 8H), 2.45 (t, J = 7.5 Hz, 2H), 2.36-1.96 (m, 18H), 1.81-1.51 (m, 12H), 1.45-1.32 (m, 15H), 1.12-0.89 (m, 12H) ppm.

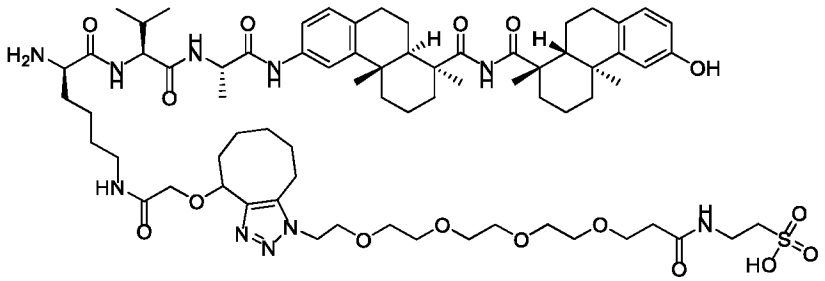
Example 16. Synthesis of LP18 (FIG. 6)

1-Azido-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (L18-2)



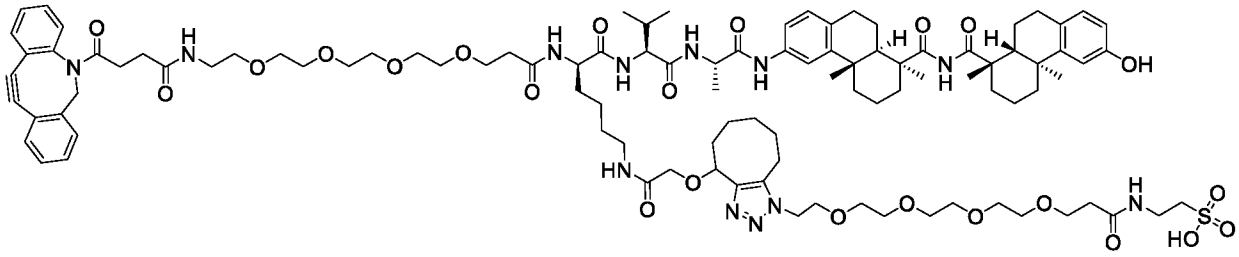
[0603] To a solution of azido-PEG_4-NHS (**L18-1**, 0.10 g, 0.26 mmol) in anhydrous DMF (4 mL) were added taurine (39 mg, 0.31 mmol) and DIPEA (15 mg, 0.52 mmol). The mixture was stirred at 25 °C overnight. The mixture was filtered and the filtrate was purified by prep-HPLC (method A) to give compound **LP18-2** (80 mg, 78% yield) as colorless oil. ESI m/z: 399.1 $(M + H)^+$. ^1H NMR (500 MHz, D_2O) δ 3.69 (t, J = 6.0 Hz, 2H), 3.64-3.59 (m, 14H), 3.49 (t, J = 6.5 Hz, 2H), 3.41 (t, J = 4.5 Hz, 2H), 3.00 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 6.0 Hz, 2H) ppm.

1-(4-(2-((*R*)-5-Amino-6-((*S*)-1-((*S*)-1-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-ylamino)-6-oxohexylamino)-2-oxoethoxy)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (LP18-3)



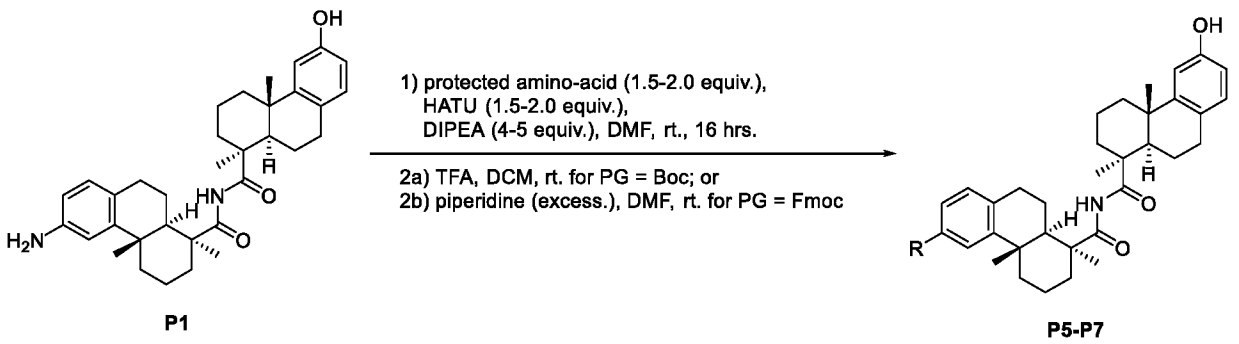
[0604] To a solution of compound **LP1-4** (40 mg, 40 μmol) in DMF (1 mL) was added azide **LP18-2** (40 mg, 0.10 mmol) at RT. The reaction was stirred at RT for 16 hours, until LCMS showed complete reaction. The reaction mixture was directly purified by prep-HPLC to give compound **LP18-3** (43 mg, 77% yield) as a white solid. ESI m/z: 695.4 $(M/2 + H)^+$.

2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP18)



[0605] To a solution of compound **LP18-3** (30 mg, 22 μmol) in DMF (1 mL) were added a solution of DIBAC-suc-PEG_4-OSu (**LP18-4**, 14 mg, 22 μmol) in DMF (1 mL) and DIPEA (4 mg, 32 μmol) successively at RT. The reaction mixture was stirred at RT for 2 hours. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP18** (15 mg, 37% yield) as a white solid. ESI m/z: 642 $(M/3 + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 9.68-9.27 (m, 1H), 8.99 (s, 1H), 8.23-7.85 (m, 4H), 7.79-7.71 (m, 2H), 7.76-7.42 (m ,6H), 7.39-7.28 (m, 3H), 7.21 (s, 1H), 7.09 (s, 1H), 6.96-6.93 (m, 2H), 6.81 (d, J = 8.4 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.50 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 5.02 (d, J = 14.0 Hz, 1H), 4.93-4.72 (m, 1H), 4.53-4.09 (m, 5H), 3.82-3.75 (m, 4H), 3.62-3.53 (m, 3H), 3.51-3.38 (m, 23H), 3.30-3.27 (m, 6H), 3.12-2.67 (m, 10H), 2.61-2.54 (m, 4H), 2.39-1.52 (m, 31H), 1.45-1.08 (m, 18H), 1.01-0.98 (m, 6H), 0.90-0.82 (m, 6H) ppm.

Example 17. Synthesis of Payload P5, Payload P6, and Payload P7

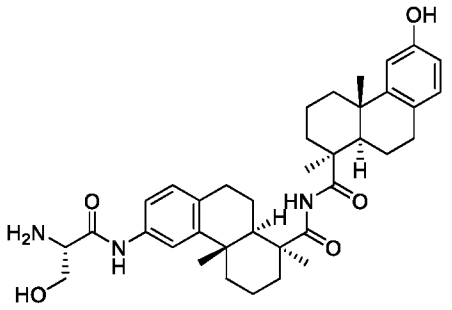


P1 P5-P7

Cpd	R	PG	Yield
P5	NHC(O)(*S*)-CH(CH_2OH)NH_2 (Ser)	Fmoc	51%
P6	NHC(O)(*S*)-CH(CH_2CH_2COOH)NH_2 (Glu)	tBu, Boc	46%
P7	NHC(O)(*S*)-CH($(CH_2)_4NH_2$)NH_2 (Lys)	Boc, Boc	49%

Payload P5

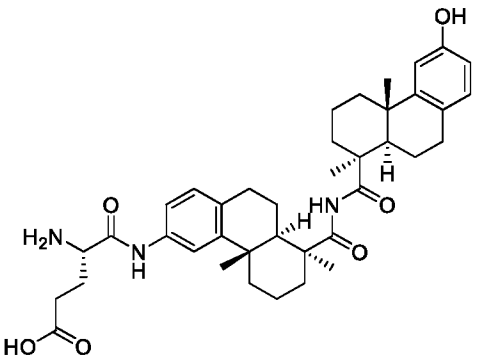
(1*S*,4a*S*,10a*R*)-6-((*S*)-2-Amino-3-hydroxypropanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (P5)



[0606] To a solution of Fmoc-Ser-OH (33 mg, 0.1 mmol) in DMF (1 mL) were added HATU (38 mg, 0.1 mmol) and DIPEA (39 mg, 0.3 mmol) at 25 °C. The resulting mixture was stirred at this temperature for an hour. To the mixture was then added **P1** (30 mg, 0.06 mmol). After the reaction mixture was stirred at 25 °C for 16 hours and **P1** was totally consumed (monitored by LCMS), piperidine (0.2 mL) was added into the mixture, which was stirred for another 30 min at room temperature. The residue was directly purified by prep-HPLC (method B) to give **P5** (18 mg, 51% yield) as a white solid. ESI m/z: 616 $(M + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 9.74 (br s, 1H), 9.00 (s, 1H), 8.11 (s, 1H,), 7.58 (s, 1H), 7.41 (dd, J = 8.2, 2.0 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 2.3 Hz, 1H), 6.50 (dd, J = 8.2, 2.4 Hz, 1H), 4.82 (t, J = 5.5 Hz, 1H), 3.62-3.45 (m, 2H), 2.97-2.67 (m, 4H), 2.67-2.61 (m, 2H), 2.33-2.21 (m, 2H), 2.21-2.03 (m, 4H), 1.96-1.77 (m, 4H), 1.70-1.50 (m, 4H), 1.43-1.37 (m, 1H), 1.36-1.20 (m, 8H), 1.23-1.06 (m, 2H), 1.06-0.93 (m, 6H) ppm.

Payload P6

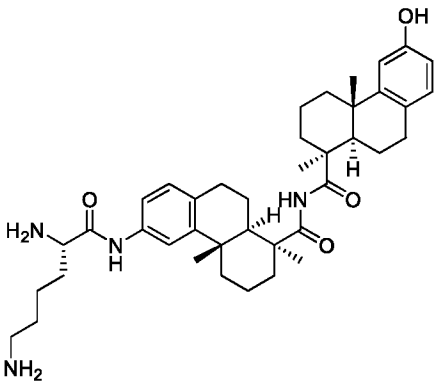
(4*S*)-4-Amino-4-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}butanoic acid; trifluoroacetic acid salt (P6)



[0607] To a solution of OtBu-*N*-Boc-Glu-OH (15 mg, 0.05 mmol) in DMF (1 mL) were added HATU (19 mg, 0.05 mmol) and DIPEA (13 mg, 0.1 mmol) at 25 °C. The resulting mixture was stirred at this temperature for an hour. To the mixture was then added **P1** (14 mg, 0.026 mmol). After stirring at 25 °C for 16 hours and **P1** was totally consumed (monitored by LCMS), the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic solution was dried over sodium sulfate and concentrated *in vacuo*. The residue was dissolved in DCM (1 mL) and to the solution was added TFA (0.1 mL) slowly at room temperature. The mixture was stirred at room temperature for 2 hours. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method A) to give **P6** (8 mg, 46% yield) as a white solid. ESI m/z: 658.3 $(M + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.32 (s, 1H), 9.00 (s, 1H), 8.12 (s, 1H), 7.48 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 2.1 Hz, 1H), 6.50 (dd, J = 8.2, 2.3 Hz, 1H), 3.87 (t, J = 6.5 Hz, 1H), 2.97-2.67 (m, 4H), 2.41-2.22 (m, 4H), 2.22-2.08 (m, 4H), 2.05-1.97 (m, 2H), 1.94-1.80 (m, 4H), 1.69-1.52 (m, 4H), 1.42-1.22 (m, 8H), 1.22-1.06 (m, 2H), 1.02 (s, 3H), 0.99 (s, 3H) ppm. ^{19}F NMR (376 MHz, $DMSO_{d6}$) δ -73.50 ppm.

Payload P7

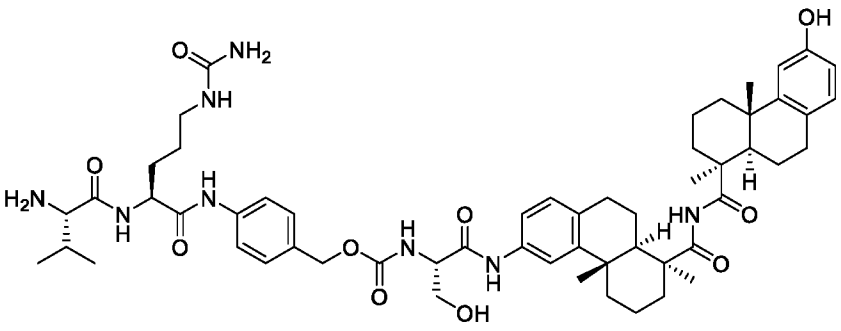
(1*S*,4a*S*,10a*R*)-*N*-[(1*S*,4a*S*,10a*R*)-6-Hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]-6-[(2*S*)-2,6-diaminohexanamido]-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide; trifluoroacetic acid salt (P7)



[0608] To a solution of Boc-Lys-OH (15 mg, 0.05 mmol) in DMF (1 mL) were added HATU (19 mg, 0.05 mmol) and DIPEA (13 mg, 0.1 mmol) at 25 °C. The resulting mixture was stirred at this temperature for an hour. To the mixture was then added **P1** (15 mg, 0.028 mmol). After stirring at 25 °C for 16 hours and **P1** was totally consumed (monitored by LCMS), the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic solution was dried over sodium sulfate and concentrated *in vacuo*. The residue (**Boc-P7**) was dissolved in DCM (1 mL) and to the solution was added TFA (0.1 mL) slowly at room temperature. The mixture was stirred at room temperature for 2 hours. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method A) to give **P7** (9 mg, 49% yield) as a white solid. ESI m/z: 657.5 $(M + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.33 (s, 1H), 9.01 (s, 1H), 8.13 (s, 1H), 7.78 (br s, 6H), 7.51 (s, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.02 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.6 Hz, 1H), 6.63 (s, 1H), 6.51 (d, J = 8.3 Hz, 1H), 3.82 (s, 1H), 2.89 (s, 1H), 2.82-2.67 (m, 5H), 2.29 (s, 2H), 2.15 (s, 4H), 1.85 (s, 6H), 1.64-1.51 (m, 6H), 1.28 (d, J = 6.8 Hz, 10H), 1.13 (s, 2H), 1.01 (s, 3H), 0.99 (s, 3H) ppm. ^{19}F NMR (376 MHz, $DMSO_{d6}$) δ -73.53 ppm.

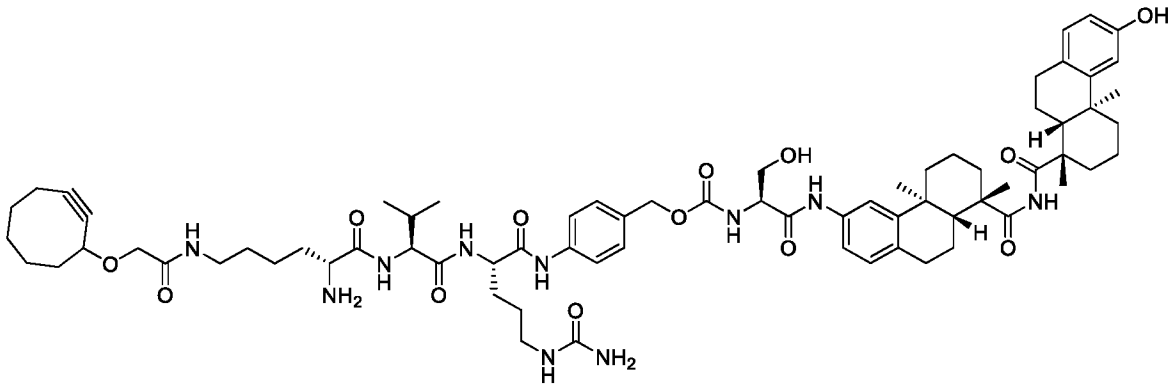
Example 18. Synthesis of LP4 (FIG. 7)

{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (LP4-2)



[0609] To a solution of Fmoc-vc-PAB-PNP (**LP4-1**, 58 mg, 76 μmol) and **P5** (36 mg, 58 μmol) in DMF (3 mL) were added HOBt (7.9 mg, 58 μmol) and DIPEA (15 mg, 0.12 mmol), and the mixture was stirred at 30 °C for 16 hours. Compound **P5** was then totally consumed according to LCMS. To the resulting mixture was added diethylamine (0.1 mL), and the reaction was stirred at RT for an hour until Fmoc was removed, as monitored by LCMS. After the reaction was filtered, the filtrate was directly purified by prep-HPLC (method B) to give compound **LP4-2** (36 mg, 48% yield) as a light yellow solid. ESI m/z: 1021 $(M + 1)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 10.02 (s, 1H), 9.82 (s, 1H), 9.00 (s, 1H), 8.69-8.65 (m, 1H), 8.11-8.00 (m, 4H), 7.65-7.53 (m, 3H), 7.40-7.30 (m, 3H), 7.30-7.20 (m, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.65-6.61 (m, 1H), 6.50 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.00-5.95 (m, 1H), 5.48 (s, 2H), 5.00-4.95 (m, 3H), 4.60-4.40 (m, 1H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 4H), 3.15-2.55 (m, 10H), 2.40-2.20 (m, 3H), 2.20-2.00 (m, 5H), 2.00-1.80 (m, 4H), 1.86-1.55 (m, 6H), 1.27 (d, J = 4.8 Hz, 9H), 1.20-1.10 (m, 2H), 0.97-0.90 (m, 6H) ppm.

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (LP4)

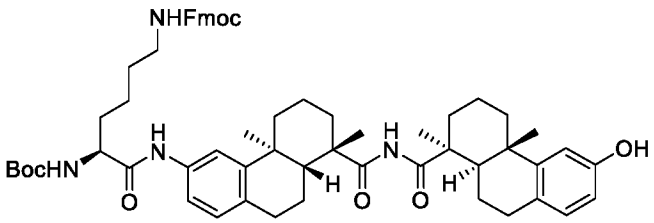


[0610] To a solution of compound **LP4-3** (24 mg, 44 μmol) in DMF (2 mL) were added HATU (17 mg, 44 μmol) and compound **LP4-2** (35 mg, 34 μmol) in succession at RT. The mixture was stirred for a few minutes at RT until the mixture was homogenous. To this mixture was added DIPEA (8.8 mg, 68 μmol) at RT by syringe. The resulting mixture was stirred at RT for 2 hours until the **LP4-2** was mostly consumed according to LCMS. To this reaction mixture was then added diethylamine or piperidine (0.1 mL, excess) dropwise at RT and the mixture was stirred for an hour until Fmoc group was removed, as monitored by LCMS. (Note: Both diethylamine and piperidine were equally effective.) The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP4** (15 mg, 33% yield) as a white solid. ESI m/z: 1313.6 (M +

H)+. 1H NMR (500 MHz, methanol$_{d4}$) δ 7.59 (d, J = 8.5 Hz, 2H), 7.51 (s, 1H), 7.36-7.26 (m, 3H), 7.01 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.72-6.71 (m, 1H), 6.57-6.54 (m, 1H), 5.09 (s, 2H), 4.64-4.52 (m, 1H), 4.35-4.28 (m, 2H), 4.21 (d, J = 7.0 Hz, 1H), 4.01-3.98 (m, 1H), 3.88-3.84 (m, 3H), 3.43 (t, J = 6.5 Hz, 1H), 3.26-3.10 (m, 4H), 3.00-2.76 (m, 3H), 2.38-2.24 (m, 7H), 2.19-2.02 (m, 9H), 1.98-1.78 (m, 4H), 1.74-1.54 (m, 12H), 1.45-1.26 (m, 14H), 1.13 (s, 6H), 1.00 (t, J = 7.5 Hz, 6H) ppm.

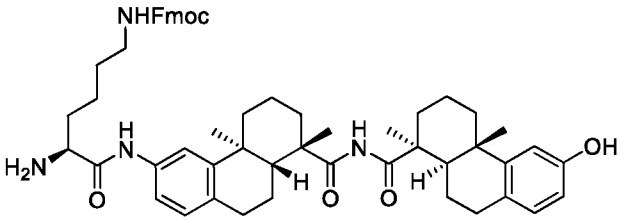
Example 19. Synthesis of LP11 (FIG. 8)

***tert*-Butyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10, 10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentyl] carbamate (LP11-1)**



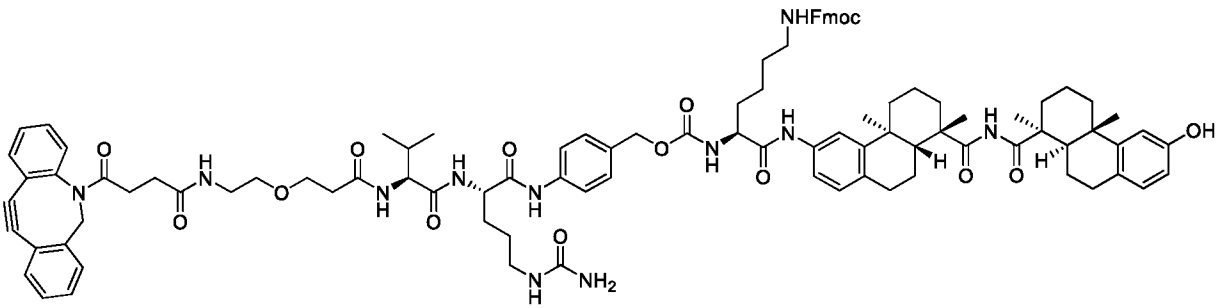
[0611] To a solution of *N*-Boc-*N'*-Fmoc-*L*-Lysine (0.21 g, 0.45 mmol) in DMF (2 mL) were added HATU (0.24 g, 0.64 mmol) and DIPEA (0.15 g, 1.1 mmol) at RT. The resulting mixture was stirred at RT for 3 minutes. To the mixture was then added payload **P1** (0.20 g, 0.38 mmol). The reaction mixture was stirred at RT for 15 minutes until the reaction completed, as monitored by LCMS. The reaction mixture was filtered and purified by prep-HPLC (method B) to give compound **LP11-1** (0.10 g, 27% yield) as a white solid. ESI m/z: 979 (M + 1)+.

9*H*-Fluoren-9-ylmethyl *N*-[(5*S*)-5-amino-5-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}pentyl]carbamate (LP11-2)



[0612] To a solution of compound **LP11-1** (0.10 g, 0.10 mmol) in DCM was added TFA (2 mL) at RT. The resulting mixture was stirred at RT for an hour until Boc was totally removed according to LCMS. The volatiles were removed *in vacuo*. The residue was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **LP11-2** (77 mg, 86% yield) as a white solid. ESI m/z: 879 $(M + 1)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 9.87-9.52 (m, 1H), 9.00 (s, 1H), 8.11 (s, 1H), 7.92-7.81 (m, 2H), 7.71-7.48 (m, 3H), 7.44-7.22 (m, 6H), 7.00-6.90 (m, 1H), 6.82 (d, J = 8.3 Hz, 1H), 6.66-6.60 (m, 1H), 6.54-6.47 (m, 1H), 4.38-4.14 (m, 3H), 3.27-3.17 (m, 1H), 3.01-2.93 (m, 2H), 2.90-2.66 (m, 4H), 2.33-2.06 (m, 7H), 1.94-1.78 (m, 4H), 1.67-1.51 (m, 5H), 1.48-1.07 (m, 16H), 1.03-0.92 (m, 6H) ppm.

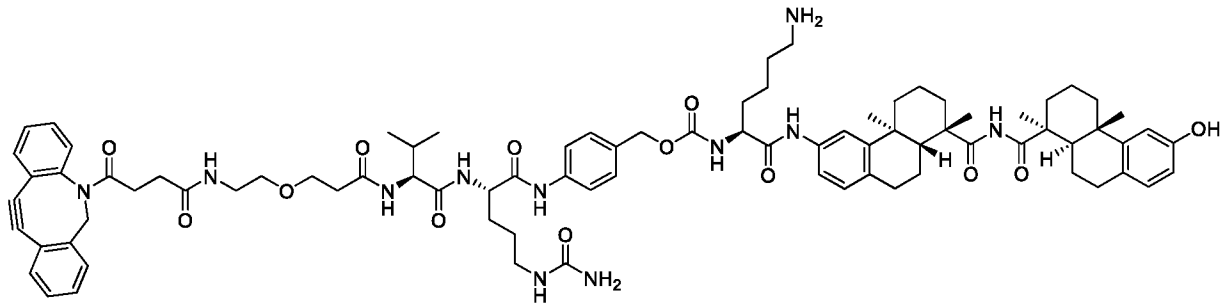
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentyl]carbamate (LP11-3)



[0613] To a mixture of compound **LP11-2** (57 mg, 65 μmol) and compound **LP9-5** (0.10 g, 96 μmol) in DMF (3 mL) were added HOBt (30 mg, 0.22 mmol) and DIPEA (0.11 g, 0.81 mmol), and the mixture was stirred at RT for an hour, which was monitored by LCMS. The reaction

mixture was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **LP11-3** (97 mg, 81% yield) as a white solid. ESI m/z: 909 $(M/2 + 1)^+$.

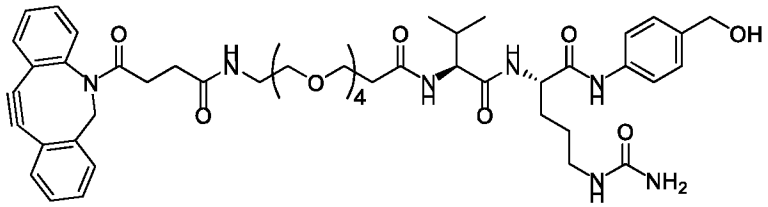
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-5-amino-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}pentyl]carbamate (LP11)



[0614] To a solution of compound **LP11-3** (97 mg, 53 μmol) in DMF (3 mL) was added diethylamine (45 mg, 0.62 mmol). The mixture was stirred at RT for 2 hours until Fmoc was totally removed according to LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP11** (40 mg, 47% yield) as a white solid. ESI m/z: 798 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.99 (s, 1H), 9.85 (s, 1H), 9.00 (s, 1H), 8.21-8.10 (m, 2H), 7.88 (d, J = 8.5 Hz, 1H), 7.82-7.59 (m, 6H), 7.55-7.43 (m, 5H), 7.41-7.29 (m, 5H), 6.98 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 6.66 (s, 1H), 6.53 (d, J = 8.1 Hz, 1H), 6.06-5.96 (m, 1H), 5.50-5.38 (m, 2H), 5.09-4.92 (m, 3H), 4.45-4.35 (m, 1H), 4.28-4.21 (m, 1H), 4.14-4.04 (m, 1H), 3.67-3.58 (m, 4H), 3.52-3.45 (m, 13H), 3.14-2.69 (m, 9H), 2.63-2.56 (m, 1H), 2.50-2.44 (m, 1H), 2.43-2.37 (m, 1H), 2.33-2.13 (m, 7H), 2.06-1.85 (m, 6H), 1.82-1.56 (m, 9H), 1.51-1.23 (m, 17H), 1.21-1.13 (m, 2H), 1.10-0.80 (m, 12H) ppm.

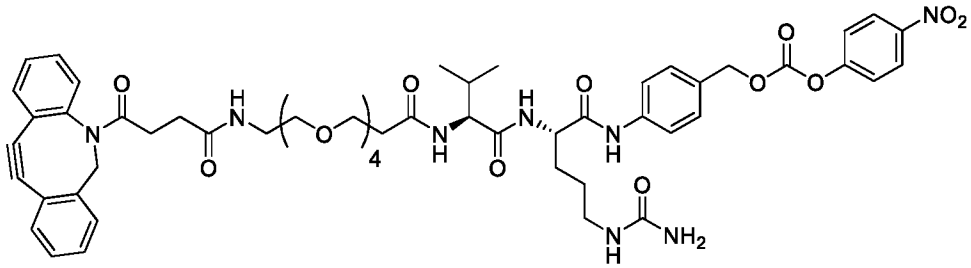
Example 20. Synthesis of LP9 (FIG. 9)

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-(hydroxymethyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide (LP9-3)



[0615] To a solution of compound **LP9-2** (0.30 g, 0.54 mmol) in DMF (10 mL) were added HATU (0.31 g, 0.81 mmol) and DIPEA (0.14 g, 1.1 mmol) successively at room temperature. The mixture was stirred at room temperature for 15 minutes. To the reaction solution was added VC-PAB-OH (**LP9-1**, CAS: 159857-79-1, 0.21 g, 0.54 mmol) at RT, and the resulting mixture was stirred at RT for 3 hours until **LP9-2** was consumed, as monitored by LCMS. The reaction mixture was filtered through filtering a membrane and the filtrate was directly purified by reversed flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give compound **LP9-2** (0.30 g, 60% yield) as a white solid. ESI m/z: 617 $(M + H)^+$.

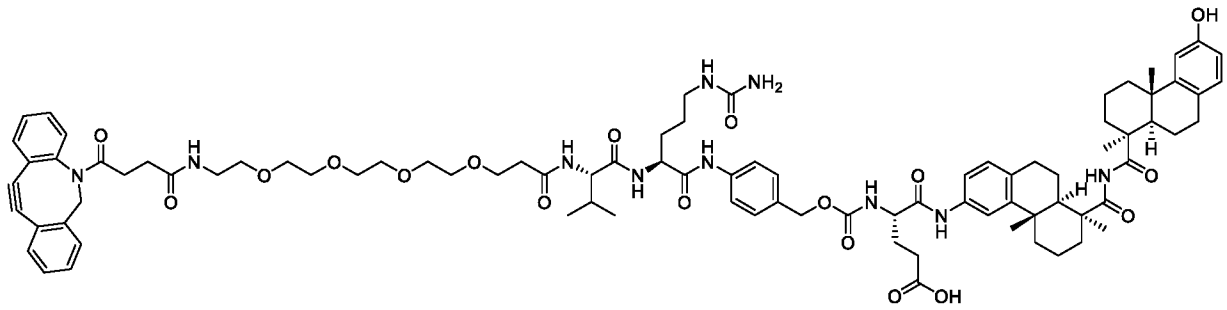
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-nitrophenyl carbonate (LP9-5)



[0616] To a solution of compound **LP9-3** (0.15 g, 0.16 mmol) in DMF (10 mL) were added bis(4-nitrophenyl) carbonate (**LP9-4**, 0.15 g, 0.49 mmol) and DIPEA (63 mg, 0.49 mmol) successively at 0 °C. The mixture was then stirred at RT for 3 hours until **LP9-3** was mostly consumed, as monitored by LCMS. The reaction mixture was filtered through a filtering membrane and the filtrate was directly purified by reversed flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give compound **LP9-5** (50 mg, 28% yield) as a white solid. ESI m/z: 1079 $(M + H)^+$.

(4*S*)-4-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-4-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl] carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-

octahydrophenanthren-3-yl]carbamoyl}butanoic acid (LP9)



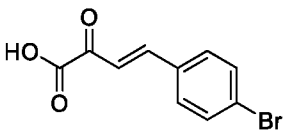
[0617] To a mixture of compound **P6** (50 mg, 76 μmol) and compound **LP9-5** (0.10 g, 96 μmol) in DMF (1 mL) were added HOBt (16 mg, 0.12 mmol) and DIPEA (39 mg, 0.31 mmol), and the mixture was stirred at RT for an hour, which was monitored by LCMS. The reaction mixture was purified by prep-HPLC (method B) to give compound **LP9** (40 mg, 33% yield) as a white solid. ESI m/z: 799 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 10.02 (s, 1H), 9.03 (s, 1H), 8.20-8.07 (m, 2H), 7.94-7.87 (m, 1H), 7.82-7.76 (m, 1H), 7.70-7.57 (m, 4H), 7.54-7.42 (m, 4H), 7.40-7.26 (m, 6H), 6.99-6.91 (m, 1H), 6.85-6.79 (m, 1H), 6.63 (s, 1H), 6.53-6.47 (m, 1H), 6.03 (s, 1H), 5.44 (s, 2H), 5.07-4.88 (m, 3H), 4.42-4.32 (m, 1H), 4.27-4.20 (m, 1H), 4.11-4.02 (m, 1H), 3.63-3.55 (m, 3H), 3.51-3.41 (m, 13H), 3.11-2.58 (m, 10H), 2.40-2.10 (m, 11H), 2.00-1.55 (m, 15H), 1.46-1.09 (m, 14H), 1.05-0.94 (m, 6H), 0.99-0.77 (m, 7H) ppm.

Example 21. Synthesis of Payload P9 and Linker-payload LP12 (FIG. 10)

Payload P9

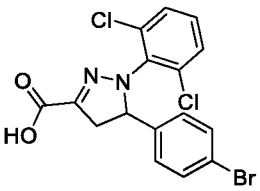
[0618] Synthesis of Payload P9 is based, at least in part, on US 2015/0291563A1, incorporated herein by reference in its entirety.

4-(4-Bromophenyl)-2-oxobut-3-enoic acid (P9-3)



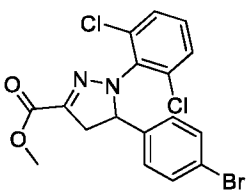
[0619] To a solution of 4-bromobenzaldehyde **P9-2** (5.5 g, 30 mmol) and pyruvic acid **P9-1** (2.8 g, 32 mmol) in methanol (100 mL) was added a solution of potassium hydroxide (2.5 g, 45 mmol) in methanol (100 mL) at 0 °C. Precipitation occured during addition of the base and the mixture was allowed to reach RT for an hour. The mixture was allowed to stirred at RT for 16 hours and then concentrated *in vacuo* to remove volatiles. The residue was diluted with water (200 mL) and acidified with *aq.* HCl (1 M) to pH 3. The mixture was extracted with ethyl acetate (100 mL x 3). The combined organic solution was dried over sodium sulfate and concentrated *in vacuo* to give compound **P9-3** (7.1 g, 93% yield) as viscous oil. ESI m/z: 255 $(M + 1)^+$.

5-(4-Bromophenyl)-1-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid (P9-5)



[0620] A mixture of compound **P9-3** (5.1 g, 20 mmol) and (2,6-dichlorophenyl)hydrazine hydrochloride **P9-4** (4.1 g, 20 mmol) in glacial acetic acid (100 mL) were refluxed under a nitrogen atmosphere for 4 hours. After cooled to 25 °C, the reaction mixture was poured into ice-water, whereby a sticky mass was obtained, which was extracted with DCM. The combined organic solutions were washed with water, dried with sodium sulfate and concentrated. The residue was dried in air to give compound **P9-5** (5.4 g, 65% yield) as a pale yellow solid. ESI m/z: 413 $(M + 1)^+$.

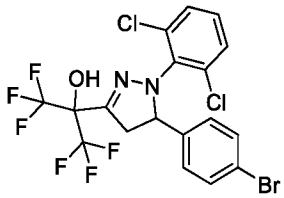
Methyl 5-(4-bromophenyl)-1-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylate (P9-6)



[0621] To the solution of compound **P9-5** (8.2 g, 20 mmol) in methanol (200 mL) was added sulfuric acid (0.5 mL) dropwise. The mixture was refluxed for 4 hours, which was monitored by

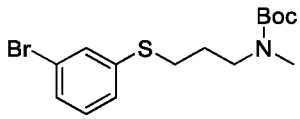
LCMS. The reaction mixture was cooled to RT and the volatiles were removed *in vacuo*. The residue was extracted with ethyl acetate (300 mL) and subsequently washed with *sat. aq.* ammonium chloride and water. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/n-hexane = 1/4) to give compound **P9-6** (2.1 g, 25% yield) as yellow oil. ESI m/z 429 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 7.50-7.45 (m, 4H), 7.35-7.25 (m, 3H), 5.70-5.52 (m, 1H), 3.77 (s, 3H), 1.75-1.65 (m, 1H), 3.32-3.24 (m, 1H) ppm.

2-(5-(4-Bromophenyl)-1-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (P9-7)



[0622] To a mixture of compound P9-6 (0.49 g, 1.2 mmol) in toluene (20 mL) were added (trifluoromethyl)trimethylsilane (0.68 mL, 4.6 mmol), 4 Å molecular sieves (100 mg) and TBAF (1 M in THF, 4.6 mL) slowly under nitrogen atmosphere. The resulting mixture was stirred at 46 ^{o}C for 16 hours. The resulting mixture was filtered through celite pad and the filtrate was diluted with DCM. The solution was washed with distilled water and brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The yellow residue was purified by silica gel column chromatography (ethyl acetate/n-hexane = 1/8) to give compound P9-7 (0.15 g, 25% yield) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.38 (m, 2H), 7.25 (s, 1H), 7.24 (s, 1H), 7.22-7.17 (m, 2H), 7.12-7.06 (m, 1H), 5.75-5.95 (m, 1H), 4.88 (s, 1H), 3.65-3.55 (m, 1H), 3.26-3.16 (m, 1H) ppm.

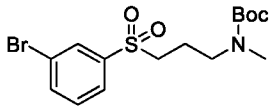
***tert*-Butyl 3-(3-bromophenylthio)propyl(methyl)carbamate (P9-11)**



[0623] To the solution of tert-butyl 3-hydroxypropyl(methyl)carbamate **P9-8** (1.9 g, 10 mmol) in Pyridine (5 mL) was added tosyl chloride (2.1 g, 11 mmol) dropwise at 0 ^{o}C. The mixture was stirred at RT for 2 hours, which was monitored by LCMS. The mixture was concentrated *in vacuo* to remove Pyridine to give crude product **P9-9** (ESI m/z: 244 $(M - Boc + 1)^+$), which was dissolved in acetonitrile (100 mL). To the solution were added 3-bromobenzenethiol **P9-10** (1.9 g, 10 mmol) and cesium carbonate (6.5 g, 20 mmol). The mixture was refluxed for 4 hours, which was monitored by LCMS. After cooled to RT, the reaction mixture was filtered and the

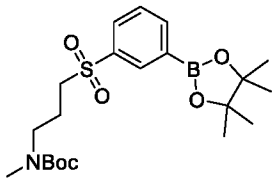
filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (8-10% ethyl acetate in petroleum ether) to give compound **P9-11** (2.2 g, 61% yield) light yellow oil. ESI m/z: 260 $(M - Boc + 1)^+$.

***tert*-Butyl 3-(3-bromophenylsulfonyl)propyl(methyl)carbamate (P9-12)**



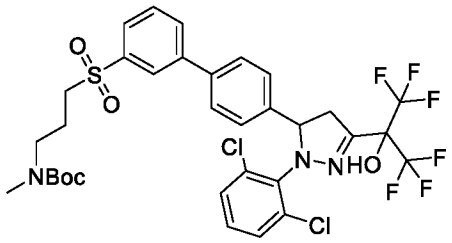
[0624] To a solution of compound **P9-11** (0.36 g, 1.0 mmol) in DCM (20 mL) was added mCPBA (1.0 g, 0.4 mmol) slowly at 0 °C. The mixture was stirred at RT for 4 hours, which was monitored by LCMS. The reaction mixture was washed with *sat. aq.* sodium carbonate (20 mL x 3) and brine. The organic solution was dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (8-10% ethyl acetate in petroleum ether) to give compound **P9-12** (0.22 g, 56% yield) as light yellow oil. ESI m/z: 294 $(M - Boc + 1)^+$.

***tert*-Butyl methyl(3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenylsulfonyl)propyl)carbamate (P9-13)**



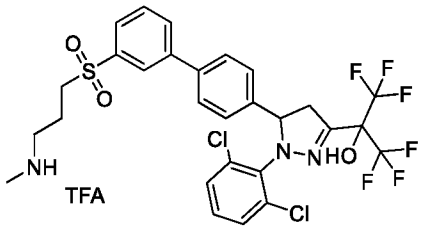
[0625] To a solution of compound P9-12 (0.20 g, 0.51 mmol) in 1,4-dioxane (20 mL) were added bis(pinacolato)diboron (0.26 g, 1.0 mmol), $Pd(dppf)Cl_2$ (37 mg, 0.051 mmol) and potassium acetate (0.20 g, 2.0 mmol) under nitrogen atmosphere. The reaction mixture was stirred at 80 °C for 16 hours under nitrogen atmosphere. After cooled to RT, the mixture was filtered through celite pad. To the filtrate was added distilled water and the mixture was extracted with diethyl ether three times. The combined extracts were washed with distilled water and brine, dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to give a yellow liquid residue. The residue was purified by silica gel column chromatography (8-10% ethyl acetate in petroleum ether) to give compound **P9-13** (0.20 g, 87% yield) as colorless oil. ESI m/z: 340 $(M - Boc + 1)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 8.10 (s, 1H), 8.05-8.0 (m, 2H), 7.70 (t, J =7.5 Hz, 1H), 3.30-3.25 (m, 2H), 3.20-3.14 (m, 2H), 2.70 (s, 3H), 1.75-1.67 (m, 2H), 1.32 (s, 9H), 1.16 (s, 12H) ppm.

***tert*-Butyl 3-(4'-(1-(2,6-dichlorophenyl)-3-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)biphenyl-3-ylsulfonyl)propyl(methyl)carbamate (P9-14)**



[0626] To a mixture of compound **P9-13** (0.20 g, 0.51 mmol) and compound **P9-7** (0.27 g, 0.51 mmol) in 1,4-dioxane (20 mL) and water (3 mL) were added $Pd(dppf)Cl_2$ (37 mg, 0.051 mmol) and potassium carbonate (0.28 g, 2.0 mmol) under nitrogen protection. The reaction mixture was exchanged with nitrogen 3 times and was stirred at 80 °C under nitrogen atmosphere for 16 hours. After cooled to RT, the reaction mixture was filtered through celite pad. The filtrate was diluted with distilled water and extracted with diethyl ether three times. The combined extracts were washed with distilled water and brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (8-10% ethyl acetate in petroleum ether) to give compound **P9-14** (0.33 g, 84% yield) as colorless oil. ESI m/z: 667 $(M - Boc + 1)^+$.

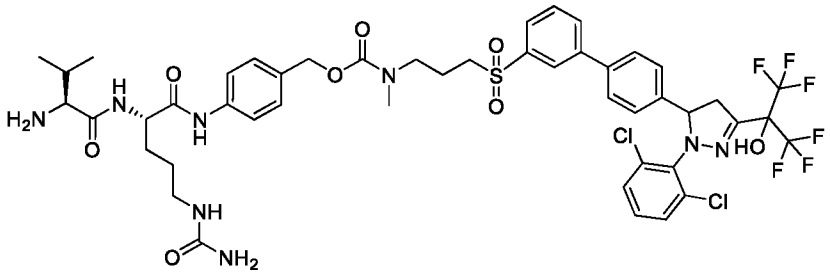
2-(1-(2,6-Dichlorophenyl)-5-(3'-(3-(methylamino)propylsulfonyl)biphenyl-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol, trifluoroacetic acid salt (P9)



[0627] To a solution of compound **P9-14** (0.20 g, 0.26 mmol) in DCM (10 mL) was added TFA (3 mL). The reaction mixture was stirred at RT for an hour, which was monitored by LCMS. The volatiles were removed *in vacuo*. The resulting yellow residue was purified by prep-HPLC (method A) to give compound **P9** (55 mg, 32% yield as its TFA salt) as a white solid. ESI m/z: 667 $(M + 1)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 8.77 (s, 1H), 8.34 (s, 2H), 8.10-8.00 (m, 2H), 7.90-7.83 (m, 1H), 7.80-7.77 (m, 3H), 7.50-7.40 (m, 4H), 7.30-7.20 (m, 1H), 5.58 (t, J = 10.4 Hz, 1H), 3.80-3.66 (m, 1H), 3.52 (t, J = 8.0 Hz, 2H), 3.26-3.18 (m, 1H), 3.00-2.90 (m, 2H), 2.56-2.52(m, 3H), 1.92-1.82 (m, 2H) ppm. (H of TFA was not revealed).

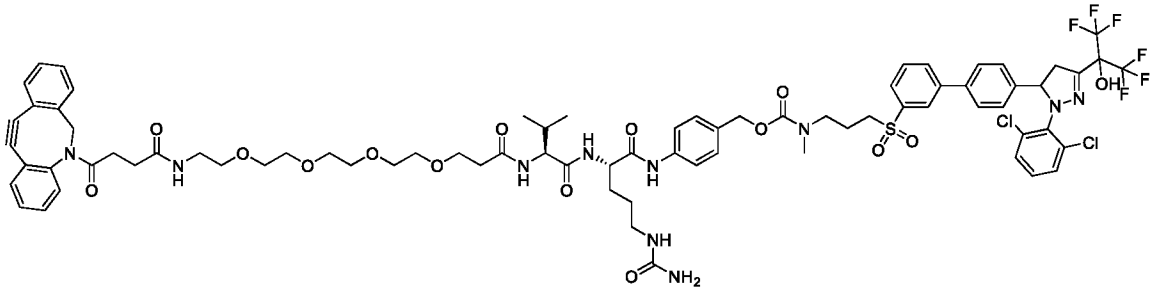
Linker-payload LP12

4-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)benzyl 3-(4'-(1-(2,6-dichlorophenyl)-3-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)biphenyl-3-ylsulfonyl)propyl(methyl)carbamate (LP12-2)



[0628] To a solution of compound **P9** (50 mg, 0.075 mmol) in DMF (5 mL) were added Fmoc-VC-PAB-PNP **P12-1** (63 mg, 0.083 mmol), DIPEA (19 mg, 0.15 mmol) and HOBt (15 mg, 0.11 mmol). The reaction mixture was stirred at RT for 4 hours, which was monitored by LCMS. Piperidine (1 mL, excess) was then added into the reaction mixture. The mixture was stirred at RT for an hour until Fmoc was totally removed according to LCMS. The mixture was directly purified by reversed phase flash chromatography (0-50% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **LP12-2** (48 mg, 60% yield) as a white solid. ESI m/z: 1073 $(M + 1)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[3-(3-{4-[1-(2,6-dichlorophenyl)-3-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl]phenyl}benzenesulfonyl)propyl]-*N*-methylcarbamate (LP12)



[0629] To a solution of DIBAC-PEG4-acid **LP12-3** (18 mg, 32 μmol) in DMF (5 mL) were added HATU (15 mg, 39 μmol) and DIPEA (14 mg, 0.11 mmol) at RT. The resulting mixture was stirred at RT for 30 minutes. To the mixture was then added **LP12-2** (29 mg, 27 μmol). The reaction mixture was stirred at RT for an hour until the reaction completed, which was monitored by LCMS. The reaction mixture was filtered and purified by prep-HPLC (method B) to give **LP12** (25 mg, 58% yield) as a white solid. ESI m/z: 805 $(M/2 + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.99 (s, 1H), 8.75 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 8.09 (s, 1H), 8.03 (d, J = 7.0 Hz, 1H), 7.90-7.80 (m, 2H), 7.80-7.65 (m, 5H), 7.65-7.60 (m, 1H), 7.60-7.52 (m, 2H), 7.52-7.43 (m, 5H), 7.43-7.39 (m, 2H), 7.39-7.31 (m, 2H), 7.31-7.20 (m, 4H), 5.98 (t, J = 5.5 Hz, 1H), 5.57 (t, J = 10.5 Hz, 1H), 5.41 (s, 2H), 5.03 (d, J = 14.0Hz, 1H), 5.0-4.90 (m, 2H), 4.43-4.34 (m, 1H), 4.23 (t, J = 7.0

Hz, 1H), 3.75-3.65 (m, 1H), 3.65-3.53 (m, 3H), 3.53-3.40 (m, 12H), 3.32-3.20 (m, 6H), 3.10-3.05 (m, 2H), 3.05-3.00 (m, 1H), 3.00-2.90 (m, 1H), 2.80-2.70 (m, 3H), 2.60-2.53 (m, 1H), 2.49-2.42 (m, 1H), 2.40-2.30 (m, 1H), 2.30-2.20 (m, 1H), 2.04-1.90 (m, 2H), 1.80-1.65 (m, 4H), 1.65-1.50 (m, 1H), 1.50-1.30 (m, 2H), 1.23 (s, 1H), 0.90-0.80 (m, 6H) ppm.

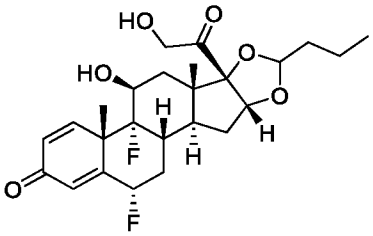
Example 22. Synthesis of Payload P3 and Payload P4 (FIG. 11)

[0630] This example refers to FIG. 11. Certain steroidal payloads were prepared starting from commercial fluocinolone acetonide **P3-1** (CAS: 67-73-2). Compound **P3-2**, obtained from **P3-1** by ketal-exchange with butyraldehyde in the presence of perchloric acid, was converted to mesylate **P3-3** followed by replacement of the mesylate group with azide moiety to form **P4-4** that was reduced to amine **P4**. Otherwise, the mesylate moiety in **P3-3** was also replaced by 4-amino-phenol to afford aniline **P3**.

[0631]

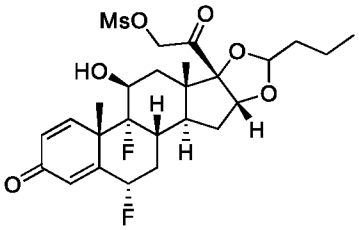
Payload P3

(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (P3-2)



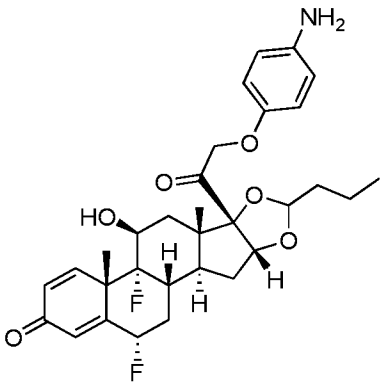
[0632] To a mixture of fluocinolone acetonide (**P3-1**, 0.90 g, 2.0 mmol) and silica gel (18 g) in heptanes (90 mL) was added butyraldehyde (0.27 mL, 3.0 mmol) at 10 °C and the suspension was stirred at 10-20 °C for 10 minutes. To the mixture was added perchloric acid (70%, 0.68 mL, 8.3 mmol) dropwise at 0 °C. The reaction mixture was then stirred at 10-20 °C overnight. Most of fluocinolone acetonide **P3-1** was consumed according to TLC and LCMS. The reaction mixture was diluted with petroleum ether and quenched with *sat. aq.* Na_2CO_3. The suspension was filtered and the solid was washed with DCM/methanol (v/v = 1). The combined filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (0-100% ethyl acetate in petroleum ether) to give compound **P3-2** (0.15 g, 16% yield) as a white solid. ESI m/z: 467.1 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl methanesulfonate (P3-3)



[0633] To a solution of compound **P3-2** (0.28 g, 0.65 mmol)) and triethylamine (0.13 g, 1.3 mmol) in DCM (3 mL) was added methanesulfonyl chloride (89 mg, 0.78 mmol) at 0 °C. After stirred at 0 °C for 0.5 h, the reaction mixture was diluted with DCM (20 mL). The mixture was washed with H_2O (20 mL×2), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography on silica gel (0-50% ethyl acetate in petroleum ether) to give compound **P3-3** (0.26 g, >99% yield) as a white solid. ESI m/z: 545 $(M + H)^+$.

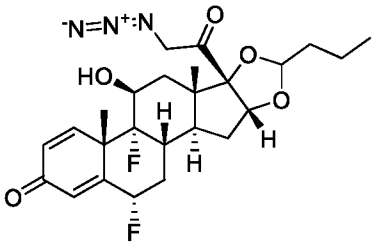
(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-[2-(4-Aminophenoxy)acetyl]-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (P3)



[0634] A mixture of compound **P3-3** (93 mg, 0.17 mmol), 4-aminophenol (37 mg, 0.34 mmol) and cesium carbonate (0.11 g, 0.34 mmol) in acetone (0.5 mL) was refluxed for 2 hours. The mixture was cooled to RT and diluted with H_2O (10 mL). The mixture was extracted with ethyl acetate (10 mL×3). The combined organic layer was washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by prep-HPLC to give payload **P3** (6.0 mg, 6.3% yield) as a white solid. ESI m/z: 298 $(M/2 + H)^+$, 558 $(M + H)^+$ (10%). ^1H NMR (500 MHz, $MeOD_{d4}$) δ 7.34 (d, J = 10.0 Hz, 1H), 6.78-6.71 (m, 4H), 6.37-6.33 (m, 2H), 5.63-5.49 (m, 1H), 5.10-4.99 (m, 1H), 4.77-4.63 (m, 2H), 4.33 (d, J = 9.1 Hz, 1H), 2.74-2.57 (m, 1H), 2.39-2.13 (m, 3H), 1.98-1.31 (m, 12H), 1.03-0.93 (m, 6H) ppm. *Anal.* HPLC: purity 97.4%, Retention time: 7.55 min (method B).

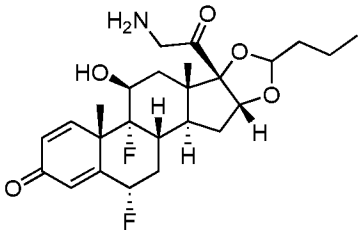
Payload P4

(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-(2-Azidoacetyl)-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (P4-4)



[0635] A suspension of compound **P3-3** (1.0 g, 1.8 mmol) and sodium azide (1.2 g, 18 mmol) in acetone (15 mL) was stirred at 50 °C overnight. The mixture was cooled to RT and poured into water (80 mL). The aqueous mixture was extracted with ethyl acetate (50 mL x 3). The combined organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford crude azido precursor compound **P4-4** (0.90 g, > 99% yield) as a yellow solid, which was used for the next step without further purification. ESI m/z: 492 $(M + H)^+$.

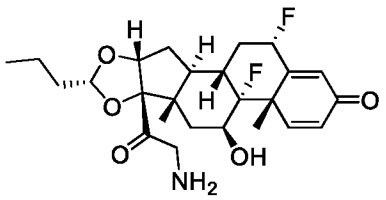
(1*S*,2*S*,4*R*,6*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-(2-Aminoacetyl)-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one; trifluoroacetic acid salt (P4)



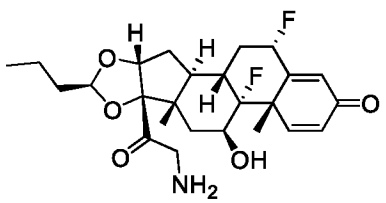
[0636] To a solution of compound **P4-4** (0.85 g, 1.7 mmol) in THF (20 mL) was added *aq.* hydrochloride (1 N, 10 mL). The mixture was stirred at 28-32 °C until it turned clear, to the mixture was then added triphenylphosphine (0.68 g, 2.6 mmol). The resulting yellow clear solution was stirred at RT for 18 h. The mixture was concentrated *in vacuo* and the residue was purified by reversed phase flash chromatography (0-50% acetonitrile in *aq.* TFA (0.05%)) to give compound **P4 (*R/S*)** (0.56 g, 57% yield, TFA salt) as an off-white solid. ESI m/z: 466 $(M + H)^+$. ^{1}H NMR (400 MHz, $MeOD_{d4}$) δ 7.33 (d, J = 9.9 Hz, 1H), 6.40-6.29 (m, 2H), 5.69-5.45 (m, 1H), 4.93-4.92 (m, 1H), 4.71 (t, J = 4.3 Hz, 1H), 4.35-4.27 (m, 2H), 3.90-3.84 (m, 1H), 2.81-2.54 (m, 1H), 2.42-2.06 (m, 3H), 1.82-1.32 (m, 11H), 1.09-0.87 (m, 6H) ppm. ^{19}F NMR (376 MHz,

CD_3OD) δ -77.01, -166.24, -166.92, -188.81, -188.83 ppm. *Anal.* HPLC: 100%, Retention time: 6.86 min (method A).

[0637] Payload **P4** is optionally subjected to chiral separation by HPLC and/or supercritical fluid chromatography (SFC). Stereochemically pure epimers of **P4,** i.e., **P4-1 (*R*)** and **P4-2 (*S*)** or enriched mixtures thereof, **P4-3**, were obtained by chiral separation from a mixture of their corresponding ***R/S*** isomers. The absolute stereochemistry for each compound was determined by 2D–*NOESY*. Reference is made to US Application Publication Number US 2018/0155389, which shows that in the 2D–NOESY spectra for compounds **11-*5R/S*** therein, H^{22} and H^{18} were correlated for a compound **11-*5R***, and that there was no correlation between H^{22} and H^{18} in **11-*5S***. Using a similar analysis as depicted in US 2018/0155389 for compounds **11-*5R/S***, the chiral centers at C^{22}–position are identified for compounds **P4-1 and P4-2** herein by 2D–NOESY.



P4-1 (R isomer)



P4-2 (S isomer)

P4-3: R:S 3:1 to 4:1

Alternate synthesis (1*S*,2*S*,4*R*,6*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-(2-Aminoacetyl)-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one; trifluoroacetic acid salt P4-1 (*R*)

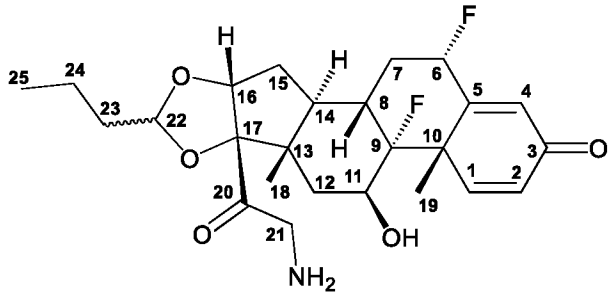
[0638] **Step 1**: Using the same procedure described above, the azido precursor P4-4 (***R***) (0.12 g, 87% yield) was obtained from compound (**P3-3*R***) as a white solid after purification by flash chromatography (0-50% ethyl acetate in petroleum ether). ESI m/z: 492 $(M + H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 7.10 (dd, J = 10.2, 1.3 Hz, 1H), 6.44 (s, 1H), 6.38 (dd, J = 10.2, 1.8 Hz, 1H), 5.48-5.31 (m, 1H), 4.92 (d, J = 5.4 Hz, 1H), 4.62 (t, J = 4.4 Hz, 1H), 4.43 (dd, J = 5.6, 2.7 Hz, 1H), 4.22 (d, J = 18.7 Hz, 1H), 3.94 (d, J = 18.7 Hz, 1H), 2.56-2.39 (m, 2H), 2.32-2.18 (m, 2H), 1.85-1.71 (m, 3H), 1.67-1.54 (m, 7H), 1.46-1.37 (m, 2H), 0.97-0.90 (m, 6H) ppm.

[0639] **Step 2:** Using the same procedure described above, compound **P4-1** (***R***) (30 mg, 66% yield) was obtained as a white solid after purification by prep-HPLC (method A). ESI m/z: 466 $(M + H)^+$. ^1H NMR (500 MHz, $MeOD_{d4}$) δ 7.34 (d, J = 10.0 Hz, 1H), 6.40-6.30 (m, 2H), 5.65-5.46 (m, 1H), 4.94-4.91 (m, 1H), 4.72 (t, J = 4.3 Hz, 1H), 4.34-4.28 (m, 2H), 3.88 (d, J = 18.8 Hz, 1H),

2.78-2.60 (m, 1H), 2.39-2.34 (m, 1H), 2.33-2.18 (m, 2H), 1.77-1.54 (m, 9H), 1.53-1.40 (m, 2H), 0.99-0.95 (m, 6H) ppm. *Anal.* HPLC: 100%, Retention time: 6.85 min (method A).

[0640] **P4-2** (***S***) is obtained using a similar procedure.

[0641] The Table below provides a comparison of ^{1}H NMR chemical shifts (ppm in *d6*-DMSO) of ***R*-P4** (**P4-1** herein) and ***S*-P4** (**P4-2** herein) isolated by SFC of **P4** (***R*/*S***).



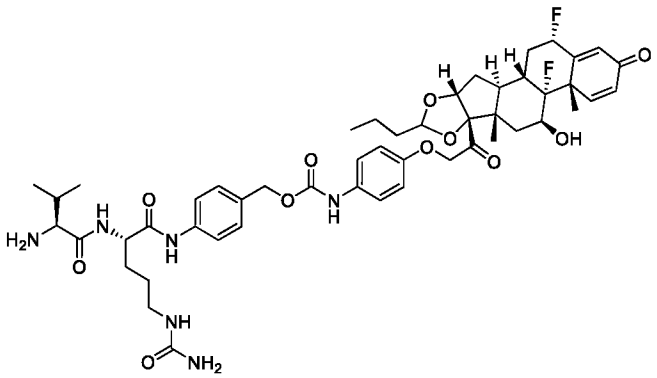
Proton #	**P4 (rac-) in DMSO-d6**	**P4-1 *(R)* in DMSO-d6**	**P4-2 *(S)*-in DMSO-d6**
	8.14 (s, 3H)	8.14 (s, 3H)	8.12 (brs, 3H),
C^{1}-H	7.31 (d, *J* = 10.2 Hz, 1H)	7.30 (d, *J* = 10.0 Hz, 1H)	7.30 (d, *J* = 10.0 Hz, 1H),
C^{2}-H	6.31 (dd, *J* = 10.1 Hz, 1.6 Hz, 1H)	6.31 (dd, *J* = 10.0 Hz, 1.6 Hz, 1H)	6.30 (dd, *J* = 1.6 Hz, 10.0 Hz, 1H),
C^{4}-H	6.11 (s, 1H)	6.11 (s, 1H)	6.11 (s, 1H),
C^{6}-H	5.71-5.54 (m, 1H)	5.71-5.55 (m, 1H)	5.71-5.54 (m, 1H),
C^{7}-H_2	2.28-2.26 (m, 1H), 1.68-1.56 (m, 1H)	2.28-2.26 (m, 1H), 1.68-1.56 (m, 1H)	2.25-2.22 (m, 1H), 1.72-1.52 (m, 1H)
C^{8}-H	1.87-1.79 (m, 1H)	1.44-1.31 (m, 1H)	1.87-1.79 (m, 1H)
C^{11}-H	4.18 (m, 1H)	4.22 (m, 1H)	4.18 (m, 1H)
C^{12}-H_2	2.09-2.01 (m, 1H), 1.68-1.56 (m, 1H)	2.09-2.01 (m, 1H), 1.68-1.56 (m, 1H)	2.03-1.97 (m, 1H), 1.72-1.52 (m, 1H)
C^{14}-H	2.09-2.01 (m, 1H)	2.09-2.01 (m, 1H)	2.03-1.97 (m, 1H)
C^{15}-H_2	2.67-2.50 (m, 1H), 1.68-1.56 (m, 1H)	2.67-2.50 (m, 1H), 1.68-1.56 (m, 1H)	2.58-2.53 (m, 1H), 1.72-1.52 (m, 1H)
C^{16}-H	4.78 (m, 0.6H)	4.78 (m, 1H)	5.15 (d, J=7.6 Hz, 1 H)

Proton #	**P4 (rac-) in DMSO-d6**	**P4-1 *(R)* in DMSO-d6**	**P4-2 *(S)*-in DMSO-d6**
	5.15 (d, J=7.6 Hz, 0.4 H),		
C^{18}-H_3	0.85 (s, 3H)	0.85 (s, 3H)	0.91 (s, 3H)
C^{19}-H_3	1.48 (s, 3H)	1.48 (s, 3H)	1.48 (s, 3H)
C^{21}-H_2	4.20 (m, 1H), 3.77 (m, 1H)	4.20 (d, J=19 Hz, 1H), 3.77 (d, J=19 Hz, 1H)	4.15 (d, J=19 Hz, 1H), 3.74 (d, J=19 Hz, 1H)
C^{22}-H	4.67 (t, *J* = 4.4 Hz, 0.6H) 5.22 (t, J=4.8 Hz, , 0.4H),	4.67 (t, *J* = 4.4 Hz, 1H)	5.22 (t, J=4.8 Hz, , 1H)
C^{23}-H_2	1.68-1.56 (m, 2H)	1.68-1.56 (m, 2H)	1.48 (m, 2H)
C^{24}-H_2	1.44-1.31 (m, 2H)	1.44-1.31 (m, 2H)	1.34-1.28 (m, 1H)
C^{25}-H_3	0.87 (t, J=7.2 Hz, 3H)	0.87 (t, J=7.2 Hz, 3H)	0.88 (t, J=7.6 Hz, 3 H)
***S*-isomer:** ESI m/z: 522 $(M+H)^+$; chiral SFC (CC4): 99.5 d.e.%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 10.1 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 8.8 Hz, 2H), 6.24 (dd, *J* = 10.1, 1.6 Hz, 1H), 6.02 (s, 1H), 5.20 (d, *J* = 6.8 Hz, 1H), 5.18 (t, *J* = 4.8 Hz, 1H), 4.99 (d, *J* = -17.9 Hz, 1H), 4.61 (d, *J* = -17.9 Hz, 1H), 4.43 (s, 1H), 3.46 (s, 2H), 2.57 (td, *J* = 13.2, 4.4 Hz, 1H), 2.34 (dd, *J* = 13.4, 3.2 Hz, 1H), 2.16-2.01 (m, 4H), 1.85-1.68 (m, 3H), 1.59-1.49 (m, 3H), 1.44 (s, 3H), 1.44-1.26 (m, 2H), 1.18-1.09 (m, 2H), 1.00 (s, 3H), 0.91 (t, *J* = 7.3 Hz, 3H) ppm. ***R*-isomer:** ESI m/z: 522 $(M+H)^+$; chiral SFC (CC4): 98.1 d.e.%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 10.1 Hz, 1H), 6.79 (dd, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.8 Hz, 2H), 6.27 (dd, *J* = 10.1, 1.7 Hz, 1H), 6.04 (s, 1H), 4.94 (d, *J* = 4.4 Hz, 1H), 4.89 (d, *J* = 18.0 Hz, 1H), 4.65 (d, *J* = 18.0 Hz, 1H), 4.61 (t, *J* = 4.4 Hz, 1H), 4.48 (d, *J* = 2.1 Hz, 1H), 3.51 (s, 2H), 2.58 (td, *J* = 13.3 Hz, 4.9 Hz, 1H), 2.35 (dd, *J* = 13.4 Hz, 2.8 Hz, 1H), 2.23-1.99 (m, 4H), 1.79-1.61 (m, 6H), 1.46-1.38 (m, 2H), 1.44 (s, 3H), 1.23-1.09 (m, 2H), 0.95 (s, 3H), 0.93 (t, *J* = 7.3 Hz, 3H) ppm.			

Example 23. Synthesis of Linker-payload LP3 (FIG. 12)

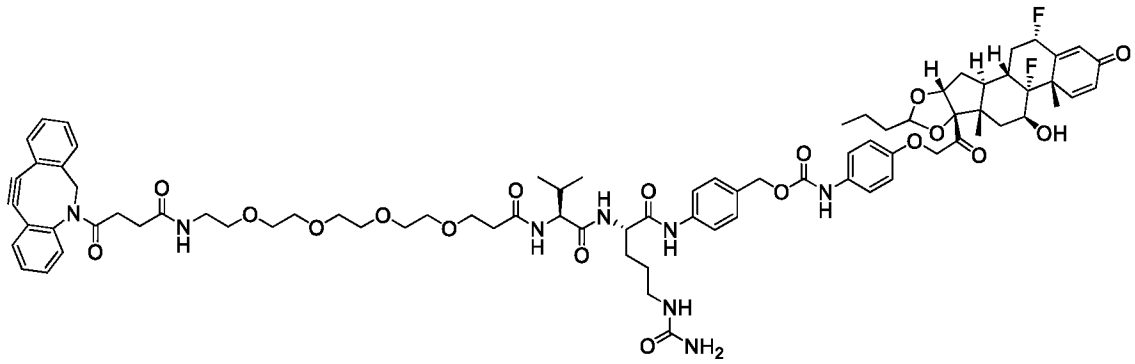
{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-

(carbamoylamino)pentanamido]phenyl}methyl *N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamate (LP3-2)



[0642] To a solution of Fmoc-vc-PAB-PNP (**LP3-1**, 100 mg, 0.13 mmol) and compound **P3** (87 mg, 0.15 mmol) in DMF (5 mL) was added with DIPEA (67 mg, 0.52 mmol) at RT by syringe. The mixture was stirred at RT for 3 hours and most of **LP3-1** was consumed according to LCMS. To the resulting mixture was added piperidine (1 mL, excess) and it was stirred at 25 °C for 2 hours until Fmoc was totally removed, which was monitored by LCMS. After filtering through a membrane, the filtrate was directly purified by prep-HPLC (method B) to give a compound **LP3-2** (80 mg, 64% yield) as a white solid. ESI m/z: 963 $(M + 1)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 10.22 (s, 1H), 9.57 (s, 1H), 8.69 (d, J = 7.5 Hz, 1H), 8.08 (s, 3H), 7.61 (d, J = 6.8 Hz, 2H), 7.36 (d, J = 6.8 Hz, 3H), 7.27 (d, J = 8.0 Hz, 1H), 7.22-7.00 (m, 1H), 6.84 (d, J = 7.2 Hz, 2H), 6.30 (dd, J = 8.0 Hz, 1.6Hz, 1H), 6.11 (s, 1H), 6.10-6.00 (m, 1H), 5.72-5.55 (m, 1H), 5.52 (s, 1H), 5.48 (s, 1H), 5.16-5.05 (m, 3H), 4.88-4.80 (m, 1H), 4.80-4.76 (m, 1H), 4.75-4.70 (m, 1H), 4.55-4.48 (m, 1H), 4.25-4.20 (m, 1H), 3.70-3.60 (m, 1H), 3.12-2.90 (m, 2H), 2.70-2.55 (m, 1H), 2.40-2.20 (m, 1H), 2.15-2.00 (m, 3H), 1.86-1.75 (m, 1H), 1.75-1.65 (m, 1H), 1.64-1.54 (m, 5H), 1.49 (s, 4H), 1.46-1.34 (m, 4H), 0.97-0.91 (m,5H), 0.90-0.85 (m, 4H), 0.85-0.80 (m, 3H) ppm.

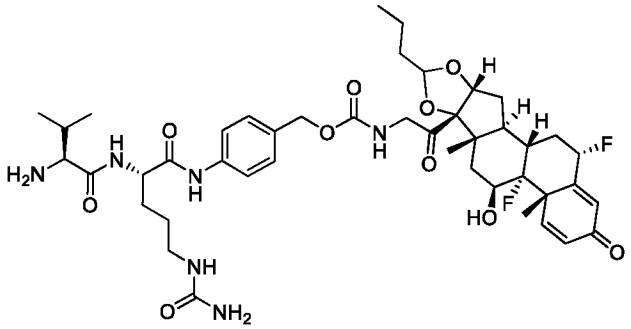
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamate (LP3)



[0643] To a solution of acid **LP3-3** (37 mg, 67 μmol) in DMF (5 mL) were added DIPEA (15 mg, 0.12 mmol) and HATU (34 mg, 90 μmol) at RT successively. The resulting mixture was stirred at this temperature for 0.5 hour before the amine **LP3-2** (58 mg, 60 μmol) was added. The reaction mixture was stirred at RT for 3 hours until the amine was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtrate was then separated by prep-HPLC to give compound **LP3** (20 mg, 22% yield) as a white solid. ESI m/z: 1499 $(M + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.02 (s, 1H), 9.59 (s, 1H), 8.14 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.80-7.75 (m, 1H), 7.70-7.66 (m, 1H), 7.65-7.60 (m, 3H), 7.53-7.45 (m, 3H), 7.40-7.28 (m, 7H), 6.84 (d, J = 9.2 Hz, 2H), 6.30 (dd, J = 10.4 Hz, 1.6 Hz, 1H), 6.11 (s, 1H), 6.10-6.00 (m, 1H), 5.72-5.55 (m,1H), 5.52 (s, 1H), 5.43 (s, 2H), 5.16-5.05 (m, 4H), 4.88-4.70 (m, 3H), 4.43-4.33 (m, 1H), 4.25-4.20 (m, 2H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 12H), 3.30-3.25 (m, 2H), 3.12-2.90 (m, 4H), 2.70-2.55 (m, 2H), 2.48-2.43 (m, 1H), 2.40-2.35 (m, 1H), 2.30-2.20 (m, 2H), 2.15-1.95 (m, 4H), 1.86-1.75 (m, 2H), 1.64-1.54 (m, 5H), 1.49 (s, 4H), 1.46-1.34 (m, 4H), 1.23 (s, 2H), 0.90-0.80 (m, 12H) ppm. *Anal. HPLC*: 100%, Retention time: 7.99 min (method B). Solubility: <0.01 mg/mL water.

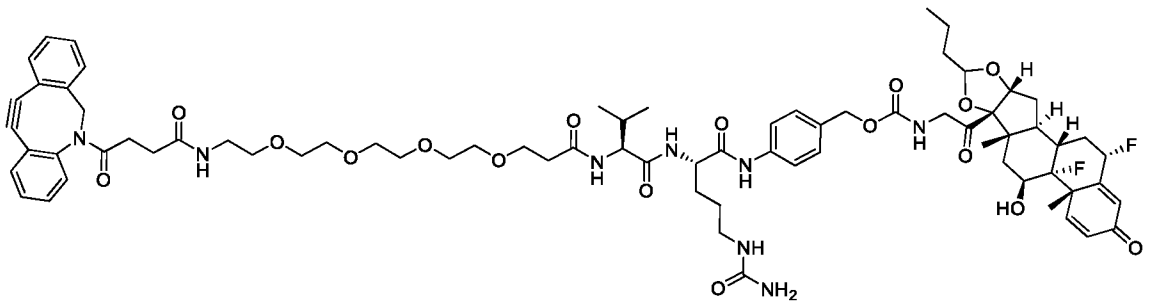
Example 24. Synthesis of Linker-payload LP13 (FIG. 13)

{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (LP13-1)



[0644] To a solution of Fmoc-VC-PAB-PNP (**LP3-1**, 0.17 g, 0.22 mmol) and compound **P4** (93 mg, 0.20 mmol) in DMF (3 mL) was added with DIPEA (51 mg, 0.40 mmol) at RT by syringe. The mixture was stirred at RT for 3 hours and most of materials were consumed according to LCMS. To the resulting mixture was added piperidine (0.3 mL, excess) and it was stirred at RT for an hour until Fmoc was totally removed, which was monitored by LCMS. After filtering through a membrane, the filtrate was directly purified by prep-HPLC (method B) to give compound **LP13-1** (0.13 g, 73% yield) as a white solid. ESI m/z: 871 $(M + 1)^+$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (LP13)

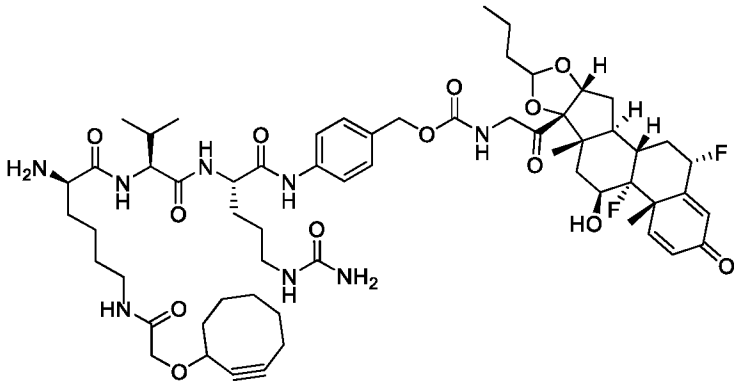


[0645] To a solution of acid **LP3-3** (30 mg, 54 μmol) in DMF (5 mL) were added DIPEA (13 mg, 0.10 mmol) and HATU (31 mg, 81 μmol) at RT successively. The resulting mixture was stirred at this temperature for 0.5 hour before the amine **LP13-1** (43 mg, 50 μmol) was added. The reaction mixture was stirred at RT for 3 hours until the amine was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtrate was then separated by prep-HPLC (method B) to give compound **LP13** (16 mg, 23% yield) as a white solid. ESI m/z: 1406 $(M + H)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.99 (s, 1H), 8.11 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.80-7.75 (m, 1H), 7.70-7.66 (m, 1H), 7.65-7.60 (m, 3H), 7.53-7.33 (m, 6H), 7.33-7.28 (m, 3H), 6.30 (dd, J = 10.0 Hz, 1.5 Hz, 1H), 6.11 (s, 1H),6.10-6.00 (m, 1H), 5.72-5.55 (m, 2H), 5.41 (s, 2H), 5.05-5.01 (m, 1H), 4.97 (s, 2H), 4.80-4.72 (m, 1H), 4.60-

4.58 (m, 1H), 4.43-4.33 (m, 1H), 4.25-4.10 (m,3H), 3.88-3.80 (m,1H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 12H), 3.30-3.25 (m, 2H), 3.12-2.90 (m, 4H), 2.70-2.55 (m, 2H), 2.48-2.35 (m, 2H), 2.30-2.20 (m, 2H),2.15-1.95 (m, 4H), 1.86-1.65 (m, 3H), 1.64-1.54 (m, 5H), 1.49 (s, 4H), 1.46-1.34 (m, 5H), 0.90-0.80 (m, 12H) ppm. *Anal. HPLC*: 100%, Retention time: 7.40 min (method B). Solubility: 0.02 mg/mL water.

Example 25. Synthesis of Linker-payload LP14 (FIG. 14)

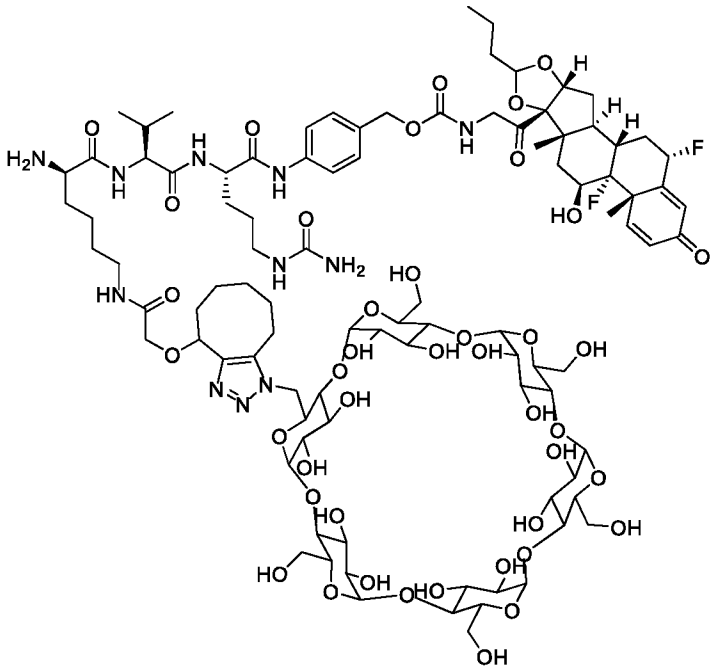
{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (LP14-1)



[0646] To a solution of acid **LP1-3** (0.12 g, 0.22 mmol) in DMF (5 mL) were added HATU (0.11 g, 0.30 mmol) and DIPEA (77 mg, 0.60 mmol) at RT. After the mixture was stirred at RT for 30 minutes, a solution of amine **LP13-1** (0.17 g, 0.20 mmol) in DMF (5 mL) was added by syringe. The resulting mixture was stirred at RT for 3 hours until the amine was mostly consumed according to LCMS. To the mixture was then added piperidine (1 mL, excess), and the mixture was stirred at RT for half an hour until Fmoc was totally removed, which was monitored by LCMS. The reaction mixture was filtered through a membrane and the filtrate was directly purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **LP14-1** (0.12 g, 52% yield) as a white solid. ESI m/z: 1163 (M + 1)$^+$. ^{1}H NMR (400 MHz, MeOD$_{d4}$) δ 7.65-7.55 (m, 2H), 7.40-7.26 (m, 3H), 6.39-6.27 (m, 2H), 5.65-5.45 (m, 1H), 5.13-5.01 (m, 2H), 4.71-4.50 (m, 2H), 4.40-4.14 (m, 4H), 4.11-3.82 (m, 3H), 3.46-3.39 (m, 1H), 3.29-3.09 (m, 4H), 2.76-2.54 (m, 1H), 2.41-2.10 (m, 7H), 2.09-1.99 (m, 1H), 1.96-1.80 (m, 5H), 1.78-1.21 (m, 23H), 1.06-0.82 (m, 12H) ppm.

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-

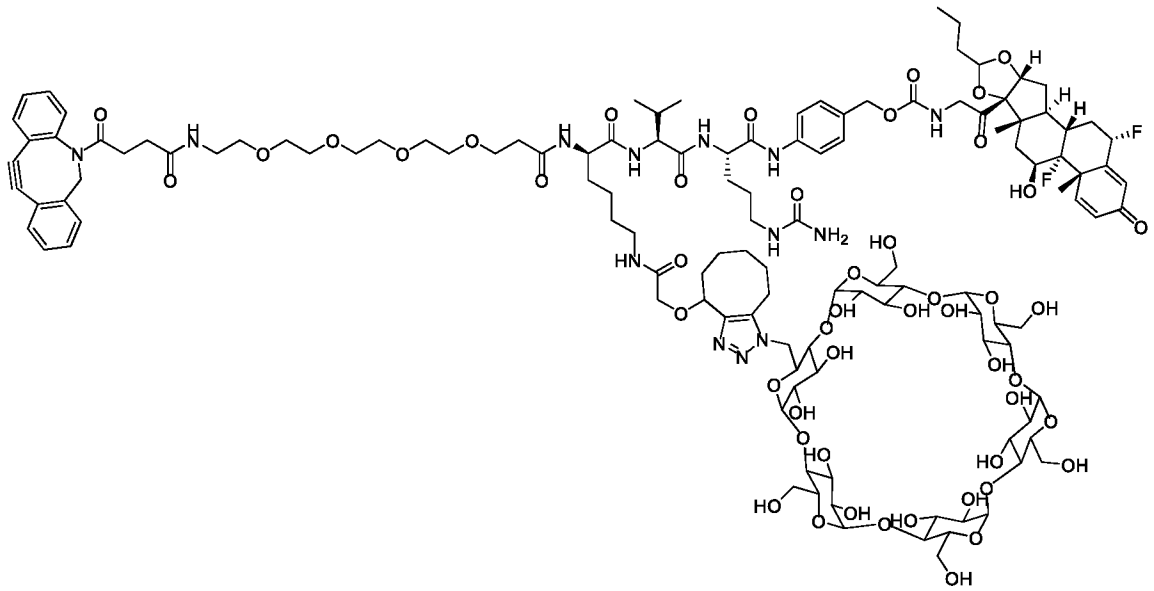
dodecaoxaheptacyclo[26.2.2.2^{3},^{6}.2^{8},^{11}.2^{13},^{16}.2^{18},^{21}.2^{23},^{26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (LP14-2)



[0647] To a solution of alkyne **LP14-1** (0.12 g, 0.10 mmol) in DMF (5 mL) was added α-cyclodextrin-azide (0.30 g, 0.30 mmol). The resulting mixture was then stirred at 50 °C for 3 hours until the compound **LP14-1** was mostly consumed and desired mass was detected, which was monitored by LCMS. After filtered, the resulting mixture was directly purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **LP14-2** (0.11 g, 51% yield) as a white solid. ESI m/z: 1081 $(M/2 + 1)^{+}$. ^{1}H NMR (500MHz, $DMSO_{d6}$) δ 10.05 (s, 1H), 8.30-7.80 (m, 3H), 7.80-7.55 (m, 2H), 7.50-7.40 (m, 1H), 7.40-7.25 (m, 3H), 6.30 (d, J = 12.5 Hz, 1H), 6.11 (s, 1H), 6.00 (s, 1H), 5.80-5.35 (m, 16H), 5.25-5.05 (m, 1H), 4.97 (s, 2H), 4.90-4.50 (m, 13H), 4.50-4.00 (m, 5H), 3.95-3.55 (m, 22H), 3.30-3.20 (m, 8H), 3.20-3.00 (m, 4H), 3.00-2.85 (m, 5H), 2.25-2.20 (m, 2H), 2.10-1.95 (m, 4H), 1.80-1.00 (m, 30H), 1.00-0.90 (m, 4H),0.90-0.80 (m, 14H) ppm.

{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4},^{9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-{2-[(1-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-

dodecaoxaheptacyclo[26.2.2.2^{3},^{6}.2^{8},^{11}.2^{13},^{16}.2^{18},^{21}.2^{23},^{26}]dotetracontan-5-yl]methyl}-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl)oxy]acetamido}hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (LP14)

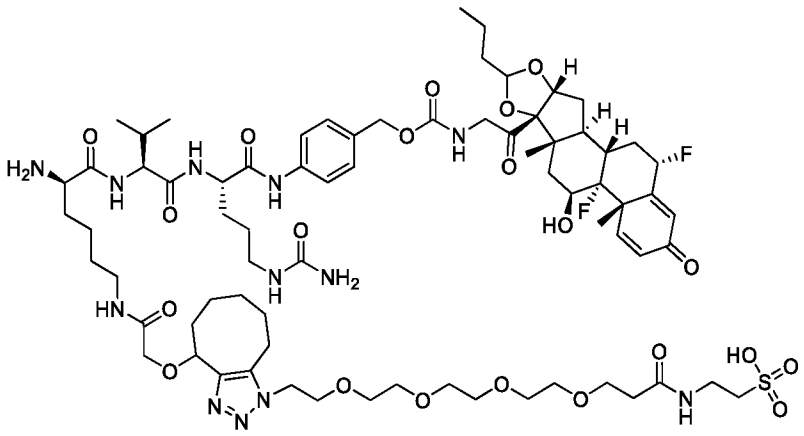


[0648] To a solution of acid **LP14-3** (30 mg, 54 μmol) in DMF (5 mL) were added DIPEA (18 mg, 0.14 mmol) and HATU (26 mg, 69 μmol) at RT successively. The resulting mixture was stirred at this temperature for 0.5 hour before the amine **LP14-2** (0.10 g, 46 μmol) was added. The reaction mixture was stirred at RT for 4 hours until the amine was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtrate was then separated by prep-HPLC (method B) to give compound **LP14** (26 mg, 22% yield) as a white solid. ESI m/z: 1349 $(M + H)^+$. ^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 9.71 (s, 1H), 8.30-8.00 (m, 3H), 8.00-7.74 (m, 2H), 7.70-7.58 (m, 5H), 7.52-7.20 (m, 12H), 6.35-6.20 (m, 2H), 6.15-5.85 (m, 3H), 5.80-5.35 (m, 18H), 5.25-4.90 (m, 6H), 4.90-4.50 (m, 14H), 4.40-4.25 (m, 4H), 4.25-4.10 (m, 3H), 4.10-3.95 (m, 2H), 3.95-3.55 (m, 22H), 3.55-3.40 (m, 22H), 3.20-3.00 (m, 6H), 3.00-2.85 (m, 3H), 2.65-2.55 (m, 1H), 2.25-2.20 (m, 4H), 2.10-1.95 (m, 6H), 1.80-1.70 (m, 5H), 1.70-1.50 (m, 10H), 1.50-1.45 (m, 9H), 0.90-0.80 (m, 14H) ppm. *Anal. HPLC*: 100%, Retention time: 6.23 min (method B). Solubility: 0.026 mg/mL water.

Example 26. Synthesis of Linker-payload LP15 (FIG. 15)

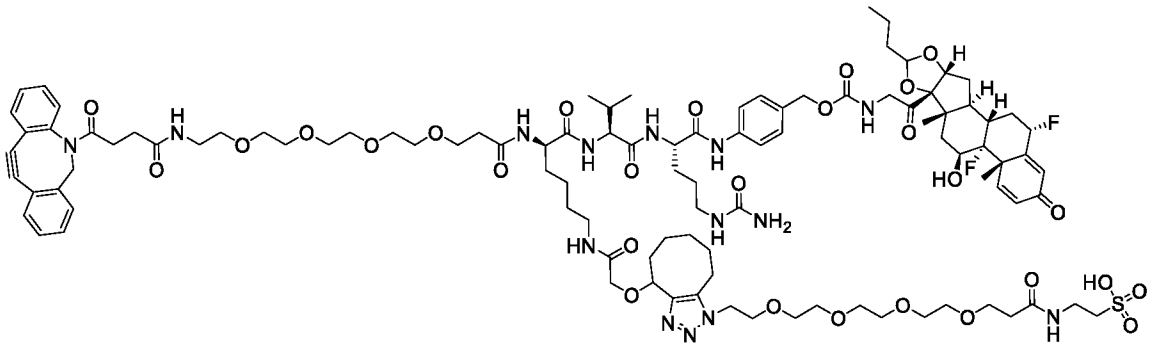
2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-

oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP15-2)



[0649] To a solution of compound **LP14-1** (60 mg, 52 μmol) in DMF (2 mL) was added azido compound **LP15-1** (62 mg, 0.16 mmol). The reaction was stirred at 30 °C overnight. LCMS showed the completion of reaction. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP15-2** (60 mg, 74% yield) as a white solid. ESI m/z: 781 (M/2 + H)$^+$. ^{1}H NMR (400 MHz, MeOD$_{d4}$) δ 7.61 (d, J = 8.5 Hz, 2H), 7.39-7.28 (m, 3H), 6.39-6.30 (m, 2H), 5.66-5.46 (m, 1H), 5.29-5.13 (m, 1H), 5.12-5.04 (m, 3H), 4.72-4.60 (m, 2H), 4.56-4.49 (m, 2H), 4.36-3.84 (m, 8H), 3.76-3.70 (m, 2H), 3.66-3.54 (m, 14H), 3.30-3.23 (m, 2H), 3.21-3.04 (m, 3H), 3.03-2.97 (m, 2H), 2.96-2.84 (m, 1H), 2.75-2.52 (m, 1H), 2.50-2.42 (m, 2H), 2.39-2.01 (m, 6H), 1.99-1.78 (m, 6H), 1.74-1.22(m, 22H), 1.03-0.87 (m, 12H) ppm.

2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (LP15)



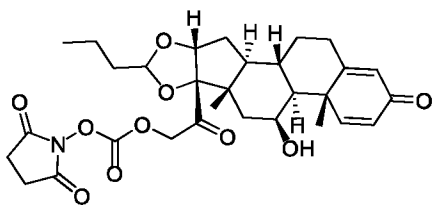
[0650] To a solution of compound **LP15-2** (47 mg, 30 μmol) in DMF (1 mL) were added compound **LP15-3** (21 mg, 33 μmol) and DIPEA (19 mg, 0.15 mmol) at RT. The reaction mixture was stirred at RT for 3 hours. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **LP15** (28 mg, 45% yield) as a white solid. ESI m/z: 1049 $(M/2 + H)^+$. 1H **NMR (500 MHz, MeODd4) δ 7.75**-7.57 (m, 4H), 7.48-7.43 (m, 3H), 7.38-7.23 (m, 6H), 6.37-6.28 (m, 2H), 5.65-5.43 (m, 1H), 5.17-5.03 (m, 3H), 4.69-4.59 (m, 2H), 4.52-4.47 (m, 1H), 4.45-4.41 (m, 1H), 4.34-4.25(m, 2H), 4.23-4.13 (m, 2H), 4.07-3.86 (m, 5H), 3.77-3.68 (m, 4H), 3.64-3.54 (m, 22H), 3.52-3.47 (s, 3H), 3.46-3.40 (m, 4H), 3.29-3.21 (m, 4H), 3.20-3.14 (m, 2H), 3.10-3.01 (m, 1H), 3.00-2.95 (m, 2H), 2.92-2.84 (m, 1H), 2.76-2.60 (m, 2H), 2.47-2.42 (m, 2H), 2.40-2.25 (m, 5H), 2.23-2.13 (m, 3H), 2.07-1.97 (m, 3H), 1.90-1.79 (m, 4H), 1.71-1.55 (m, 14H), 1.52-1.42 (m, 3H), 1.40-1.29 (m, 8H), 1.07-0.85 (m, 12H) ppm. *Anal. HPLC*: 98%, Retention time: 5.88 min (method B).

Example 26A: Synthesis of Budenoside spacers (See FIG. 22)

[0651] FIG. 22 shows Scheme 1 for the synthesis of Budesonide-spacers containing the reactive groups, suc-acid (compound **1c**), carbamate analogues (**1d**, **1e**), THP-analogues (**1g** and **1h**), glucose analogues (**1i** and **1j**), phosphate analogues (**1k** and **1l**), and a commercial phosphate analogue (**1m**).

Intermediate 3a

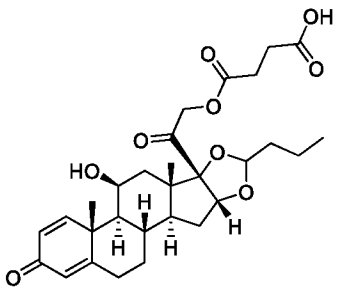
1-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]pyrrolidine-2,5-dione (**3a**)



[0652] To a solution of budesonide **1a** (0.10 g, 0.26 mmol) in DCM (1 mL) were added bis(2,5-dioxopyrrolidin-1-yl) carbonate (71 mg, 0.30 mmol), triethylamine (47 mg, 0.52 mmol) and DMAP (3.0 mg, cat.). The reaction mixture was stirred at 15-25 °C for 12 hours until budesonide was consumed, which was monitored by LCMS. The reaction mixture was then diluted with DCM and washed by water. The organic solution was dried over sodium sulfate. After filtered, the solution was concentrated in vacuo and the residue (crude **3a**) was used for the next step directly without purification. (93 mg, yield 71%). ESI m/**z**: 572.2 $(M + H)^+$.

Example 1c

4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}-4-oxobutanoic acid (1c)

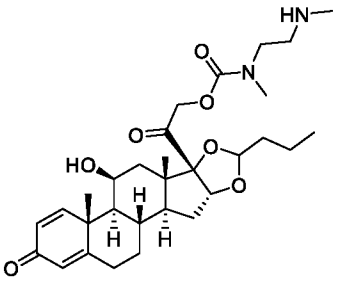


[0653] See WO2015005459; WO2013074988; *Research in Pharmaceutical Science*, **2011**, 6(2), 107-116; and *International Journal of Pharmaceutics*, **2009**, 365(1-2), 69-76.

[0654] To a solution of budesonide (**1a**, 0.10 g, 0.26 mmol) in DCM (1 mL) were added succinic anhydride (30 mg, 0.30 mmol), triethylamine (47 mg, 0.52 mmol) and DMAP (3 mg, catalyst, 0.02 mmol). The mixture was stirred at RT for 12 hours until budesonide was consumed, which was monitored by TLC and LCMS. The reaction mixture was then diluted with DCM and washed with water. The organic solution was dried over sodium sulfate. After filtered, the solution was concentrated in vacuo and the residue was purified by prep-HPLC to give title compound **1c** (22 mg, yield 18%) as a white solid. ESI m/**z**: 531.3 $(M + H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, J = 10 Hz, 1H), 6.28-6.26 (m, 1H), 6.04-6.02 (m, 1H), 5.23-5.11 (m, 1H), 5.06-4.99 (m, 1H), 4.83-4.68 (m, 1H), 4.45-4.43 (m, 1H), 2.76-2.63 (m, 5H), 2.41-2.39 (m, 1H), 2.26-2.12 (m, 2H), 1.99-1.66 (m, 6H), 1.57-1.38 (m, 6H), 1.32 (s, 1H), 1.16-0.94 (m, 8H) ppm.

Example 1d

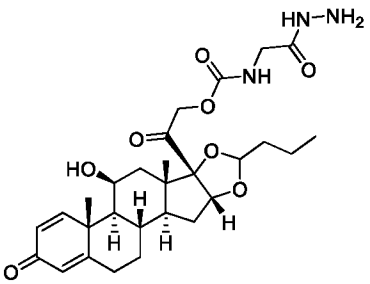
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-methyl-*N*-[2-(methylamino)ethyl]carbamate (1d)



[0655] To a solution of crude intermediate **3a** (63 mg, 0.11 mmol) in DCM (5 mL) were added *N,N'*-dimethylethane-1,2-diamine (28 mg, 0.32 mmol) and triethylamine (38 mg, 0.38 mmol) at RT. The resulting mixture was stirred at RT for 2 hours until most of **3a** was consumed, which was monitored by LCMS. The volatiles were removed in vacuo and the residue was purified by prep-HPLC (method B) to give **1d** (4 mg, yield 4.5%) as a white solid. ESI m/**z**: 545.3 $(M + H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.30 (m, 1H), 6.28-6.25 (m,1H), 6.02-6.01 (m, 1H), 4.42 (br s, 1H), 3.50-3.23 (m, 3H), 3.10-3.02 (m, 3H), 2.82-2.71 (m, 3H), 2.57-2.55 (m, 1H), 2.35-2.33 (m, 1H), 2.15-2.06 (m, 2H), 1.96-1.79 (m, 12H), 1.60-1.56 (m, 3H), 1.46 (m, 3H), 1.37-1.33 (m, 2H), 1.23-1.04 (m, 4H), 0.92-0.86 (m, 3H) ppm.

Example 1e

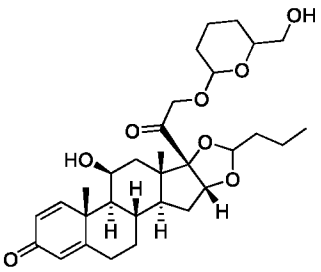
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-[(hydrazinecarbonyl)methyl]carbamate (1e)



[0656] To a mixture of crude **3a** (0.16 g, 0.28 mmol) in DMF (3 mL) were added Boc-2-(2-aminoacetyl)hydrazine (80 mg, 0.42 mmol) and triethylamine (85 mg, 0.84 mmol). The reaction mixture was stirred at 25 °C for 16 hours and intermediate **3a** was consumed, which was monitored by LCMS. The mixture was then quenched with water and extracted with ethyl acetate. The combined organic solution was dried over anhydrous sodium sulfate. After filtered, the solution was concentrated in vacuo. The residue was purified by silica gel column chromatography (30-50% ethyl acetate in petroleum ether) to give **Boc-1e** (70 mg) as a white solid (ESI m/**z**: 646 $(M + H)^+$), which was dissolved in DCM (2 mL). To the solution was added dropwise TFA (1 mL) at 0 °C. The mixture was stirred at 25°C for 2 hours until **Boc-1e** was consumed, which was monitored by LCMS. The volatiles were removed in vacuo and the residue was purified by prep-HPLC to give title compound **1e** (7 mg, yield 12%) as a white solid. ESI m/**z**: 546 $(M + H)^+$.

Example 1g

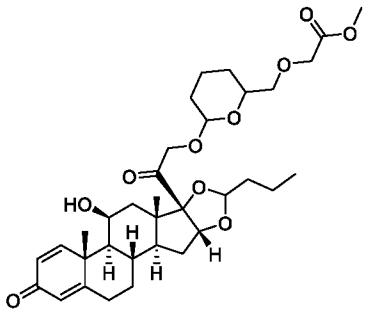
(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-8-(2-{[6-(hydroxymethyl)oxan-2-yl]oxy}acetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (1g)



[0657] To a solution of budesonide (**1a**, 50 mg, 0.12 mmol) in anhydrous DCM (4 mL) were added (3,4-dihydro-2H-pyran-2-yl)methanol (0.11 g, 0.93 mmol) and *p*-toluenesulfonic acid (31 mg, 0.18 mmol) at 0 °C. The reaction mixture was stirred at RT for 4 days, which was monitored by LCMS. The volatiles were removed in vacuo and the residue was dissolved in DMF and separated by prep-HPLC (method B) to give title compound **1g** (13 mg, yield 20%) as a white solid. ESI m/**z**: 545 $(M + H)^+$.

Example 1h

Methyl 2-[(6-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}oxan-2-yl)methoxy]acetate (1h)

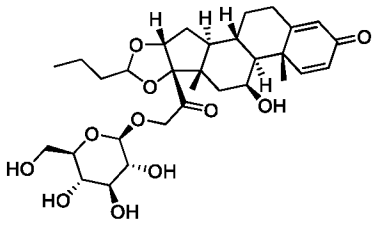


[0658] To a solution of (3,4-dihydro-2*H*-pyran-2-yl)methanol (2.0 g, 18 mmol) in THF (30 mL) was added sodium hydride (60% in mineral oil, 0.88 g, 22 mmol) portion wise at 0 °C under nitrogen protection. The suspension was stirred at 0 °C until the end of hydrogen evolution. To the resulting mixture was then added a solution of ethyl 2-bromoacetate (4.0 g, 26 mmol) in THF (18 mL). The mixture was stirred at RT overnight until the starting material was mostly consumed according to LCMS. After cooled to 0 °C, the reaction was quenched with water under nitrogen protection and extracted with ethyl acetate. The combined organic solution was washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (0-8% ethyl acetate in petroleum ether) to give ethyl 2-((3,4-dihydro-2*H*-pyran-2-yl)methoxy)acetate (0.70 g, 21% yield) as colorless oil. (^{1}H NMR (500 MHz, $DMSO_{d6}$) δ 6.36 (d, J = 6.0 Hz, 1H), 4.67 (m, 1H), 4.16 (s, 2H), 3.92 (m, 1H), 3.65 (s, 3H), 3.57 (d, J = 5.5 Hz, 2H), 2.50-1.99 (m, 1H), 1.93-1.82 (m, 1H), 1.80 (m, 1H), 1.60 (m, 1H) ppm.)

[0659] To a solution of budesonide (**1a**, 0.11 g, 0.25 mmol) in anhydrous DCM (8 mL) were added ethyl 2-((3,4-dihydro-2*H*-pyran-2-yl)methoxy)acetate (0.35 g, 1.9 mmol) obtained above and *p*-toluenesulfonic acid (66 mg, 0.35 mmol) at 0 °C. The reaction mixture was stirred at RT for 4 hours, which was monitored by LCMS. The volatiles were removed in vacuo and the residue was dissolved in DMF and separated by prep-HPLC (method A) to give title compound **1h** (46 mg, yield 29%) as a white solid. ESI m/**z**: 617 $(M + H)^+$.

Example 1i

(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-6-propyl-8-(2-{[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}acetyl)-5,7-dioxapentacyclo[10.8.0$0^{2,9}$.$0^{4,8}$.$0^{13,18}$]icosa-14,17-dien-16-one (1i, with epimers)

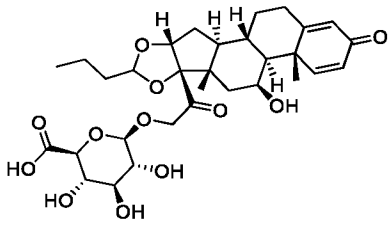


[0660] To a mixture of budesonide (**1a**, 0.22 g, 0.50 mmol) and acetobromo-**α**-D-Glucose (0.33 g, 0.80 mmol) in DCM (15 mL) was added 4 Å molecular sieves (1.0 g), and the mixture was stirred at RT for half an hour followed by the addition of silver trifluoromethanesulfonate (0.19 g, 0.75 mmol) at 0 °C. The suspension was stirred in dark at RT over weekend (72 hours) under nitrogen protection. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by prep-HPLC (method B) to give **Ac-1i** (25 mg, yield 9.2%) as a white solid. ESI m/**z**: 761 $(M + H)^+$. ^{1}H NMR ($DMSO_{d6}$, 400 MHz) (with epimers) δ 7.34-7.30 (m, 1H), 6.19-6.14 (m, 1H), 5.91 (s, 1H), 5.29 (t, J = 9.2 Hz, 1H), 5.17-5.15 (m, 0.5H), 5.03-5.01 (m, 0.5H), 4.96-4.90 (m, 1H), 4.85-4.74 (m, 3H), 4.70-4.67 (m, 1H), 4.64-4.63 (m, 0.5H), 4.59-4.56 (m, 0.5H), 4.39-4.28 (m, 2H), 4.20-4.16 (m, 1H), 4.05-3.99 (m, 2H), 2.56-2.40 (m, 1H), 2.32-2.26 (m, 1H), 2.13-1.89 (m, 14H), 1.83-1.63 (m, 3H), 1.59-1.48 (m, 3H), 1.44-1.21 (m, 6H), 1.15-0.91 (m, 2H), 0.86-0.81 (m, 6H) ppm.

[0661] To a solution of the compound **Ac-1i** (30 mg, 39 μmol) obtained above in water (1 mL) and methanol (3 mL) was added lithium hydroxide monohydrate (17 mg, 0.40 mmol) at 0 °C. After stirred at 0 °C for an hour, the mixture was directly purified by prep-HPLC (method B) to give **1i** (24 mg, 98% yield) as a white solid. ESI m/**z**: 593 $(M + H)^+$. ^{1}H NMR ($MeOD_{d4}$, 400 MHz) (with epimers) δ 7.46 (t, J = 10.4 Hz, 1H), 6.26 (dt, J = 10.0 and 2.0 Hz, 1H), 6.02 (s, 1H), 5.21 and 4.64 (t, J = 4.8 Hz, 1H), 5.16 (t, J = 7.2 Hz, 0.5H), 4.94-4.80 (m, 2.5H), 4.57-4.47 (m, 1H), 4.44-4.41 (m, 1H), 4.34-4.31 (m, 1H), 3.90-3.87 (m, 1H), 3.70-3.64 (m, 1H), 3.39-3.24 (m, 4H), 2.70-2.62 (m, 1H), 2.41-2.36 (m, 1H), 2.27-2.08 (m, 2H), 2.00-1.93 (m, 1H), 1.88-1.81 (m, 1H), 1.74-1.32 (m, 9H), 1.22-0.90 (m, 8H) ppm.

Example 1j

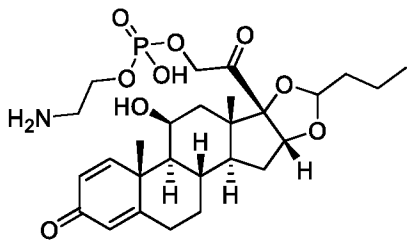
(2*S*,3*S*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0$0^{2,9}$.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}oxane-2-carboxylic acid (1j, with epimers)



[0662] Following the similar procedures as **1i** except substituting acetobromo-*α*-*D*-Glucuronic acid methyl ester for acetobromo-α-D-Glucose, the compound **1j** (24 mg, 9.2% yield in 2 steps) as a white solid was obtained. ESI m/**z**: 607 (M + H)$^+$. ^{1}H NMR (MeOD$_{d4}$, 400 MHz) δ 7.48 (t, J = 10.0 Hz, 1H), 6.28-6.24 (m, 1H), 6.02 (s, 1H), 5.22-5.15 (m, 1H), 4.98-4.81 (m, 2.5H), 4.65-4.56 (m, 1.5H), 4.45-4.39 (m, 2H), 3.62-3.59 (m, 1H), 3.52-3.46 (m, 1H), 3.41 (t, J = 8.8 Hz, 1H), 3.30-3.28 (m, 1H), 2.70-2.62 (m, 1H), 2.40-2.36 (m, 1H), 2.27-2.10 (m, 2H), 1.95-1.92 (m, 2H), 1.75-1.54 (m, 3H), 1.50 (s, 3H), 1.51-1.32 (m, 3H), 1.12-0.90 (m, 8H) ppm.

Example 1k

(2, 2113ethoxy)({2 1H)*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)13oxy)({2 1H), 3.30-3.28 (m, 1H), 2.70-2.62 (m, 1H), 2.40-2.36 (m, 1H)^{2,9}.0^{4,8}.0^{13,18}]icosa)({2 1H), 3.30-3.28 oxoethoxy})phosphinic acid (1k)



[0663] To a solution of triethylamine (0.67 g, 6.6 mmol) in chloroform (4 mL) was added phosphorus oxychloride (0.51 mg, 3.3 mmol) at 0 °C, followed by the addition of a solution of budesonide (**1a**, 1.3 g, 3.0 mmol) in chloroform (4 mL). After stirred at 20 °C for 2 hours, the mixture was cooled to 0 °C and was added a solution of Boc-ethanolamine (0.41 mg, 2.6 mmol) in chloroform (4 mL) and pyridine (3 mL). After stirred at 20 °C for an hour until the reaction was completed according to LCMS, the reaction mixture was quenched by the addition of water (2 mL) at 0 °C. The mixture was stirred at 20 °C overnight. The volatiles were removed in vacuo and the residue was purified by prep-HPLC (method B) to afford crude **Boc-1k** (330 mg, 17%) as yellow film. ESI m/**z**: 676 (M + Na)$^+$.

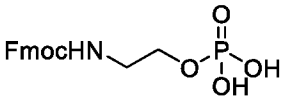
[0664] To a solution of **Boc-1k** (0.18 g, 0.28 mmol) in DCM (5 mL) was added trifluoroacetic acid (0.5 mL) at 0 °C. The resulting mixture was stirred at 23 °C for 2 hours until Boc was totally

removed according to LCMS. The volatiles were removed *in vacuo*. The residue was purified by prep-HPLC (method B) to afford **1k** as a white solid (90 mg, 59% yield). ESI m/**z**: 554 $(M + H)^+$. ^1H NMR (500 MHz, $MeOD_{d4}$) δ 7.49 (d, J = 10.1 Hz, 1H), 6.28 (d, J = 10.1 Hz, 1H), 6.03 (s, 1H), 5.24-5.15 (m, 1H), 4.87-4.62 (m, 3H), 4.45 (d, J = 6.1 Hz, 1H), 4.18-4.15 (m, 2H), 3.22-3.20 (m, 2H), 2.71-2.64 (m, 1H), 2.40 (d, J = 13.4 Hz, 1H), 2.29-1.71 (m, 6H), 1.65-1.32 (m, 9H), 1.22-0.91 (m, 7H) ppm. Anal. HPLC: > 99.9%, Retention time: 3.90 min (method B).

Example 1l

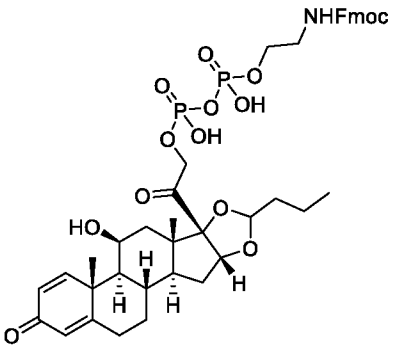
(2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethoxy)phosphonic acid

[0665] See J. Am. Chem. Soc., **2016**, 138(4), 1430-1445; WO2015153401; and Phosphorus, Sulfur and Silicon and the Related Elements, **2000**, 165, 83-90.



[0666] To a mixture of Fmoc-ethanolamine (1.1 g, 3.9 mmol) in THF (16 mL) was added diphosphoryl chloride (2.2 g, 8.8 mmol) by syringe at -40 °C. After stirred at -40 °C for an hour until the starting material was totally consumed, which was monitored by LCMS, the reaction mixture was quenched with water (1 mL) at -40 °C, treated with saturated aqueous sodium bicarbonate solution (200 mL) and kept at 10-20 °C overnight. The resulting mixture was acidified with *conc.* HCl to pH 2 and was then extracted with ethyl acetate. The combined organic solution was dried over sodium sulfate and concentrated to afford the crude title compound (1.6 g, crude) as a white solid, which was used for the next step without purification. ESI m/**z**: 364 $(M + H)^+$.

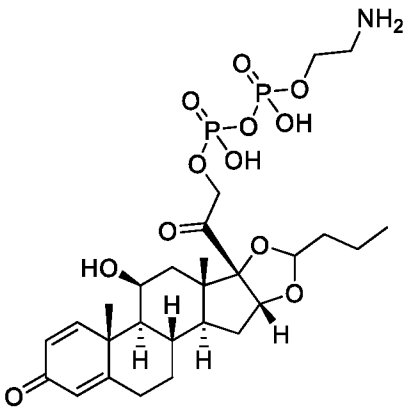
(2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethoxy)({[hydroxy({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy})phosphoryl]oxy})phosphinic acid (Fmoc-1l)



[0667] Following the above procedure except substituting budesonide (**1a**, 0.86 g, 20 mmol) for Fmoc-ethanolamine, the phosphonic budesonide intermediate **1a-PO_3H_2** (1.1 g, crude) as a white solid (ESI m/**z**: 551 $(M + H)^+$) was obtained.

[0668] To a solution of crude (2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethoxy)phosphonic acid (0.29 g, 0.80 mmol) in DMF (5 mL) were added triethylamine (81 mg, 0.80 mmol) and 1,1'-carbonyldiimidazole (CDI, 0.32 g, 2.0 mmol) at 10 °C. The mixture was stirred at 10-20 °C for 30 minutes before the phosphonic budesonide intermediate **1a-PO_3H_2** obtained above (0.41 g, 0.80 mmol) and zinc chloride (0.87 g, 6.4 mmol) were added into the reaction mixture. The resulting mixture was stirred at 10-20 °C overnight and starting material was totally consumed according to LCMS. The reaction was then quenched with diluted *aq.* HCl (1 N, 50 mL) and extracted with ethyl acetate. The combined organic solution was concentrated and the residue was purified by prep-HPLC (method B) to afford **Fmoc-1l** (0.32 g, yield 47%) as a white solid. ESI m/**z**: 856 $(M + H)^+$.

(2-Aminoethoxy)({[hydroxy({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy})phosphoryl]oxy})phosphinic acid (1l)

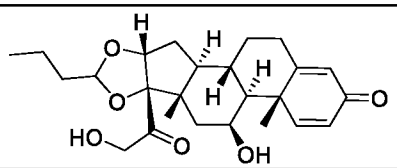
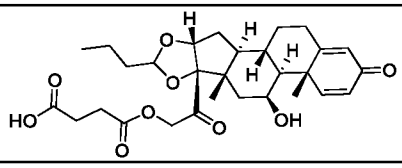
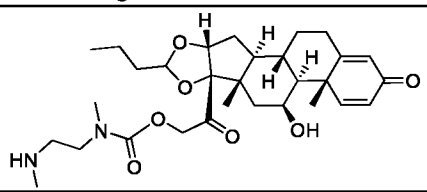
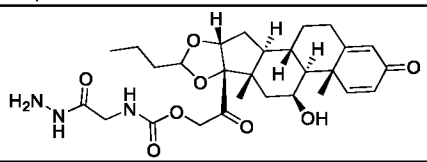
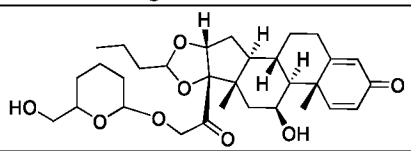
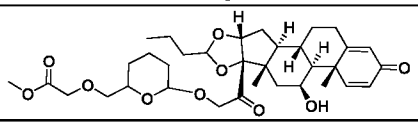


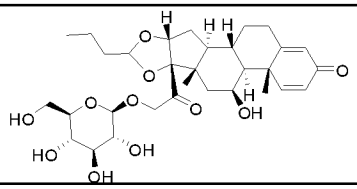
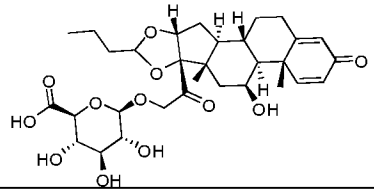
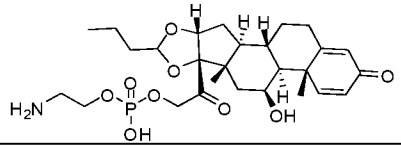
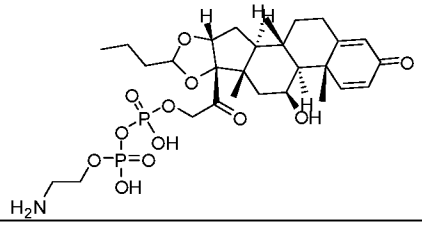
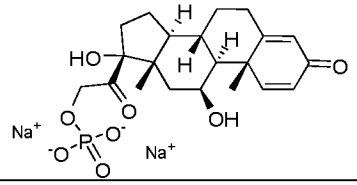
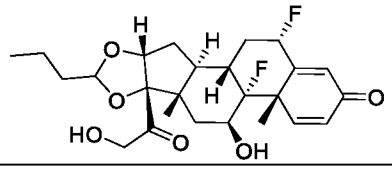
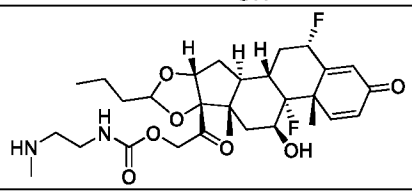
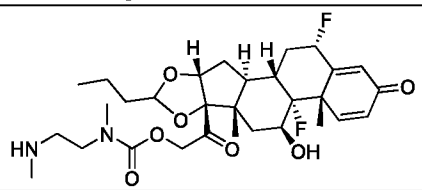
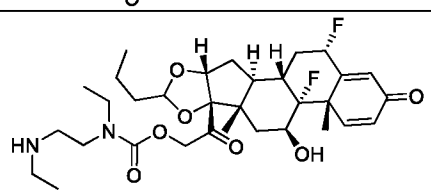
[0669] See WO2015153401.

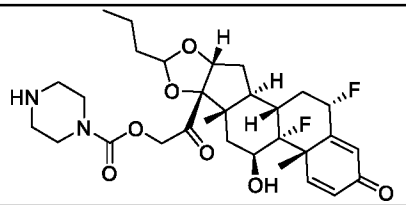
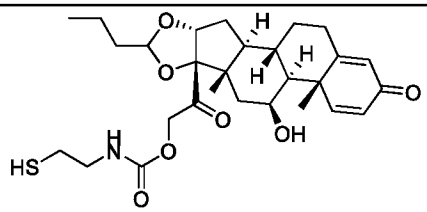
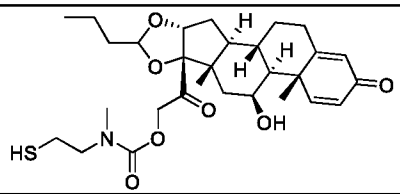
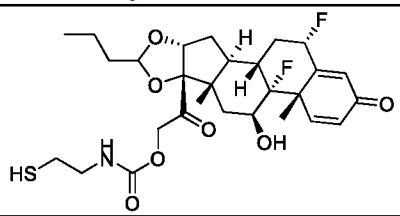
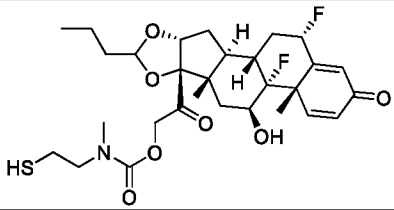
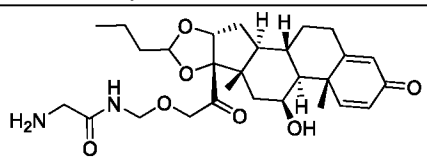
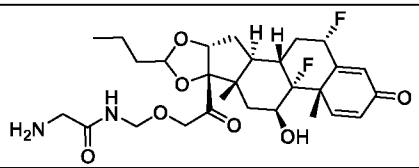
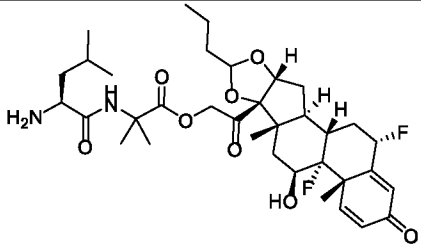
[0670] To a solution of **Fmoc-1l** (0.10 g, 0.12 mmol) in DCM (2 mL) was added piperidine (67 mg, 0.79 mmol) at 10 °C. The reaction mixture was stirred at 10-20 °C for 16 hours. Compound **Fmoc-1l** was totally consumed according to LCMS. The volatiles were removed in vacuo and the residue was purified by prep-HPLC (method B) to afford **1l** (50 mg, yield 68%) as a white solid. ESI m/**z**: 634 $(M + H)^+$. ^1H NMR (500 MHz, $MeOD_{d4}$) δ 7.50 (d, J = 10.0 Hz, 1H), 6.28 (d, J = 9.9 Hz, 1H), 6.03 (s, 1H), 5.23-5.16 (m, 1H), 5.02-4.97 (m, 1H), 4.88-4.67 (m, 1H), 4.45 (d, J = 3.4 Hz, 1H), 4.23 (s, 2H), 3.24 (s, 2H), 2.67 (dd, J = 13.5, 8.3 Hz, 1H), 2.40 (d, J = 11.3 Hz, 1H), 2.28-1.32 (m, 15H), 1.23-0.92 (m, 8H) ppm. Anal. HPLC: > 99.9%, Retention time: 2.74 min (method B).

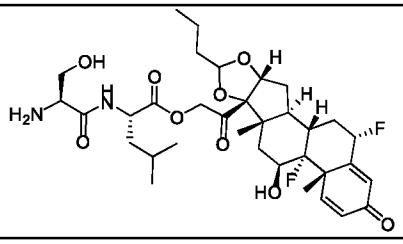
[0671] Table 1a below presents steroids made using the methods described herein.

Table 1a – Structure and Chemical-Physical Properties of Compounds

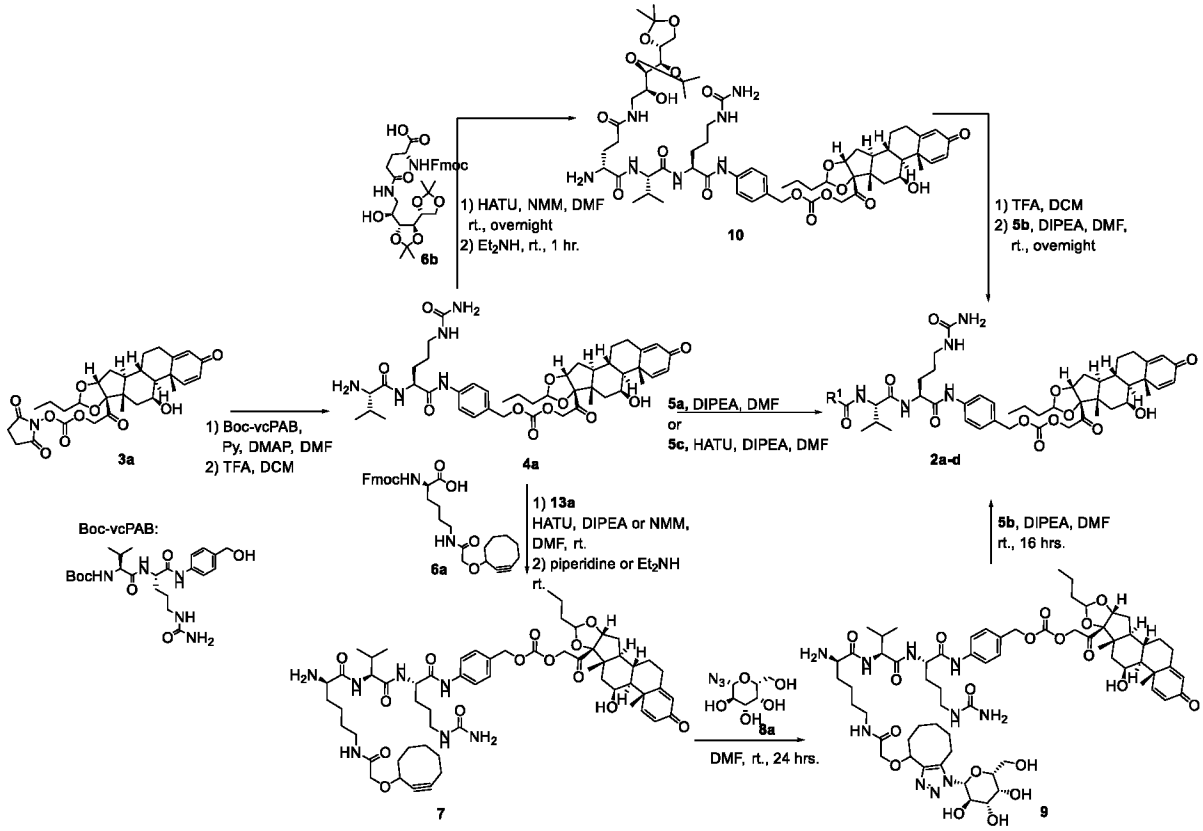
#	Structure	HPLC purity	cLogP	MF	MW (Cal.)	MS (M+H)
1a		95	2.73	$C_{25}H_{34}O_6$	430.55	431.3
1c		98	3.00	$C_{29}H_{38}O_9$	530.25	531.2
1d		93	2.92	$C_{30}H_{44}N_2O_7$	544.69	545.3
1e		100	1.44	$C_{28}H_{39}N_3O_8$	545.62	546.2
1g		92	3.65	$C_{31}H_{44}O_8$	544.68	545.3
1h		98	3.19	$C_{34}H_{48}O_{10}$	616.32	616.32

#	Structure	HPLC purity	cLogP	MF	MW (Cal.)	MS (M+H)
1i		100	0.96	$C_{31}H_{44}O_{11}$	592.67	593.4
1j		98	1.28	$C_{31}H_{42}O_{12}$	606.66	607.3
1k		100	1.18	$C_{27}H_{40}NO_9P$	553.58	554.1
1l		100	0.55	$C_{27}H_{41}NO_{12}P_2$	633.56	634.0
1m		100	1.15	$C_{21}H_{27}Na_2O_8P$	484.39	590.3
100		100	2.44	$C_{25}H_{32}F_2O_6$	466.51	467
101a		>95	2.40	$C_{29}H_{40}F_2N_2O_7.C_2HF_3O_2$	663.66	567
101b		>95	2.63	$C_{30}H_{42}F_2N_2O_7.C_2HF_3O_2$	678.69	581
101c		100	3.34	$C_{32}H_{46}F_2N_2O_7.C_2HF_3O_2$	722.74	609

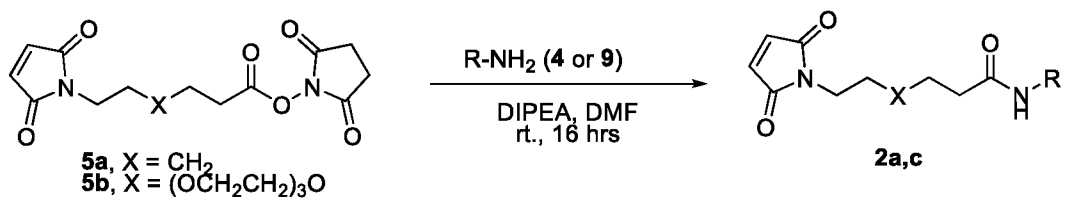
#	Structure	HPLC purity	cLogP	MF	MW (Cal.)	MS (M+H)
101d		100	2.46	$C_{30}H_{40}F_2N_2O_7$	578.64	579
102c		>95	3.47	$C_{28}H_{39}NO_7S$	533.68	531
102d		>95	3.69	$C_{29}H_{41}NO_7S$	547.71	548
102e		>95	3.17	$C_{28}H_{37}F_2NO_7S$	569.66	570
102f		>95	3.40	$C_{29}H_{39}F_2NO_7S$	583.69	584
103a		98	1.58	$C_{28}H_{40}N_2O_7$	516.64	517
103b		98	1.29	$C_{28}H_{38}F_2N_2O_7$	552.62	553
104a		>90	3.67	$C_{35}H_{50}F_2N_2O_8$	664.79	665

#	Structure	HPLC purity	cLogP	MF	MW (Cal.)	MS (M+H)
104b		>95	2.19	$C_{34}H_{48}F_2N_2O_9$	666.76	667

Scheme 2. Synthesis of VC-PAB Spacer-Budesonide



General procedure A for synthesis of MC- Spacer-Budesonide (2a and 2c)

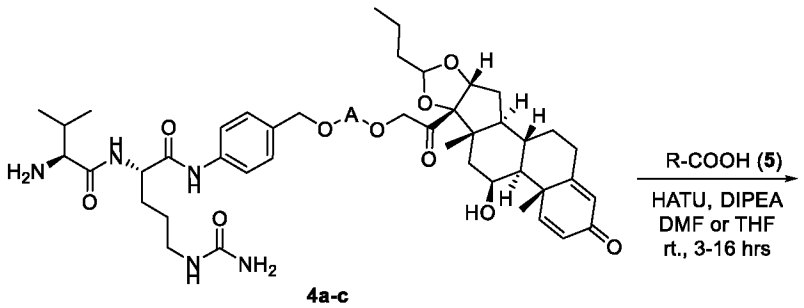


[0672] To a solution of vcPAB-Budesonide (**4a** A = CO, 1.0 equiv.) or amine **9** (1.0 equiv.) in Scheme 2 in DMF (*ca.* 1 mL per 10 mg amine) were added activated NHS ester **5** (1.5-3.0 equiv.) in the table below and DIPEA (2.0 equiv.) at RT. The reaction mixture was stirred at RT overnight when NHS ester and most of amine were consumed according to LCMS spectra. After filtration, the reaction solution was directly purified by prep-HPLC or reversed phase flash chromatography to give the desired amide **2** (*ca.* 17% yield) as a white solid.

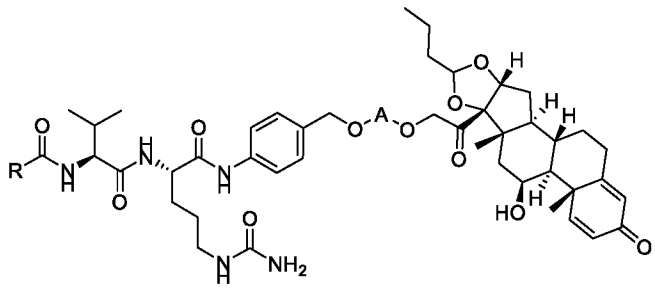
Amines		Activated ester 5		Base/reagents	Solvent	Time (hr)	Purification	Product 2
4a[c]	50 mg, 56 µmol	**5a**	28 mg, 91 µmol	DIPEA (23 mg, 0.18 mmol)	DMF (3 mL)	16	Prep-HPLC (method A)	**2a** (10 mg, 17%)
rxn solution of **9**		**5b**	8.8 mg, 20 µmol	DIPEA (4.0 mg, 31 µmol)	DMF (1 mL)	16	Prep-HPLC (method A)	**2d** (3.5 mg, 4%)

c. TFA salt

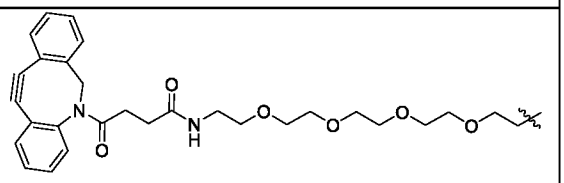
General procedure B for synthesis of amides 2b, 2l, 2m-precursor from acid

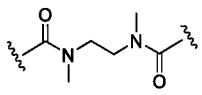
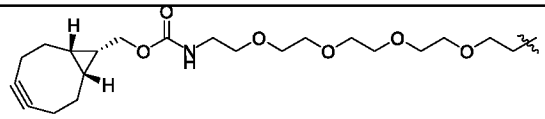
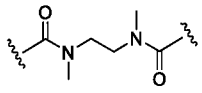
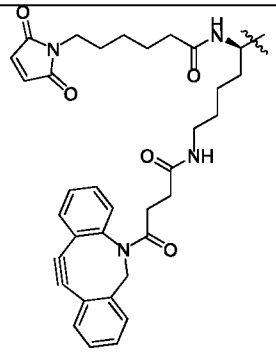


4a-c



2b, 2l, 2m-precursor

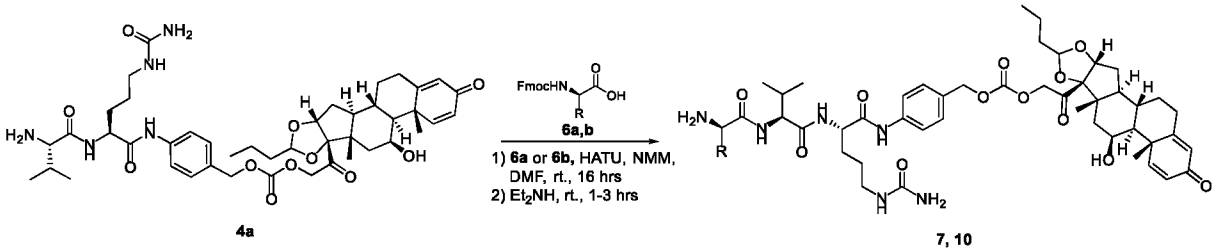
Products	Amines	Acids	A	R
2b	**4a**	**5c**		

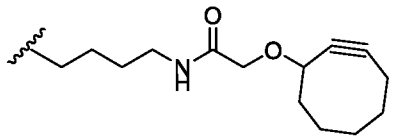
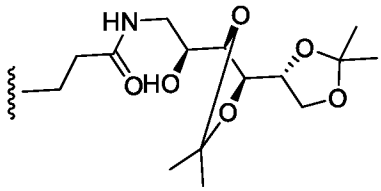
Products	Amines	Acids	A	R
2l	**4c**	**5d**		
2m - precursor	**4c**	**5e**		

[0673] To a solution of acid **5** (1.0-1.5 equiv.) in DMF or DCM or THF (1 mL per 5 mg **5**) were added DIPEA (2.0-5.0 equiv.) and HATU (1.4-2.2 equiv.) at RT. The resulting mixture was stirred at this temperature for 0.5-1 hour before the vcPAB-Budesonide (**4**, 1.0 equiv.) was added. The reaction mixture was stirred at RT for 3-16 hours until the amine was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtration was then separated by prep-HPLC or reversed phase flash chromatography to give the amide **2** (21-54% yield) as a white solid. In the table below are additional details.

Amines		acid		Base/reagents	Solvent	Time (hr)	Purification	Product 2
4b	10 mg, 11 µmol	**5c**	10 mg, 18 µmol	DIPEA (6.2 mg, 48 µmol) HATU (9.0 mg, 24 µmol)	DMF (1 mL)	16	Prep-HPLC (method B)	**2b** (3.0 mg, 21%)
4c	58 mg, 61 µmol	**5c**	37 mg, 67 µmol	DIPEA (15 mg, 0.12 mmol) HATU (34 mg, 89 µmol)	DMF (5 mL)	3	Prep-HPLC (method B)	**2i** (21 mg, 24%)
4c	8.9 mg, 9.4 µmol	**5d**	6.3 mg, 14 µmol	DIPEA (3.6 mg, 27 µmol) HATU (8.0 mg, 20 µmol)	DMF (1 mL)	16	Prep-HPLC (method B)	**2l** (7 mg, 54%)
4c	20 mg, 21 µmol	**5e**	13 mg, 21 µmol	DIPEA (8.0 mg, 62 µmol) HATU (12 mg, 31 µmol)	THF (5 mL)	16	Prep-HPLC (method A)	**2m - precursor** (5 mg)

General procedure C for synthesis of intermediate 7 and 10



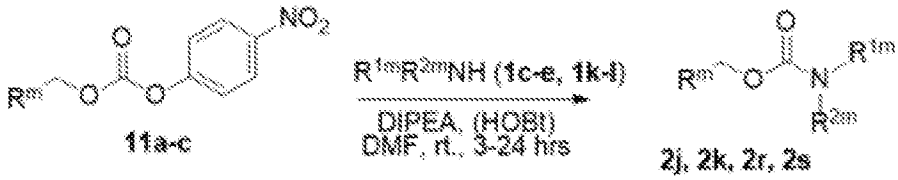
Fmoc-Aminoacid **6**	Product	R
6a	**7**	
6b	**10**	

[0674] To a solution of intermediate **6** (1.2-1.4 equiv.) in DMF was added HATU (1.5-1.7 equiv.) at RT. The solution obtained was stirred at RT for an hour. To this suspension were added a solution of vcPAB-budesonide (**4a**, 1.0 equiv.) in DMF (0.10 mL per mg of **4a**) and subsequently NMM (2.3-3.0 equiv.). The reaction mixture was stirred at RT for 16 hours and turned clear. The reaction was monitored by LCMS until compound **4a** was totally consumed. To the reaction mixture was then added diethylamine (excess), and the resulting mixture was then stirred at RT for 1-3 hours until Fmoc was removed according to LCMS. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method A) to give compound **7** (18% yield from compound **4a**) or purified by prep-HPLC (method B) to give compound **10** (5-% yield) as a white solid. In the table below are additional details.

Amines		**Intermediate 6**		**Base/reagents**	**Solvent**	**Time**	**Purification**	**Product**
4a	60 mg, 64 µmol	**6a**	46 mg, 86 µmol	NMM (15 mg, 0.15 mmol) HATU (41 mg, 0.11 mmol) then Et_2NH (0.5 mL)	DMF (3 mL)	16 hrs then 3 hrs	Prep-HPLC (method A)	**7** (13 mg, 18%)
4a	0.19 g,	**6b**	0.15 g, 0.24 mmol	NMM (61 mg, 0.60 mmol)	DMF (5 mL)	16 hrs	Prep-HPLC (method B)	**10** (13 mg, 5%)

Amines		Intermediate 6		Base/reagents	Solvent	Time	Purification	Product
	0.20 mmol			HATU (0.11 g, 0.29 mmol) then Et_2NH (1 mL)		then 1 hr		

General procedure D for synthesis of carbamates 2j, 2k, 2r, and 2s



[0675] The mixture was stirred at RT for 3-24 hours until **PNP activated ester** was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtrate was separated directly by prep-HPLC to give compound **2** (with or without diastereoisomers, 11-63% yield) as a white solid(s). In the table below are additional details.

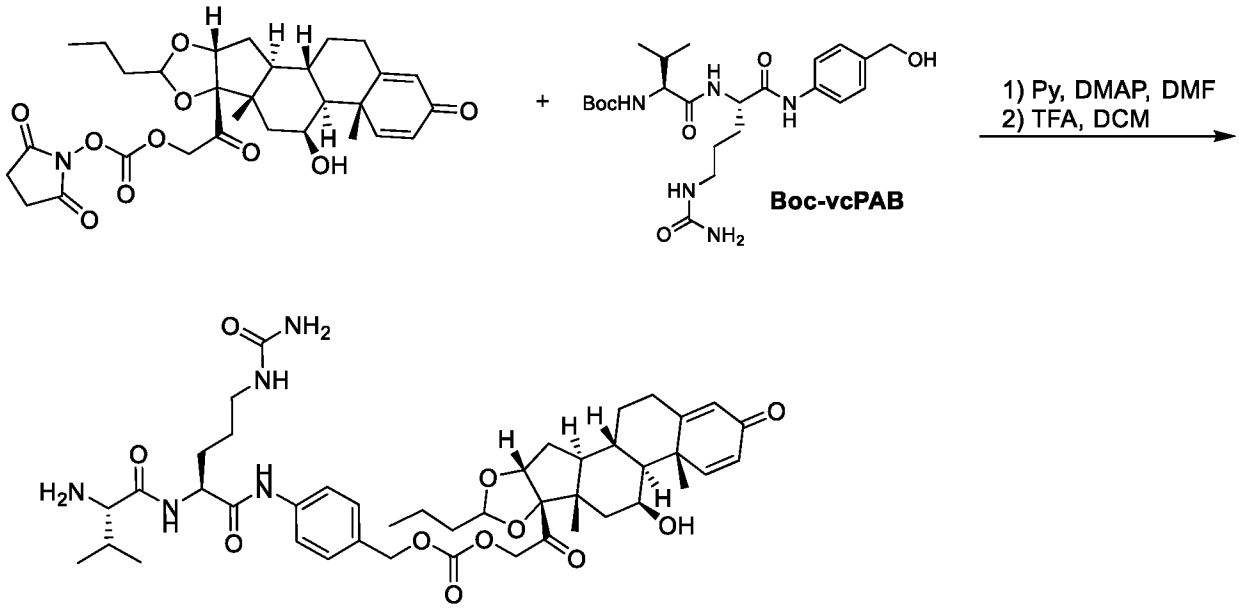
Spacer-budesonide 21		Activated ester 23		Base/reagents	Solvent	Time (hr)	Purification	Product 2
1e	15 mg, 28 µmol	**11a**	15 mg, 20 µmol	DIPEA (12 mg, 93 µmol) HOBt (4.0 mg, 30 µmol)	DMF (1 mL)	12	Prep-HPLC (method A)	**2j** (3.0 mg, 13%)
1d	20 mg, 37 µmol	**11a**	22 mg, 30 µmol	DIPEA (12 mg, 93 µmol) HOBt (6.0 mg, 44 µmol)	DMF (1 mL)	12	Prep-HPLC (method A)	**2k-A** (3.3 mg, 10%) **2k-B** (4.1 mg, 12%)
1k	50 mg, 90 µmol	**11c**	30 mg, 95 µmol	DIPEA (50 mg, 0.39 mmol)	DMF (1 mL)	3	Prep-HPLC (method B)	**2r** (40 mg, yield 61%)

Synthesis of 2a-d

[0676] Budesonide-Linkers **2a-d** were prepared from three approaches according to Scheme 2. The first approach was directly amide coupling reactions from vcPAB-Budesonide (**4a**), which was obtained from the activated ester of budesonide **3a** with linkers **5**. The second approach was via initial amide coupling reactions from vcPAB-Budesonide (**4a**) with intermediate **6a** to generate Budesonide-Linkers **7**, followed by 3+2 cyclization with galactose-azide (**8a**) to generate intermediates**9**, and finally by secondary amide coupling reactions with **5**. The third

approach was via first amide coupling reactions of **4** with intermediate **6b**, followed by amide formation with **5b** and sequentially deprotection of acetone to give **2d**.

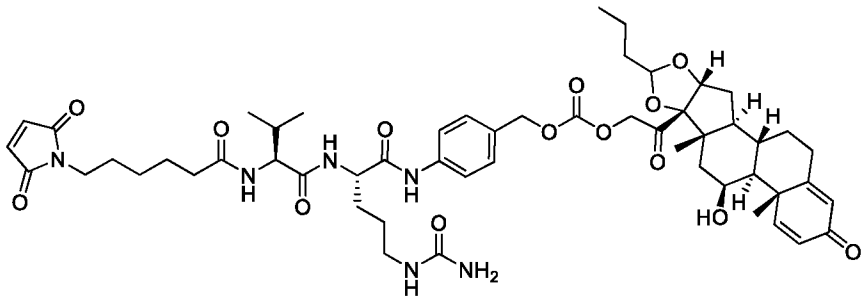
Intermediate 4a



[0677] 1.4 mmol) in dry DMF (10 mL) were added Boc-vc-PAB (**12a**) [WO2008/34124 A2] (0.59 g, 1.2 mmol), DMAP (0.30 g, 2.4 mmol) and pyridine (0.29 g, 3.7 mmol). The mixture was stirred at RT for 16 hours until **3a** was totally consumed according to LCMS. The reaction mixture was directly purified by reversed phase flash chromatography (50-80% acetonitrile in water) to give intermediate **Boc-4a** (0.74 g, yield 38%, ESI m/**z**: 936 $(M + H)^+$) as a white solid, which was dissolved in DCM (40 mL). To 5 mL of the DCM solution (containing 94 mg **Boc-4a**) was added TFA (0.5 mL) dropwise at 0 °C. After stirred at RT for 1.5 hours until the **Boc-4a** was consumed, which was monitored by LCMS, the resulting mixture was concentrated in vacuo to give crude title product **4a** (83 mg, yield 34% from budesonide) as its TFA salt as colorless oil, which can be used without purification for next step synthesis. 20 mg of the crude **4a** was purified by prep-HPLC (method B) to give pure **4a** (8 mg) as free base for plasma stability test. ESI m/**z**: 836 $(M + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.17 (s, 1H), 8.12 (br s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 10.2 Hz, 3H), 6.17 (d, J = 10.1 Hz, 1H), 5.97 (t, J = 5.7 Hz, 1H), 5.92 (s, 1H), 5.40 (s, 2H), 5.22-5.01 (m, 4H), 4.89-4.62 (m, 3H), 4.53-4.40 (m, 1H), 4.30 (s, 1H), 3.09-2.87 (m, 3H), 2.36-2.22 (m, 1H), 2.14-1.87 (m, 4H), 1.81 (d, J = 5.6 Hz, 2H), 1.75 (s, 2H), 1.62-1.52 (m, 4H), 1.51-1.41 (m, 2H), 1.40-1.19 (m, 8H), 1.18-0.92 (m, 2H), 0.92-0.82 (m, 9H), 0.78 (d, J = 6.8 Hz, 3H) ppm.

Example 2a

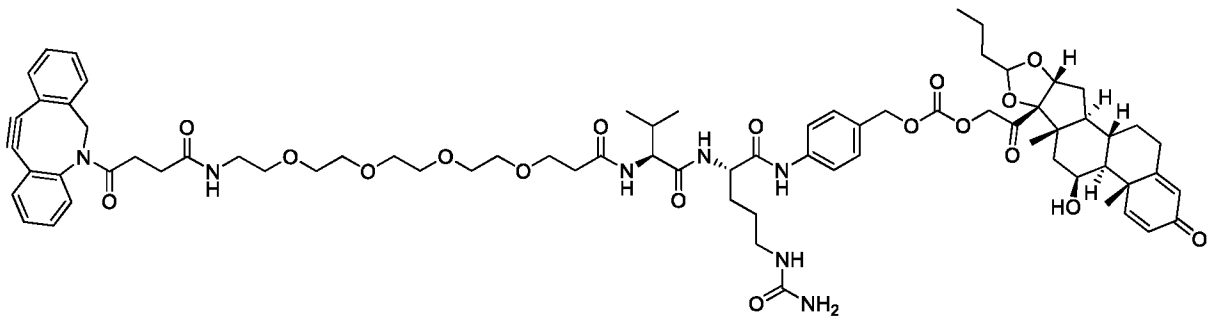
***N*-[(1*S*)-1-{[(1*S*)-4-(Carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamide (2a)**



[0678] Following the general procedure A, the title compound **2a** (10 mg, 17% yield) was obtained as a white solid. ESI m/**z**: 1029 $(M + H)^+$. Anal. HPLC: 92.5%, Retention time: 7.66 min (method A).

Example 2b

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanam-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-ido)-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide (2b)

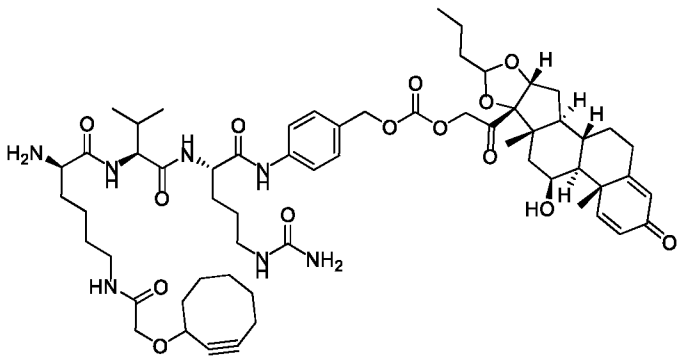


[0679] Following the general procedure B, the title compound **2b** (3.0 mg, 21% yield) was obtained as a white solid. ESI m/**z**: 686 $(M/2 + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.05 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.77 (t, J = 5.7 Hz, 1H), 7.71-7.66 (m,

1H), 7.66-7.59 (m, 3H), 7.52-7.27 (m, 9H), 6.17 (d, *J* = 10.0 Hz, 1H), 6.03-5.95 (m, 1H), 5.92 (s, 1H), 5.79-5.74 (m, 1H), 5.43 (s, 2H), 5.22-4.99 (m, 6H), 4.88-4.84 (m, 1H), 4.84-4.60 (m, 2H), 4.42-4.34 (m, 1H), 4.33-4.26 (m, 1H), 4.26-4.20 (m, 1H), 3.65-3.55 (m, 3H), 3.48-3.44 (m, 12H), 3.32-3.26 (m, 2H), 3.12-2.91 (m, 4H), 2.63-2.54 (m, 1H), 2.41-2.19 (m, 3H), 2.03-1.94 (m, 4H), 1.84-1.78 (m, 2H), 1.63-1.22 (m, 14H), 1.03-0.82 (m, 15H) ppm. Anal. HPLC: 96.9%, Retention time: 8.10 min (method B).

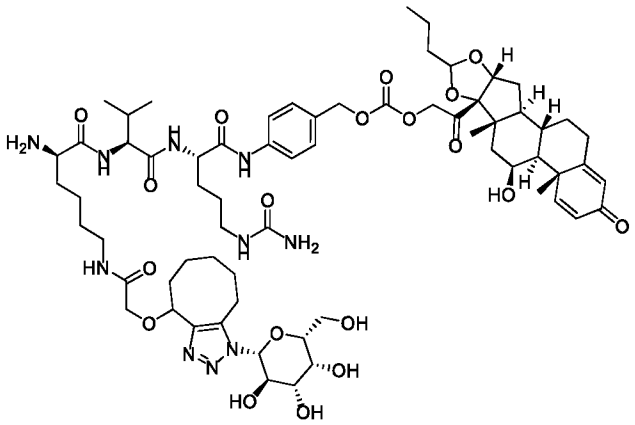
Example 2c

(2*R*)-2-Amino-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamide (7)



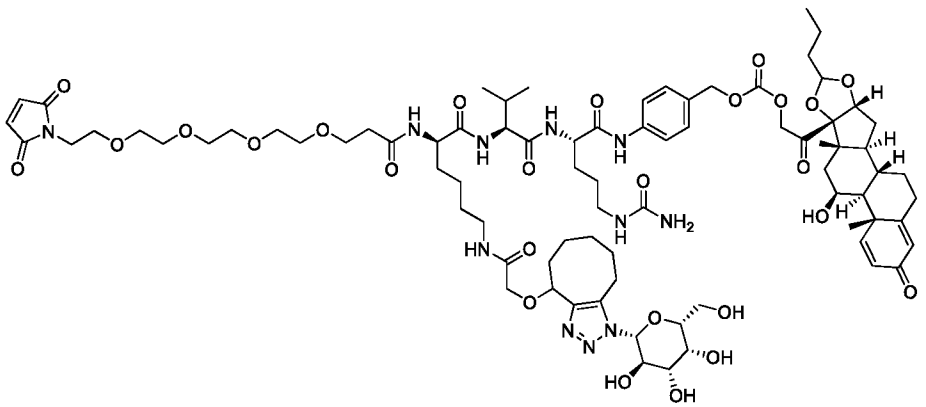
[0680] ESI m/**z**: 565 (M/2 + H)$^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 10.17-10.00 (m, 1H), 8.47-7.73 (m, 2H), 7.69-7.57 (m, 3H), 7.38-7.26 (m, 3H), 6.17 (d, *J* = 10.0 Hz, 1H), 5.99 (s, 1H), 5.92 (s, 1H), 5.76 (s, 1H), 5.42 (s, 2H), 5.23-5.01 (m, 4H), 4.88-4.61 (m, 3.4H), 4.43-4.16 (m, 4.6H), 3.85 (d, *J* = 14.7 Hz, 1H), 3.73 (d, *J* = 14.5 Hz, 1H), 3.23-3.18 (m, 1H), 3.09-2.90 (m, 4H), 2.33-2.18 (m, 3H), 2.18-2.04 (m, 3H), 2.03-1.66 (m, 10H), 1.64-1.50 (m, 7H), 1.49-1.22 (m, 13H), 1.17-0.90 (m, 4H), 0.90-0.79 (m, 12H) ppm.

(2*R*)-2-Amino-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-6-[2-({1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]hexanamide (9)



[0681] After filtration, the resulting solution of **9** was directly used directly for the next step. ESI m/**z**: 667 $(M/2 + H)^+$

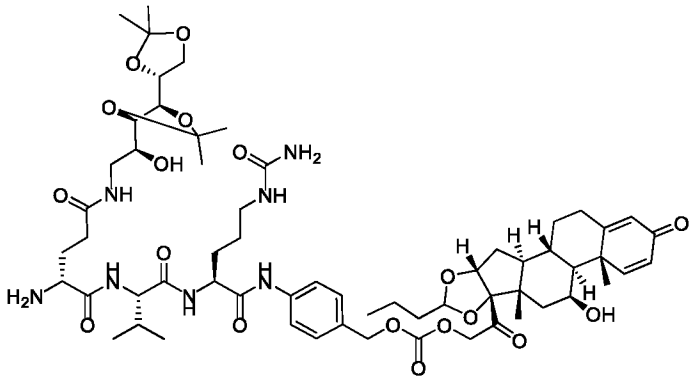
***N*-[(1*R*)-1-{[(1*S*)-1-{[(1*S*)-4-(Carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-[2-({1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]pentyl]-1-(2,5-dioxopyrrol-1-yl)-3,6,9,12-tetraoxapentadecan-15-amide (Mc-PEG$_4$-*N*(sugar-COT)Lys-vc-PAB-Budesonide) (2c)**



[0682] ESI m/**z**: 831 (M/2 + H)$^+$. ^{1}H NMR (400 MHz, DMSO$_{d6}$) δ 9.73 (s, 1H), 8.21-8.07 (m, 3H), 7.94-7.82 (m, 1H), 7.71-7.59 (m, 3H), 7.39-7.26 (m, 4H), 7.02 (s, 1H), 6.17 (d, *J* = 10.0 Hz, 1H), 6.09-5.98 (m, 1H), 5.92 (s, 1H), 5.56-5.28 (m, 4H), 5.25-5.01 (m, 4H),4.90-4.62 (m, 5H), 4.39-4.13 (m, 5H), 3.84-3.69 (m, 4H), 3.59-3.40 (m, 16H), 3.17-2.79 (m, 9H), 2.43-1.71 (m, 13H), 1.71-1.42 (m, 15H), 1.40-1.19 (m, 11H), 1.04-0.77 (m, 15H) ppm. Anal. HPLC: 97.6%, Retention time: 7.63 min (method A).

Example 2d

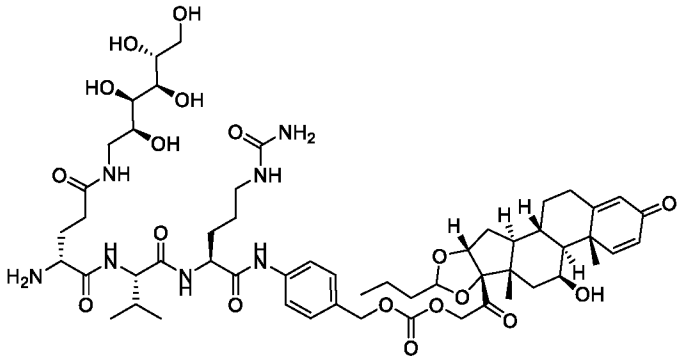
(2*R*)-2-Amino-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-*N*'-[(2*S*)-2-[(4*R*,5*R*)-5-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethyl-1,3-dioxolan-4-yl]-2-hydroxyethyl]pentanediamide (10)



[0683] Following the general procedure C, compound **10** (13 mg, 5% yield) was obtained as a white solid. ESI m/**z**: 605 (M/2 + H)$^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 10.08 (s, 1H), 8.34-8.17 (m, 1H), 7.98 (s, 1H), 7.93-7.81 (m, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.42-7.26 (m, 3H), 6.17 (d, *J* = 10.1 Hz, 1H), 5.99 (s, 1H), 5.92 (s, 1H), 5.76 (s, 1H), 5.42 (s, 2H), 5.23-5.01 (m, 4H), 4.91-4.61 (m, 4H), 4.43-4.20 (m, 3H), 4.10-3.72 (m, 6H), 3.60-3.54 (m, 1H), 3.24-3.09 (m, 2H), 3.07-2.91 (m, 2H), 2.32-2.25 (m, 1H), 2.22-2.10 (m, 2H), 2.04-1.93 (m, 2H), 1.88-1.67 (m, 5H), 1.64-1.22 (m, 27H), 1.17-0.93 (m, 3H), 0.91-0.81 (m, 12H) ppm.

(2*R*)-2-Amino-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-

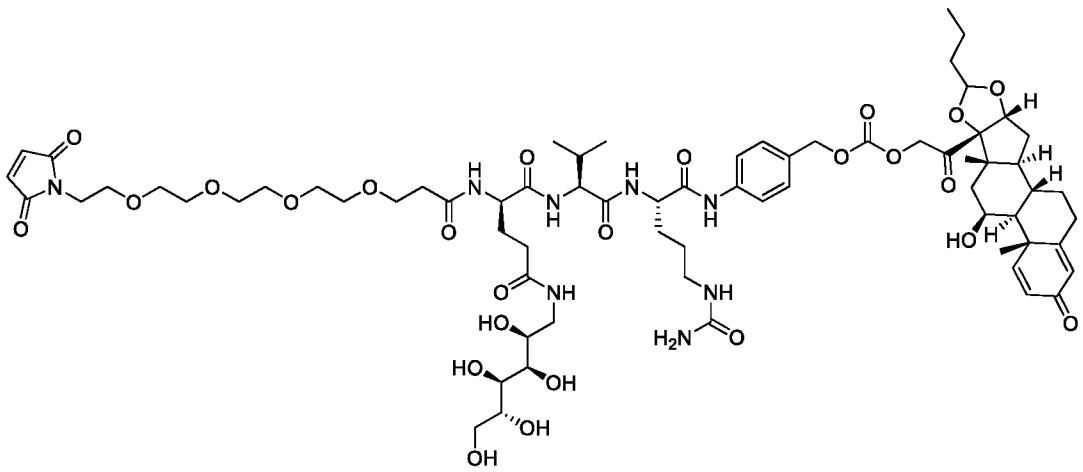
oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-*N'*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]pentanediamide (**10A**)



[0684] To a mixture of compound **10** (13 mg, 11 μmol) in DCM (1 mL) was added TFA (1 mL) dropwise. The mixture was stirred at RT for an hour which was monitored by LCMS. The volatiles were removed *in vacuo* to give crude de-protection product **10A** (13 mg) as a light yellow oil, which was used for the next step without further purification. ESI m/**z**: 565 $(M/2 + H)^+$.

(2*R*)-*N*-[(1*S*)-1-{[(1*S*)-4-(Carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-

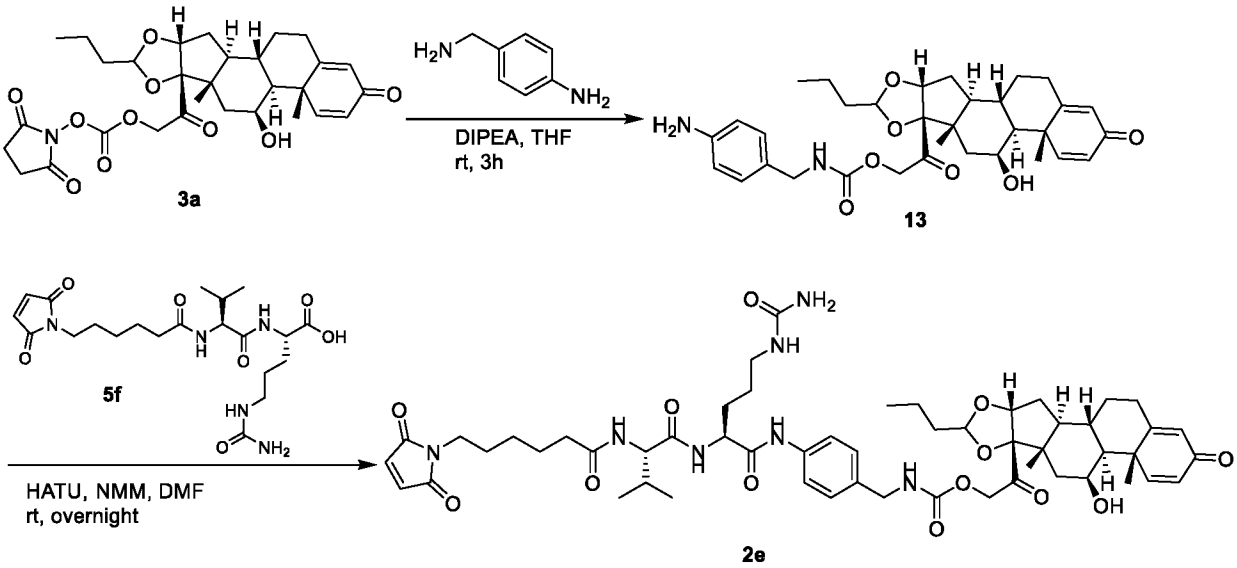
9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.$0^{2,9}$.$0^{4,8}$.$0^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-2-[1-(2,5-dioxopyrrol-1-yl)-3,6,9,12-tetraoxapentadecan-15-amido]-*N'*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]pentanediamide (**2d**)



[0685] Following the general procedure A, the title compound **2d** (3.5 mg, 1% total yield from **4a**) was obtained as a white solid. ESI m/**z**: 829.7 $(M/2 + H)^+$. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 10.10 (s, 0.25H), 9.79 (s, 0.75H), 8.22 (d, J = 7.1 Hz, 1H), 8.12 (d, J = 7.5 Hz, 1H), 8.07 (d, J =

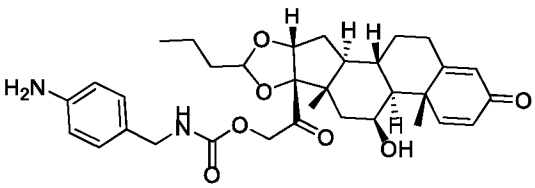
7.8 Hz, 1H),7.79-7.71 (m, 1H), 7.69-7.60 (m, 2H), 7.38-7.27 (m, 4H), 7.02 (d, *J* = 1.4 Hz, 2H), 6.17 (dt, *J* = 7.6, 1.6 Hz, 1H), 6.03-5.97 (m, 1H), 5.92 (s, 1H), 5.46-5.40 (m, 2H), 5.35-5.02 (m, 4H), 4.89-4.61 (m, 4H), 4.50-4.45 (m, 1H), 4.40-4.16 (m, 7H), 3.65-3.37 (m, 21H), 3.07-2.90 (m, 3H), 2.44-2.23 (m, 4H), 2.17-1.93 (m, 6H), 1.90-1.71 (m, 4H), 1.62-1.20 (m, 15H), 1.06-0.76 (m, 15H) ppm. Anal. HPLC: >99%, Retention time: 6.40 min (method A).

Scheme 3. Synthesis of 2e



Example 2e

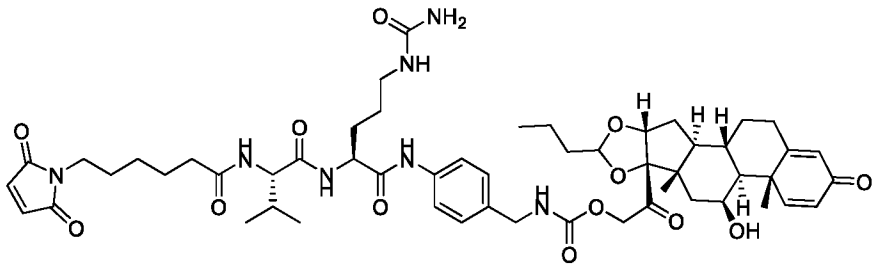
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-[(4-aminophenyl)methyl]carbamate (13)



[0686] To a solution of 4-aminobenzylamine (9.7 mg, 79 μmol) and DIPEA (12 mg, 93 μmol) in THF (10 mL) was added a solution of **3a** (24 mg, 44 μmol) in THF (5.0 mL) dropwise at RT. The mixture was stirred at RT for 3 hours until **3a** was totally consumed, which was monitored by LCMS. The volatiles were removed in vacuo and residue was purified via prep-HPLC (method B) to give compound **13** (5.6 mg, yield 22%) as a white solid. ESI m/**z**: 579.2 $(M + H)^+$. ^{1}H NMR (400 MHz, $MeOD_{d4}$) δ 7.48-7.45 (m, 3H), 7.31-7.28 (m, 2H), 6.27-6.24 (m, 1H), 6.02 (s,

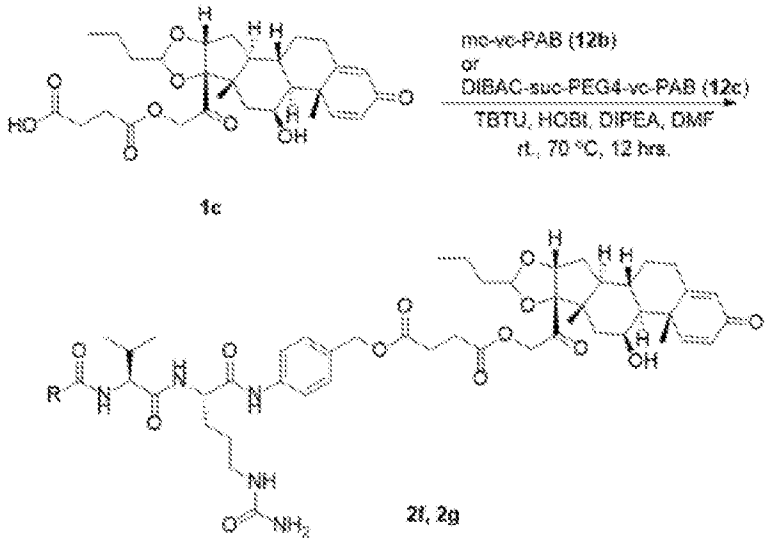
1H), 5.22 (m, 1H), 5.12-5.11 (m, 1 H), 4.96 (m, 1 H), 4.86-4.81 (m, 2 H), 4.66 (m, 1 H), 4.44-4.35 (m, 3 H), 2.66-2.65 (m, 1H), 2.40-2.36 (m, 1 H), 2.15-2.10 (m, 2 H), 1.97-1.60 (m, 6H), 1.53-1.36 (m, 6H), 1.09-0.92 (m, 7H) ppm.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-3-methylbutanamido]pentanamido]phenyl}methyl)carbamate (2e)



[0687] To a mixture of MC-VC-OH **5f** (WO2014/191578 A1) (5.7 mg, 12 μmol) in dry DMF (1.5 mL) were added HATU (4.6 mg, 12 μmol) and NMM (4.0 mg, 40 μmol) at RT. The mixture was stirred at RT for 10 minutes before compound **13** (4.7 mg, 8.1 μmol) was added into the reaction mixture. The resulting mixture was stirred at RT overnight. Compound **13** was totally consumed and desired product was detected as major product according to LCMS. The mixture was directly purified by prep-HPLC to give title compound **2e** (2.9 mg, yield 35%) as a white solid. ES m/**z**: 1029.1 $(M + H)^+$, 514.7 $(M/2 + H)^+$. Anal. HPLC: >99%, Retention time: 7.54 min (method A).

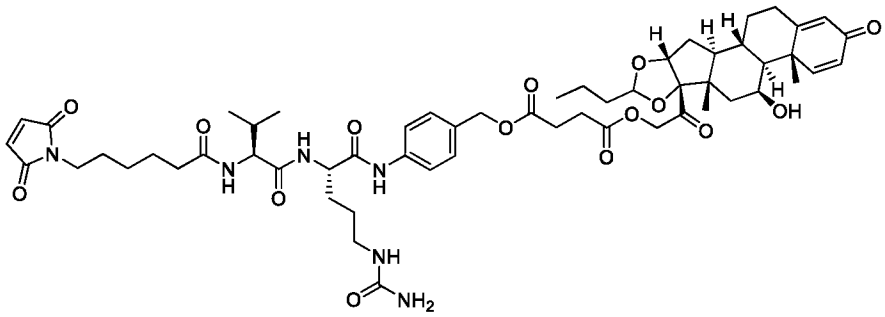
Scheme 4. Synthesis of linker-spacer-budesonide (via ester) 2f and 2g



Compd #	R
2f	MC
2g	DIBAC-suc-PEG4

Example 2f

1-{4-[(2*S*)-5-(Carbamoylamino)-2-[(2*S*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-3-methylbutanamido]pentanamido]phenyl}methyl 4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl} butanedioate (2f)

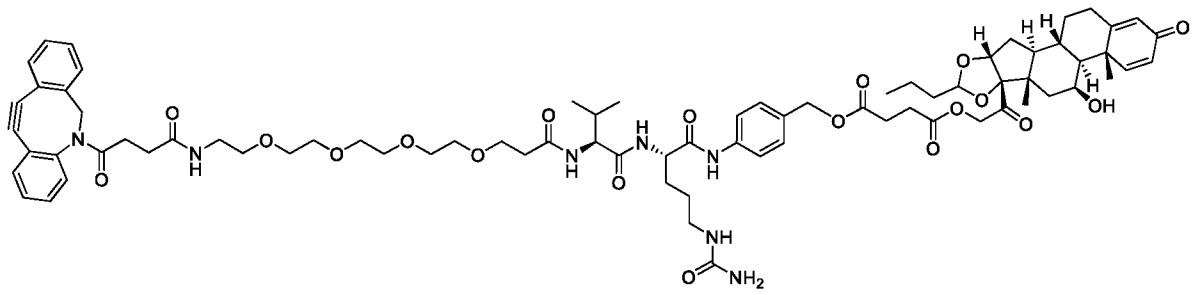


[0688] To a solution of **1c** (20 mg, 38 μmol) in DMF (2 mL) were added MC-VCPAB (**12b**, 0.11 g, 0.19 mmol), TBTU (63 mg, 0.19 mmol), HOBt (26 mg, 0.19 mmol) and DIPEA (41 mg, 0.19 mmol) at RT. The resulting mixture was stirred at 70 °C for 12 hours. No more product **2f** was formed, which was monitored by LCMS. The reaction mixture was directly purified by prep-HPLC (method A) to give title compound **2f** (2.6 mg, yield 6%) as a white solid. ESI m/**z**: 1085.3

(M + H)$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-7.52 (m, 1H), 7.34-7.30 (m,1H), 6.67 (br s, 1H), 6.30-6.27 (m, 1H), 6.03 (br s, 1H), 5.16-5.11 (m, 2H), 5.09-4.87 (m, 2H), 4.80-4.61 (m, 1H), 4.60-4.30 (m, 2H), 3.47-3.44 (m, 1H), 3.21-3.10 (m, 1H), 3.03-2.96 (m, 2H), 2.79-2.55 (m, 4H), 2.35-1.73 (m, 28H), 1.71-1.32 (m, 15H), 1.29-1.07 (m, 4H), 0.99-0.85 (m, 9H) ppm.

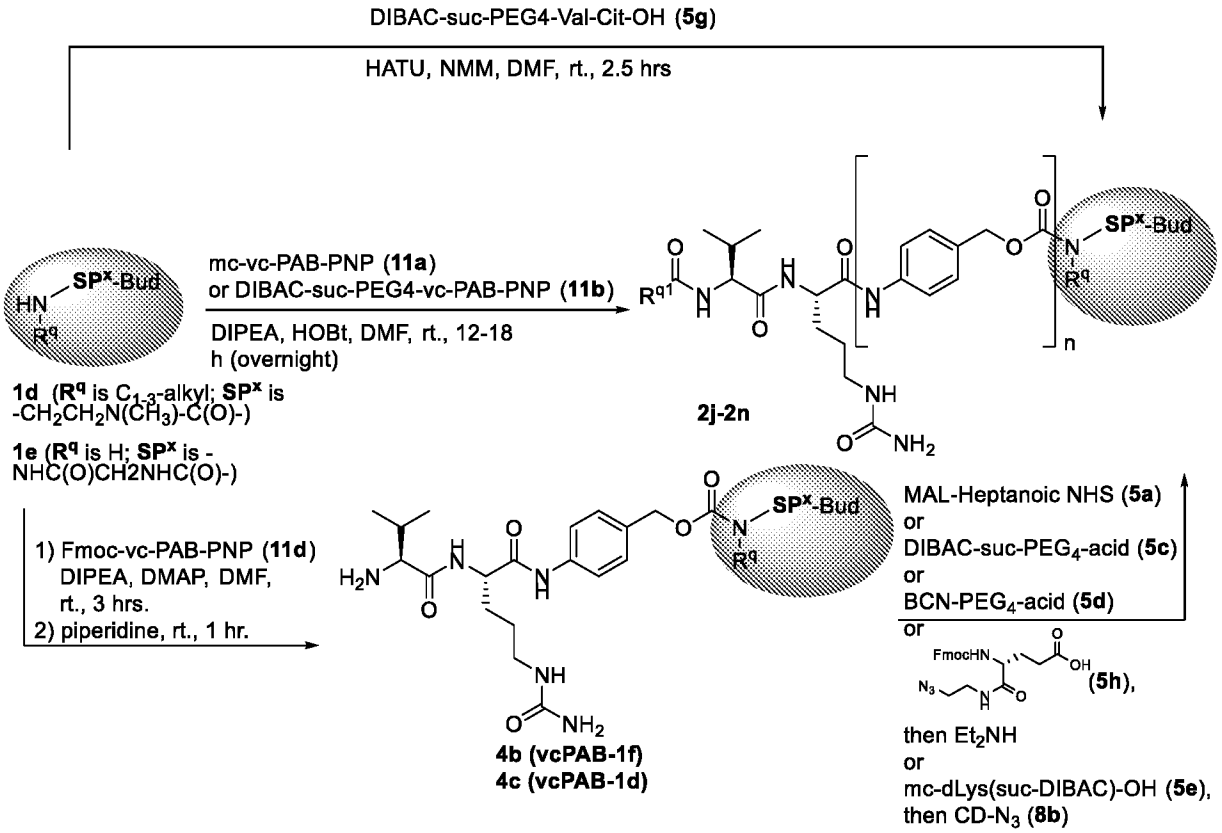
Example 2g

1-{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl} butanedioate (2g)



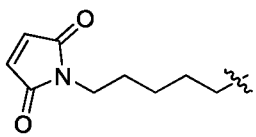
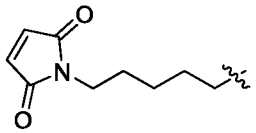
[0689] Following the procedures of **example 2f** except substituting DIBAC-suc-PEG4-vc-PAB (**12c**) for **12b**, the title compound **2g** (15 mg, yield 19%) as a white solid. ESI m/**z**: 714 (M/2 + H)$^+$. ^{1}H NMR (500 MHz, $MeOD_{d4}$) δ 7.67-7.60 (m, 4H), 7.48-7.46 (m, 4H), 7.38-7.32 (m, 4H), 7.26 (d, *J* = 6.8 Hz, 1H), 6.27 (d, *J* = 10.0 Hz, 1H), 6.03 (s, 1H), 5.24-5.10 (m, 4H), 5.02-4.97 (m, 1H), 4.85-4.66 (m, 2H), 4.61 (s, 1H), 4.53 (dd, *J* = 9.1, 4.9 Hz, 1H), 4.45-4.43 (m, 1H), 4.22 (dd, *J* = 6.7, 4.7 Hz, 1H), 3.78-3.69 (m, 3H), 3.60-3.55 (m, 11H), 3.47-3.40 (m, 2H), 3.26-3.10 (m, 4H), 2.78-2.54 (m, 8H), 2.41-2.35 (m, 2H), 2.25-2.11 (m, 4H), 2.02-1.31 (m, 17H), 1.21-0.92 (m, 14H) ppm. Anal. HPLC: 99.5%, Retention time: 7.86 min (method B).

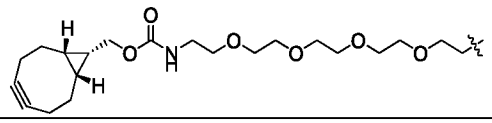
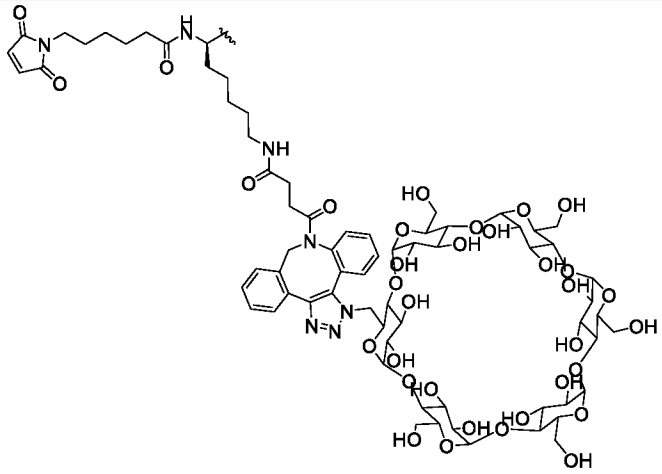
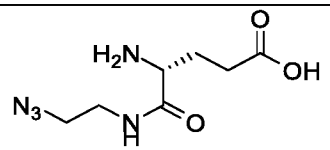
Scheme 5. Synthesis of linker-spacer-budesonide 2j-2n (via amide or carbamate)



[0690] In Scheme 5, Bud refers to budesonide.

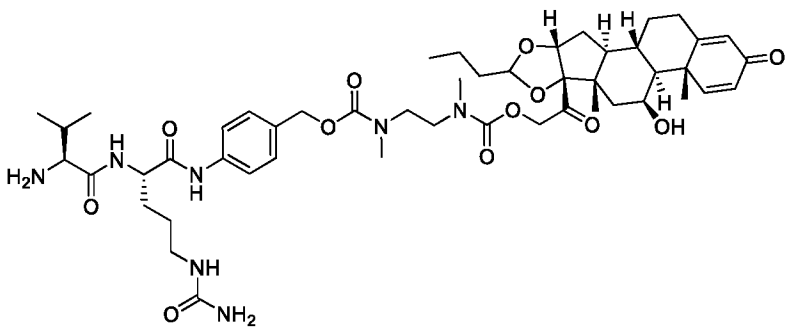
[0691] Most Budesonide-Linkers (**2**) having VC-PAB moiety were synthesized from three approaches via amide or carbamate (Scheme 5). The compounds **1d**, **1e** were separately conjugated with linkers by amide coupling to give amide or carbamate (Scheme 5). In the table below are additional details.

Compd No	**R^{q1} in 2j, 2k, 2l, 2m, 2n**	**n in 2j-n**	R^q-NH-Spacer-Budesonide
2j		1	**1e**
2k		1	**1d**

Compd No	R^{q1} in 2j, 2k, 2l, 2m, 2n	n in 2j-n	R^{q}-NH-Spacer-Budesonide
2l		1	**1d**
2m		1	**1d**
2n		1	**1d**

Intermediate 4c

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)-*N*-methylcarbamate (4c)

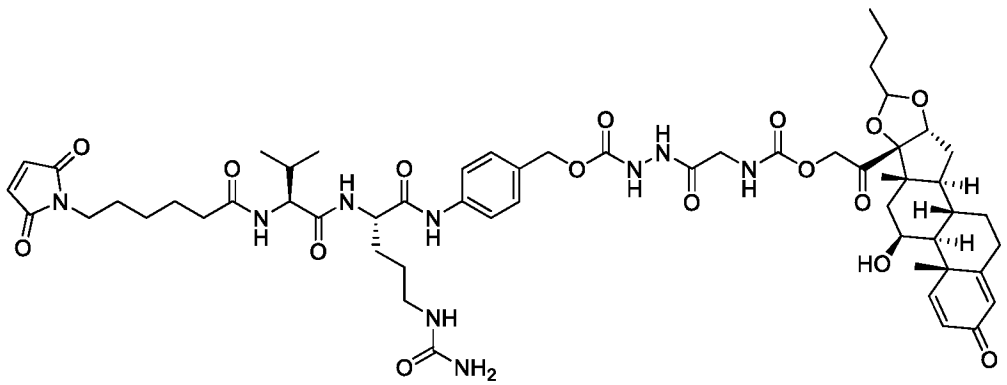


[0692] To a solution of compound **1d** (40 mg, 73 μmol) in DMF (3 mL) were added Fmoc-vcPAB-PNP (**11d**, 60 mg, 78 μmol), DMAP (9.0 mg, 74 μmol) and DIPEA (20 mg, 0.16 mmol) at

room temperature. The mixture was stirred at room temperature for 3 hours until most of starting materials were consumed, which was monitored by LCMS. To the reaction mixture was then added piperidine (1 mL). After stirred at room temperature for an hour until the de-Fmoc reaction was completed, which was monitored by LCMS, the reaction mixture was directly purified by prep-HPLC (method B) to give compound **4c** (9.0 mg, yield 13%, the 2nd peak in LC) as a white solid. ESI m/**z**: 951 $(M + H)^+$, 973 $(M + Na)^+$.

Example 2j

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-({*N'*-[({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-3-methylbutanamido]pentanamido]phenyl}methoxy)carbonyl]hydrazinecarbonyl}methyl)carbamate (2j)

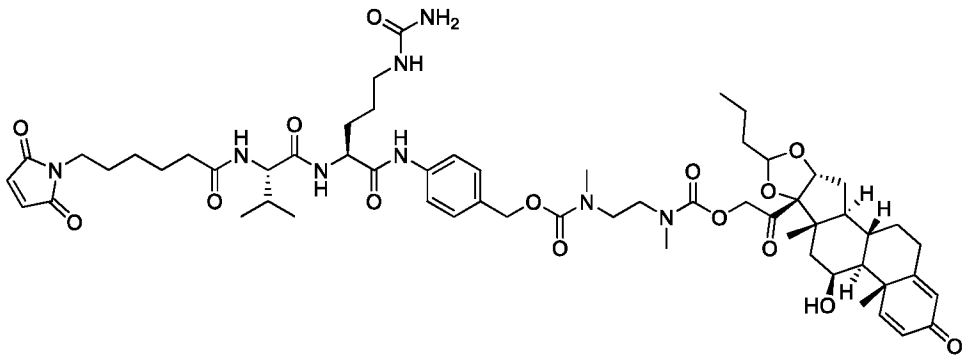


[0693] ESI m/**z**: 1144.3 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 9.77 (s, 1H), 9.18 (s, 1H), 8.12 (d, J = 7.2 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.73-7.70 (m, 1H), 7.61-7.59 (m, 2H), 7.32-7.29 (m, 3H), 7.01 (s, 2H), 6.19-6.16 (m, 1H), 6.00-5.92 (m, 2H), 5.05-5.00 (m, 2H), 4.95-4.85 (m, 2H), 4.71-4.62 (m, 2H), 4.40-4.17 (m, 3H), 3.71-3.58 (m, 2H), 3.45-3.39 (m, 2H), 3.06-2.89 (m, 3H), 2.33-2.28 (m, 2H), 2.20-2.07 (m, 3H), 2.02-1.91 (m, 3H), 1.81-1.75 (m, 2H), 1.75-1.62 (m, 2H), 1.60-1.44 (m, 11H), 1.42-1.20 (m, 6H), 1.19-1.12 (m, 4H), 1.00-0.92 (m, 2H), 0.88-0.81 (m, 10H) ppm.

Example 2k

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-3-

methylbutanamido]pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)-*N*-methylcarbamate (2k-A and 2k-B)



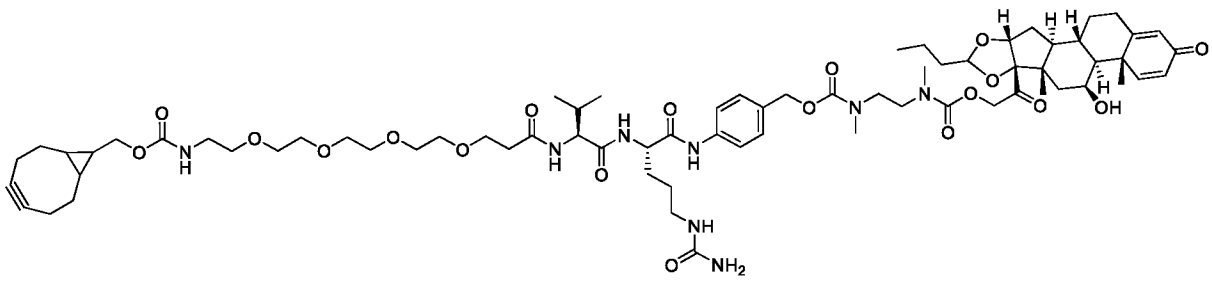
[0694] Following the general procedure D, the title compounds **2k-A** (3.3 mg, yield 10%, the second peak in LC) and **2k-B** (4.1 mg, yield 12%, the first peak in LC) as diastereoisomers were obtained as white solids.

2k-A: ESI m/**z**: 1143.4 $(M + H)^+$, Retention time: 1.70 min (method A).

2k-B : ESI m/**z**: 1143.4 $(M + H)^+$, Retention time: 1.65 min (method A).

Example 2l

Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(14-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)(methyl)amino]ethyl}(methyl)carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}-3,6,9,12-tetraoxatetradecan-1-yl)carbamate (2l)

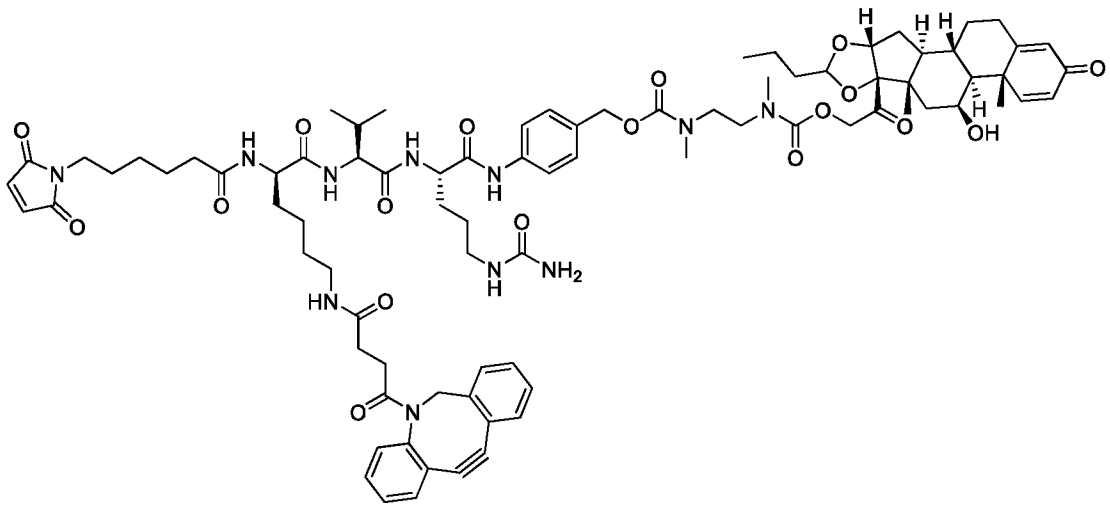


[0695] ESI m/**z**: 687.5 $(M/2 + H)^+$, 1396.8 $(M + Na)^+$ (50%), ^{1}H NMR (500 MHz, $MeOD_{d4}$) δ 7.66 (d, J = 7.5 Hz, 2H), 7.50 (d, J = 10.0 Hz, 1H), 7.36 (d, J = 7.0 Hz, 2H), 6.28 (d, J = 10.0 Hz, 1H), 6.04 (s, 1H), 5.15-5.05 (m, 2H), 4.87-4.62 (m, 4H), 4.53(s, 1H), 4.46(s, 1H), 4.21 (d, J = 6.5

Hz, 1H), 4.16 (d, *J* = 8.0 Hz, 2H), 3.83-3.72 (m, 2H), 3.66-3.60 (m, 12H), 3.56-3.52 (m, 3H), 3.49-3.42 (m, 1H), 3.30 (s, 3H), 3.25-3.09 (m, 3H), 3.02-2.96 (m, 4H), 2.87 (dd, *J* = 16.6, 4.8 Hz, 2H), 2.72-2.64 (m, 1H), 2.58 (t, *J* = 6.0 Hz, 2H), 2.40 (d, *J* = 13.1 Hz, 1H), 2.31-2.10 (m, 9H), 2.01-1.91 (m, 3H), 1.81-1.70 (m, 2H), 1.63 (s, 7H), 1.51 (s, 3H), 1.48-1.30 (m, 4H), 1.11 (s, 1H), 1.02-0.91 (m, 15H) ppm. Anal. HPLC: > 99.9%, Retention time: 9.40 min (method A).

Example 2m

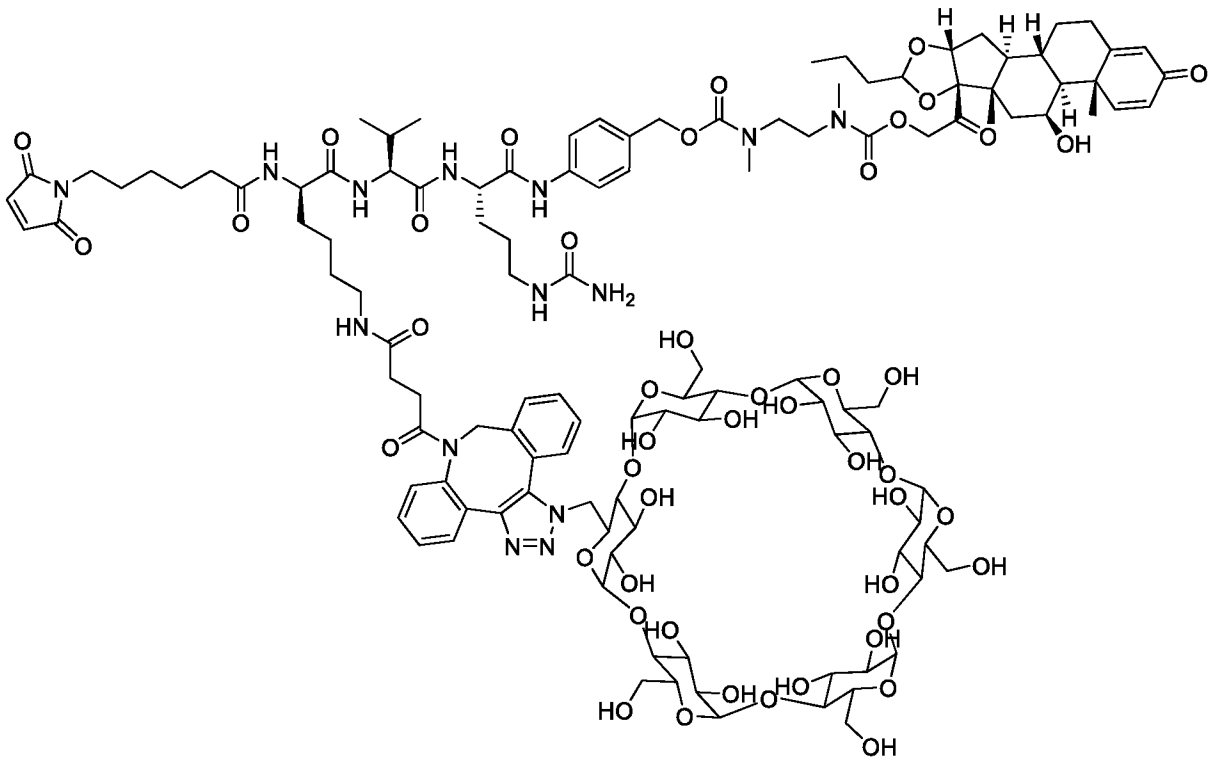
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-6-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl) amino}ethyl)-*N*-methylcarbamate (2m-precursor)



[0696] Following the general procedure B, the crude compound **2m-precursor** (the precursor of **2m**) (5 mg) was obtained as light yellow oil, which was used directly for the next step. ESI m/**z**: 780 $(M/2 + H)^+$

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-[(2*R*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-6-[4-(3-{[31,32,33,34,35,36,37,38,39,40,41,42-dodecahydroxy-10,15,20,25,30-pentakis(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29-dodecaoxaheptacyclo[26.2.2.2^{3,6}.2^{8,11}.2^{13,16}.2^{18,21}.2^{23,26}]dotetracontan-5-yl]methyl}-3,4,5,13-

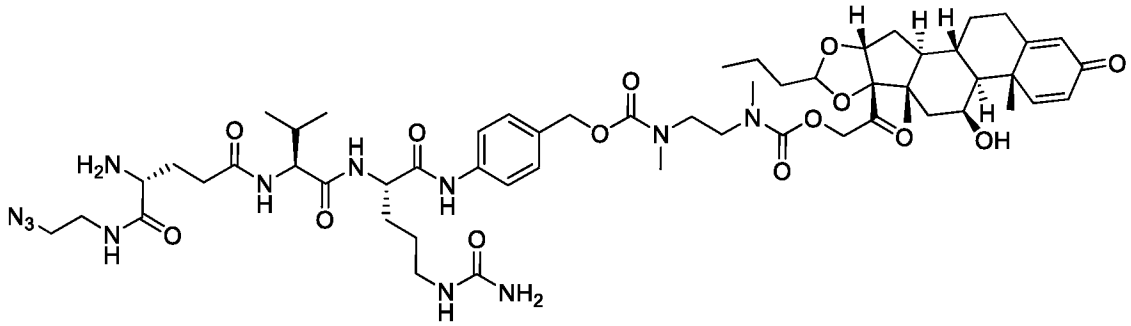
tetraazatetracyclo[13.4.0.0^{2,6}.0^{7,12}]nonadeca-1(15),2(6),4,7(12),8,10,16,18-octaen-13-yl)-4-oxobutanamido]hexanamido]-3-methylbutanamido]pentanamido]phenyl}methoxy) carbonyl](methyl)amino}ethyl)-*N*-methylcarbamate (2m)



[0697] To a solution of crude **2m** (5 mg) in DMF (1 mL) was added CD-N_3 (**8b**, 63 mg, 63 μmol). The resulting mixture was stirred at RT for 72 hours, which was monitored by LCMS. The resulting mixture was directly purified by prep-HPLC (method A) to give compound **2m** (2 mg, 4% yield from **4c**) as a white solid. ESI m/**z**: 1278.8 $(M/2 + H)^+$. Anal. HPLC: 97.9%, Retention time: 6.49 min (method A).

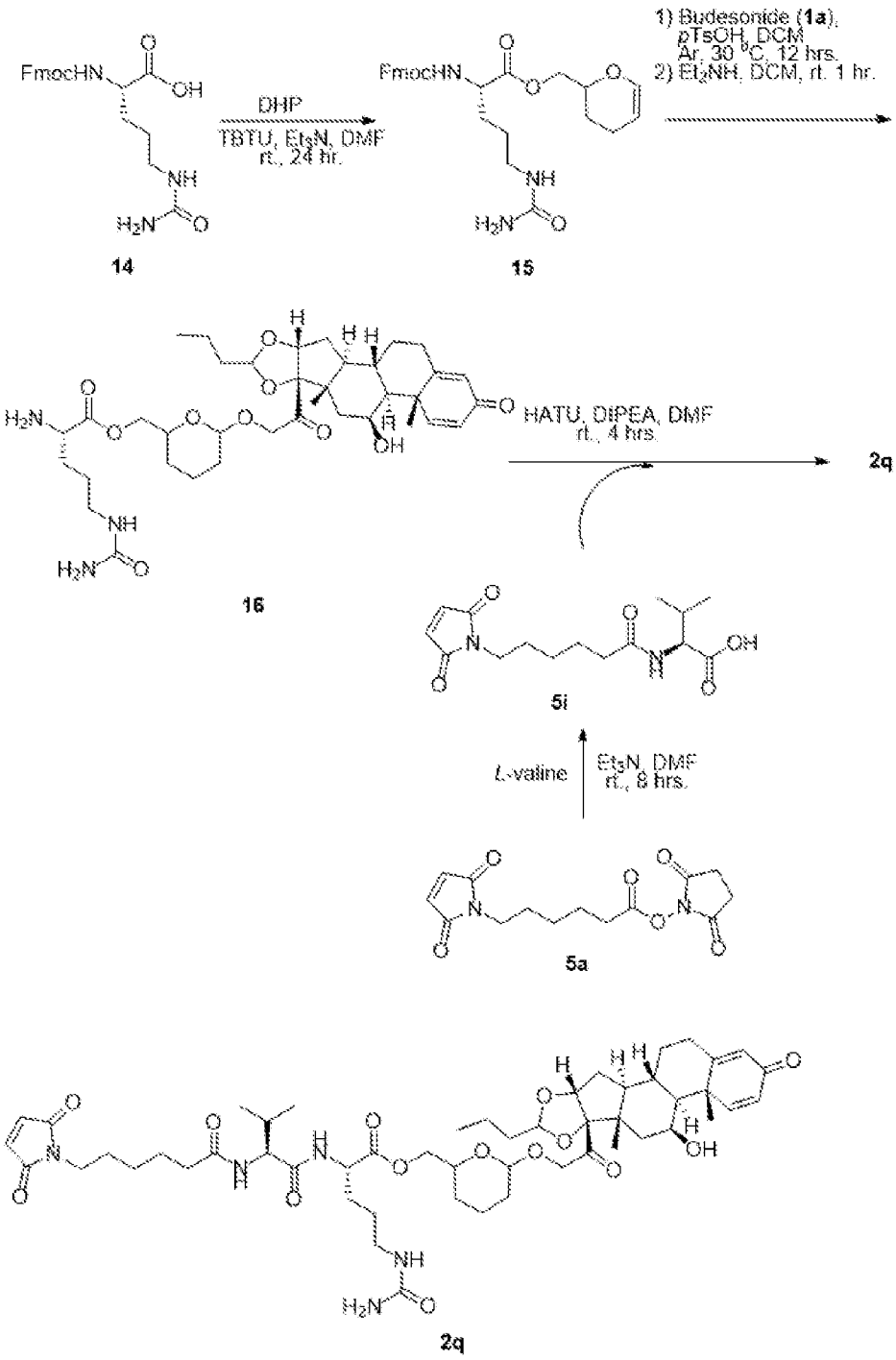
Example 2n

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-[(4*R*)-4-amino-4-[(2-azidoethyl)carbamoyl]butanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)-*N*-methylcarbamate (2n)



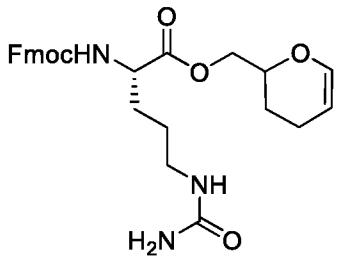
[0698] The reaction mixture was then stirred at RT overnight. Compound **4c** was totally consumed according to LCMS. The mixture was separated by prep-HPLC (method A) and after lyophilization, **Fmoc-2n** was obtained (20 mg), which was dissolved in DMF (2 mL). To the DMF solution was added diethylamine (4 drops, *c.a.* 0.08 mL). The reaction mixture was stirred at RT for 2 hours until de-Fmoc reaction was completed, which was monitored by LCMS. The mixture was filtered through filter membrane and the solution was purified by prep-HPLC (method A) to give the title compound **2n** (10 mg, yield 8.4%) as a white solid. ESI m/**z**: 1147 $(M + H)^+$. Anal. HPLC: 99.6%, Retention time: 5.67 min (method A); 7.72 min (method B).

Scheme 6. Synthesis of Linker-Budesonide 2q (THP analog)



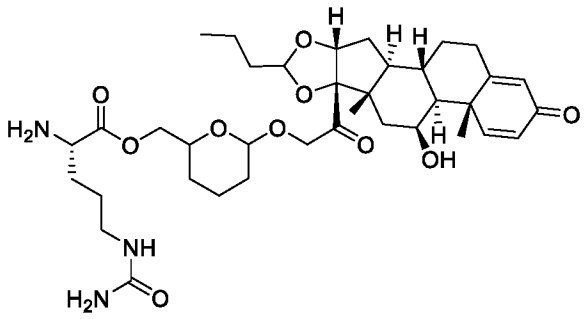
Example 2q

(2*S*)-(3,4-Dihydro-2*H*-pyran-2-yl)methyl 2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-5-ureidopentanoate (15)



[0699] To a mixture of Fmoc-Cit-OH (**14**, 0.29 g, 0.73 mmol) in DMF (5 mL) were added DHP (0.10 mg, 0.88 mmol), TBTU (0.70 g, 2.2 mmol) and triethylamine (0.37 g, 3.7 mmol) at RT. The resulting mixture was stirred at RT for 24 hours. 15% of desired mass was detected by LCMS. The reaction was quenched with water (30 mL) and extracted with ethyl acetate (10 mL x 3). The combined organic solution was washed with water and brine, dried over sodium sulfate and concentrated in vacuo. The crude product was purified by reversed phase flash chromatography (0-100% acetonitrile in water) to give title compound **15** (110 mg, yield 30%) as colorless oil. ESI m/**z**: 494 $(M + H)^+$.

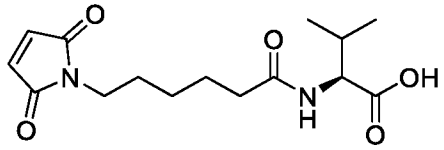
(6-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}oxan-2-yl)methyl (2*S*)-2-amino-5-(carbamoylamino)pentanoate (16)



[0700] To a solution of compound **15** (80 mg, 0.16 mmol) in anhydrous DCM (3 mL) was added a solution of budesonide (**1a**, 70 mg, 0.16 mmol) and *p*-toluenesulfonic acid (42 mg, 0.24 mmol) in anhydrous DCM (2 mL) by syringe under protection of argon balloon at RT. The reaction mixture was stirred at 30 °C under argon balloon for 12 hours. Most of compound **15** was consumed according to LCMS. The reaction mixture was cooled to RT, and to the reaction

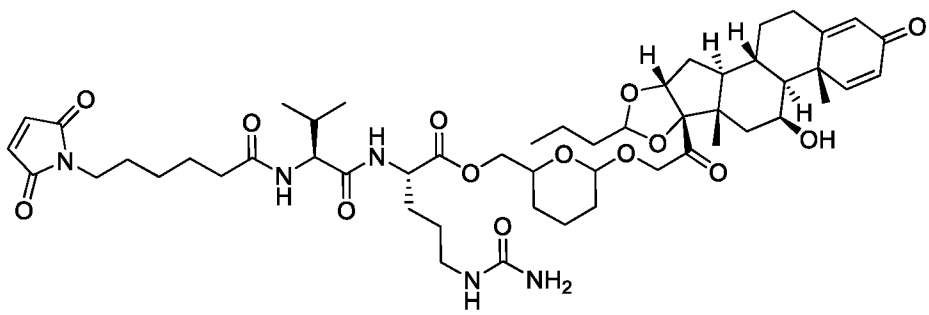
mixture was added diethylamine (1 mL). The reaction mixture was stirred at RT for 1 hours until de-Fmoc reaction was completed, which was monitored by LCMS. The volatiles were removed in vacuo and the residue was purified by prep-HPLC (method B) to give the title compound **16**(17 mg, yield 31%) as a white solid. ESI m/**z**: 702 $(M + H)^+$.

(*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanoic acid (5i)



[0701] To a solution of L-valine (0.12 g, 1.0 mmol) in anhydrous DMF (2 mL) were added compound **5a** (0.31 g, 1.0 mmol) and triethylamine (0.51 g, 5.0 mmol) at RT. The reaction mixture was stirred at RT for 8 hours until compound **5a** was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through filtering membrane and the filtrate was directly purified by prep-HPLC (method A) to give the title compound **5i**(0.14 g, yield 46%) as colorless oil. ESI m/**z**: 311 $(M + H)^+$.

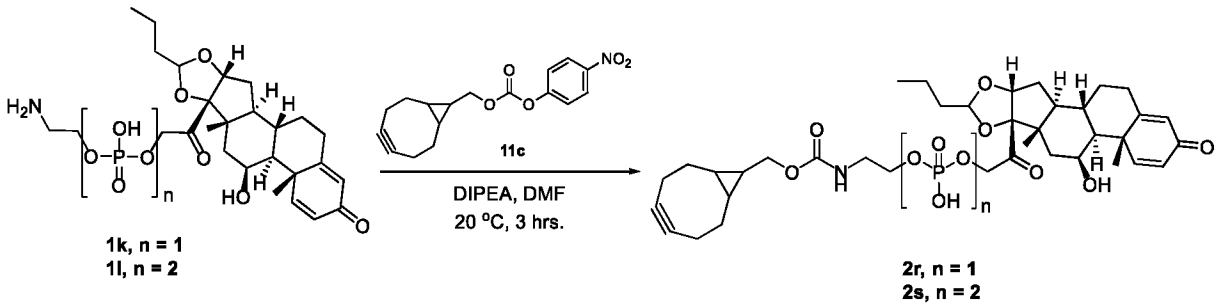
6-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-Hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}oxan-2-yl)methyl (2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]-3-methylbutanamido]pentanoate (2q)



[0702] To a solution of compound **5i**(5.8 mg, 19 μmol) in DMF (1 mL) were added HATU (10 mg, 26 μmol) and DIPEA (6.6 mg, 51 μmol) at RT. The mixture was stirred at RT for 15 minutes, and to the solution was then added compound **16** (12 mg, 17 μmol) at RT. The resulting mixture was stirred at RT for 4 hours until compound **5i** was totally consumed, which was monitored by LCMS. The reaction mixture was then filtered though filtering membrane and the filtrate was directly purified by prep-HPLC (method A) twice to give the title compound **2q** (2 mg, yield 12%)

as a white solid. ESI m/**z**: 995.3 $(M + H)^+$. Anal. HPLC: 83.5%, Retention time: 10.36 min (method B).

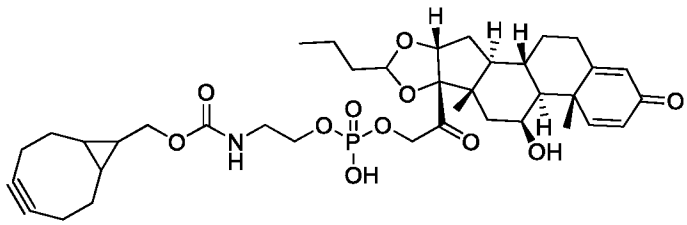
Scheme 7. Synthesis of linker-phosphate-budesonide 2r and 2s



[0703] Phosphate-budesonides were coupled with BCN-PNP (**11c**) to give BCN-phosphate-Budesonides (compound **2r** and **2s**) (scheme 7).

Example 2r

{2-[({Bicyclo[6.1.0]non-4-yn-9-ylmethoxy}carbonyl)amino]ethoxy}({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy})phosphinic acid (**2r**)

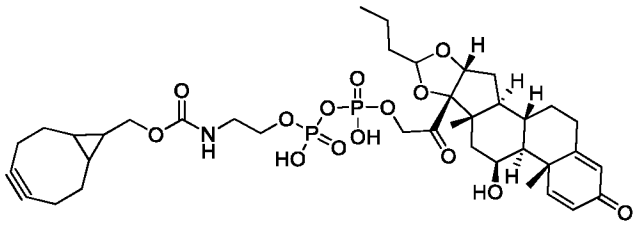


[0704] Following the general procedure D, compound **2r** (40 mg, 61% yield) was obtained as a white solid. ESI m/**z**: 730 $(M + H)^+$. ^{1}H NMR (500 MHz, $MeOD_{d4}$) δ 7.49 (d, J = 10.1 Hz, 1H), 6.29-6.26 (m, 1H), 6.03 (s, 1H), 5.23-5.16 (m, 1H), 4.88-4.64 (m, 3H), 4.45 (dd, J = 8.0, 3.1 Hz, 1H), 4.23-4.13 (m, 2H), 3.97 (dd, J = 11.9, 5.7 Hz, 2H), 3.36 (t, J = 4.7 Hz, 2H), 2.67 (td, J = 13.4, 5.3 Hz, 1H), 2.40 (d, J = 9.6 Hz, 1H), 2.30-1.34 (m, 23H), 1.21-0.92 (m, 10H) ppm. Anal. HPLC: > 99.9%, Retention time: 5.26 min (method B).

Example 2s

{2-[({Bicyclo[6.1.0]non-4-yn-9-ylmethoxy}carbonyl)amino]ethoxy}({[hydroxy({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-

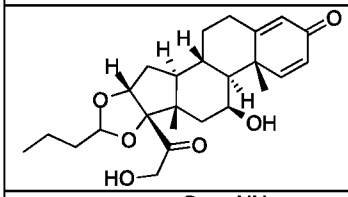
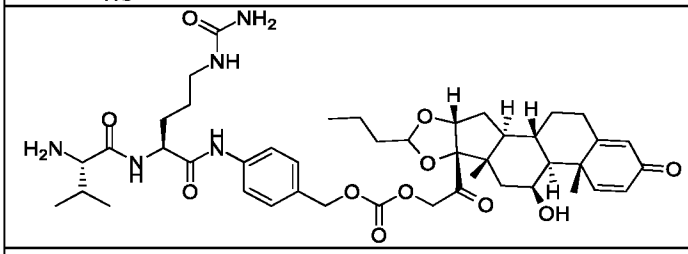
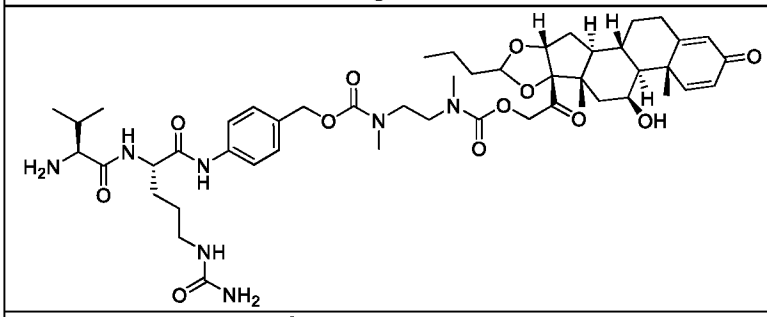
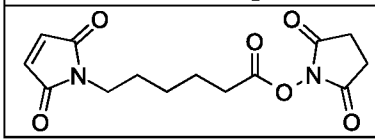
dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy})phosphoryl]oxy})phosphinic acid (2s)

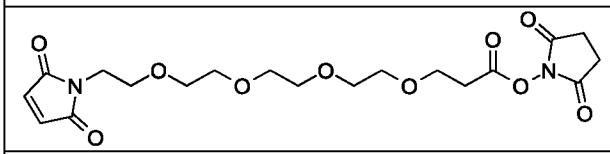
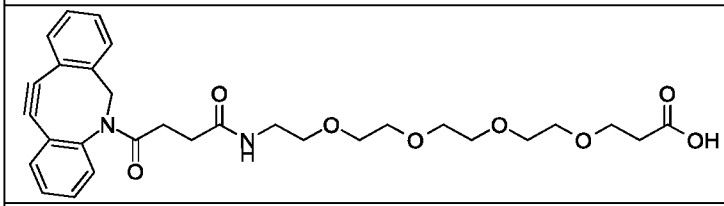
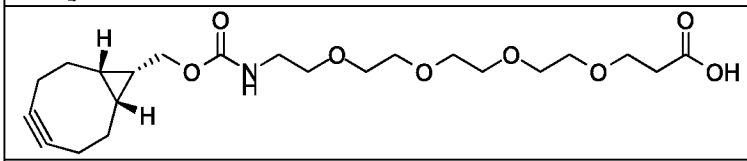
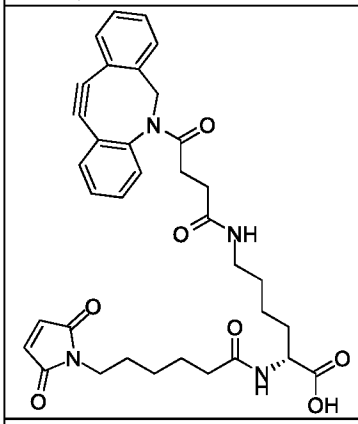
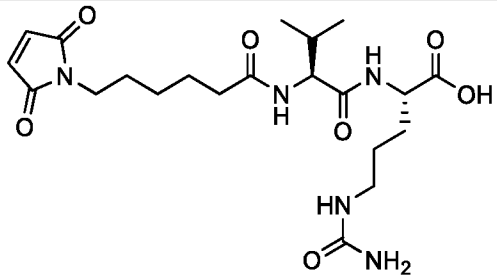
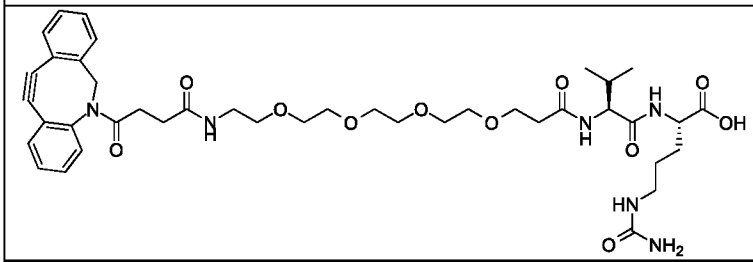
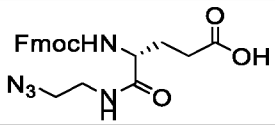
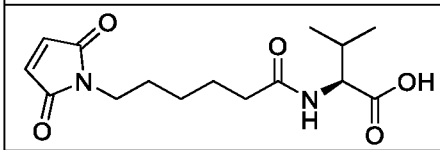


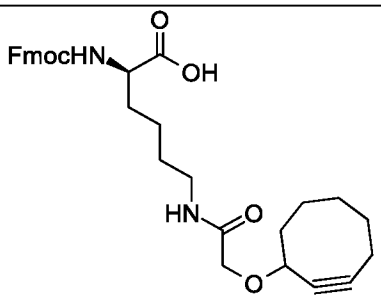
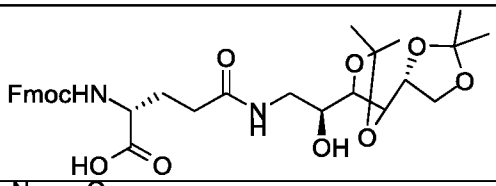
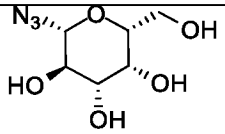
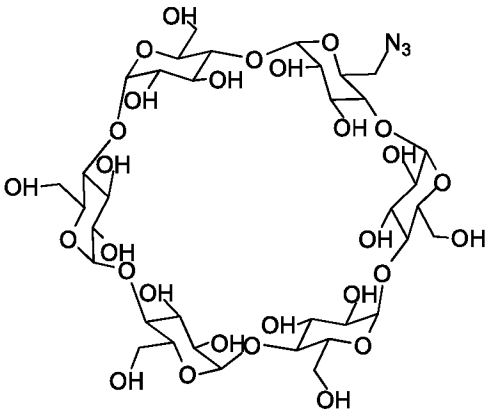
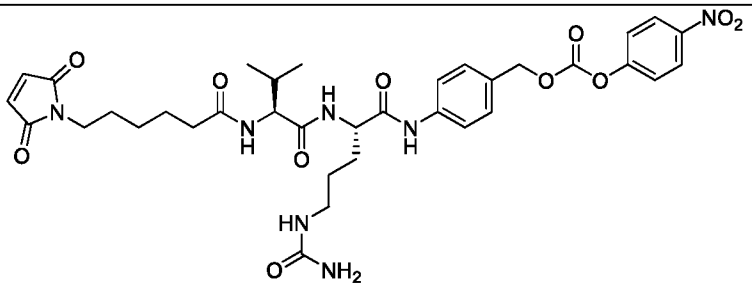
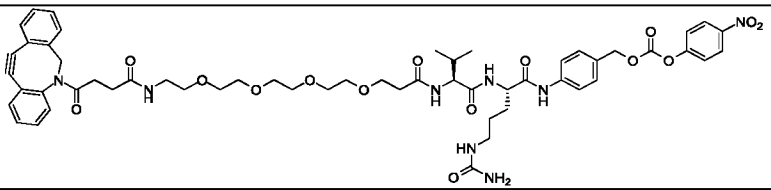
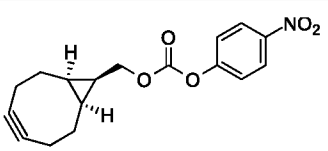
[0705] ESI m/**z**: 810 (M + H)$^+$. ^{1}H NMR (500 MHz, MeOD$_{d4}$) δ 7.51 (d, J = 10.1 Hz, 1H), 6.28 (d, J = 10.0 Hz, 1H), 6.03 (s, 1H), 5.22-5.16 (m, 1H), 4.98-4.66 (m, 3H), 4.46 (s, 1H), 4.12 (dt, J = 45.0, 13.0 Hz, 4H), 3.39 (t, J = 11.9 Hz, 2H), 2.67 (dd, J = 13.3, 8.0 Hz, 1H), 2.40 (d, J = 11.2 Hz, 1H), 2.30-1.32 (m, 23H), 1.22-0.92 (m, 10H) ppm. Anal. HPLC: > 99.9%, Retention time: 4.03 min (method B).

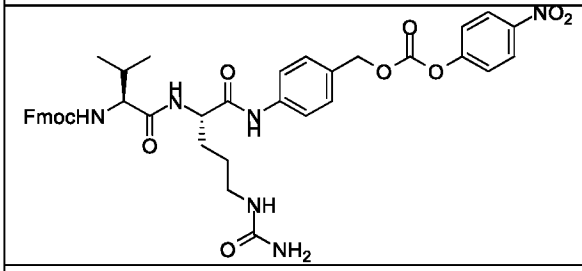
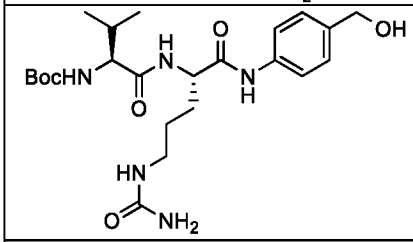
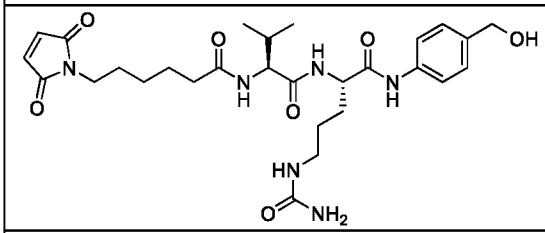
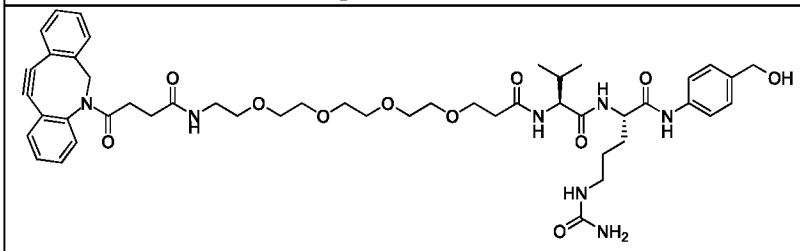
Experimental procedures for Intermediates

Table 4. Key intermediates and starting materials

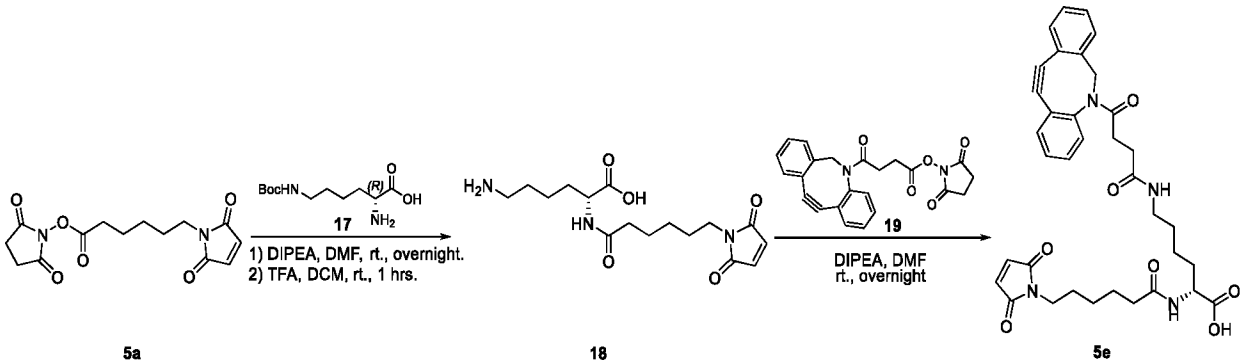
Structures	Intermediate No.	References or synthesis
	**1a** (budesonide)	Commercially available (51333-22-3)
	**4a** (vcPAB-budesonide)	See scheme 2
	**4c** (vcPAB-**1d**)	See scheme 5
	**5a** (mc-NHS)	Commercially available (55750-63-5)

Structures	Intermediate No.	References or synthesis
	**5b** (MAL-PEG4-NHS)	Commercially available (1325208-25-0)
	**5c** (DIBAC-suc-PEG4-acid)	Commercially available (1537170-85-6)
	**5d** (BCN-PEG4-acid)	Commercially available (1421932-54-8)
	**5e**	Scheme 8
	**5f** (mc-Val-Cit-OH)	WO2014/191578 A1
	**5g** (DIBAC-suc-PEG4-Val-Cit-OH)	Scheme 9
	**5h**	Scheme 10
	**5i**	See scheme 6

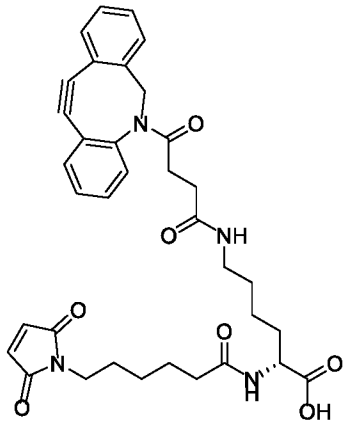
Structures	Intermediate No.	References or synthesis
	**6a**	Scheme 11
	**6b**	*J. Org. Chem.* **2010**, 75, 3685-3691
	**8a**	Commercially available (35899-89-9)
	**8b**	*Synth. Commun.*, **2002**, 32(21), 3367-3372 *J. Am. Chem. Soc.*, **2012**, 134(46), 19108-19117 *J. Med. Chem.*, **1997**, 40(17), 2755-2761 *J. Am. Chem. Soc.*, **1993**, 115(12), 5035-5040
	**11a** (MC-VC-PAB-PNP)	Commercially available (159857-81-5) WO2014/191578 A1
	**11b** (DIBAC-suc-PEG4-vc-PAB-PNP)	Scheme 12
	**11c** (BCN-PNP)	WO2013/181697A1 *Angew. Chem. Int. Ed.*, **2010**, 49 (49), 9422-

Structures	Intermediate No.	References or synthesis
		9425
	**11d** (Fmoc-vc-PAB-PNP)	Commercially available 863971-53-3
	**12a** (Boc-vc-PAB)	Commercially available (870487-09-5) WO2008/341244 A2
	**12b** (MC-VC-PAB)	Commercially available 159857-80-4
	**12c** (DIBAC-suc-PEG4-vc-PAB)	Scheme 12

Scheme 8. Synthesis of 5e

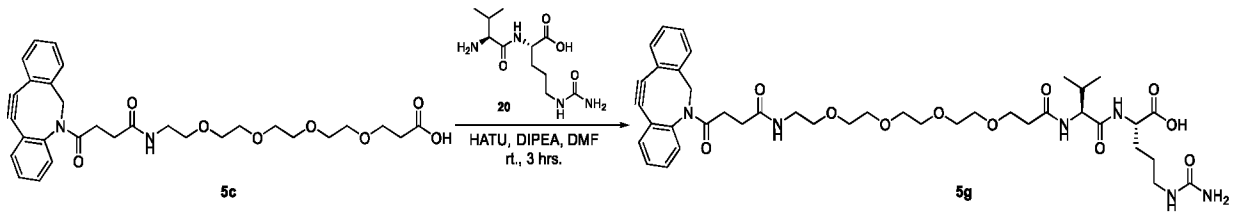


(2*R*)-6-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4,6,8,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido]hexanoic acid (5e)

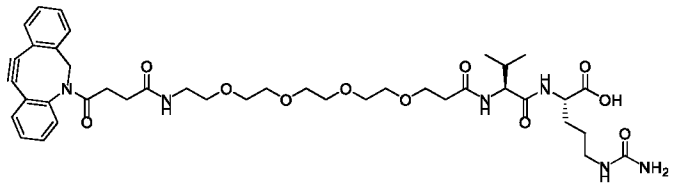


[0706] To a mixture of H-*D*-Lys(Boc)-OH (**17**, 0.25 g, 1.0 mmol) in DMF (10 mL) were added 6-Maleimidocarproic acid-NHS (**5a**, 0.31 g, 1.0 mmol) and diisopropylethylamine (DIPEA, 0.26 g, 2.0 mmol) at RT. After the reaction was stirred at RT overnight, compound **5a** was totally consumed. The mixture was directly separated by reversed phase flash chromatography (0-100% acetonitrile in water (0.05% TFA)) to give intermediate **Boc-18** (ESI m/**z**: 440 $(M + H)^+$) as colorless oil, which was dissolved in DCM (5 mL). To the solution was added TFA (0.5 mL) dropwise at 0 °C, and the mixture was stirred at RT for an hour. The reaction was monitored by LCMS and intermediate **Boc-18** was totally consumed. The volatiles were removed in vacuo to give crude **18** (ESI m/**z**: 340 $(M/2 + H)^+$), which was used for the next step without further purification. To the mixture of crude compound **18** (0.21 g, 0.62 mmol) in DMF (5 mL) was added activated ester **19** (0.20 g, 0.50 mmol) and DIPEA (50 mg, 0.39 mmol) at RT. After the reaction mixture was stirred at RT overnight, compound **19** was totally consumed, which was monitored by LCMS. The reaction mixture was then directly separated by reversed phase flash chromatography (0-100% acetonitrile in water) to give title compound **5e** (0.10 g, 20% yield in 3 steps from **5a**) as a white solid. ESI m/**z**: 627 $(M + H)^+$. ^{1}H NMR ($DMSO_{d6}$, 400 MHz): δ 7.89 (d, J = 13.2 Hz, 1H), 7.69-7.62 (m, 3H), 7.48-7.47 (m, 3H), 7.36-7.28 (m, 3H), 6.99 (s, 2H), 5.03 (d, J = 13.6 Hz, 1H), 4.09-4.04 (m, 1H), 3.60 (d, J = 13.6 Hz, 1H), 3.38-3.34 (m, 1H), 2.90-2.87 (m, 2H), 2.61-2.55 (m, 1H), 2.25-2.17 (m, 1H), 2.08-1.51 (m, 5H), 1.46-1.15 (m, 12H) ppm.

Scheme 9. Synthesis of 5g

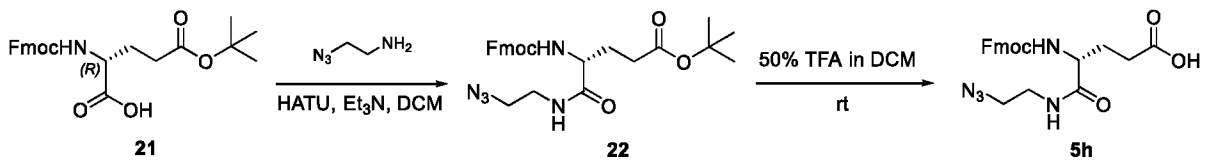


(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanoic acid (5g)

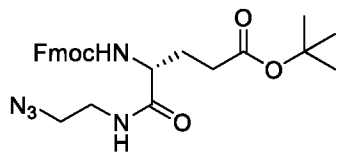


[0707] To a solution of compound **5c** (0.30 g, 0.54 mmol) in DMF (10 mL) were added HATU (0.31 g, 0.81 mmol) and DIPEA (0.14 g, 1.1 mmol) at RT. The mixture was stirred at RT for 15 minutes. To the reaction solution was added Val-Cit-OH (**20** (CAS# 159858-33-0), 0.21 g, 0.76 mmol) at RT, and the resulting mixture was stirred at RT for 3 hours until most materials were consumed, which was monitored by LCMS. The reaction mixture was filtered through filtering membrane and the filtrate was directly purified by reversed flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give title intermediate **5g** (0.25 g, yield 57%) as a white solid. ESI m/**z**: 809.5 $(M + H)^+$.

Scheme 10. Synthesis of intermediates 5h



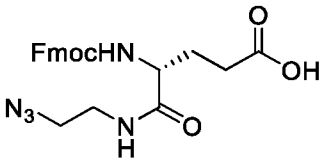
***tert*-Butyl (4*R*)-4-[(2-azidoethyl)carbamoyl]-4-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butanoate (22)**



[0708] To a mixture of Fmoc-*D*-Glu(OTBU)-OH (**21**, (104091-08-9), 0.30 g, 0.71 mmol) and 2-azidoethanamine (87156-40-9, 73 mg, 0.85 mmol) in DCM (50 mL) were added HATU (0.41 g, 1.1 mmol) and triethylamine (0.3 mL) at RT. The reaction mixture was stirred at RT overnight. Most of compound **21** was then consumed according to TLC and LCMS. After the reaction

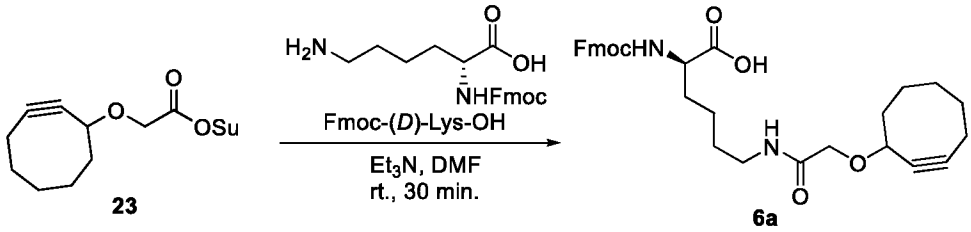
mixture was quenched with water (50 mL) at RT, the organic solution was washed with water and brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by prep-TLC (silica gel, eluting with petroleum ether / ethyl acetate (v/v = 1)) to give the title compound **22** (0.30 g, yield 76%) as yellow viscous oil. ESI m/**z**: 494 $(M + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$): δ 8.09 (m, 1H), 7.84-7.90 (m, 4H), 7.33-7.44 (m, 4H), 6.28 (s, 2H), 3.35 (m, 6H), 3.26 (m, 1H), 2.50 (m, 2H), 2.25 (m, 1H), 1.80 (m, 1H), 1.39 (s, 9H) ppm.

(4*R*)-4-[(2-Azidoethyl)carbamoyl]-4-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butanoic acid (5h)

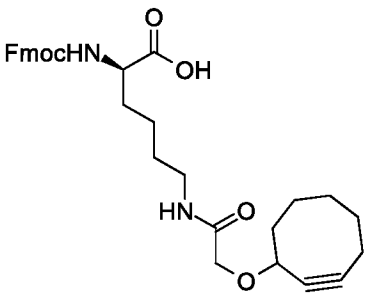


[0709] The resulting mixture was stirred at RT for 2 hours until compound **22** was totally consumed, which was monitored by TLC and LCMS. The volatiles were removed in vacuo to give crude compound **5h** (17 mg, yield 96%) as yellow oil, which was used for the next step without further purification. ESI m/**z**: 438 $(M + H)^+$.

Scheme 11. Synthesis of Intermediates 6a

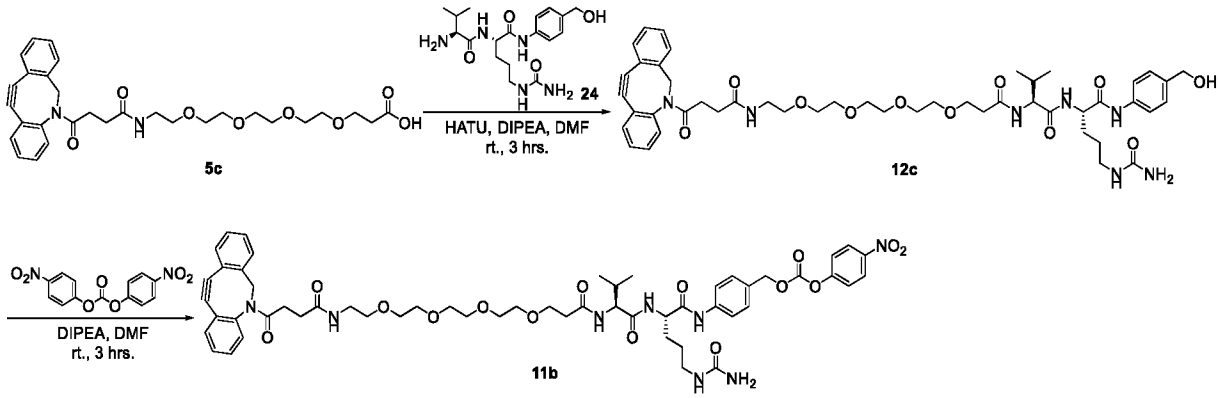


(2*R*)-6-[2-(Cyclooct-2-yn-1-yloxy)acetamido]-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic acid (6a)

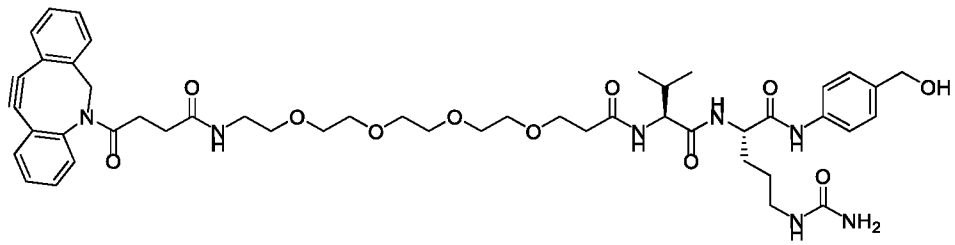


[0710] To a mixture of commercial compound **23** (65 mg, 0.23 mmol, CAS: 1425803-45-7) in DMF (2 mL) were added Fmoc-*D*-Lys-OH (85 mg, 0.23 mmol) and triethylamine (52 mg, 0.51 mmol). The reaction mixture was stirred at RT for 30 minutes. The mixture was directly separated by reversed phase flash chromatography (0-100% acetonitrile in water (0.05% TFA)) to give intermediate **6a** (85 mg, yield 70%) as a white solid. ESI m/**z**: 533 $(M + H)^+$. ^{1}H NMR ($MeOD_{d4}$, 500 MHz): δ 7.70 (d, J = 7.5 Hz, 2H), 7.59 (t, J = 8.0 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 7.4 Hz, 2H), 4.35-4.22 (m, 2H), 4.22-4.09 (m, 2H), 4.09-3.99 (m, 1H), 3.94-3.81 (m, 1H), 3.79-3.67 (m, 1H), 3.15 (t, J = 6.9 Hz, 2H), 2.17-1.96 (m, 3H), 1.96-1.86 (m, 1H), 1.85-1.66 (m, 4H), 1.66-1.41 (m, 5H), 1.41-1.25 (m, 3H) ppm.

Scheme 12. Synthesis of intermediates 11b and 12c



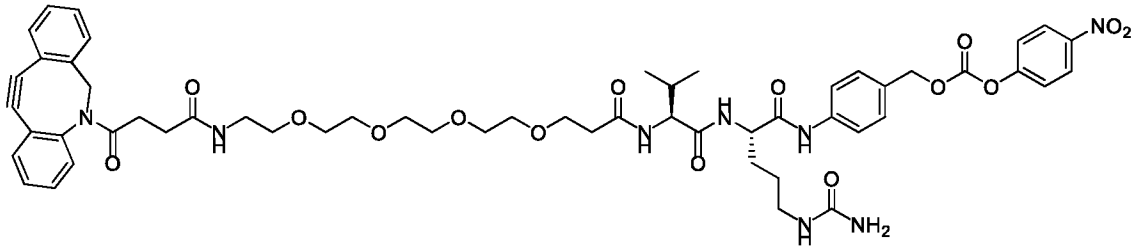
1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-(hydroxymethyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide (12c)



[0711] The mixture was stirred at RT for 15 minutes. To the reaction solution was added vc-PAB (**24** (159857-79-1), 0.21 g, 0.54 mmol) at RT, and the resulting mixture was stirred at RT for 3 hours until most materials were consumed, which was monitored by LCMS. The reaction mixture was filtered through filtering membrane and the filtrate was directly purified by reversed

flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give title intermediate **12c** (0.30 g, yield 60%) as a white solid. ESI m/**z**: 617 $(M + H)^+$.

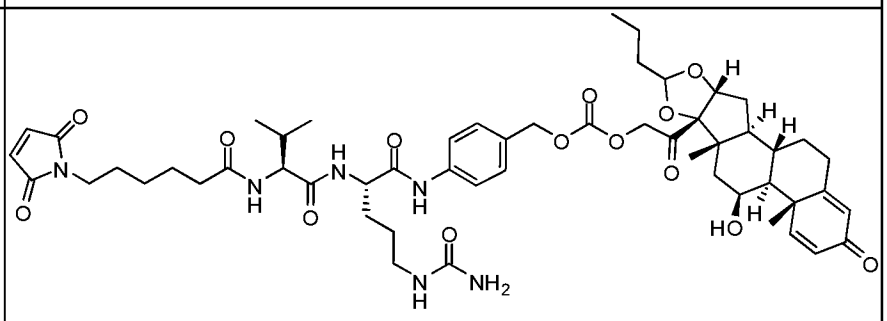
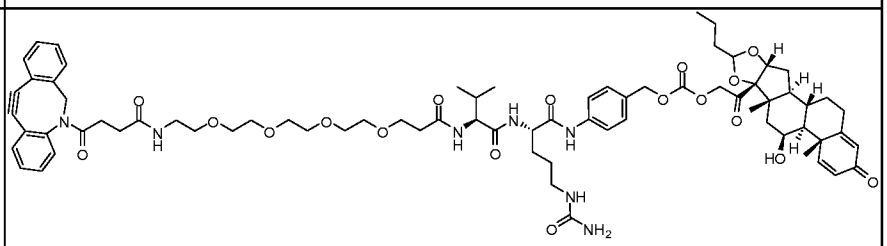
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-nitrophenyl carbonate (11b)

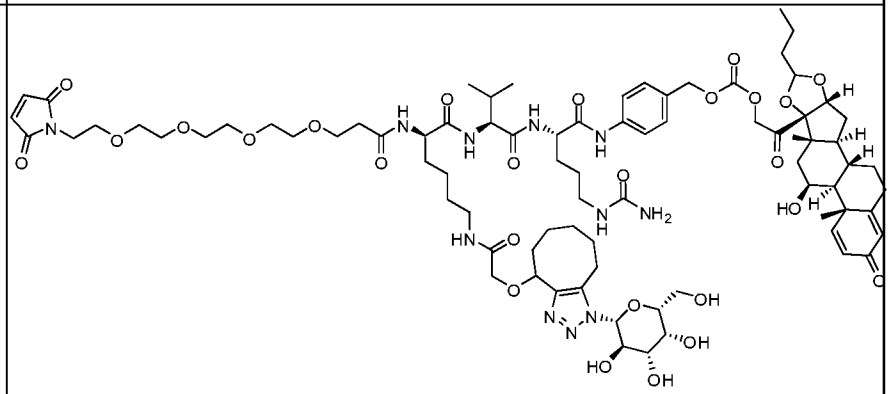
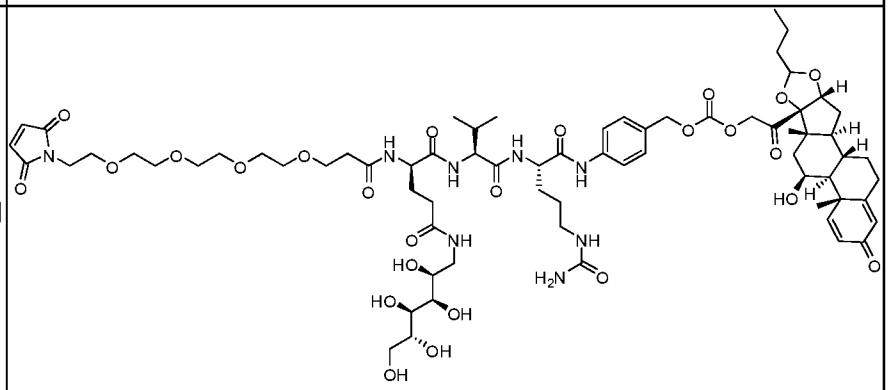
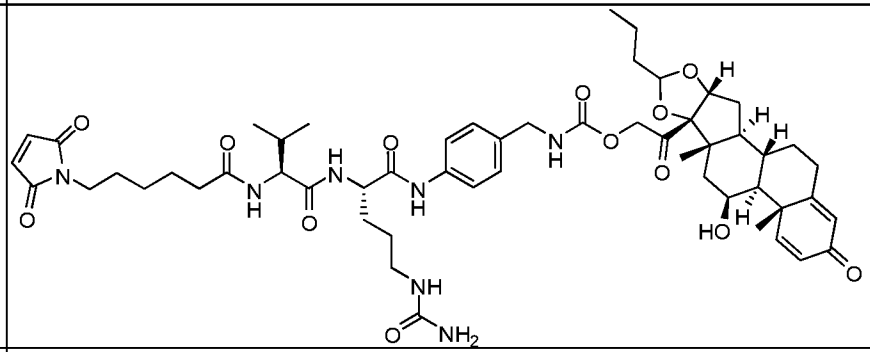
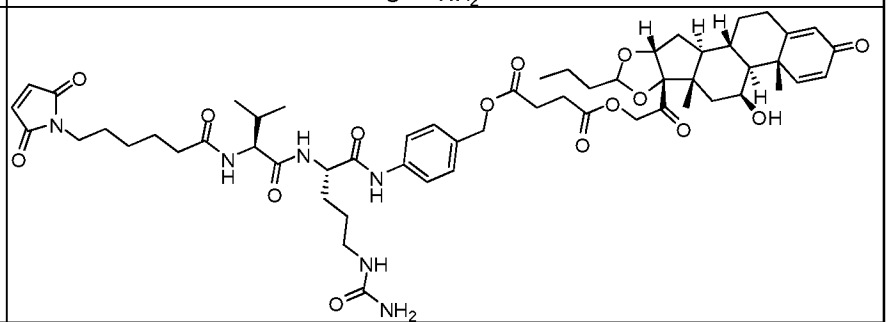
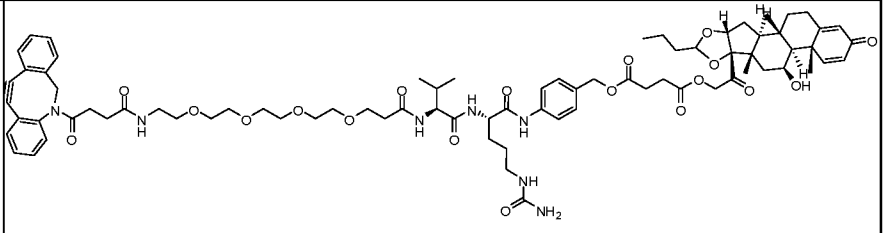


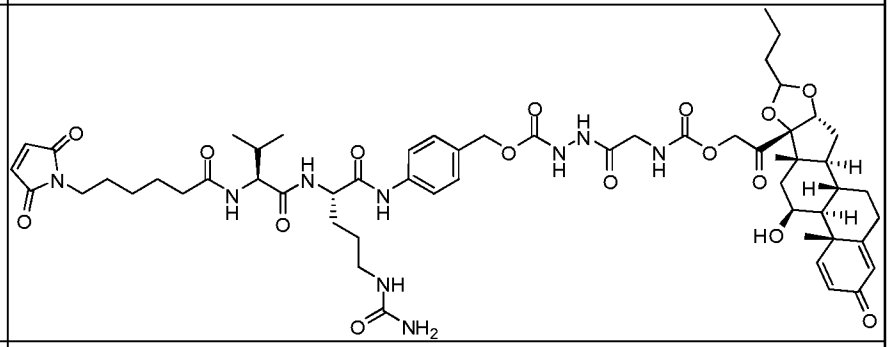
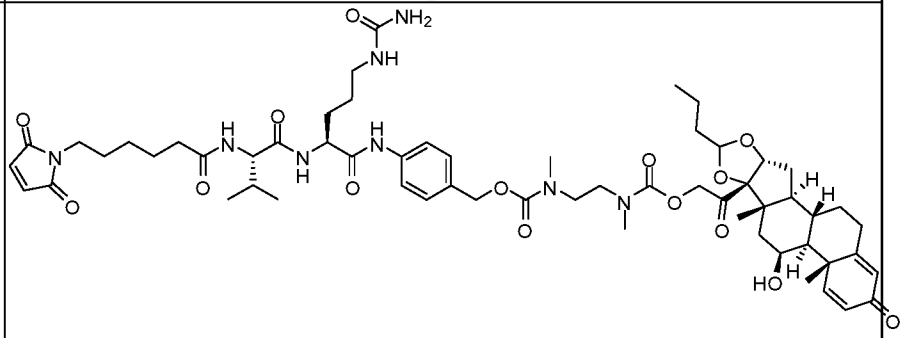
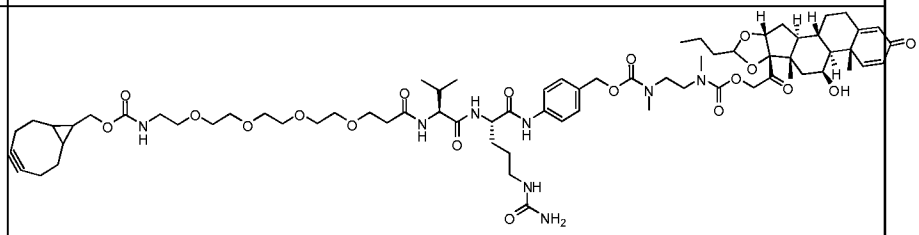
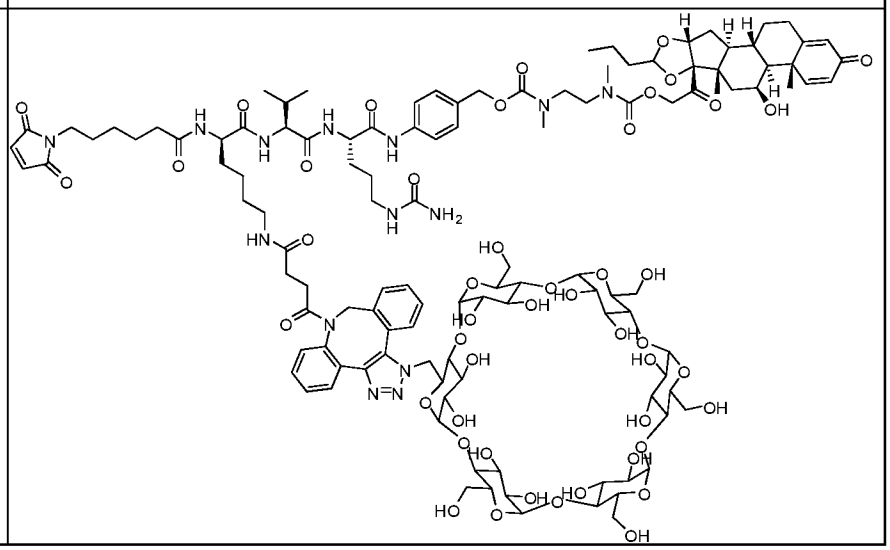
[0712] To a solution of compound **12c** (0.15 g, 0.16 mmol) in DMF (10 mL) were added bis(4-nitrophenyl) carbonate (0.15 g, 0.49 mmol) and DIPEA (63 mg, 0.49 mmol) at 0 °C. The mixture was then stirred at RT for 3 hours until **12c** was mostly consumed, which was monitored by LCMS. The reaction mixture was filtered through filtering membrane and the filtrate was directly purified by reversed flash chromatography (0-100% acetonitrile in water (with 10 mmol/L ammonium bicarbonate)) to give title intermediate **11b** (50 mg, yield 28%) as a white solid. ESI m/**z**: 1079 $(M + H)^+$.

[0713] **Table 5A** below presents linker payloads made using the methods described herein.

Table 5A. Examples of Linker–Payloads

LP	Payload	Linker name	Structures
2a	**1a**	MC-VC-PAB	
2b	**1a**	DIBAC-suc-vc-PAB	

LP	Payload	Linker name	Structures
2c	1a	mc-PEG4-(sugar-COT)dLys-vc-PAB	
2d	1a	mc-PEG4-(sugar2)dGlu-vc-PAB	
2e	1a	MC-VC-PABA	
2f	1c	MC-VC-PAB	
2g	1c	DIBAC-suc-vc-PAB	

LP	Payload	Linker name	Structures
2j	1e	MC-VC-PAB	
2k	1d	MC-VC-PAB	
2l	1d	BCN-PEG4-vc-PAB	
2m	1d	mc-N5(CD-DIBAC-suc)Lys-vc-PAB	

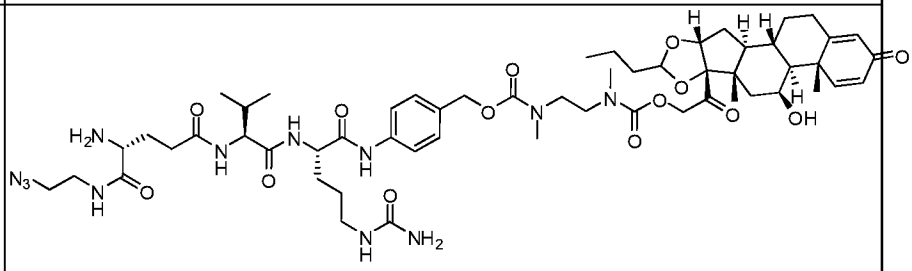
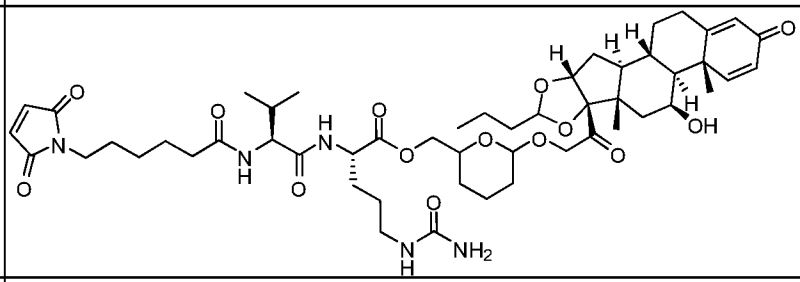
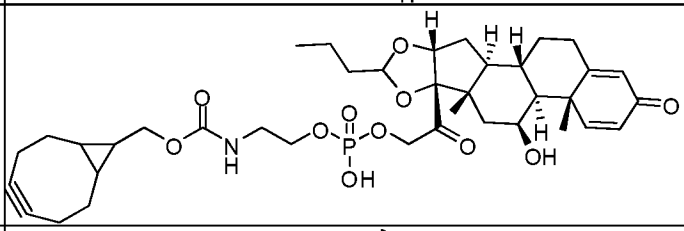
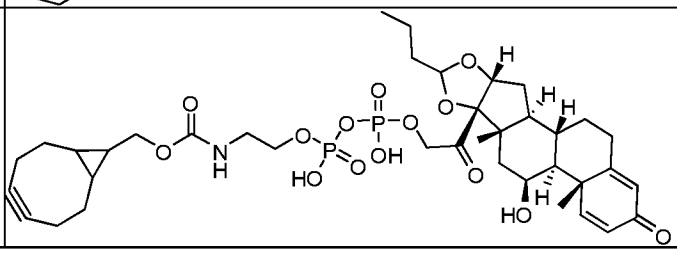
LP	Payload	Linker name	Structures
2n	1d	azidoethyl-GLu-vc-PAB	
2q	1g	MC-VC	
2r	1k	BCN	
2s	1l	BCN	

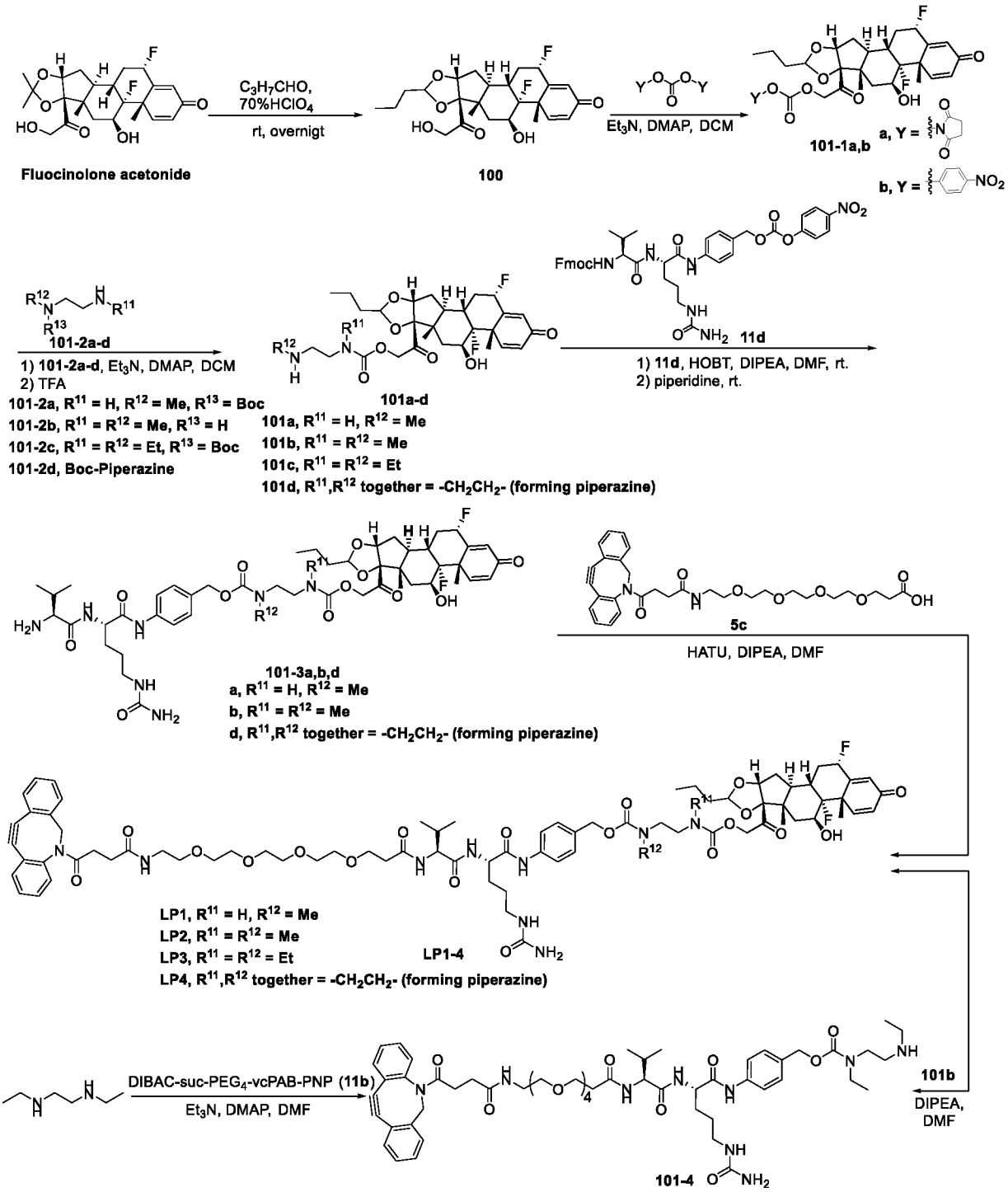
Table 6A. Characterization Data for Linker–Payloads

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
2a	4.18	$C_{54}H_{72}N_6O_{14}$	1029.2	92	7.6 (A)	1029 (M+H)	1029 (M+H)
2b	5.42	$C_{74}H_{95}N_7O_{18}$	1370.6	97	8.1 (B)	685.8 (M/2+H)	685.8 (M/2+H)
2c	1.03	$C_{81}H_{117}N_{11}O_{26}$	1660.9	97	7.0 (A)	830.8 (M/2+H)	830.8 (M/2+H)
2d	-1.87	$C_{70}H_{102}N_8O_{25}$	1455.6	100	6.48 (A)	728 (M/2+H)	1456 (M+H) (10%)

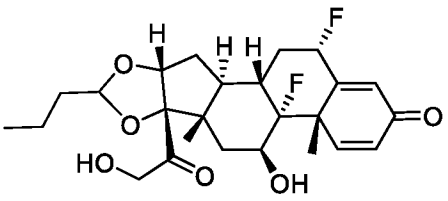
LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
2e	3.45	$C_{54}H_{73}N_7O_{13}$	1028.2	100	7.54 (A)	1028.3 (M+H)	1028.3 (M+H)
2f	3.54	$C_{57}H_{76}N_6O_{15}$	1085.2	98	1.71 (LCMS)	1085.3 (M+H)	1085.3 (M+H)
2g	4.77	$C_{77}H_{99}N_7O_{19}$	1426.7	> 99	7.86 (B)	714.0 (M/2+H)	1427 (M+H) (15%)
2j	2.16	$C_{57}H_{77}N_9O_{16}$	1144.3	98	1.57 (LCMS)	1144.3 (M+H)	1144.3 (M+H)
2k	3.54	$C_{59}H_{82}N_8O_{15}$	1143.3	98	1.70 (LCMS)	1143.4 (M+H)	1143.4 (M+H)
2l	4.66	$C_{71}H_{104}N_8O_{19}$	1373.6	100	9.40 (A)	687.5 (M/2+H)	1396.8 (M+Na) (50%)
2m	-4.89	$C_{120}H_{166}N_{14}O_{47}$	2556.7	100	6.49 (A)	1278.8 (M/2+H)	1278.8 (M/2+H)
2n	1.68	$C_{56}H_{82}N_{12}O_{14}$	1147.3	100	7.72 (B)	1147.5 (M+H)	1147.5 (M+H)
2q	3.52	$C_{52}H_{75}N_5O_{14}$	994.2	79	10.36 (B)	995.3 (M+H)	995.3 (M+H)
2r	4.88	$C_{38}H_{52}NO_{11}P$	729.8	100	5.26 (B)	686.2 (weak mass)	1459.1 (2M+H)
2s	0.55	$C_{38}H_{53}NO_{14}P_2$	809.8	100	4.03 (B)	766.1 (M-44)	810.1 (M+H) (70%)

Synthesis of Linker-payloads LP1A-4A

Scheme 101. Synthesis of Payload 100, spacer-payload 101a-d and linker-payloads LP1A-4A

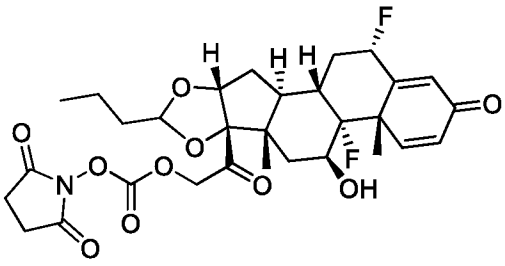


(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (100)



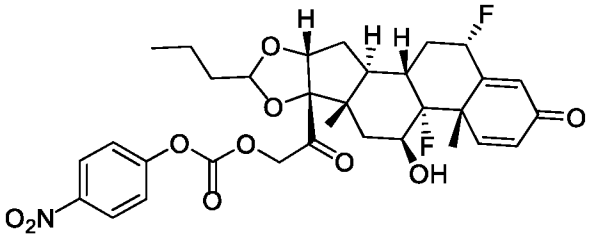
[0714] To a mixture of fluocinolone acetonide (10 g, 22 mmol) and silica gel (100 g) in heptanes (900 mL) was added butyraldehyde (3.0 mL, 33 mmol) below 10 °C and the suspension was stirred at 10-20 °C for 30 minutes. To the mixture was added perchloric acid (70%, 7.6 mL, 91 mmol) dropwise at 0 °C. The reaction mixture was then stirred at RT overnight. Most of fluocinolone acetonide was consumed according to TLC and LCMS. The reaction mixture was diluted with petroleum ether and quenched with *sat. aq.* sodium carbonate. The suspension was filtered and the solid was washed with DCM/methanol (v/v = 1:1). The combined filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography (0-1.5% methanol in DCM) to give compound **100** (8.0 g, yield: 78%) as a white solid. ESI m/z: 467.1 $(M + H)^+$.

1-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)oxy]pyrrolidine-2,5-dione (101-1a)



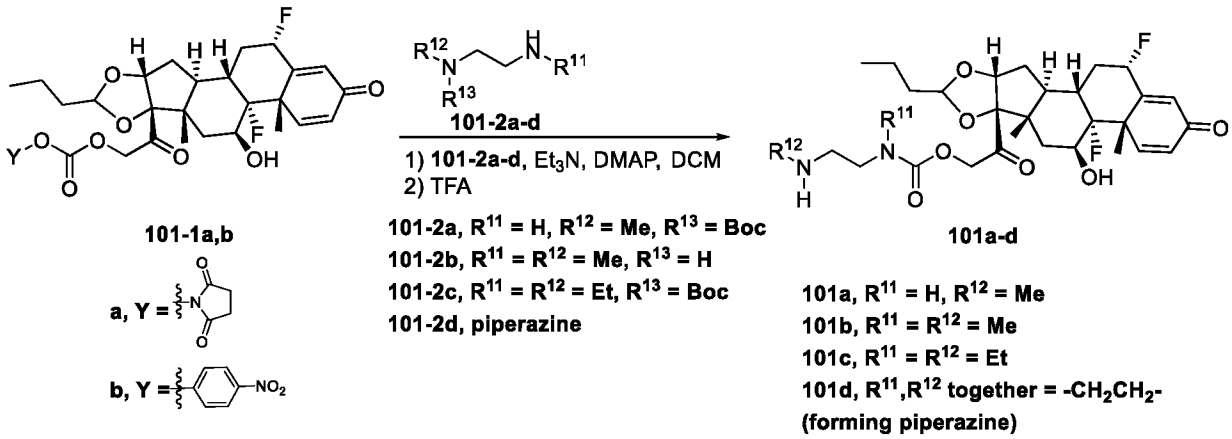
[0715] To a solution of compound **100** (0.47 g, 1.0 mmol) in DCM (20 mL) were added bis(2,5-dioxopyrrolidin-1-yl) carbonate (0.28 g, 1.1 mmol), triethylamine (0.40 g, 4.0 mmol) and DMAP (3.0 mg, cat.). The reaction mixture was stirred at RT for 4 hours until compound **100** was consumed, which was monitored by LCMS. The reaction mixture was then diluted with DCM and washed by water. The organic solution was dried over sodium sulfate. After filtered, the solution was concentrated *in vacuo* and the residue (crude **101-1a**) was used for the next step directly without purification. ESI m/z: 608.2 $(M + H)^+$.

(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-8-{2-[(4-nitrophenoxycarbonyl)oxy]acetyl}-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one (101-1b)



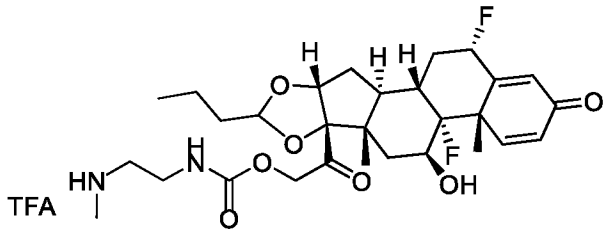
[0716] A mixture of compound **100** (0.80 g, 1.7 mmol), bis(4-nitrophenyl) carbonate (1.6 g, 5.2 mmol) and DIPEA (1.1 g, 8.6 mmol) in THF (20 mL) was stirred at RT for 4 hours, which was monitored by LCMS. The mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (50% ethyl acetate in petroleum ether) to give compound **101-1b** (0.75 g, 69 % yield) as colorless oil. ESI m/z: 632 $(M + H)^+$.

General Procedure for compound 101



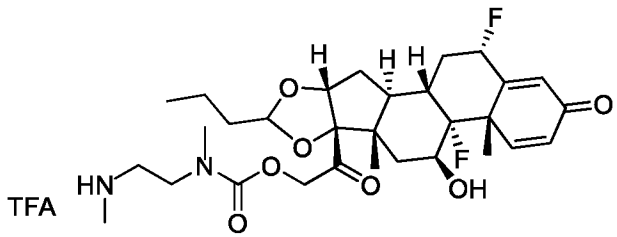
[0717] To a solution of compound **101-1a** or **101-1b** (crude, calculated as 1.0 mmol) in DCM (20 mL) were added diamine **101-2(a, b, c, or d)** (1.1 mmol) and DMAP (0.1 mmol). The reaction mixture was stirred at RT for 4 hours before TFA (1 mL) was added into. The mixture was stirred at RT for half an hour (for R^{13} = Boc, the reaction was stirred until Boc was totally removed, which monitored by LCMS). The resulting mixture was concentrated *in vacuo* and the residue was purified by reversed phase flash chromatography (50-80% acetonitrile in *aq.* TFA (0.5%)) to give compound **101(a, b, c, or d)** as colorless oil.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-[2-(methylamino)ethyl]carbamate (101a)



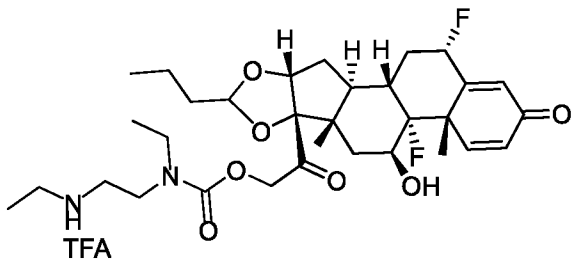
[0718] Following the general procedure and using compound **101-1a** with Boc-diamine **101-2a**, compound **101a** (0.38 g, 57% in 2 steps, TFA salt) was obtained as colorless oil. ESI m/z: 567 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-methyl-*N*-[2-(methylamino)ethyl]carbamate (101b)



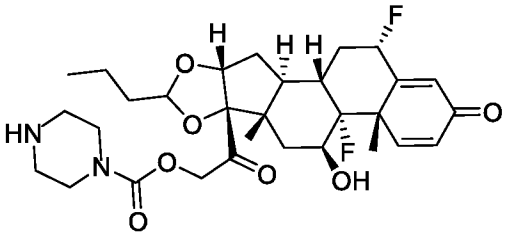
[0719] Following the general procedure and using compound **101-1a** with diamine **101-2b**, compound **101b** (0.45 g, 66% yield in 2 steps, TFA salt) was obtained as colorless oil. ESI m/z: 581 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-ethyl-*N*-[2-(ethylamino)ethyl]carbamate (101c)



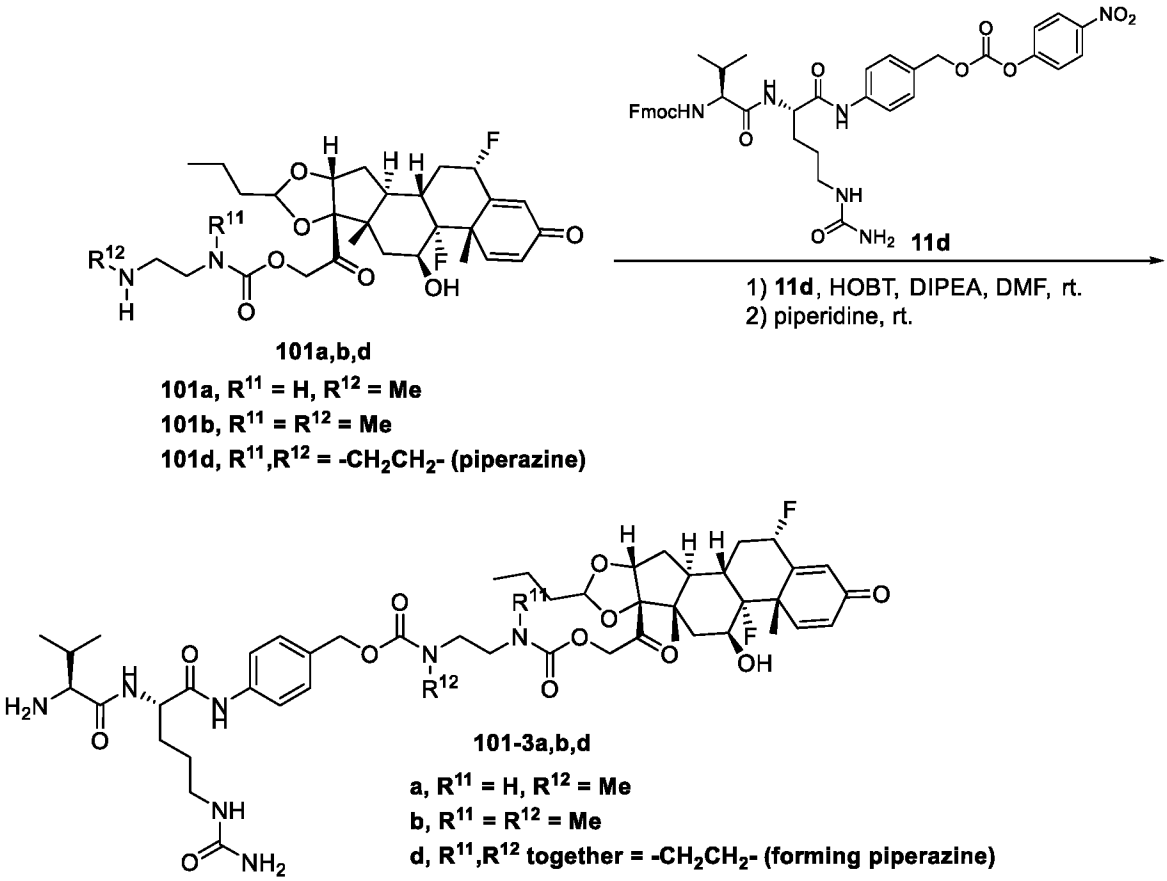
[0720] Following the general procedure and using compound **101-1b** with diamine **101-2c**, compound **101c** (24 mg, 18% yield in 2 steps, TFA salt) was obtained as colorless oil. ESI m/z: 609 $(M + H)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 8.38 (s, 2H), 7.30 (d, J = 10.5 Hz, 1H), 6.24 (dd, J = 10.5 Hz, 2.0 Hz, 1H), 6.11 (s, 1H), 5.70-5.54 (m, 2H), 5.10-4.91 (m, 1H), 4.85-4.65 (m, 3H), 4.25-4.16 (m, 1H), 3.60-3.40 (m, 2H), 3.33-3.20 (m, 4H), 3.20-2.90 (m, 4H), 2.67-2.53 (m, 1H), 2.30-2.20 (m, 1H), 2.10-2.00 (m, 2H), 1.80-1.70 (m, 1H), 1.64-1.51 (m, 4H), 1.49 (s, 3H), 1.40-1.30 (m, 2H), 1.20-1.10 (m, 4H), 1.10-1.00 (m, 1H), 0.90-0.80 (m, 6H) ppm.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl piperazine-1-carboxylate (**101d**)



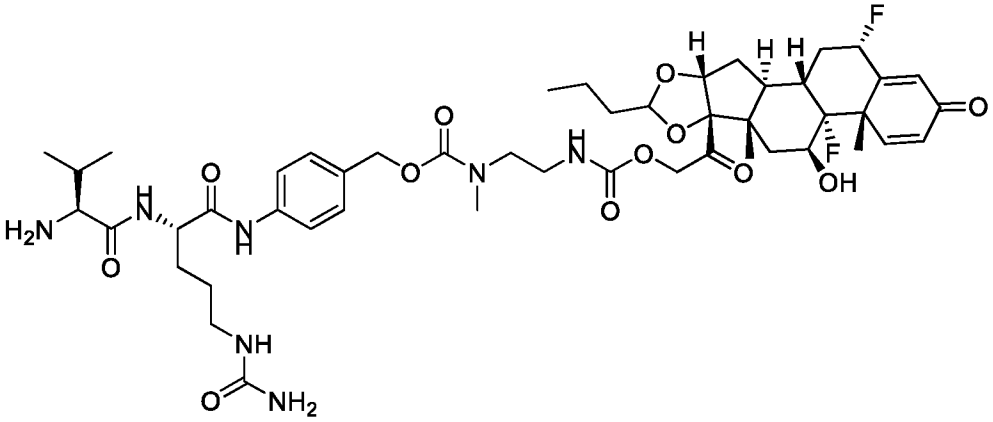
[0721] Following the general procedure and using compound **101-1b** with piperazine (**101-2d**) without treatment with TFA and purified by silica gel column chromatography (0-10% methanol in DCM), compound **101d** (0.21 g, 49% yield in 2 steps, free base) was obtained as colorless oil. ESI m/z: 579 $(M + H)^+$.

General Procedure for compound 101-3



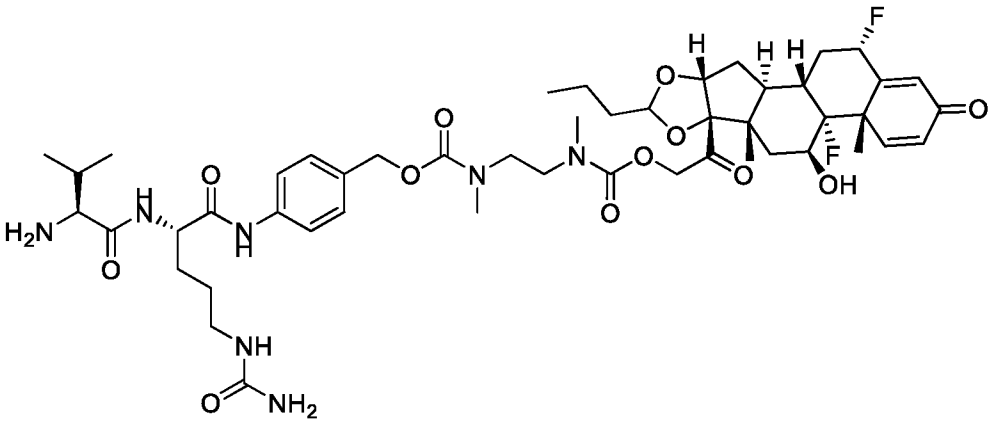
[0722] To a solution of compound **101a, b or d** (0.20 mmol) in DMF (3 mL) were added Fmoc-VC-PAB-PNP **11d** (0.17 g, 0.22 mmol), HOBT (41 mg, 0.30 mmol) and DIPEA (77 mg, 0.60 mmol). The mixture was stirred at RT for 3 hours, which was monitored by LCMS. To the mixture was then added piperidine (0.3 mL). The reaction mixture was stirred at RT for an hour until Fmoc was totally removed according to LCMS. The resulting mixture was directly purified by reversed phase flash chromatography (50-80% acetonitrile in *aq.* NH_4HCO_3 (10 mM)) to give compound **101-3a, b or d** as a white solid.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)carbamate (101-3a)



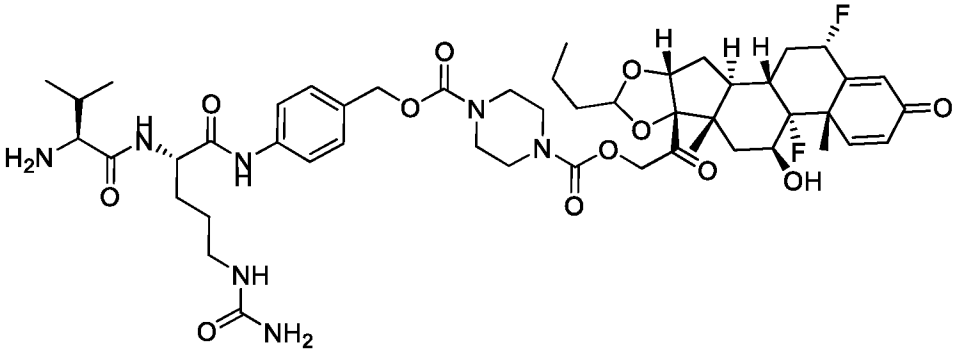
[0723] Following the general procedure and using compound **101a**, compound **101-3a** (0.11 g, 57% yield) was obtained as a white solid. ESI m/z: 972 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-methyl-*N*-[2-(methylamino)ethyl]carbamate (101-3b)



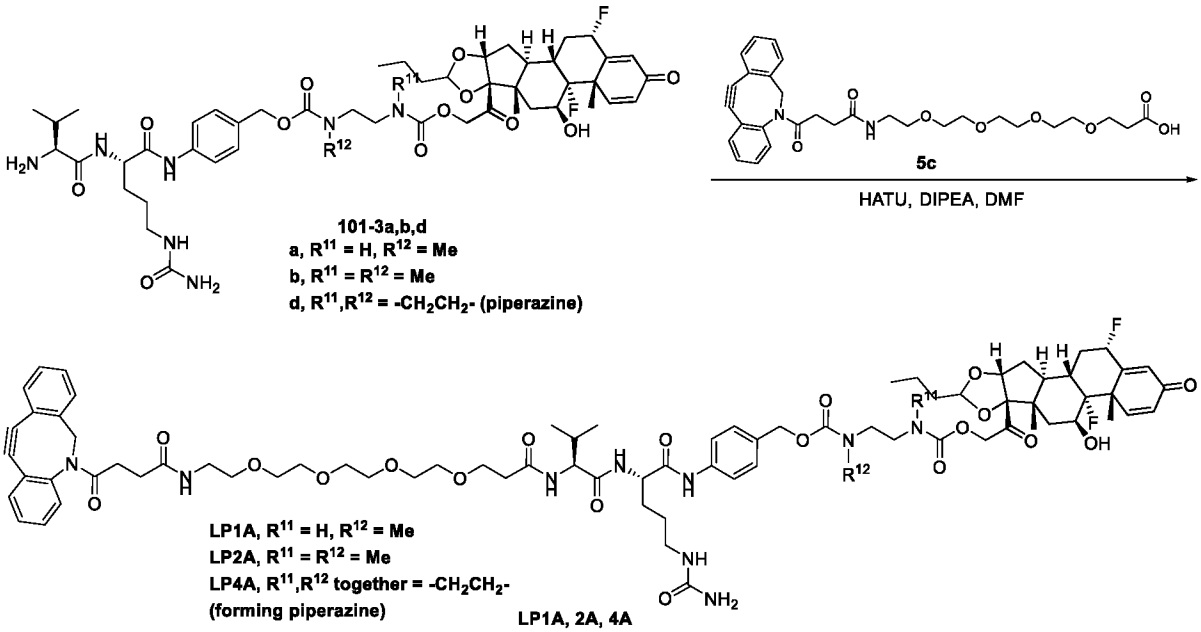
[0724] Following the general procedure and using compound **101b**, compound **101-3b** (0.13 g, 65% yield) was obtained as a white solid. ESI m/z: 986 $(M + H)^+$.

1-{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl} piperazine-1,4-dicarboxylate (101-3d)



[0725] Following the general procedure and using compound **101d** (58 mg, 0.10 mmol), compound **101-3d** (52 mg, 53% yield) was obtained as a white solid. ESI m/z: 984 $(M + H)^+$. ^1H NMR (500 MHz, $DMSO_{d6}$) δ 10.0 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H),7.60 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 8.5 Hz, 1H), 6.30 (dd, J = 1.5 Hz, 10.5 Hz, 1H), 6.11 (s, 1H), 6.02-5.88 (m, 1H), 5.70-5.50 (m, 2H), 5.42 (s, 2H), 5.20-4.92 (m, 3H), 4.80-4.62 (m, 3H), 4.50-4.45 (m, 1H), 4.25-4.15 (m, 1H), 3.50-3.35 (m, 8H), 3.05-2.90 (m, 3H), 2.70-2.54 (m, 2H), 2.32-2.20 (m, 1H), 2.10-1.50 (m, 10H), 1.46 (s, 3H), 1.45-1.30 (m, 5H), 0.95 (d, J = 7.5 Hz, 1H), 0.90-0.80 (m, 9H), 0.77 (d, J = 7.0 Hz, 3H) ppm.

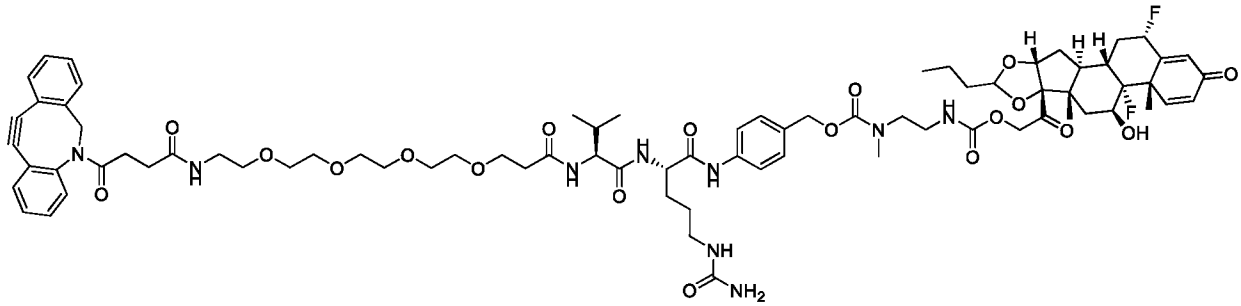
General Procedure for compounds LP1A, LP2A and LP4A



[0726] To a solution of compound **5c** (34 mg, 60 μmol) in DMF (1 mL) were added HATU (27 mg, 71 μmol) and DIPEA (20 mg, 0.15 mmol). The mixture was stirred at RT for 5 minutes before compound **101-3a, b, or d** (50 μmol) was added into the mixture. The mixture was stirred

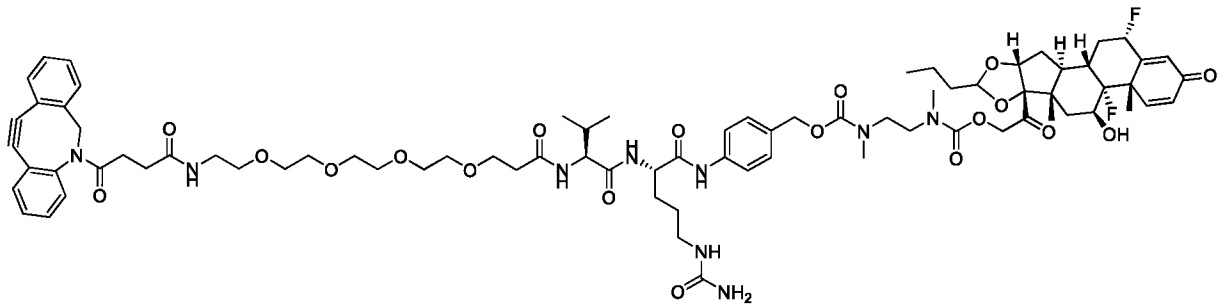
at RT for 2 hours, which was monitored by LCMS. The resulting mixture was directly purified by prep-HPLC (method B) to give compound **LP1A, 2A or 4A** as a white solid.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)carbamate (LP1A)



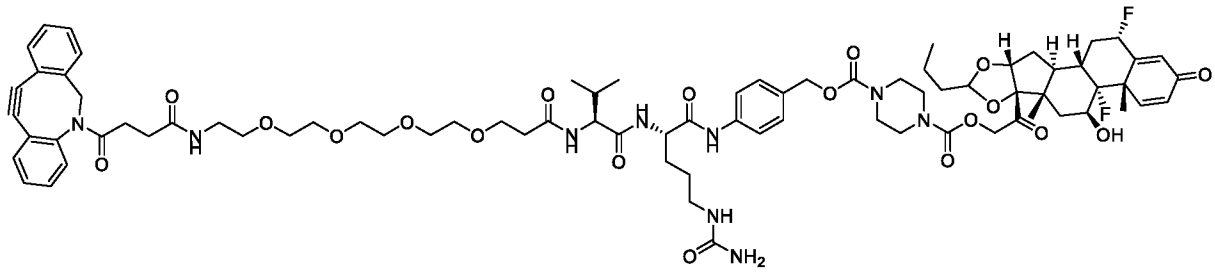
[0727] Following the general procedure and using compound **101-3a**, linker-payload **LP1A** (20 mg, 26% yield) was obtained as a white solid. ESI m/z: 754 (M/2 + H)$^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](methyl)amino}ethyl)-*N*-methylcarbamate (LP2A)



[0728] Following the general procedure and using compound **101-3b**, linker-payload **LP2A** (20 mg, 26% yield) was obtained as a white solid. ESI m/z: 761 (M/2 + H)$^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 9.98 (s, 1H), 8.12 (d, *J* = 7.4 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.76 (t, *J* = 5.4 Hz, 1H), 7.69-7.66 (m, 1H), 7.64-7.57 (m, 3H), 7.51-7.44 (m, 3H), 7.40-7.33 (m, 2H), 7.32-7.25 (m, 4H), 6.33-6.27 (m, 1H), 6.11 (s, 1H), 5.98 (t, *J* = 5.5 Hz, 1H), 5.70-5.55 (m, 2H), 5.41 (s, 2H), 5.08-4.91 (m, 4H), 4.79-4.66 (m, 2H), 4.41-4.35 m, 1H), 4.25-4.17 (m, 2H), 3.64-3.56 (m, 3H), 3.49-3.41 (m, 15H), 3.30-3.27 (m, 2H), 3.13-3.00 (m, 3H), 2.98-2.75 (m, 8H), 2.65-2.54 (m, 2H), 2.48-2.43 (m, 1H), 2.41-2.34 (m, 1H), 2.28-2.19 (m, 2H), 2.07-1.94 (m, 4H), 1.81-1.67 (m, 3H), 1.63-1.53 (m, 5H), 1.48 (s, 3H), 1.45-1.31 (m, 5H), 0.88-0.82 (m, 13H) ppm.

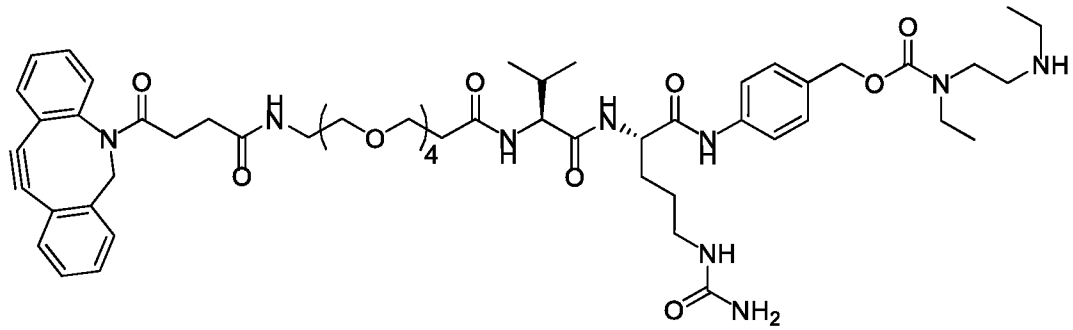
1-{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl 4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl} piperazine-1,4-dicarboxylate (**LP4A**)



[0729] Following the general procedure and using compound **101-3d**, linker-payload **LP4A** (35 mg, 44% yield) was obtained as a white solid. ESI m/z: 760 (M/2 + H)+. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 10.0 (s, 1H), 8.14 (d, *J* = 7.5 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.77 (t, J = 5.0 Hz, 1H), 7.70-7.66 (m, 1H), 7.63-7.55 (m, 3H), 7.52-7.43 (m, 3H), 7.40-7.27 (m, 6H), 6.30 (d, *J* = 10.5 Hz, 1H), 6.11 (s, 1H), 6.02-5.88 (m, 1H), 5.70-5.50 (m, 2H), 5.42 (s, 2H), 5.20-4.92 (m, 4H), 4.80-4.62 (m, 3H), 4.40-4.35 (m, 1H), 4.26-4.20 (m, 2H), 3.64-3.55 (m, 3H), 3.50-3.35 (m, 17H), 3.30-3.25 (m, 3H), 3.15-2.90 (m, 4H), 2.64-2.54 (m, 2H), 2.50-2.45 (m, 1H), 2.41-2.34 (m,1H), 2.32-2.20 (m, 2H), 2.10-1.92 (m, 4H), 1.80-1.68 (m, 3H), 1.62-1.50 (m, 5H), 1.46 (s, 3H), 1.45-1.40 (m, 2H), 1.40-1.30 (m, 3H), 1.22-1.20 (m, 1H), 0.90-0.80 (m, 13H) ppm.

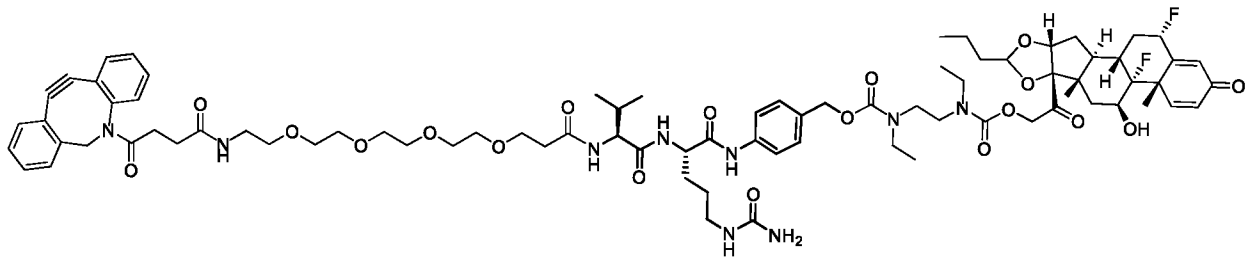
{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-

5-(carbamoylamino)pentanamido]phenyl}methyl *N*-ethyl-*N*-[2-(ethylamino)ethyl]carbamate (101-4)



[0730] To a solution of DIBAC-suc-PEG$_4$-vcPAB-PNP **11b** (0.11 g, 0.10 mmol) in DMF (5 mL) were added *N*,*N*'-diethylethylenediame (58 mg, 0.50 mmol) and DMAP (1 mg, 0.01 mmol). The reaction mixture was stirred at RT for 4 hours, which was monitored by LCMS. The resulting mixture was directly purified by prep-HPLC (method B) to give compound **101-4** as colorless oil. ESI m/z: 1056.6 $(M + H)^+$, 529 $(M/2 + H)^+$.

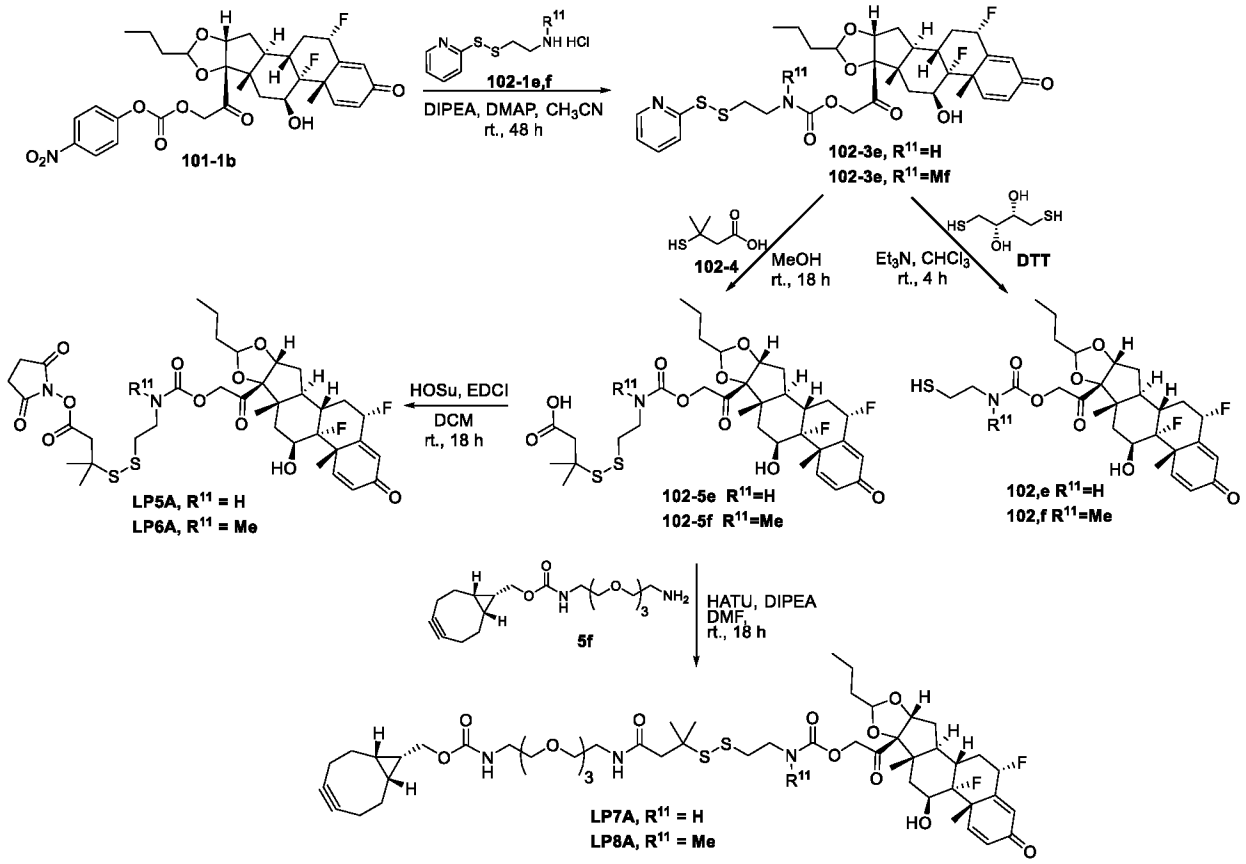
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-{[({4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl](ethyl)amino}ethyl)-*N*-ethylcarbamate (LP3A)



[0731] A mixture of compound **101-4** (16 mg, 15 μmol), **101-1b** (11 mg, 17 μmol) and DIPEA (4.0 mg, 31 μmol) in DMF (5 mL) was stirred at RT for 4 hours, which was monitored by LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give linker-payload **LP3A** (3 mg, 13% yield) as a white solid. ESI m/z: 775 $(M/2 + H)^+$. ^{1}H NMR (500 MHz, DMSO$_{d6}$) δ 10.0 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.77 (t, J = 5.0 Hz, 1H), 7.70-7.66 (m, 1H), 7.63-7.55 (m, 3H), 7.52-7.43 (m, 3H), 7.40-7.27 (m, 7H), 6.30 (d, J = 10.5 Hz, 1H), 6.11 (s, 1H), 6.02-5.88 (m, 1H), 5.70-5.50 (m, 2H), 5.42 (s, 2H), 5.20-4.92 (m, 4H), 4.80-4.62

(m, 3H), 4.40-4.35 (m, 1H), 4.26-4.20 (m, 2H), 3.64-3.55 (m, 4H), 3.50-3.35 (m, 18H), 3.12-2.90 (m, 7H), 2.64-2.54 (m, 3H), 2.41-2.34 (m, 2H), 2.32-2.20 (m, 3H), 2.10-1.92 (m, 5H), 1.88-1.68 (m, 3H), 1.62-1.50 (m, 6H), 1.50-1.40 (m, 6H), 1.40-1.30 (m, 4H), 1.22-1.20 (m, 1H), 1.02-0.90 (m, 4H), 0.90-0.78 (m, 6H) ppm.

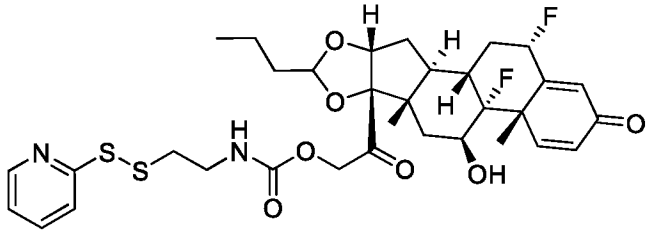
Synthesis of Cysteamine Prodrugs: Linker-payloads LP5A, 6A, 7A and LP8A



[0732] **2-(pyridin-2-yldisulfanyl)ethanamine hydrochloride** (**102-1e**) was commercially available with CAS 83578-21-6. ***tert*-butyl (2-mercaptoethyl)(methyl)carbamate** (**102-1f**) was synthesized according to *Eur. J. Med. Chem.*, **2015**, 95, 483-491.

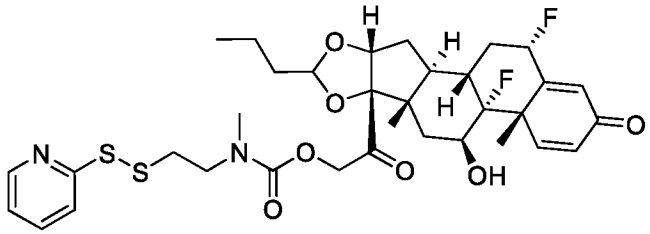
[0733] ***endo*-bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-(2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethyl)carbamate** (**5f**) was synthesized according to WO2016168769.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-[2-(pyridin-2-yldisulfanyl)ethyl]carbamate (102-3e)



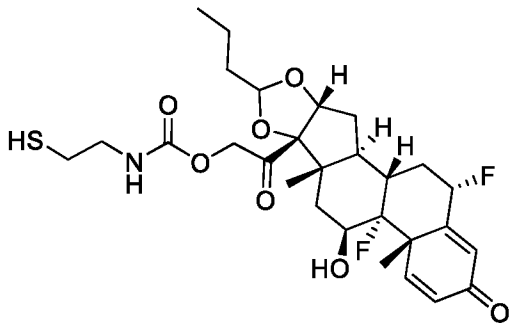
[0734] To a solution of compound **101-1b** (0.54 g, 0.86 mmol) in acetonitrile (10 mL) were added DIPEA (0.22 g, 1.7 mmol), **102-1e** (0.19 g, 0.86 mmol) and DMAP (10 mg, 86 μmol). The reaction mixture was stirred at RT for 48 hours until **101-1b** was totally consumed according to LCMS. The mixture was then filtered and the filtrate was concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (20-50% ethyl acetate in hexane) to give compound **102-3e** (0.47 g, 80% yield) as a white solid. ESI m/z: 679.2 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-methyl-*N*-[2-(pyridin-2-yldisulfanyl)ethyl]carbamate (**102-3f**)



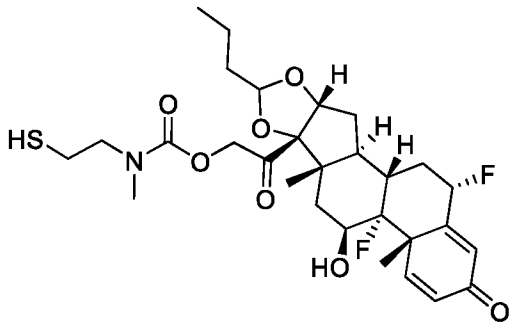
[0735] Following the similar procedure as **102-3e** except substituting **102-1f** for **102-1e**, compound **102-3f** (0.45 g, 75% yield) was obtained as a white solid. ESI m/z: 693.2 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-(2-sulfanylethyl)carbamate (**102e**)



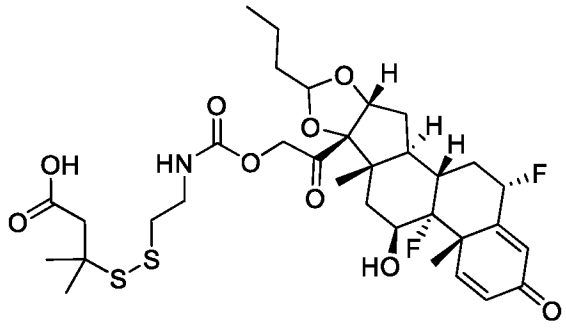
[0736] To a solution of compound **102-3e** (1.4 g, 2.1 mmol) in chloroform (30 mL) were added triethylamine (0.82 mL, 5.9 mmol) and 1,4-dithiothreitol (Cleland's reagent, DTT) (1.2 g, 7.8 mmol). The reaction mixture was stirred at RT under nitrogen for 4 hours, which was monitored by LCMS. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50-70% ethyl acetate in hexane) to give compound **102e** (0.95 g, 83% yield) as a white solid. ESI m/z: 570.2 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl *N*-methyl-*N*-(2-sulfanylethyl)carbamate (102f)



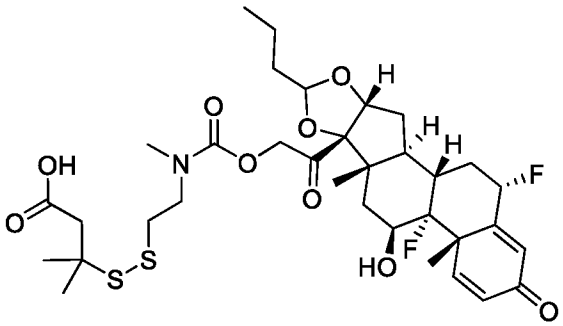
[0737] Following the similar procedure as **102e** except substituting **102-3f** for **102-3e**, compound **102f** (0.97 g, 83% yield) was obtained as a white solid. ESI m/z: 584.3 $(M + H)^+$.

3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)amino]ethyl}disulfanyl)-3-methylbutanoic acid (102-5e)



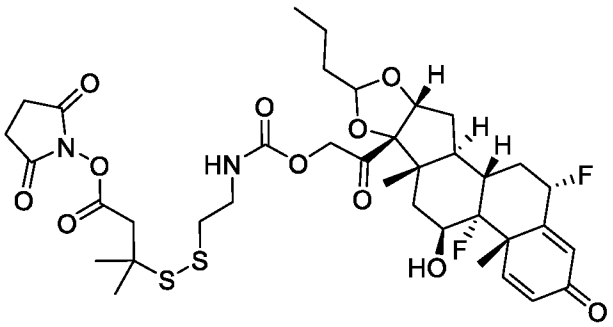
[0738] To a solution of compound **102-3e** (0.68 g, 1.0 mmol) in methanol (5 mL) were added compound **102-4** (0.13 g, 1.0 mmol). The reaction mixture was stirred at RT for 18 hours, which was monitored by LCMS. The resulting mixture was then directly purified by prep-HPLC (method B) to give compound **102-5e** (0.39 g, 55% yield) as a white solid. ESI m/z: 702.2 $(M + H)^+$.

3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)(methyl)amino]ethyl}disulfanyl)-3-methylbutanoic acid (**102-5f**)



[0739] Following the similar procedure as **102-5e** except substituting **102-3f** for **102-3e**, compound **102-5f** (0.29 g, 40% yield) was obtained as a white solid. ESI m/z: 716.3 $(M + H)^+$.

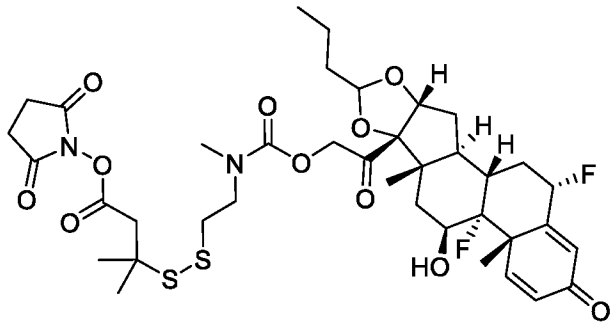
2,5-Dioxopyrrolidin-1-yl 3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)amino]ethyl}disulfanyl)-3-methylbutanoate (**LP5A**)



[0740] To a solution of compound **102-5e** (0.20 g, 0.29 mmol) in DCM (10 mL) were added HOSu (73 mg, 0.64 mmol) and EDCI (0.12 g, 0.64 mmol), and the mixture was stirred at RT for 24 hours, which was monitored by LCMS. The resulting mixture was diluted with DCM (50 mL) and the organic solution was washed with water and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by prep-HPLC (method A) to give **LP5A** (85 mg, 37% yield) as colorless oil. ESI m/z: 799.3 $(M + H)^+$.

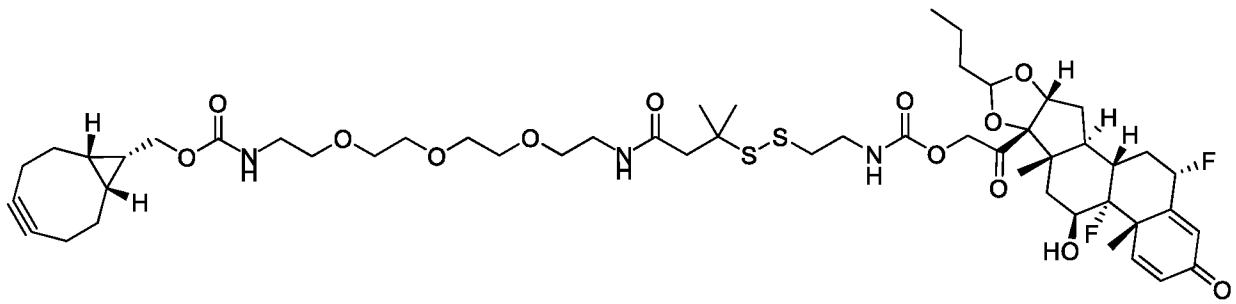
2,5-Dioxopyrrolidin-1-yl 3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-

14,17-dien-8-yl]-2-oxoethoxy}carbonyl)(methyl)amino]ethyl}disulfanyl)-3-methylbutanoate (LP6A)



[0741] Following the similar procedure as **LP5A** except substituting **102-5f** for **102-5e**, compound **LP6A** (86 mg, 36% yield) was obtained as colorless oil. ESI m/z: 813.3 $(M + H)^+$.

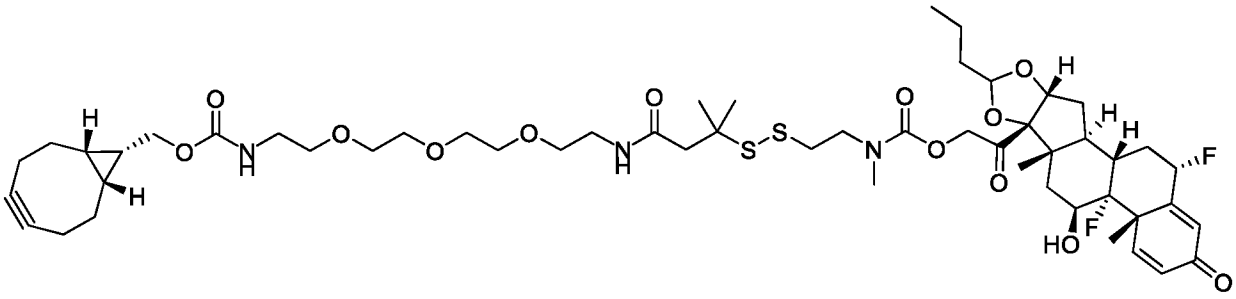
***endo*-Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-{2-[2-(2-{2-[3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}carbonyl)amino]ethyl}disulfanyl)-3-methylbutanamido]ethoxy}ethoxy)ethoxy]ethyl}carbamate (LP7A)**



[0742] To a solution of compound **102-5e** (0.20 g, 0.29 mmol) in DMF (10 mL) were added HATU (0.11 g, 0.29 mmol) and DIPEA (75 mg, 0.58 mmol). The reaction mixture was stirred at RT for 10 minutes before compound **5f** (0.11 g, 0.29 mmol) was added into. The reaction mixture was stirred at RT for 18 hours, which was monitored by LCMS. The resulting mixture was then directly purified by prep-HPLC (method B) to give compound **LP7A** (0.16 g, 55% yield) as a white solid. ESI m/z: 526.7 $(M/2 + H)^+$.

***endo*-Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-{2-[2-(2-{2-[3-({2-[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-**

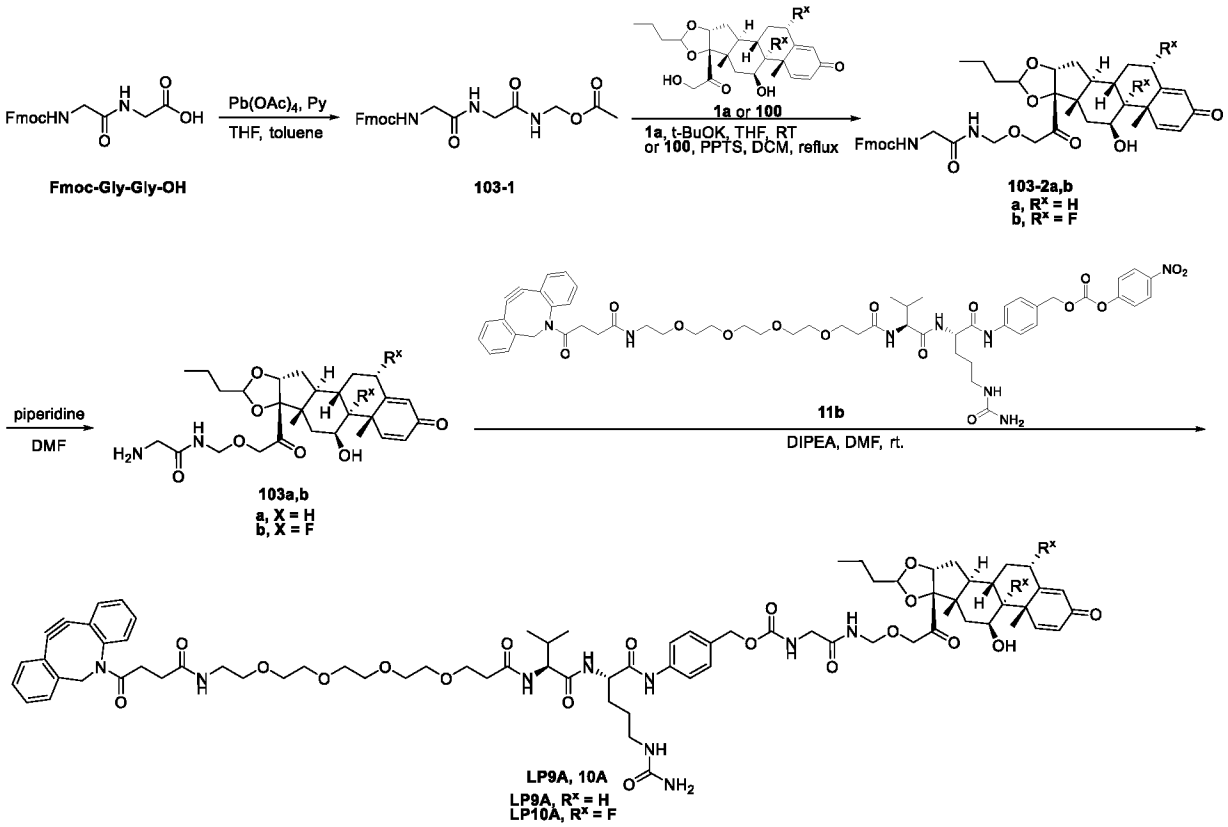
oxoethoxy}carbonyl)(methyl)amino]ethyl}disulfanyl)-3-methylbutanamido]ethoxy}ethoxy)ethoxy]ethyl}carbamate (LP8A)



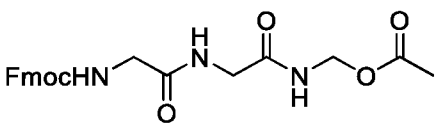
[0743] Following the similar procedure as **LP7A** except substituting **102-5f** for **102-5e**, compound **LP8A** (0.13 g, 41% yield) was obtained as a white solid. ESI m/z: 533.8 $(M/2 + H)^+$.

Synthesis of AMO linker-payloads LP9A-11A

Scheme 103A. Synthesis of Payloads 103a,b and Linker-payloads LP9A-10A

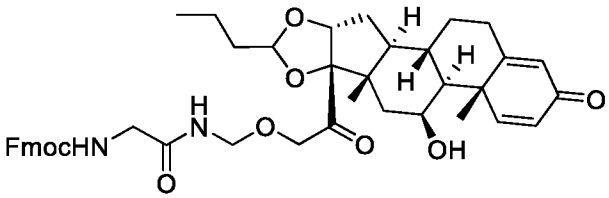


(2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)acetamido)methyl acetate (103-1)



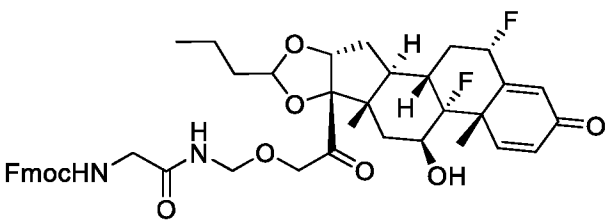
[0744] To a mixture of Fmoc-Gly-Gly-OH (4.3 g, 12 mmol) in THF (0.12 L) and toluene (40 mL) were added pyridine (1.2 mL, 15 mmol) and lead tetraacetate (6.8 g, 15 mmol). The reaction mixture was refluxed for 5 hours, which was monitored by LCMS. After cooled to RT, the reaction mixture was filtered through Celite to remove the insoluble material, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed with water and brine. The organic solution was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to provide compound **103-1** (3.0 g, 67% yield) as a colorless solid. ESI m/z: 391 $(M + Na)^+$.

9*H*-Fluoren-9-ylmethyl *N*-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamate (**103-2a**)



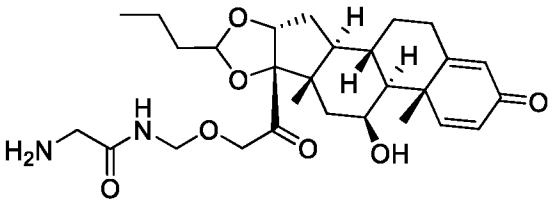
[0745] To a solution of compound **103-1** (0.37 g, 1.0 mmol) and budesonide **1a** (1.3 g, 3.0 mmol) in THF (4 mL) was added potassium *tert*-butoxide (0.22 g, 2.0 mmol) at 0 °C. The reaction mixture was stirred at RT for 15 minutes, which was monitored by TLC. The reaction solution was charged with ethyl acetate and water at 0 °C, and extracted with ethyl acetate and chloroform. The combined organic solution was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (30-35% ethyl acetate in petroleum ether) to give compound **103-2a** (0.19 g, 40% yield) as a white solid. ESI m/z: 739 $(M + H)^+$.

9*H*-Fluoren-9-ylmethyl *N*-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamate (**103-2b**)



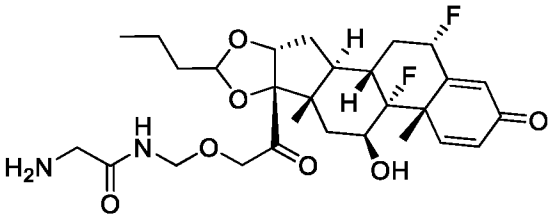
[0746] To a sealed tube were added a solution of compound **103-1** (0.30 g, 0.81 mmol) and compound **100** (0.38 g, 0.81 mmol) in DCM (4 mL) and pyridinium *p*-toluenesulfonate (21 mg, 81 μmol) at RT. The reaction tube was sealed and stirred at 50 °C for 24 hours, which was monitored by LCMS. After cooled, the reaction mixture was concentrated *in vacuo* and the residue was purified by prep-HPLC (method A) to give compound **103-2b** (0.23 g, 37% yield) as a white solid. ESI m/z: 775 $(M + H)^+$.

2-Amino-*N*-({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)acetamide (**103a**)

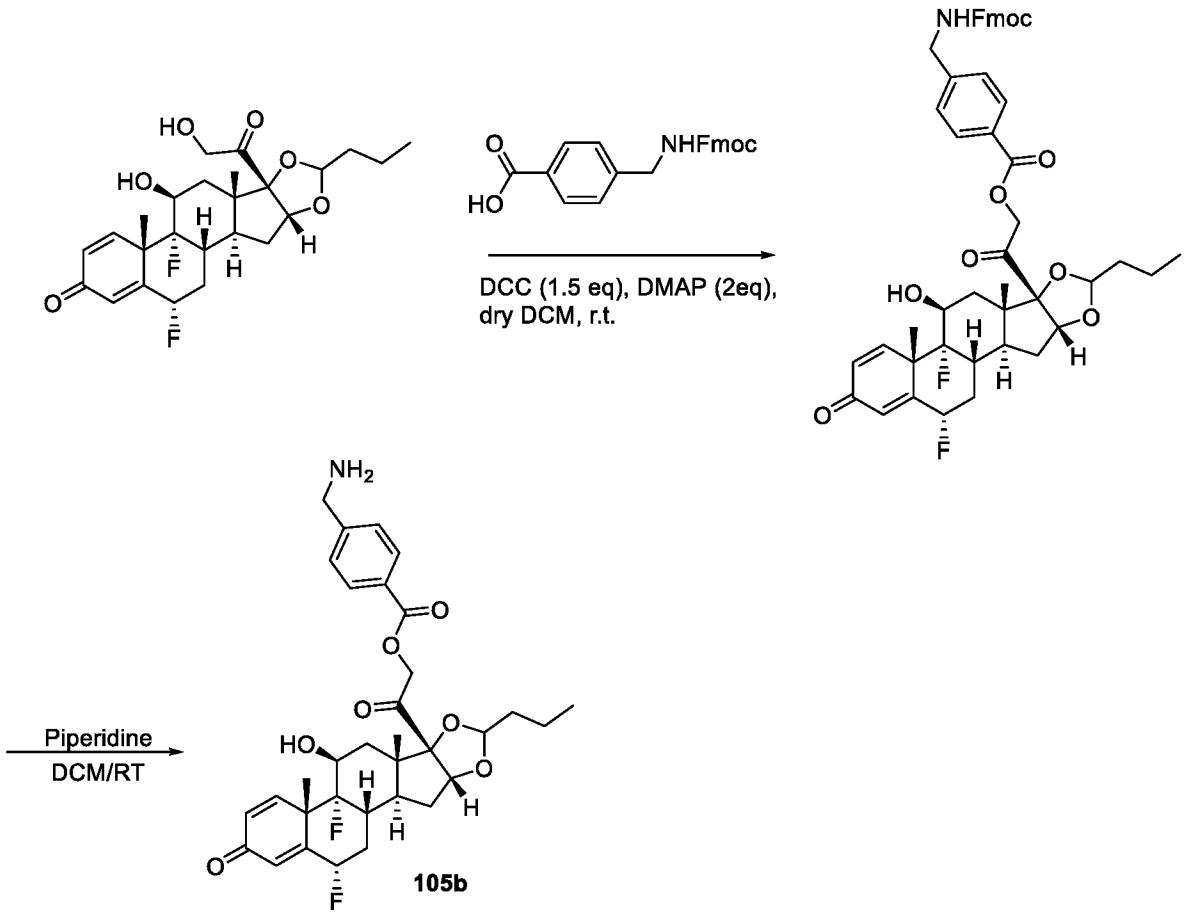


[0747] To a solution of compound **103-2a** (0.10 g, 0.14 mmol) in DMF (2 mL) was added piperidine (35 mg, 0.41 mmol). The reaction mixture was stirred at RT for 2 hours until Fmoc was totally removed, which was monitored by LCMS. The resulting mixture was directly purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.05%)) to give compound **103a** (50 mg, 70% yield) as a white solid. ESI m/z: 517.6 $(M + H)^+$.

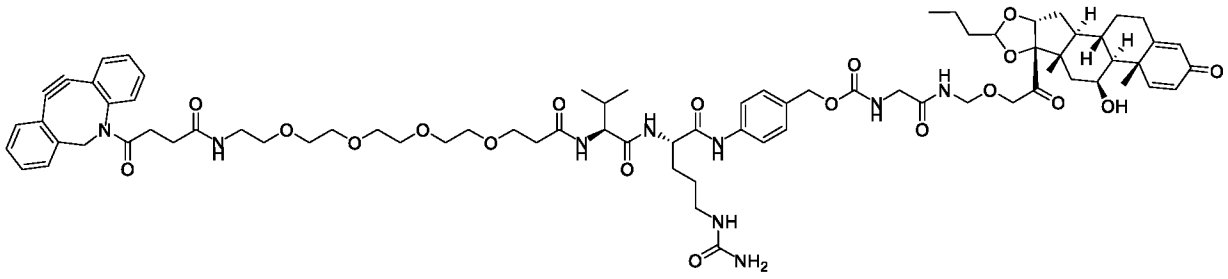
2-Amino-*N*-({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)acetamide (**103b**)



[0748] Following the similar procedure as **103a** except substituting **103-2b** for **103-2a**, compound **103b** (0.26 g, 65% yield) was obtained as a white solid. ESI m/z: 553.2 $(M/2 + H)^+$.

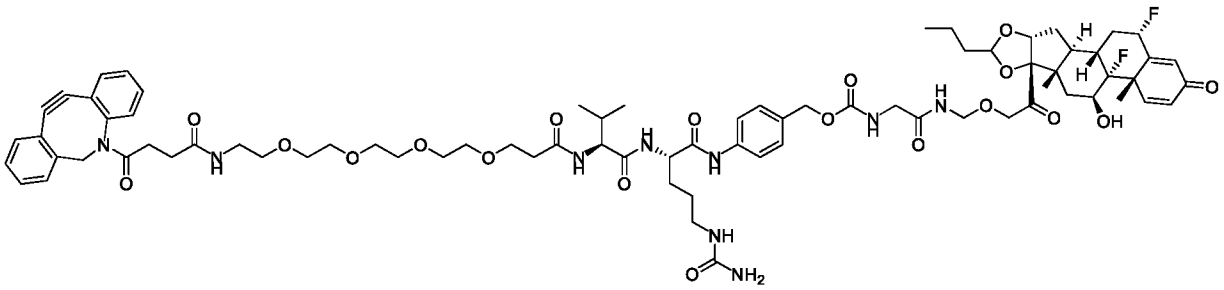


[0749] Compound 105(b) was prepared according to the above procedure. **{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4},^{9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*S*,13*R*)-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamate (LP9A)**



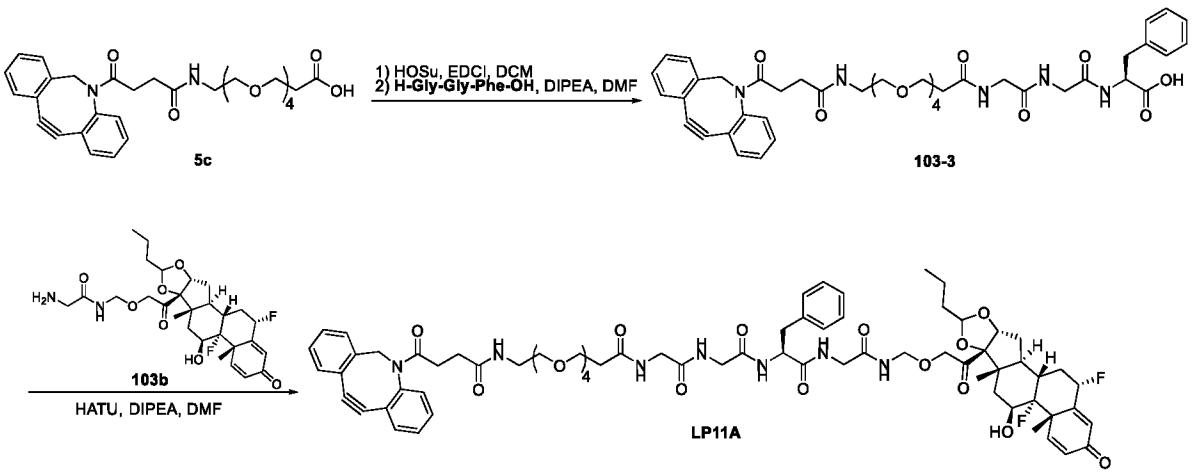
[0750] To a solution of compound **103a** (15 mg, 29 μmol) in DMF (1.0 mL) were added compound **11b** (30 mg, 28 μmol), HOBT (2.0 mg, 15 μmol) and DIPEA (7.7 mg, 60 μmol). The reaction mixture was stirred at RT for 2 hours, which was monitored by LCMS. The mixture was directly purified by prep-HPLC (method B) to give linker-payload **LP9A** (5.0 mg, 11% yield) as a white solid. ESI m/z: 729 $(M/2 + H)^{+}$.

{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamate (LP10A)

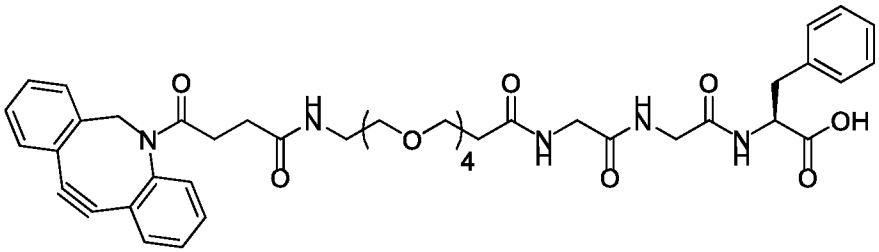


[0751] Following the similar procedure as **LP9A** except substituting **103b** for **103a**, linker-payload **LP10A** (23 mg, 58% yield) was obtained as a white solid. ESI m/z: 747.0 $(M/2 + H)^+$; 513.8 (fragment cleaved by LCMS, piece cleaved at $NHCH_2$-O) ^{1}HNMR (400 MHz, $DMSO_{d6}$) δ 10.02 (s, 1H), 8.80-8.72 (m, 1H), 8.15 (d, J = 6.8 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.80-7.75 (m, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.64-7.58 (m, 3H), 7.54-7.22 (m, 9H), 6.29 (d, J = 10.0 Hz, 1H), 6.10 (s, 1H), 6.04-5.96 (m, 1H), 5.78-4.10 (m, 15H), 3.66-3.54 (m, 5H), 3.54-3.42 (m, 13H), 3.32-3.26 (m, 2H), 3.20-2.50 (m, 7H), 2.42-2.20 (m, 4H), 2.05-1.90 (m, 4H), 1.85-1.25 (m, 15H), 0.90-0.80 (m, 13H) ppm.

Scheme 103B. Synthesis of Linker-payload LP11A

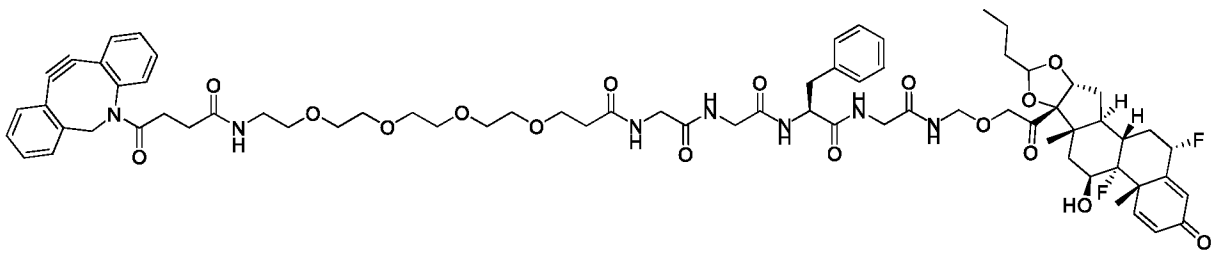


(2*S*)-2-(2-{2-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]acetamido}acetamido)-3-phenylpropanoic acid (103-3)



[0752] To a solution of compound **5c** (0.16 g, 0.29 mmol) in DCM (20 mL) were added HOSu (73 mg, 0.64 mmol) and EDCI (0.12 g, 0.64 mmol). The mixture was stirred at RT for 24 hours until compound **5c** was totally consumed according to LCMS. The resulting mixture was diluted with DCM (50 mL) and the organic solution was washed with water (50 mL) and brine (50 mL), dried with anhydrous sodium sulfate and concentrated *in vacuo* to give the OSu active ester (0.16 g, 84% yield) as colorless oil, which was used for next step directly.
To a solution of **H-Gly-Gly-Phe-OH** (10 mg, 36 μmol) in DMF (0.5 mL) were added the OSu active ester (23 mg, 36 μmol) obtained above and DIPEA (9.0 mg, 72 μmol). The reaction mixture was stirred at RT overnight. The mixture was directly purified by prep-HPLC (method B) to give compound **103-3** (15 mg, 51% yield) as a white solid. ESI m/z: 408.2 $(M/2 + H)^+$.

1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-{[({[(1*S*)-1-({[({2-[(19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamoyl)-2-phenylethyl]carbamoyl}methyl)carbamoyl]methyl}-3,6,9,12-tetraoxapentadecan-15-amide (LP11A)

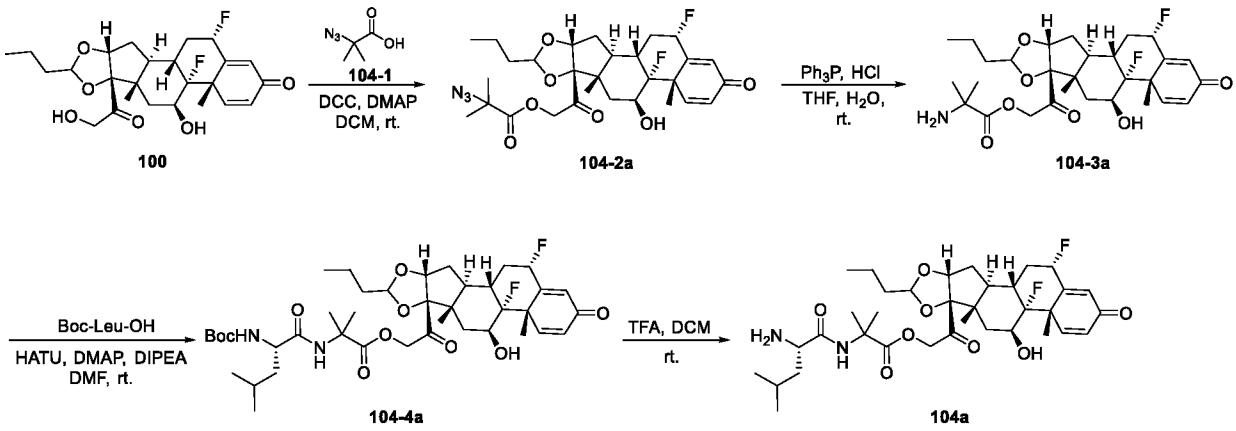


[0753] To a solution of compound **103-3** (15 mg, 18 μmol) in DMF (0.5 mL) were added HATU (14 mg, 36 μmol) and DIPEA (9.3 mg, 72 μmol). The reaction mixture was stirred at RT for 10 min before compound **103b** (10 mg, 18 μmol) was added into the mixture. The reaction mixture

was then stirred at RT overnight. The resulting mixture was directly purified by prep-HPLC (method B) to give linker-payload **LP11A** (5.0 mg, 20% yield) as a white solid. ESI m/z: 420.2 $(M/3 + H)^+$.

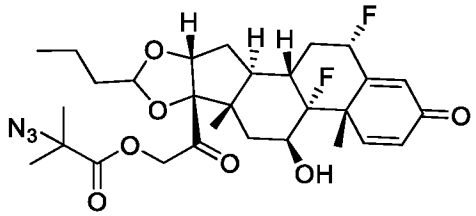
Synthesis of dipeptide prodrug: Linker-payload LP12A

Scheme 104A. Synthesis of payload 104a



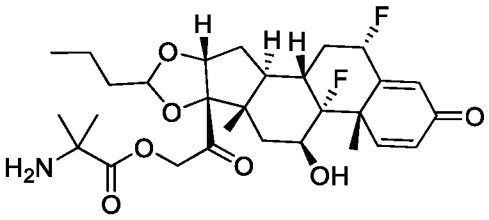
[0754] ***Alpha*-Azidoisobutyric acid 104-1** was synthesized according to *Org. Lett.*, **2001**, 3(5), 781-783.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-azido-2-methylpropanoate (104-2a)



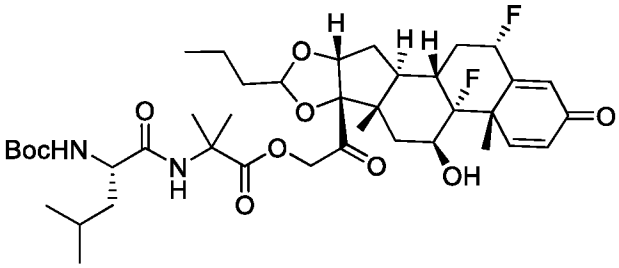
[0755] To a solution of compound **100** (0.10 g, 0.21 mmol) in DCM (30 mL) were added compound **104-1** (52 mg, 0.40 mmol), DCC (90 mg, 0.44 mmol) and DMAP (10 mg) and the mixture was stirred at RT overnight, which was monitored by LCMS. The resulting mixture was concentrated *in vacuo* and the residue was purified by prep-HPLC (method A) to give compound **104-2a** (80 mg, 70% yield) as a white solid. ESI m/z: 578.3 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-amino-2-methylpropanoate (**104-3a**)



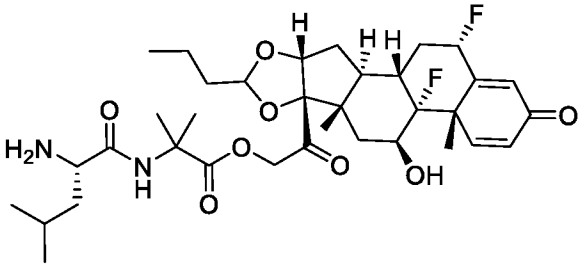
[0756] To a solution of compound **104-2a** (75 mg, 0.15 mmol) in THF (5 mL) were added PPh_3 (0.15 g, 0.57 mmol), water (5 mL) and *conc.* HCl (1 drop), and the reaction mixture was stirred at RT overnight. The mixture was concentrated *in vacuo* and diluted with DMF. The precipitated was removed off by filtration. The filtrate was purified by prep-HPLC (method A) to give compound **104-3a** (45 mg, 54% yield) as a white solid. ESI m/z: 552.3 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-4-methylpentanamido]-2-methylpropanoate (**104-4a**)



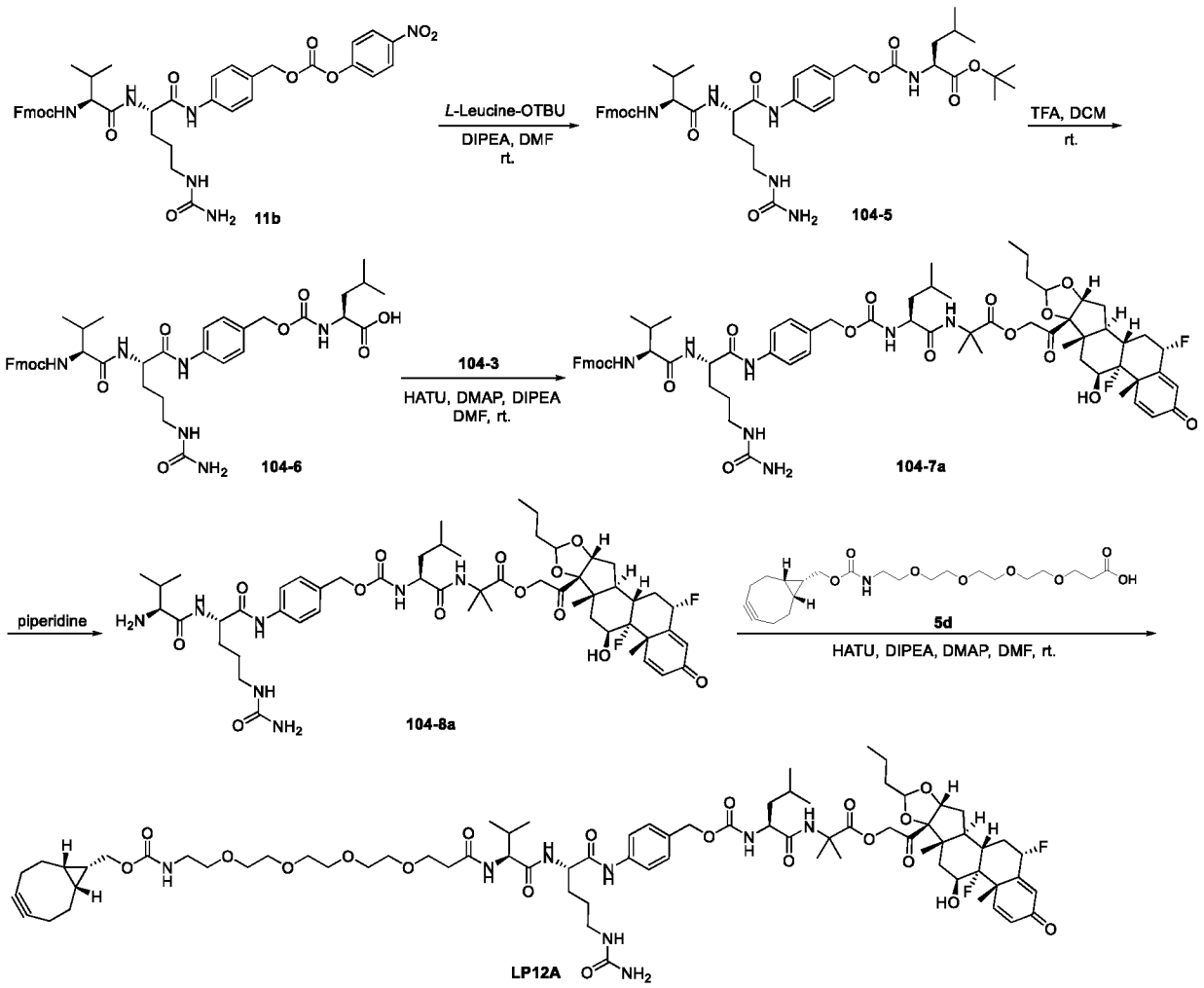
[0757] To a mixture of Boc-Leu-OH (20 mg, 87 μmol) and HATU (50 mg, 0.13 mmol) in DMF (3 mL) were added DIPEA (30 mg, 0.23 mmol), DMAP (2 mg) and compound **104-3a** (30 mg, 54 μmol). The mixture was stirred at RT for an hour, which was monitored by LCMS. The mixture was directly purified by prep-HPLC (method A) to give compound **104-4a** (25 mg, 55% yield) as a white solid. ESI m/z: 787.4 $(M + Na)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-[(2*S*)-2-amino-4-methylpentanamido]-2-methylpropanoate (**104a**)

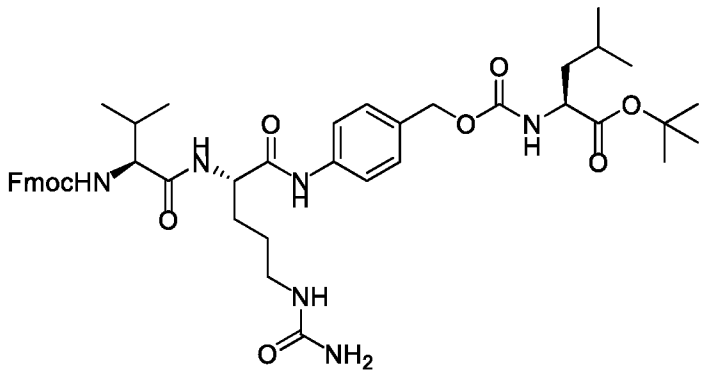


[0758] To a mixture of compound **104-4a** (25 mg, 33 μmol) in DCM (1.5 mL) was added TFA (0.15 mL), and the mixture was stirred at RT for an hour until Boc was totally removed according to LCMS. The reaction mixture was concentrated *in vacuo* and the residue was purified by prep-HPLC (method A) to give compound **104a** (13 mg, 61% yield) as a white solid. ESI m/z: 665.4 $(M + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 8.39 (s, 1H), 8.26 (s, 1H), 7.27 (d, J = 10.0 Hz, 1H), 6.31 (d, J = 10.1 Hz, 1H), 6.11 (s, 1H), 5.63 (t, J = 24.2 Hz, 2H), 5.30-5.05 (m, 1H), 4.96 (d, J = 17.7 Hz, 1H), 4.81-4.65 (m, 2H), 4.20 (s, 1H), 2.69-2.54 (m, 1H), 2.36-2.15 (m, 1H), 2.12-1.94 (m, 2H), 1.85-1.63 (m, 2H), 1.63-1.53 (m, 3H), 1.52-1.41 (m, 11H), 1.41-1.10 (m, 6H), 0.97-0.76 (m, 12H) ppm.

Scheme 104B. Synthesis of Linker-payload LP12A



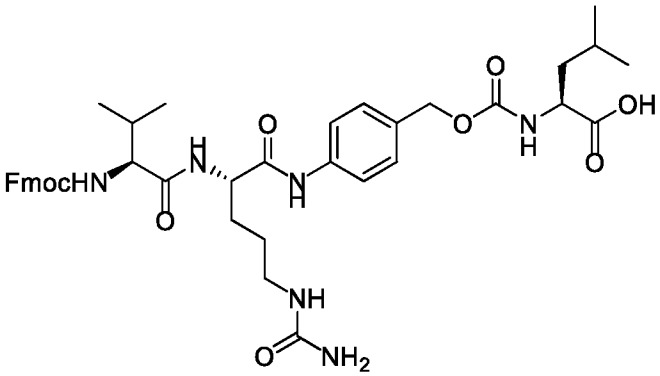
***tert*-Butyl (2*S*)-2-{[({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-3-methylbutanamido]pentanamido]phenyl}methoxy)carbonyl]amino}-4-methylpentanoate (104-5)**



[0759] To a solution of compound **11b** (0.30 g, 0.39 mmol) and H-Leu-OTBU-OH (0.14 g, 0.63 mmol) in DMF (3 mL) was added DIPEA (0.26 g, 2.0 mmol), and the reaction mixture was

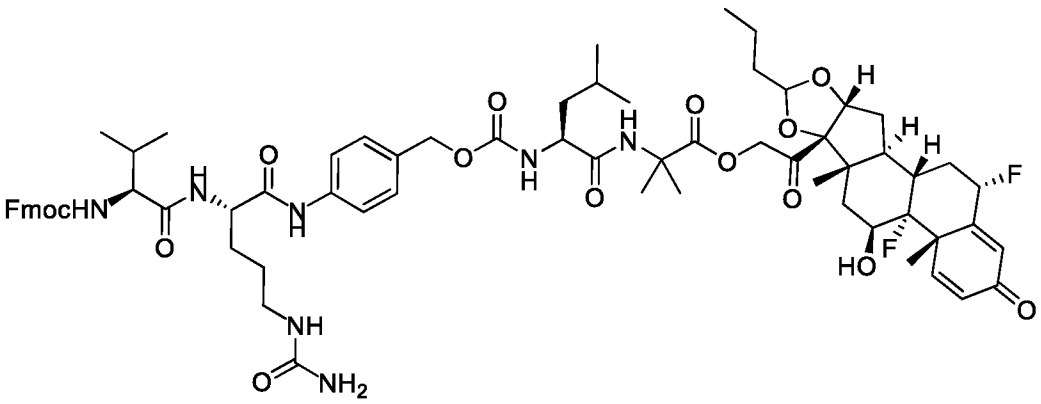
stirred at RT for 2 hours. The resulting mixture was directly purified by prep-HPLC (method A) to give compound **104-5** (0.11 g, 35% yield) as a white solid. ESI m/z: 815.4 $(M + H)^+$.

(2*S*)-2-{[({4-[(2*S*)-5-(Carbamoylamino)-2-[(2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-3-methylbutanamido]pentanamido]phenyl}methoxy)carbonyl]amino}-4-methylpentanoic acid (104-6)



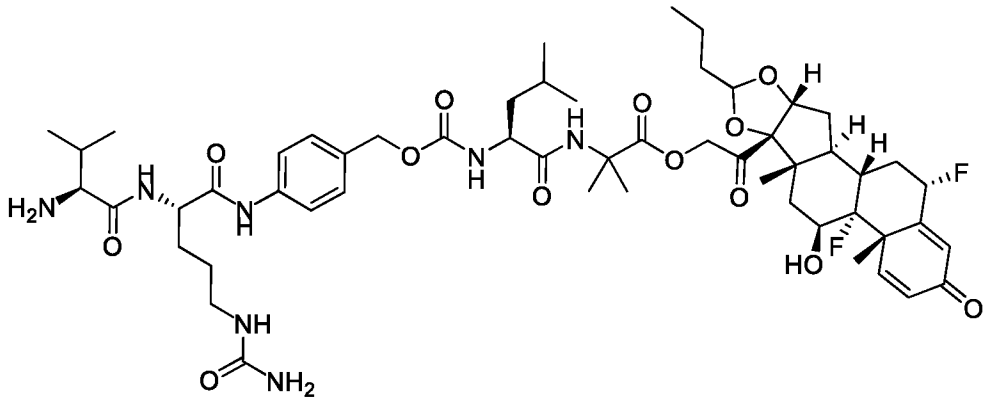
[0760] To a solution of compound **104-5** (25 mg, 31 μmol) in DCM (4 mL) was added TFA (0.8 mL) and the mixture was stirred at RT for 2 hours. The resulting mixture was concentrated *in vacuo* and the residue was purified by prep-HPLC (method A) to give compound **104-6** (15 mg, 67% yield) as a white solid. ESI m/z: 759.3 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-[(2*S*)-2-{[({4-[(2*S*)-5-(carbamoylamino)-2-[(2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-3-methylbutanamido]pentanamido]phenyl}methoxy)carbonyl]amino}-4-methylpentanamido]-2-methylpropanoate (104-7a)



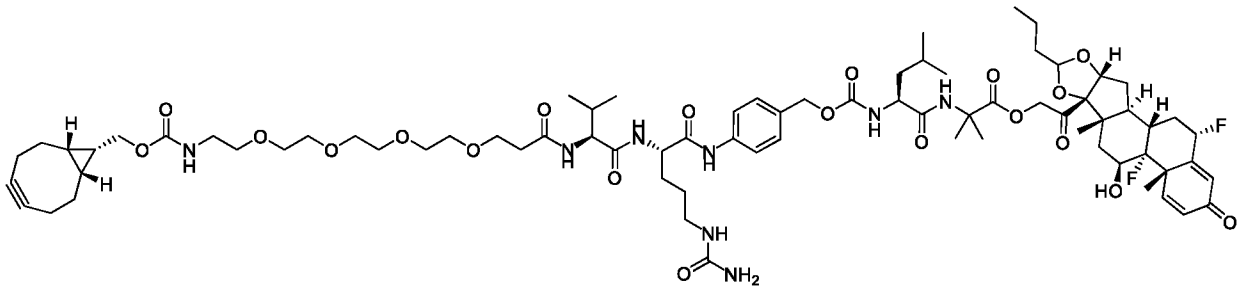
[0761] To a solution of compound **104-5** (20 mg, 26 μmol) and compound **104-3** (17 mg, 32 μmol) in DMF (2 mL) were added HATU (20 mg, 52 μmol), DIPEA (13 mg, 0.10 mmol) and DMAP (1 mg), and the reaction mixture was stirred at RT for an hour, which was monitored by LCMS. The resulting mixture was directly purified by prep-HPLC (method A) to give compound **104-7a** (30 mg, 89% yield) as a white solid. ESI m/z: 1293 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-[(2*S*)-2-{[({4-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-4-methylpentanamido]-2-methylpropanoate (**104-8a**)



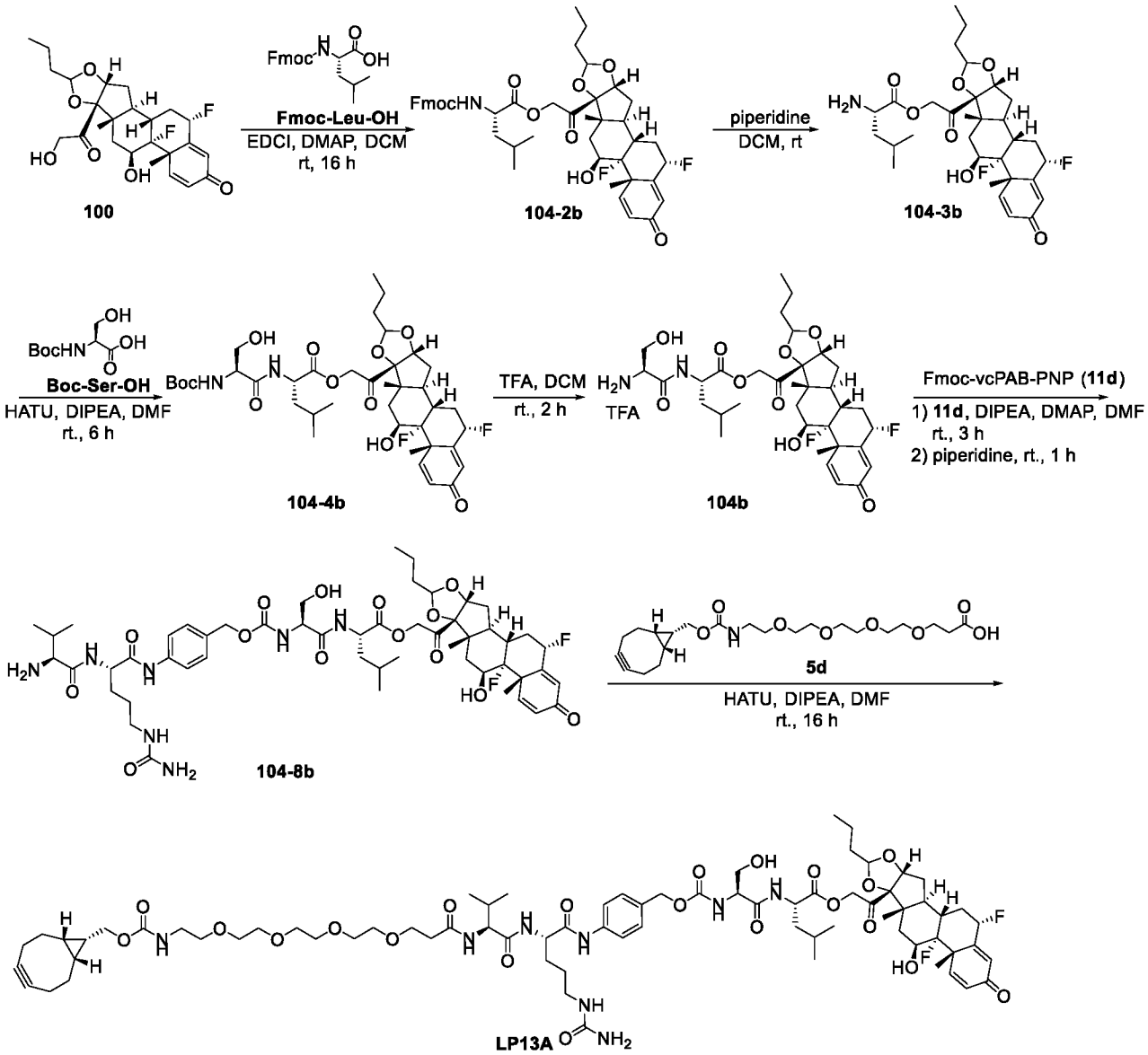
[0762] To a solution of compound **104-7a** (25 mg, 19 μmol) in DMF (2 mL) was added piperidine (0.2 mL), and the mixture was stirred at RT for half an hour until Fmoc was totally removed according to LCMS. The reaction mixture was immediately purified by reversed phase flash chromatography (0-50% acetonitrile in *aq.* TFA (0.03%)) to give compound **104-8a** (20 mg, 95% yield) as a white solid. ESI m/z: 1070.5 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-[(2*S*)-2-{[({4-[(2*S*)-2-[(2*S*)-2-[1-({[*endo*-bicyclo[6.1.0]non-4-yn-9-ylmethoxy]carbonyl}amino)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-4-methylpentanamido]-2-methylpropanoate (**LP12A**)

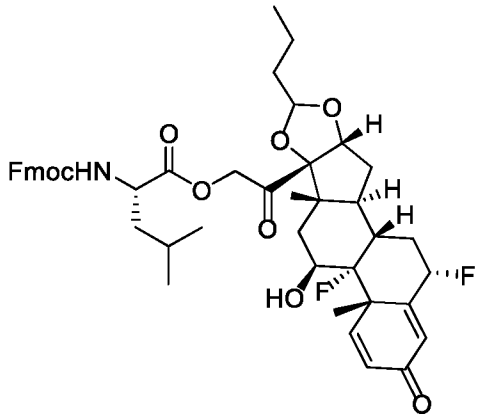


[0763] To a solution of compound **104-8a** (20 mg, 26 μmol) and compound **5d** (14 mg, 32 μmol) in DMF (2 mL) were added HATU (22 mg, 58 μmol), DIPEA (15 mg, 0.12 mmol) and DMAP (1 mg), and the reaction mixture was stirred at RT for 2 hours, which was monitored by LCMS. The resulting mixture was directly purified by prep-HPLC (method A) to give linker-payload **LP12A** (10 mg, 21% yield) as a white solid. ESI m/z: 747.6 $(M/2 + H)^+$. ^1H NMR (400 MHz, $DMSO_{d6}$) δ 9.97 (s, 1H), 8.35-8.25 (m, 1H), 8.11 (d, J = 7.4 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 8.2 Hz, 2H), 7.34-7.18 (m, 4H), 7.17-7.04 (m, 1H), 6.30 (d, J = 10.1 Hz, 1H), 6.11 (s, 1H), 6.03-5.90 (s, 1H), 5.72-5.50 (m, 2H), 5.41 (s, 2H), 5.34-3.91 (m, 12H), 3.66-3.38 (m, 15H), 3.15-2.88 (m, 4H), 2.73-2.53 (m, 1H), 2.50-2.45 (m, 1H), 2.41-2.31 (m, 1H), 2.30-1.90 (m, 9H), 1.83-1.62 (m, 2H), 1.63-1.17 (m, 27H), 0.92-0.77 (m, 20H) ppm.

Scheme 104C. Synthesis of Payload 104b and its linker-payload LP13A

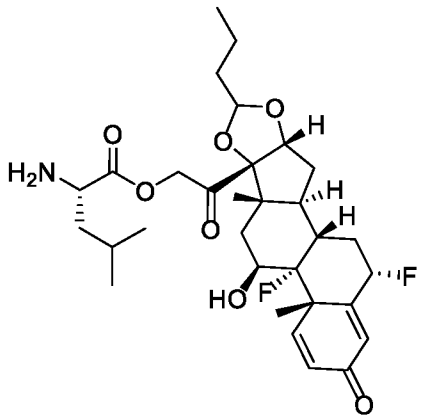


2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-methylpentanoate (104-2b)



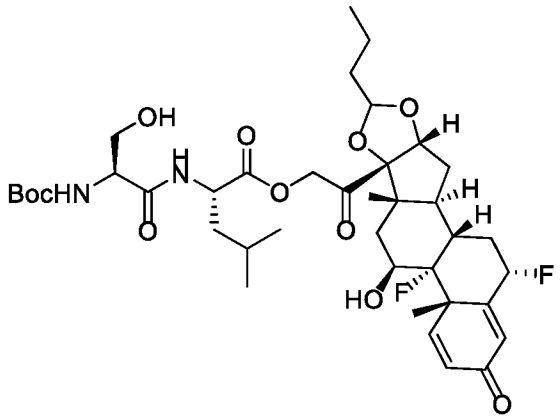
[0764] To a solution of compound **100** (0.47 g, 1.0 mmol) and **Fmoc-Leu-OH** (0.39 g, 1.1 mmol) in DCM (10 mL) were added EDCI (0.23 g, 1.2 mmol) and DMAP (12 mg, 0.10 mmol). The mixture was stirred at RT for 16 hours, which was monitored by LCMS. The resulting mixture was diluted with water (50 mL) and extracted with DCM (50 mL x 2). The combined organic solution was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified silica gel column chromatography (10-50% ethyl acetate in petroleum ether) to give compound **104-2b** (0.52 g, 65% yield) as a white solid. ESI m/z: 802.4 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-amino-4-methylpentanoate (**104-3b**)



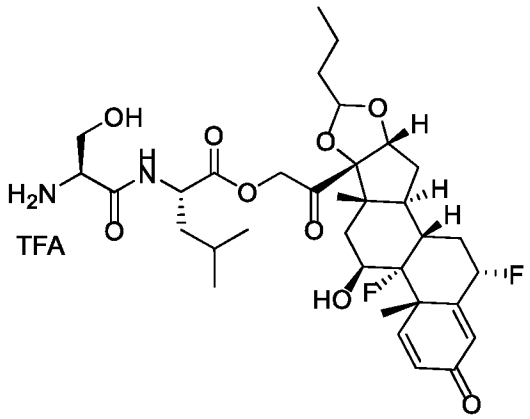
[0765] To a solution of compound **104-2b** (0.50 g, 0.62 mmol) in DCM (9 mL) was added piperidine (1 mL) and the reaction mixture was stirred at RT for 30 minutes until Fmoc was totally removed according to LCMS. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5-10% methanol in DCM) to give compound **104-3b** (0.32 g, 90% yield) as a white solid. ESI m/z: 580.3 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-hydroxypropanamido]-4-methylpentanoate (104-4b)



[0766] To a mixture of compound **104-3b** (0.32 g, 0.55 mmol) and **Boc-Ser-OH** (0.14 g, 0.66 mmol) in DMF (3 mL) were added HATU (0.25 g, 0.66 mmol) and DIPEA (0.21 g, 1.6 mmol) at RT. The mixture was stirred at RT for 6 hours, which was monitored by LCMS. The resulting mixture was concentrated *in vacuo*, and the residue was purified silica gel column chromatography (10-50% ethyl acetate in petroleum ether) to give compound **104-4b** (0.34 mg, 80% yield) as a white solid. ESI m/z: 767.4 $(M + H)^+$.

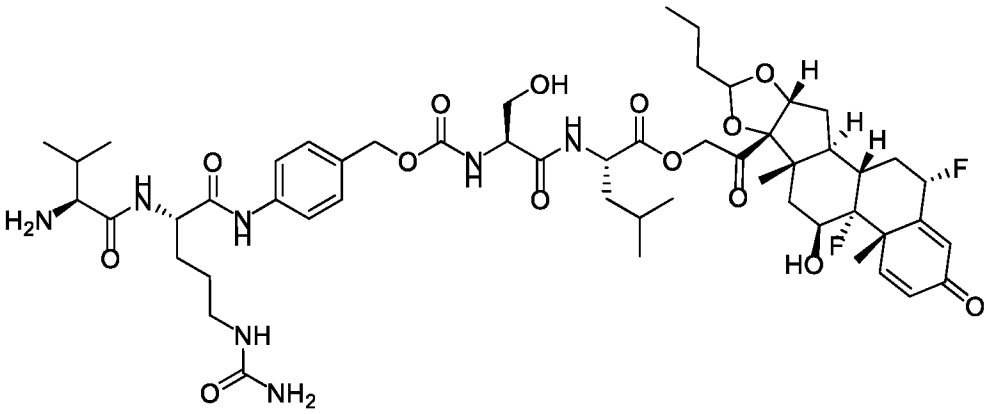
2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-[(2*S*)-2-amino-3-hydroxypropanamido]-4-methylpentanoate trifluoroacetic acid salt (104b)



[0767] To a solution of compound **104-4b** (0.34 g, 0.44 mmol) in DCM (5 mL) was added TFA (1 mL). The reaction mixture was stirred at RT for 2 hours until Boc was totally removed according to LCMS. The resulting mixture was concentrated and the residue (0.33 g, 95% yield)

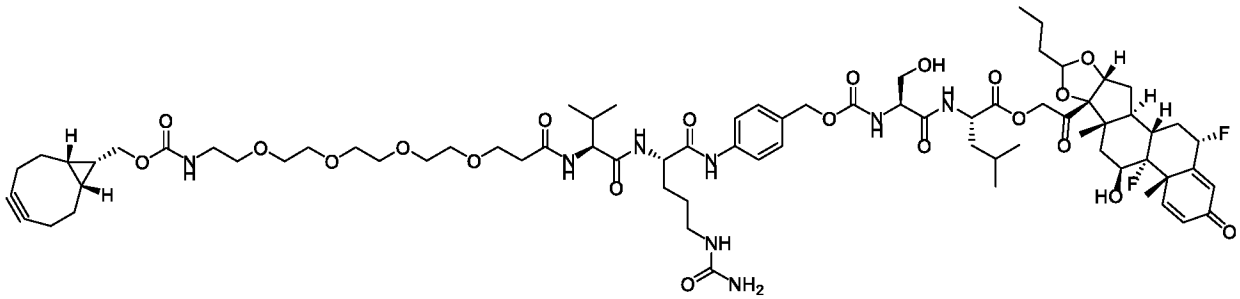
was pure enough for the next step. 100 mg of crude product was further purified by prep-HPLC (method A) to give compound **104b** (80 mg, 80% yield) as a yellow solid. ESI m/z: 667.3 $(M + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-[(2*S*)-2-{[({4-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-3-hydroxypropanamido]-4-methylpentanoate (104-8b)



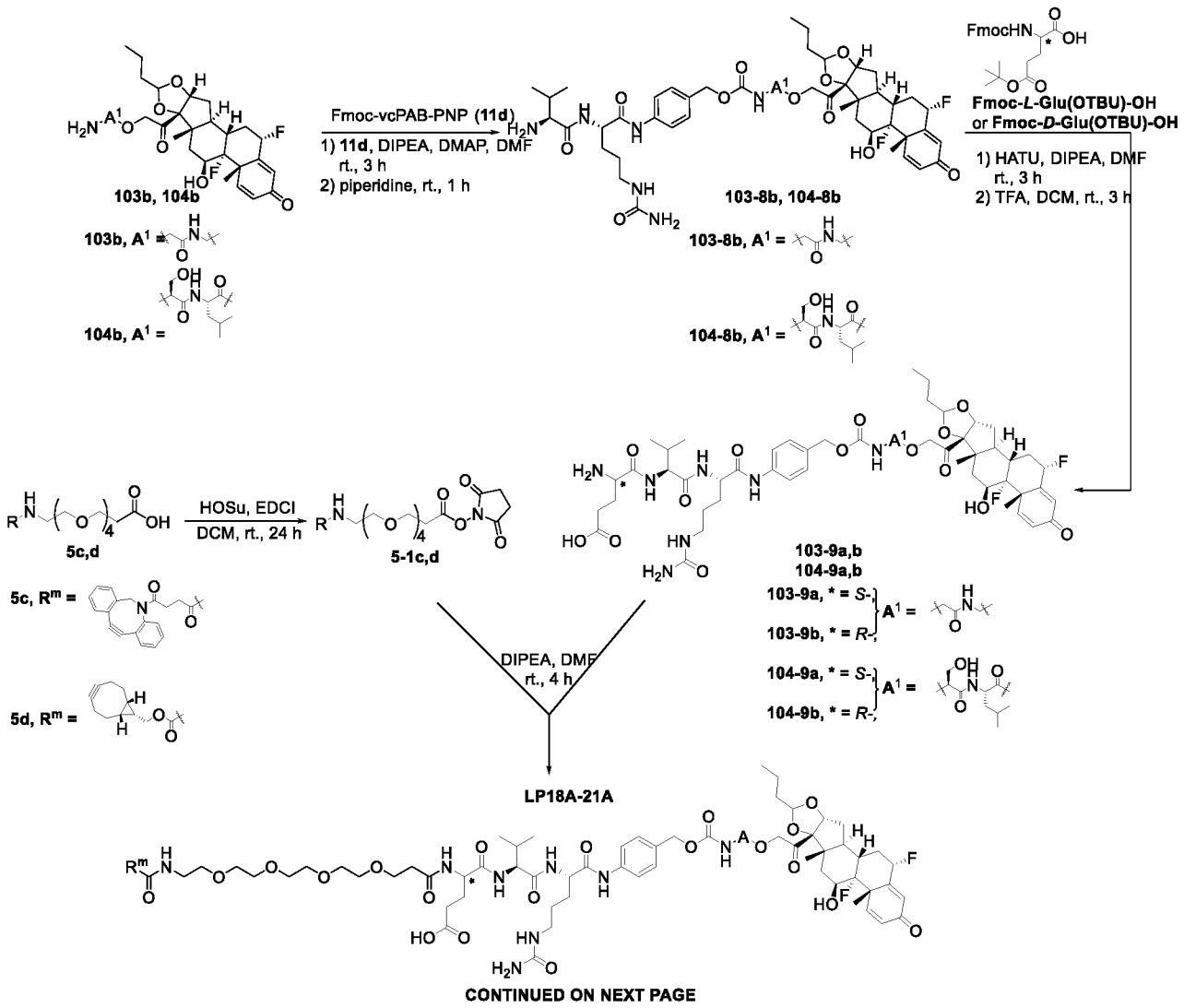
[0768] To a solution of crude compound **104b** (0.10 g, 0.13 mmol) freshly obtained above in DMF (3 mL) were added Fmoc-vcPAB-PNP (**11d**) (0.12 g, 0.16 mmol), DMAP (16 mg, 0.13 mmol) and DIPEA (50 mg, 0.39 mmol) at RT. The mixture was stirred at RT for 3 hours until most of starting materials were consumed, which was monitored by LCMS. To the reaction mixture was then added piperidine (1 mL). After stirred at room temperature for an hour until the Fmoc was totally removed according to LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **104-8b** (28 mg, 20% yield) as a white solid. ESI m/z: 536.8 $(M/2 + H)^+$.

2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl (2*S*)-2-[(2*S*)-2-{[({4-[(2*S*)-2-[(2*S*)-2-[1-({[*endo*-bicyclo[6.1.0]non-4-yn-9-ylmethoxy]carbonyl}amino)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methoxy)carbonyl]amino}-3-hydroxypropanamido]-4-methylpentanoate (LP13A)



[0769] To a solution of compound **5d** (10 mg, 22 μmol) in DMF (1 mL) were added HATU (9 mg, 22 μmol) and DIPEA (8.0 mg, 62 μmol) successively at RT. The mixture was stirred at RT for half an hour followed by the addition of a solution of compound **104-8b** (20 mg, 19 μmol) in DMF (1 mL). The resulting mixture was stirred at RT for 2 hours until compound **104-8b** was consumed, which was monitored by LCMS. After filtered through membrance, the filtrate was directly purified by prep-HPLC (method B) to give linker-payload **LP13A** (10 mg, 35% yield) as a white solid. ESI m/z: 748.4 $(M/2 + H)^+$.

Scheme 105. Synthesis of LP18A-21A



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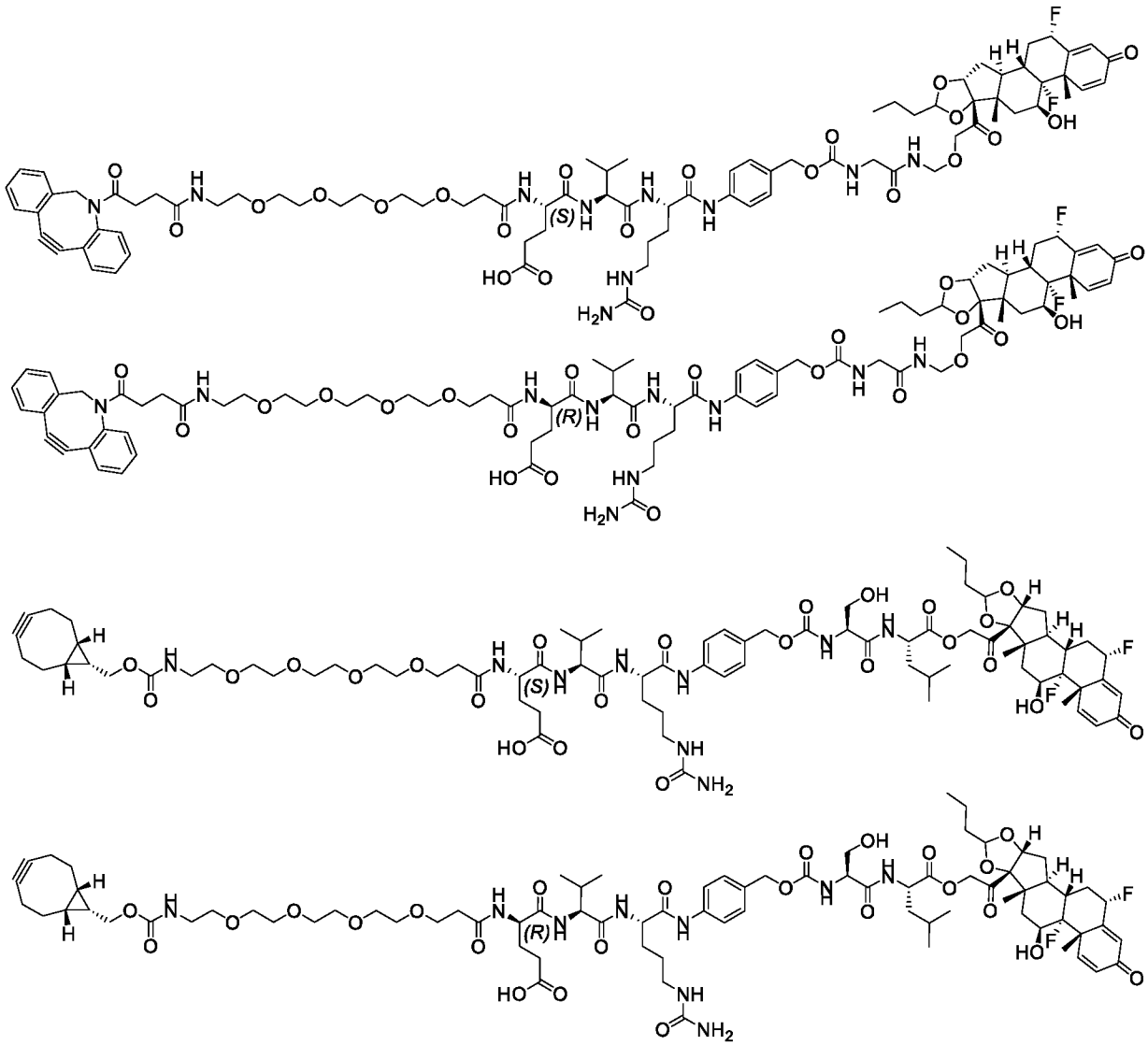
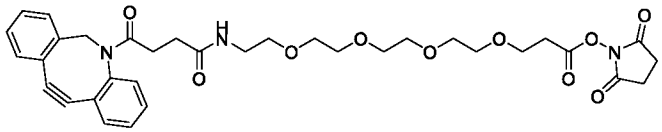
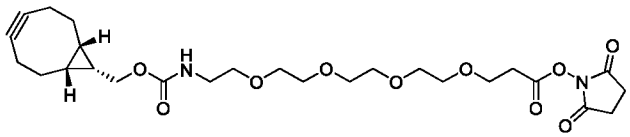


2,5-Dioxopyrrolidin-1-yl 1-(4-{2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-oate (5-1c)



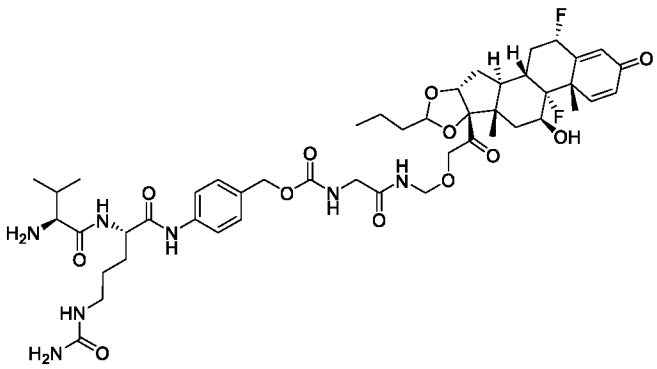
[0770] To a solution of compound **5c** (160 mg, 0.290 mmol) in DCM (20 mL) were added HOSu (73.3 mg, 0.637 mmol) and EDCI (122 mg, 0.637 mmol), and the mixture was stirred at RT for 24 hours, which was monitored by LCMS. The reaction mixture was diluted with DCM (50 mL) and the organic layer was washed with water (50mL) and brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to give compound **5-1c** (159 mg, 84% yield) as colorless oil, which was used for the next step directly. ESI m/z: 650 $(M + H)^+$.

2,5-Dioxopyrrolidin-1-yl 1-[({*endo*-bicyclo[6.1.0]non-4-yn-9-ylmethoxy}carbonyl)amino]-3,6,9,12-tetraoxapentadecan-15-oate (**5-1d**)



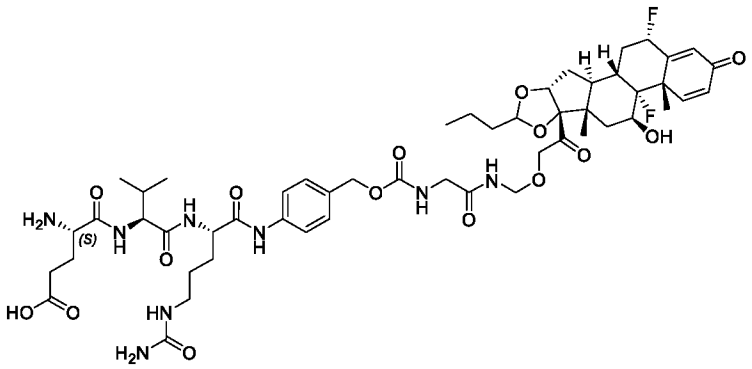
[0771] Following the similar procedure as **5-1c** except substituting **5d** for **5c**, compound **5-1d** (150 mg, 54% yield) was obtained as colorless oil, which was used for the next step directly without further purification. ESI m/z: 539 $(M + H)^+$.

{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamate (**103-8b**)



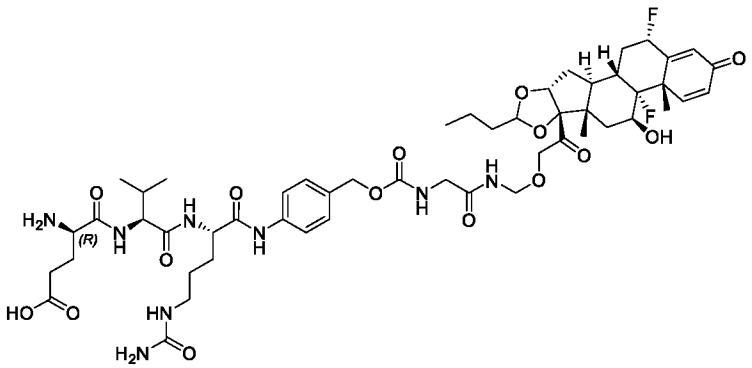
[0772] Following the similar procedure as **104-8b** (see scheme 104C) except substituting **103b** for **104b**, compound **103-8b** (28 mg, 20% yield) was obtained as a white solid. ESI m/z: 480 $(M/2 + H)^+$.

(4*S*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (**103-9a**)



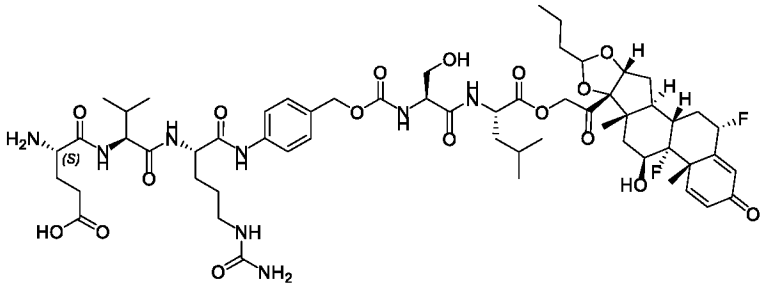
[0773] To a solution of compound **Boc-*L*-Glu(OTBU)-OH** (0.15 g, 0.50 mmol) in DMF (5 mL) were added HATU (0.19 g, 0.50 mmol) and DIPEA (0.13 g, 1.0 mmol). The reaction mixture mixture was stirred at RT for 10 minutes, before compound **103-8b** (0.48 g, 0.50 mmol) was added into the reaction mixture. The reaction mixture was then stirred at RT for 3 hours until **103-8b** was totally consumed according to LCMS. The mixture was extracted with EtOAc, and the combined organic solution was washed with water, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was dissolved into DCM (10 mL). To the solution was added TFA (2 mL), and the reaction mixture was stirred at RT for 3 hours, which was monitored by LCMS. The reaction mixture was concentrated, and the residue was directly purified by prep-HPLC (method B) to give compound **103-9a** (0.41 g, 75% yield) as a white solid. ESI m/z: 536.8 $(M/2 + H)^+$.

(4*R*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamoyl)oxy]methyl}phenyl) carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (103-9b)



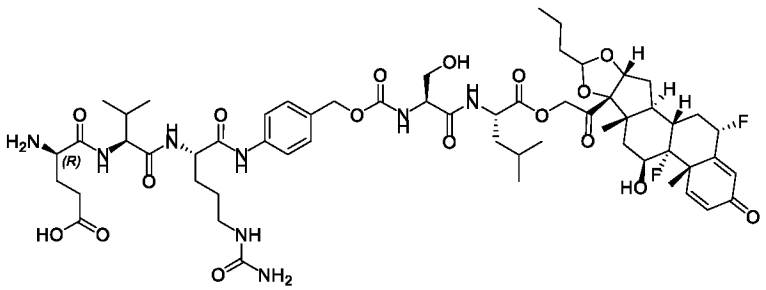
[0774] Following the similar procedure as **103-9a** except substituting **Boc-*D*-Glu(OTBU)-OH** for **Boc-*L*-Glu(OTBU)-OH**, compound **103-9b** (0.40 g, 74% yield) as a white solid. ESI m/z: 536.8 $(M/2 + H)^+$.

(4*S*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(2*S*)-1-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}-4-methyl-1-oxopentan-2-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (104-9a)



[0775] Following the similar procedure as **103-9a** except substituting **104-8b** for **103-8b**, compound **104-9a** (0.39 g, 65% yield) as a white solid. ESI m/z: 601.3 (M/2 + H)$^+$.

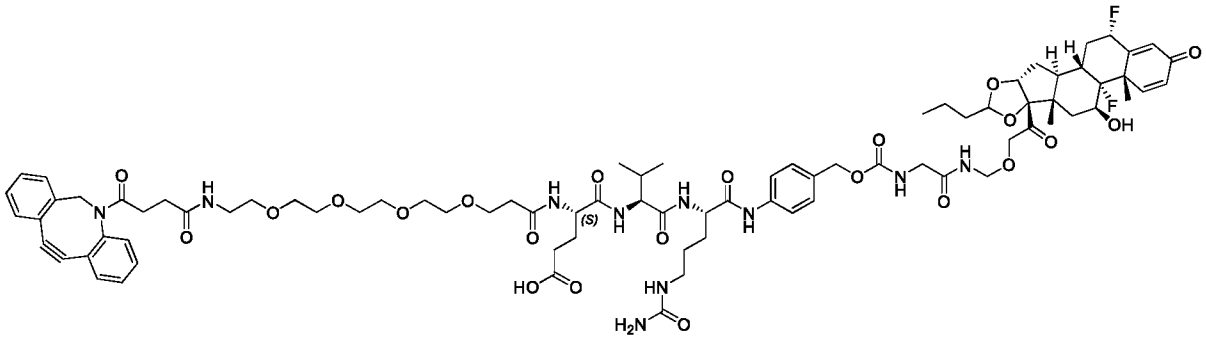
(4*R*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(2*S*)-1-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}-4-methyl-1-oxopentan-2-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (104-9b)



[0776] Following the similar procedure as **103-9a** except substituting **104-8b** for **103-8b**, **Boc-*D*-Glu(OTBU)-OH** for **Boc-*L*-Glu(OTBU)-OH**, compound **104-9b** (0.39 g, 65% yield) as a white solid. ESI m/z: 601.3 (M/2 + H)$^+$.

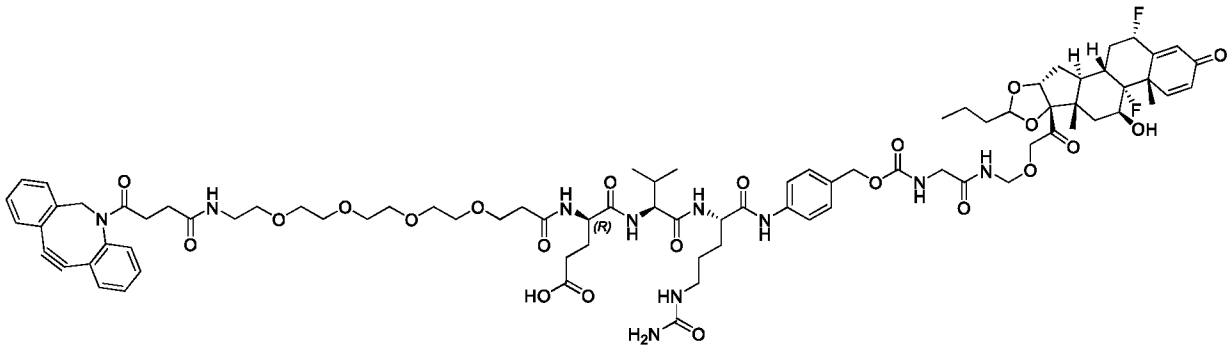
(4*S*)-4-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(1*S*)-1-{[(1*S*)-4-

(carbamoylamino)-1-[(4-{[({[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (LP18A)



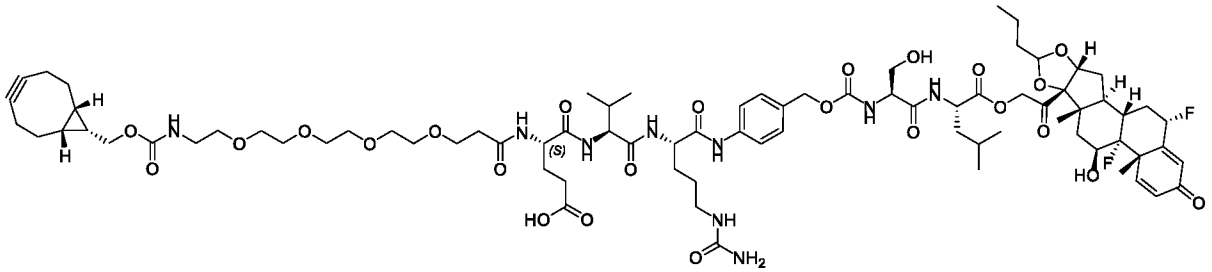
[0777] To a solution of compound **103-9a** (57 mg, 53 μmol) in DMF (1 mL) were added compound **5-1c** (36 mg, 56 μmol) and DIPEA (27 mg, 0.21 mmol). The reaction mixture was stirred at RT for 4 hours, which was monitored by LCMS. The resulting mixture was then directly purified by prep-HPLC (method B) to give compound **LP18A** (12 mg, 15% yield) as a white solid. ESI m/z: 811.4 (M/2 + H)$^+$.

(4*R*)-4-[1-(4-{2-Azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}methyl)carbamoyl]methyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (LP19A)



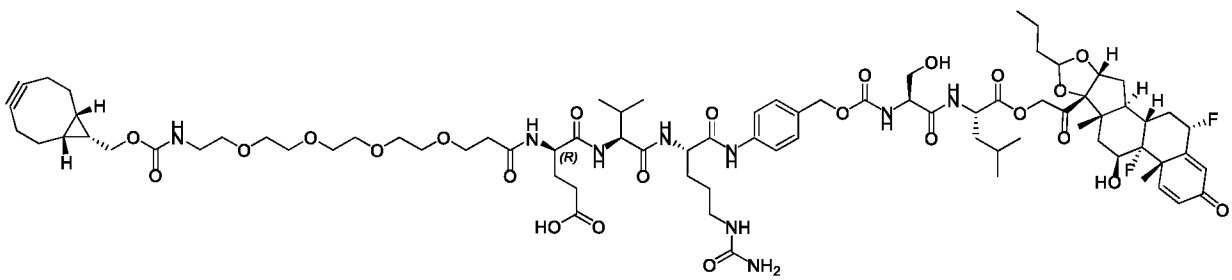
[0778] Following the similar procedure as **LP18A** except substituting **103-9b** for **103-9a**, compound **LP19A** (14 mg, 17% yield) as a white solid. ESI m/z: 811.4 (M/2 + H)$^+$.

(4*S*)-4-{1-[({*endo*-Bicyclo[6.1.0]non-4-yn-9-ylmethoxy}carbonyl)amino]-3,6,9,12-tetraoxapentadecan-15-amido}-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(2*S*)-1-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}-4-methyl-1-oxopentan-2-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (LP20A)



[0779] To a solution of compound **104-9a** (64 mg, 53 μmol) in DMF (1 mL) were added compound **5-1d** (30 mg, 56 μmol) and DIPEA (27 mg, 0.21 mmol). The reaction mixture was stirred at RT for 4 hours, which was monitored by LCMS. The resulting mixture was then directly purified by prep-HPLC (method B) to give compound **LP20A** (17 mg, 20% yield) as a white solid. ESI m/z: 813.0 (M/2 + H)$^+$.

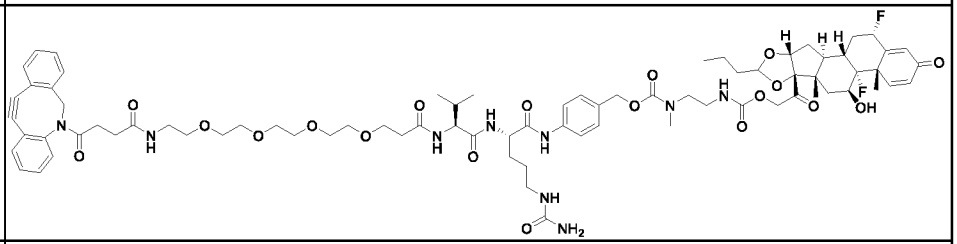
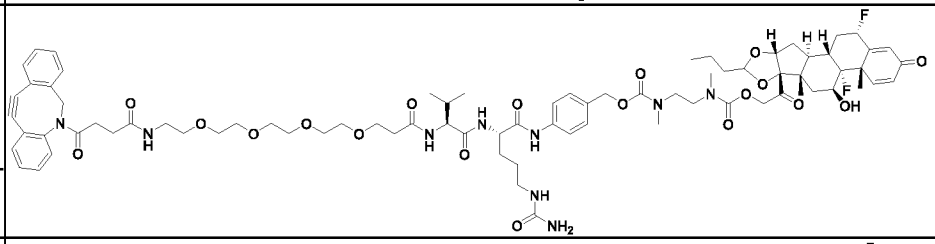
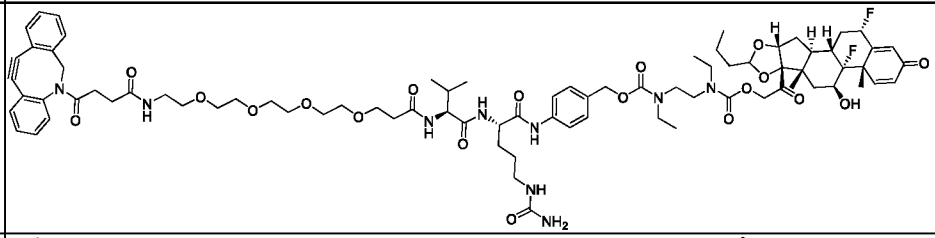
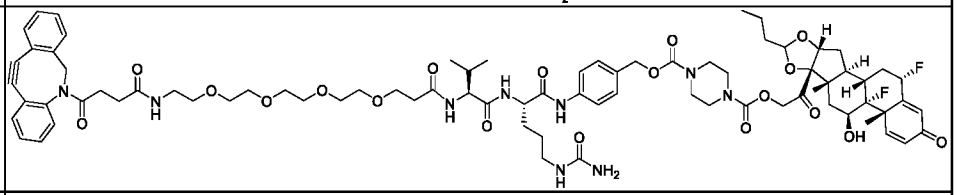
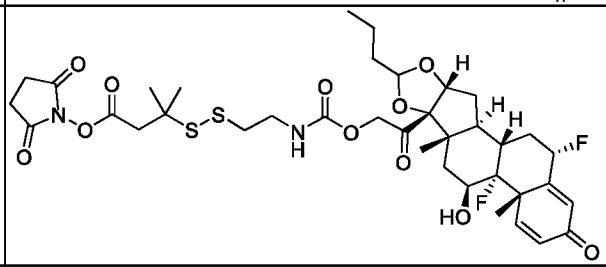
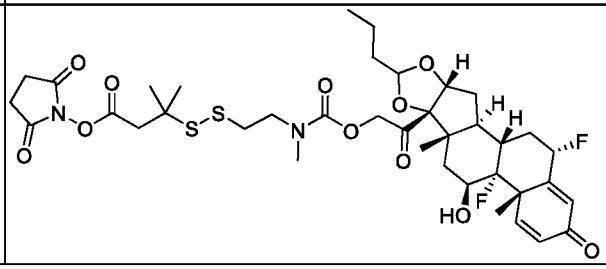
(4*R*)-4-{1-[({*endo*-Bicyclo[6.1.0]non-4-yn-9-ylmethoxy}carbonyl)amino]-3,6,9,12-tetraoxapentadecan-15-amido}-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(2*S*)-1-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethoxy}-4-methyl-1-oxopentan-2-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanoic acid (LP21A)

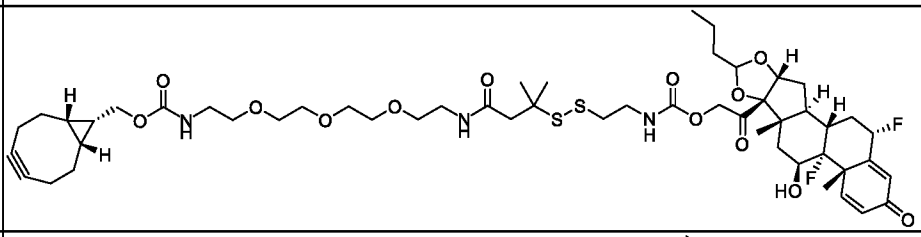
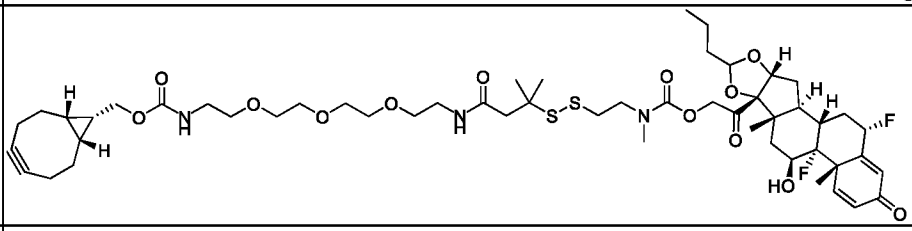
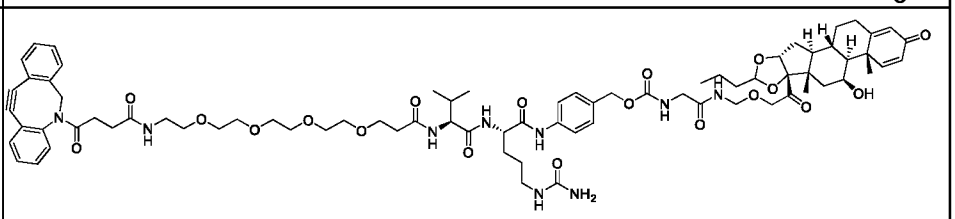
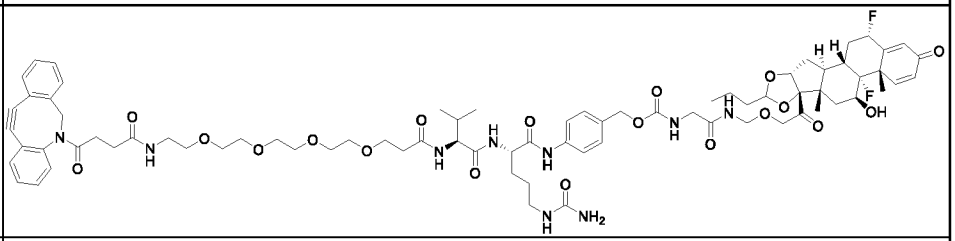
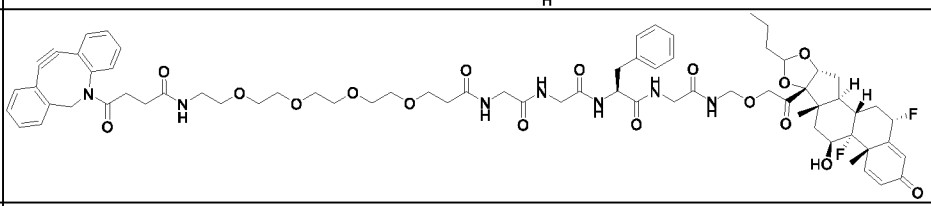
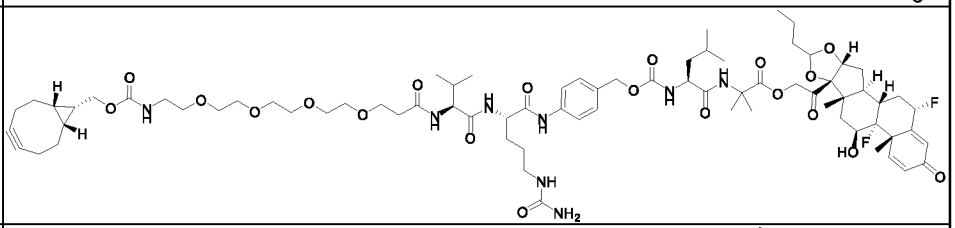
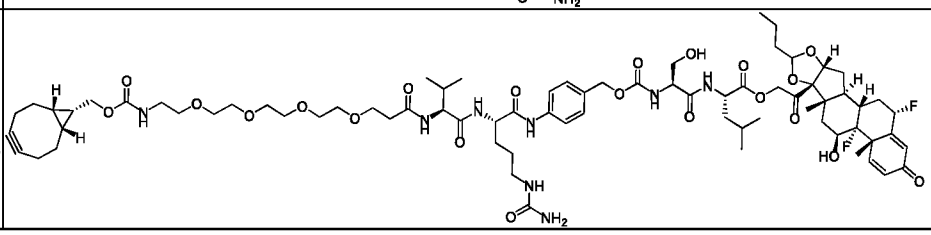


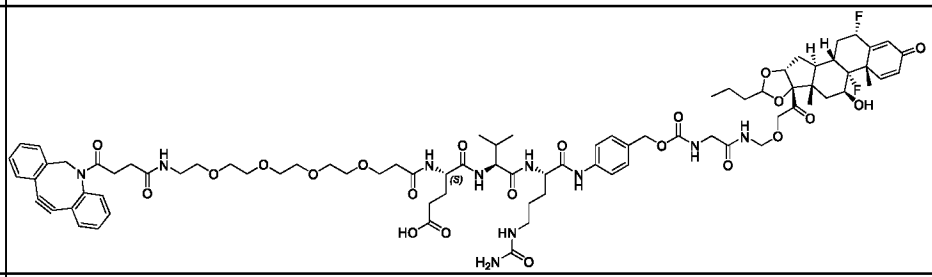
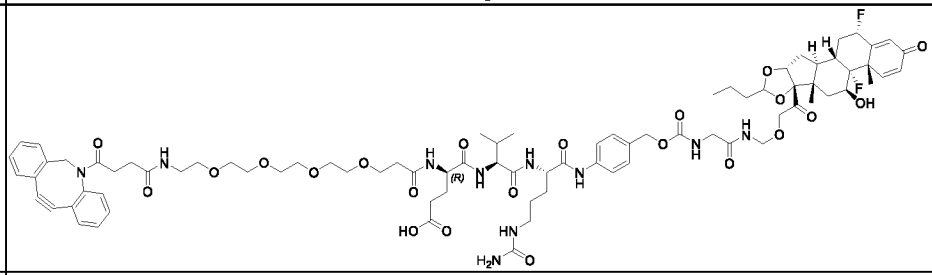
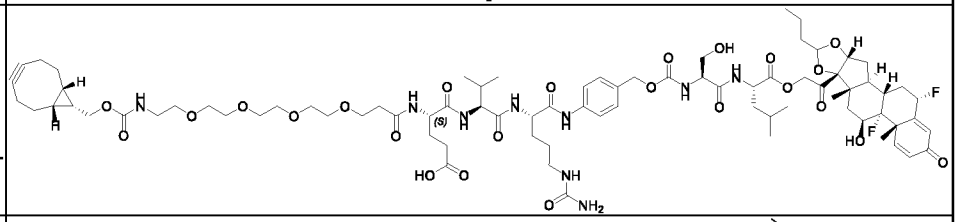
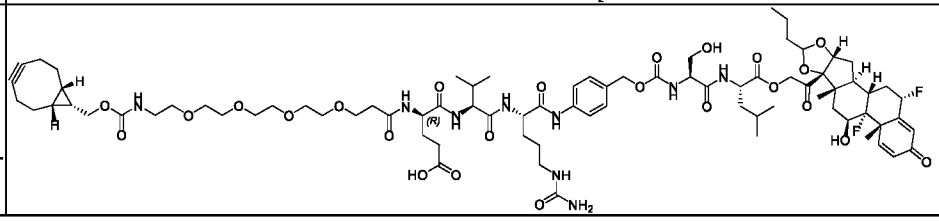
[0780] Following the similar procedure as **LP20A** except substituting **104-9b** for **104-9a**, compound **LP21A** (14 mg, 17% yield) as a white solid. ESI m/z: 813.0 (M/2 + H)$^+$.

[0781] Table 5B below presents linker payloads made using the methods described herein.

Table 5B. Examples of Linker–Payloads

LP	Payload	Linker name	Structures
LP1A	**101a**	DIBAC-suc-PEG4-vcPAB-MEDA-**100**	
LP2A	**101b**	DIBAC-suc-PEG4-vcPAB-DMEDA-**100**	
LP3A	**101c**	DIBAC-suc-PEG4-vcPAB-DEEDA-**100**	
LP4A	**101d**	DIBAC-suc-PEG4-vcPAB-Pip-**100**	
LP5A	**102e**	NHS-disulfide-SEA-**100**	
LP6A	**102f**	NHS-disulfide-SEMA-**100**	

LP	Payload	Linker name	Structures
LP7A	102e	BCN-PEG3-disulfide-SEA-100	
LP8A	102f	BCN-PEG3-disulfide-SEMA-100	
LP9A	103a	DIBAC-suc-PEG4-vcPAB-Gly-AMO-Bud	
LP10A	103b	DIBAC-suc-PEG4-vcPAB-Gly-AMO-100	
LP11A	103b	DIBAC-suc-PEG4-GGFG-AMO-100	
LP12A	104a	BCN-PEG4-vcPAB-Leu-Aib-100	
LP13A	104b	BCN-PEG4-vcPAB-Ser-Leu-100	

LP	Payload	Linker name	Structures
LP18A	**103b**	DIBAC-suc-PEG4-EVC-PAB-Gly-AMO-**100**	
LP19A	**103b**	DIBAC-suc-PEG4-dEVC-PAB-Gly-AMO-**100**	
LP20A	**104b**	BCN-PEG4-EVC-PAB-Ser-Leu-**100**	
LP21A	**104b**	BCN-PEG4-dEVC-PAB-Ser-Leu-**100**	

Example 27. Characterization of Linker-payloads

[0782] Table 16 summarizes the results of the Linker-Payloads HPLC purity and LC retention time on HPLC, M/Z from mess spectra. The analytical HPLC and MS methods were described in general procedures as disclosed herein.

Table 16: Chemical-physical property of Linker-payloads

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)[1]	MS (m/z) 100%	Highest m/z peak
13	**4.39**	C74H94F2N8O17	1405.60	>99	7.40[1]	703.5 (M/2+H)	703.5 (M/2+H)
15	**2.98**	C103H144F2N14O28S	2096.38	98	5.88	699.6 (M/3+H)	1049.0 (M/2+H) (67%)

3	6.25	$C_{80}H_{98}F_2N_8O_{18}$	1497.70	99	7.99[1]	1498.7 (M/2+H)	1498.7 (M+H) (10%)
6	6.59	$C_{115}H_{160}N_{16}O_{28}S$	2245.13	99	7.24	749.5 (M/3+H)	1123.8 (M/2 + H)$^+$

[1] Method B for HPLC-MS

Table 17: Linker-Payload Properties

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
LP1	-0.38	$C_{124}H_{175}N_{11}O_{44}$	2523.76	98	6.45, 6.55 (B)	841.8 [M/3+H]	1262.4 [M/2+H] (30%)
LP2	-3.92	$C_{136}H_{195}N_{11}O_{54}$	2848.04	96	7.48 (B)	955.0 [(M+18)/3] 949.5 (M/3+H)	1424.2 (M/2+H) (30%)
LP3	6.25	$C_{80}H_{98}F_2N_8O_{18}$	1497.67	100	7.99 (B)	749.5 (M/2+H)	1497.7 (M+H) (5%)
LP4	7.53	$C_{72}H_{100}N_{10}O_{13}$	1313.62	96	8.16 (B)	657.5 (M/2+H)	1313.6 (M+H) (5%)
LP5	-1.48	$C_{138}H_{193}N_{15}O_{49}$	2846.08	100	6.11, 6.21 (B)	949.0 (M/3+H)	1423.3 (M/2+H) (5%)
LP6	6.59	$C_{115}H_{160}N_{16}O_{28}S$	2246.66	100	7.24 (B)	749.5 (M/3+H)	1123.8 (M/2+H) (5%)
LP9	8.70	$C_{88}H_{112}N_{10}O_{18}$	1597.89	95	5.67 (B)	799.0 (M/2+H)	799.0 (M/2+H)
LP11	8.17	$C_{89}H_{117}N_{11}O_{16}$	1596.95	95	8.51 (B)	798.5 (M/2+H)	798.5 (M/2+H)
LP12	8.77	$C_{77}H_{86}Cl_2F_6N_{10}O_{15}S$	1608.53	99	9.36 (B)	804.2 (M/2+H)	804.2 (M/2+H)
LP13	4.39	$C_{74}H_{94}F_2N_8O_{17}$	1405.58	100	7.40 (B)	703.5 (M/2+H)	1405.7 (M+H) (5%)
LP14	-5.09	$C_{126}H_{177}F_2N_{13}O_{49}$	2695.81	100	6.23 (B)	899.2 (M/3+H)	1348.6 (M/2+H) (40%)
LP15	2.98	$C_{103}H_{144}F_2N_{14}O_{28}S$	2096.38	98	5.88 (B)	699.6 (M/3+H)	1049.0 (M/2+H) (67%)

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
LP18	6.49	$C_{101}H_{142}N_{12}O_{23}S$	1924.34	97	7.57 (B)	642.2 (M/3+H)	962.5 (M/2+H) (70%)

Table 17A Characterization Data for Linker–Payloads

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
LP1A	4.26	$C_{78}H_{101}F_2N_9O_{19}$	1506.71	95		753.9 (m/2+H)	753.9 (m/2+H)
LP2A	4.48	$C_{79}H_{103}F_2N_9O_{19}$	1520.71	97		761.0 (m/2+H)	761.0 (m/2+H)
LP3A	5.20	$C_{81}H_{107}F_2N_9O_{19}$	1548.76	95		775.0 (m/2+H)	775.0 (m/2+H)
LP4A	4.31	$C_{79}H_{101}F_2N_9O_{19}$	1518.69	99		760.0 (m/2+H)	760.0 (m/2+H)
LP5A	3.40	$C_{37}H_{48}F_2N_2O_{11}S_2$	798.91	99		799.3 (m+H)	799.3 (m+H)
LP6A	3.62	$C_{38}H_{50}F_2N_2O_{11}S_2$	812.94	97		813.3 (m+H)	813.3 (m+H)
LP7A	5.20	C52H75F2N3O13S2	1052.29	97		526.7 (m/2+H)	526.7 (m/2+H)
LP8A	5.42	C53H77F2N3O13S2	1066.32	98		533.8 (m/2+H)	533.8 (m/2+H)
LP9A	3.65	$C_{77}H_{101}N_9O_{19}$	1456.70	96		729.0 (m/2+H)	729.0 (m/2+H)
LP10A	3.35	$C_{77}H_{99}F_2N_9O_{19}$	1492.68	95		513.8 (fragment)	747.0 (m/2+H)
LP11A	1.96	$C_{71}H_{87}F_2N_7O_{17}$	1348.51	95		450.2 (m/3+H)	675.2 (m/2+H)
LP12A	5.61	$C_{76}H_{110}F_2N_8O_{20}$	1493.75	90		747.6 (m/2+H)	747.6 (m/2+H)
LP13A	4.13	$C_{75}H_{108}F_2N_8O_{21}$	1495.70	95		748.4 (m/2+H)	748.4 (m/2+H)
LP18A	2.46	$C_{82}H_{106}F_2N_{10}O_{22}$	1621.77	95		811 (m/2+H)	811 (m/2+H)

LP	cLogP	MF	MW	HPLC purity (%)	HPLC RT (min)	MS (m/z) 100%	Highest m/z peak
LP19A	2.46	$C_{82}H_{106}F_2N_{10}O_{22}$	1621.77	95		811 (m/2+H)	811 (m/2+H)
LP20A	3.25	$C_{80}H_{115}F_2N_9O_{24}$	1624.81	95		813 (m/2+H)	813 (m/2+H)
LP21A	3.25	$C_{80}H_{115}F_2N_9O_{24}$	1624.81	95		813 (m/2+H)	813 (m/2+H)

Example 28. ADC Conjugation

[0783] This example demonstrates a method for site–specific conjugation, generally, of a payload to an antibody or antigen–binding fragment thereof. See FIG. 16.

[0784] Generated anti-MSR1 antibodies (Table 4) were mutated (N297Q) to incorporate a transglutaminase site for conjugation with a therapeutic payload. The site–specific aglycosylated antibodies containing an N297Q mutation were conjugated with amine–PEG_3–N_3 to generate the azido–functionalized antibody conjugates (mAb-N_3), including anti MSR1 Ab–PEG_3–N_3.

[0785] The present example demonstrates a method for making the conjugates. Aglycosylated antibody with a human IgG1 isotype in BupH™ (pH 7-8) was mixed with ≥200 molar equivalents of azido–$dPEG_3$–amine (MW. 218.26 g/mol). The resulting solution was mixed with transglutaminase (25 U/mL; 5U MTG per mg of antibody) resulting in a final concentration of the antibody at 0.5–10 mg/mL, and the solution was then incubated at 37 °C for 4–24 hours while gently shaking. The reaction was monitored by SDS–PAGE or ESI–MS. Upon the completion, the excess amine and MTG were removed by Size Exclusion Chromatography (SEC) to generate the azido–functionalized antibody (mAb-N_3). This product was analyzed on SDS–PAGE and ESI–MS. The azido–$dPEG_3$–amine added to two sites – Q295 and Q297– of the antibody resulting in an 804 Da increase for the 4DAR aglycosylated antibody–PEG_3–azide conjugate. The conjugation sites were identified and confirmed at $EEQ^{Linker}YQ^{Linker}STYR$ for the 4DAR azido–functionalized antibody via peptide sequence mapping of trypsin digested heavy chains.

[0786] The site–specific aglycosylated antibody drug conjugates (ADCs) with a human IgG1 containing an N297Q mutation were prepared by a [2+3] click reaction between the azido–functionalized antibody (mAb-N_3) with an alkyne containing linker–payload (LP).

[0787] A site-specific antibody conjugate with linker–payload (**LP**) was prepared by incubating **mAb–PEG_3–N_3** (1–12 mg / mL) in an aqueous medium (e.g., PBS, PBS containing 5% glycerol,

HBS) with ≥6 molar equivalents of an **LP** dissolved in a suitable organic solvent, such as DMSO, DMF or DMA (i.e., the reaction mixture contains 5-20% organic solvent, v/v) at 24 °C to 37 °C for 30 min to 24 hr. The progress of the reaction was monitored by ESI–MS and the absence of **mAb–PEG$_3$–N$_3$** indicated the completion of the conjugation. The excess amount of the **LP** and organic solvent were removed by SEC via elution with PBS, or via protein A column chromatography via elution with acidic buffer followed by neutralization with Tris (pH 8.0). The purified conjugates were analyzed by SEC, SDS–PAGE, and ESI–MS. Shown in Table 19 is a list of the MMAE antibody conjugates, steroid antibody conjugates, and LXR agonist antibody conjugates from the corresponding LPs, their molecular weights and ESI–DAR values.

[0788] In a specific example, the azido-functionalized antibody (1 mg) in 0.800 mL PBSg (PBS, 5% glycerol, pH 7.4) was treated with six molar equivalents of linker payload (**LP**) in Table 1 (conc. 10 mg/mL in DMSO) for 2 hours at RT and the excess linker payload (**LP**) was removed by size exclusion chromatography (SEC, Superdex 200 HR, GE Healthcare). The final product was concentrated by ultra-centrifugation and characterized by UV, SEC, SDS-PAGE and ESI-MS.

Example 28A: ADC Conjugation

[0789] This example demonstrates a method for making a non–site–specific conjugation of a drug to an antibody using a thiol–maleimide reaction.

[0790] Conjugation through antibody cysteines is performed in two steps using the methods described similar to those described in *Mol Pharm.* 2015 Jun 1;12(6):1863-71.

[0791] A monoclonal antibody (mAb, 10 mg/ml in 50 mM HEPES, 150 mM NaCl) at pH 7.5 is reduced with 1 mM dithiothreitol (0.006 mg per mg of antibody) or TCEP (2.5 molar equivalent to antibody) at 37 °C for 30 minutes. The concentration of the antibody was calculated based on the absorbance at 280 nm on Nanodrop (ThermoFisher Scientific) and an extinction coefficient of the antibody. After gel filtration (G–25, pH 4.5 sodium acetate), the LP compound in DMSO (10 mg/mL) is added to the reduced antibody, and the mixture was adjusted to pH 7.0 with 1 M HEPES (pH 7.4). The reaction is allowed to react for 3–14 hours. The resulting conjugate was purified by SEC. The DAR (UV) values are determined using the measured absorbances of the ncADC and the extinction coefficients of the antibody and **LP** compound.

Example 29. Characterization of ADCs by LC–ESI–MS

[0792] Measurement of intact mass for the ADC samples by LC–ESI–MS was performed to determine the drug-payload distribution profile and to calculate the average DAR. Each testing sample (20-50 ng, 5uL) was loaded onto an Acquity UPLC Protein BEH C4 column (10K psi, 300 Å, 1.7 µm, 75µm × 100 mm; Cat No. 186003810). After 3 minutes of desalting, the protein

was eluted and mass spectra were acquired by a Waters Synapt G2–Si mass spectrometer. As summarized in Table 18, most site-specific ADCs have 3.9 – 4 DAR for the site specific conjugates.

Table 18: ADC Properties

LP #	MW (LP)	Ab, Ab–N_3, or ncADC	MS m/z (ncADC)	DAR (ESI–MS)
		H1H27729P		
		H1H27729P-N_3	144166	4
1	2523.8	H1H27729P-LP1		
		H1H27731P	144620	4
		H1H27731P-N_3		
1	2523.8	H1H27731P-LP1		
		H1H27732P	144176	5.7
		H1H27732P-N_3		
1	2523.8	H1H27732P-LP1		
		H1H27734P	145110	4.4
		H1H27734P-N_3		
1	2523.8	H1H27734P-LP1		
		H1H27736P	145940	4
		H1H27736P-N_3		
1	2523.8	H1H27736P-LP1		
		H1H27739P	145251	4
		H1H27739P-N_3		
1	2523.8	H1H27739P-LP1		
		H1H27747P	145214	5.3
		H1H27747P-N_3		
1	2523.8	H1H27747P-LP1		
		H1H27749P	143441	4
		H1H27749P-N_3		
1	2523.8	H1H27749P-LP1		
		H1H17751P	146092	3.8
		H1H17751P-N_3		
1	2523.8	H1H17751P-LP1		

LP #	MW (LP)	Ab, Ab–N_3, or ncADC	MS m/z (ncADC)	DAR (ESI–MS)
		H1H27754P	145477	4.2
		H1H27754P-N_3		
1	2523.8	H1H27754P-LP1		
		H1H27756P	145503	
		H1H27756P-N_3	146310	4
1	2523.8	H1H27756P-LP1	156424	4
		H1H17759P2	145126	
		H1H17759P2-N_3	145930	4
1	2523.8	H1H17759P2-LP1	156046	4
		H1H17760P	145611	
		H1H17760P2-N_3	146431	4
1	2523.8	H1H17760P2-LP1	156533	4
		H1H17761P2	145508	
		H1H17761P2-N_3	146717	6
1	2523.8	H1H17761P-LP1	161884 159158	5.34
		H1H27762P2	144371	
		H1H27762P2-N_3	145177	4
1	2523.8	H1H27762P2-LP1	155294	4
		H1H27766P2	146314	
		H1H27766P2-N_3	147121	4
1	2523.8	H1H27766P2-LP1	157236	4
		H1H27771P2	145966	
		H1H27771P2-N_3	146774	4
1	2523.8	H1H27771P2-LP1	156890	4
		H1H27773P2	145533	
		H1H27773P2-N_3	146337	4
1	2523.8	H1H27773P2-LP1	156453	4
		H1H27778P2	145310	
		H1H27778P2-N_3	146115	4
1	2523.8	H1H27778P2-LP1	156227	4
		H1H21231N		
		H1H21231N-N_3	Lot2	2

LP #	MW (LP)	Ab, Ab–N_3, or ncADC	MS m/z (ncADC)	DAR (ESI–MS)
1	2523.8	H1H21231N-LP1	Lot4	1.9
		H1H21227N		
		H1H21227N -N_3	Lot2	4
1	2523.8	H1H21227N -LP1	Lot3	3.7
		H1H21231N		
		H1H21231N -N_3	Lot2	6
1	2523.8	H1H21231N -LP1	Lot3	5.9
		H1H21235N	145487	
		H1H21235N -N_3	146288	4
1	2523.8	H1H21235N -LP1	156390	4.1
		H1H25700N	145484	
		H1H25700N -N_3	146688	6
1	2523.8	H1H25700N -LP1	161873	6
		H1H25690N	145157	
		H1H25690N -N_3	145969	4
1	2523.8	H1H25690N -LP1	156060	4.1
		H1H25695N	145736	
		H1H25695N -N_3	146537	4
1	2523.8	H1H25695N -LP1	156637	3.9
		H1H25685N	145380	
		H1H25685N -N_3	146582	6
1	2523.8	H1H25685N -LP1	161767	5.8
		H1H21228N	144830	
		H1H21228N -N_3	145631	4
1	2523.8	H1H21228N -LP1	155732	4.2
		H1H21234N	145790	
		H1H21234N -N_3	146583	4
1	2523.8	H1H21234N -LP1	156691	4
2	2848.1	H1H21234N -LP2	157983	3.9
4	1313.7	H1H21234N -LP4	151841	3.9
5	2846.2	H1H21234N -LP5	157963	3.9
6	2246.7	H1H21234N -LP6	155570	3.9
9	1597.9	H1H21234N -LP9	152975	3.9

LP #	MW (LP)	Ab, Ab–N_3, or ncADC	MS m/z (ncADC)	DAR (ESI–MS)
11	1597.0	H1H21234N -LP11	152979	3.9
12	1608.6	H1H21234N -LP12	153019	3.9
13	1405.6	H1H21234N -LP13	152218	3.9
14	2695.9	H1H21234N -LP14	157369	3.6
15	2096.4	H1H21234N -LP15	154964	3.9
3	1497.7	H1H21234N -LP3	152568	3.9
Fel D1		Isotype Control-N297Q		
		Isotype Control-N297Q-N_3	146251	4
1	2523.8	Isotype Control-N297Q-LP1	156352	3.9
13	1405.6	Isotype Control-N297Q-LP13	152218	3.9
14	2695.9	Isotype Control-N297Q-LP14	157369	3.6
15	2096.4	Isotype Control-N297Q-LP15	154964	3.9
3	1496.7	Isotype Control-N297Q-LP3	152568	4

Example 30. Biacore Surface Plasmon Resonance Derived Binding Kinetics of Anti-MSR1 Antibody-Drug Conjugates

[0793] The MSR1 antibodies disclosed herein were conjugated to various liver X receptor (LXR) payloads or various steroid payloads. This example describes how equilibrium dissociation constant (K_D) values for human MSR1 reagents binding to human anti-MSR1 antibody-drug conjugates and their corresponding unconjugated parental antibodies were determined using a real-time surface plasmon resonance-based Biacore T200 biosensor.

[0794] All binding studies were performed in 10 mM HEPES, 300 mM NaCl, 3 mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C. The Biacore CM4 sensor chip surface was first derivatized by amine coupling with the goat anti-human Fcγ specific polyclonal antibody (Jackson ImmunoResearch Laboratories, Cat# BR-1008-39) to capture anti-MSR1 monoclonal antibodies. Binding studies were performed on human MSR1 extracellular domain expressed with a N-terminal nonahistidine tag (His9-hMSR1; R&D Systems, Cat# 2708-MS). Different concentrations of His9-hMSR1 (100 nM – 3.7 nM or 30 nM –

3.33 nM; 3-fold serial dilution) were first prepared in HBS-ET running buffer and were injected over anti-human Fcγ captured anti-MSR1 monoclonal antibody surface for 3 minutes at a flow rate of 50 µL/minute, while the dissociation of monoclonal antibody bound MSR1 reagent was monitored for about 8-10 minutes in HBS-ET running buffer.

[0795] The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life (t½) were calculated from the kinetic rates as:

$$K_D\ (M) = \frac{kd}{ka}, \text{ and } t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[0796] Binding kinetics parameters for His9-hMSR1 binding to different anti-MSR1 antibody-LXR ADCs and their unconjugated parental antibodies at 25°C are shown in Table 19. "LP1" represents a linker-payload for which the payload structure is provided in Example 11.

Table 19: Binding kinetics of His-hMSR1 binding to MSR1 Antibody-LXR ADCs and Corresponding Unconjugated Antibodies at 25 °C

Antibody Captured	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (Molar)	t½ (min)
H1H27729P-N297Q	1.05E+05	9.06E-04	8.67E-09	12.8
H1H27729P-N297Q-LP1	5.47E+04	7.18E-04	1.31E-08	16.1
H1H27731P-N297Q	1.17E+05	1.00E-05	8.55E-11	1155.2
H1H27731P-N297Q-LP1	1.28E+05	1.00E-05*	7.80E-11	1155.2
H1H27732P-N297Q	2.20E+05	1.00E-05*	4.50E-11	1155.2
H1H27732P-N297Q-LP1	1.50E+05	1.00E-05*	6.60E-11	1155.2
H1H27734P-N297Q	7.72E+04	5.96E-04	7.72E-09	19.4
H1H27734P-N297Q-LP1	6.93E+04	4.49E-04	6.49E-09	25.7
H1H27736P-N297Q	1.14E+05	1.47E-04	1.29E-09	78.7
H1H27736P-N297Q-LP1	9.59E+04	1.21E-04	1.26E-09	95.5
H1H27739P-N297Q	1.19E+05	5.55E-04	4.68E-09	20.8
H1H27739P-N297Q-LP1	8.88E+04	7.22E-04	8.14E-09	16.0
H1H27747P-N297Q	1.17E+05	2.62E-04	2.24E-09	44.1
H1H27747P-N297Q-LP1	1.36E+05	2.03E-04	1.49E-09	56.9
H1H27749P-N297Q	1.43E+05	1.00E-05*	6.99E-11	1155.2
H1H27749P-N297Q-LP1	1.40E+05	1.00E-05*	7.16E-11	1155.2
H1H27751P-N297Q	2.10E+05	1.75E-04	8.33E-10	66.1
H1H27751P-N297Q-LP1	2.29E+05	1.52E-04	6.64E-10	76.1
H1H27754P-N297Q	2.00E+05	1.00E-05*	4.99E-11	1155.2
H1H27754P-N297Q-LP1	1.77E+05	1.00E-05*	5.64E-11	1155.2

Antibody Captured	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (Molar)	t½ (min)
H1H27756P-N297Q	7.21E+04	1.19E-04	1.65E-09	97.4
H1H27756P-N297Q-LP1	6.27E+04	1.10E-04	1.76E-09	104.7
H1H27759P-N297Q	1.03E+05	4.35E-04	4.23E-09	26.6
H1H27759P-N297Q-LP1	1.30E+05	5.95E-04	4.57E-09	19.4
H1H27760P-N297Q	2.31E+05	3.83E-04	1.66E-09	30.2
H1H27760P-N297Q-LP1	2.59E+05	4.34E-04	1.67E-09	26.6
H1H27761P-N297Q	5.95E+05	3.62E-04	6.09E-10	31.9
H1H27761P-N297Q-LP1	2.53E+05	5.11E-04	2.02E-09	22.6
H1H27762P-N297Q	4.05E+05	5.60E-04	1.38E-09	20.6
H1H27762P-N297Q-LP1	4.83E+05	6.23E-04	1.29E-09	18.5
H1H27766P-N297Q	1.72E+05	1.00E-05*	5.83E-11	1155.2
H1H27766P-N297Q-LP1	4.16E+05	2.70E-05	6.49E-11	427.4
H1H27771P-N297Q	3.83E+05	3.55E-04	9.26E-10	32.6
H1H27771P-N297Q-LP1	3.38E+05	4.42E-04	1.31E-09	26.1
H1H27773P-N297Q	5.49E+04	7.52E-04	1.37E-08	15.4
H1H27773P-N297Q-LP1	2.72E+04	9.47E-04	3.48E-08	12.2
H1H27778P-N297Q	1.66E+05	2.71E-04	1.63E-09	42.6
H1H27778P-N297Q-LP1	2.85E+05	2.76E-04	9.70E-10	41.8
H1H21234N-N297Q	2.20E+05	1.00E-05*	4.54E-11	1155.2
H1H21234N-N297Q-LP1	4.90E+05	1.00E-05*	2.04E-11	1155.2
H1xH29273P2	8.20E+04	7.63E-03	9.30E-08	1.5
H1xH29282P2	8.28E+04	3.62E-03	4.37E-08	3.2
H1xH29283P2	1.39E+05	1.85E-03	1.34E-08	6.2

* indicates that no dissociation of His9-hMSR1 was observed under the current experimental conditions and the k_d value was manually fixed at 1.00E-05 while fitting the data

$ indicates that no binding was observed under the current experimental conditions.

[0797] At 25°C, different anti-MSR1 antibody-LXR conjugates bound to 9xHis-hMSR1 with K_D values ranging from less than or equal to 0.6 pM to 34.8 nM, while the unconjugated parental antibodies bound to 9xHis-hMSR1 with K_D values ranging from less than or equal to 0.6 pM to 13.7 nM as shown in Table 19.

[0798] The binding studies were repeated with anti-MSR1 antibody H1H21234-N297Q and steroid linker payloads (LPs). Binding kinetics parameters for His9-hMSR1 binding at 25°C are shown in Table 20. "LP3" represents a linker-payload for which the structure is provided in Example 23, and "LP13" represents a linker-payload for which the structure is provided in Example 24.

Table 20: Binding kinetics of His-hMSR1 binding to MSR1 Antibody-Steroid ADCs and Corresponding Unconjugated Antibody at 25 °C

Antibody Captured	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (Molar)	t½ (min)

Antibody Captured	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (Molar)	t½ (min)
H1H21234N-N297Q *	3.00E+05	1.00E-05	3.33E-11	1155.2
H1H21234N-N297Q-LP3 *	4.17E+05	1.00E-05	2.40E-11	1155.2
H1H21234N-N297Q-LP13 *	4.00E+05	1.00E-05	2.50E-11	1155.2

* indicates that no dissociation of His9-hMSR1 was observed under the current experimental conditions and the k_d value was manually fixed at 1.00E-05 while fitting the data

$ indicates that no binding was observed under the current experimental conditions.

[0799] At 25°C, an anti-MSR1 monoclonal antibody conjugated with LP3 and LP13 bound to His9-hMSR1 with K_D values ranging from 25.0 pM and 24.0 pM, respectively, as shown in Table 20. In comparison, the corresponding unconjugated anti-MSR1 monoclonal antibody bound to His9-hMSR1 with a K_D value of 33.3 pM.

Example 31. Anti-MSR1 Antibody-LXR Conjugates Activate LXR Signaling in a LXR-Luciferase Reporter Bioassay

[0800] *Generation of assay cell line.* To test the efficacy of anti-MSR1 antibody-LXR conjugates *in vitro*, a cell-based LXR responsive luciferase reporter assay was developed. To generate the assay cell line, a LXR regulated luciferase reporter gene [Cignal Lenti LXR Reporter (luc) kit (Qiagen, Cat# CLS-001L)] was transduced into THP1 cells, and cells were selected for two weeks in puromycin. The lentivirus expresses the firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the LXR transcriptional response element. The resulting cell line is referred to as THP1/LXR-Luc cells.

[0801] *Assay protocol*. THP1/LXR-Luc cells were seeded onto 96 well plates at 40,000 cells/ well in media containing RPMI supplemented with 10% FBS and pencillin/streptomycin and subsequently differentiated with 200 nM Phorbol Myristate Acetate (PMA) for 3 days. After the 3-day differentiation period, three-fold serial dilutions of antibody drug conjugates, unconjugated antibodies, or free payloads in fresh media were added to the cells at final concentration ranging from 100 nM to 0.01 nM. Media alone served as a blank control. Forty-eight hours later, luciferase activity was determined after the addition of One-Glo™ reagent (Promega, Cat# E6130) to each well. Relative light units (RLUs) were measured on a Victor luminometer (PerkinElmer) and EC_{50} values were determined using a four-parameter logistic equation over a 10-point dose response curve (GraphPad Prism). The EC_{50} value of each reagent tested is shown in Table 21. The signal to noise (S/N) was determined by calculating the ratio of RLU of standard one over RLU of standard eight for each of the unconjugated anti-MSR1 antibodies or free payloads. "LP#" represents a linker-payload for which the corresponding structures are

provided elsewhere herein, and "P#" represents a payload for which the corresponding structures are provided elsewhere herein.

Table 21: Agonist Activity of Anti-MSR1 Antibody-LXR Conjugates, Payloads, and Unconjugated Antibodies

Molecule tested	EC_{50} (M)	S/N
H1H21231N-N297Q-LP1	8.6E-10	14.5
H1H21227N-N297Q-LP1	8.9E-10	31.0
H1H21231N-N297Q-LP1	1.73E-09	38.6
H1H21234N-N297Q-LP1	3.65E-09	18.4
H1H21234N-N297Q-LP4	6.3E-10	9.0
H1H21234N-N297Q-LP5	9.8E-10	9.6
H1H21234N-N297Q-LP6	1.02E-09	9.2
H1H21234N-N297Q-LP12	No activation	1.5
H1H21234N-N297Q-LP9	1.12E-09	8.3
H1H21234N-N297Q-LP11	8.1E-10	10.0
H1H21234N-N297Q-LP2	8.7E-10	9.1
H1H27729P-N297Q-LP1	1.64E-09	14.5
H1H27731P-N297Q-LP1	1.46E-09	15.5
H1H27732P-N297Q-LP1	8.2E-10	19.1
H1H27734P-N297Q-LP1	5.19E-09	17.8
H1H27736P-N297Q-LP1	1.01E-09	16.4
H1H27739P-N297Q-LP1	1.60E-09	21.0
H1H27747P-N297Q-LP1	4.77E-09	20.6
H1H27749P-N297Q-LP1	1.46E-09	13.1
H1H27751P-N297Q-LP1	1.54E-09	17.9
H1H27754P-N297Q-LP1	1.30E-09	17.3
H1H27756P-N297Q-LP1	1.61E-09	18.6
H1H27759P-N297Q-LP1	5.94E-09	18.9
H1H27760P-N297Q-LP1	6.34E-09	21.5
H1H27761P-N297Q-LP1	5.72E-09	20.7
H1H27762P-N297Q-LP1	7.02E-09	16.4
H1H27766P-N297Q-LP1	1.277E-08	11.3
H1H27771P-N297Q-LP1	2.41E-09	17.3
H1H27773P-N297Q-LP1	>4.05E-08	12.9
H1H27778P-N297Q-LP1	1.51E-09	16.1
H1H21235N-N297Q-LP1	8.3E-10	8.2
H1H25700N-N297Q-LP1	1.13E-09	7.4
H1H25690N-N297Q-LP1	2.5E-10	8.0
H1H25695N-N297Q-LP1	1.87E-09	9.8
H1H25685N-N297Q-LP1	7.8E-10	8.3
H1H21228N-N297Q-LP1	1.8E-09	8.6
Isotype Control-N297Q-LP1	>6.6E-08	2.7
H1H21234N-N297Q	No activation	No activation
P1	1.14E-09	15.9
P5	4.6E-10	5.7
P7	2.09E-09	8.9
P6	3.7E-10	8.3

[0802] As shown in Table 21, at 48-hour time point, all of the anti-MSR1 antibodies conjugated with **LP1** demonstrated stimulation of the THP1/Luc cells with EC_{50} values ranging from 0.2 nM to greater than 140.5 nM with S/N values ranging from 7.4 to 38.6. One exemplary anti-MSR1 antibody-LXR conjugate (H1H21234N-N297Q-LP1) demonstrated stimulation of the THP1/Luc cells with an EC_{50} value of 3.7 nM and S/N of 18.4. The free payload, **P1**, demonstrated stimulation of the THP1/Luc cells with an average EC_{50} of 15.9 nM. The isotype control antibody conjugated with **LP1 (**Isotype control-LP1) had an average EC_{50} value of >66 nM and S/N of 2.7. Additionally, H1H21234N-N297Q conjugated with additional LXR agonist linker-payloads (H1H21234N-N297Q-LP4, H1H21234N-N297Q-LP5, H1H21234N-N297Q-LP6, H1H21234N-N297Q-LP12, H1H21234N-N297Q-LP9, H1H21234N-N297Q-LP11, and H1H21234N-N297Q-LP2) tested demonstrated stimulation of the THP1/Luc cells with EC_{50} values ranging from 0.63 nM to greater than 73 nM and S/N ranging from 1.4 to 10. In contrast, an anti-MSR1 antibody conjugated to an LXR agonist linker-payload (H1H21234N-N297Q-LP12), which acted as comparators in the assay, did not demonstrate any activation of the THP1/Luc cells. Additional LXR agonist payloads tested (P5, P7, and P6) demonstrated stimulation of the THP1/Luc cells with EC_{50} values ranging from 0.37 nM to 2.09 nM and S/N ranging from 5.7 to 8.9. One unconjugated anti-MSR1 antibody (H1H21234N) alone did not have any impact on stimulation of the THP/Luc cells.

Example 32. Anti-MSR1 Antibody-Steroid Conjugates Inhibit Release of LPS-mediated IL-1β In Vitro

[0803] To test the anti-inflammatory properties of anti-MSR1 antibody-steroid conjugates *in vitro*, a lipopolysaccharide (LPS)-mediated IL-1β release assay was performed. For the assay, THP-1 cells were seeded onto 96 well plates at 40,000 cells/ well in media containing RPMI supplemented with 10% FBS and pencillin/streptomycinin and differentiated with 200 nM Phorbol Myristate Acetate (PMA) for 3 days. After the 3-day differentiation period, three-fold serial dilutions of antibody drug conjugates, unconjugated antibodies, or free payloads in fresh media were added to the cells at final concentration ranging from 100 nM to 0.01 nM. Media alone served as a blank control. Subsequently at 24, 48, or 72 hours later, cells were treated with 100 ng/ml LPS (InVivoGen, Cat# tlrl-eklps) for 5 hours. The cell media was then collected, and IL-1β levels were measured using a V-PLEX Proinflammatory Panel 1 human kit (Meso Scale Diagnostics, Cat # 15049D-2) as per manufacturer's instructions. Subsequently, the plate was read on a Meso Scale Diagnostics instrument.

[0804] IC_{50} values were determined from a four- parameter logistic equation over a 10-point response curve (GraphPad Prism). All IC_{50} values are expressed in M (molar) concentration.

The IC_{50} values and IL-1β amount at the maximum concentration of antibody-steroid conjugates or controls are shown in Table 22 each of the time point tested.

Table 22: LPS-induced IL-1β Release of Anti-MSR1 Antibody-Steroid Conjugates

	24 hour		48 hour		72 hour	
Molecule	**EC_{50} (M)**	**IL1beta released at max concentration tested (pg/mL)**	**EC_{50} (M)**	**IL1beta released at max concentration tested (pg/mL)**	**EC_{50} (M)**	**IL1beta released at max concentration tested (pg/mL)**
H1H21234N-N297Q-LP13	6.287E-09	119.43	3.586E-09	69.3	1.7E-09	70.31
Isotype control-N297Q-LP13	NA	209.6	NA	279.4	NA	276.27
H1H21234N-N297Q	NA	234.21	NA	267.7	NA	355.76
P4	8.486E-10	43.39	1.017E-09	9.3	1.199E-09	12.95
Dexamethasone	4.199E-10	45.33	7.941E-10	11.8	5.433E-10	13.34

[0805] As shown in Table 22, at 24-hour time point, the anti-MSR1 antibody-steroid conjugate (H1H21234N-N297Q-LP13) inhibited LPS-induced IL-**1β secretion with an IC_{50}** of 6.287 nM. The free payload **P4** and free payload control **Dexamethasone**, inhibited LPS-induced IL-1β secretion with IC_{50} values of 0.8486 nM and 0.4199 nM, respectively. Neither the isotype control antibody conjugated with **LP13** (Isotype control-N297Q-LP13) nor the unconjugated anti-MSR1 antibody (H1H21234N-N297Q) had any impact on LPS-induced IL-1β release.

[0806] As shown in Table 22, at 48-hour time point, the anti-MSR1 antibody-steroid conjugate (H1H21234N-N297Q-LP13) inhibited LPS-induced IL-**1β secretion with an IC_{50}** of 3.586 nM. The free payload **P4** and free payload control **Dexamethasone**, inhibited LPS-induced IL-**1β** secretion with IC_{50} values of 1.017 nM and 0.7941 nM, respectively. Neither the isotype control antibody conjugated with **LP13** (Isotype control-N297Q-LP13) nor the unconjugated anti-MSR1 antibody (H1H21234N-N297Q) had any impact on LPS-induced IL-1β release.

[0807] As shown in Table 22, at 72-hour time point, the anti-MSR1 antibody-steroid conjugate (H1H21234N-N297Q-LP13) inhibited LPS-induced IL-1β secretion with an IC_{50} of 1.7 nM. The free payload **P4** and free payload control **Dexamethasone** inhibited LPS-induced IL-1β secretion with IC_{50} values of 1.199 nM and 0.5433 nM, respectively. Neither the isotype control antibody conjugated with **LP13** (Isotype control-N297Q-LP13) nor the unconjugated anti-MSR1 antibody (H1H21234N-N297Q) had any impact on LPS-induced IL-1β release.

Example 33. Anti-MSR1 Antibody-Steroid Conjugates Inhibit Release of LPS-mediated Inflammatory Responses In Vivo

[0808] To examine the anti-inflammatory effect of the anti-MSR1 antibody-steroid conjugates, an *ex-vivo* and *in vivo* LPS-induced inflammatory immune response model was performed.

[0809] *Generation of a mouse inflammatory immune response model.* Mice homozygously expressing human MSR1 extracellular domain in place of the mouse MSR1 extracellular domain and with a homozygous deletion of the ApoE gene were utilized (resulting mice referred to as *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice).

[0810] *Assay protocol for ex vivo LPS-induced responses.* In *ex vivo* LPS challenge experiments, mice were intraperitoneally administered with 10 **μL/g of anti**-MSR1 antibody-steroid conjugates (H1H21234N-N297Q-LP13), isotype control steroid conjugate (Isotype control-LP13), unconjugated anti-MSR1 antibody (H1H21234N-N297Q) and isotype control antibodies at different time points (24, 48, and 72 hours) prior to harvesting the peritoneal cells from peritoneal cavity. PBS and free steroids **P4** and Dexamethasone (Henry Schein Animal Health, Cat # NDC#11695-4017-1), were intraperitoneally injected at 2 hour prior to peritoneal

cell isolation. Experimental dosing and treatment protocol for groups of mice are shown in Table 23. All mice were sacrificed at 0 hour time point and peritoneal cells were obtained from the peritoneum by the peritoneal lavage technique. In brief, 5mL of ice cold 1xPBS (ThermoFisher Scientific, Cat #20012027) containing 2% BSA (Gibco, Cat #A9576) and 0.6mM EDTA (ThermoFisher Scientific, Cat #15575020) were injected into the peritoneal cavity using 27g needle. Peritoneal cells were collected into 15 mL tubes with 20g needle attached to 5-mL syringe, centrifuged at 300xg for 8 minutes and adjusted to $1x10^6$ cells/mL in RPMI-1640 (ThermoFisher Scientific, #15140122) containing 10% of FBS (ThermoFisher Scientific, Cat #10082147) and 1% of penicillin-streptomycin (ThermoFisher Scientific, Cat #11875093). The resuspended cells were plated at 500,000 cells per well in 24-well tissues culture plates and incubated with or without 10 ng/mL of LPS for 18 hours.

Table 23: Experiment Design, Dosing, and Treatment Protocol for Ex Vivo LPS Challenge Experiment

	In vivo				*Ex vivo*
	Group	**Mice (n)**	**Molecule tested**	**Dose (mg/kg)**	**LPS Challenge (10 ng/mL)**
72 hour	1	3	H1H21234N-N297Q-LP13	96.08	+/- LPS
	2	3	Isotype control-N297Q-LP13	97.07	+/- LPS
	3	3	H1H21234N-N297Q	96.08	+/- LPS
	4	3	Isotype control-N297Q	97.07	+/- LPS
48 hour	5	3	H1H21234N-N297Q-LP13	96.08	+/- LPS
	6	3	Isotype control-N297Q-LP13	97.07	+/- LPS
	7	3	H1H21234N-N297Q	96.08	+/- LPS
	8	3	Isotype control-N297Q	97.07	+/- LPS
24 hour	9	3	H1H21234N-N297Q-LP13	96.08	+/- LPS
	10	3	Isotype control-N297Q-LP13	97.07	+/- LPS
	11	3	H1H21234N-N297Q	96.08	+/- LPS
	12	3	Isotype control-N297Q	97.07	+/- LPS
2 hour	13	3	PBS	-	+/- LPS
	14	3	Dexamethasone	1	+/- LPS
	15	3	P4	1.48	+/- LPS

[0811] *Assay protocol for in vivo LPS-induced responses.* In *in vivo* LPS challenge experiments, mice were intraperitoneally administered with 10 µL/g of decreasing concentrations of anti-MSR1 antibody-steroid conjugates (H1H21234N-N297Q-LP13) and isotype control steroid conjugate (Isotype control-N297Q-LP13) at 24 hours prior to *in vivo* LPS challenge. PBS and free steroid **P4** were intraperitoneally injected at 2 hours prior to *in vivo* LPS challenge. Experimental dosing and treatment protocol for groups of mice are shown in Table 24. At 0 hour time point, three large drops of blood (volume ~ 100 µL) were collected from the maxillary vein of each mouse into serum gel separator tubes. The tubes were centrifuged at 2200-2500 rpm for 15 minutes, and the serum was collected into 96-well plates for subsequent

measurement of cytokines. At 18 hours post *in vivo* LPS challenge, the peritoneal cells were obtained from periotenal cavity as aforementioned for subsequent flow cytometry analysis.

Table 24: Experiment Design, Dosing, and Treatment Protocol for In Vivo LPS Challenge Experiment

	In vivo				*In vivo*
	Group	**Mice (n)**	**Molecule tested**	**Dose (mg/kg)**	**LPS Challenge (1µg/mouse)**
24 hour	1	5	H1H21234N-N297Q-LP13	96.08	+
	2	5	Isotype control-N297Q-LP13	97.07	+
	3	5	H1H21234N-N297Q-LP13	32.03	+
	4	5	Isotype control-N297Q-LP13	32.36	+
	5	5	H1H21234N-N297Q-LP13	10.67	+
	6	5	Isotype control-N297Q-LP13	10.79	+
2 hour	7	3	PBS	-	-
	8	5	PBS	-	+
	9		P4	1.48	+
	10	5	P4	0.49	+
	11	5	P4	0.16	+

[0812] *Measurement of cytokines at 18 hours post-LPS ex vivo challenge.* Supernatants were collected into 96-well round bottom tissue culture plates at 18 hours post-*ex vivo* LPS challenge and stored at 20°C until further analysis. Cytokine concentrations in the supernatants were measured using a Proinflammatory Panel 1 (mouse) multiplex immunoassay kit (MesoScale Diagnostics, Cat #K15048D) according to manufacturer's instructions. Electrochemiluminescence was read on a Meso Scale Diagnostics SECTOR® instrument. Data analysis was performed using GraphPad Prism™ software. Statistical significance within the groups was determined by one-way Anova with Turkey's multiple comparison post-test (*p<0.01; **p<0.001; ***p<0.0001).

[0813] *Analysis of expression of activation markers on peritoneal macrophages after LPS ex vivo challenge.* Upon collection of supernatants for cytokine analysis, the plates were washed twice with 1xPBS w/o Ca^{++}/$Mg2^{++}$ (ThermoFisher Scientific, Cat #14190144), and the adherent peritoneal cells were harvested by addition of 250-300 µL of cold Macrophage Detachment Solution DXF (PromoCell, Cat #C41330) for 40 minutes. The detached cells were collected into 50-mL tubes and centrifuged at 350xg for 15 minutes. After two washes with 1xPBS supplemented with 2mM EDTA, the cells were resuspended in Cell Staining Buffer (Biolegend, #420201) and plated at $1x10^6$ cells per well in a 96-well V bottom plate. Cells were centrifuged and cell pellets were resuspended in 100 µL of LIVE/DEAD Blue Fluorescent Reactive Dye (Life Technologies, Cat #L34962) diluted at 1:600 in 1xPBS to determine cell viability. Cells were incubated at room temparature for 20 minutes in dark. After one wash with 1xPBS, cells were

incubated in Cell Staining Buffer containing 10μg/mL of purified rat anti-mouse CD16/CD32 Fc Block (Clone: 2.4G2; BD Biosciences, Cat #553142) for 10 minutes at 4°C. The cells were washed once and incubated in the appropriate antibody mixture diluted in Brilliant Stain Buffer (BD Biosciences, Cat #566345) for 30 minutes at 4°C in dark. The antibody panel used for flow cytometry analysis is shown in Table 25. The cells were subsequently washed with 1xPBS, resuspended in Cell Staining Buffer and acquired on FACS LSRFortessa X-20 flow cytometer (Becton Dickinson). Data were analyzed using FlowJo (Tree Star).

Table 25: Antibodies used in flow cytometry analysis

Antibody	Fluorochrome	Manufacturer	Catalog Number	Final Dilution
anti-mouse CD45	BV510	Biolegend	103138	1/200
anti-mouse B220	APC-Cy7	Biolegend	103224	1/200
anti-mouse TCRβ	BV711	Biolegend	109243	1/200
anti-mouse CD11b	PercP-Cy5.5	Biolegend	101228	1/300
anti-mouse F4/80	FITC	Biolegend	123108	1/200
anti-human SR-AI/MSR1	PE	R&D Systems	FAB2708P	1/30
anti-mouse TLR4	APC	Biolegend	145405	1/200
anti-mouse MHCII	BV421	Biolegend	107631	1/200
anti-mouse CD80	PE-Cy7	Biolegend	104733	1/200

[0814] Results are provided in Tables 26 to 28. Flow cytometry analysis of peritoneal cells derived from *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice revealed that hMSR1 and TLR4 are predominantly expressed on the surface of F4/80^{+}CD11b^{+} peritoneal macrophages suggesting that MSR1^{+} cells are the main responders to LPS stimulation.

Table 26: Ex Vivo LPS-induced Cytokine Production for Anti-MSR1 Antibody-steroid Conjugates and Controls

Antibody/Reagent	Time	Cytokine [ng/mL]		
		IL-6	KC-GRO	TNF-α
PBS	2 hours	224.8 ± 11.04	26.4 ± 2.1	1.8 ± 0.3
Dexamethasone		134.5 ± 18.2*	19.9 ± 1.5	0.63 ± 0.11*
P4		12.98 ± 5.8****	2.6 ± 1.3**	0.43 ± 0.06**
H1H21234N-N297Q-LP13	24 hours	21.1 ± 1.38****	9.3 ± 1.7	0.26 ± 0.08***
Isotype Control-N297Q-LP13		165.6 ± 20.7	17.8 ± 1.7	0.96 ± 0.14
H1H21234N-N297Q		261.9 ± 18.3	36.4 ± 2.9	2.02 ± 0.4
Isotype Control-N297Q		215.8 ± 40.1	21.9 ± 5.2	1.9 ± 0.1
H1H21234N-N297Q-LP13	48 hours	34.02 ± 7.8**	6.3 ± 2.3*	0.58 ± 0.06*
Isotype Control-N297Q-LP13		113.4 ± 11.3	22.2 ± 4.2	1.2 ± 0.2
H1H21234N-N297Q		158.3 ± 11.6	15.2 ± 0.8	1.6 ± 0.3
Isotype Control-N297Q		143.4 ± 12.4	18.8 ± 1.8	1.12 ± 0.1
H1H21234N-N297Q-LP13	72 hours	50.7 ± 14.1*	13.1 ± 1.1	0.47 ± 0.1**
Isotype Control-N297Q-LP13		132.1 ± 18.9	20.8 ± 2.7	0.92 ± 0.07
H1H21234N-N297Q		228.6 ± 19.7	27.1 ± 3.5	1.09 ± 0.2
Isotype Control-N297Q		284.5 ± 14.9	29.4 ± 8.2	1.2 ± 0.2

[0815] As shown in Table 26, *ex vivo* LPS challenge induced robust production of IL-6, KC-GRO and TNF-α by peritoneal cells derived from *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice pre-treated with PBS. On the contrary, *in vivo* administration of MSR1-ncADC conjugates (H1H21234N-N297Q-LP13) at the dose equivalent to 1mg/kg of Dexamethasone was efficacious in inhibiting LPS-induced cytokine production by peritoneal cells in a time-dependent manner. Comparable inhibition of LPS-induced immune responses was observed between administration of antibody-steroid conjugates at 24, 48, and 72 hours and administration of free payload **P4** at 2 hours (Table 26). A similar effect on inhibition of LPS-induced immune response was observed between administration of isotype control-steroid conjugates at 24, 48, and 72 hours and Dexamethasone at 2 hours (Table 26). No effect on LPS-induced cytokine production was observed with isotype control-steroid conjugate (Isotype control-N297Q-LP13), unconjugated anti-MSR1 antibody (H1H21234N-N297Q) and isotype control antibodies administered at different time points (Table 26).

Table 27: In Vivo LPS-induced Cytokine Production in Serum for Anti-MSR1 Antibody-steroid Conjugates and Controls

Antibody/Reagent	Administered Concentration (mg/kg)	Time	Cytokine [ng/mL]		
			IL-6	KC-GRO	TNF-α
PBS	-	2 hours	56.94 ± 9.3	13.97 ± 1.7	1.7 ± 0.3
P4	1.48		3.51 ± 0.7***	4.1 ± 1.5*	0.09 ± 0.02***

Antibody/Reagent	Administered Concentration (mg/kg)	Time	Cytokine [ng/mL]		
			IL-6	KC-GRO	TNF-α
P4	0.49		11.89 ± 7.7**	6.7 ± 2.3	0.2 ± 0.04***
P4	0.16		19.15 ± 1.1*	15.1 ± 1.2	0.6 ± 0.05***
H1H21234N-N297Q-LP13	96.08	24 hours	7.7 ± 3.1***	5.1 ± 1.5*	0.42 ± 0.14***
	32.03		15.34 ± 5.2***	10.6 ± 2.8	0.48 ± 0.18***
	10.67		33 ± 8.1	13.3 ± 1.2	0.51 ± 0.08***
Isotype Control-N297Q-LP13	97.07	24 hours	33.6 ± 4.1	19.2 ± 0.6	2.6 ± 0.92
	32.36		65.9 ± 7.8	18.2 ± 1.7	2 ± 0.67
	10.79		76.3 ± 9.9	19.9 ± 2.1	1.9 ± 0.62

[0816] *In vivo* administration of MSR1-steroid conjugates (H1H21234N-N297Q-LP13) was also efficacious in inhibiting *in vivo* LPS-induced cytokine production in a dose-dependent manner in *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice 2 hours post LPS challenge (Table 27). No effect on LPS-induced cytokine production *in vivo* was observed with isotype control-ncADC (Isotype control-N297Q-LP13, Table 27).

Table 28: Ex Vivo LPS-induced Cytokine Production for Anti-MSR1 Antibody-steroid Conjugates and Controls

Antibody/Reagent	Time	LPS-mediated expression of CD80 [% relative to PBS/LPS-treatment]	
		pMΦ Cells	non-pMΦ Cells
Dexamethasone	2 hours	77.69 ± 2.6	99.6 ± 5.8
P4		54.7 ± 3.8****	93.02 ± 4.4
H1H21234N-N297Q-LP13	24 hours	61.1 ± 1.3***	95.5 ± 2.6
Isotype Control-N297Q-LP13		94.9 ±7.2	90.22 ± 5.2
H1H21234N-N297Q		76.8 ± 2.3	98.9 ± 3.1
Isotype Control-N297Q		75.6 ± 4	100.8 ± 8.5
H1H21234N-N297Q-LP13	48 hours	58.7 ± 4.2****	99 ± 1.8
Isotype Control-N297Q-LP13		75.9 ± 3.8	95.4 ± 5.2
H1H21234N-N297Q		81.9 ± 2	100.4 ± 3.3
Isotype Control-N297Q		87.4 ± 0.6	107.7 ± 6.7
H1H21234N-N297Q-LP13	72 hours	63.9 ± 1.01***	102.3 ± 0.14
Isotype Control-N297Q-LP13		85.3 ± 12.04	95.6 ± 2.2
H1H21234N-N297Q		82.9 ± 5.9	96.3 ± 3.3
Isotype Control-N297Q		86.4 ± 4.3	96.8 ± 8.8

[0817] In addition, as illustrated in Table 28, *in vivo* administration of anti-MSR1 antibody-steroid conjugate (H1H21234N-N297Q-LP13*)* led to a significant reduction of LPS-mediated expression of CD80 on hMSR1^{+}F4/80^{+}CD11b^{+}peritoneal macrophages (pMΦ), but not on hMSR1^{-}F4/80^{-}CD11b^{-}

cells (non-pMΦ). No effect on CD80 expression was observed with Isotype control antibodies (Table 28).

Example 34. Anti-MSR1 Antibody-LXR Conjugates Activate Cholesterol Efflux in THP-1 Cells

[0818] The ability of anti-MSR1 antibody-LXR conjugates to activate cholesterol efflux in a human macrophage cell line (THP-1; ATCC Catalog # TIB-202), was assessed using a fluorescent cholesterol analog.

[0819] Briefly, THP-1 cells were seeded onto 96-well poly-lysine coated plates (Corning, Catalog # 354640) at 100,000 cells / well in RPMI 1640 media (Irvine Scientific, Catalog # 9160) containing 10% FBS (Gibco, Catalog # 1043010), 10 µg/mL penicillin-streptomycin (Gibco, Catalog # 15140122) and incubated at 5% CO_2 at 37 °C. Cells were differentiated into macrophages by addition of 100 nM Phorbol-12 myristate 13-acetate (Sigma, Catalog # P8139) to the media and subjected to further incubation for 96 hours. Differentiated macrophages were then incubated in phenol red free RPMI 1640 media (Gibco, Catalog # 32404-014) containing 25 µM BODIPY-cholesterol (Avanti Polar Lipids, Catalog # 810255P), 0.2% bovine serum albumin (BSA; Sigma, Catalog # A7211), and 10 µg/mL penicillin-streptomycin for 24 hours, followed by a 24-hour treatment with serial dilutions of ranging from 1×10^{7} M to 5×10^{-14} M of either free payload, anti-MSR1 antibody-LXR conjugate (H1H21234N-N297Q-LP1), Isotype control-LXR conjugate (Isotype control-N297Q-LP1), and unconjugated anti-MSR1 antibody (H1H21234N-N297Q) in phenol red free RPMI 1640 media containing 0.2% BSA. Cells were washed with phenol red free RPMI 1640 media and incubated with 100 µL of acceptor media containing 50 µg/mL high density lipoprotein (Millipore, Catalog # 437641), 10 µg/mL apolipoprotein A1 (Millipore, Catalog # ALP10) in phenol red free RPMI 1640 media for 5 hours, after which, the acceptor media was collected and cells were lysed in 100 µL of RIPA buffer (Millipore, Catalog #20-188) for 2 hours with gentle agitation at room temperature. Fluorescence was measured in these fractions at excitation 482 nm, emission 515 nm in SpectraMax i3 plate reader (Molecular Devices).

[0820] Percentage of BODIPY-cholesterol efflux was calculated using the following formula: [fluorescence in acceptor media / (fluorescence in acceptor media + fluorescence in cell lysate)] x 100. Table 29 provides activated cholesterol efflux for the tested articles, and FIG. 17 illustrates the data in graph form.

Table 29: Activation of cholesterol efflux by antibody-LXR conjugates and comparators

Molecule tested	Cholesterol Efflux activation EC_{50} (M)	Maximum efflux (%)
P1	1.5E-10	36.7
H1H21234N-N297Q-LP1	5.0E-11	36.7
Isotype control-N297Q-LP1	> 6.4E-8	30.3
H1H21234N-N297Q	N/A	18.7

[0821] As shown in Table 29, after 24 hours, H1H21234N-N297Q-LP1 conjugate demonstrated the largest amount of cholesterol efflux with a maximum percent efflux of 36.6% and an EC_{50} value of 50 pM. The free payload **P1** demonstrated the second largest amount of cholesterol efflux with a maximum percent efflux of 36.6% and an EC_{50} value of 150 pM. The Isotype control-N297Q-LP1 conjugate demonstrated a minimal amount of cholesterol efflux with a maximum percent efflux of 30.3%. The unconjugated antibody, H1H21234N-N297Q, did not demonstrated any measurable cholesterol efflux.

Example 34A activity of LXR agonist payloads in a cell based LXR responsive luciferase reporter assay

[0822] The activity of certain LXR agonist payloads described herein were assessed in a cell based LXR responsive luciferase reporter assay. To generate the assay cell line, an LXR regulated luciferase reporter gene (Cignal Lenti LXR Reporter (luc) kit (Qiagen, Cat# CLS-001L)) was transduced into THP1 cells and the cells were selected for two weeks in puromycin. The lentivirus expresses the firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the LXR transcriptional response element. The resulting cell line is referred to as THP1/LXR-Luc cells. For the assay, THP1/LXR-Luc cells were seeded onto 96 well plates at 40,000 cells/ well in media containing RPMI supplemented with 10% FBS and penicillin/streptomycin and were then differentiated with 200 nM Phorbol Myristate Acetate (PMA) for 3 days. The media was subsequently removed and replaced with 80uL of fresh media without PMA. Three-fold serial dilutions of free payloads were prepared in 100% DMSO, transferred to fresh media, and 20uL were added to the cells at a final constant DMSO concentration of 0.2% and free payloads at final concentrations ranging from 100 nM to 0.015 nM. The last well in the plate served as blank control containing only the media and 0.2% DMSO (untreated well) and was plotted as a continuation of the 3-fold serial dilution. Forty-eight hours later, luciferase activity was determined after the addition of One-Glo™ reagent (Promega, Cat# E6130) to each well. Relative light units (RLUs) were measured on a Victor luminometer (PerkinElmer) and EC_{50} values were

determined using a four- parameter logistic equation over a 10-point dose response curve (GraphPad Prism). The EC_{50} value of each molecule tested is shown in the Table 1. The signal to noise (S/N) was determined by taking the ratio of the highest RLU on the dose response curve to the RLU in the untreated wells. As shown in Table 5, all of the tested payload compounds increased LXR-dependent luciferase activity in THP1/LXR-Luc cells with EC_{50} values ranging from 112 pM to 3.51 nM and S/N values ranging from 10.4 to 13.8.

Table 30: LXR-Reporter Activity by Payload Compounds in Differentiated THP-1/LXR-Luc cells

Payload Compound	EC_{50} (M)	S/N
P1	1.14E-09	11.4
P2B	2.92E-10	11.3
P4B	1.25E-10	10.4
P6B	3.34E-09	10.9
P5B	1.74E-10	13.8
P7B	2.53E-10	12.5
P9B	2.34E-10	12.8
P8B	3.51E-09	10.8
P10B	2.22E-10	11.1
P12B	2.96E-10	11.4
P11B	1.12E-10	10.7

Example 34B Antibody conjugation

[0823] Conjugation through antibody cysteines is performed in two steps using methods similar to those described in *Mol. Pharm.* **2015**, 12(6), 1863-71. In an exemplary procedure, a monoclonal antibody (mAb) is reduced with dithiothreitol or TCEP. After gel filtration, the appropriate linker-payload in DMSO solution is added to the reduced antibody, and the mixture is adjusted to appropriate pH. The reaction is allowed to stir. The resulting conjugate is purified by SEC. The DAR (UV) values are determined using the measured absorbances of the ADC and the extinction coefficients of the antibody linker-payload.

[0824] Site-specific antibody conjugation can be performed, *e.g.*, in two steps: (1) microbial transglutaminase *e.g.*, (MTG EC 2.3.2.13, Zedira, Darmstadt, Germany)-based enzymatic attachment of an azido-PEG-amine to a site-specifically mutated antibody and (2) attachment of an apprioriate linker–payload to the azido–PEG-amine functionalized antibody via a [2+3] cycloaddition, *e.g.*, 1,3–dipolar cycloaddition between the azido moiety of the functionalized

antibody and appropriate cyclooctyne moiety of the linker-payload, *e.g.*, copper–free click chemistry. See, *Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. PNAS* ***2007****, 104 (43), 16793–7.* For example, aglycosylated human antibody IgG (IgG1, IgG4, etc.) or a human IgG1 isotype in BupH™ (pH 6.5–8.0) is mixed with ≥200 molar equivalents of azido–dPEG$_3$–amine (MW= 218.26 g/mol). The resulting solution is mixed with transglutaminase (25 U/mL; 5U MTG per mg of antibody, from Zedira, Darmstadt, Germany, or Ajinomoto, Japan) resulting in a final concentration of the antibody at 0.5–5 mg/mL, and the solution is kept at pH 6.5–8.0 and then incubated at 37 °C for 4–24 h while gently shaking. The reaction is monitored by ESI–MS. Upon reaction completion, excess amine and MTG is removed by SEC or protein A column eluting with acidic buffer and then neutralizing with Tris buffer (pH 8) to generate the azido–functionalized antibody. This product is analyzed by SDS–PAGE and ESI. The azido–dPEG$_3$–amine adds to two sites – Q295 and Q297– of the antibody resulting in an 804 Da increase for the 4DAR aglycosylated antibody–PEG$_3$–azide conjugate. The conjugation sites are identified and confirmed at EEQ^{Linker}YQ^{Linker}STYR for the 4DAR azido–functionalized antibody via peptide sequence mapping of trypsin digested heavy chains.

[0825] In another example, site–specific aglycosylated antibody drug conjugates with a human IgG (IgG1, IgG4, etc.) containing an N297Q mutation (EU numbering) are prepared by a [2+3] click reaction between azido–functionalized antibodies with an alkyne containing linker–payload. Specifically, an azido–functionalized aglycosylated human IgG1 antibody (mAb–PEG$_3$–N$_3$) is conjugated to an appropriate linker payload by incubating mAb–PEG$_3$–N$_3$ (1–3 mg / mL) in an aqueous medium (e.g., PBS, PBS containing 5% glycerol, HBS) with ≥6 molar equivalents of a linker payload dissolved in a suitable organic solvent, such as DMSO, DMF or DMA (i.e., the reaction mixture contains 5-20% organic solvent, v/v) at 24 °C to 37 °C for over 6 h. The progress of the reaction is monitored by ESI–MS and the absence of mAb–PEG$_3$–N$_3$ indicated the completion of the conjugation. The excess amount of the linker payload and organic solvent are removed by SEC via elution with PBS, or via protein A column eluting with acidic buffer followed by neutralization with Tris (pH 8). The purified conjugates are analyzed by SEC, SDS–PAGE, and ESI–MS.

[0826] The antibody and antibody-drug conjguates can be characterized by SDS–PAGE, SEC, and MS (ESI). In one method, SDS–PAGE conditions including non–reduced and reduced samples (2-4 µg) along with BenchMark Pre–Stained Protein Ladder (Invitrogen, cat# 10748–010; L#

1671922.) are loaded per lane in (1.0 mm x 10 well) Novex 4-20% Tris–Glycine Gel and are ran at 180 V, 300 mA, for 80 min. An analytic sample is prepared using Novex Tris–Glycine SDS buffer (2X) (Invitrogen, Cat# LC2676) and the reducing sample is prepared with SDS sample buffer (2X) containing 10% 2-mecaptoethanol.

Example 34C

[0827] Several linker-payloads (LPs) were derived from the payload compounds in Table C and conjugated to an anti-MSR1 antibody (H1H21234N-N297Q) or a non-binding control using the techniques described in the prevous example. The resulting anti-MSR1 LXR agonist antibody drug conjugates (ADCs) were tested for activity in the THP1/LXRLuc reporter assay as described above for the payload compounds. As shown in Table 31, all of the tested anti-MSR1 LXR agonist ADCs demonstrated stimulation of the THP1/LXR-Luc cells with EC_{50} values ranging from 414 pM to 2.11 nM and S/N values ranging from 9.4 to 13.7. The unconjugated anti-MSR1 had no impact on LXR-Luc activity and a nonbinding control antibody conjugated to LP1 (Control ADC-LP1) had EC_{50} values of > 100 nM and maximum S/N values < 5.0.

[0828] Anti-MSR1 antibody H1H21234N has the HCVR according to SEQ ID NO:50 and the LCVR according to SEQ ID NO:58. It comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 according to SEQ ID NOS:52, 54, 56, 60, 62, and 64, respectively. The polypeptide sequences can be encoded by the polynucleotide sequences SEQ ID NOS: 49, 51, 53, 55, 57, 59, 61, and 63. N297Q indicates that residue 297 is mutated from asparagine (N) to glutamine (Q). All numbering is according to the EU numbering system.

Table 31: Anti-MSR1-LXR Agonist Conjugate Activity in Differentiated THP-1/LXR-Luc cells

Anti-MSR1-LXR agonist ADC	LXR agonist LP	LXR Agonist Payload	EC_{50} (M)	S/N
H1H21234N-N297Q-LP1	LP1	P2B	7.38E-10	11.8
H1H21234N-N297Q- LP4B	LP4B	P2B	9.23E-10	11.9
H1H21234N-N297Q-LP5B	LP5B	P2B	1.20E-09	11.0
H1H21234N-N297Q-LP2B	LP2B	P2B	8.68E-10	13.7
H1H21234N-N297Q-LP12B	LP12B	P2B	1.30E-09	11.6
H1H21234N-N297Q-LP6B	LP6B	P4B	6.85E-10	12.5
H1H21234N-N297Q-LP7B	LP7B	P4B	5.60E-10	11.6
H1H21234N-N297Q-LP8B	LP8B	P4B	6.19E-10	12.5
H1H21234N-N297Q-LP9B	LP9B	P6B	4.14E-10	11.2

Anti-MSR1-LXR agonist ADC	LXR agonist LP	LXR Agonist Payload	EC_{50} (M)	S/N
H1H21234N-N297Q-LP10B	LP10B	P6B	9.60E-10	12.5
H1H21234N-N297Q-LP11B	LP11B	P8B	9.78E-10	12.4
Unconjugated anti-MSR1	NA	NA	>1.00E-07	0.8
Control ADC-LP1	LP1	P2	>1.00E-07	4.4

Example 35. In vivo Effect of Anti-MSR1 Antibody-LXR Conjugates on Atherosclerosis in a Mouse Model

[0829] The effect of an anti-MSR1 antibody-LXR agonist conjugate, H1H21234N-N297Q-LP1, on atherosclerosis development was evaluated *in vivo* in mice homozygous for the expression of human MSR1 extracellular domain in place of the mouse MSR1 extracellular domain and homozygous for deletion of the *apoE* gene (referred to herein as *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice).

[0830] The *Msr1*$^{hu/hu}$ *ApoE*$^{-/-}$ mice were pre-bled 6 days before the start of the experiment after 4-hour fast and were then placed on an atherogenic western diet (Research Diets, Cat #106452). The mice were sorted into groups (n=7-9 each) based on their baseline triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) values. An MSR1 antibody (H1H21234N-N297Q) or MSR1 antibody-LXR agonist conjugate (H1H21234N-N297Q-LP1) were administered by weekly subcutaneous injections at 25 mg/kg dose (based on the antibody concentration) starting on day 0 for 16 weeks. Serum was collected at 4, 8, and 16 weeks of the study after 4-hour fast to evaluate serum lipids using AdviaXPT Chemistry System (Siemens). Average serum lipid values were calculated for each time point. Results, expressed as (mean ± SEM) are shown in FIG. 18. FIG. 18 illustrates that administration of the MSR1 antibody-LXR agonist conjugate H1H21234N-N297Q-LP1 did not have an effect on serum lipid levels.

[0831] Mice were sacrificed at the end of the study under nonfasted conditions 6 days after the last injection anti-MSR1 antibody or MSR1 antibody-LXR agonist ncADC, and their heart and liver were collected. Hearts were imbedded in Optimal cutting temperature compound (OCT), and sectioned perpendicular to the axis of the aorta, starting within the heart and working in the direction of the aortic arch. Once the aortic root was identified by the appearance of aortic valve leaflets, serial cross sections (12 µm thick) were taken and mounted on consecutive slides (VWR International, Cat# 16004-406). These sections were stained with hematoxylin and eosin stain (H&E stain), Oil Red O lipid stain, and rat-anti-CD68 antibody, (Abcam, Cat# ab201844) to label macrophages for analysis. An Aperio AT2 slide scanner (Leica Biosystems, Illinois) was used to

scan the slides and to generate images. For each mouse, the lipid area was measured using HALO software (Indica Labs, New Mexico) in 7 subsequent cross sections based on Oil Red O staining, and subsequently the average of total lesion lipid area per mouse was calculated using these measurements. All measurements were conducted by an analyst who was blinded to the treatment groups. Results, expressed as (mean ± SEM) are shown in FIG. 19A. FIG. 19A illustrates that administration of the MSR1 antibody-LXR agonist conjugate H1H21234N-N297Q-LP1 led to reduction in atherosclerotic lesion area.

[0832] In addition, H&E stained slides were used to calculate Intima/Media ratio, which represents the normalized value of plaque size. The internal and external elastic laminas of arterial media and lumen areas were measured in 7 subsequent cross sections for each mouse using the H&E stained sections and the average values were calculated per mouse. Intima/media ratio were calculated using the equation:

Intima/media ratio = (Internal elastic lamina area - Lumen area) / (External elastic lamina area - Internal elastic lamina area)

Results, expressed as (mean ± SEM) are shown in FIG. 19B.

[0833] The macrophage content in the sections was measured using slides stained with rat anti-CD68 antibody. For each mouse, macrophage positive area was measured using HALO software in at least 5 subsequent cross sections, and the average of total macrophage content per mouse was calculated using these measurements. Results, expressed as (mean ± SEM) are shown in FIG. 19C. FIG. 19C illustrates that administration of the MSR1 antibody-LXR agonist conjugate H1H21234N-N297Q-LP1 led to reduction in macrophage content.

[0834] Livers collected at sacrifice were used for qRT-PCR and lipid extraction. One piece of liver from each mouse was placed in RNAlader (Invitrogen, Cat #AM7023) for RNA extraction and then the expression of lipogenic genes (*Srebf1*, *Acc*, *Fasn*) to evaluated *de novo* lipogenesis was evaluated by qRT-PCR using standard methods. Results, expressed as (mean ± SEM) are shown in FIG. 21. FIG. 21 illustrates that administration of the MSR1 antibody-LXR agonist conjugate H1H21234N-N297Q-LP1 has no effect on hepatic triglyceride or cholesterol level.

[0835] Lipids were extracted from the second piece of liver from each mouse by Folch's method and solubilized by Carr's method. The levels of TG, total and free cholesterol were measured using enzymatic assays for detection (Teco Diagnostics, Cat# T532-480 (TG); Thermo Fisher Scientific, Cat#TR13421 (total cholesterol); Waco Diagnostics, Cat# 993-02501 (free cholesterol)) and

normalized to wet tissue weight. Results, expressed as (mean ± SEM) are shown in FIG. 20. FIG. 20 illustrates that administration of the MSR1 antibody-LXR agonist conjugate H1H21234N-N297Q-LP1 has no effect on hepatic *de novo* lipogenesis.

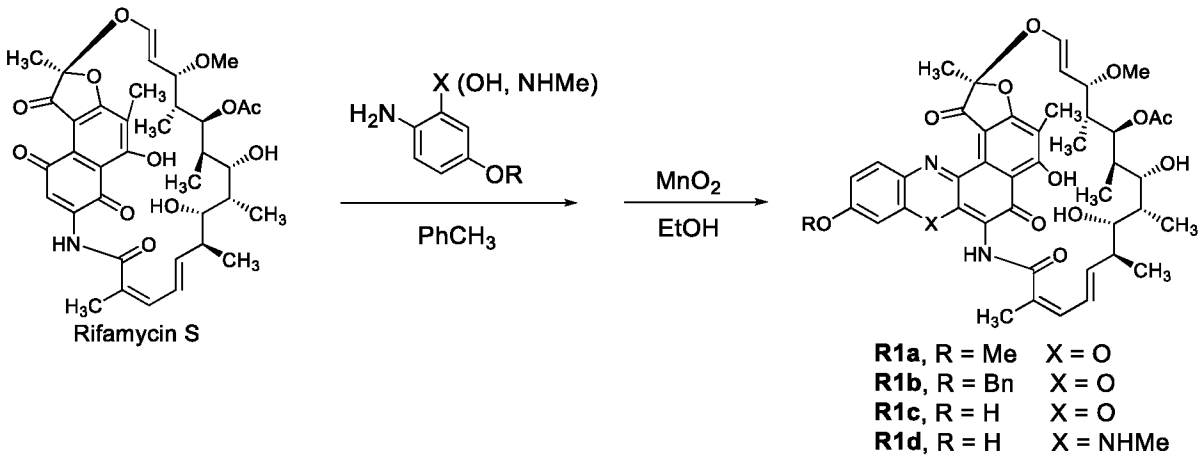
General Methods for Examples 36-41:

[0836] All the solvents used were purchased either from Sigma Aldrich or Fisher Scientific and were used as is. Rifamycin S was purchased from Bosche Scientific. ^{1}H-NMR spectra were recorded on a Varian Inova 300 MHz and 500 MHz NMR instruments. The chemical shifts (δ) are reported in ppm with respect to the NMR solvents used for analysis and are reported as s – singlet, d – doublet, t – triplet, q – quartet, dd – doublet of doublet, dt – doublet of triplet, dq – doublet of quartet, and m - multiplet. Coupling constants (*J*) are reported in hertz (Hz). Chromatographic purities were determined on an Agilent 1100, 1260 Infinity, or 1200 Series LC/MS systems using Chromolith® FastGradient RP-18e analytical columns (50 × 2 mm, Merck KGaA, P/N 1.52007.0001) and the following analytical HPLC method: injection volume 5 or 10 μL; flow rate 1 mL/min; 5-95% acetonitrile in water over 4 min; Agilent diode array detector at λ = 254 nm; room temperature. Low resolution mass spectrometry was performed on an Agilent system using electrospray ionization sources and analyzed with either single quadrupole or ion trap mass detectors.

Example 36. Synthesis of analogs **R1a-R1d**

[0837] **Scheme R1**, below, depicts the synthesis of exemplary compounds **1a-1d** according to the disclosure from commercially available starting materials.

Scheme R1



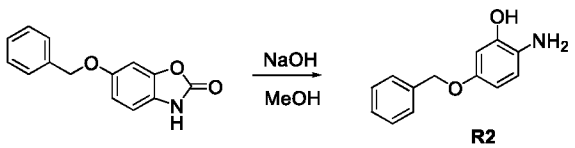
EXAMPLE 36A: Rifamycin 4-MeO-Phenol analog (R1a):

[0838] *The general coupling procedure of Example 36 is used to prepare the title compound:* To a stirring solution under argon of rifamycin S (200 mg, 0.287 mmol) in 15 mL of toluene at room temperature was added 2-amino-5-methoxyphenol (44 mg, 0.316 mmol). The mixture solution was stirred for 3 days at room temperature. The progress of reaction was monitored by LC/MS, then the mixture was evaporated to dryness. The dark residue was dissolved in 10 mL of ethanol, and 100 mg (1.14 mmol) of manganese oxide (MnO_2) was added in one portion to the ethanol solution. The sluggish mixture was stirred for 15h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 40 g HP silica gel Gold RediSep column *via* **ISCO (gradient elution: 5 → 95% EA in** hexanes) and the pure fractions evaporated and dried *in vacuo* giving the title compound **R1a** as a **dark reddish solid (85 mg, 37%). MS (ESI, pos.): calc'd for** $C_{44}H_{50}N_2O_{13}$, 814.33; found 815.3 (M+H), 837.3 (M+Na). ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.96 (d, J = 9.0 Hz, 1H), 7.47 (s, 1H), 7.05 - 7.01 (m, 2H), 6.86 (s, 1H), 5.99 (s, 2H), 4.97 (dd, J = 12.4, 7.4 Hz, 2H), 3.93 (s, 3H), 3.08 (s, 3H), 3.00 - 2.99 (m, 1H), 2.30 (s, 3H), 2.13 (s, 3H), 2.03 (d, J = 18.1 Hz, 3H), 1.81 (s, 3H), 1.70 - 1.67 (m, 1H), 1.59 - 1.54 (m, 16H), 1.53 (s, 3H), 0.96 - 0.95 (m, 3H).

EXAMPLE 36B: Rifamycin 4-BnO-Phenol analog (R1b):

[0839] Analog **R1b** was prepared using intermediate **R2**, the synthesis of which is depicted in **Scheme R2**, below.

Scheme R2



R2

[0840] *Synthesis of compound **R2***. The mixture of 6-(benzyloxy)benzo[d]oxazol-2(3H)-one (500 mg, 2.07 mmol) and methanol (6 mL) was treated with a solution of 1.2 g of NaOH in 6 mL of water. The suspension was heated at 90 °C overnight. After cooling at room temperature, the mixture was treated with 6*N* HCl (5 mL) then filtered. The filtrate was adjusted to afford pH = 8-9 with sat. aq. $NaHCO_3$ and the precipitate was filtered, washed with water to give a dark solid, which was purified by **40 g HP silica gel Gold RediSep column (0 → 90% EA in hexanes) to afford 220 mg (49%) of** compound **R2**. **MS (ESI, pos.): calc'd for** $C_{13}H_{13}NO_2$, 215.09; found 216.1 (M+H). ^{1}H NMR (500

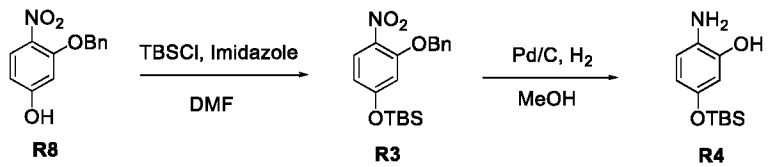
MHz; DMSO-d_6) δ 7.39 - 7.37 (m, 5H), 7.31 (d, J = 7.0 Hz, 1H), 6.49 (d, J = 8.4 Hz, 1H), 6.37 (d, J = 2.7 Hz, 1H), 6.25 (dd, J = 8.4, 2.7 Hz, 1H), 4.91 (s, 2H).

[0841] *Synthesis of analog **R1b**.* To a stirred solution of rifamycin S (20 mg, 0.0287 mmol) under argon in 1 mL of toluene at room temperature was treated with 2-amino-5-(benzyloxy)phenol **R2** (6.8 mg, 0.0316 mmol). The solution was stirred for 3 days at room temperature and additional 2-amino-5-(benzyloxy)phenol (6.8 mg) was added. The progress of reaction was monitored by LC/MS. After 5 days, the mixture was evaporated to dryness. The dark residue was dissolved in 3.5 mL of ethanol and 10 mg (0.11 mmol) of manganese oxide (MnO_2) was added in one portion to the ethanol solution. The sluggish mixture was stirred for 3h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 24 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: **5 → 98% EA in hexanes) and the pure fractions evaporated and dried** *in vacuo* giving the title compound **R1b as a dark reddish solid (8.5 mg, 33%). MS (ESI, pos.): calc'd for** $C_{50}H_{54}N_2O_{13}$, 890.36; found 891.3 (M+H). ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.97 (s, 1H), 7.49 (s, 1H), 7.44 - 7.41 (m, 5H), 7.14 - 7.11 (m, 2H), 7.07 (s, 1H), 6.94 (s, 1H), 5.32 (s, 1H), 5.23 (s, 1H), 5.18 (d, J = 11.9 Hz, 2H), 4.99 (s, 2H), 3.11 (s, 3H), 3.04 (dd, J = 2.0, 0.6 Hz, 1H), 2.32 (s, 3H), 2.07 (s, 6H), 1.83 (s, 3H), 1.71 - 1.69 (m, 1H), 1.61 (d, J = 0.4 Hz, 9H), 1.58—1.52 (m, 6H), 1.28 (s, 1H), 0.97 (td, J = 1.9, 1.2 Hz, 3H), 0.79 (t, J = 0.8 Hz, 1H).

EXAMPLE 36C: Rifamycin 4-OH-Phenol analog (36c):

[0842] Analog **R1c** was prepared using intermediate **R4**, the synthesis of which is depicted in **Scheme R3**, below.

Scheme R3



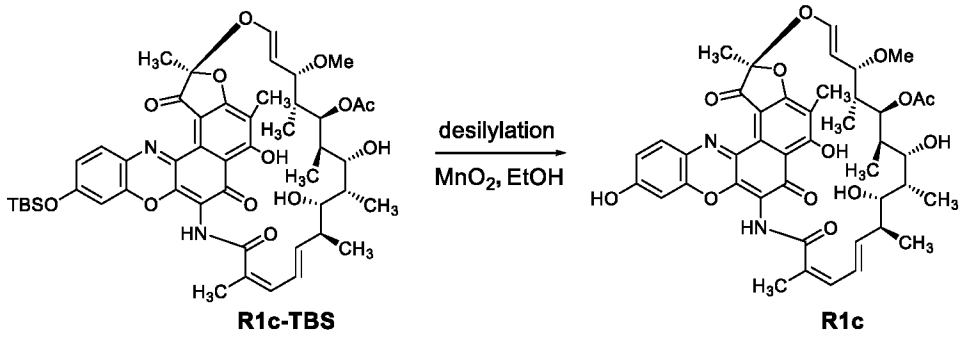
[0843] 2-amino-5-((tert-butyldimethylsilyl) oxy)phenol (**R4**):

[0844] *Synthesis of compound **R3**.* Compound **R8** was prepared from the product in Example 36B. To the solution of 3-benzyloxy-4-nitrophenol **R8** (400 mg, 1.63 mmol) under argon in DMF (2 mL) was added TBSCl (0.247 mL, 2.44 mmol), imidazole (222 mg, 3.26 mmol), and DMAP (0.5 mg). The mixture was stirred at room temperature overnight then diluted with ethyl acetate (25

mL), washed with water (2 x 10 mL), brine solution (10 mL), and dried over sodium sulfate. The crude was purified by 40g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 → 20 % EA in hexanes) and the pure fractions evaporated to afford the desired compound **R3** (540 mg, 92%). MS (ESI, pos.): calc'd for $C_{19}H_{25}N_2O_4Si$, 359.16; found 382.1 (M+Na).

[0845] *Synthesis of compound **R4.*** To the solution under argon of compound **R3** (120 mg, 0.33 mmol) in 3 mL of methanol (degassed with argon three times) was added 10% Pd/C (10 mg). The mixture was again degassed and bubbled with hydrogen from a balloon. A hydrogen balloon was inserted through the septa and the mixture was aged for overnight. The mixture was filtered through Celite and concentrated to give a dark greenish solid (71 mg, 90%). MS (ESI, pos.): calc'd for $C_{12}H_{21}NO_2Si$, 239.13; found 240.2 (M+H).

[0846] *Synthesis of analog **R1c.*** To a stirring solution under argon of rifamycin S (120 mg, 0.172 mmol) in 10 mL of toluene at room temperature was added compound **R4** (46 mg, 0.192 mmol). The mixture solution was stirred for 3 days at room temperature. The progress of the reaction was monitored by LC/MS, then the mixture was evaporated to dryness. The dark residue was dissolved in 10 mL of ethanol, and 50 mg (0.6 mmol) of manganese oxide (MnO_2) was added in one portion to the ethanol solution. The sluggish mixture was stirred for 12 h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 24 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 5 → 95% EA in hexanes). The pure fractions were evaporated and dried *in vacuo* giving the title compound **R1c as a dark reddish solid (48 mg, 35%). MS: calc'd for** $C_{43}H_{48}N_2O_{13}$, 800.32; found 801.3 (M+H), 799.2 (M-H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 11.43 (d, J = 1.7 Hz, 1H), 9.33 - 9.32 (m, 1H), 7.82 (dt, J = 2.0, 1.0 Hz, 1H), 7.02 - 7.01 (m, 1H), 6.89 (t, J = 1.3 Hz, 1H), 6.04 (dd, J = 2.5, 0.9 Hz, 1H), 5.83 (dt, J = 1.9, 1.0 Hz, 1H), 5.25 - 5.24 (m, 1H), 4.78 - 4.77 (m, 1H), 4.14 - 4.14 (m, 1H), 3.52 (d, J = 0.8 Hz, 1H), 3.07 (d, J = 0.7 Hz, 1H), 3.03 (t, J = 0.6 Hz, 3H), 2.89 (s, 1H), 2.78 (t, J = 2.7 Hz, 1H), 2.19 (d, J = 16.7 Hz, 3H), 1.99 (d, J = 12.2 Hz, 4H), 1.95 (t, J = 0.5 Hz, 4H), 1.67 (d, J = 1.9 Hz, 3H), 1.24 (s, 2H), 0.89 (dd, J = 2.5, 1.1 Hz, 2H), 0.85 (d, J = 6.5 Hz, 6H), 0.69 (d, J = 1.5 Hz, 3H).

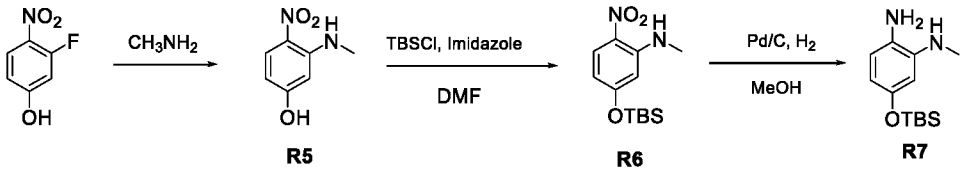


R1c-TBS R1c

EXAMPLE 36D: Rifamycin 4-OH-Phenol N-Methyl analogs (36d):

[0847] Analog **36d** was prepared using intermediate **R7**, the synthesis of which is depicted in **Scheme R4**, below.

Scheme R4



R5 R6 R7

[0848] 5-((tert-butyldimethylsilyl)oxy)-N1-methylbenzene-1,2-diamine (**R7**):

[0849] *Synthesis of compound **R5**.* The title compound was prepared using the method disclosed in PTC *Int. Appl.* 2008051805. In a sealed tube were placed a mixture of 3-fluoro-4-nitrophenyl (1 g, 6.36 mmol) and 2 mL of a 40% methylamine aqueous solution. The flask was sealed via septum, purged with argon, and heated at 80° C in an oil-bath for 18 h. The reaction was complete by LCMS analysis and cooled to room temperature. The solution was dissolved by the addition of water (15-20 mL) and extracted using ethyl acetate (3 x 30 mL). The combined organic layer was then washed with water, brine, dried (Na_2SO_4), and then concentrated to give a crude product, brown white solid (900 mg, 84%) of **R5**, which was used in the next step without further purification. **MS (ESI, pos.): calc'd for $C_7H_8N_2O_3$**, 168.05; found 169.1 (M+H).

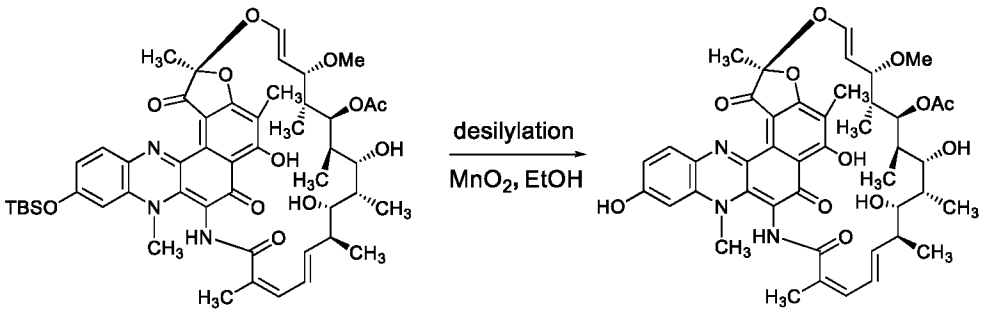
[0850] *Synthesis of compound **R6**.* Under argon 3-(methylamino)-4-nitrophenol **R5** (200 mg, 1.19 mmol) and imidazole (162 mg, 2.38 mmol) were dissolved in anhydrous DMF in the presence of catalytic DMAP (0.7 mg). The stirred yellow solution was cooled in an ice-bath and TBSCl (269 mg, 1.79 mmol) was added in one portion to the yellow solution. After 5 min the bath was removed and the solution was allowed to warm to room temperature overnight. The mixture was quenched

by saturated $NaHCO_3$ solution and extracted with ethyl acetate (2 x 25 mL). The combine organics were dried by addition of Na_2SO_4 and then concentrated to give a crude product. The residue was purified on a 24g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 → 90% EA in hexanes) and the pure fractions evaporated then dried *in vacuo* giving the title compound **R6** as a yellow solid (220 mg, 66%). MS: calc'd for $C_{13}H_{22}N_2O_3Si$, 282.14; found 283.1 (M+H).

[0851] *Synthesis of compound **R7***. Under argon 5-((tert-butyldimethylsilyl)oxy)-N1-methylbenzene-1,2-diamine **6** (50 mg, 0.177 mmol) was dissolved in 2 mL of methanol. The solution was degassed with argon three times followed by addition of Pd/C (5 mg). The mixture was further degassed with argon and connected to a hydrogen balloon via septum. After 2.5 h, the analysis by LC/MS from an in-process aliquot indicated the reaction was complete. The mixture was filtered through Celite and concentrated to afford 46 mg of compound **R7** quantitatively, which was used in the next step instantly without further purification. MS: calc'd for $C_{13}H_{24}N_2OSi$, 252.17; found 253.2 (M+H).

[0852] *Synthesis of analog **R1d.*** To a stirring solution under argon of rifamycin S (58 mg, 0.083 mmol) in 3 mL of toluene at room temperature was added compound **R7** (21 mg, 0.083 mmol). The solution was stirred for 2 days at room temperature. The progress of the reaction was monitored by LC/MS, then the solution was evaporated to dryness. The dark residue was dissolved in 5 mL of ethanol and 10 mg of manganese oxide (MnO_2) was added in one portion to the ethanol solution. The sluggish mixture was stirred for 12 h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 12 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 5 → 95% EA in hexanes) and the pure fractions evaporated then dried *in vacuo* giving the title compound **R1d** as a dark reddish solid (22.3 mg, 33%). This was found to be impure by LC/MS, so it was dissolved in MeCN/water and repurified on a 15.5 g C18 Aq Gold column (gradient elution: 10 – 95% MeCN in water, 0.05% acetic acid in both, over 20 min). The product fractions were combined, frozen on dry ice, and lyophilized giving the title compound **R1d** as a white solid (13.5 mg, 20%). MS: calc'd for $C_{44}H_{51}N_3O_{12}$, 813.35; found 814.3 (M+H), 812.3 (M-H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 11.31 (b, J = 0.8 Hz, 2H), 9.41 (s, 1H), 9.22 (s, 1H), 8.86 (s, 1H), 8.01 - 7.95 (m, 2H), 7.19 - 7.13 (m, 2H), 7.04 (s, 2H), 6.79 - 6.74 (m, 1H), 6.39 - 6.37 (m, 1H), 6.19 (t, J = 11.4 Hz, 2H), 6.08 (d, J = 12.4 Hz, 1H), 6.02 - 5.92 (m, 1H), 5.73 (d, J = 26.4 Hz, 1H), 5.49 (d, J = 11.2 Hz, 1H), 5.28 (d, J = 0.6 Hz, 1H), 5.09 - 5.02 (m, 2H), 4.82 (dd, J = 11.5, 10.2 Hz, 1H), 4.54 (d, J = 6.6 Hz, 1H), 4.36 (d, J = 2.6 Hz, 1H), 3.96 (d, J = 4.4 Hz, 1H), 3.88 (s, 1H), 3.83 (s, 1H), 3.79 (s, 1H), 3.70 (s, 1H), 3.09 (s,

1H), 2.91 (s, 3H), 2.21 (s, 3H), 2.15 (d, *J* = 5.9 Hz, 1H), 1.97 (s, 2H), 1.72 (s, 2H), 1.64 (s, 2H), 1.59 (s, 2H), 0.90 (d, *J* = 7.0 Hz, 1H), 0.70 (d, *J* = 6.6 Hz, 1H), 0.62 (d, *J* = 6.8 Hz, 1H), 0.20 - 0.18 (m, 1H), 0.07 (d, *J* = 0.7 Hz, 1H).

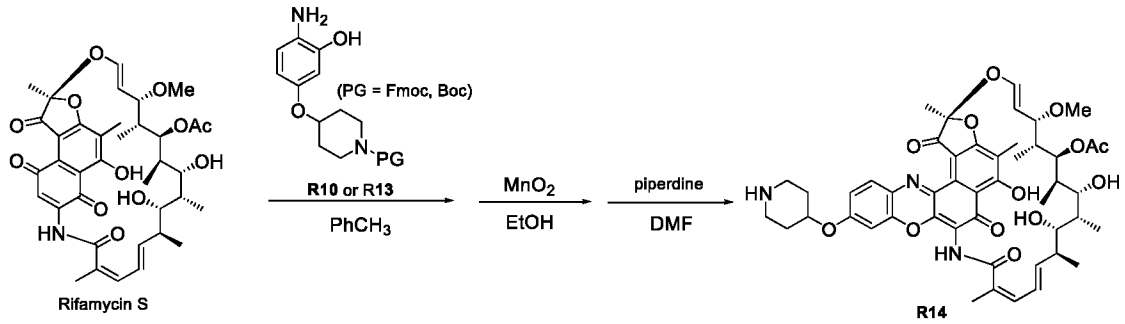


R1d-TBS **R1d**

EXAMPLE 37: Synthesis of analog R14

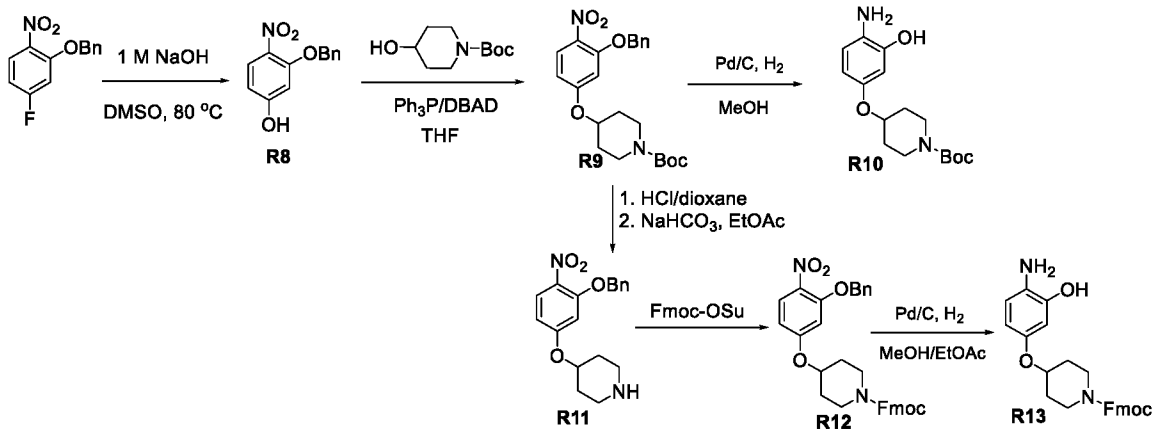
[0853] Rifamycin analog **R14** was synthesized from rifamycin S as shown in **Scheme R5**, below, and as described below.

Scheme R5



EXAMPLE 37A: Preparation of compounds (R10 and R13):

[0854] Intermediates **R10** and **R13** were prepared according to **Scheme R6**, shown below.



[0855] *Synthesis of compound **R8**.* The title compound was prepared using a slightly modified method reported by Otten *et. al.* (*Bioconjugate Chem.* **2001**, *12*, 76−83). To a solution of 3-fluoro-4-nitrophenyl (2.09 g, 8.45 mmol) in DMSO (10 mL) was added 1M NaOH (10 mL) and heated to 80 °C on a heating block for 18 h. The reaction was complete by LCMS and cooled to room temperature. The reaction was acidified with 1M HCl (15-20 mL) until the pH = 3-4 and the resultant solution was extracted using ethyl acetate (3 x 30 mL). The combined organic layers were washed with water, brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude oil was then purified on an 80 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 → 100% ethyl acetate in hexanes) and the pure fractions evaporated then dried *in vacuo* giving the title compound **R8** as a yellowish white solid (1.51 g, 73%). MS (ESI, pos.): calc'd for $C_{13}H_{11}NO_4$, 245.1; found 268.1 (M+Na), 244.1 (M-H).

[0856] *Synthesis of compound **R9**.* To a stirring solution under argon of compound **R8** (1.51 g, 6.157 mmol) in THF (16 mL) at room temperature were added the BOC-piperidin-4-ol (1.61 g, 8.005 mmol) and PPh_3 (2.91 g, 11.083 mmol). A solution of DBAD (2.55 g, 11.083 mmol) in THF (9 mL) was added to the reaction mixture dropwise. After stirring for 16 h, the mixture was evaporated to dryness and the residue was purified on a 40 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 → 100% ethyl acetate in hexanes) and the pure fractions evaporated then dried *in vacuo* giving the title compound **R9** as a yellowish white solid (2.41 g, 91%). MS: calc'd for $C_{23}H_{28}N_2O_6$, 428.2; found 451.1 (M+Na).

[0857] *Synthesis of compound **R10**.* To a degassed solution under argon of compound **R9** (100 mg, 0.233 mmol) in 3 mL of methanol was added 5 mg of 10% Pd/C. The mixture was further degassed and connected to a hydrogen balloon. After 2.5 h, the analysis by LC/MS from in-process

aliquot indicated the reaction was complete. The mixture was filtered through Celite and concentrated to afford 75 mg of compound **R10** quantitatively, which was used in the next step instantly without further purification. MS: calc'd for $C_{16}H_{24}N_2O_4$, 308.17; found 331.2 (M+Na), 307.1 (M-H).

[0858] *Synthesis of compound **R11**.* To a solution of compound **R9** (1100 mg, 2.561 mmol) in 1,4-dioxane (15 mL) was added 4 M HCl in 1,4-dioxane (5 mL). After stirring for 15 h an in-process aliquot indicated the reaction was complete. To the solution was added diethyl ether (50 mL), then the mixture was stirred vigorously for 1 h until a white precipitate formed. The solid was filtered and washed with ether to afford the HCl salt of **R11**. To the white solid was added EtOAc (10 mL) and sat. $NaHCO_3$ (15 mL) until pH = 8-9 and stirred for 15 min. The two layers were separated and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give compound **R11** (372 mg, 44%) which was used in the next step instantly without further purification. MS: calc'd for $C_{18}H_{21}N_2O_4$, 328.1; found 329.1 (M+H).

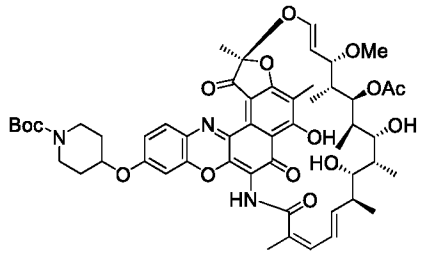
[0859] *Synthesis of compound **R12**.* To a solution under argon of compound **R11** (372 mg, 1.128 mmol) in 1,4-dioxane/water (v/v, 10:1, 11 mL) was added Fmoc-OSu (399 mg, 1.184 mmol). After stirring for 15 h an in-process LC/MS analysis indicated the reaction was complete. The reaction mixture was concentrated *in vacuo* to give compound **R12** which was used in the next step instantly without further purification. MS: calc'd for $C_{33}H_{30}N_2O_6$, 550.2; found 551.2 (M+H).

[0860] *Synthesis of compound **R13**.* To a solution under argon of compound **R12** (72 mg, 0.131 mmol) in 2 mL of methanol and degassed with argon was added 9 mg of 10% Pd/C. The mixture was further degassed with argon and connected to a hydrogen balloon. After 45 min, analysis by LC/MS from an in-process aliquot indicated the reaction was complete. The mixture was filtered through Celite and concentrated *in vacuo* to afford 55 mg of compound **R13** quantitatively, which was used in the next step instantly without further purification. MS: calc'd for $C_{26}H_{26}N_2O_4$, 430.1; found 431.2 (M+H).

EXAMPLE 37B: Preparation of analog R14 from intermediate R10

[0861] *Synthesis of compound **R14-Boc**:* To a stirring solution of rifamycin S (100 mg, 0.143 mmol) in 5 mL of toluene at room temperature was added compound **R10** (44 mg, 0.143 mmol). The mixture solution was stirred for 4 days at room temperature. The progress of the reaction was monitored by LC/MS until complete, then the mixture was evaporated to dryness. The dark residue was dissolved in 10 mL of ethanol and 62 mg (0.715 mmol) of manganese oxide (MnO_2)

was added at one portion to the ethanol solution. The sluggish mixture was stirred for 15 h at room temperature. After filtration of insoluble materials using Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 12g HP silica gel Gold RediSep column *via* **ISCO (gradient elution: 5% → 95% ethyl acetate in hexanes).** After concentrating under reduced pressure the crude product (*ca.* 85% pure) was repurified on a 50 g C18 Aq Gold column (gradient elution: 10 – 95% MeCN in water, 0.05% acetic acid in both). The pure fractions were combined, frozen on dry ice, and lyophilized to afford **R14-Boc** as a dark reddish solid (36 mg, 26%). **MS: calc'd for $C_{53}H_{65}N_3O_{15}$**, 983.44; found 984.4 (M+H).



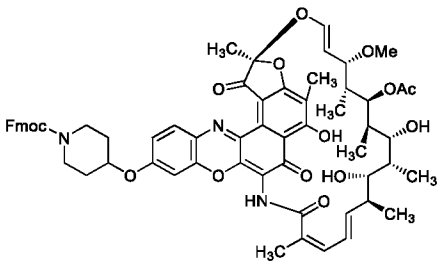
R14 -Boc

[0862] *Synthesis of compound **R14:*** **R14-Boc** (30 mg, 0.03 mmol) was treated with a mixture of TFA/acetonitrile/water (0.25 mL/5 mL/5 mL) at room temperature for 20 h to afford compound **R14**. The reaction mixture was purified on a 15.5g C18 Aq. Gold column via ISCO system (gradient elution: 10% – 100% MeCN in water, 0.05% acetic acid in both, over 30 min). The product-containing fractions were combined, frozen on dry ice, and lyophilized overnight giving the title compound **R14** (10 mg, 37%) as dark reddish solid. **MS: calc'd for $C_{48}H_{57}N_3O_{13}$**, 883.4; found 884.3 (M+H).

EXAMPLE 37C: Preparation of analog R14 from intermediate R13

[0863] *Synthesis of compound **R14**:* To a round-bottom flask with hydroxyaniline **R13** (55 mg, 0.1278 mmol) were added toluene (1.5 mL) and rifamycin S (67 mg, 0.0956 mmol). The reaction mixture was sonicated for 1 min to dissolve the reaction mixture, sealed via rubber septum, purged with argon, and the reaction stirred at ambient temperature. After 2 days another portion of hydroxyaniline (45 mg, 0.1045 mmol) was added and stirred for 1 d. The reaction was concentrated *in vacuo* to remove toluene, dissolved in EtOH (6 mL) and MnO_2 (20 mg) was added. After stirring for 3 d, the reaction was concentrated *in vacuo* and purified by chromatography on a 40 g HP silica gel Gold RediSep column *via* **ISCO (gradient elution: 0 → 100% ethyl acetate in hexanes). The** pure fractions were evaporated and dried *in vacuo* giving the title compound **R14-Fmoc** as a dark

reddish solid (35 mg, 33%). **MS (ESI, pos.): calc'd for $C_{63}H_{67}N_3O_{15}$**, 1105.4; found 1106.5 (M+H), 1128.5 (M+Na).

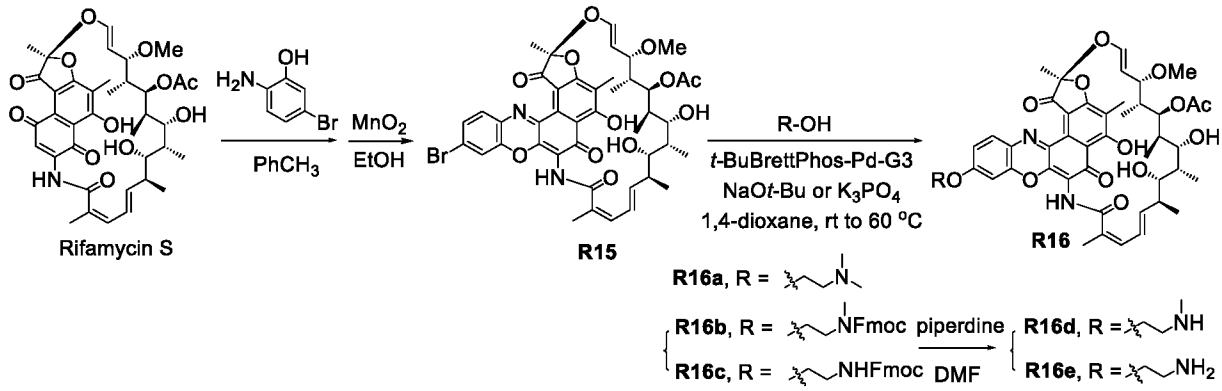


R14 -Fmoc

[0864] To a stirred solution under argon of Fmoc-rifamycin-piperidine-O-phenol **R14-Fmoc** of the preceding step (35 mg, 0.0361 mmol) in *N,N*-dimethylformamide (DMF, 1 mL), was treated with a solution of 2% piperidine (3.5 mg, 0.2 mL, 0.0411 mmol) in DMF and the reaction stirred at ambient temperature. After 2 h, the reaction was purified directly on a 50 g C18 RediSep Gold column via ISCO system (gradient elution: 0 – 100% MeCN in water, 0.05% acetic acid in both, over 30 min). The product-containing fractions were combined, frozen on dry ice, and lyophilized overnight giving the title compound **R14** as dark reddish solid (12 mg, 43%). **MS: calc'd for $C_{48}H_{57}N_3O_{13}$**, 883.4; found 884.3 (M+H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 9.40 (s, 1H), 7.87 (d, J = 8.9 Hz, 1H), 7.16 – 7.23 (m, 4H), 5.99 – 6.05 (m, 2H), 5.76 – 5.85 (m, 2H), 5.18 – 5.23 (m, 2H), 4.83 – 4.95 (m, 2H), 4.80 (br. s, 2H), 4.12 (br. S., 1H), 2.91 – 3.18 (m, 13H), 2.88 (s, 1H), 2.78 (t, J = 0.9 Hz, 2H), 2.67 (s, 2H), 2.22 (d, J = 3.7 Hz, 4H), 2.15 (s, 2H), 2.02 (s, 2H), 1.96 (d, J = 1.2 Hz, 2H), 1.90 (s, 1H), 1.68 (s, 2H), 0.85 – 0.92 (m, 12H), 0.69 (br. s, 9H).

EXAMPLE 38: Synthesis of analogs R16a-R16e

[0865] Rifamycin analogs **R16a-R16e** were synthesized from rifamycin S as shown in **Scheme R7**, below, and as described below.



EXAMPLE 38A: Pd-catalyzed O-alkylation (R16a-R16c):

[0866] *Synthesis of compound **R15**.* To a stirring solution under argon of rifamycin S (2.0 g, 2.87 mmol) in 80 mL of toluene at room temperature was added 2-amino-5-bromophenol (0.54 g, 2.87 mmol). The solution was stirred for 2 days at room temperature. The reaction mixture was then evaporated to dryness and the dark residue dissolved in 20 mL of ethanol and 300 mg of manganese oxide (MnO_2) was added in one portion to the ethanol solution. The sluggish mixture was stirred under argon for 15 h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated under reduced pressure. The dark residue was purified on a 120 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 5 → 95% EA in hexanes). The pure fractions were evaporated and dried *in vacuo* giving the title compound **R15** as a dark reddish solid (1.6 g, 65%). MS (ESI, pos.): calc'd for $C_{43}H_{47}BrN_2O_{13}$, 862.23; found 863.1 and 865.1 (M+H), 885.1 and 888.0 (M+Na). ^{1}H NMR (500 MHz; DMSO-d_6): δ 9.49 (d, J = 6.0 Hz, 1H), 7.92 (ddd, J = 3.6, 2.9, 1.8 Hz, 1H), 7.86 - 7.85 (m, 1H), 7.75 - 7.74 (m, 1H), 6.06 - 6.05 (m, 1H), 5.84 (dt, J = 2.6, 1.4 Hz, 2H), 5.25 - 5.23 (m, 2H), 4.80 (dt, J = 2.5, 1.0 Hz, 1H), 4.23 (td, J = 2.4, 1.0 Hz, 1H), 3.49 (d, J = 1.1 Hz, 1H), 3.10 - 3.09 (m, 2H), 3.03 (s, 3H), 2.79 (s, 1H), 2.19 (s, 3H), 2.01 (s, 4H), 1.96 (s, 4H), 1.81 (d, J = 2.2 Hz, 1H), 1.68 (s, 3H), 1.60 (dq, J = 2.8, 0.9 Hz, 1H), 1.48 (t, J = 1.4 Hz, 1H), 0.90 (dt, J = 2.1, 1.1 Hz, 2H), 0.84 (d, J = 7.1 Hz, 4H), 0.69 (dd, J = 2.2, 1.2 Hz, 5H).

[0867] *Synthesis of compound **R16a**.* Using a similar method reported by Buchwald S. L. *et al.* (*Org. Lett.* **2018**, *20*, 1580), a palladium-catalyzed C-O coupling of primary alcohols with compound **R15** was employed for title compounds **R16a**-**R16c**. To a 2 dram screw-top oven-dried test tube, equipped with a stir bar, and sealed with a screw cap was charged compound **R15** (40 mg, 0.0463 mmol, 1.00 eq.), 2-(dimethylamino)ethan-1-ol (42 mg, 0.462 mmol, 10 eq.), tBuBrettPhos Pd G3-palladacycle (11.8 mg, 30 mol%), and NaO*t*-Bu (5 mg, 0.051 mmol, 1.1 eq.). The reaction tube was

recapped with a septum and pierced with a needle attached to evacuate and backfilled with argon (this process was repeated twice) followed by addition of 1,4-dioxane (2.0 mL) via syringe. The reaction was heated at 55°C ± 5 in an oil bath under argon pressure for 15 h, the reaction was allowed to cool to room temperature before filtration through a pad of Celite® and rinsed with EtOAc. The crude material was concentrated in vacuo and purified on a 15.5 g C18 Aq Gold column (gradient elution: 10 – 95% MeCN in water, 0.05% acetic acid in both). The product fractions were combined, frozen on dry ice, and lyophilized giving the title compound **R16a** as a dark reddish solid (12.5 mg, 32%). **MS: calc'd for $C_{47}H_{57}N_3O_{13}$**, 871.39; found 872.3 (M+H), 870.2 (M-H). ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.40 (s, 1H), 7.87 (d, J = 8.9 Hz, 1H), 7.23—7.16 (m, 2H), 6.83 (dt, J = 2.3, 1.1 Hz, 1H), 6.23 (d, J = 4.6 Hz, 1H), 6.06 (dd, J = 5.9, 1.1 Hz, 1H), 5.82 (dd, J = 2.3, 1.5 Hz, 2H), 5.24 (dt, J = 1.4, 0.7 Hz, 1H), 4.83—4.75 (m, 1H), 4.24 (d, J = 29.9 Hz, 3H), 3.80 (d, J = 1.3 Hz, 1H), 3.03 (t, J = 0.5 Hz, 3H), 2.88 (s, 1H), 2.78 (t, J = 0.9 Hz, 2H), 2.67 (s, 2H), 2.22 (d, J = 3.7 Hz, 4H), 2.15 (s, 2H), 2.02 (s, 2H), 1.96 (d, J = 1.2 Hz, 2H), 1.90 (s, 1H), 1.68 (s, 2H), 0.85 (d, J = 6.7 Hz, 3H), 0.69 (t, J = 1.2 Hz, 3H).

[0868] *Synthesis of compound **R16b**.* To a 8 mL screw-top oven-dried vial, equipped with a stir bar, and sealed with a screw cap was charged compound **R15** (40 mg, 0.0463 mmol, 1.00 eq.), Fmoc-glycinol (131 mg, 0.463 mmol, 10 eq.), *t*-BuBrettPhos-Pd-G3-palladacycle (16 mg, 0.4 eq.), and K_3PO_4 (20 mg, 0.0942 mmol, 2.0 eq.). The reaction vial was capped with a rubber septum, pierced with a needle attached to evacuate and backfilled with argon (this process was repeated twice), followed by the addition of 1,4-dioxane (2.0 mL). The reaction was heated at 60 °C in a heating block under argon pressure for 15h, the reaction was allowed to cool to room temperature before filtration through a pad of Celite® and rinsed with MeOH. The crude material was concentrated *in vacuo* and purified on a 50 g C18 Aq Gold column (gradient elution: 5 – 100% MeCN in water, 0.05% acetic acid in both). The product fractions were combined, frozen on dry ice, and lyophilized giving the title compound **R16b** as a dark reddish solid (19 mg, 38%). MS (ESI, **pos.): calc'd for $C_{60}H_{63}N_3O_{15}$**, 1065.4; found 1066.4 (M+H).

[0869] *Synthesis of compound **R16d**.* Compound **R16b** of the preceding step (26 mg, 0.0244 mmol) was dissolved in DMF (1 mL), treated with a solution of 2% piperidine (3.1 mg, 0.2 mL, 0.0366 mmol) in DMF and the reaction stirred under argon at ambient temperature. After 2 h, the reaction was purified directly on a 50 g C18 Aq Gold column via ISCO system (gradient elution: 0 – 100% MeCN in water, 0.05% acetic acid in both, over 30 min). The product-containing fractions were combined, frozen on dry ice, and lyophilized overnight giving the title compound **R16d** as dark

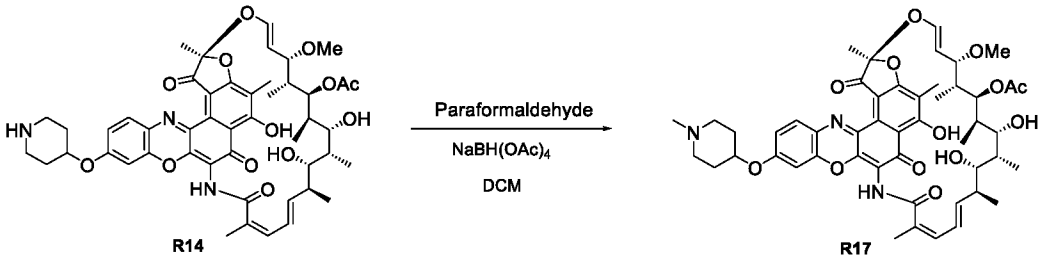
blue solid (9 mg, 44%). **MS: calc'd for** $C_{45}H_{53}N_3O_{13}$, 843.4; found 844.4 (M+H), 842.3 (M-H). ^{1}H NMR (500 MHz; CD_3OD): **δ 7.83 (d,** *J* = 8.8 Hz, 1H), 6.91 - 7.03 (m, 2H), 6.55 (s, 1H), 6.43 (d, *J* = 11.2 Hz, 1H), 6.21 - 6.30 (m, 2H), 4.98 - 5.08 (m, 2H), 3.76 (br. s, 3H), 3.43 - 3.47 (m, 1H), 3.41 (d, *J* = 5.37 Hz, 2H), 3.12 (br. s, 1H), 2.97 - 3.04 (m, 4H), 2.39 (br. s, 1H), 2.19 - 2.32 (m, 4H), 2.09 - 2.14 (m, 4H), 1.95 - 2.07 (m, 4H), 1.78 (s, 4H), 1.67 (d, *J* = 6.84 Hz, 1H), 1.31 (br. s., 2H), 0.97 (br. s, 8H), 0.66 - 0.85 (m, 4H), 0.08 (d, *J* = 5.5 Hz, 3H), -0.26 (d, *J* = 6.5 Hz, 3H).

[0870] *Synthesis of compound **R16c**.* To a 8 mL screw-top oven-dried vial, equipped with a stir bar and sealed with a screw cap was charged compound **R15** (80 mg, 0.0926 mmol, 1.00 eq.), Fmoc-sarcosinol (275 mg, 0.9262 mmol, 10 eq.), *t*-BuBrettPhos-Pd-G3-palladacycle (40 mg, 0.5 eq.), and K_3PO_4 (39 mg, 0.1852 mmol, 2 eq.). The reaction vial was capped with a rubber septum, pierced with a needle attached to evacuate and backfilled with argon (this process was repeated twice) followed by addition of 1,4-dioxane (3.0 mL) via syringe. The reaction was heated at 60 °C in a heating block under argon pressure for 15 h, the reaction was allowed to cool to room temperature before filtration through a pad of Celite® and rinsed with MeOH. The crude material was concentrated *in vacuo* and purified on a 50 g C18 Aq Gold column (gradient elution: 5 – 100% MeCN in water, 0.05% acetic acid in both). The product fractions were combined, frozen on dry ice, and lyophilized to give the title compound **R16c** as a dark reddish solid (49 mg, 49%). MS: **calc'd for** $C_{61}H_{65}N_3O_{15}$, 1079.4; found 1080.5 (M+H).

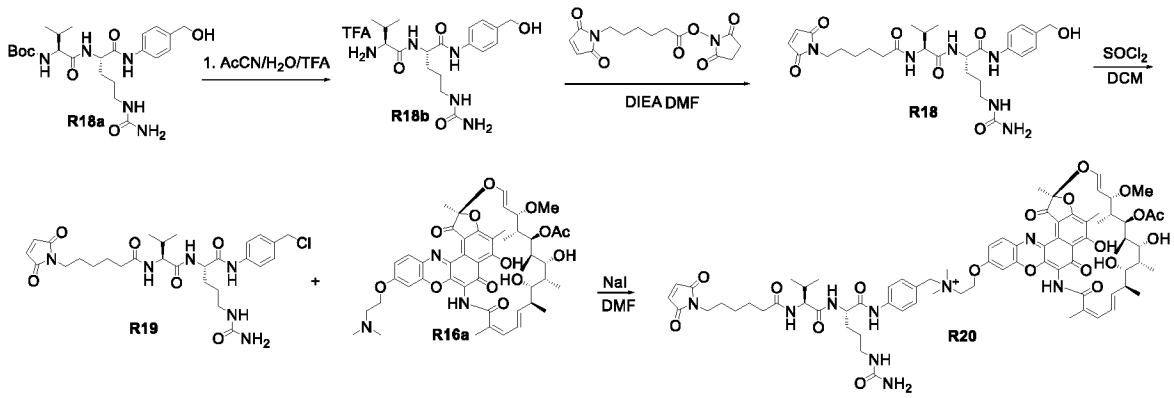
[0871] *Synthesis of compound **R16e**.* Compound **R16c** of the preceding step (49 mg, 0.045 mmol) was dissolved in DMF (1 mL), treated with a solution of 2% piperidine (7.7 mg, 0.45 mL, 0.091 mmol) in DMF and the reaction stirred under argon at ambient temperature. After 2 h, the reaction was purified directly on a 50 g C18 Aq Gold column *via* ISCO system (gradient elution: 0 – 100% MeCN in water, 0.05% acetic acid in both, over 30 min). The product-containing fractions were combined, frozen on dry ice, and lyophilized overnight giving the title compound **R16e** as a dark blue solid (18 mg, 46%). **MS: calc'd for** $C_{46}H_{55}N_3O_{13}$, 857.3; found 858.3 (M+H). ^{1}H NMR (500 MHz; CD_3OD): **δ 7.84 (d,** *J* = 8.79 Hz, 1H), 7.11 - 7.20 (m, 1H), 6.88 - 6.96 (m, 1H), 6.64 (s, 1H), 6.42 (d, *J* = 10.26 Hz, 1H), 6.17 - 6.28 (m, 2H), 4.93 - 5.06 (m, 2H), 3.86 (br. s, 1H), 3.66 - 3.84 (m, 8H), 3.18 - 3.31 (m, 7H), 3.10 (br. s, 2H), 2.94 - 3.05 (m, 6H), 2.37 (br. s, 1H), 2.25 (d, *J* = 4.88 Hz, 4H), 2.05 - 2.22 (m, 7H), 1.85 - 2.05 (m, 7H), 1.78 (s, 6H), 1.65 (br. s, 1H), 1.30 (br. s., 2H), 0.95 (br. s, 8H), 0.82 - 0.92 (m, 4H), 0.78 (br. s., 1H), 0.70 (br. s, 1H), 0.03 (d, *J* = 5.86 Hz, 3H), -0.28 (d, *J* = 5.86 Hz, 3H).

EXAMPLE 39: Synthesis of Rifamycin analogs R17

Rifamycin analogs (**R17**) were synthesized from compound **R14** by use of reductive amination. To a solution of compound **R14** (9 mg, 0.0102 mmol) and paraformaldehyde (1.52 mg, 0.051 mmol) in 1.0 mL of anhydrous DCM at room temperature was added $NaBH(OAc)_3$ (4.3 mmol, 0.0204 mmol). The mixture was stirred for 1 h. The reaction progress was monitored by LC/MS to afford the desired product. The crude reaction mixture was quenched by addition of 2-3 drops of water. All volatiles were removed under reduced pressure, then diluted with DMSO (0.5 mL). The crude mixture was purified by preparative HPLC (Gemini, 5µm, 150 mm x 30 mm, eluents: 10 - 95% MeCN in water, 0.05% AcOH) pure fractions combined and lyophilized to give 6 mg (66%) of **R17** as a reddish solid. **MS (ESI, pos.): calc'd for** $C_{49}H_{59}N_3O_{13}$, 897.40; found 898.4 (M+H). ^{1}H NMR (500 MHz; DMSO-d_6): δ 9.38 (br. s., 1H), 7.86 (br. s., 1H), 7.17 - 7.25 (m, 4H), 6.04 (d, J = 6.35 Hz, 1H), 5.81 (br. s., 2H), 4.79 (br. s., 2H), 4.70 (br. s., 2H), 4.15 (br. s., 1H), 3.53 (br. s., 1H), 3.30 (br. s., 5H), 3.09 (br. s., 3H), 3.03 (br. s., 4H), 2.87 (s, 1H), 2.78 (br. s., 2H), 2.58 - 2.66 (m, 6H), 2.54 (br. s., 8H), 2.37 (d, J = 1.47 Hz, 2H), 2.15 - 2.27 (m, 20H), 2.12 (br. s., 1H), 2.03 - 2.09 (m, 3H), 2.00 (s, 9H), 1.95 (br. s., 10H), 1.91 (s, 3H), 1.72 (br. s., 3H), 1.67 (br. s., 8H), 1.58 (s, 1H), 1.50 (br. s., 1H), 1.24 (br. s., 2H), 0.81 - 0.94 (m, 15H), 0.78 (d, J = 6.84 Hz, 3H), 0.69 (br. s., 9H).



EXAMPLE 40



[0872] This example describes linker payload chemistry which is used to prepare the title compound **R20**.

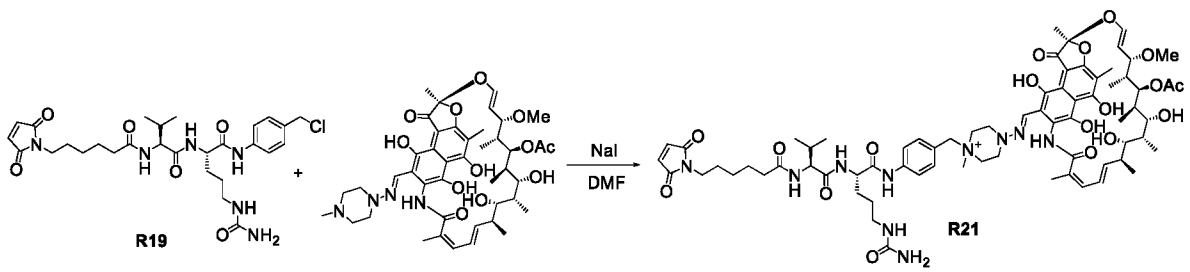
[0873] *Compound **R18**:* The title compound was prepared using a procedure in PCT Int. Appl., 2014145090. tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl) phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **R18a**, (500 mg, 1.04 mmol) was dissolved in a mixture of CH_3CN/H_2O/TFA (3:1:1 = v/v/v, 12 mL/4 mL/4 mL). The reaction mixture was stirred at room temperature for 48 h. The progress of the reaction was determined to be complete by LCMS. After concentrating *in vacuo*, the crude product **R18b** (0.9 g wet) was used directly for the next step without further purification. **MS (ESI, pos.): calc'd for $C_{18}H_{29}N_5O_4$**, 379.22; found 380.2 (M+H).

[0874] A solution of **R18b** (700 mg, 1.47 mmol, 1.0 eq) in water (8 mL) was diluted with 2 mL of aqueous $NaHCO_3$ solution at 4° C and the mixture (pH = 8.0) was treated with commercially available 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (408 mg, 0.9 eq) in 10 mL of acetonitrile. The suspension was stirred at room temperature for 16 h until the reaction was complete. The crude product was concentrated under reduced pressure and diluted with DMSO (5 mL). The crude product was purified by an ISCO 150g C18 column (eluents: 10 - 95% MeCN in water, 0.05 % in AcOH). Pure fractions were combined and lyophilized to afford 368 mg (44%) of compound **R18 as a white solid. MS (ESI, pos.): calc'd for $C_{28}H_{40}N_6O_7$**, 572.30; found 573.6 (M+H), (2M+H), 1145.9. ^{1}H NMR (500 MHz; DMSO-d_6): δ 9.89 (s, 1H), 8.05 (d, *J* = 7.33 Hz, 1H), 7.81 (d, *J* = 8.79 Hz, 1H), 7.52 - 7.57 (m, *J* = 8.79 Hz, 2H), 7.21 - 7.26 (m, *J* = 8.79 Hz, 2H), 6.99 - 7.02 (m, 2H), 5.98 (br. s., 1H), 5.40 (s, 2H), 5.10 (t, *J* = 5.62 Hz, 1H), 4.35 - 4.45 (m, 3H), 4.18 (dd, *J* = 6.84, 8.30 Hz, 1H), 3.26 - 3.33 (m, 2H), 2.91 - 3.06 (m, 2H), 2.08 - 2.22 (m, 2H), 1.93 - 2.01 (m, 1H), 1.66 - 1.74 (m, 1H), 1.59 (dd, *J* = 4.40, 9.28 Hz, 1H), 1.43 - 1.55 (m, 5H), 1.32 - 1.43 (m, 1H), 1.19 (quin, *J* = 7.57 Hz, 2H), 0.84 (d, *J* = 8.30 Hz, 3H), 0.80 - 0.89 (m, 3H).

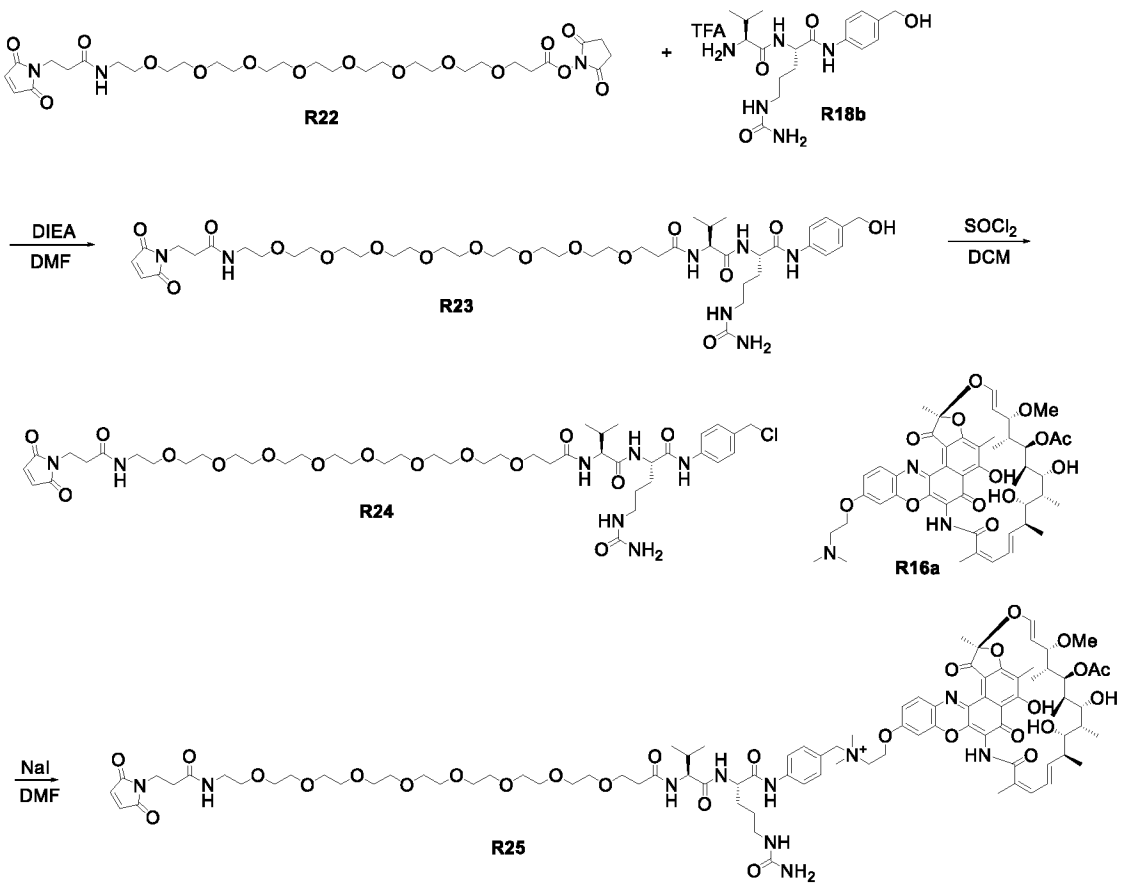
[0875] *Compound **R19**:* To a stirred suspension of **R18** (100 mg, 0.174 mmol, 1.0 eq) at room temperature was slowly added $SOCl_2$ (14 µL, 0.192 mmol, 1.1 eq) using a micro syringe. The slurry reaction mixture was stirred for 1.5 h and an aliquot analyzed by LC/MS indicated the formation of the desired. The crude mixture was concentrated to remove all volatiles under reduced pressure. The mixture was diluted with 2 mL of DMSO and loaded on to an ISCO C18 Aq 50g column for purification (10 - 95% MeCN in water, 0.05% AcOH). The pure fractions were combined and lyophilized to give 72 mg (71%) of **R19** as an off-white solid. **MS (ESI, pos.): calc'd**

for $C_{28}H_{39}ClN_6O_6$, 590.26; found 591.3 (M+H), 1181.5 (2M+H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.03 (s, 1H), 8.03 - 8.11 (m, 1H), 7.79 (d, *J* = 8.30 Hz, 1H), 7.57 - 7.63 (m, *J* = 8.79 Hz, 2H), 7.34 - 7.38 (m, *J* = 8.79 Hz, 2H), 6.99 - 7.02 (m, 2H), 5.97 (br. s., 1H), 5.40 (br. s., 2H), 4.71 (s, 2H), 4.34 - 4.43 (m, 2H), 4.16 - 4.21 (m, 1H), 3.36 - 3.42 (m, 3H), 2.90 - 3.06 (m, 3H), 2.07 - 2.22 (m, 3H), 1.91 - 2.00 (m, 1H), 1.66 - 1.73 (m, 1H), 1.31 - 1.41 (m, 1H), 1.18 (quin, *J* = 7.69 Hz, 3H), 0.79 - 0.89 (m, 7H).

[0876] *Compound **R20**:* The mixture of **R19** (13.5 mg, 0.0228 mmol, 1.2 eq), **R16a** (16.6 mg, 0.0190 mmol, 1.0 eq), and NaI (14.2 mg, 0.095 mmol) in a 2 dram vial was dissolved in 1 mL of anhydrous DMF. A catalytic amount (10 µL) of 0.5 M DIPEA solution in DMF was added by syringe. The mixture was heated at 55 °C in an oil bath overnight. The reaction was complete by LC/MS to afford the desired product. The mixture was cooled to 4° C and diluted with 1 mL of water. After filtration, the dark crude mixture was purified by an EZ preparative HPLC column (Gemini, 5µm, 150 mm x 30 mm, eluents: 10 - 95% MeCN in water, 0.05% AcOH). Pure fractions were combined and lyophilized to afford 14.6 mg (55%) of **R20** as a dark red solid. MS (ESI, pos.): **calc'd for** $C_{75}H_{96}N_9O_{19}^+$, 1426.68; found 1427.3 (M+1) and 1425.5 (M-1). ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.25 (s, 1H), 8.19 (d, *J* = 6.84 Hz, 1H), 7.82 (d, *J* = 8.30 Hz, 2H), 7.76 (d, *J* = 8.79 Hz, 3H), 7.50 (d, *J* = 8.30 Hz, 3H), 7.00 (s, 2H), 6.12 (d, *J* = 12.70 Hz, 1H), 6.03 (br. s., 1H), 5.43 (s, 2H), 4.70 - 4.80 (m, 2H), 4.58 (br. s., 3H), 4.36 - 4.41 (m, 1H), 4.18 (t, *J* = 7.82 Hz, 1H), 3.77 (br. s., 2H), 3.36 - 3.45 (m, 8H), 3.13 (d, *J* = 8.30 Hz, 1H), 2.89 - 3.06 (m, 12H), 2.78 (t, *J* = 9.04 Hz, 1H), 2.06 - 2.22 (m, 3H), 2.03 (br. s., 1H), 1.91 - 2.00 (m, 10H), 1.85 (s, 3H), 1.66 - 1.74 (m, 1H), 1.56 - 1.64 (m, 6H), 1.42 - 1.56 (m, 8H), 1.38 (d, *J* = 6.84 Hz, 2H), 1.15 - 1.25 (m, 3H), 0.75 - 0.88 (m, 14H), 0.07 (s, 1H).



[0877] *Compound **R21**:* A mixture of **R19** (20 mg, 0.0349 mmol, 1.0 eq), rifampicin (25.8 mg, 0.0314 mmol, 0.9 eq), and NaI (39 mg, 0.261 mmol) in 2 dram vial was dissolved in 1 mL of anhydrous DMF. The mixture was heated at 55 °C in an oil bath overnight. The reaction was determined to be complete by LC/MS. The mixture was cooled to 4° C and diluted with 1 mL of water. After filtration, the dark crude mixture was purified by an EZ preparative HPLC column (Gemini, 5µm, 150 mm x 30 mm, eluents: 10 - 95% MeCN in water, 0.05% AcOH). Pure fractions were combined and lyophilized for 30 h to afford 22 mg (51%) of **R21** as a reddish yellow solid. MS (ESI, pos.): calc'd for $C_{71}H_{97}N_{10}O_{18}^{+}$, 1377.70; found 1377.6 (M+H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 12.55 (s, 1H), 10.20 (s, 1H), 8.81 (s, 1H), 8.13 (s, 2H), 7.77 (dd, *J* = 23.3, 8.4 Hz, 4H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.13 - 7.07 (m, 1H), 7.00 (s, 2H), 6.25 - 6.20 (m, 2H), 5.98 (d, *J* = 0.4 Hz, 1H), 5.90 (dd, *J* = 15.9, 6.9 Hz, 1H), 5.42 (s, 2H), 5.08 - 5.06 (m, 1H), 5.02 - 5.02 (m, 1H), 4.95 - 4.91 (m, 1H), 4.58 (dd, *J* = 1.0, 0.4 Hz, 2H), 4.39 - 4.38 (m, 1H), 4.20 - 4.15 (m, 2H), 3.74 - 3.73 (m, 1H), 3.56 - 3.54 (m, 3H), 3.03 - 3.01 (m, 2H), 2.96 - 2.93 (m, 6H), 2.90 (s, 5H), 2.81 - 2.81 (m, 1H), 2.17 (dd, *J* = 9.5, 5.3 Hz, 3H), 1.97 (s, 5H), 1.91(d, *J* = 11.0 Hz, 8H), 1.63 (s, 6H), 1.58 (dd, *J* = 6.5, 0.8 Hz, 2H), 1.48 (t, *J* = 7.4 Hz, 8H), 1.37 - 1.32 (m, 2H), 1.20 - 1.17 (m, 2H), 1.04 (dd, *J* = 6.1, 0.6 Hz, 1H), 0.86 (td, *J* = 12.9, 6.9 Hz, 14H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.43 (d, *J* = 6.5 Hz, 3H).

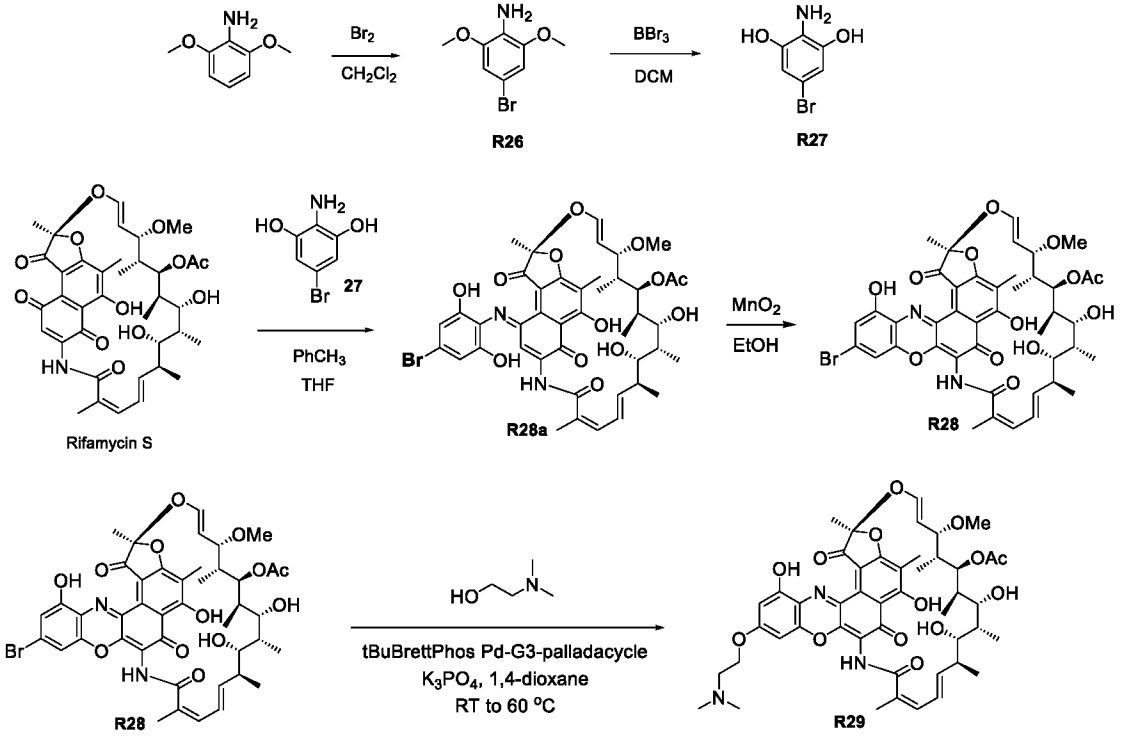


[0878] *Compound **R23**:* To a mixture of **R22** (100 mg, 0.144 mmol) and **R18b** (82 mg, 0.217 mmol) in anhydrous DMF (1.5 mL) was then treated with DIEA (50 µL, 0.288 mmol) *via* micro syringe. The reaction mixture was stirred for 2 h at room temperature and determined to afford **R23** by LC/MS. The crude mixture was purified by an ISCO C18 100g Aq column (eluents: 10 - 95% MeCN in water, 0.05 % in AcOH), pure factions combined and lyophilized to yield 84.4 mg (62%) of **23**. MS (ESI, pos.): calc'd for $C_{44}H_{71}N_7O_{16}$, 953.50; found 954.4 (M+H), 976.4 (M+Na), 952.4 (M-H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 9.88 (s, 1H), 8.08 (d, J = 7.2 Hz, 1H), 8.00 (s, 1H), 7.86 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.3 Hz, 2H), 7.00 (s, 2H), 5.98 (s, 1H), 5.40 (s, 1H), 4.43 (s, 2H), 4.39 (d, J = 5.4 Hz, 1H), 4.23 (dd, J = 8.3, 6.8 Hz, 2H), 3.60 (d, J = 6.9 Hz, 6H), 3.44 - 3.54 (m, 30H), 3.37 (t, J = 5.8 Hz, 3H), 3.15 (d, J = 5.7 Hz, 2H), 2.99 (d, J = 29.6 Hz, 2H), 2.33 (t, J = 7.3 Hz, 3H), 1.98 (d, J = 6.7 Hz, 1H), 1.71 - 1.70 (m, 1H), 1.61 - 1.58 (m, 1H), 1.44 - 1.36 (m, 2H), 0.85 (dd, J = 15.5, 6.7 Hz, 7H).

[0879] *Compound **R24**:* To a stirred suspension of **R23** (15 mg, 0.0157 mmol, 1.0 eq) in a vial at room temperature was slowly added $SOCl_2$ (1.3 µL, 0.0173 mmol, 1.1 eq) *via* a micro syringe. After 1 h, an aliquot analyzed by LC/MS indicated the formation of the desired product. The crude mixture was concentrated to remove all volatiles under reduced pressure. The crude mixture was diluted with 0.8 mL of MeCN and loaded onto an EZ preparative HPLC column and eluted (Gemini, 5µm, 150 mm x 30 mm, eluents: 10 - 95% MeCN in water, 0.05% AcOH). Pure fractions were combined and lyophilized to afford 9.5 mg (63%) of **R24** as an off-white solid. MS (ESI, pos.): **calc'd for** $C_{44}H_{70}ClN_7O_{15}$, 971.46; found 972.4 (M+H), 994.4 (M+Na), 970.3 (M-1).

[0880] *Compound **R25**:* To a mixture of **R24** (9.5 mg, 0.00976 mmol, 1.0 eq), **R16a** (8.51 mg, 0.00976 mmol, 1.0 eq), and NaI (7.3 mg, 0.0488 mmol) in a 1 dram vial was dissolved in 1 mL of anhydrous DMF. A catalytic amount (20 µL) of 0.5M DIEA solution in DMF was added by syringe. The mixture was heated at 55° C in an oil bath overnight. The reaction was complete by LC/MS to afford the desired product. The mixture was cooled in an ice-bath and diluted with 1 mL of water. After filtration, the dark crude mixture was purified by an EZ preparative HPLC column (Gemini, 5µm, 150 mm x 30 mm, eluents: 10 - 95% MeCN in water, 0.05% AcOH). Pure fractions were combined and lyophilized to afford 7.4 mg (42%) of **R25** as **a dark red solid. MS (ESI, pos.): calc'd** for $C_{91}H_{127}N_{10}O_{28}^+$, 1807.88; found 1808.8 (M+H) and 1806.5 (M-1). ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.23 (s, 1H), 8.22—8.17 (m, 1H), 8.03—7.98 (m, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 4H), 7.50 (d, *J* = 8.3 Hz, 3H), 7.00 (s, 2H), 6.01 (s, 1H), 5.76 (s, 1H), 5.43 (s, 1H), 4.80—4.80 (m, 1H), 4.58 (s, 1H), 4.43—4.41 (m, 1H), 4.27—4.23 (m, 1H), 3.76 (t, *J* = 0.6 Hz, 2H), 3.59 (t, *J* = 7.3 Hz, 5H), 3.49 (d, *J* = 2.9 Hz, 54H), 3.14 (d, *J* = 5.8 Hz, 4H), 3.03 (s, 8H), 2.90 (t, *J* = 0.7 Hz, 3H), 2.77 (d, *J* = 0.7 Hz, 1H), 2.33 (t, *J* = 7.3 Hz, 4H), 2.08 (d, *J* = 6.1 Hz, 4H), 1.94 (d, *J* = 18.9 Hz, 10H), 1.83 (s, 5H), 1.59 (s, 8H), 0.85 (dd, *J* = 16.1, 6.7 Hz, 8H).

EXAMPLE 41



[0881] *Compound **R26***: To a stirred solution of 2,6-dimethoxyaniline (9.0 g, 58.7 mmol, 1.0 eq) in 350 mL of anhydrous DCM was dropwise added over 30 min a Br_2 solution in 50 mL of anhydrous DCM at 4° C. An additional 200 mL was added to the slurry to achieve a semi-homogeneous solution. The reaction mixture was stirred overnight at room temperature. The dark brown mixture was cooled to 4° C and basified by addition of 1.0 M NaOH solution (*ca.* 100 mL) to pH = 10-11. The mixture was diluted with 200 mL of DCM and the layers are separated. The aqueous layer was extracted with DCM (200 mL total). The combined DCM layers were washed with water, brine, and dried over Na_2SO_4. After concentration *in vacuo*, the crude product was obtained as a slightly reddish solid. The residue was dissolved in DCM (8 mL) and loaded onto a 220 g HP silica gel Gold RediSep column and purified *via* ISCO (gradient elution: 5 - 95% EA in hexanes), pure fractions combined, and the solvent evaporated *in vacuo*. The solid was triturated with DCM and hexanes and filtered. The off-while solid was dried *in vacuo* giving the title compound **R26** as an off-white solid (9.4 g, 70%). MS (ESI, pos.): calc'd for $C_8H_{10}BrNO_2$, 230.99; found 231.9 and 234.0 (M+H). ^1H NMR (500 MHz; $CDCl_3$) δ 6.66 (s, 2H), 3.84 (s, 6H)

[0882] *Compound **R27***: Compound **R26** (2.2 g, 9.47 mmol, 1.0 eq) was dissolved in 10 mL of anhydrous DCM and a BBr_3 solution was added dropwise over 10 min (10 mL, 1.0 M solution in DCM) at 4° C. The reaction was exothermic and produced a precipitate. An additional amount of BBr_3 (9 mL, 94.7 mmol, 10 eq) was added and the reaction mixture was stirred at room temperature overnight. The reddish suspension was checked by LC/MS to confirm the desired product. The reaction mixture was transferred to a 250 mL flask and cooled to 4° C. The mixture was carefully quenched with water followed by treatment with aqueous saturated $NaHCO_3$ solution to give a pH = 7-8. The mixture was extracted with DCM and the aqueous layer cooled to 4° C to afford a dark brown precipitate. The mixture was filtered and the brown solid was dissolved in 10 mL of methanol and dried over Na_2SO_4 to provide the desired product **R27** (1.9 g, 100%). MS (ESI, pos.): calc'd for $C_6H_6BrNO_2$, 202.96; found 204.0 and 206.1 (M+H). ^1H NMR (500 MHz; CD_3OD) δ 6.45 (s, 2H), 4.87 (s, 2H).

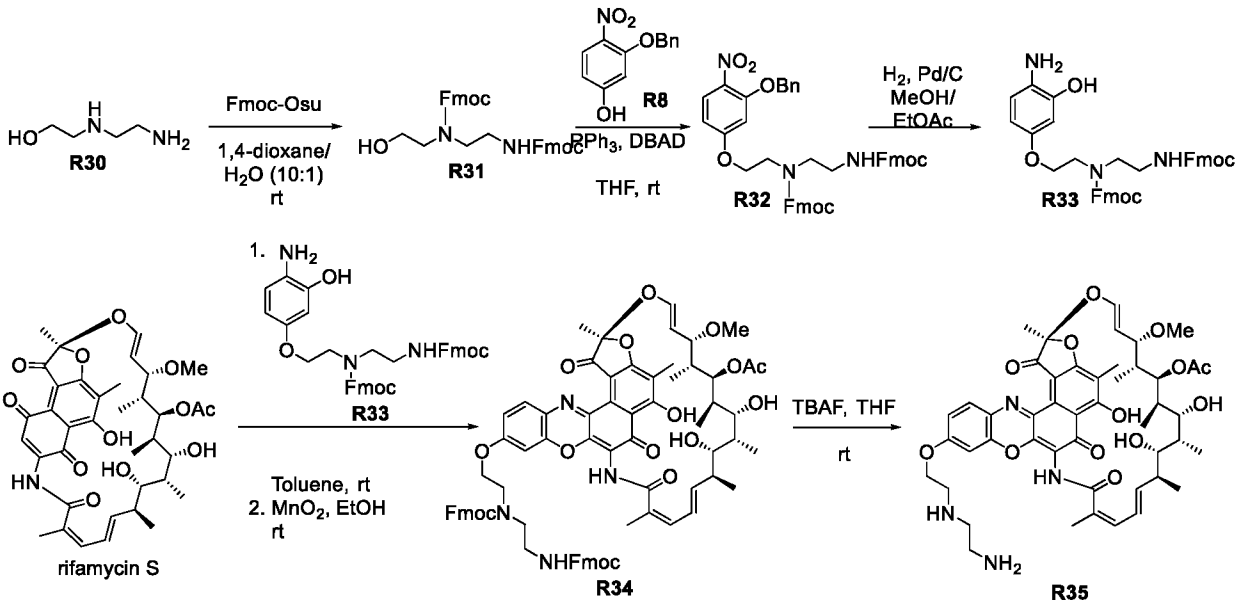
[0883] *Compound **R28**:* To a stirred solution of compound **R27** (0.146 g, 0.72 mmol) in a mixture of toluene (20 mL) and THF (20 mL) at room temperature was added rifamycin S (0.5 g, 0.72 mmol). The solution was stirred for 3 days at room temperature to afford the desired product. The solvents were removed *in vacuo*, the dark residue was dissolved in 10 mL of ethanol followed by 100 mg of manganese dioxide (MnO_2). The sluggish mixture was stirred for 5 h at room temperature. After filtration of insoluble materials using a Celite pad, the filtrate was evaporated *in vacuo*. The dark residue was purified on a 120 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 5 - 95% EA in hexanes). The pure fractions combined and evaporated *in vacuo* giving the title compound **R28 as a dark reddish solid (270 mg, 43%). MS (ESI, pos.): calc'd for** $C_{43}H_{47}BrN_2O_{13}$, 878.23; found 879.2 and 880.2 (M+H), 878.1 and 879.1 (M-1). ^1H NMR (500 MHz; DMSO-d_6) δ 10.22 (br. s., 1H), 9.52 (br. s., 1H), 7.43 (br. s., 1H), 7.35 (br. s., 1H), 6.04 (br. s., 1H), 5.83 (br. s., 2H), 5.21 (d, J = 6.35 Hz, 2H), 4.89 (t, J = 10.50 Hz, 1H), 4.16 (br. s., 1H), 3.51 (br. s., 1H), 3.15 (br. s., 2H), 3.02 (br. s., 4H), 2.80 (t, J = 8.55 Hz, 1H), 2.21 (br. s., 3H), 2.08 (br. s., 1H), 1.96 (s, 4H), 1.99 (s, 4H), 1.78 (br. s., 1H), 1.71 (br. s., 3H), 1.60 (br. s., 1H), 1.47 (br. s., 1H), 0.84 (d, J = 6.84 Hz, 6H), 0.69 (br. s., 6H).

[0884] *Compound **R29**:* To a 8 mL screw-top oven-dried vial, equipped with a stir bar was charged with compound **R15** (60 mg, 0.069 mmol, 1.00 eq), 2-(dimethylamino)ethan-1-ol (61 mg, 0.69 mmol, 10 eq), *t*-BuBrettPhos-Pd-G3-palladacycle (31 mg, 0.0345 mmol, 0.5 eq), and K_3PO_4 (30 mg, 0.141 mmol, 2.0 eq.). The reaction vial was capped with a rubber septum. The septum was pierced with a needle attached to evacuate and backfilled with argon (this process was repeated

twice) followed by the addition of 1,4-dioxane (1.5 mL). The reaction was heated at 60 °C under argon pressure for 15 h, the reaction was allowed to cool to room temperature, filtered through a pad of Celite®, and rinsed with MeOH. The crude material was concentrated *in vacuo* and purified on a 50 g C18 Aq column (gradient elution: 10 - 95% MeCN in water, 0.05% acetic acid in both). The product fractions were combined and lyophilized giving the title compound **R29** as a dark reddish solid (21 mg, 35%). **MS (ESI, pos.): calc'd for $C_{47}H_{57}N_3O_{14}$**, 887.38; found 888.3 (M+H). ^{1}H NMR (500 MHz; DMSO-d_6) δ 10.12 (br. s., 1H), 9.39 (br. s., 1H), 6.75 (br. s., 1H), 6.70 (br. s., 1H), 6.03 (br. s., 1H), 5.77 (d, J = 15.14 Hz, 1H), 5.21 (br. s., 1H), 4.83 - 4.90 (m, 1H), 4.15 - 4.30 (m, 2H), 4.08 (br. s., 1H), 3.53 (br. s., 1H), 3.29 (s, 1H), 3.16 (br. s., 1H), 3.03 (br. s., 3H), 2.87 (br. s., 1H), 2.79 (br. s., 1H), 2.62 - 2.71 (m, 2H), 2.36 (s, 1H), 2.23 (s, 6H), 2.19 (br. s., 3H), 1.93 - 2.11 (m, 7H), 1.91 (s, 1H), 1.76 (br. s., 1H), 1.69 (br. s., 3H), 1.53 - 1.65 (m, 1H), 1.50 (br. s., 1H), 1.32 - 1.45 (m, 1H), 0.76 - 0.94 (m, 6H), 0.68 (br. s., 5H).

EXAMPLE 42: Synthesis of rifamycin analog R35

[0885] Rifamycin analog **R35** was synthesized from rifamycin S as described below.



[0886] *Compound **R31**:* To a solution of compound **R30** (200 mg, 1.920 mmol) under argon in 1,4-dioxane/water (v/v, 10:1, 11 mL) was added Fmoc-OSu (1360 mg, 4.032 mmol). After stirring for 5 h an LC/MS analysis indicated the reaction was complete. The reaction mixture was treated with sat. $NaHCO_3$ (5 mL) and extracted with EtOAc (3 X 15 mL). The combined organic layer was then treated with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude compound

R31 as a white foam (800 mg, 76%), which was used in the next step instantly without further purification. MS: calc'd for $C_{34}H_{32}N_2O_5$, 548.2; found 549.2 (M+H).

[0887] *Compound **R32**:* To a stirring solution of compound **R8** (160 mg, 0.652 mmol) under argon in THF (2 mL) at room temperature were added the alcohol **R31** (432 mg, 0.788 mmol) and PPh_3 (308 mg, 1.174 mmol). Then a solution of DBAD (270 mg, 1.174 mmol) in THF (1 mL) was added to the reaction mixture dropwise. After stirring for 15 h, the mixture was evaporated to dryness and the residue was purified on a 40 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 - 100% ethyl acetate in hexanes), and the pure fractions evaporated and dried *in vacuo* giving the title compound **R32** as a yellowish white solid (286 mg, 56%). MS: calc'd for $C_{47}H_{41}N_3O_8$, 775.3; found 776.3 (M+H), 798.2 (M+Na).

[0888] *Compound **R33**:* To a solution under argon of compound **R32** (220 mg, 0.284 mmol) in 5 mL of methanol/EtOAc (2:3) and degassed with argon was added 31 mg of 10% Pd/C. The mixture was further degassed with argon and connected to a hydrogen balloon. After 2 h, analysis by LC/MS from an in-process aliquot indicated the reaction was complete. The mixture was filtered through Celite and concentrated to afford 150 mg of compound **R33** (85% pure by LC/MS) as yellowish oil, which was used in the next step instantly without further purification. MS: calc'd for $C_{40}H_{37}N_3O_6$, 655.3; found 656.3 (M+H).

[0889] *Compound **R34**:* To a round-bottom flask with hydroxyaniline **R33** (150 mg, 0.194 mmol, 85% pure), were added toluene (2 mL) and rifamycin S (129 mg, 0.185 mmol). The reaction mixture was sonicated for 1 min to dissolve the reaction mixture, sealed *via* rubber septum, purged with argon, and the reaction stirred at ambient temperature. After 1 day another portion of hydroxyaniline **R33** (45 mg, 0.059 mmol, 86% pure, synthesized using same procedure describe before) in toluene (2 mL) was added and stirred for 5 d. The reaction was concentrated in vacuo to remove toluene, dissolved in EtOH (4 mL) and MnO_2 (20 mg) was added. After stirring for 4 d, the reaction was concentrated *in vacuo* and purified by chromatography on a 40 g HP silica gel Gold RediSep column *via* ISCO (gradient elution: 0 - 100% ethyl acetate in hexanes). The pure fractions were evaporated and dried *in vacuo* giving the title compound **R34** as a dark reddish solid (65 mg, 26%). MS (ESI, pos.): calc'd for $C_{77}H_{78}N_4O_{17}$, 1330.5; found, 1353.5 (M+Na).

[0890] *Compound **R35**:* To a stirred solution of compound **R34** (28 mg, 0.021 mmol) under argon in THF (1 mL), was treated with a solution of TBAF (13 mg, 0.05 mL, 0.050 mmol, 1M in THF) and the reaction was stirred at ambient temperature. After 2 h, the reaction was purified directly on a 50

g C18 RediSep Gold column via ISCO system (gradient elution: 0 – 100% MeCN in water, 0.05% acetic acid in both, over 30 min). The product-containing fractions were combined, frozen on dry ice, and lyophilized overnight giving the title compound **35** as dark reddish solid (9 mg, 48%). MS: calc'd for $C_{47}H_{58}N_4O_{13}$, 886.4; found 887.3 (M+H). ^{1}H-NMR (500 MHz; CD_3OD): δ 7.86-7.74 (m, 1H), 7.18-7.08 (m, 1H), 6.98-6.84 (m, 1H), 6.78-6.68 (m, 1H), 6.53-6.40 (m, 1H), 6.23-6.15 (m, 1H), 6.23-6.15 (m, 1H), 6.00-5.79 (m, 1H), 6.00-5.79 (m, 1H), 5.30-4.95 (m, 2H), 3.81-3.65 (m, 6H), 3.35 (s, 3H), 3.09-2.93 (m, 7H), 2.25-2.20 (m, 2H), 2.17-2.03 (m, 4H), 2.00-1.87 (m, 5H), 1.76-1.68 (m, 4H), 1.03-0.85 (m, 7H), 0.78-0.65 (m, 2H), 0.14-0.03 (m, 4H), 0.13-0.00 (m, 3H), -0.30 (m, 2H).

EXAMPLE 43: Broth Minimum Inhibitory Concentration (MIC) Assay

[0891] To test the efficacy of rifamycin analogs of the disclosure *in vitro*, a broth growth inhibition assay was developed. For the assay, *S. aureus* NRS384 was grown in Tryptic Soy Broth (TSB) overnight, then sub-cultured 1:50 in fresh TSB and grown for an additional two hours. The culture was then pelleted via centrifugation and washed twice in PBS. The culture was then diluted to $1x10^4$ cfu/mL in TSB and 50 µL of the suspension was added per well to a 96 well microtiter dish in duplicate. A dilution series of the indicated antibiotic (an analog according to the disclosure or a previously known analog Rifampicin) was added 1:1 for a final starting concentration of $1x10^{-5}$ M with 1:3 dilutions. The plates were incubated at 37°C with shaking for 24h and then the OD600 nm was read on a Spectramax i3 Minimax 300.

[0892] The reagents used and lot numbers are shown in **Table R2**, below.

Table R2: Reagents and Lot Numbers for MIC Assay

Reagent	Vendor	Catalogue #	Lot
PBS	Gibco	20012-043	2003838
S. aureus NRS384	BEI resources	NR-46070	
Tryptic Soy Broth (TSB)	Teknova	T1525	T014420G1801
Dilution plates	Greiner Bio one	780261	B17073CP

[0893] The lowest concentrations that inhibited growth of *S. aureus* (minimum inhibitory concentration, MIC) are listed in **Table R3**. A plot of the *S. aureus* inhibition assay conducted with rifamycin analogs according to the disclosure is shown as **Figure 35**.

Table R3: Minimum inhibitory concentration (MIC) of antibiotics in a broth growth inhibition assay.

Rifamycin analog tested	Mol. Wt. (Da)	*S. aureus* Broth MIC (M)
Rifampicin	**823**	**4.6E-09**
R1a	815	1.4E-08
R1b	890	1.2E-07
R1d	813	3.7E-07
R14	883	4.1E-08
R16a	823	1.5E-09
R16d	843	4.1E-08
R16e	858	4.6E-09

[0894] As shown in **Table R3**, all rifamycin analogs according to the disclosure are effective at inhibiting growth of *S. aureus* at sub-micromolar to nanomolar concentrations. Analog **16a** inhibited growth of S. aureus more potently than rifampicin with an MIC of 1.5×10^{-9}M.

EXAMPLE 44: Intracellular Assay

[0895] The rifamycin analog compounds' activity against *S. aureus* was tested in an intracellular "killing" assay.

[0896] The reagents used and lot numbers are shown in **Table R4**, below.

Table R4: Reagents and Lot Numbers for Intracellular Assay

Reagent	Vendor	Catalogue #	Lot
TSB	Teknova	T1525	T152517E1701
PBS	Gibco	20012-043	1951145
Triton X-100	Sigma	TX1568-1	
RPMI	Gibco	11875-093	1989237
FBS	Gibco	172667	138252-100
PMA	Sigma	P8139	MkBV849TV
Costa 96 well plate	Corning	3904	16618025
TSA plates	Teknova	T0144	T014420G1801
Pen/Strep	Gibco	15140-122	1953095
Dilution plates	Greiner Bio one	780261	B17073CP
Gentamicin	Gibco	10131-035	1729122

[0897] THP-1 monocytic cell line was grown in media (RMPI + 10% FBS + 1% Penicillin/Streptomycin), then seeded at a density of 1e5 cells/well in a 96 well plate and differentiated into macrophages for three days prior to infection using 200 nM PMA. An overnight culture of *S. aureus* NRS384 was grown in RPMI, washed twice with PBS and resuspended at 1e7 cfu/mL in PBS. THP-1 were washed with warm media (RMPI without FBS) to remove the Penicillin/Streptomycin and then infected with the *S. aureus* suspension at a multiplicity of infection of 10:1 (*S. aureus*: macrophages). Plates were spun at 300 xg for 5 minutes to synchronize adhesion of the bacteria, then incubated at 37°C for 2 hours. Free-floating bacteria were removed by washing 2x with warm media and remaining extracellular *S. aureus* were killed by addition of media containing gentamicin (50 ug/mL). After 1h, media was aspirated and the indicated compound was added to infected macrophages in media containing 50 µg/mL gentamicin to prevent extracellular growth of *S. aureus*. Afer 2h, plates were washed 2x with warm RPMI without FBS, and 100 ul of THP-1 lysis buffer (0.1% Triton in PBS) was added to each well. *S. aureus* survival was enumerated by colony forming units through serial dilution and plating onto TSA.

[0898] The results of the intacellular killing assay are shown in **Table R5** and **Figures 36** and **37**. The minimum inhibitory concentration (MIC) corresponds to the lowest concentration of each compound that resulted in intracellular *S. aureus* eradication.

Table R5

Rifamycin Analog Tested	Intracellular killing MIC
Rifampicin	>1.0E-06
R1a	1.0E-06
R1b	>1.0E-06
R1d	>1.0E-06
R14	1.0E-06
R16a	4.0E-08
R16d	1.0E-06
R16e	1.0E-06

[0899] As **Table R5** and **Figures 36** and **37** demostrate, compounds **R1a, R14, R16a, R16d,** and **R16e** had increased intracellular *S. aureus* killing capacity compared to rifampicin, with compound **R16a** having the highest activity.

EXAMPLE 45: Conjugation method for maleimides

[0900] The antibody (1-10 mg/ml) in 50 mM HEPES, 150 mM NaCl, pH 7.5, was treated with 1 mM dithiothreitol at 37 °C for 30 min. After gel filtration (G-25, pH 4.5 sodium acetate), the maleimido linker payload derivative compound R25 (1.2 equivalents/SH group) in DMSO (10 mg/ml) was added to the reduced antibody and the mixture adjusted to pH 7.0 with 1 M HEPES (pH 7.4). After 1 h the reaction was quenched with excess N-ethyl maleimide. The conjugates were purified using PBS with 5% glycerol by size exclusion chromatography and sterile filtered. Protein concentrations and payload to antibody ratios were determined by UV spectral analysis. Size-exclusion HPLC established that all conjugates used were >90% monomeric, and RP-HPLC established that there was <1% unconjugated linker payload. All conjugated antibodies were analyzed by HIC for linker payload loading values.

[0901] Characterization of Conjugates by Hydrophobic Interaction Chromatography (HIC):

[0902] To determine the loading of the linker-payloads on the antibody, the conjugates were run on Agilent 1260 using a TSK-NPR Butyl HIC column using a linear gradient of 1M potassium phosphate pH 8.5 to water over 60 min. The payload loading was determined by integration of peak areas corresponding to the species of conjugated and unconjugated antibody. Payload to antibody ratios are reported in **Table R6**.

Table R6

Percent yield and payload to antibody ratios for each of the antibody drug conjugates

Antibody	Yield (%)	DAR (HIC)
H1H21234N-Compound **R25**	50	3
Isotype Control -Compound **R25**	50	2

EXAMPLE 46:

[0903] An MSR1-non-cytotoxic antibody drug (ncADC) conjugate was tested in anti-type II collagen antibody-induced arthritis (CAIA) *in vivo*; cytokine detection in inflamed joints using Proinflammatory Panel 1 (mouse) Multiplex Immunoassay kit (MSD)

[0904] **Lipopolysaccharide** (LPS; 0111:B4) (Chondrex, Cat #53100, Lot #180141). Stock concentration: 2.5mg/ml; working concentration: 0.5mg/ml.

[0905] To further examine the anti-inflammatory effect of the anti-MSR1 Ab-steroid ncADC, the ncADC was evaluated in a collagen-antibody induced arthritis (CAIA) model *in vivo*. For the model, mice homozygously expressing human MSR1 extracellular domain and transmembrane domain in place of mouse MSR1 extracellular and transmembrane domains (humanized MSR1) and with a homozygous deletion of the ApoE gene (ApoE-KO) were utilized (resulting mice referred to as hMSR1/ApoE-KO mice).

[0906] **ncADC testing in CAIA in vivo:**

[0907] To induce CAIA, hMSR1/ApoE-KO mice were intraperitoneally injected with an 8 mg/kg mixture of five anti-type II collagen mAbs (Chondrex, Cat #53100, Lot #180140) on day 0, followed by intraperitoneal administration with 50 μg lipopolysaccharide (LPS; 0111:B4; Chondrex, Cat #53100, Lot #180141) on day 3. Severity of the microscopic levels of arthritis was graded up to 13 days after mAb injection in each of the four limbs per mouse on a 1-4 scale. The criteria for the grading were as follows: 0, normal; 1, mild redness and swelling of ankle/wrist or individual digits; 2, moderate redness and swelling of ankle/wrist; 3, severe redness and swelling of the entire paw including the digits; 4, maximal swelling in limbs involving multiple joints. The maximum value of the sum of the scores obtained from the four limbs of each mouse was 16. Mice developed first signs of arthritis on day 5 and were intraperitoneally treated every other day with 20 μL/g of decreasing concentrations (21 mg/kg, 63 mg/kg, and 191 mg/kg) of anti-MSR1 Ab-steroid ncADC conjugates (H1H21234N-N297Q-**P4-3**) and isotype control steroid ncADC (Isotype Control-N297Q-**P4-3**) starting from day 6. PBS, unconjugated anti-MSR1 Ab (H1H21234N-N297Q) and free steroid payload (**P4-1**), were intraperitoneally injected every other day or daily (referred to as **P4-1**ED). Experimental dosing and treatment protocol for groups of mice are shown in **Table 32**. All mice were sacrificed on day 13 and inflamed patella were collected for subsequent homogenization and cytokine detection.

[0908] **Measurement of cytokines in joint homogenates:**

[0909] Inflamed knee patella were collected in 2 mL microcentrifuge tubes containing two tungsten carbide beads (Qiagen, Cat #69997) and 500 μL of T-per buffer (Thermo Scientific, Cat #78510, Lot #QF217759) containing protease inhibitor cocktail and 0.5M EDTA (Thermo Scientific, Cat #78444, Lot #RF231047). Tissues were disrupted at the frequency of $30s^{-1}$ for 10 minutes using Qiagen Tissue Lyser II, centrifuged at 14,000 rpm for 7 minutes, and then supernatants were collected without disturbing the pellet. Cytokine concentrations in the supernatants were measured using a Proinflammatory Panel 1 (mouse) multiplex immunoassay kit (MesoScale Discovery, Cat #K15048D) according to the manufacturer's instructions. In brief, 50 μL/well of calibrators and samples (diluted in Diluent 41) were added to the plates pre-coated with capture antibodies and incubated at room temperature while shaking at 700 rpm for 2 hours. The plates were then washed 3 times with 1xPBS containing 0.05% (w/v) Tween-20, followed by the addition of 25 μL of Detection Antibody Solution diluted in Diluent 45. After 2-hour incubation at room temperature while shaking, the plates were washed 3 times, and 150 μL of 2x Read Buffer was added to each well. Electrochemiluminescence was immediately read on MSD Spector® instrument. Cytokine concentrations were normalized per total protein content in tissue homogenates measured using Bradford protein assay (BioRad, Cat #5000006, Lot #64144266). Data analysis was performed using GraphPad Prism™ software. Statistical significance within the groups was determined by one-way Anova with Turkey's multiple comparison post-test (*p<0.01; **p<0.001; ***p<0.0001).

Table 32: Experiment design, dosing, and treatment protocol for CAIA hMSR1/ApoE-KO mice

Group	Mice (n)	Molecule Tested	Dose (mg/kg)	Frequency (d=day)
1	5	PBS	-	every 2 days: d6, d8, d10, d12
2	5	**P4-1**ED	2.95	every day: d6, d7, d8, d9, d10, d11, d12
3	5	**P4-1**	2.95	every 2 days: d6, d8, d10, d12
4	3	H1H21234N-N297Q	191	every 2 days: d6, d8, d10, d12
5	5	H1H21234N-N297Q-**P4-3**	191	every 2 days: d6, d8, d10, d12
6	5	H1H21234N-N297Q-**P4-3**	63	every 2 days: d6, d8, d10, d12
7	5	H1H21234N-N297Q-**P4-3**	21	every 2 days: d6, d8, d10, d12
8	5	Isotype Control-N297Q-**P4-3**	191	every 2 days: d6, d8, d10, d12
9	5	Isotype Control-N297Q-**P4-3**	63	every 2 days: d6, d8, d10, d12

10	5	Isotype Control-N297Q-**P4-3**	21	every 2 days: d6, d8, d10, d12

[0910] As shown in **Table 33**, a mixture of anti-type II collagen mAbs induced robust CAIA in hMSR1/ApoE-KO mice treated with PBS. Therapeutic administration of anti-MSR1 Ab-steroid ncADC (H1H21234N-N297Q-**P4-3**) *in vivo* was efficacious in inhibiting the progression and ameliorating the severity of CAIA in a dose-dependent manner with all doses demonstrating a significant effect. Comparable inhibition of CAIA was observed between the anti-MSR1 Ab-steroid ncADC (H1H21234N-N297Q-**P4-3**) dosed at 21 mg/kg and the free **P4-1** dosed every day (**P4-1**ED). The anti-MSR1 Ab-steroid ncADC (H1H21234N-N297Q-**P4-3**) dosed at 191 mg/kg and 63 mg/kg demonstrated greater efficacy than the payload dosed every day. Therapeutic administration of 63 mg/kg and 21mg/kg isotype control-ncADC (Isotype Control-**P4-3**), unconjugated anti-MSR1 Ab (H1H21234N) and free **P4-1** every other day did not attenuate CAIA in hMSR1/ApoE-KO mice. The highest dose of the isotype control ncADC demonstrated efficacy in ameliorating the severity of CAIA.

[0911] As shown in **Table 34**, in *vivo* therapeutic administration of anti-MSR1 Ab-steroid ncADC (H1H21234N-N297Q-**P4-3**) was also efficacious in inhibiting *in vivo* pro-inflammatory cytokine production in hMSR1/ApoE-KO mice in a dose-dependent manner with all doses tested demonstrating a significant effect on IL-6, IL-1beta and KC-GRO; however, only the highest dose had a significant effect on TNF-alpha. No effect on pro-inflammatory cytokine levels in inflamed joints was observed with unconjugated anti-MSR1 Ab (H1H21234N-N297Q) and free **P4-1** administered every other day. The isotype control ncADC demonstrated effects on proinflammatory cytokines at the higher doses tested.

Table33: Anti-MSR1 Ab-steroid ncADC ameliorates clinical scores of CAIA in hMSR1/ApoE-KO mice *in vivo* in a dose-dependent manner. Statistical significance to PBS- and Isotype Control-**P4-3**- treated groups was determined by one-**way Anova with Turkey's** multiple comparison post-test and standard error of mean (SEM±) calculated.

Table 33

Molecule Tested	**PBS**	**P4-1ED**	**P4-1**	**H1H21234N-N297Q**	**H1H21234N-N297Q-P4-3**			**Isotype Control-N297Q-P4-3**		
Dose (mg/kg)	N/A	*2.95*	*2.95*	*191*	*191*	*63*	*21*	*191*	*63*	*21*
Clinical Score	11.3±2.36	5±1.54	9.4±2.46	13.67±2.02	0.5±0.35**	2.3±0.84**	5.1±2.1*	3.2±0.84	7.5±0.94	9.6±3.96

*p<0.05, **p<0.005, ***p<0.0005, ****p<0.00001

Table 34: Therapeutic treatment with anti-MSR1 Ab-steroid ncADC inhibits CAIA-induced pro-inflammatory cytokine production in hMSR1/ApoE-KO mice. Statistical significance to PBS- and Isotype Control-N297Q-**P4-3**- treated groups was determined by one-way **Anova with Turkey's mul**tiple comparison post-test and standard error of mean (SEM±) calculated.

Table 34

		PBS	**P4-1ED**	**P4-1**	**H1H21234N**	**H1H21234N-N297Q-P4-3**			**Isotype Ctrl-N297Q-P4-3**		
	Dose (mg/kg)		*2.95*	*2.95*	*191*	*191*	*63*	*21*	*191*	*63*	*21*
Cytokines [pg per mg of total protein]	**IL-6**	155.4±41	22.8±5.2****	131.4±40.9	116.3±23.8	18.8±3.7****	23.5±4.2****	51.9±27.8****	40.3±15.5****	75.2±24.3****	99.1±25.5
	IL-1β	23.1±6.1	1.1±0.6****	20.6±4.3	23.7±7.9	0.5±0.2****	1.6±1.3****	12.8±2.2**	6.3±2.1****	13.7±7.3*	17.3±2.9
	KC-GRO	16.6±6.8	8±4.4****	8.9±9	7.8±16	5.9±1.5****	10.8±6.9****	9.9±13*	11.7±2.7****	18.2±8.6	15±10
	TNF-α	7.9±2.8	3.5±1.4	8.3±1.9	10.2±5	1.9±0.8*	3.8±1.9	7.9±3.5	4.3±2	9.3±4.3	9.5±1.6

*p<0.05, **p<0.005, ***p<0.0005, ****p<0.00001

EXAMPLE 47:

[0912] Intracellular *S. aureus* killing assay

[0913] The following anti-MSR1 ncADcs were tested.

H1H21234N-N297Q-**R25**
Isotype-control antibody-N297Q-**R25**

[0914] To test the efficacy of an anti-MSR1 Ab-antibiotic ncADC of the invention *in vitro*, an intracellular *S. aureus* killing assay was utilized. For the assay, a THP-1 monocytic cell line was grown in media comprised of RPMI containing 10% FBS and 1% Penicillin/Streptomycin, then was seeded at a density of $1x10^5$ cells/well in a 96 well plate and differentiated into macrophages for three days prior to infection using 200 nM Phorbol Myristate Acetate (PMA). An overnight culture of *S. aureus* MRSA strain NRS384 was grown in RPMI, washed twice with PBS and subsequently resuspended at $1x10^7$ cfu/mL in PBS. THP-1 cells were washed with warm media (RPMI without FBS) to remove the Penicillin/Streptomycin and then infected with the *S. aureus* suspension at a multiplicity of infection of 10:1 (*S. aureus*: macrophages). Plates were spun at 300 xg for 5 minutes to synchronize adhesion of the bacteria, then incubated at 37°C for 2 hours. Free-floating bacteria were removed by washing twice with warm media and remaining extracellular *S. aureus* were killed by addition of media containing 100 ug/mL of gentamicin. After 1 hour, media was aspirated and the anti-MSR1 Ab-antibiotic ncADC (H1H21234N-N297Q-**R25**) at different doses (10ug/mL, 3.3ug/mL, 1.1ug/mL, 0.4ug/mL, 0.1ug/mL) and the isotype control- antibiotic ncADC (Isotype control-N297Q-**R25**) at 10ug/mL were added to infected macrophages in media containing 50 ug/mL gentamicin to prevent extracellular growth of *S. aureus*. A sample without any ncADC was also included for reference. After 24 hours, plates were washed twice with warm RPMI without FBS and then 100 uL of 0.1% Triton X-100 in PBS was added and incubated for 10 minutes to lyse the THP-1. *S. aureus* survival was enumerated by colony forming units through serial dilution in PBS and plating onto trypticase soy agar plates.

[0915] As shown in **Table R7**, the anti-MSR1 Ab-antibiotic ncADC (H1H21234N-N297Q-**R25**) at concentrations of 10, 3.3 and 1.1 ug/mL demonstrated the ability to eradicate intracellular *S. aureus* from infected macrophages *in vitro*. The lower doses of the anti-MSR1 Ab-antibiotic ncADC of 0.4ug/mL and 0.1ug/mL demonstrated the ability to reduce intracellular *S. aureus* from infected macrophages *in vitro*, but not to the limit of detection as the higher doses did. Macrophages treated with the isotype control- antibiotic ncADC (Isotype control-N297Q-**R25**) at

10 ug/mL harbored intracellular *S. aureus* at a similar level to the untreated control. These data demonstrate that an anti-MSR1 Ab-antibiotic ncADC can be used to effectively kill pathogens residing within a macrophage reservoir.

Table R7: Average colony forming units of anti-MSR1 Ab-Antibiotic

	ncADC dose (ug/mL)	Average cfu/mL	Standard Deviation
S. aureus control	none	275,000	0
Isotype Control-N297Q-**R25**	10	200,000	35,355
MSR1 ncADC H1H21234N-N297Q-**R25**	10	50 (limit of detection)	0
	3.3	50 (limit of detection)	0
	1.1	50 (limit of detection)	0
	0.4	663	124
	0.1	33,750	1,768

EXAMPLE 48:

[0916] *S. aureus* IV disseminated infection mouse model

[0917] The following ADCs were used in this experiment:

Antibody
MSR1 ncADC H1H21234N-N297Q-**R25**
Isotype Control-N297Q-**R25**
MSR1 ncADC H1H21234N 3-N297Q
Isotype control-N297Q

[0918] To test the efficacy of an anti-MSR1 Ab-antibiotic ncADC of the invention *in vivo*, an intravenous disseminated infection model was utilized. *S. aureus* MSRA strain NRS384 was grown overnight in trypic soy broth (TSB) and sub-cultured to mid-logarithmic phase. Bacteria were then washed twice with PBS and resuspended in PBS at a concentration of 1.2x10^8 cfu/mL. Mice homozygously expressing human MSR1 extracellular domain and transmembrane domain in place of mouse MSR1 extracellular and transmembrane domains (humanized MSR, MAID 7343-MSR1 HumIn delHyg) were then infected intravenously through the tail vein with 100 uL of the bacterial suspension, for a final infectious dose of 1.2x10^7 cfu/mouse. From one to three days post infection, mice were treated with 110 mg/kg vancomycin subcutaneously twice daily where indicated. Either the anti-MSR1 monoclonal antibody (H1H21234N-N297Q),

anti-MSR1 Ab-antibiotic ncADC (H1H21234N-N297Q-**R25**), the Isotype control Ab or the ncADC Isotype control (Isotype control-**R25**) was administered subcutaneously at the indicated dose, as described in **Table R8**, one day after infection. Mice were monitored for weight loss and body conditioning score throughout the infection. At four days post infection, mice were euthanized and the *S. aureus* kidney burden was quantified by tissue homogenization followed by enumeration of colony forming units through serial dilution in PBS and plating onto trypicase soy agar plates. The data from this experiment is shown in **Table R8**.

Table R8

Average *S. aureus* kidney burden in mice treated with anti-MSR1 Ab-Antibiotic ncADC in combination with vancomycin

Treatment	Vancomycin treatment	mAb or conjugate dose (mg/kg)	Average cfu/kidney pair	# of mice with *S. aureus* below the limit of detection	Standard Deviation
Infected Control	-	(-)	4.33E+08	0/5	2.32E+08
Vancomycin	+	(-)	2.07E+06	0/6	2.76E+06
Isotype Control 1mg/kg	+	1	2.44E+06	0/4	1.51E+06
ncADC Isotype control (Isotype control-N297Q-**R25**)	+	1	4.01E+05	1/5	6.49E+05
anti-MSR1 Ab (H1H21234N-N297Q)	+	1	1.55E+05	4/8	2.72E+05
anti-MSR1 Ab (H1H21234N-N297Q)	+	0.1	2.43E+05	0/6	2.64E+05
anti-MSR1 Ab-antibiotic ncADC (H1H21234N-N297Q-**R25**)	+	1	6.17E+03	7/9	1.69E+04
anti-MSR1 Ab-antibiotic ncADC (H1H21234N-N297Q-**R25**)	+	0.1	1.16E+03	6/8	1.78E+03

Limit of detection = 250 colony forming units (cfu)

[0919] As shown in **Table R8**, intravenous infection with *S. aureus* MRSA strain NRS384 results in high bacterial burden in the kidneys and treatment of mice with the standard of care antibiotic vancomycin reduces the *S. aureus* kidney burden by approximately 2 logs, but none of the mice had levels of *S. aureus* reduced below the limit of detection (LOD). The anti-MSR1 Ab-

antibiotic ncADC (H1H21234N-N297Q-**R25**) at doses of 1 and 0.1 mg/kg in combination with vancomycin resulted in 77% and 75% of mice with *S. aureus* levels below the LOD, demonstrating improvement in *S. aureus* clearance in mice treated with the Ab-antibiotic ncADC combination therapy. *S. aureus* clearance was less pronounced in mice treated with the isotype control ncADC conjugate (Isotype Control-N297Q-**R25**) plus vancomycin, demonstrating the benefit of payload targeting to macrophages.

[0920] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:

    (i) binds human MSR1 at 37ºC with a K_D of less than about 1.4 nM as measured by surface plasmon resonance;

    (ii) binds human MSR1 at 37ºC with a t½ of greater than about 4 minutes as measured by surface plasmon resonance;

    (iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC_{50} of less than about 10 nM as measured by ligand uptake assay;

    (iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay; and

    (v) exhibits binding of human MSR1 and internalization of the bound complex of more than about 10% as assayed by binding in THP-1 cells.

2. An isolated antibody or antigen-binding fragment thereof that binds to cell surface-expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC_{50} of less than 3.5 nM.

3. The isolated antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment thereof blocks blocks inhibition of modified LDL uptake by MSR1 by less than 60% with an IC_{50} of less than 1.5 nM.

4. An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 4; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in Table 4.

5. The isolated antibody or antigen-binding fragment of claim 4, wherein the antibody or antigen-binding fragment comprises an HCVR having an amino acid sequence as set forth in Table 4 and a LCVR having an amino acid sequence as set forth in Table 4.

6. The isolated antibody or antigen-binding fragment of claim 4, wherein the antibody or antigen-binding fragment comprises:

(i) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 36, 52, 92, and 284;

(ii) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 38, 54, 94, and 286;

(iii) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 40, 56, 96, and 288;

(iv) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 44, 60, 100, and 292;

(v) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 46, 62, 102, and 294; and

(vi) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 48, 64, 104, and 296.

7. The isolated antibody or antigen-binding fragment of claim 4, wherein the antibody or antigen-binding fragment comprises:

(i) a heavy chain complementarity determining region (HCDR)-1 comprising SEQ ID NO: 52; an HCDR2 comprising SEQ ID NO: 54; an HCDR3 comprising SEQ ID NO: 56; a light chain complementarity determining region (LCDR)-1 comprising SEQ ID NO: 60; an LCDR2 comprising SEQ ID NO: 62; and an LCDR3 comprising SEQ ID NO: 64;

(ii) an HCDR1 comprising SEQ ID NO: 4; an HCDR2 comprising SEQ ID NO: 6; an HCDR3 comprising SEQ ID NO: 8; an LCDR1 comprising SEQ ID NO: 12; an LCDR2 comprising SEQ ID NO: 14; and an LCDR3 comprising SEQ ID NO: 16;

(iii) an HCDR1 comprising SEQ ID NO: 36; an HCDR2 comprising SEQ ID NO: 38; an HCDR3 comprising SEQ ID NO: 40; an LCDR1 comprising SEQ ID NO: 44; an LCDR2 comprising SEQ ID NO: 46; and an LCDR3 comprising SEQ ID NO: 48;

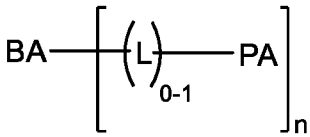
(iv) an HCDR1 comprising SEQ ID NO: 92; an HCDR2 comprising SEQ ID NO: 94; an HCDR3 comprising SEQ ID NO: 96; an LCDR1 comprising SEQ ID NO: 100; an LCDR2 comprising SEQ ID NO: 102; and an LCDR3 comprising SEQ ID NO: 104;

(v) an HCDR1 comprising SEQ ID NO: 284; an HCDR2 comprising SEQ ID NO: 286; an HCDR3 comprising SEQ ID NO: 288; an LCDR1 comprising SEQ ID NO: 292; an LCDR2 comprising SEQ ID NO: 294; and an LCDR3 comprising SEQ ID NO: 296;.

8. An isolated antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof competes for binding to human MSR1 with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOS: 2/10, 23/42, 50/58; 90/98, and 282/290.

9. An antibody-drug conjugate comprising the antibody, or antigen-binding fragment thereof, of any one of claims 1 to 8 conjugated to a payload residue optionally through a linker or through a linker-spacer.

10. The antibody-drug conjugate of claim 9, having the structure of Formula (**I**):



Formula (**I**)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form thereof, a regioisomer thereof, or mixtures of regioisomers thereof;

wherein:

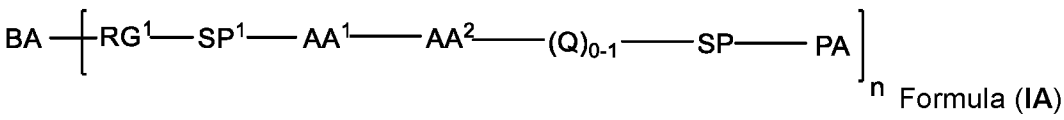
BA is a binding agent;

L is a linker;

PA is a payload moiety selected from the group consisting of a steroid residue, a LXR modulator residue, or a rifamycin analog residue; and

subscript **n** is an integer from 1 to 30.

11. The antibody-drug conjugate of claim 9 or claim 10, having the structure of Formula (**IA**):



Formula (**IA**)

wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

AA^1 is absent, or a divalent or trivalent linker comprising an amino acid residue which is optionally bonded directly or indirectly to a group **HG**;

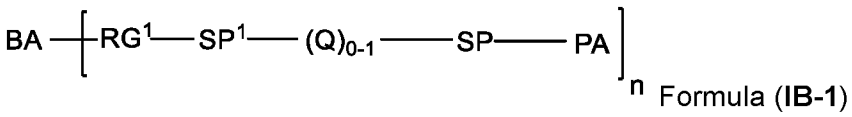
AA^2 is absent, or a dipeptide, tripeptide, or tetrapeptide residue;

Q, when present, is a connector group residue;

SP is absent, or a spacer; and

HG, when present, is a hydrophilic group.

12. The antibody-drug conjugate of any one of claims 9-11, having the structure of Formula (**IB-1**):



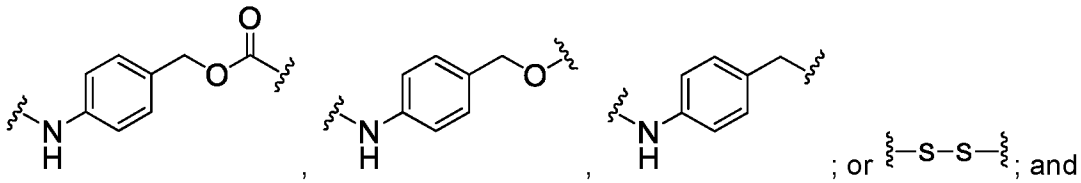
Formula (**IB-1**)

wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

Q, when present, is

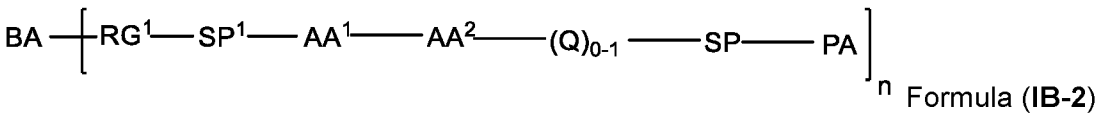


; and

SP is absent, or a spacer;

wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula.

13. The antibody-drug conjugate of any one of claims 9-11, having the structure of Formula (**IB-2**):



Formula (**IB-2**)

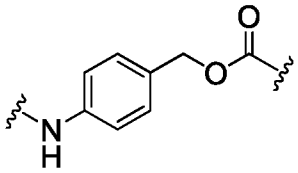
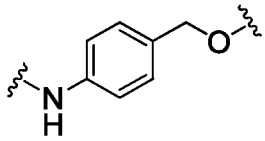
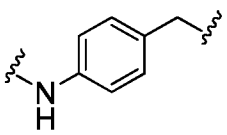
wherein

SP^1 is absent, or a spacer;

RG^1 is a reactive group residue;

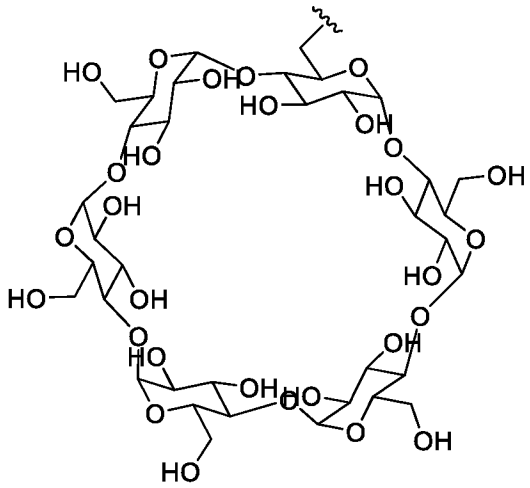
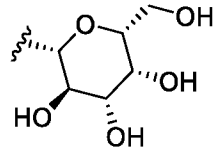
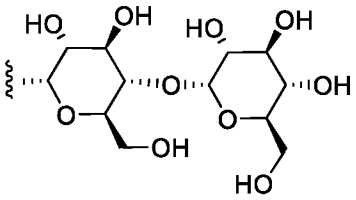
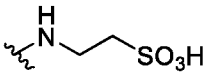
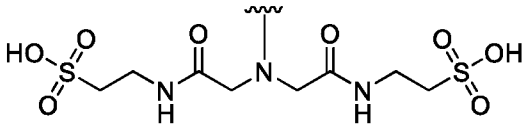
AA^1 is absent, or a divalent or trivalent linker comprising an amino acid residue which is optionally bonded directly or indirectly to a group **HG**;

AA^2 is absent, or a dipeptide, tripeptide, or tetrapeptide residue;

Q, when present, is  ,  , or  ;

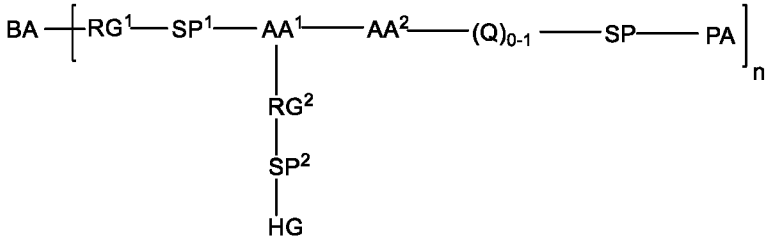
SP is absent, or a spacer; and

HG, when present, is

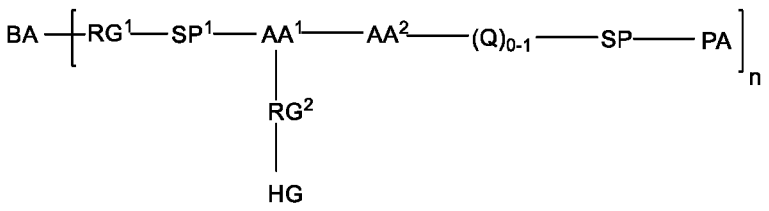
 ,  ,  ,  , or  ,

wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula.

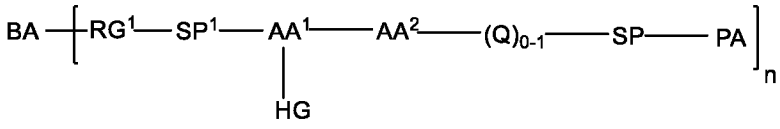
14. The antibody-drug conjugate of any one of claims 9-11 and 13, having the structure of Formula (**IC**), Formula (**ID**), or Formula (**IE**):



Formula (**IC**)



Formula (**ID**)



Formula (**IE**)

wherein

SP^1 is absent, or a spacer;

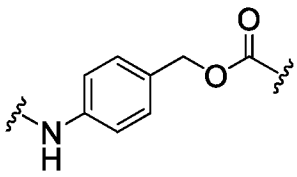
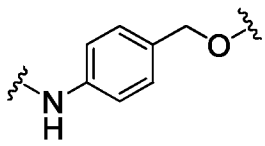
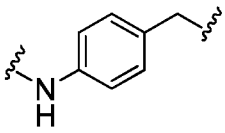
RG^1 is a reactive group residue;

SP^2 is absent, or a spacer;

RG^2 is a reactive group residue;

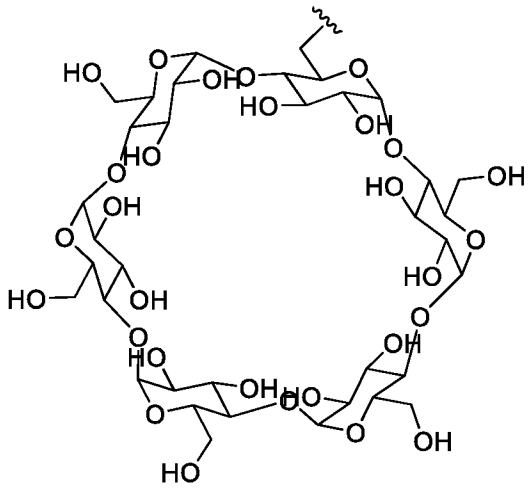
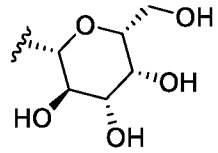
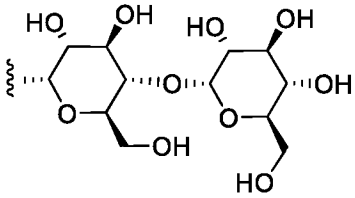
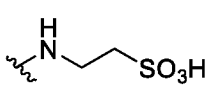
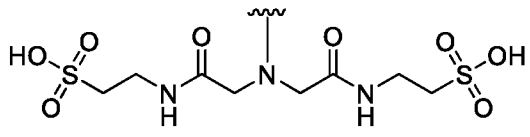
AA^1 is a divalent or trivalent linker comprising an amino acid residue;

AA^2 is a dipeptide, tripeptide, or tetrapeptide residue;

Q, when present, is  ,  , or  ;

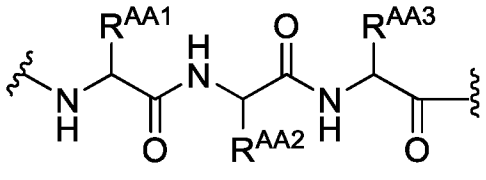
SP is absent, or a spacer; and

HG is

 ,  ,  ,  , or  ,

wherein the indicates the atoms through which the referenced group is bonded to the adjacent groups in the formula.

15. The antibody-drug conjugate of any one of claims 11, and 13-14, wherein **AA^1-AA^2** is according to Formula (**LL1**):

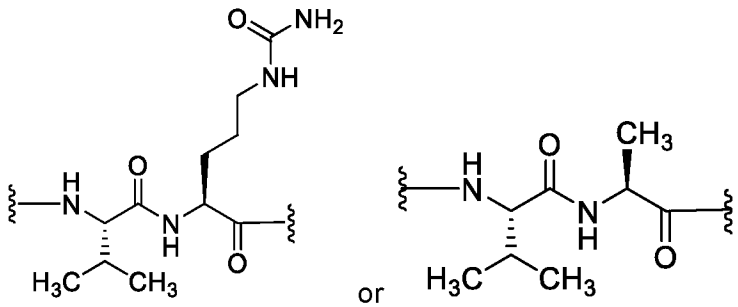


(**LL1**)

wherein **R^{AA1}**, **R^{AA2}**, and **R^{AA3}** are each, independently, amino acid side chains, at least one of which is bonded to –(**RG^2**)-**SP^2-HG**, –(**RG^2**)-**HG** or **HG;** wherein the indicates the atoms through which **AA^1-AA^2** is bonded to the adjacent groups in the formula.

16. The antibody-drug conjugate of claim 15, wherein R^{AA1} is a lysine, glutamine, glutamic acid or aspartic acid side chain bonded directly or indirectly to **HG**, and R^{AA2} and R^{AA3} are either valine and alanine or valine and citrulline sidechains respectively.

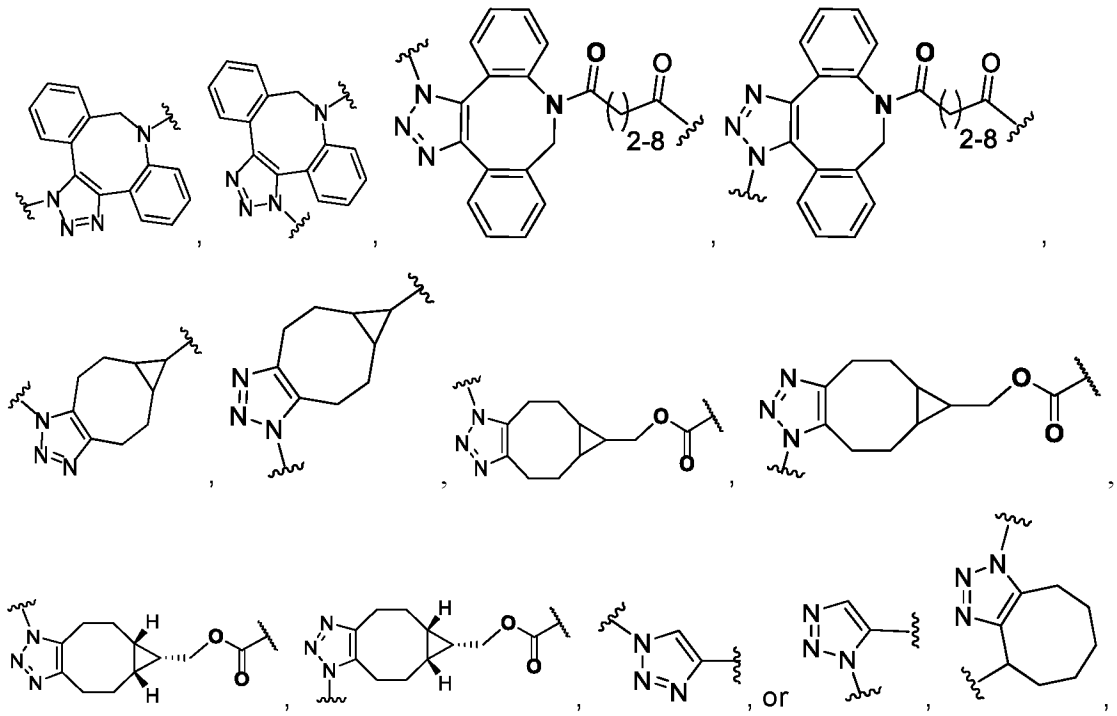
17. The antibody-drug conjugate of any one of claims 11, and 13-16, wherein AA^1-AA^2 is

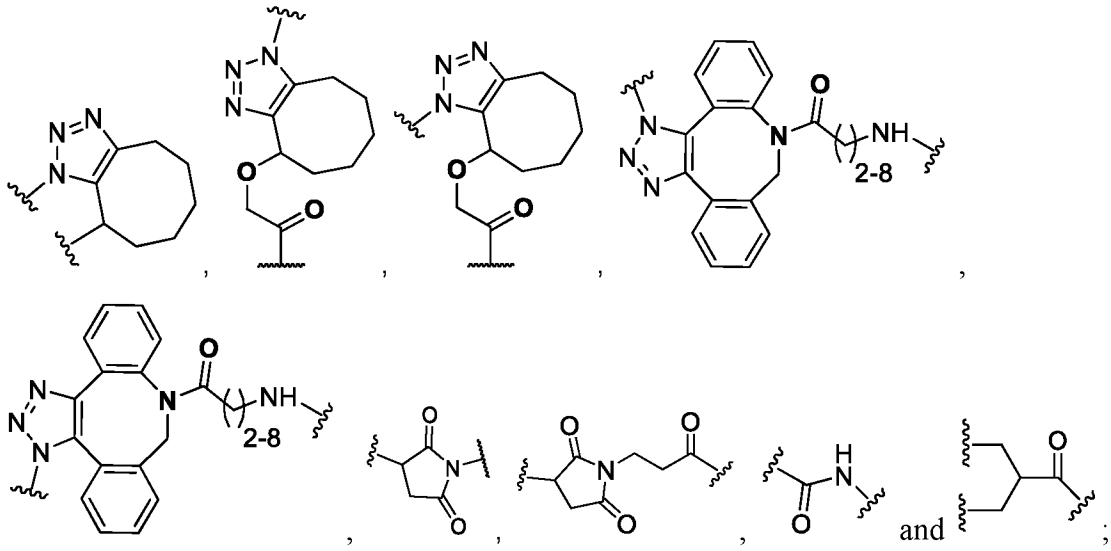


or

, wherein the indicates the atoms through which AA^1-AA^2 is bonded to the adjacent groups in the formula.

18. The antibody-drug conjugate of any one of claims 11-17, wherein the RG^1 and RG^2 residues are independently, in each instance, selected from the group consisting of:



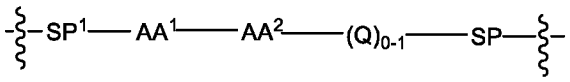


wherein the indicates the atom through which the **RG**^{1} or **RG**^{2} residue is bonded to the adjacent groups in the formula.

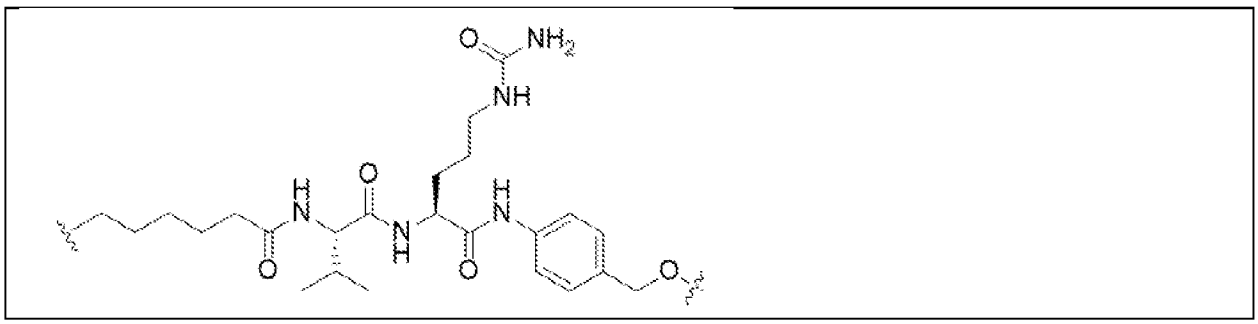
19. The antibody-drug conjugate of any one of claims 11-18, wherein **SP**, **SP**^{1} and **SP**^{2} are independently, in each instance, absent, or selected from the group consisting of C_{1-6} alkylene, -NH-, -S-, -O-, -C(O)-, $(-CH_2-CH_2-O)_e$, $-NH-CH_2-CH_2-(-O-CH_2-CH_2)_e-C(O)-$, $-C(O)-(CH_2)_u-C(O)-$, $-C(O)-NH-(CH_2)_v-$, $(glycine)_4$-serine, and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8.

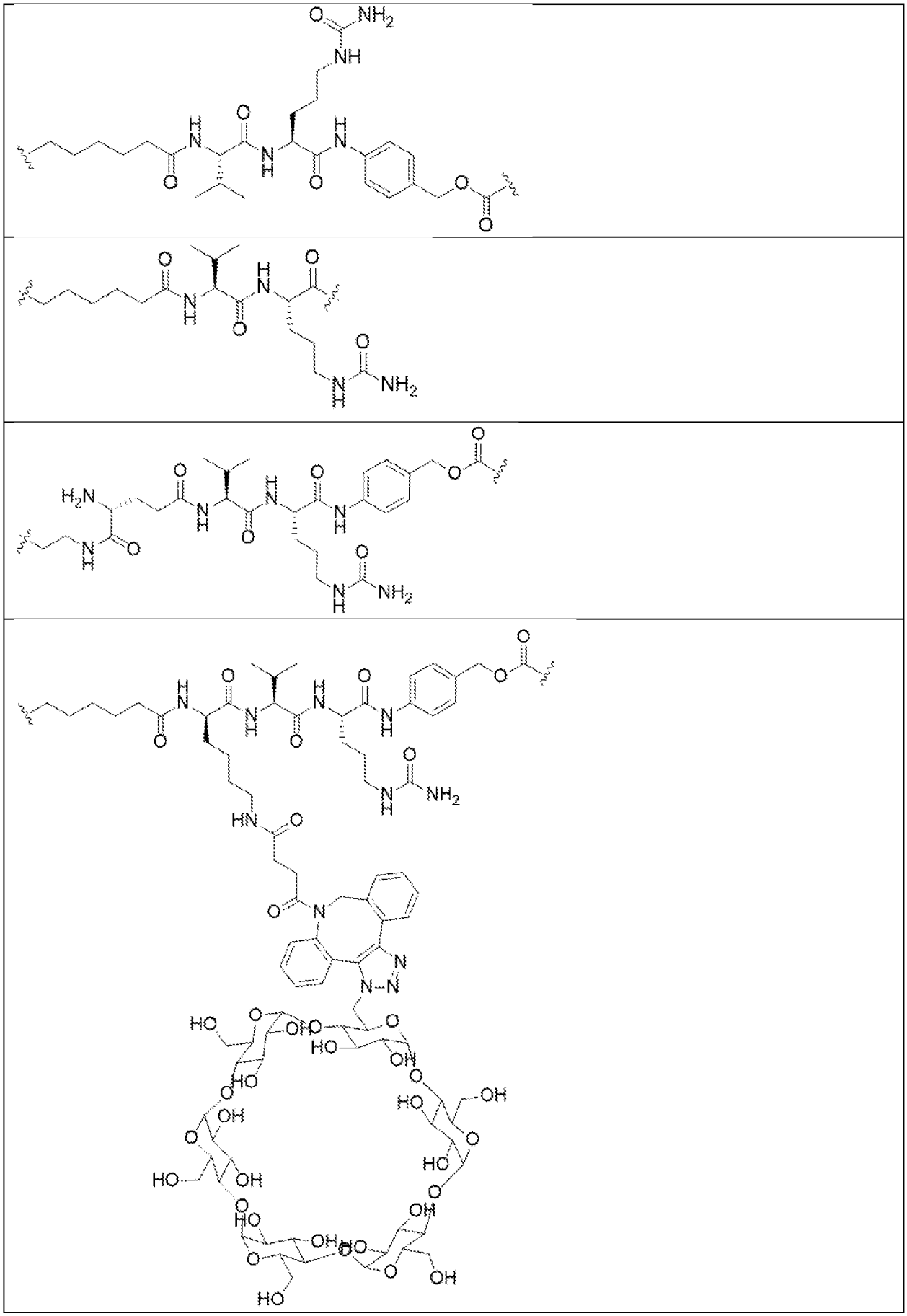
20. The antibody-drug conjugate of any one of claims 9-19, wherein n is 1, 2, 3, or 4.

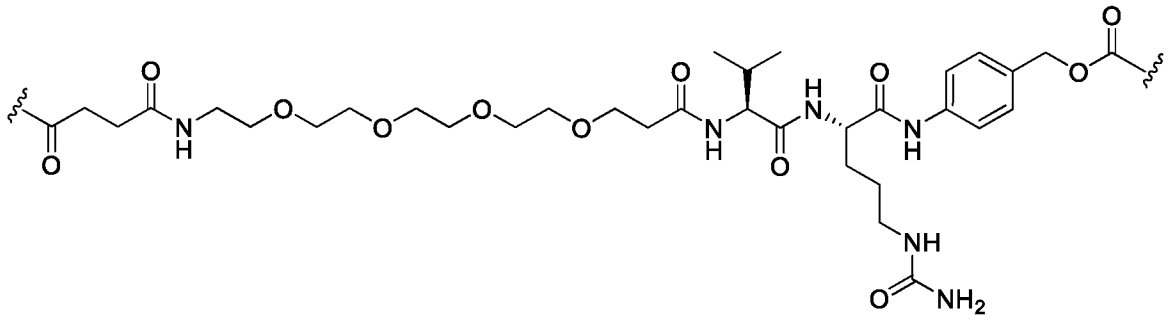
21. The antibody-drug conjugate of any one of claims 11-20, wherein

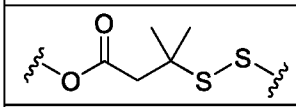


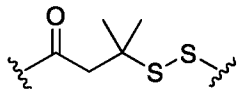
is

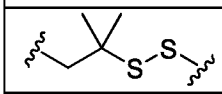


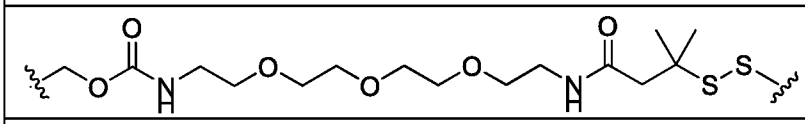


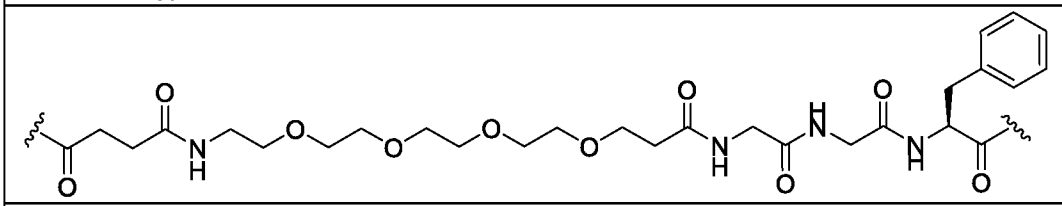


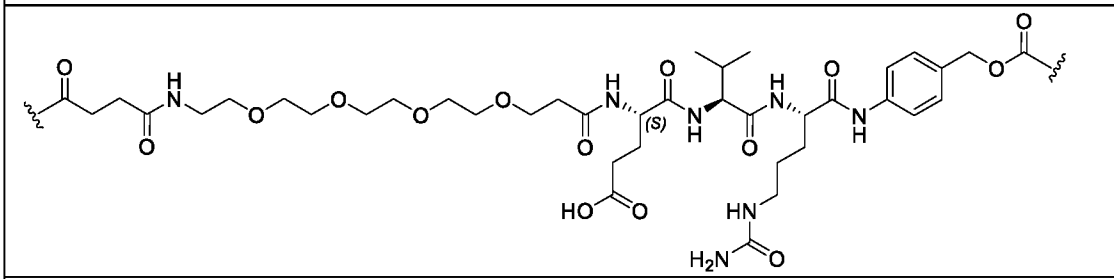


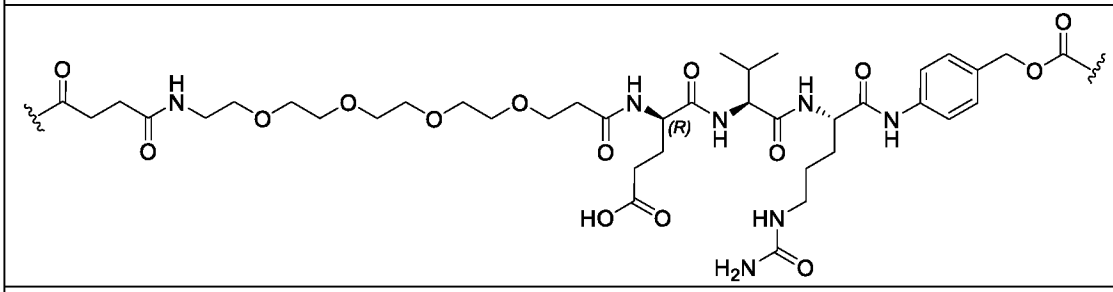


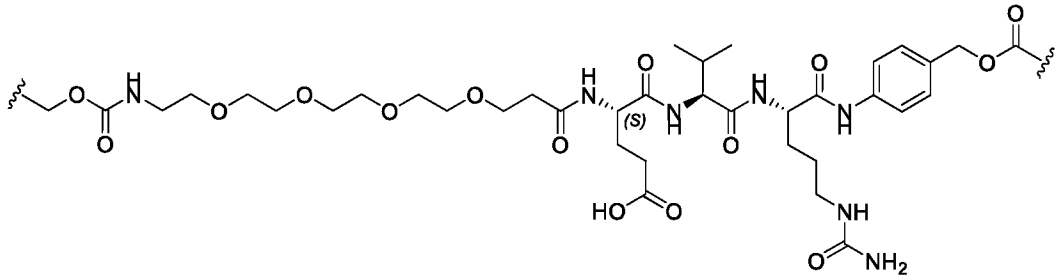


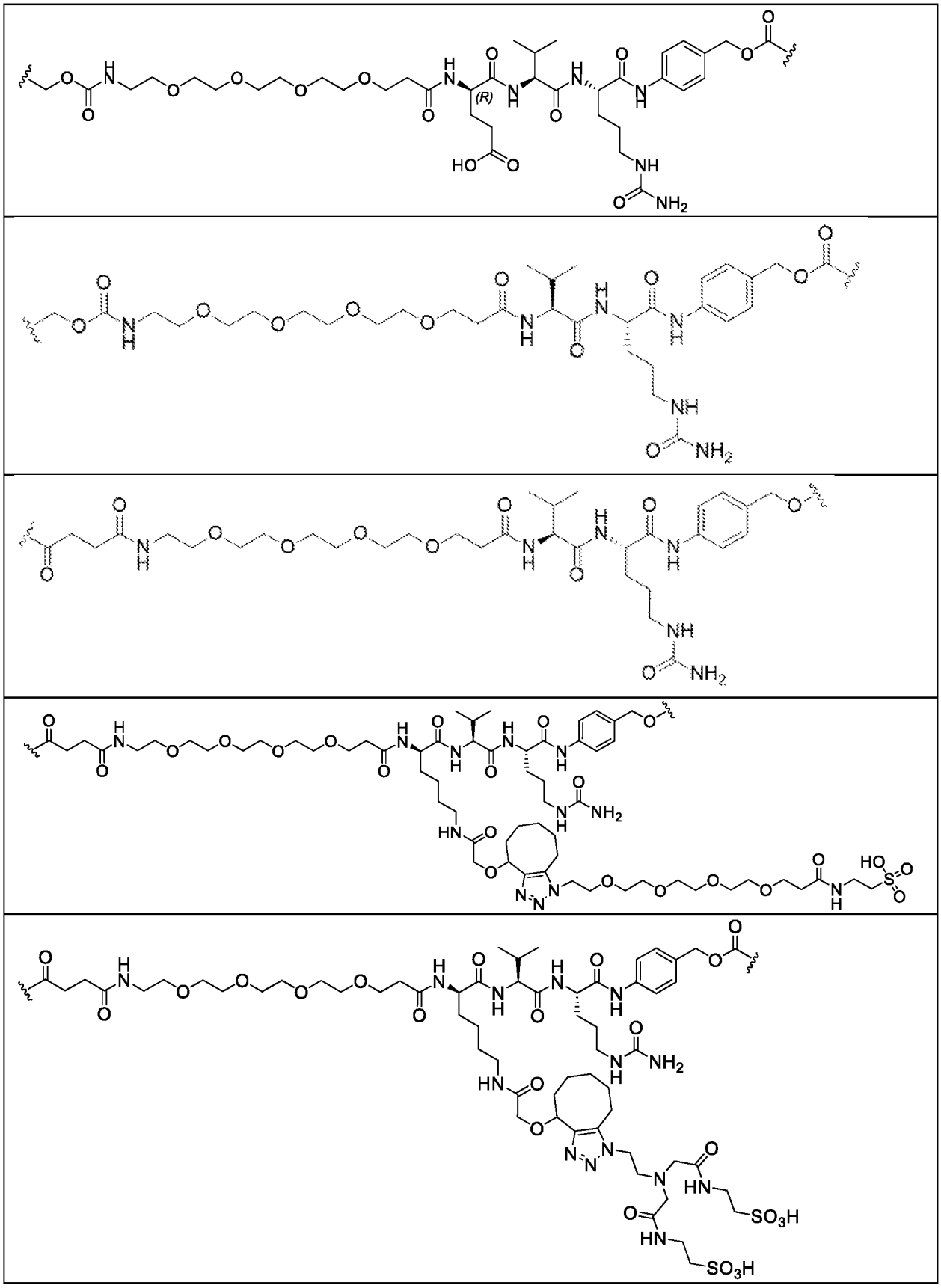


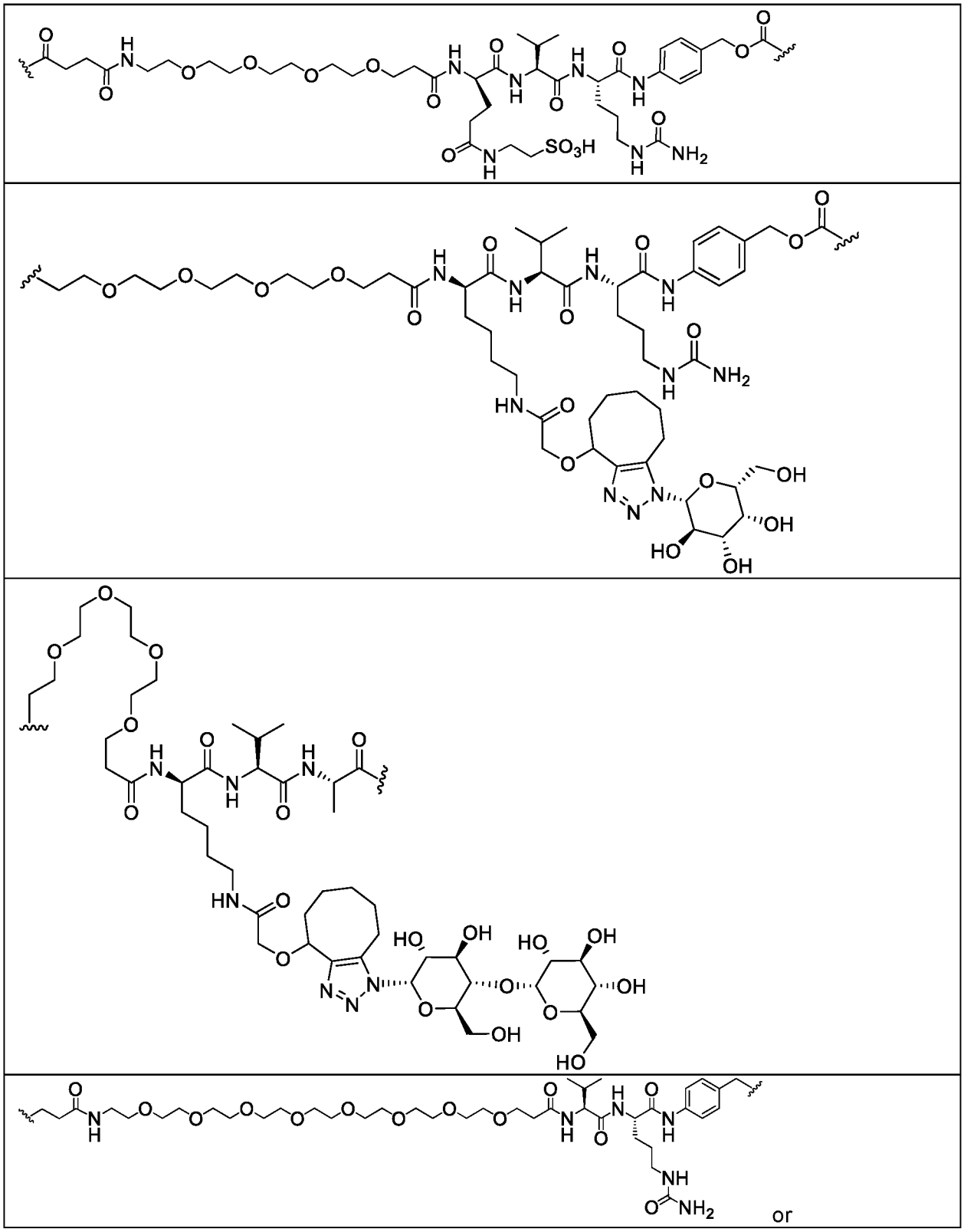








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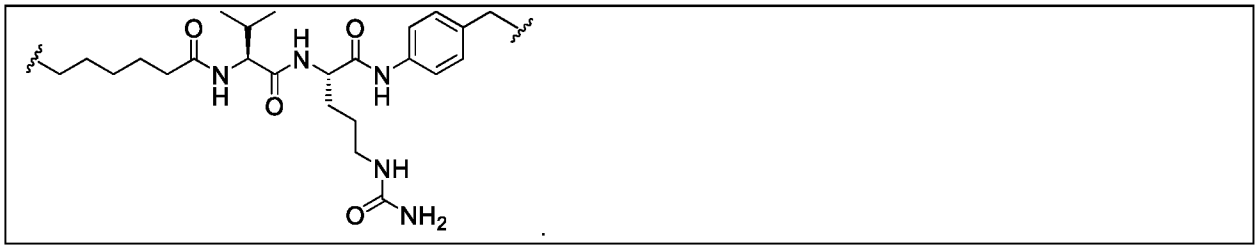
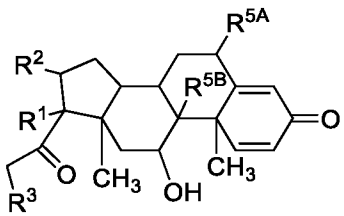


22. The antibody-drug conjugate molecule of claim 9, wherein the antibody, or antigen-binding fragment thereof, is conjugated to a steroid payload through a linker or a linker-spacer.

23. The antibody-drug conjugate molecule of claim 9, wherein the antibody, or antigen-binding fragment thereof, is conjugated to a LXR modulator payload through a linker.

24. The antibody-drug conjugate molecule of claim 9, wherein the antibody, or antigen-binding fragment thereof, is conjugated to a rifamycin analog payload through a linker.

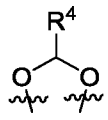
25. The antibody-drug conjugate molecule of claim 22, wherein the steroid conjugated to the antibody, or antigen-binding fragment thereof, through a linker or a linker-spacer is a compound of Formula (**A-1**)



Formula (**A-1**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein

R^1 and R^2 are, independently, –H, alkyl, alkyl-C(O)-O–, –OH, or halo; or R^1 and R^2 together form  ,

wherein R^4 is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl,

wherein the alkyl, aryl, arylalkyl, and N-containing heterocycloalkyl are, independently in each instance, optionally substituted with -$NR^{Aa}R^{Ab}$;

R^3 is –O, R^Z-C(O)-**X**–, –heteroalkyl, –piperidinyl, –$NR^{Aa}R^{Ab}$, –oxyaryl–$NR^{Aa}R^{Ab}$ or –**Z**-**A′**$(R^P)_t$;

R^Z is alkyl;

X is O or NR^{Aa};

Z is S, S(O), $S(O)_2$, SO_2NR^{Aa}, O, C(O)NR^{Aa}, C(O), or NR^{Aa};

A′ is aryl, arylalkyl, or heteroaryl;

R^P is, independently in each instance, halo, optionally substituted alkyl, –OH, or -$NR^{Aa}R^{Ab}$;

R^{Aa} and R^{Ab} are, independently in each instance, –H, optionally substituted alkyl, or optionally subtitued aryl;

subscript **a** is an integer from 0-19; and

t is an integer from 1-3;

and

R^{5A} and R^{5B} are each, independently, halo or a hydrogen atom;

wherein the group R^3 or R^4 is bonded to the linker.

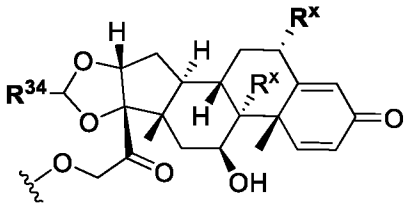
26. The antibody-drug conjugate of claim 22, having the structure of Formula (**3000**):

BA-(**L′**-**SP**-**D**)$_n$ Formula (**3000**)

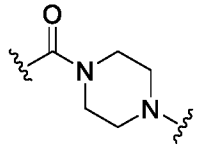
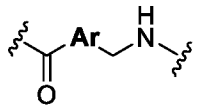
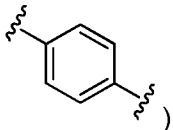
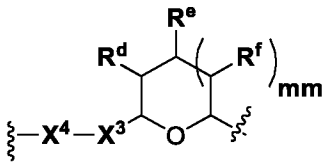
or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

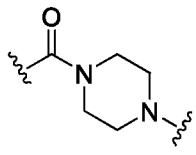
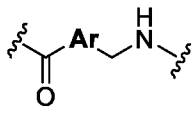
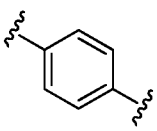
wherein

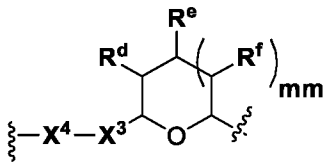
D is selected from

a)  Formula (a),

where both R^x in Formula (a) are hydrogen; R^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-X^1- where X^1 is attached to **L′** in Formula (3000), -C(O)-N(H)-(C_1-C_{10}-alkylene)-S- where S is attached to **L′** in Formula (3000), -C(O)-N(C_{1-6}alkyl)-(C_1-C_{10}-alkylene)-S-

where S is attached to **L′** in Formula (3000),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (3000), -CH_2-NH- where the N is attached to **L′** in Formula (3000),  where the N is attached to **L′** in Formula (3000) and where **Ar** is optionally substituted arylene (in some embodiments ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (3000), -C(O)-(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (3000) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N(**R**^{35})-C_1-C_{10}-alkylene-C(O)NH-**X**^{2}- where **X**^{2} is attached to **L′** in Formula (3000), or  where **X**^{4} is attached to **L′** in Formula (3000); or

where both **R**^{x} in Formula (a) are fluoro; **R**^{34} is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl; and **SP** is -C(O)-C_1-C_{10}-alkylene-C(O)-, -C(O)-N(C_{1-6}alkyl)-C_1-C_{10}-alkylene-**X**^{1b}- where **X**^{1b} is attached to **L′** in Formula (3000), -C(O)-N(H)-(C_1-C_{10}-alkylene)-**X**^{1b}- where **X**^{1b} is attached to **L′** in Formula (3000),  where the point of attachment on the right hand side (*i.e.* at N) is to **L′** in Formula (3000), -CH_2-NH- where the N is attached to **L′** in Formula (3000),  where the N is attached to **L′** in Formula (3000) and where **Ar** is optionally substituted arylene (in some embodiments ) or optionally substituted heteroarylene, -(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′** in Formula (3000), -C(O)-(C_1-C_{10}-alkylene)-N**R**^{50}C(O)-(C_1-C_{10}-alkylene)-N**R**^{50a}- where N**R**^{50a} is attached to **L′**

in Formula (3000) and where each C_1-C_{10}-alkylene is independently optionally substituted with one or more hydroxy, -C(O)-N($\mathbf{R^{35}}$)-(C_1-C_{10}-alkylene)-C(O)NH-$\mathbf{X^2}$- where $\mathbf{X^2}$ is attached to $\mathbf{L'}$ in Formula (3000), or  where $\mathbf{X^4}$ is attached to $\mathbf{L'}$ in Formula (3000); and/or

b) the compounds in Table A, where the compounds in Table A are linked to **BA** of the Compound of Formula (**III**) through the hydroxy of the -C(O)CH_2OH group, or through the hydroxy of Mapracorat;

$\mathbf{X^1}$ is -N(C_{1-6}alkyl)-;

$\mathbf{X^{1b}}$ is -S-, -NH-, or -N(C_{1-6}alkyl)-;

$\mathbf{X^2}$ is -NH-;

$\mathbf{X^3}$ is -CH_2-, $\mathbf{X^3}$ is -CH_2-O-(C_1-C_{10}-alkylene)-C(O)- where the C(O) is attached to $\mathbf{X^4}$, or $\mathbf{X^3}$ is -C(O)-;

$\mathbf{X^4}$ is -O-;

$\mathbf{R^{35}}$ is H, -OH, -OCH_3, or C_{1-6}alkyl;

$\mathbf{R^{50}}$ and $\mathbf{R^{50a}}$ are independently hydrogen or C_1-C_6-alkyl;

$\mathbf{R^d}$, $\mathbf{R^e}$, and $\mathbf{R^f}$ are independently -H, -OH, hydroxyalkyl, alkoxycarbonyl, -C(O)OH, or -CH_2O$\mathbf{R^g}$, where each $\mathbf{R^g}$ is independently -CH_2C(O)OH or -CH_2C(O)O(alkyl); and

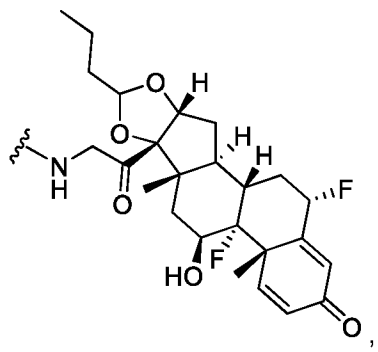
mm is 0 or 1;

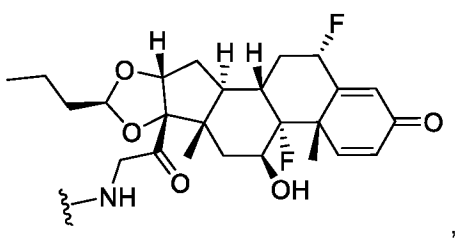
n is an integer selected from 1-30, inclusive;

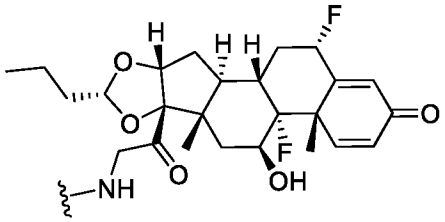
$\mathbf{L'}$ is a linker; and

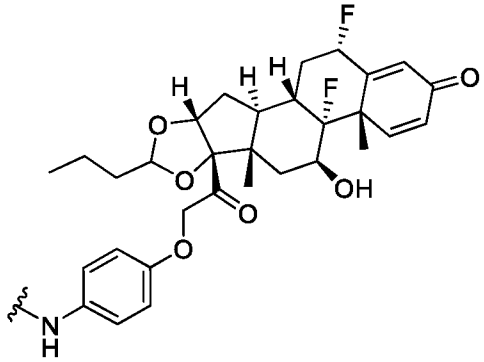
BA is a binding agent.

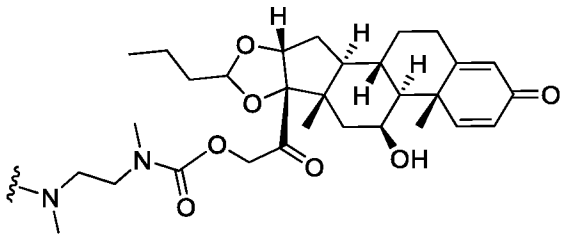
27. The antibody-drug conjugate of claim 22, wherein the steroid payload is selected from the group consisting of:

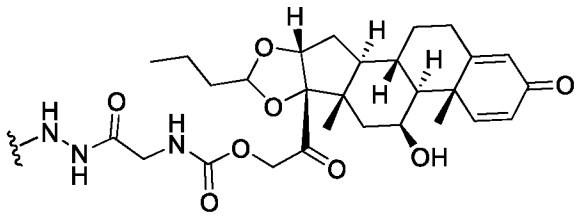


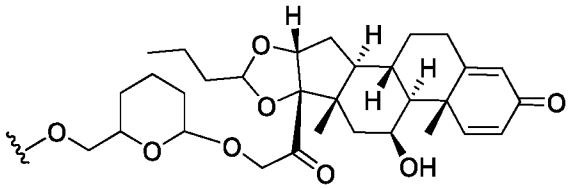


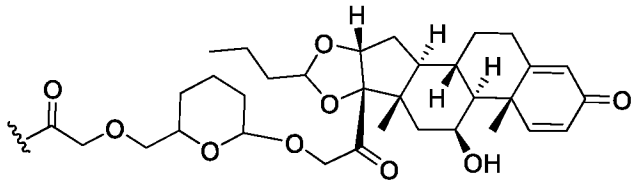


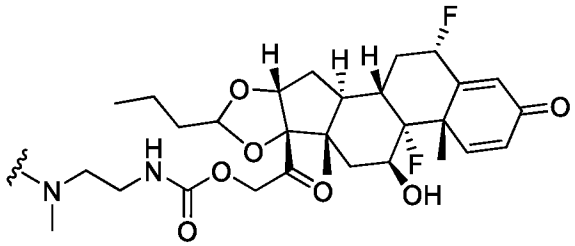


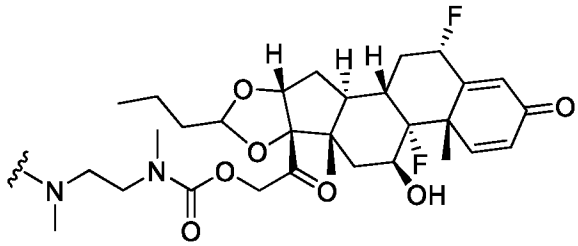


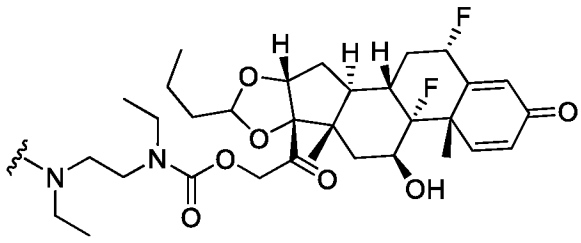


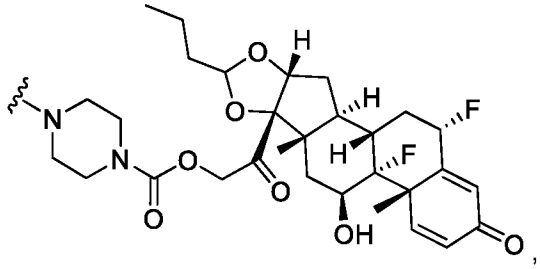


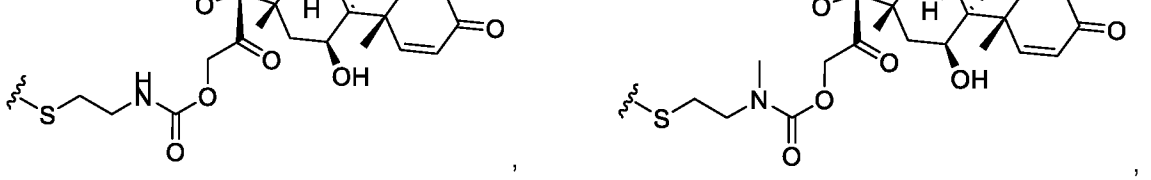


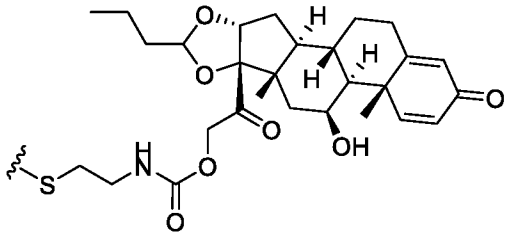


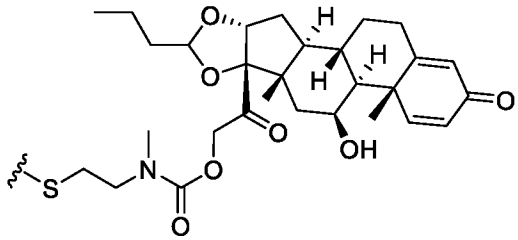


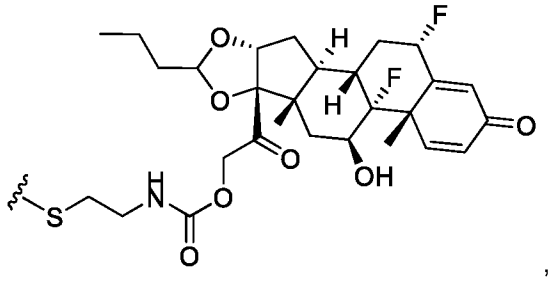








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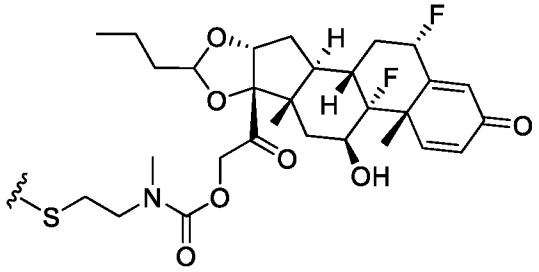
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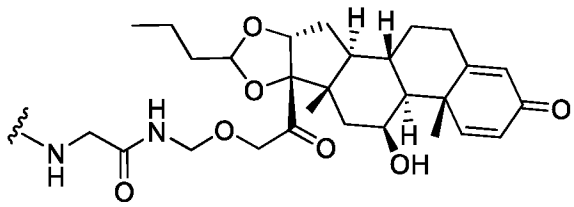
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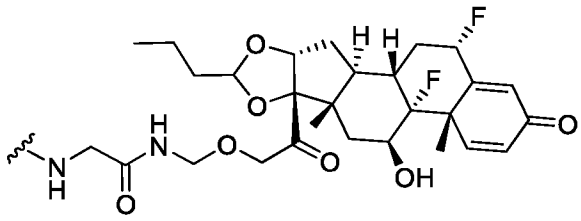
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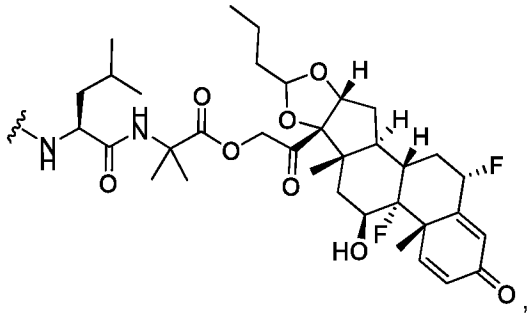
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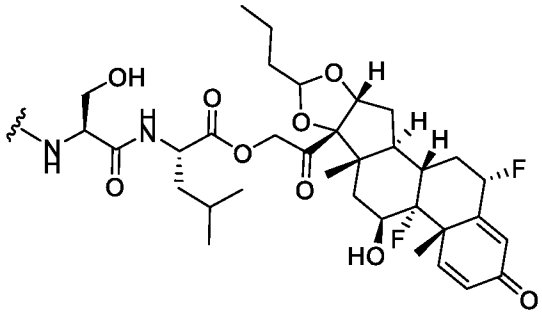
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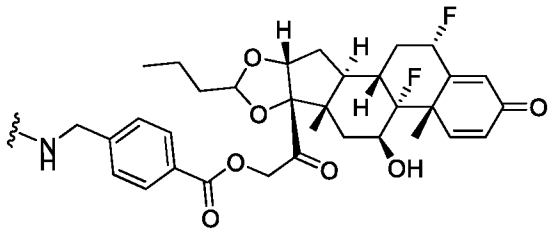
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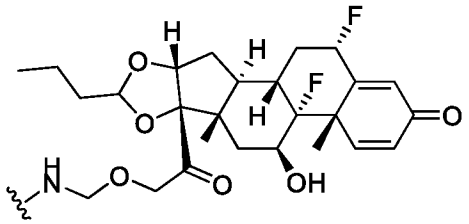
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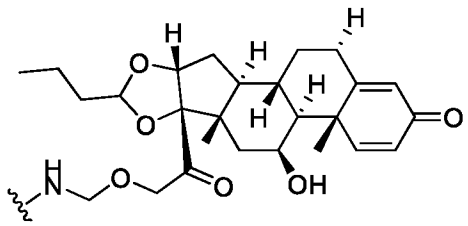
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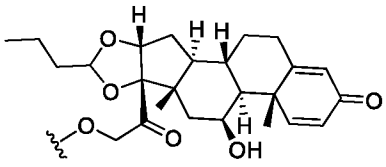
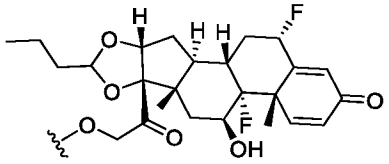
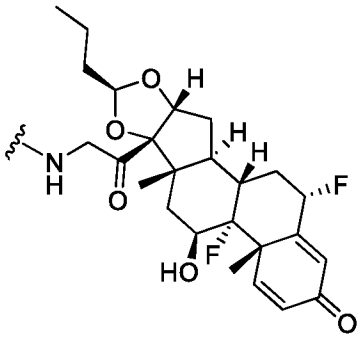
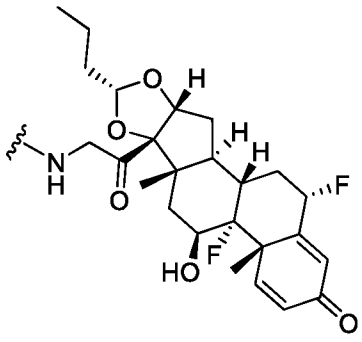
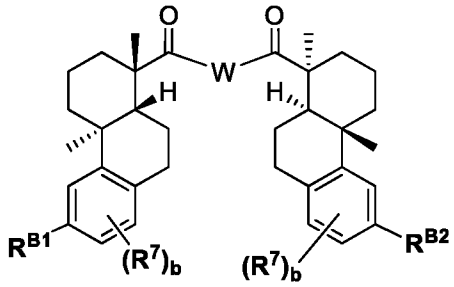

, and

,

wherein the ~— is the bond to the linker.

28. The antibody-drug conjugate of claim 22, wherein the steroid payload is selected from the group consisting of:


and

, or a mixture thereof.

29. The antibody-drug conjugate of claim 23, wherein the LXR modulator conjugated to the antibody, or antigen-binding fragment thereof, through a linker is a compound of Formula (**B**):


Formula (**B**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form, wherein

W is $–CH_2–$, –N(H)–, or –O–;

$\mathbf{R^{B1}}$ is –H, –OH, $–NH_2$, alkyl, or$–OP(O)(OR^6)_2$;

$\mathbf{R^{B2}}$ is –H, –OH, $–CH_2NH_2$, $\mathbf{R^{B3}}$, $\mathbf{R^{B4}}$, $\mathbf{R^{B5}}$, or $–O–\mathbf{R^{B5}}$, wherein $\mathbf{R^{B1}}$ and $\mathbf{R^{B2}}$ are not simultaneously –H;

$\mathbf{R^{B3}}$ is $–N(\mathbf{R^6})_2$;

$\mathbf{R^{B4}}$ is **–X–Y–Z**;

X is selected from the group consisting of –O– and –N(H)–;

Y is selected from the group consisting of alkylene, substituted alkylene (including, without limitation, oxo substitution, i.e., =O)), heteroalkylene, and substituted heteroalkylene (including, without limitation, oxo substitution (i.e., =O));

Z is selected from the group consisting of –OH and $–NH_2$;

$\mathbf{R^{B5}}$ is alkyl, heterocycloalkyl, or substituted heterocycloalkyl, wherein each heterocycloalkyl or substituted heterocycloalkyl includes one, two, or three heteroatoms selected from nitrogen

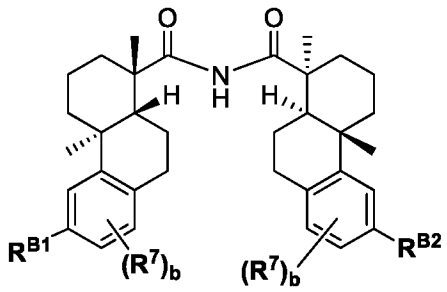
and oxygen, and includes at least one –OH and –CH_2OH substituent, or at least one primary or secondary nitrogen, for instance, O-glucose;

each $\mathbf{R^6}$ is in each instance, –H, an amino acid residue, an *N*-alkyl amino acid residue, a peptide, or alkyl; and

each $\mathbf{R^7}$ is, independently, halo, $C_{1\text{-}6}$ alkyl, $C_{1\text{-}6}$ alkoxy, –CN, O-glucose, O-amino acid residue, and O-PEG_b, wherein each subscript **b** is an integer from 0-3;

wherein the group $\mathbf{R^{B1}}$ or $\mathbf{R^{B2}}$ is bonded to the linker.

30. The antibody-drug conjugate of claim 23, wherein the LXR modulator conjugated to the antibody, or antigen-binding fragment thereof, through a linker is a compound of Formula (**B-1**):



Formula **(B-1)**

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{R^{B1}}$ is –N(H)$\mathbf{R^8}$ or –N($\mathbf{R^9}$)$_2$;

$\mathbf{R^{B2}}$ is –N(H)$\mathbf{R^8}$;

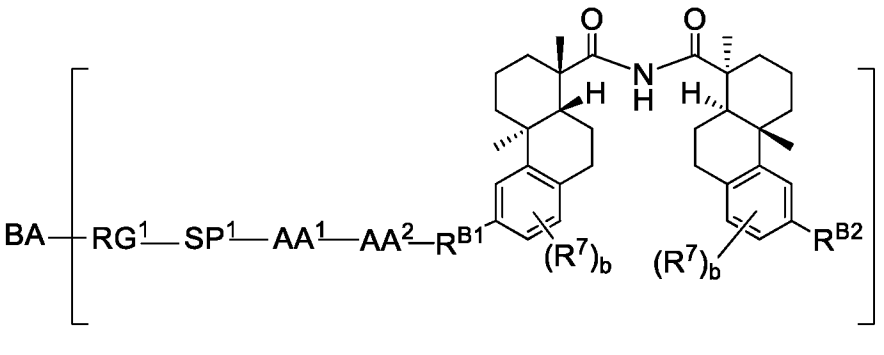
each $\mathbf{R^8}$ is, independently in each instance, hydrogen, an amino acid residue, an *N*-alkyl amino acid residue, a peptide residue, a biodegradable moiety, or alkyl;

$\mathbf{R^9}$ is alkyl, aryl, arylalkyl, heterocycloalkyl, or substituted heterocycloalkyl, wherein each heterocycloalkyl or substituted heterocycloalkyl comprises one, two, or three heteroatoms selected from nitrogen and oxygen, and when substituted includes at least one –OH and -CH_2OH, or at least one primary or secondary nitrogen;

each $\mathbf{R^7}$ is independently halo, $C_{1\text{-}6}$ alkyl, $C_{1\text{-}6}$ alkoxy, –CN, O-glucose, O-amino acid residue, or O-PEG_b, wherein each subscript **b** is an integer from 0-3;

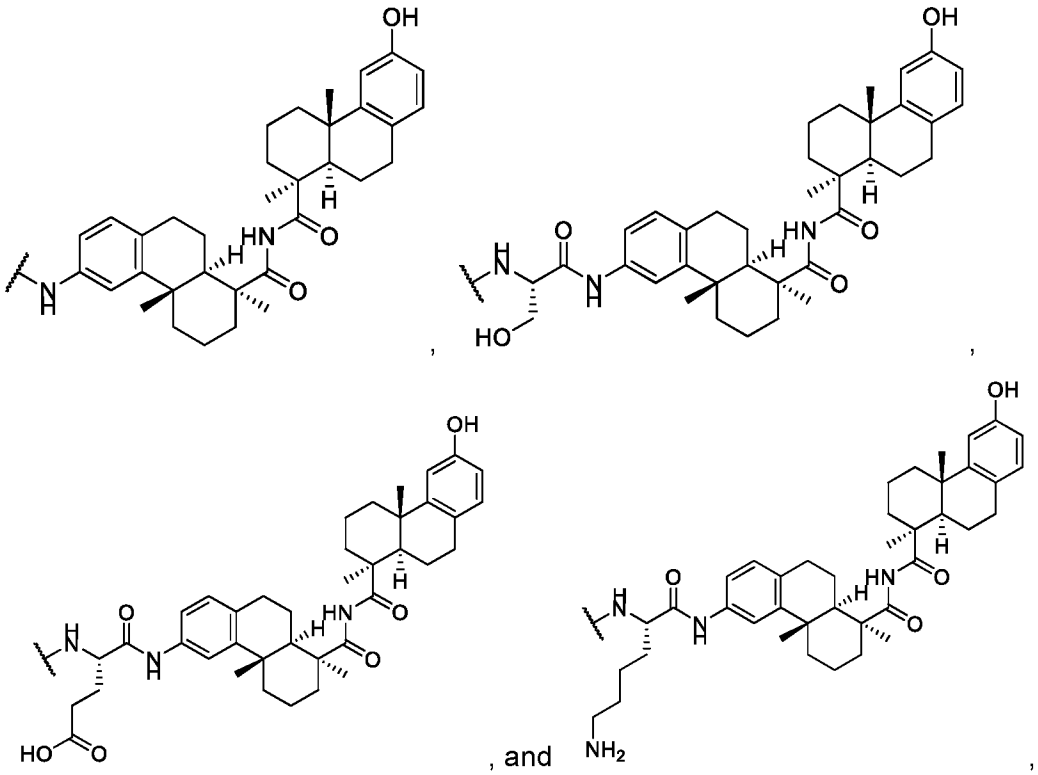
wherein the group $\mathbf{R^{B1}}$ or $\mathbf{R^{B2}}$ is bonded to the linker.

31. The antibody-drug conjugate of any one of claims 9-21, 23, and 29-30, having the structure of Formula (**6005**)



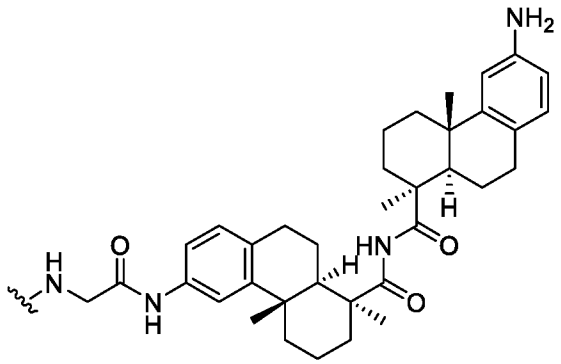
Formula (**6005**)

32. The antibody-drug conjugate of claim 23, wherein the LXR modulator is selected from the group consisting of:

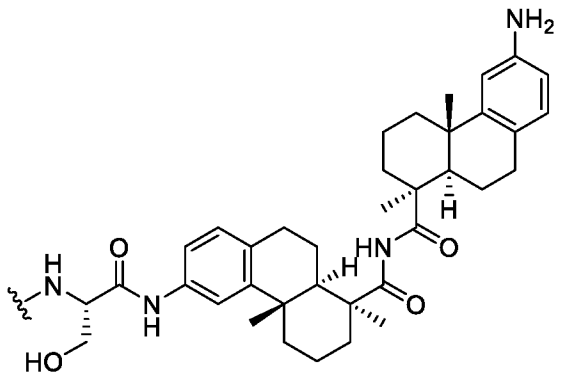


wherein the ≈— is the bond to the linker.

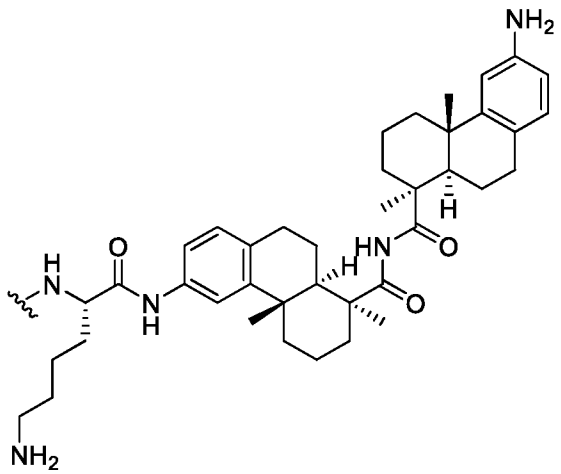
33. The antibody-drug conjugate of claim 23, wherein the LXR modulator is selected from the group consisting of:



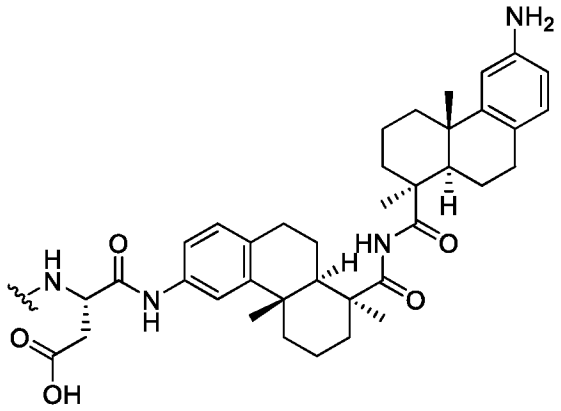
P4B;



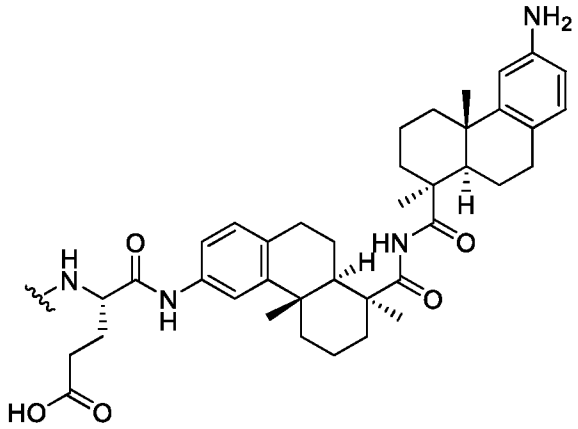
P5B;



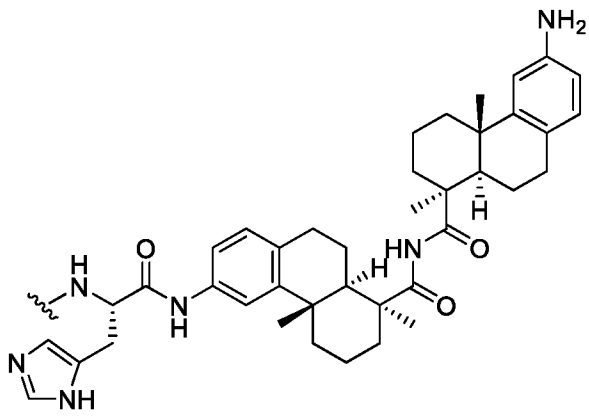
P6B;



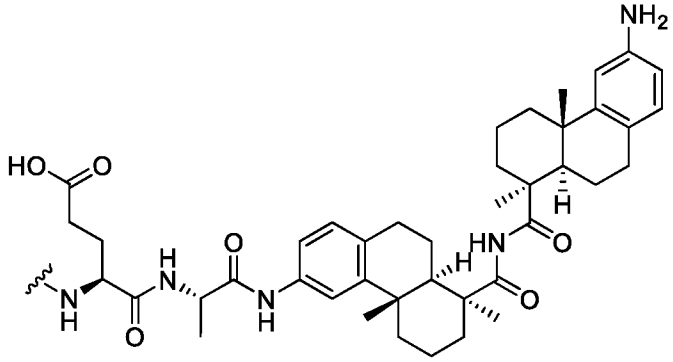
P7B;



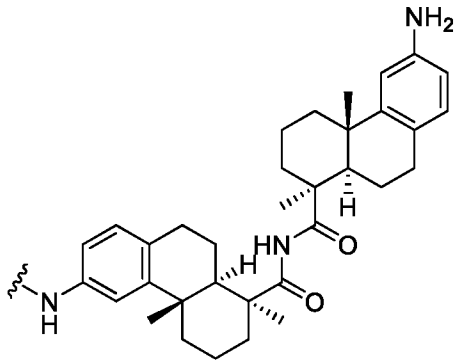
P8B;



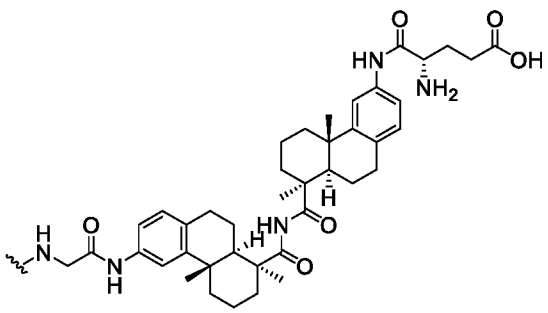
P9B;



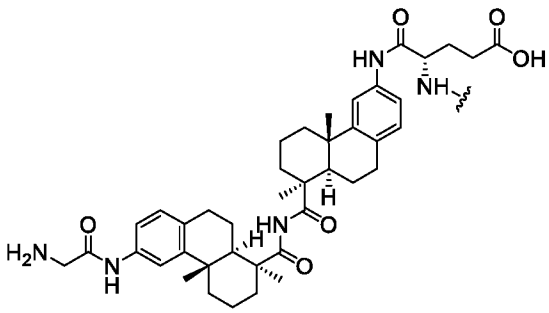
P12B;



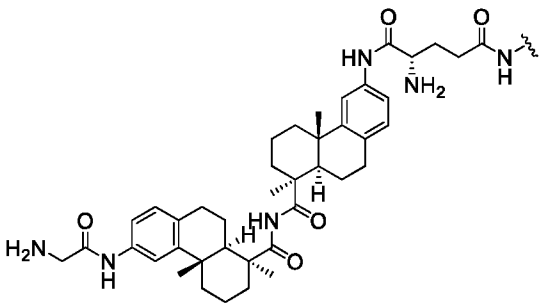
P2B;



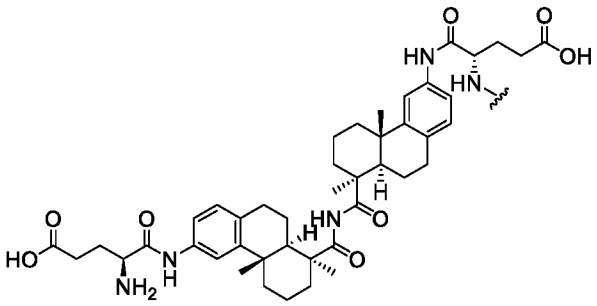
or



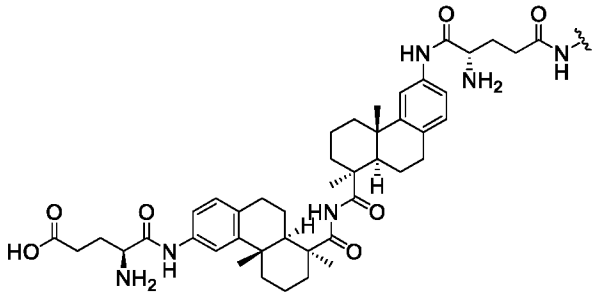
or



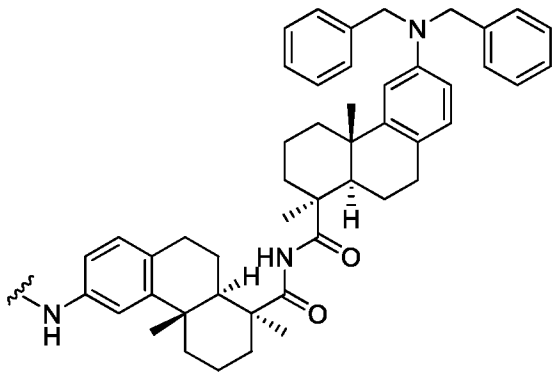
P10B;



or



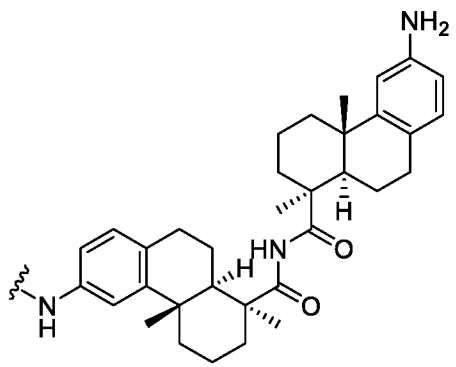
P11B;
and



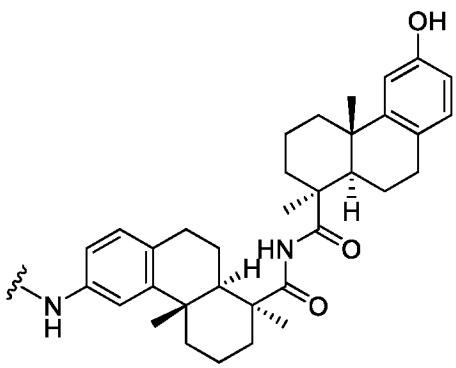
P3B;

wherein the ~— is the bond to the linker.

34. The antibody-drug conjugate of claim 23, wherein the LXR modulator is



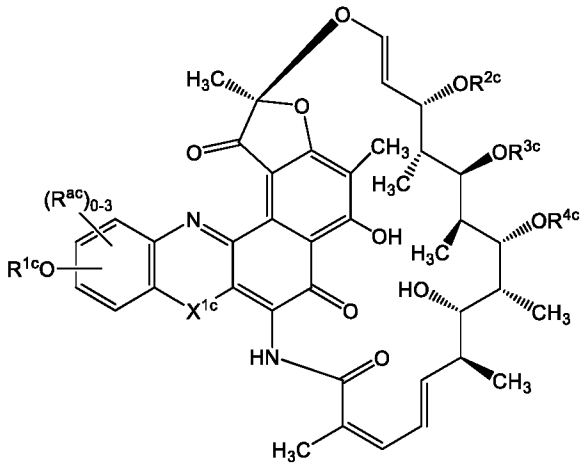
or



;

wherein the ~— is the bond to the linker.

35. The antibody-drug conjugate of claim 24, wherein the rifamycin analog is a compound of Formula (**C-1**):



Formula (**C-1**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{X^{1c}}$ is selected from –S-; -O- and –N$\mathbf{R^{5c}}$;

$\mathbf{R^{1c}}$ is amino-$C_{1\text{-}6}$alkyl; $C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; di-$C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; hydroxy-$C_{1\text{-}6}$alkyl; HS-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-N($\mathbf{R^{5c}}$)-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-O-$C_{1\text{-}6}$alkyl; $(\mathbf{R^{5c}})_2$N-$C_{1\text{-}6}$alkylene-S-$C_{1\text{-}6}$alkyl; heterocycloalkyl or heterocycloalkyl-$C_{1\text{-}6}$alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, $C_{1\text{-}6}$alkyl, -OH, =O, or –N($\mathbf{R^{5c}}$)$_2$;

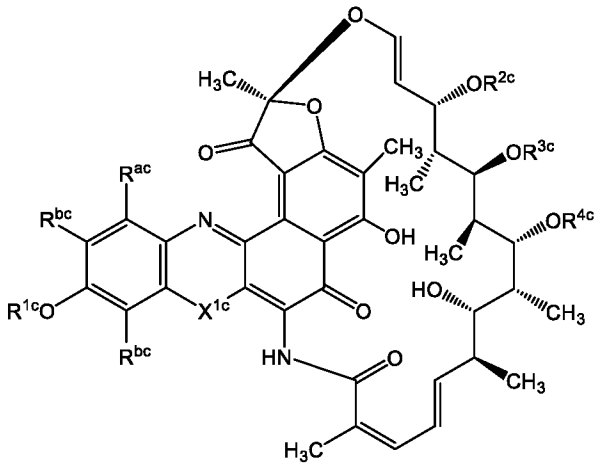
$\mathbf{R^{2c}}$, $\mathbf{R^{3c}}$, and $\mathbf{R^{4c}}$ are independently selected from hydrogen, $C_{1\text{-}6}$alkyl, and -(C=O)-$\mathbf{R^{5c}}$;

each $\mathbf{R^{ac}}$, when present, is independently selected from -F; -Cl; -Br; -I; -OH; -NH_2; and $C_{1\text{-}6}$alkoxy; and

$\mathbf{R^{5c}}$ is independently, at each occurrence, selected from hydrogen; and $C_{1\text{-}6}$alkyl;

wherein the group $\mathbf{R^{1c}}$ is bonded to the linker.

36. The antibody-drug conjugate of claim 24, wherein the rifamycin analog is a compound of Formula (**C-2**):



Formula (**C-2**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{X^{1c}}$ is selected from –S–; –O– and –N$\mathbf{R^{5c}}$;

$\mathbf{R^{1c}}$ is amino-$C_{1\text{-}6}$alkyl; $C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; di-$C_{1\text{-}6}$alkylamino$C_{1\text{-}6}$alkyl; hydroxy-$C_{1\text{-}6}$alkyl; HS-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-N($\mathbf{R^{5c}}$)-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-O-$C_{1\text{-}6}$alkyl; ($\mathbf{R^{5c}}$)$_2$N-$C_{1\text{-}6}$alkylene-S-$C_{1\text{-}6}$alkyl; heterocycloalkyl or heterocycloalkyl-$C_{1\text{-}6}$alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, $C_{1\text{-}6}$alkyl, -OH, =O, or –N($\mathbf{R^{5c}}$)$_2$;

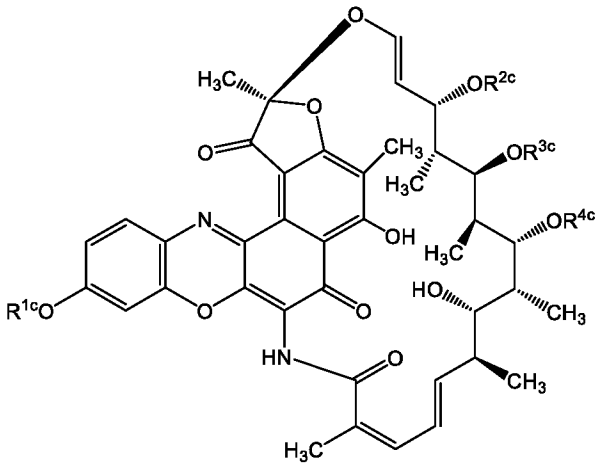
$\mathbf{R^{2c}}$, $\mathbf{R^{3c}}$, and $\mathbf{R^{4c}}$ are independently selected from hydrogen, $C_{1\text{-}6}$alkyl, and -(C=O)-$\mathbf{R^{5c}}$;

$\mathbf{R^{ac}}$and $\mathbf{R^{bc}}$ are independently selected from -F; -Cl; -Br; -I; -OH; -NH_2; and $C_{1\text{-}6}$alkoxy; and

$\mathbf{R^{5c}}$ is independently, at each occurrence, selected from hydrogen; and $C_{1\text{-}6}$alkyl;

wherein the group $\mathbf{R^{1c}}$ is bonded to the linker.

37. The antibody-drug conjugate of claim 24, wherein the rifamycin analog is a compound of Formula (**C-3**):



Formula (**C-3**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

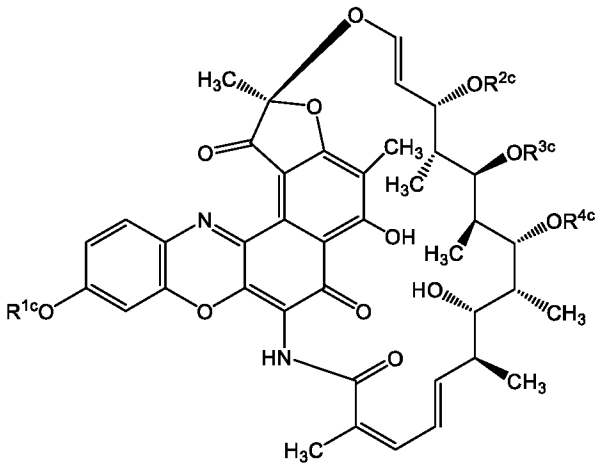
$\mathbf{R^{1c}}$ is di-C_{1-6}alkylaminoC_{1-6}alkyl; $(\mathbf{R^{5c}})_2$N-C_{1-6}alkylene-N($\mathbf{R^{5c}}$)-C_{1-6}alkyl; heterocycloalkyl or heterocycloalkyl-C_{1-6}alkyl; wherein heterocycloalkyl includes one, two, or three heteroatoms selected from O, N, and S; and wherein heterocyloalkyl is optionally substituted with halo, C_{1-6}alkyl, -OH, =O, or –N($\mathbf{R^{5c}}$)$_2$; and

$\mathbf{R^{2c}}$, $\mathbf{R^{3c}}$, and $\mathbf{R^{4c}}$ are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-$\mathbf{R^{5c}}$; and

$\mathbf{R^{5c}}$ is selected from hydrogen and C_{1-6}alkyl;

wherein the group $\mathbf{R^{1c}}$ is bonded to the linker.

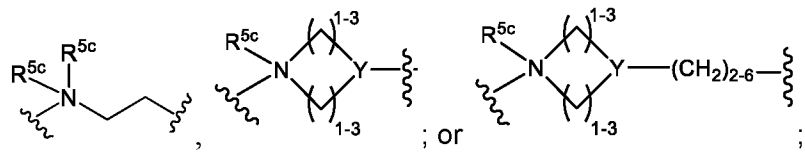
38. The antibody-drug conjugate of claim 24, wherein the rifamycin analog is a compound of Formula (**C-4**):



Formula (**C-4**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

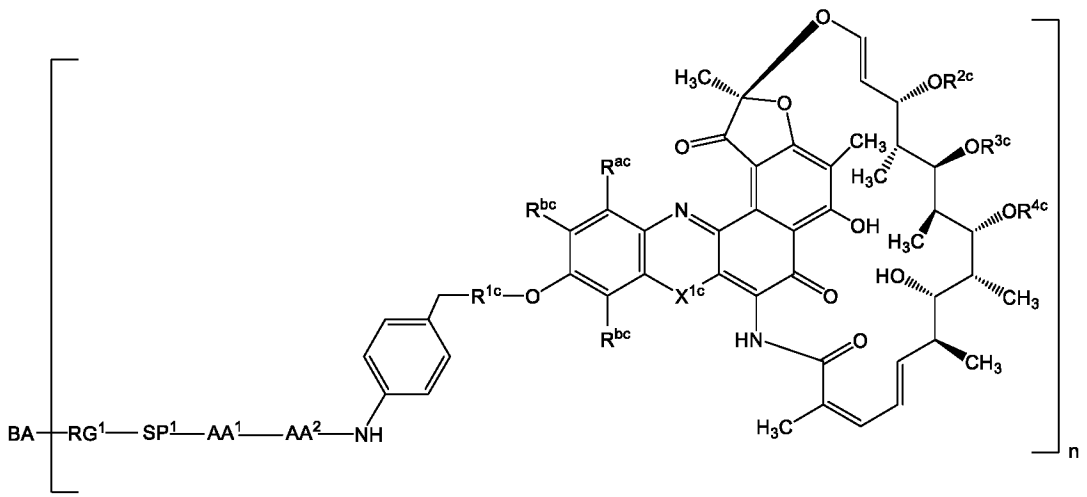
R^{1c} is  ; wherein **Y** is C or N;

R^{2c}, R^{3c}, and R^{4c} are independently selected from hydrogen, C_{1-6}alkyl, and -(C=O)-R^{5c}; and

R^{5c} is independently, at each occurrence, absent, or selected from hydrogen and C_{1-6}alkyl;

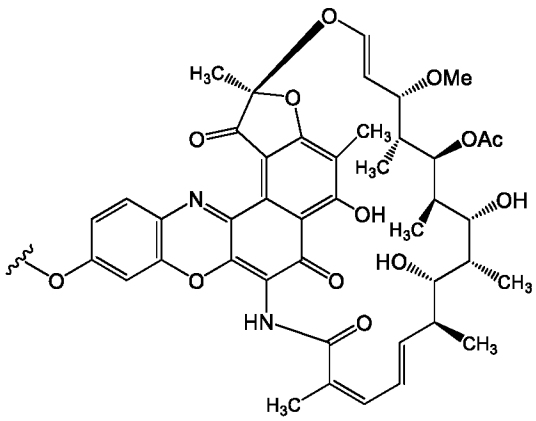
wherein the group R^{1c} is bonded to the linker via a nitrogen atom as indicated by the wavy line .

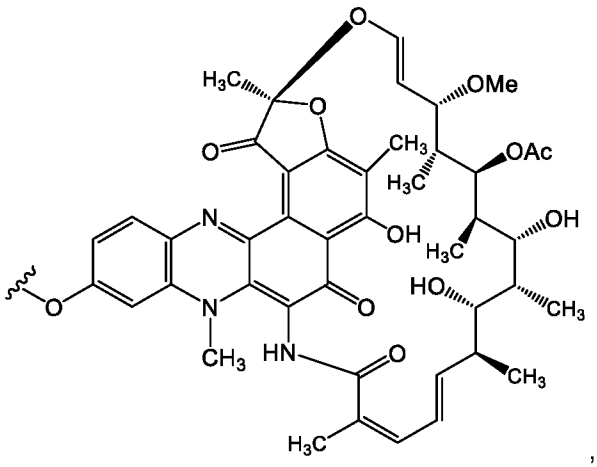
39. The antibody-drug conjugate of any one of claims 9-11, 13, 17-21, 24 and 35-38, having the structure of Formula (**7002**):



Formula (**7002**).

40. The antibody-drug conjugate of of any one of claims 9-11, 13, 17-21, 24 and 35-39, wherein the rifamycin analog is selected from the group consisting of:


,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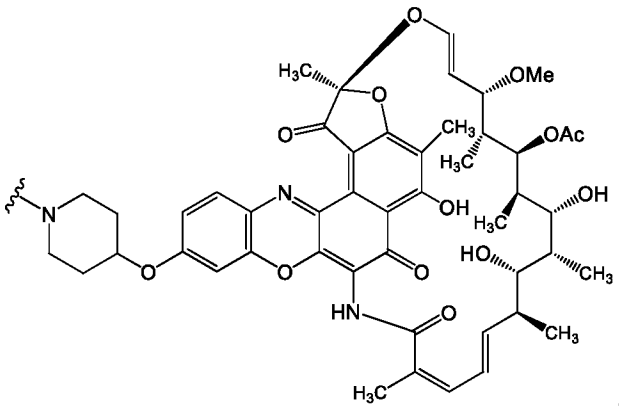
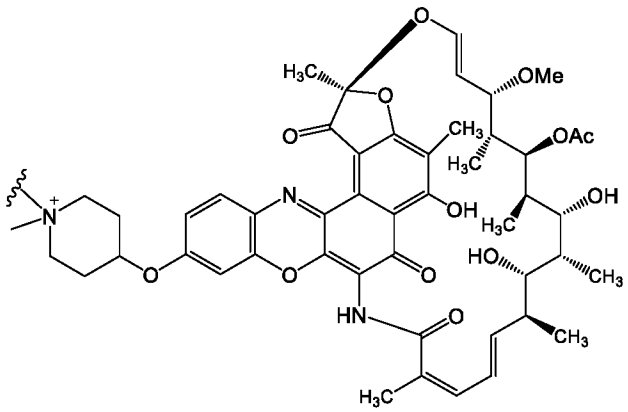

,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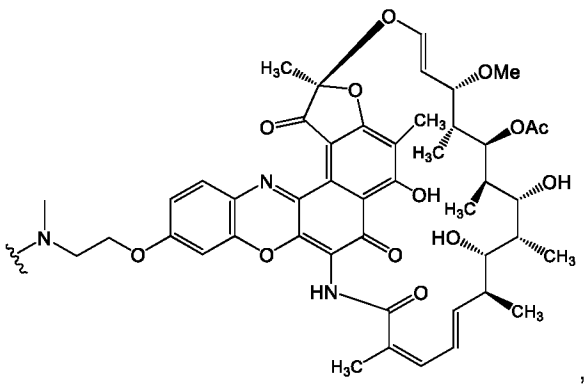
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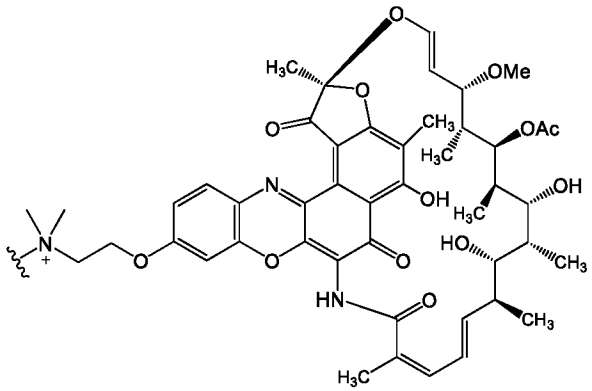

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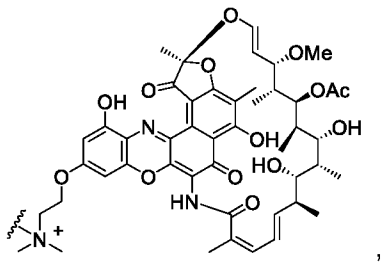

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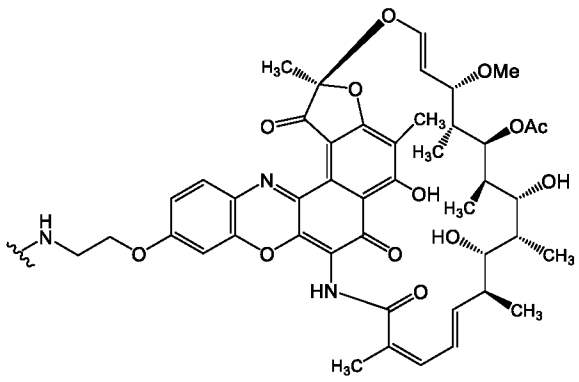
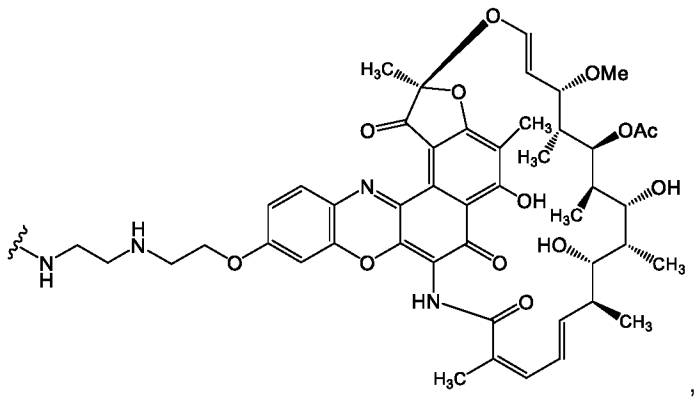


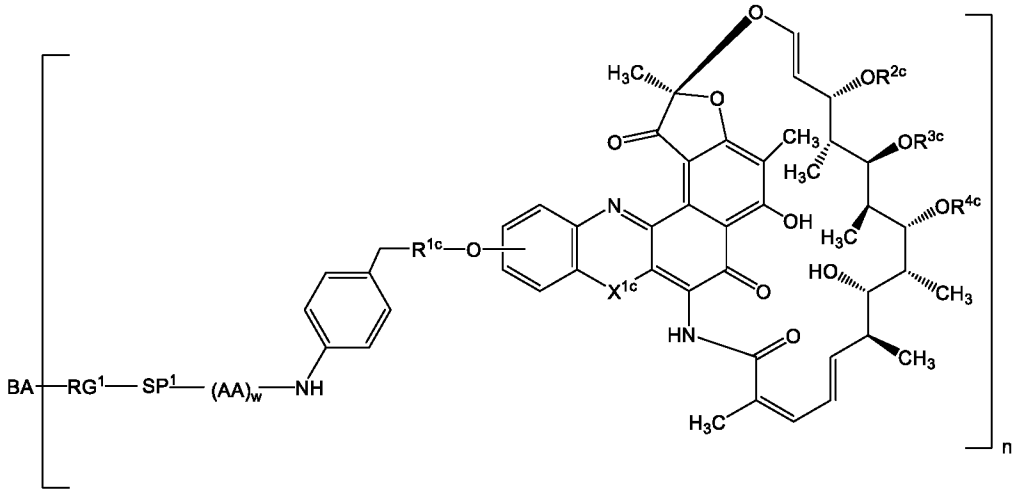
, and



,

wherein the ≈— is the bond to the linker.

41. An antibody-rifamycin analog conjugate having the structure of Formula (**7003**):

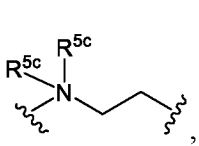
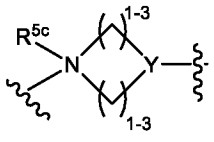
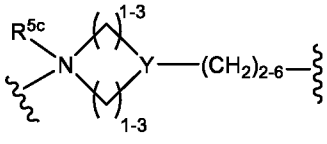


Formula (**7003**)

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof;

wherein:

$\mathbf{X^{1c}}$ is selected from –S-; -O- and –N$\mathbf{R^{5c}}$;

$\mathbf{R^{1c}}$ is  ,  ; or  ; wherein **Y** is C or N;

$\mathbf{R^{2c}}$, $\mathbf{R^{3c}}$, and $\mathbf{R^{4c}}$ are independently selected from hydrogen, $C_{1\text{-}6}$alkyl, and -(C=O)-$\mathbf{R^{5c}}$;

$\mathbf{R^{5c}}$ is independently, at each occurrence, absent, or selected from hydrogen; and $C_{1\text{-}6}$alkyl;

each **AA** is an independently selected amino acid;

$\mathbf{SP^1}$ is absent, or a spacer;

$\mathbf{RG^1}$ is a reactive group residue;

BA is an anti-MSR1 antibody or antigen binding fragment thereof;

subscript **n** is an integer from 1 to 30;

subscript **w** is 2, 3, or 4;

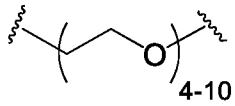
wherein $\mathbf{R^{1c}}$ is bonded to the linker via a nitrogen atom as indicated by the wavy line .

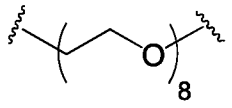
42. The antibody- rifamycin analog conjugate of claim 41, wherein $\mathbf{X^{1c}}$ is O.

43. The antibody- rifamycin analog conjugate of claim 41 or claim 42, wherein **BA** is an anti-MSR1 antibody or antigen binding fragment thereof; comprising an N297Q mutation.

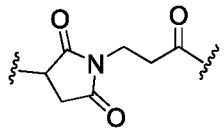
44. The antibody- rifamycin analog conjugate of any one of claims 41-43, wherein subscript **w** is 2.

45. The antibody- rifamycin analog conjugate of any one of claims 41-44, wherein $(\mathbf{AA})_2$ is valine-citrulline.

46. The antibody- rifamycin analog conjugate of any one of claims 41-45, wherein $\mathbf{SP^1}$ comprises  .

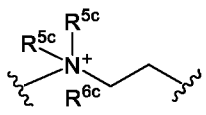
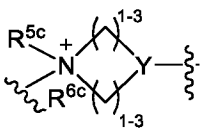
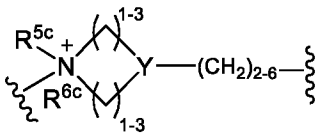
47. The antibody- rifamycin analog conjugate of any one of claims 41-45, wherein $\mathbf{SP^1}$ comprises  .

48. The antibody- rifamycin analog conjugate of any one of claims 41-47, wherein $\mathbf{RG^1}$ comprises



49. The antibody- rifamycin analog conjugate of any one of claims 41-47, wherein

$\mathbf{X^{1c}}$ is O;

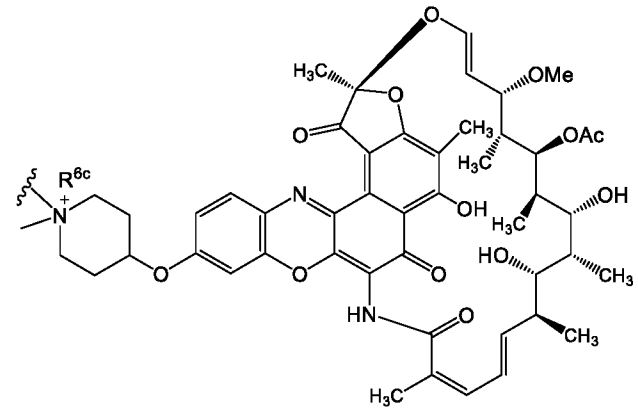
$\mathbf{R^{1c}}$ is  ,  ; or  ; wherein $\mathbf{Y}$ is C or N;

wherein $\mathbf{R^{1c}}$ is bonded to the linker via the quarternary nitrogen atom of $\mathbf{R^{1c}}$;

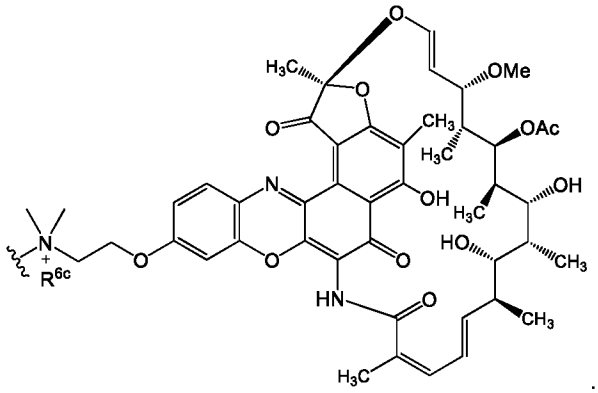
$\mathbf{R^{5c}}$ is $C_{1\text{-}6}$alkyl; and

$\mathbf{R^{6c}}$ is a counter ion.

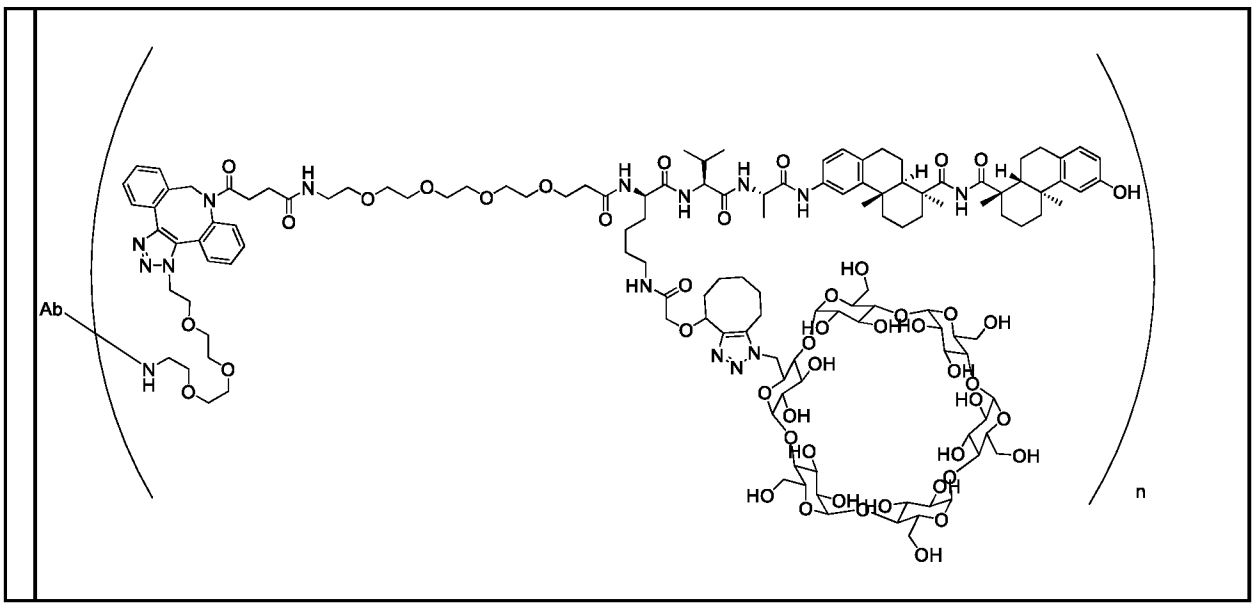
50. The antibody- rifamycin analog conjugate of any one of claims 41-49, wherein the rifamycin analog is:

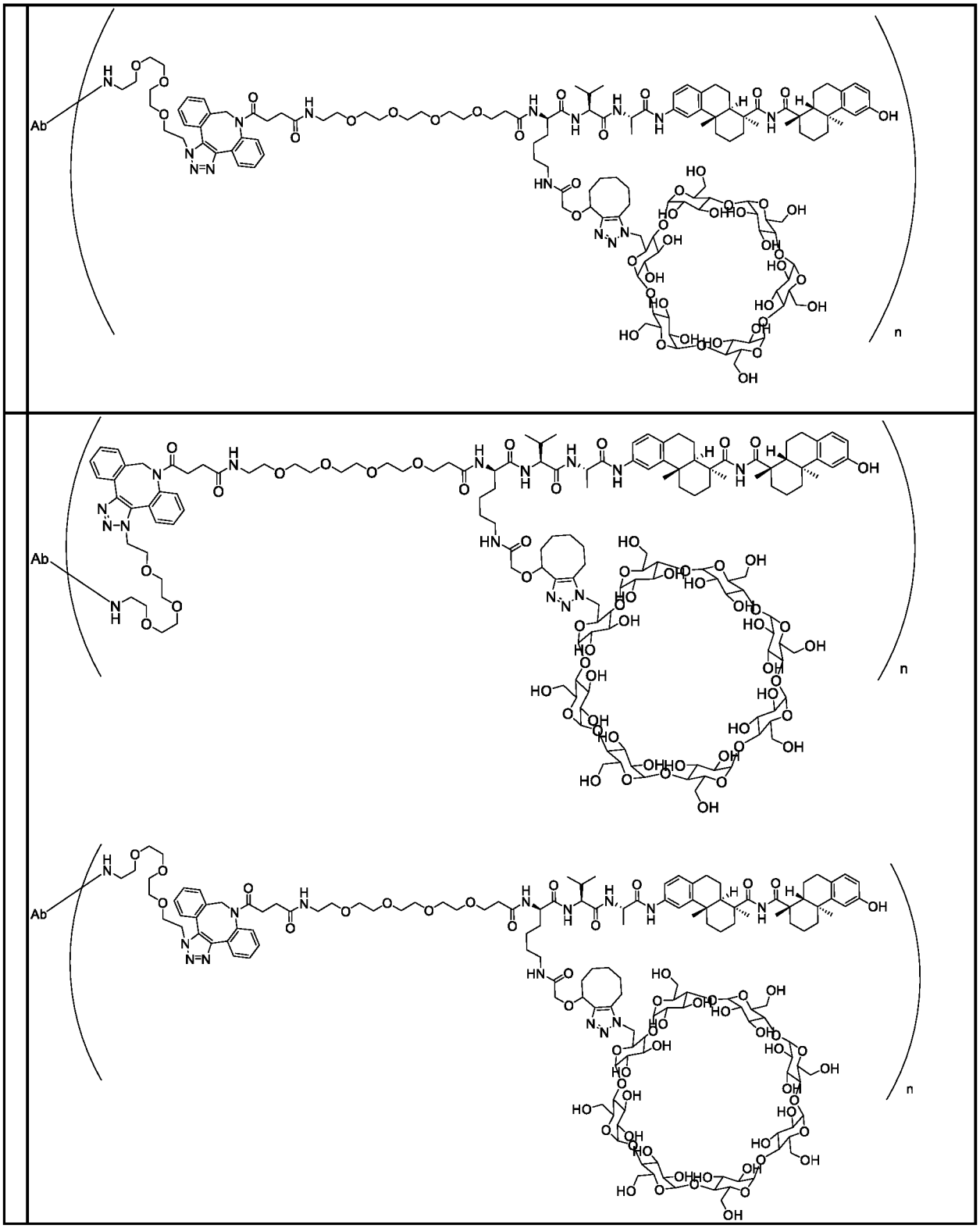


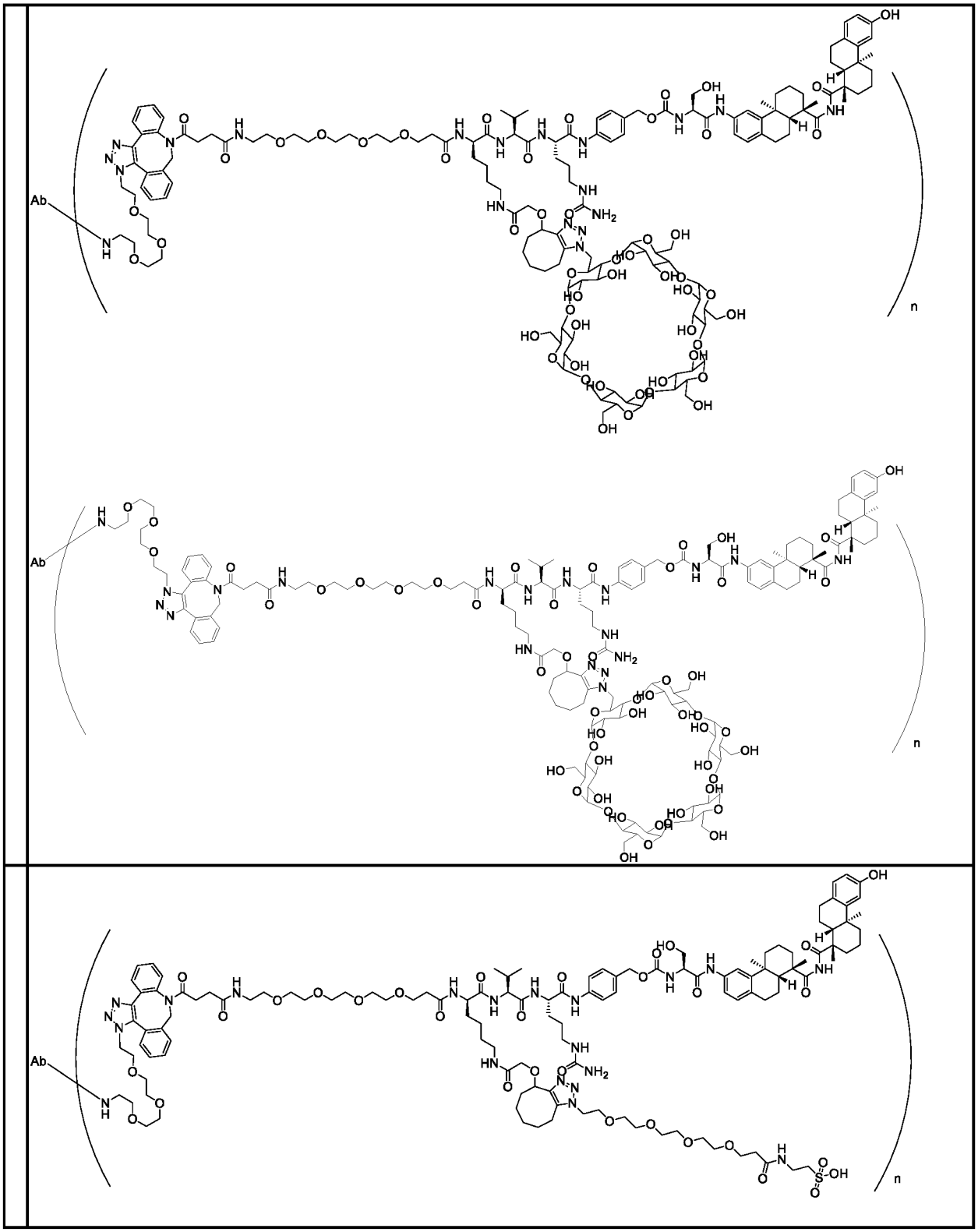
or

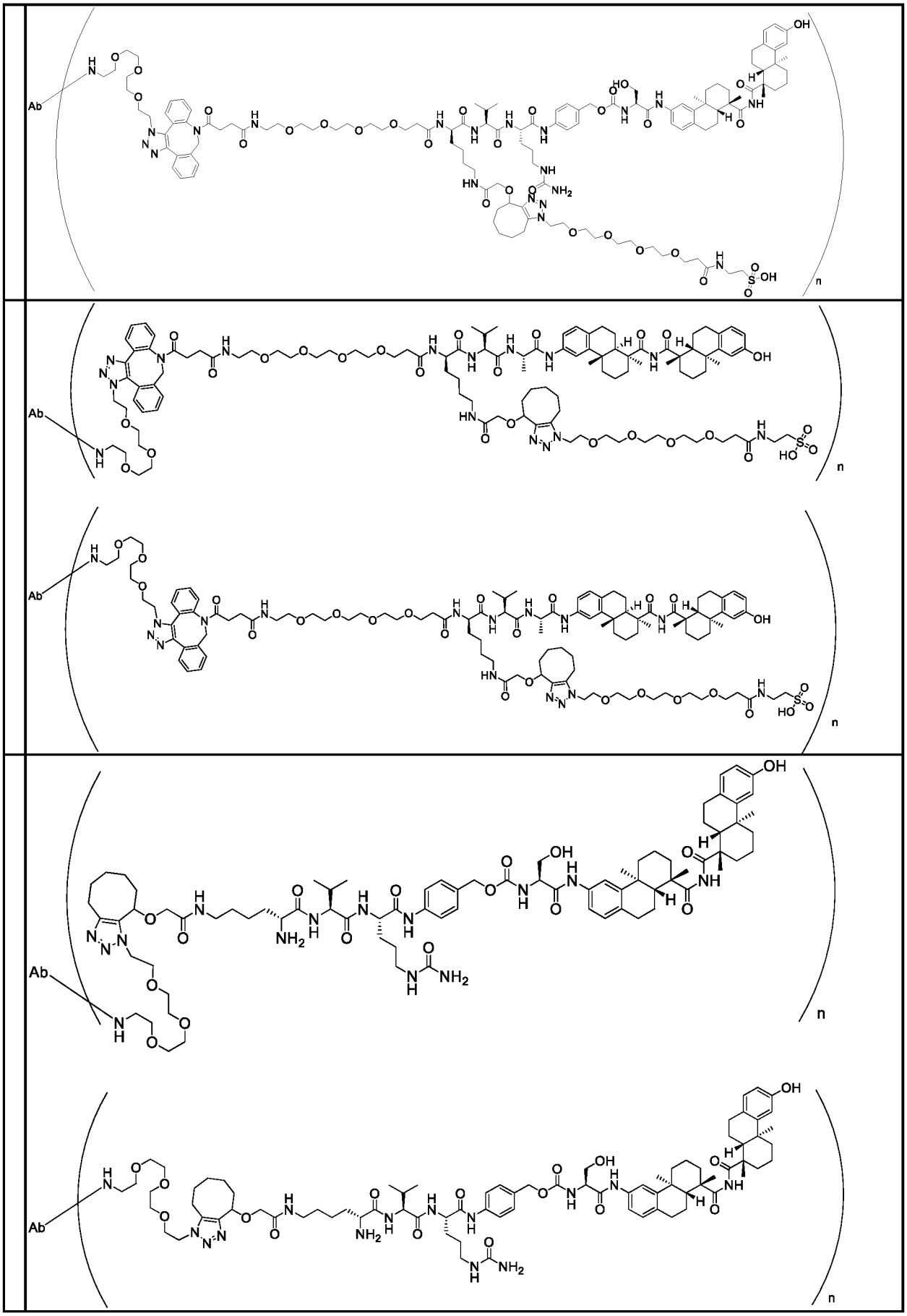


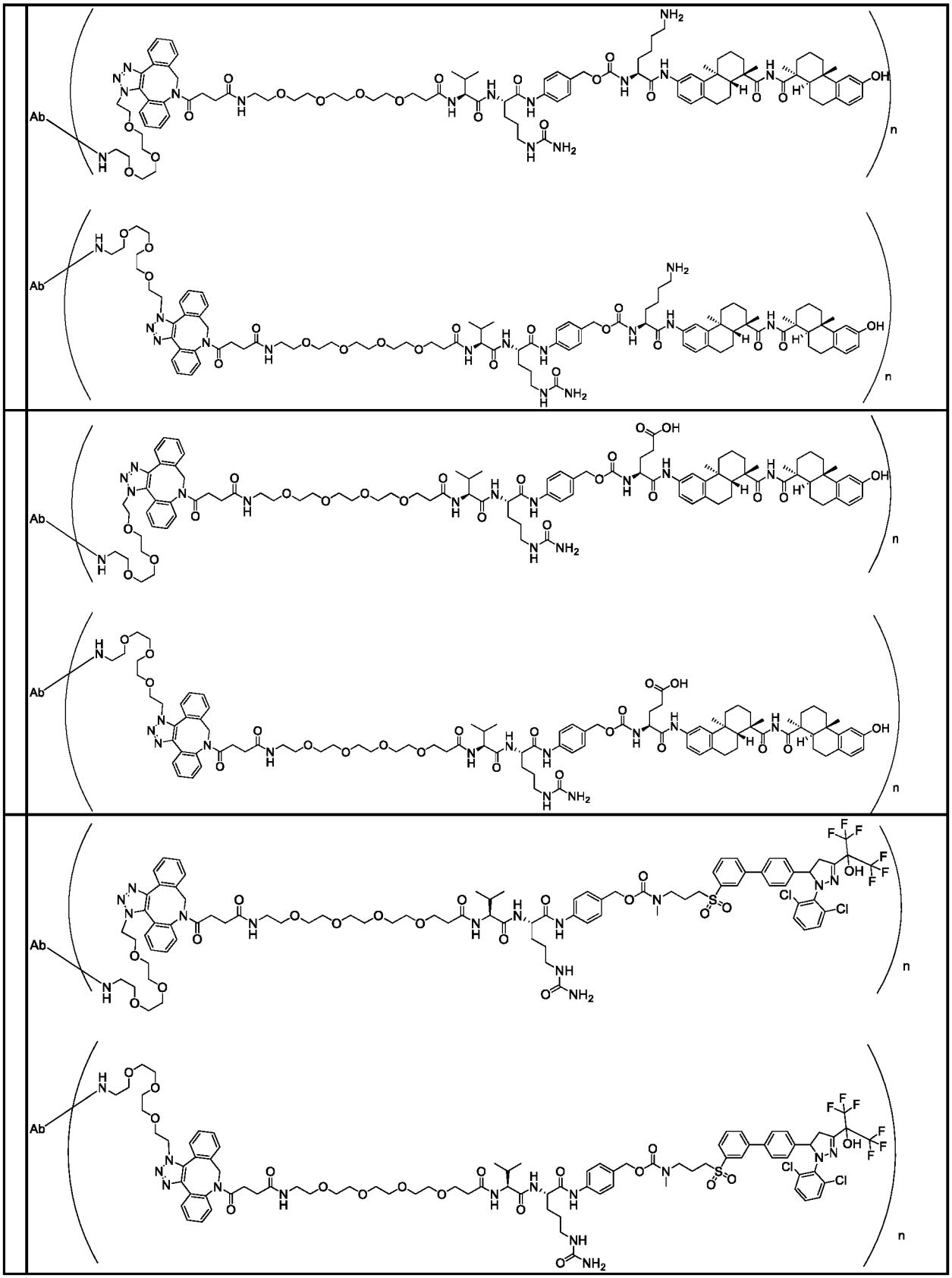
51. The antibody-drug conjugate of any one of claims 9-24, selected from the group consisting of:

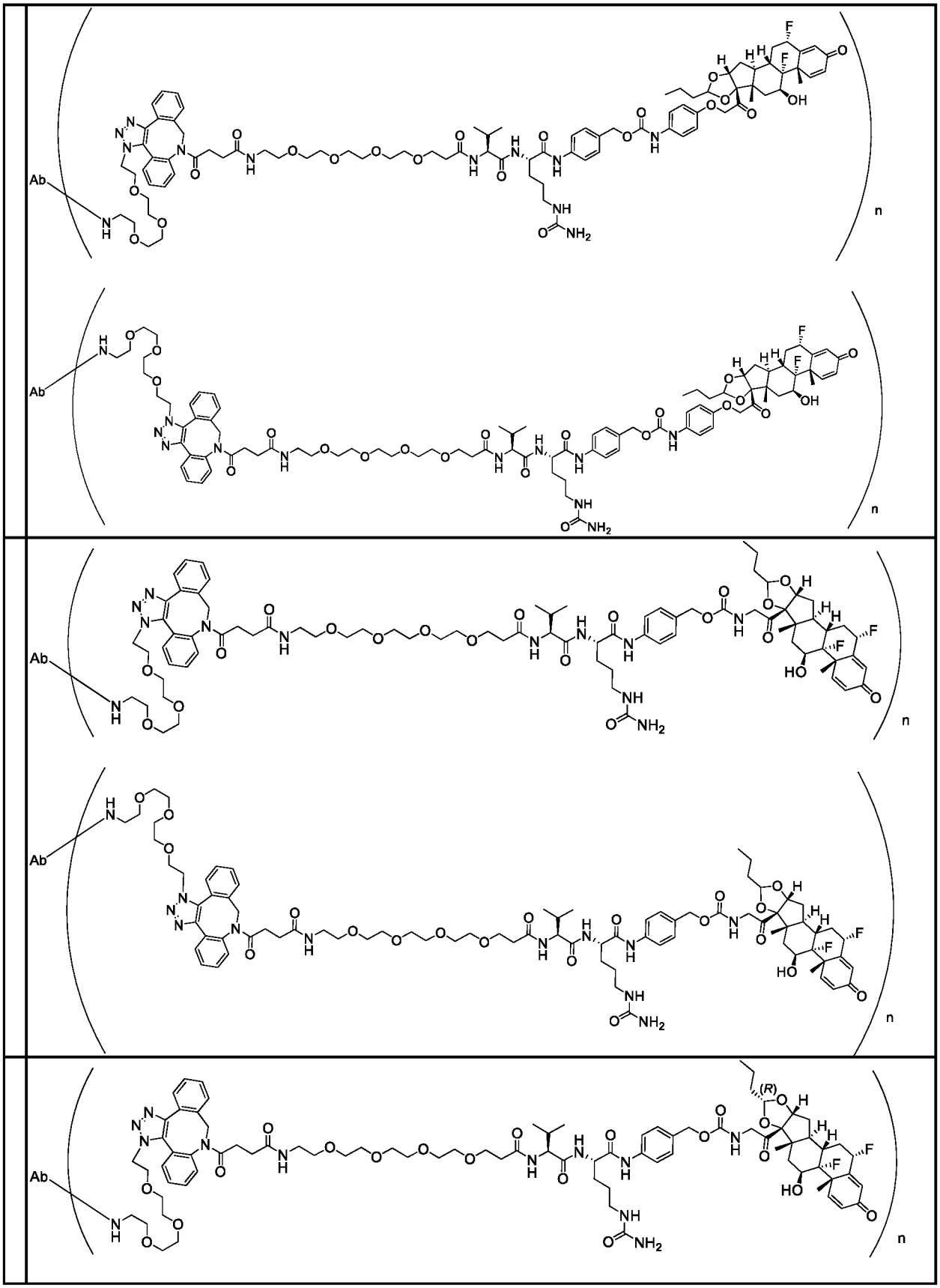


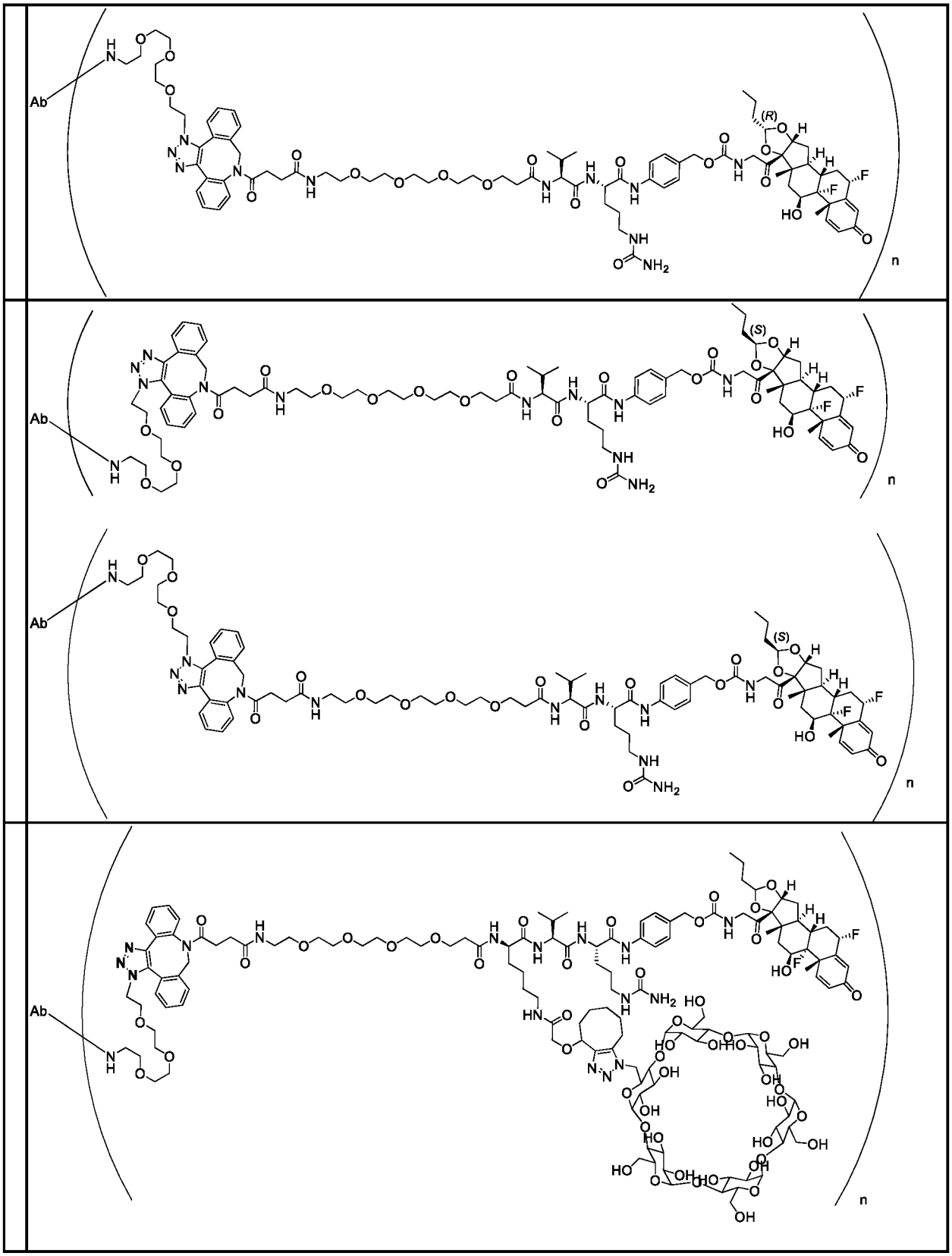


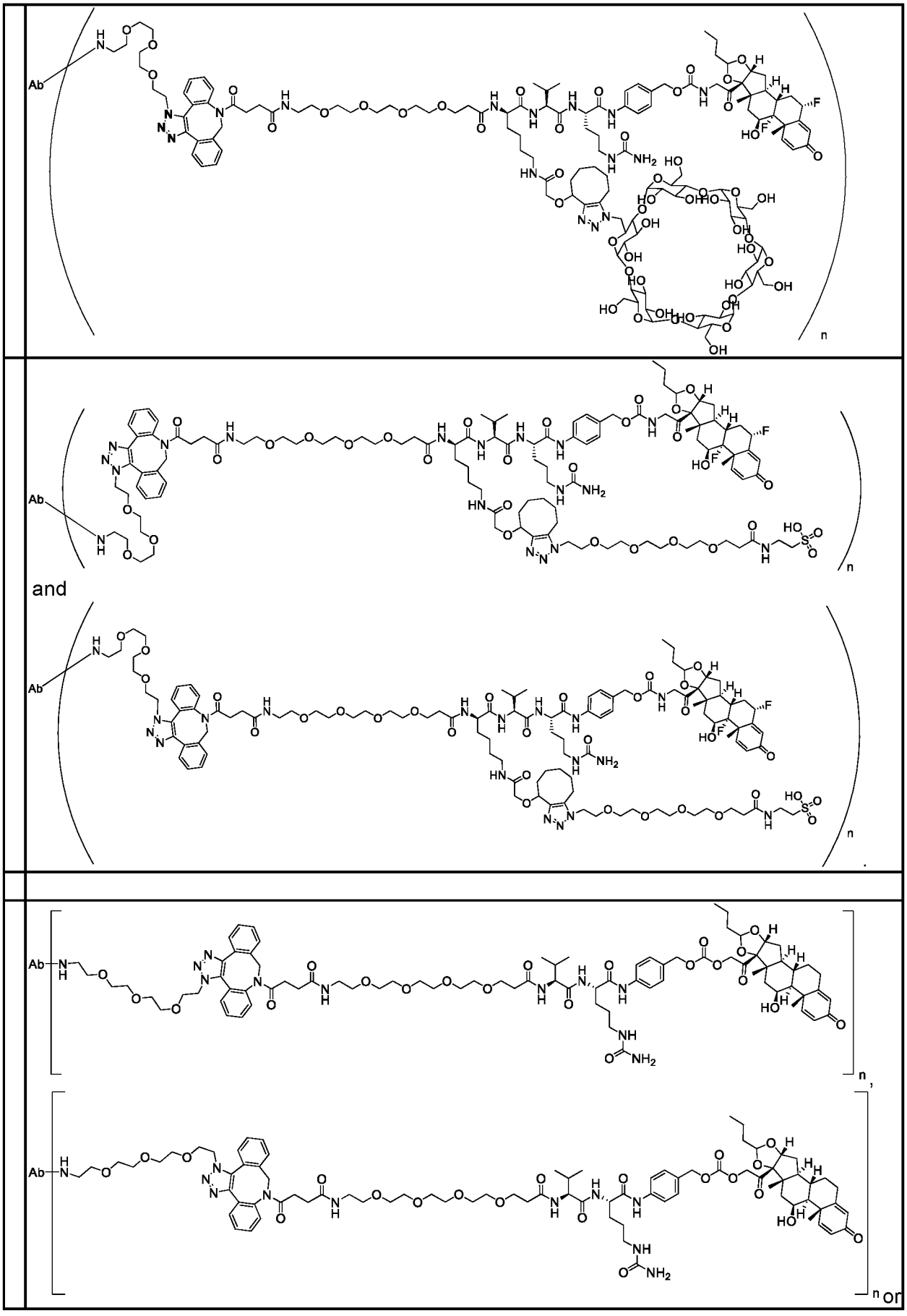

Ab
n
Ab
n
Ab
n


Ab
n
OH
NH2
HO
OH

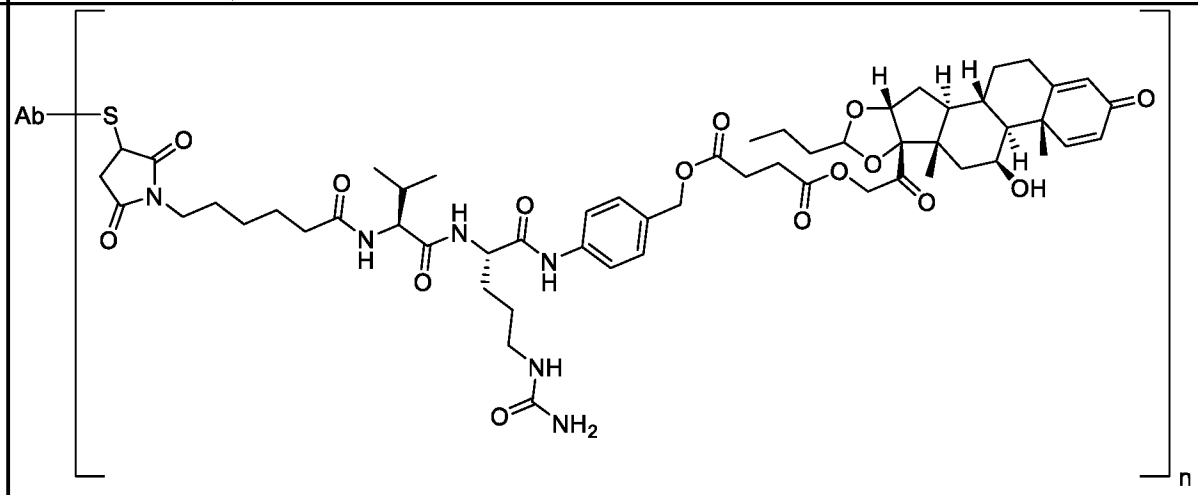




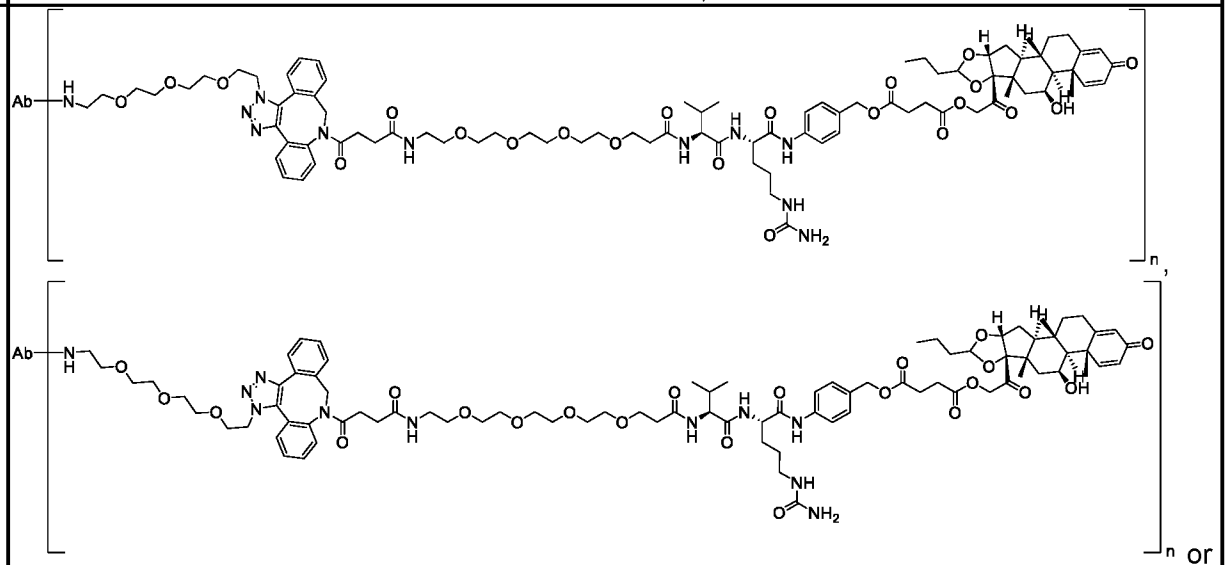



and
or

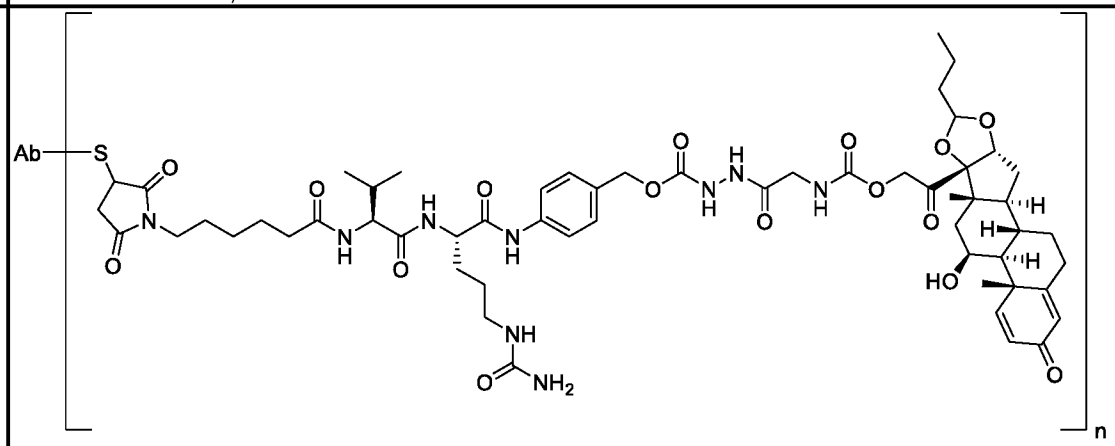
a mixture thereof;



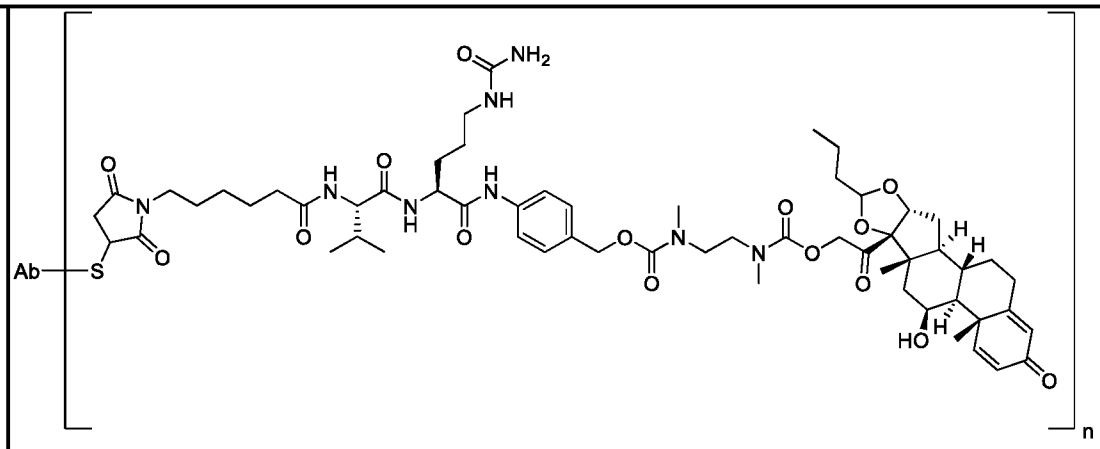
or a stereoisomer or mixture of stereoisomers thereof;



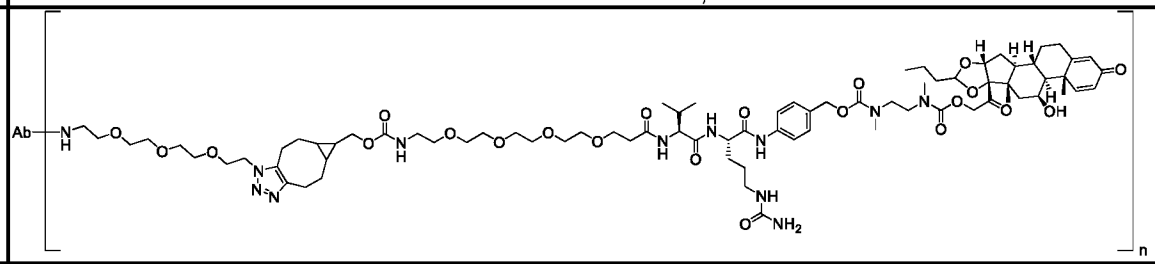
a mixture thereof; or

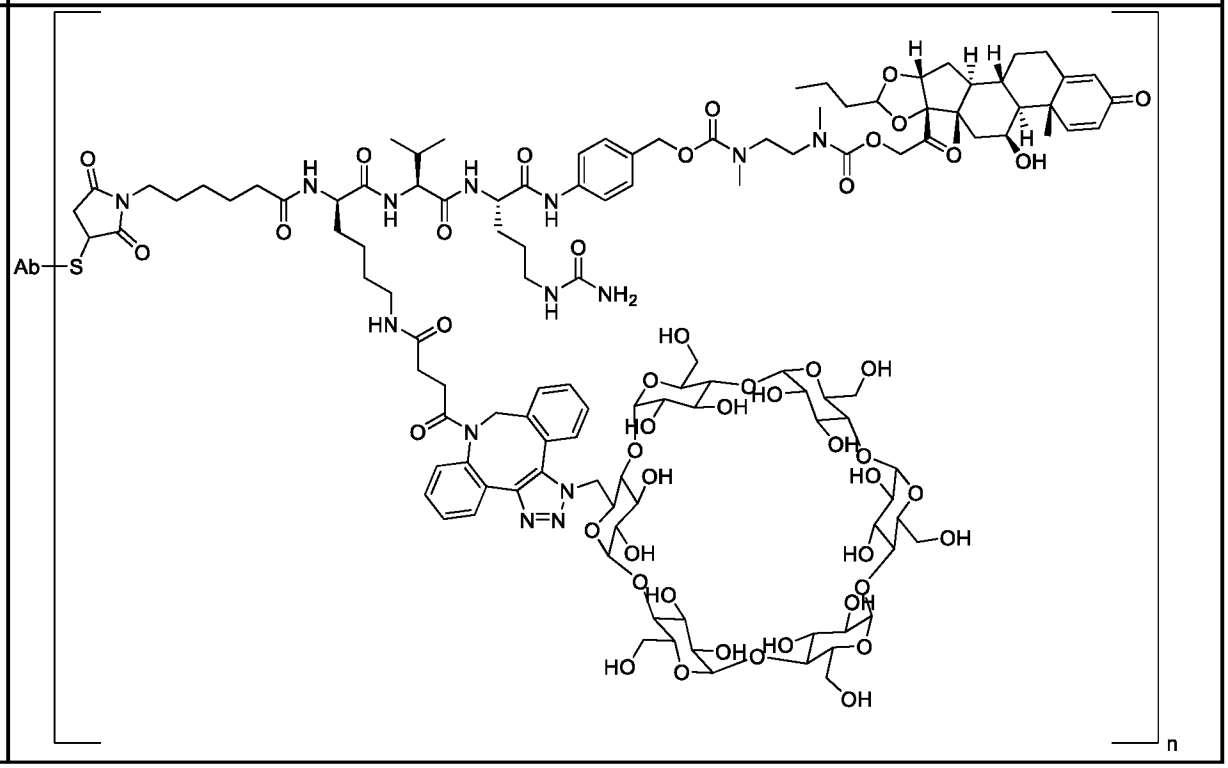


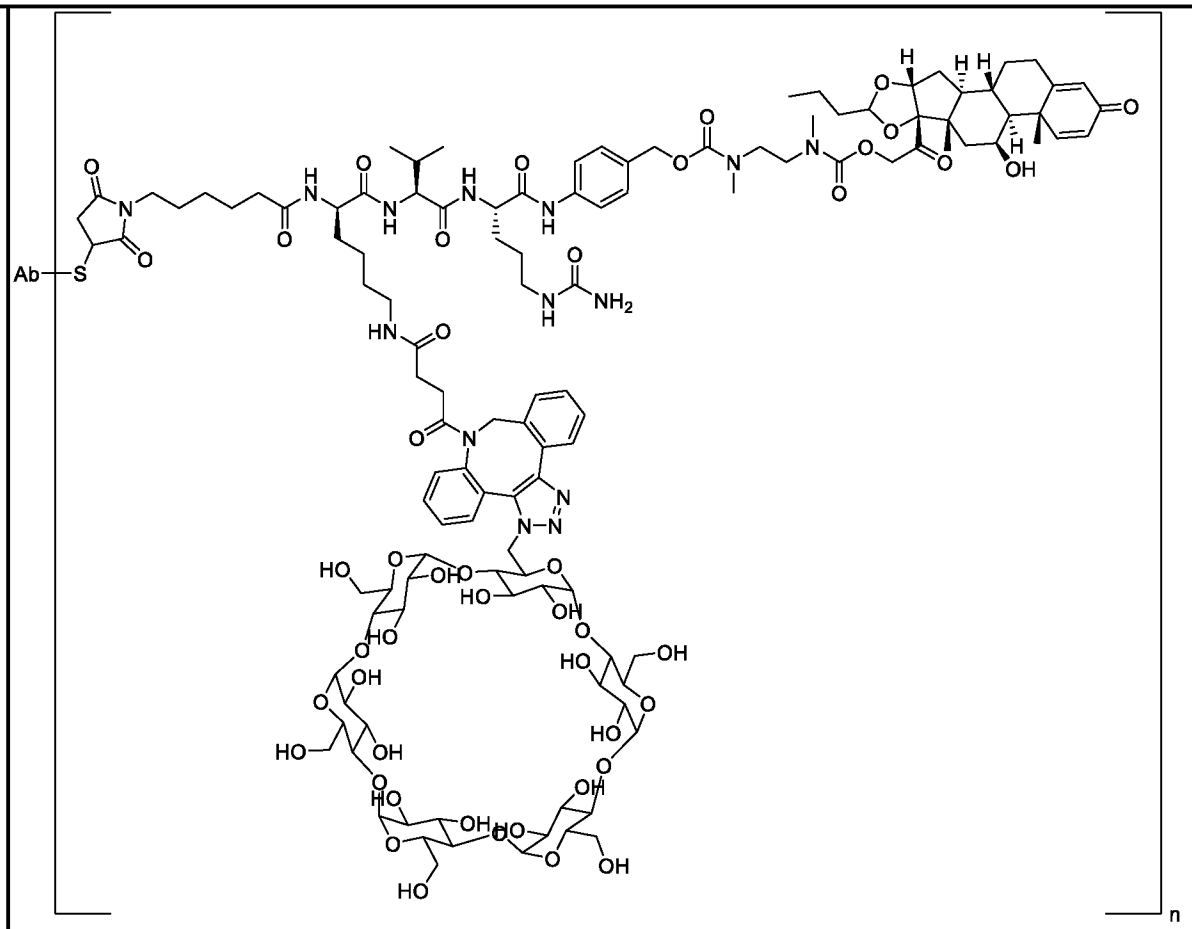
or a stereoisomer or mixture of stereoisomers thereof;



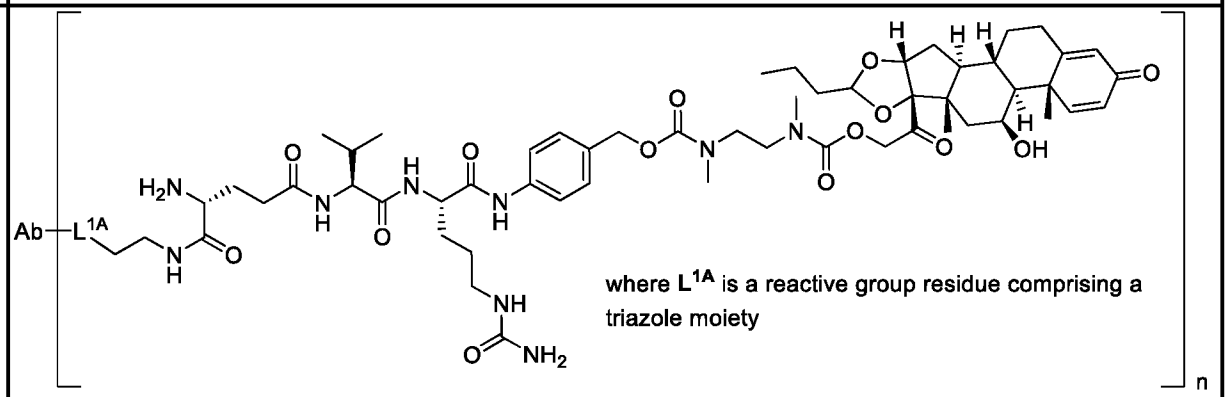
or a stereoisomer or mixture of stereoisomers thereof;



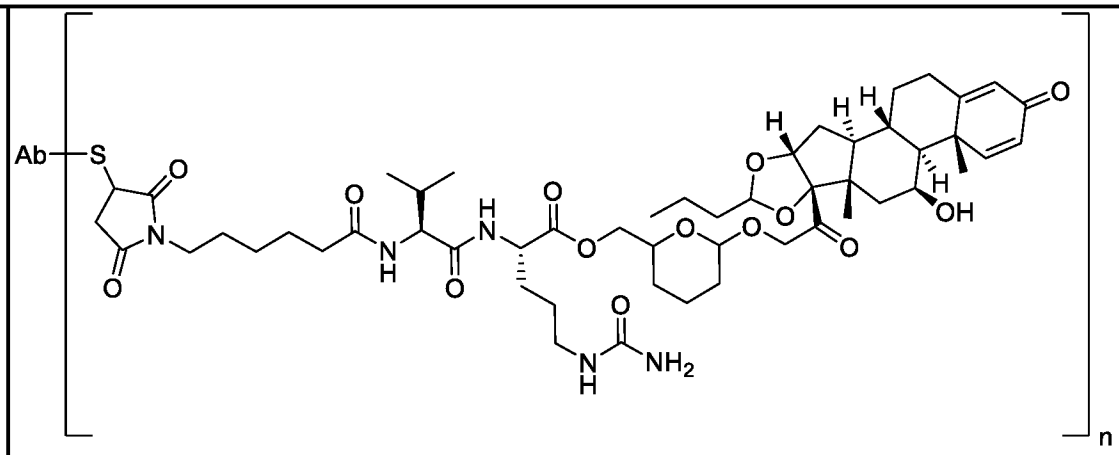




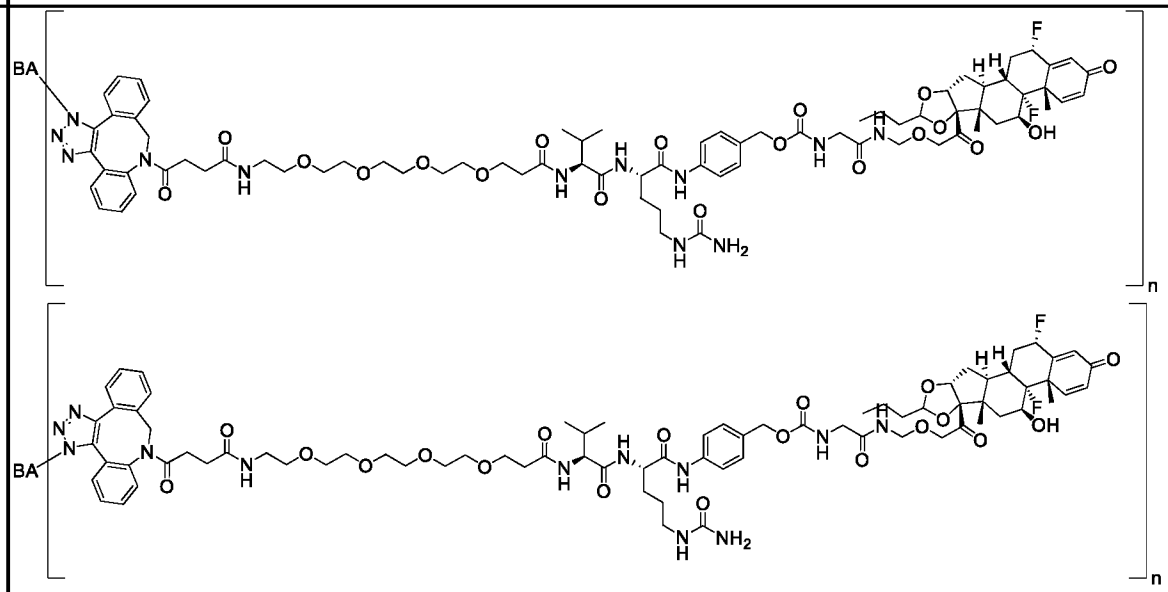
or a mixture thereof



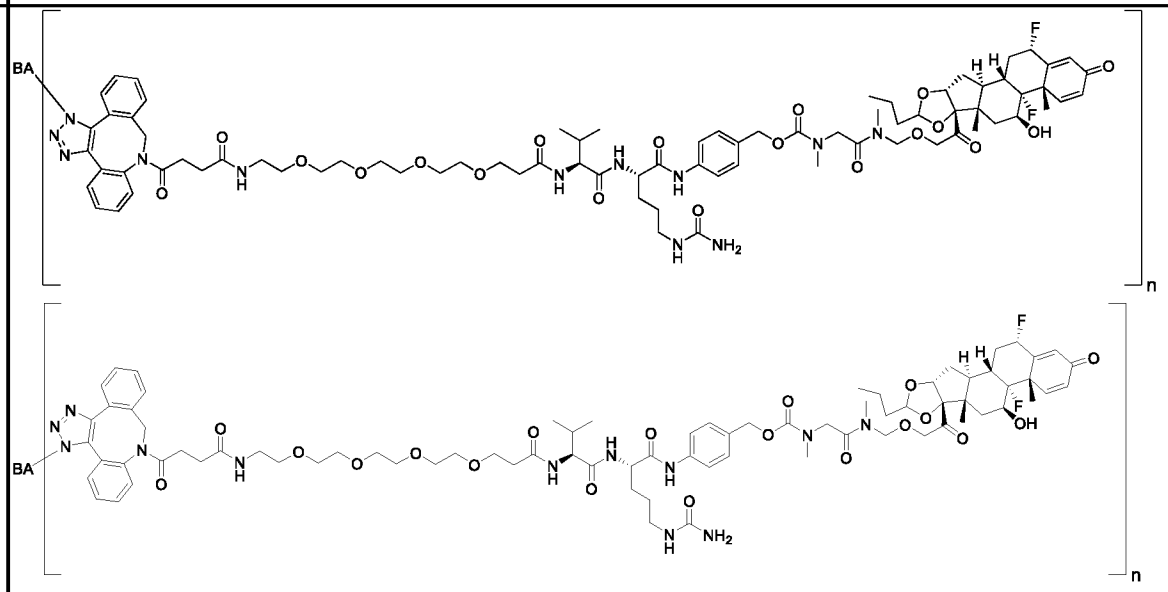
or a mixture thereof



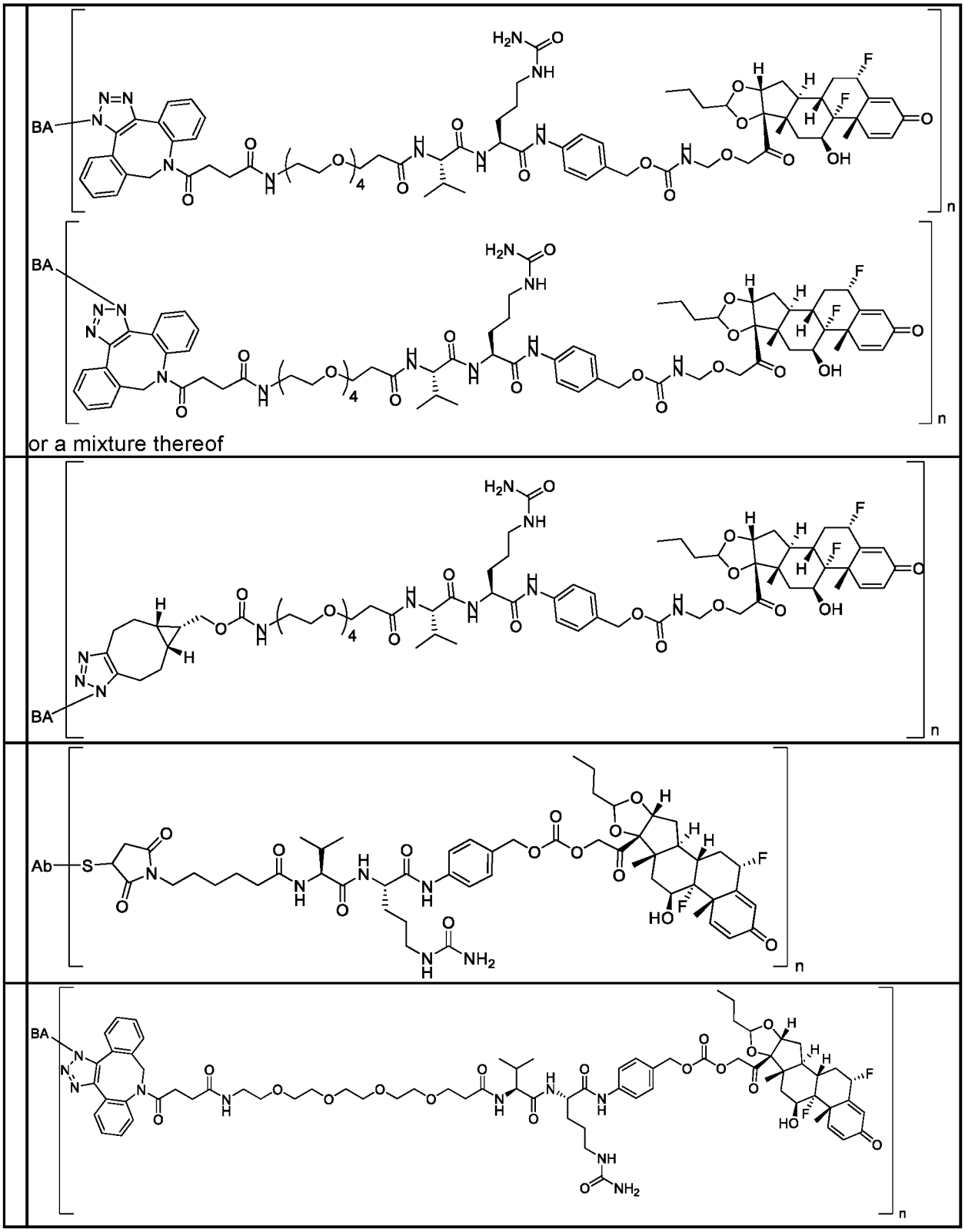
or a stereoisomer or mixture of stereoisomers thereof

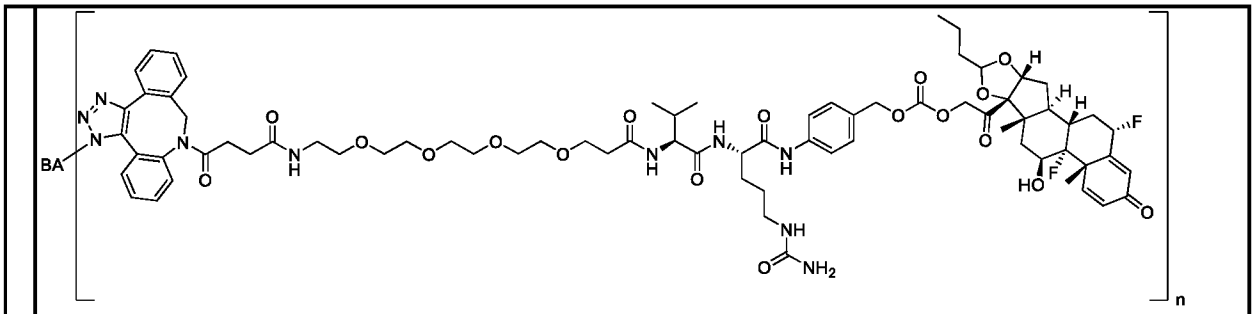


or a mixture thereof

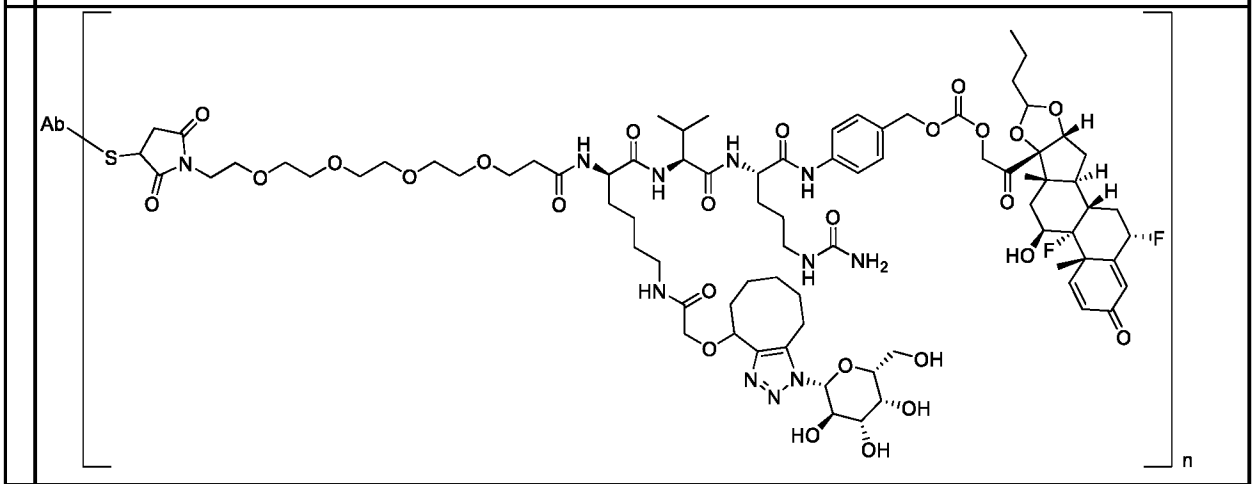


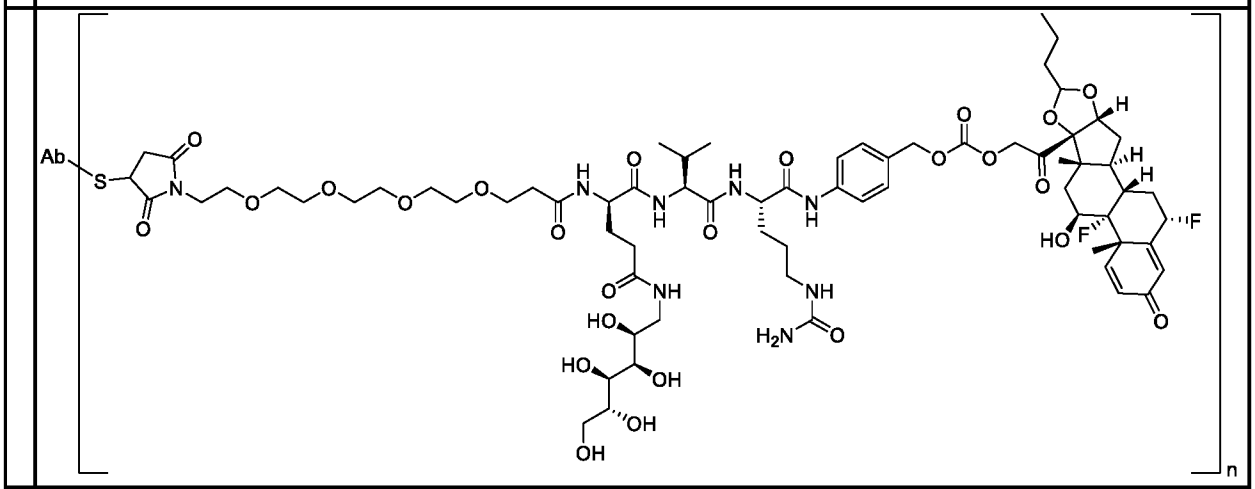
or a mixture thereof

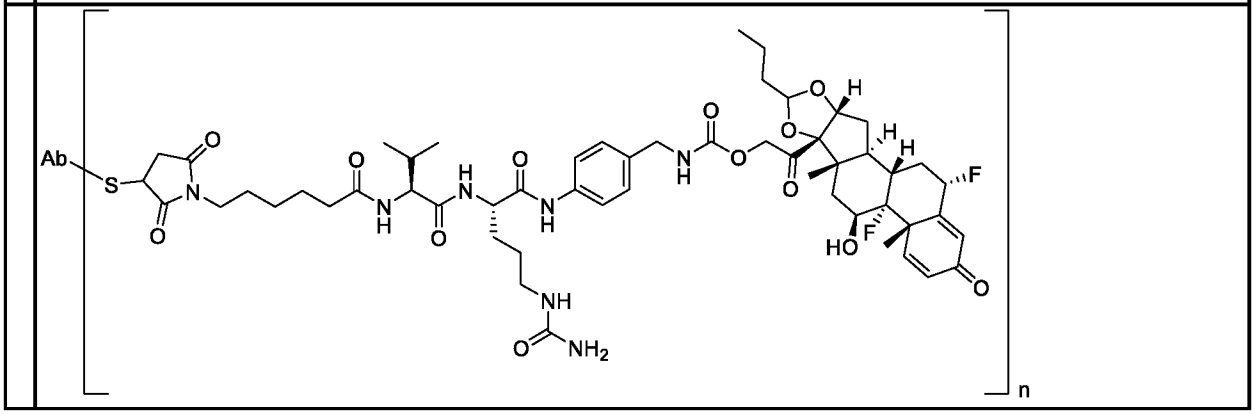

BA
Ab
or a mixture thereof

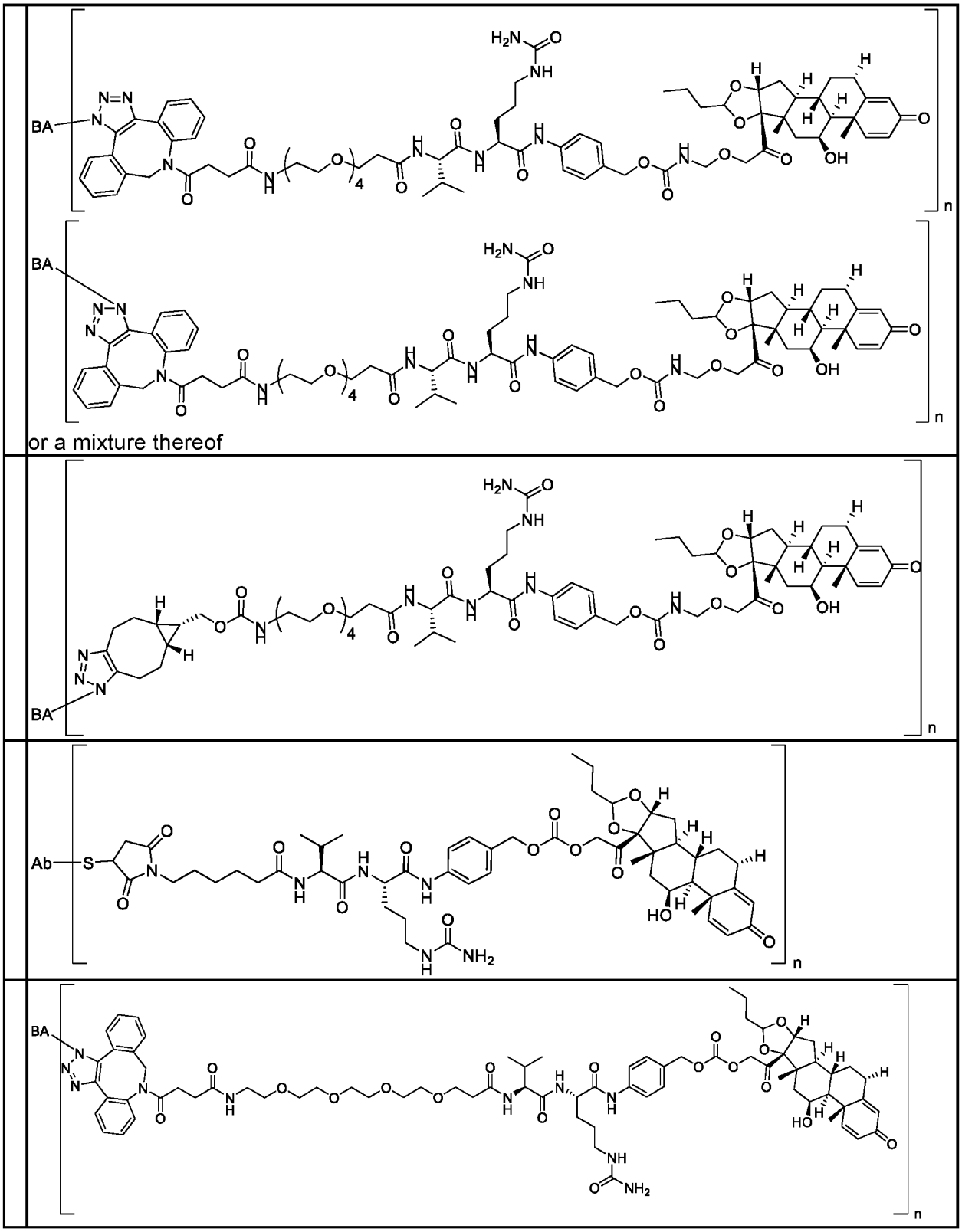


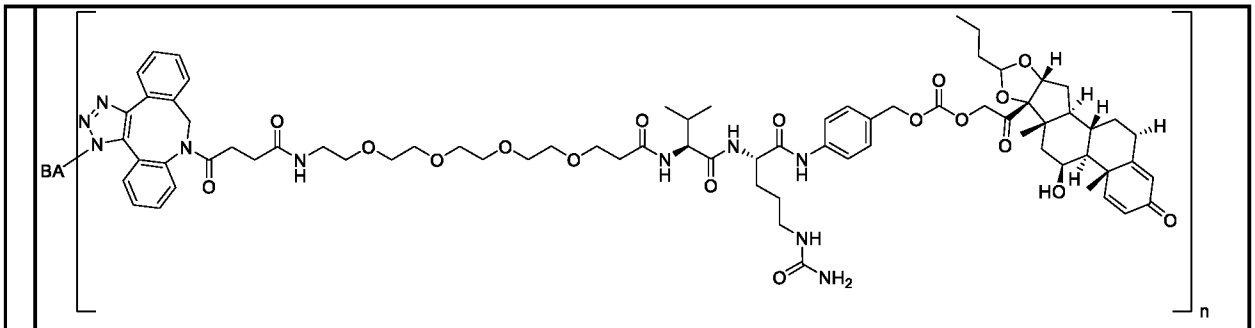
or a mixture thereof



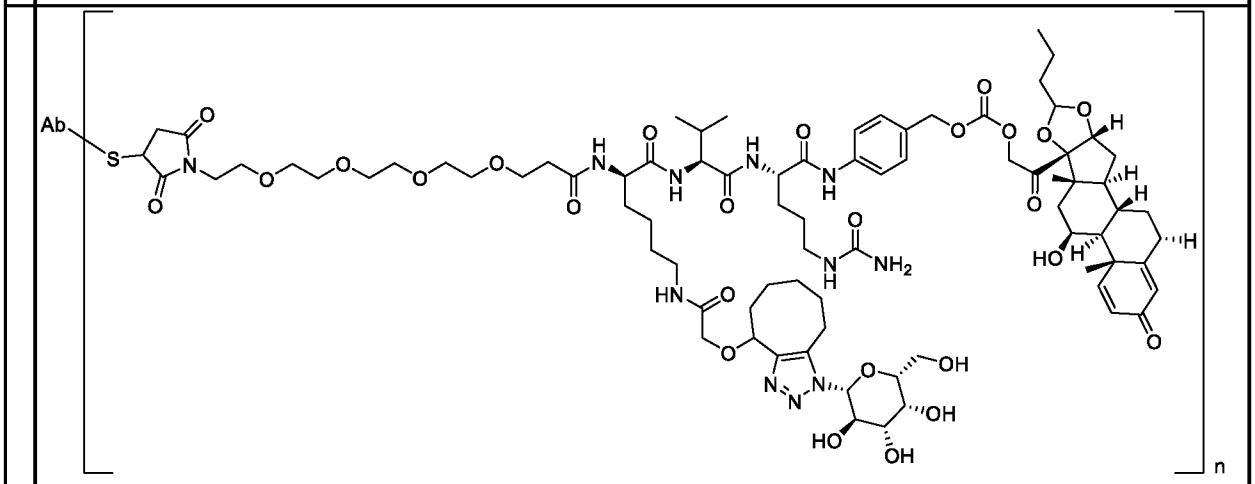


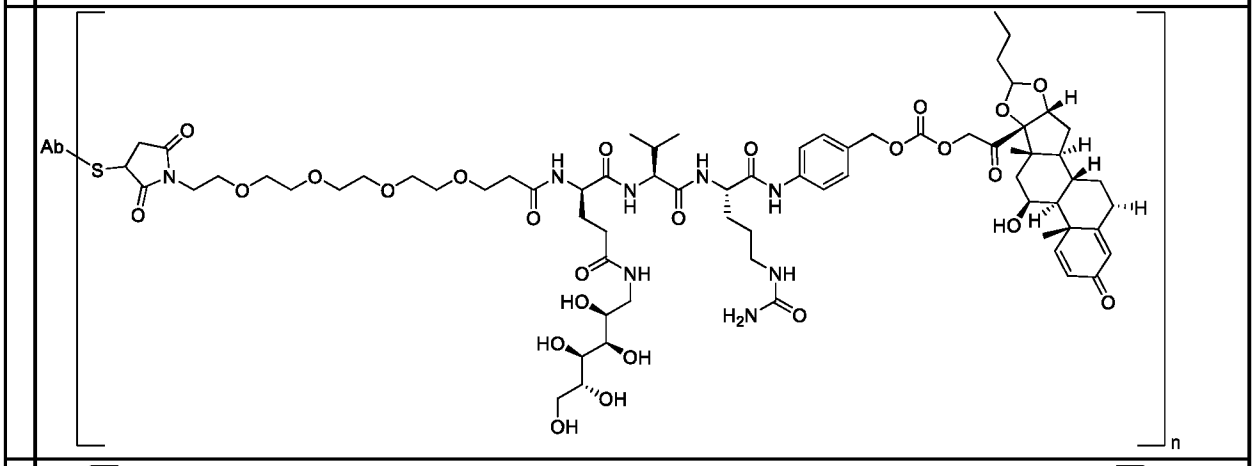


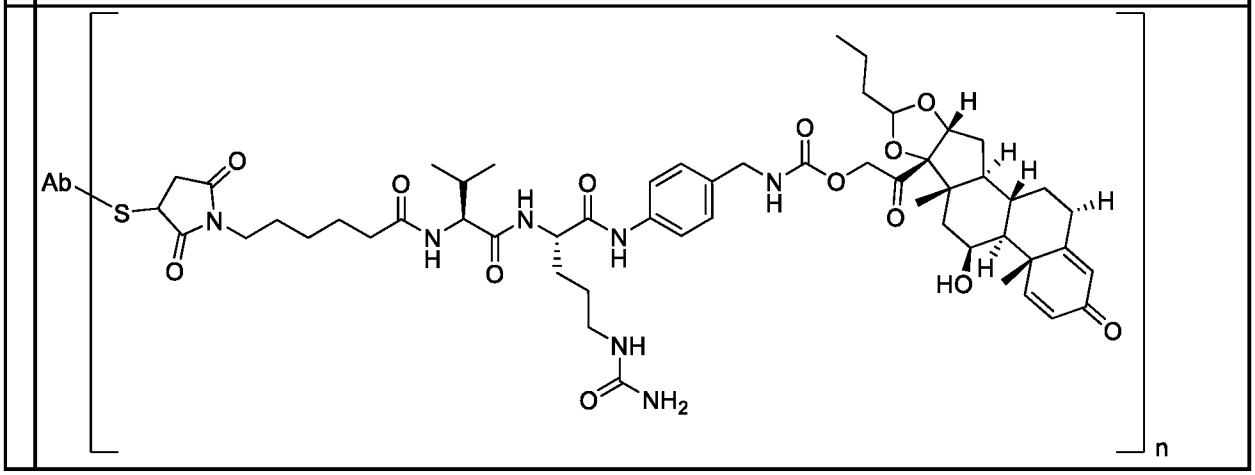

BA
Ab
or a mixture thereof

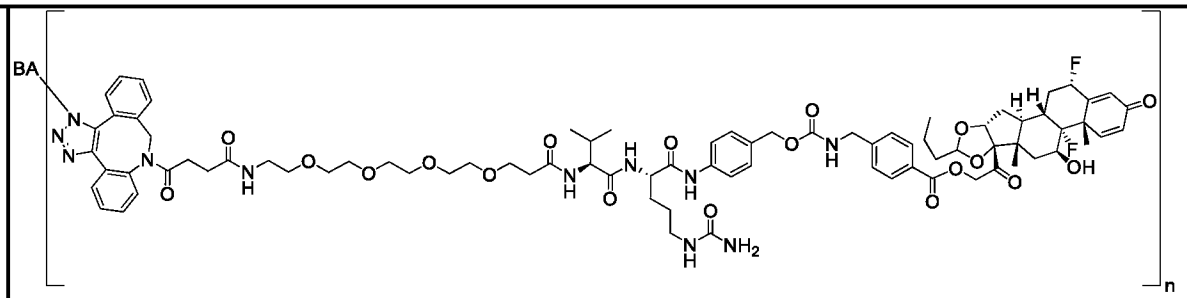


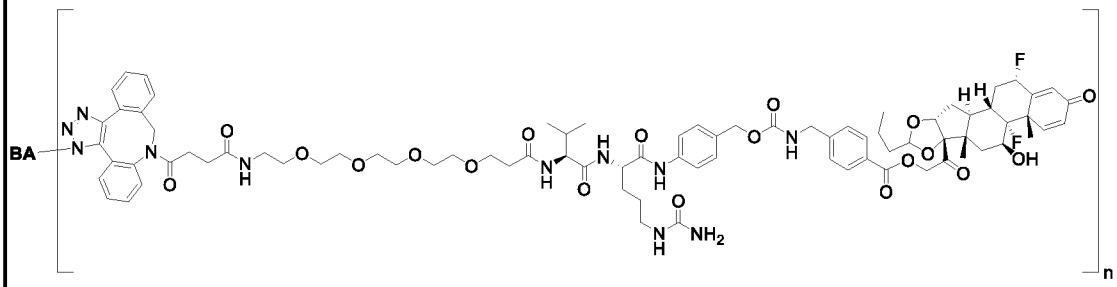
or a mixture thereof



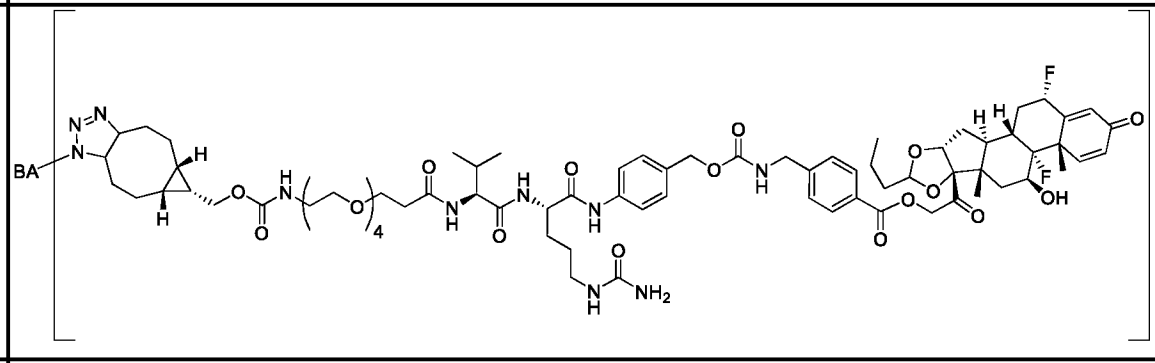


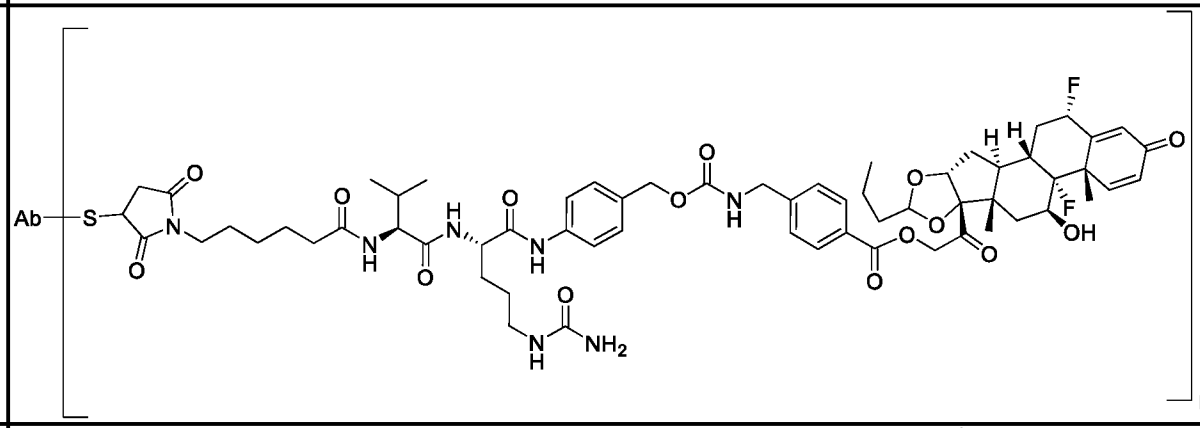


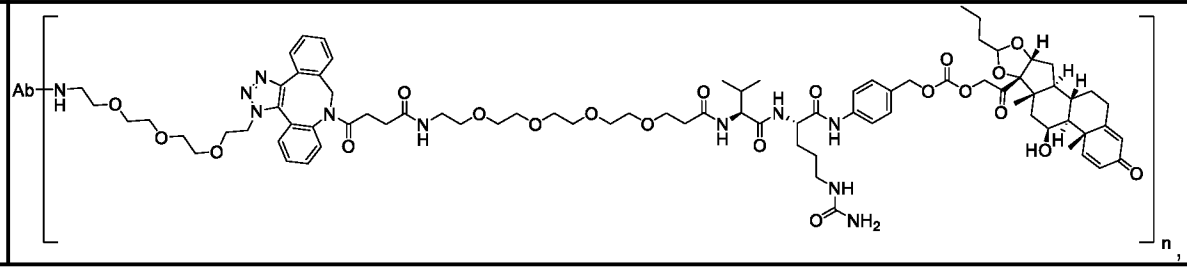


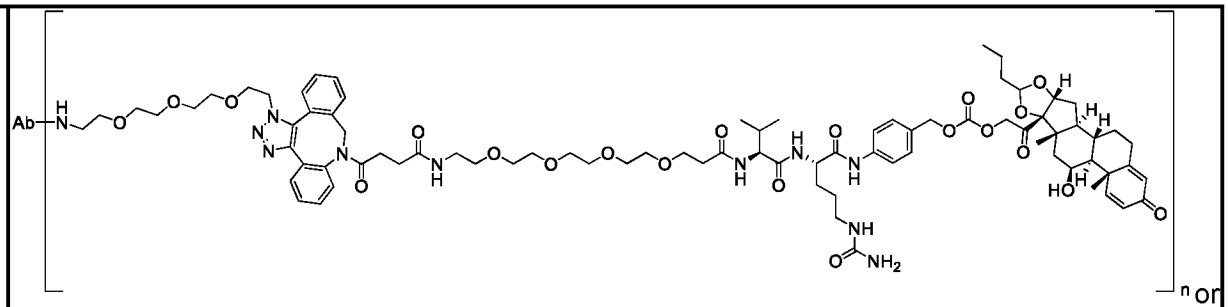


or a mixture thereof



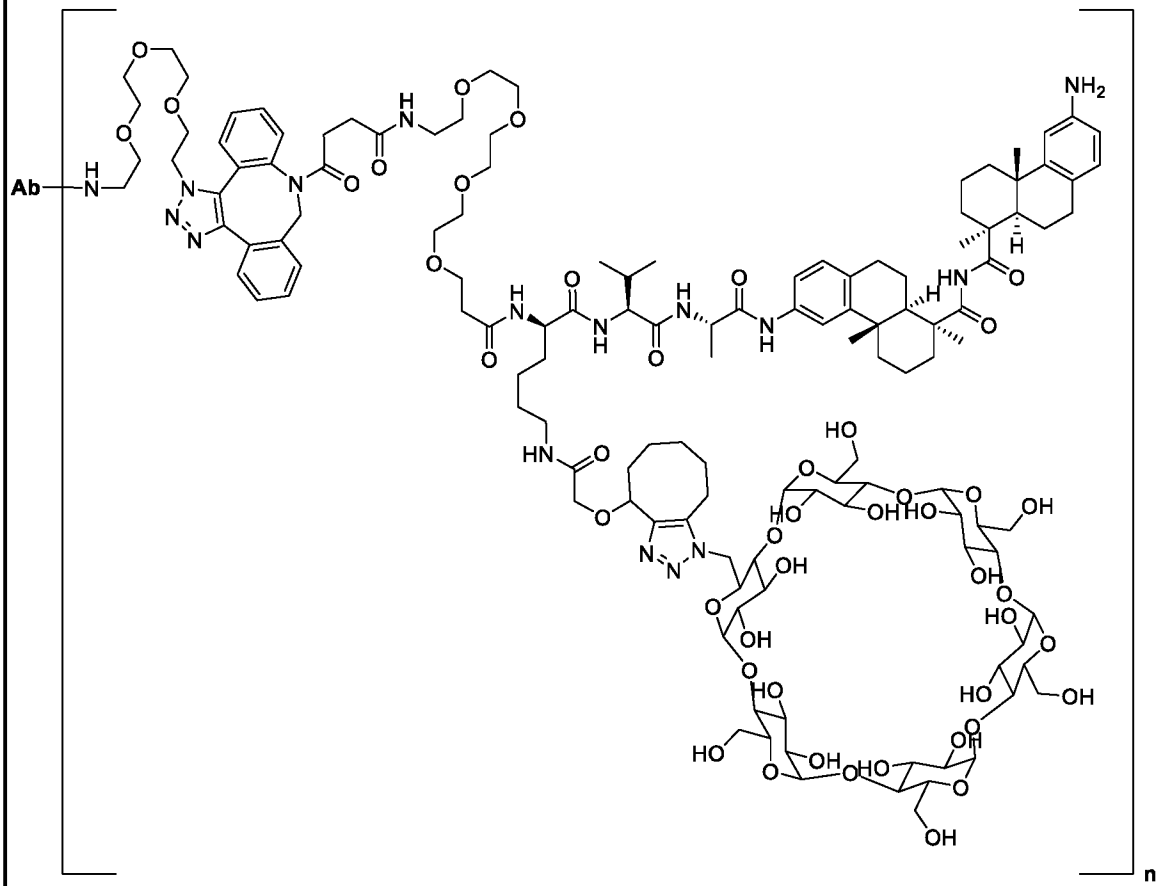


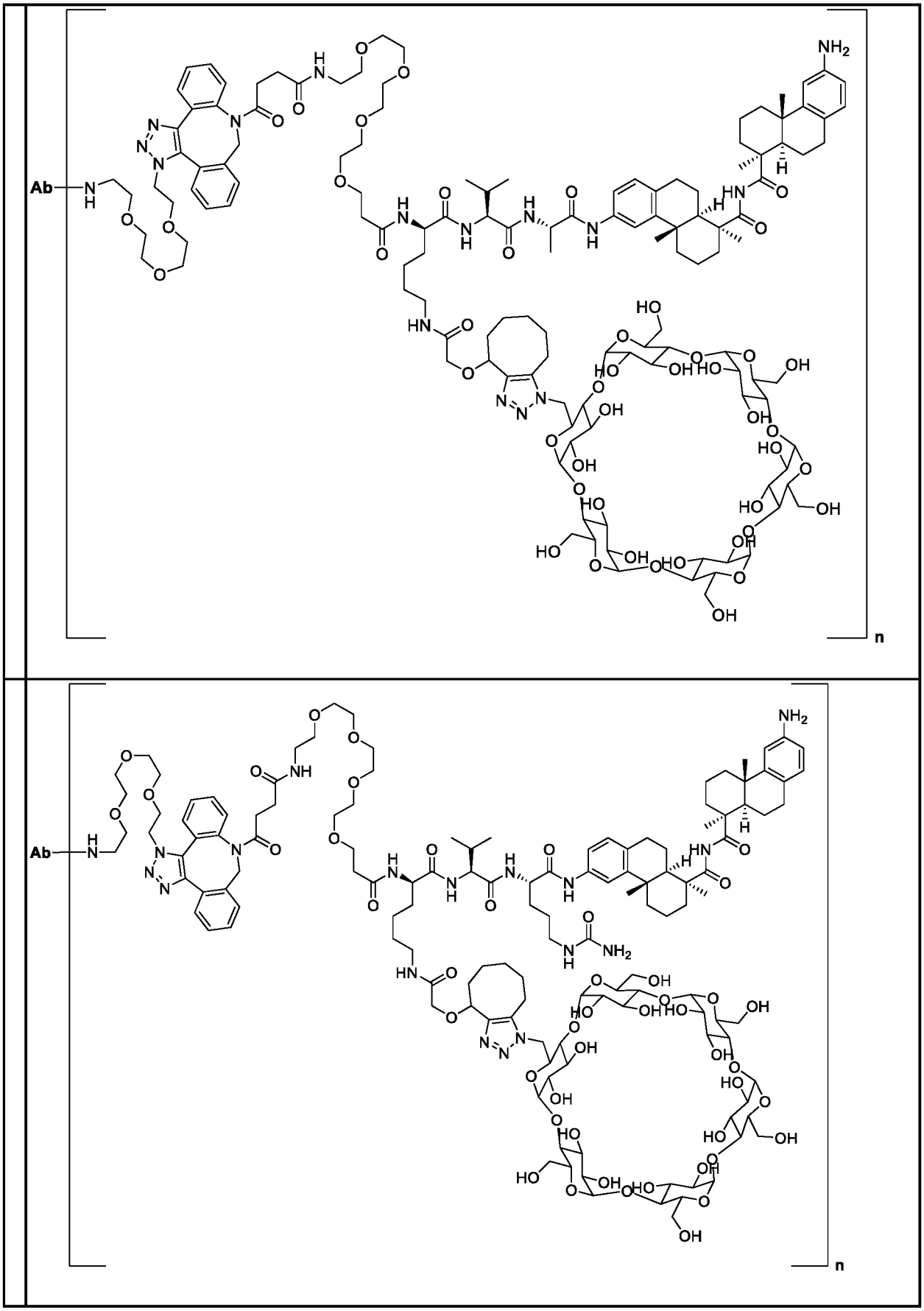


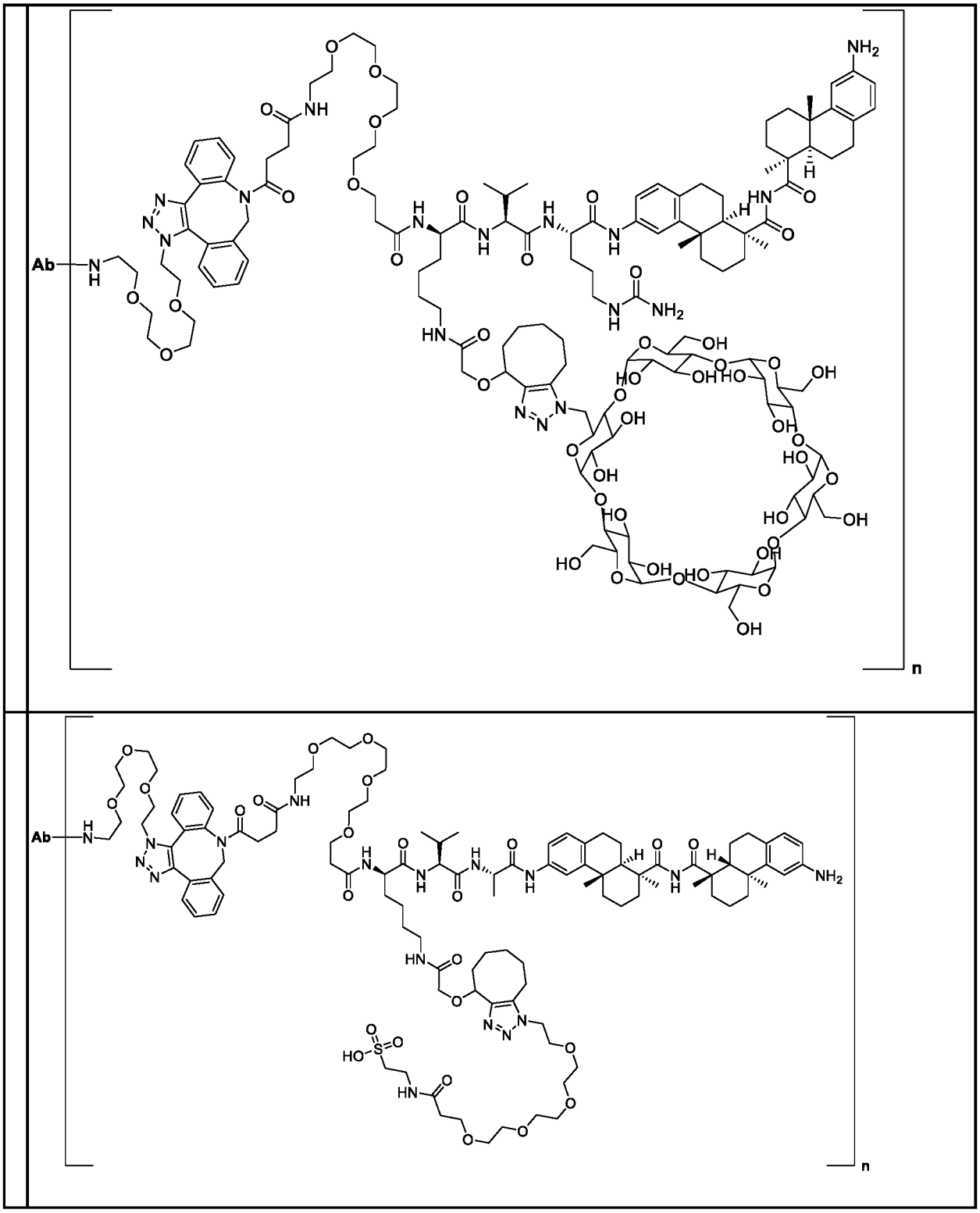


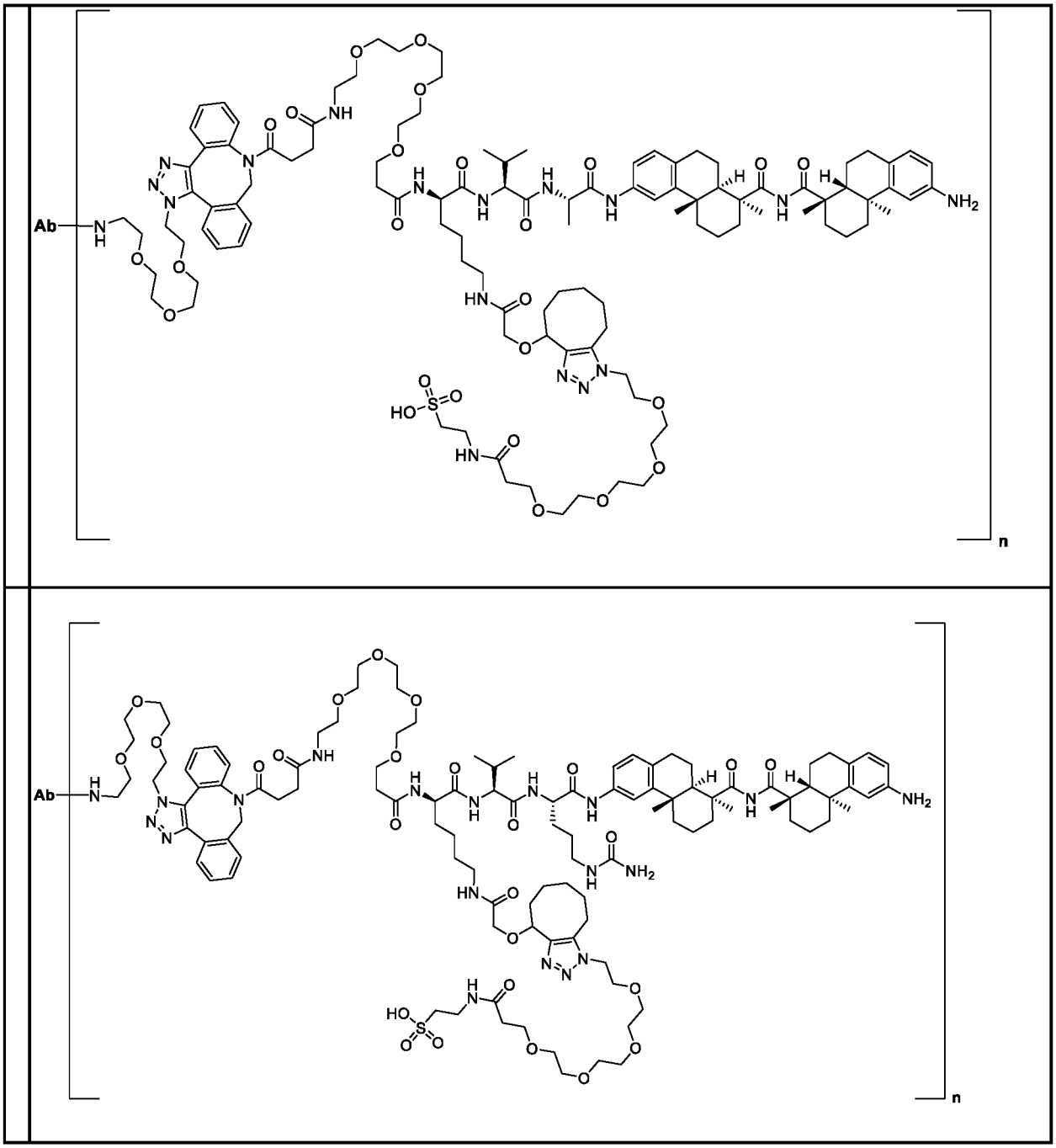
or

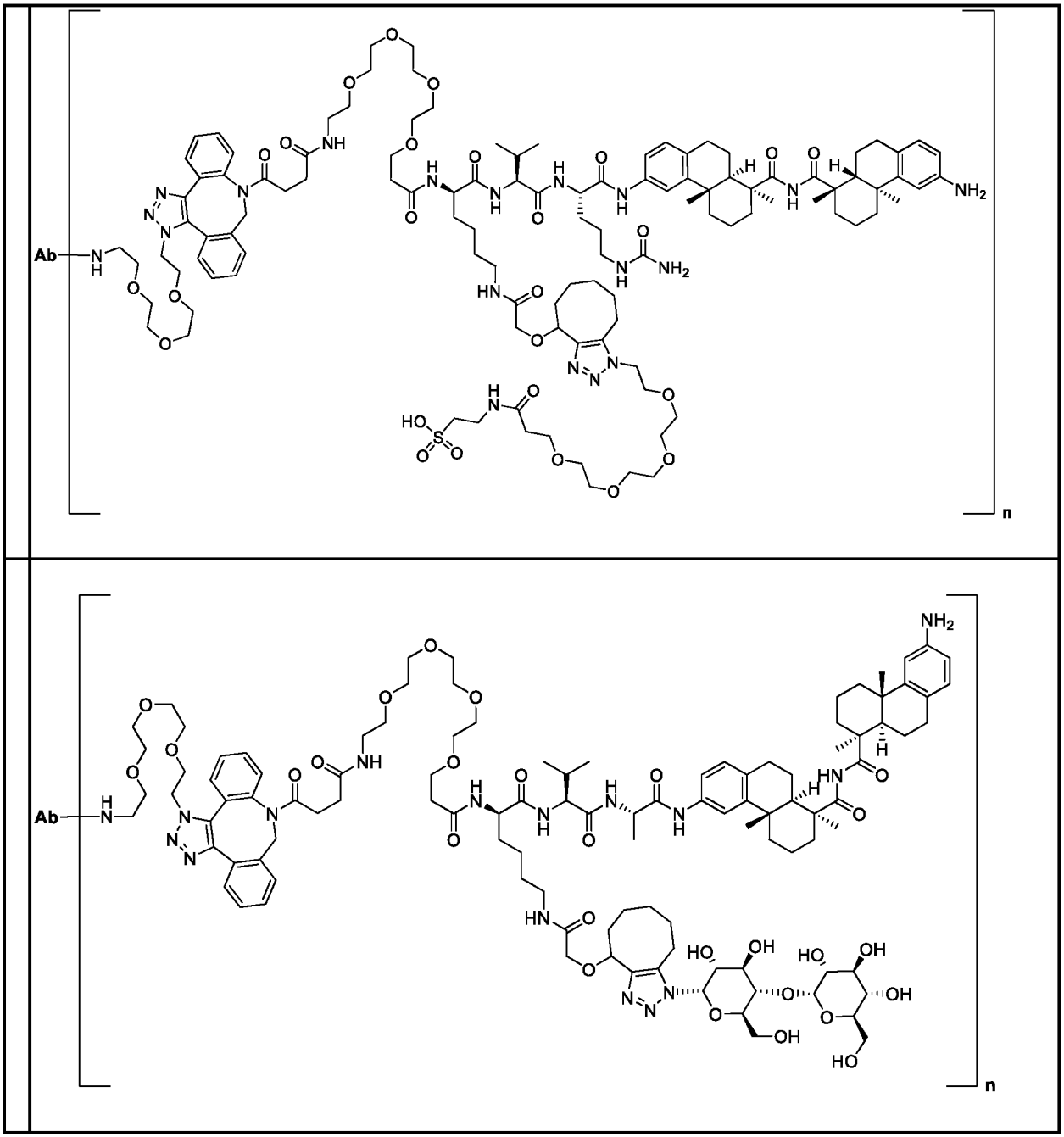
a mixture thereof;

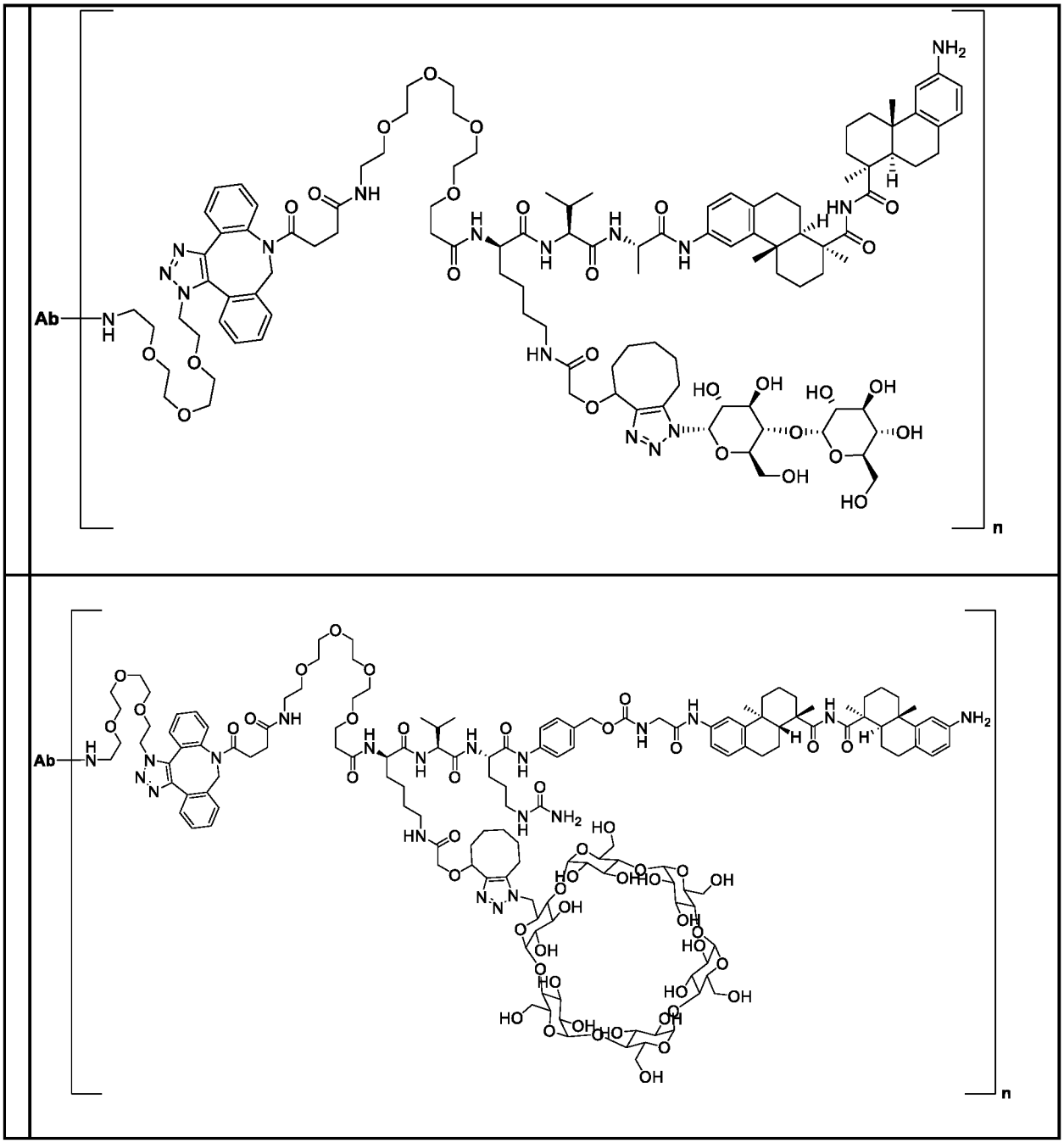


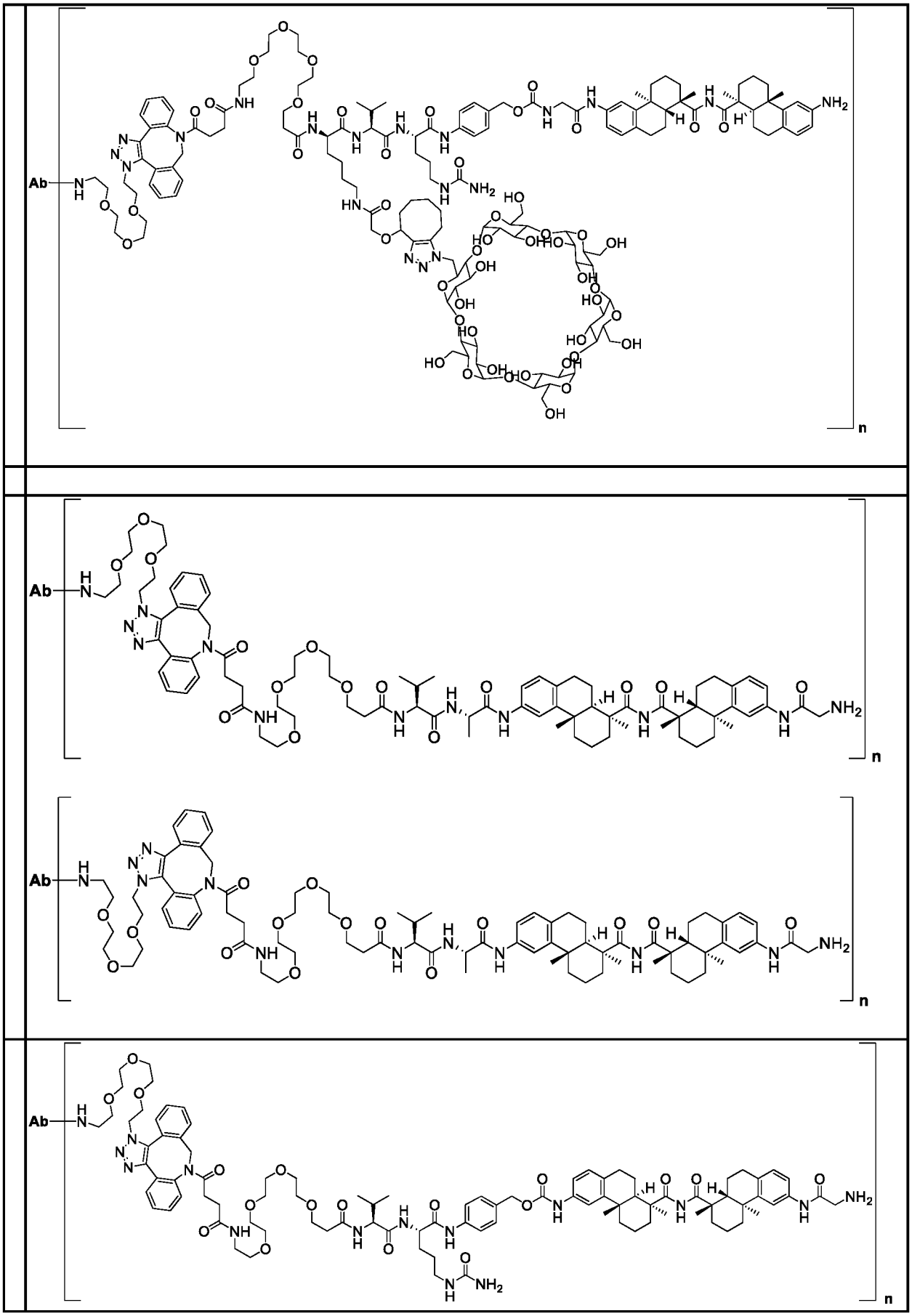

Ab
NH2
HO
OH
HN
NH
O
N
n

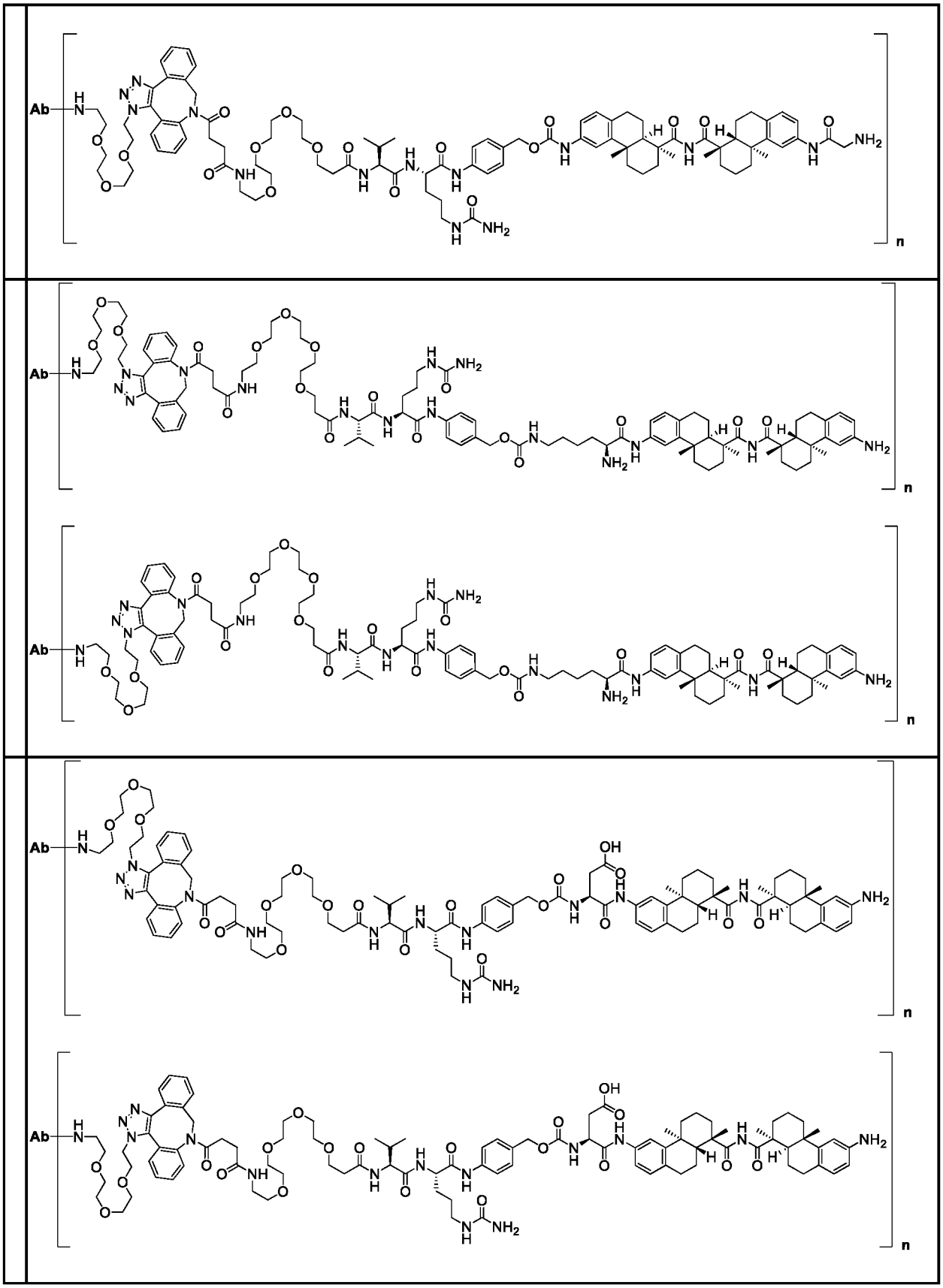

Ab
NH2
HO
OH
n
Ab
HO
OH
NH2
n

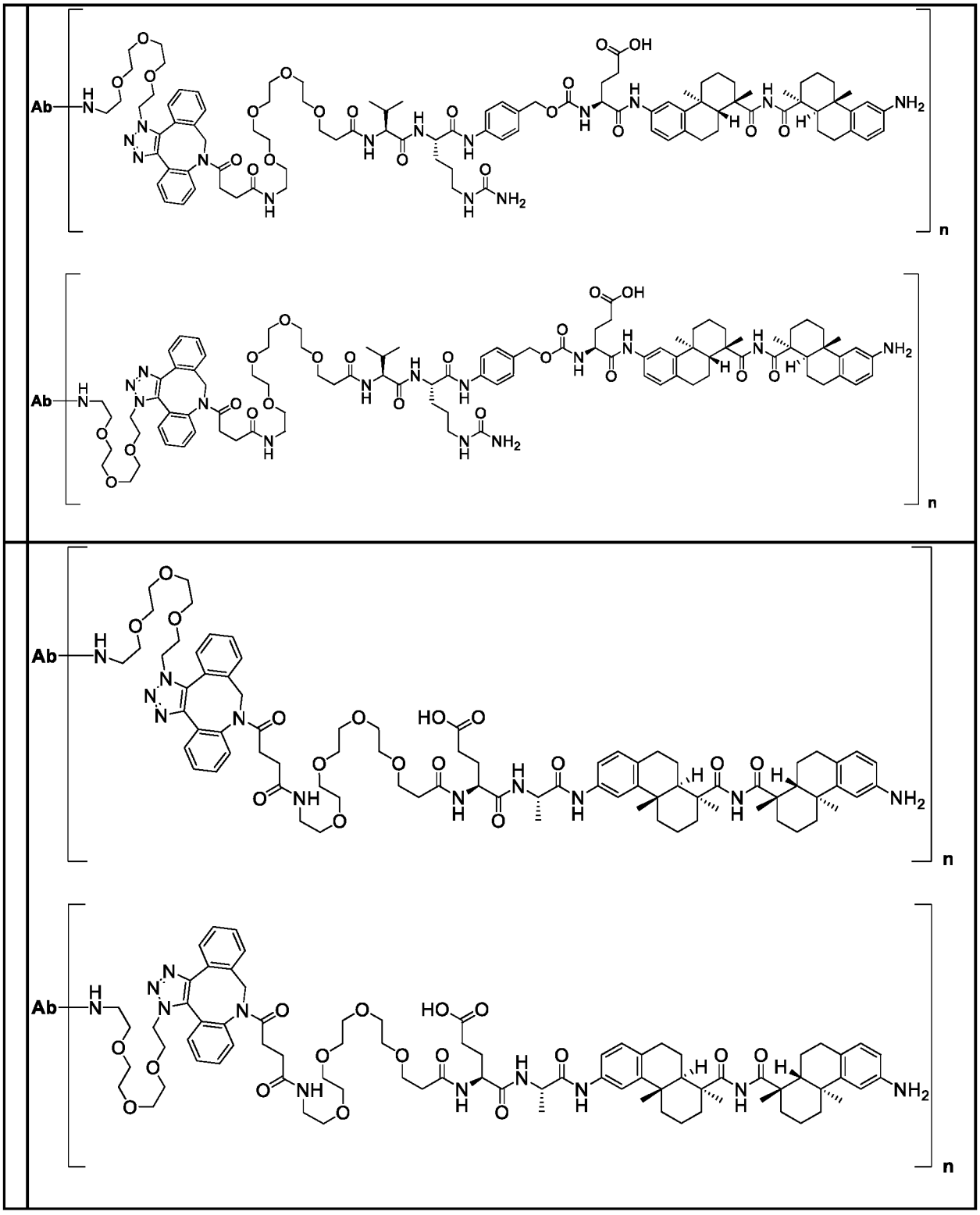


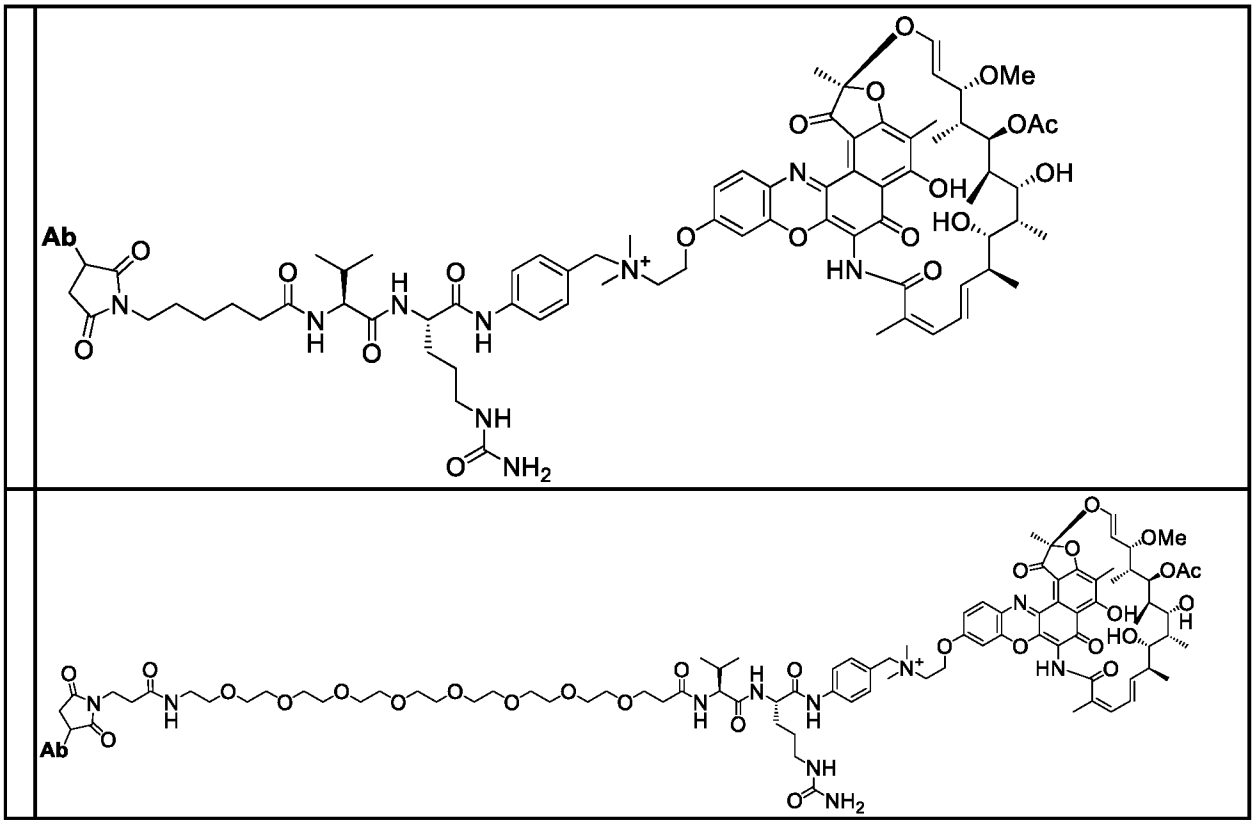



Ab
NH2
HO
OH
n
Ab
NH2
HO
OH
n


Ab
n
Ab
n
Ab
n
Ab
n

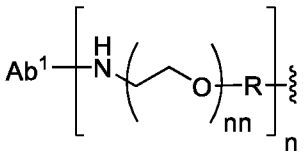



Ab
n
Ab
n
Ab
n
Ab
n



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

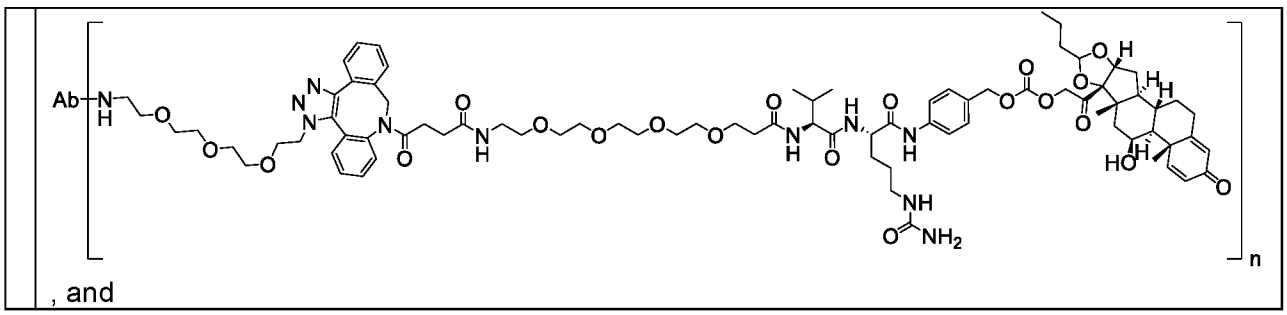
each **Ab** is an anti-MSR1 antibody, or an antigen binding fragment thereof;

each **BA** is  where **Ab**^{1} is an anti-MSR1 antibody, or an antigen-binding fragnment thereof; **R** is $C_{2\text{-}4}$-alkylene; and **nn** is an integer selected from 2 to 4, inclusive.

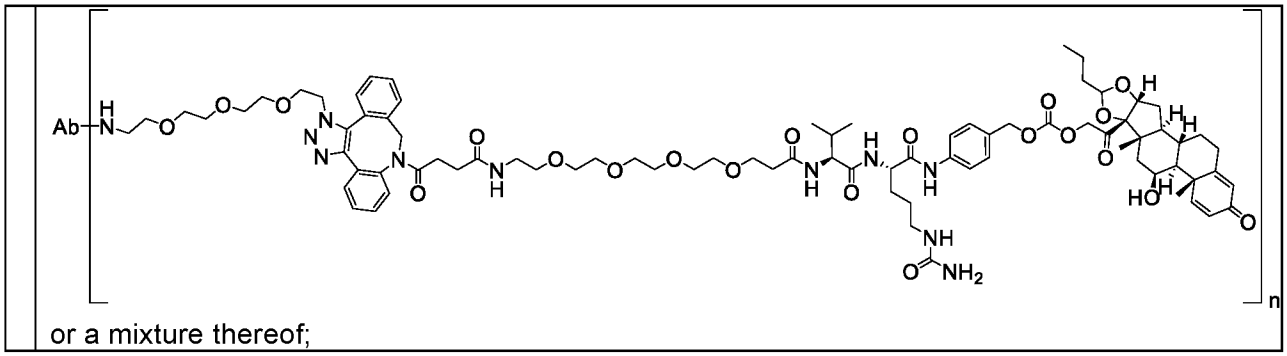
and

each **n** is an integer from 1 to 4.

52. An antibody-drug conjugate selected from



, and



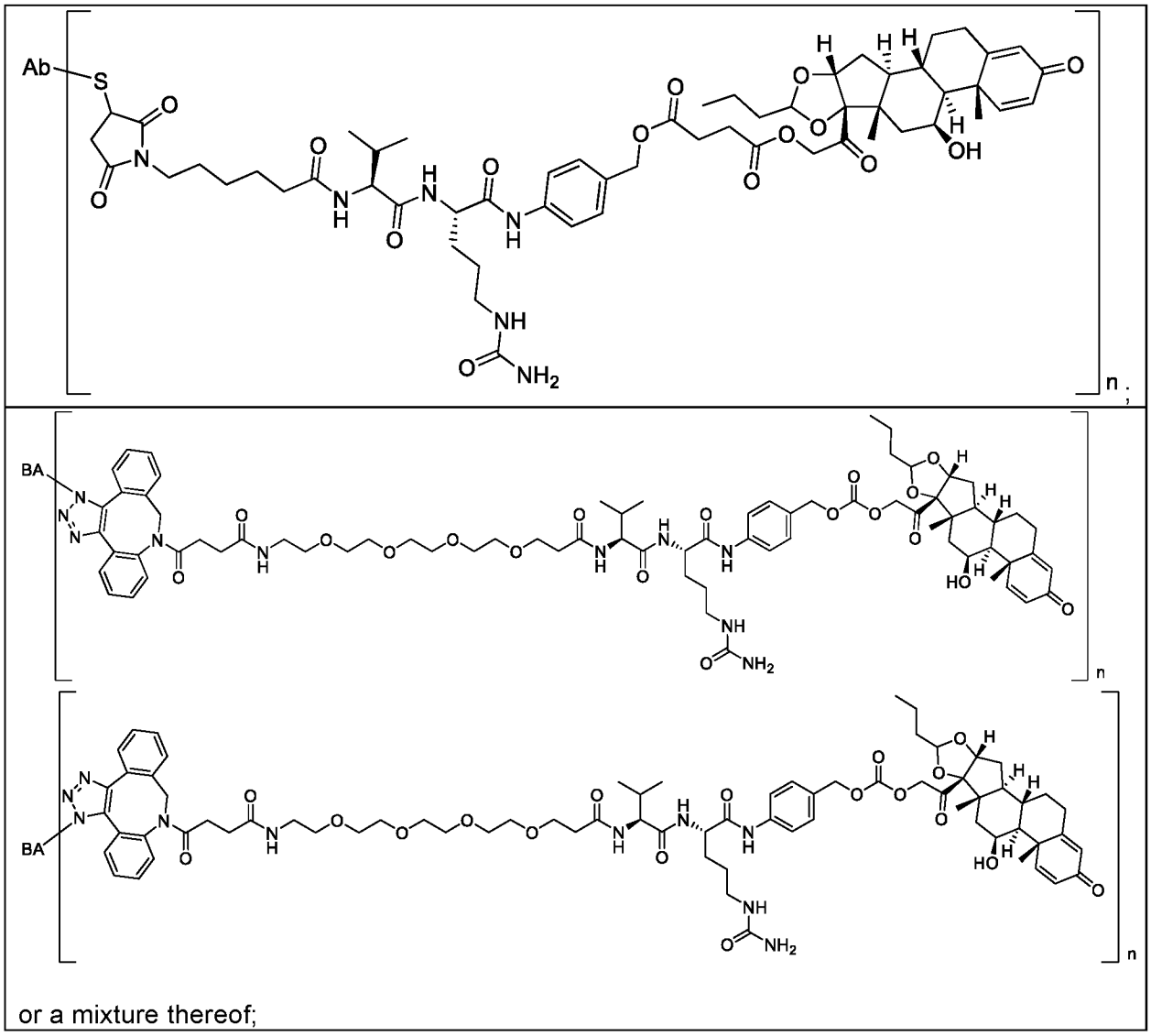
or a mixture thereof;

or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

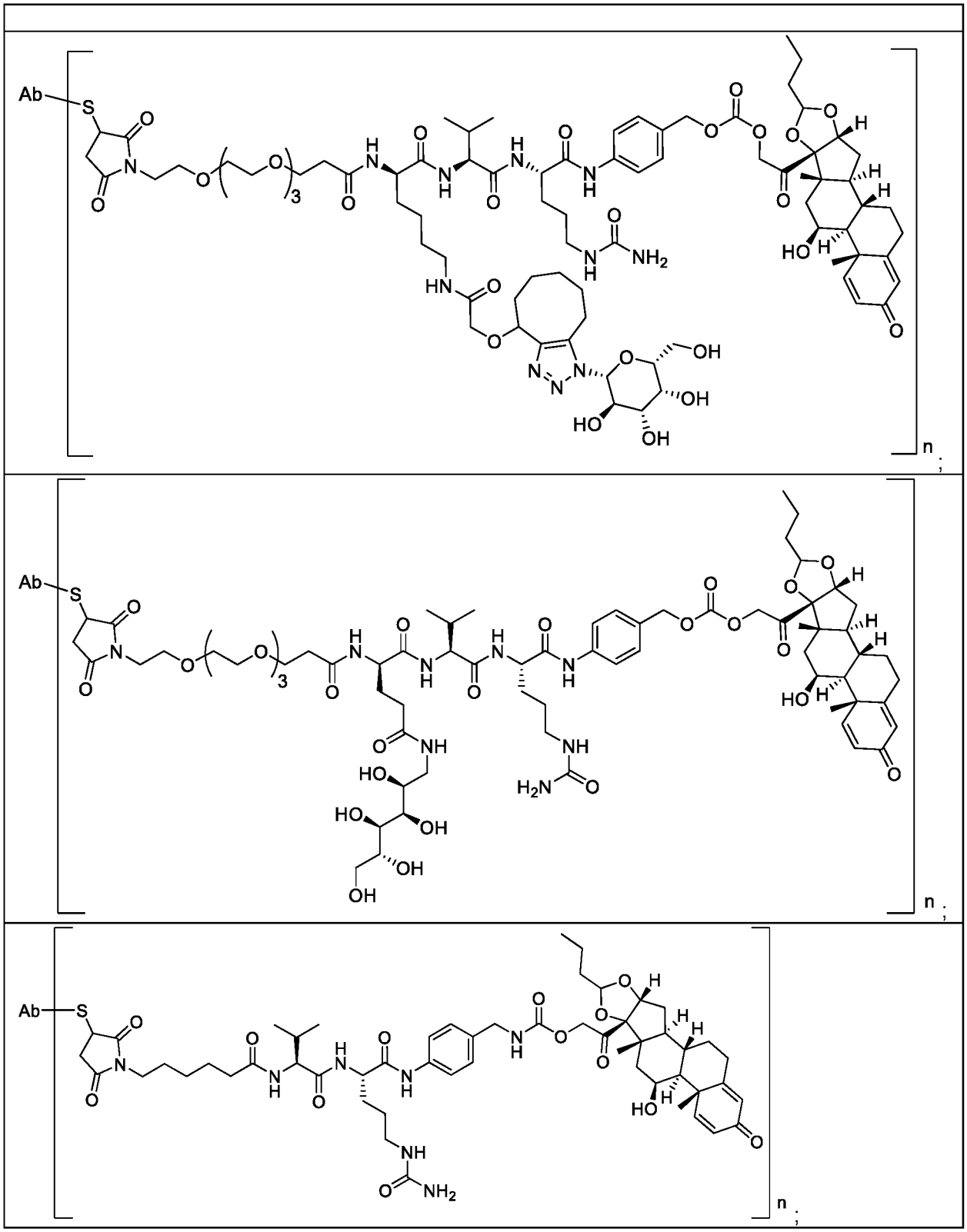
each Ab is an anti-MSR1 antibody, or an antigen binding fragment thereof; and

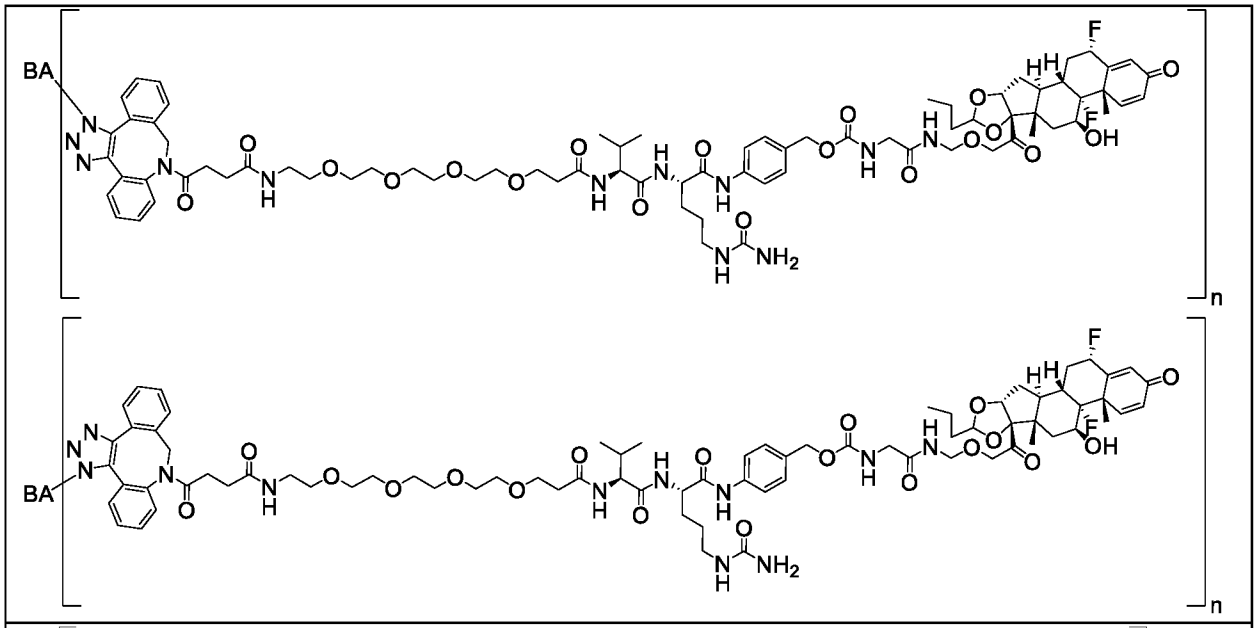
each n is an integer from 1 to 4.

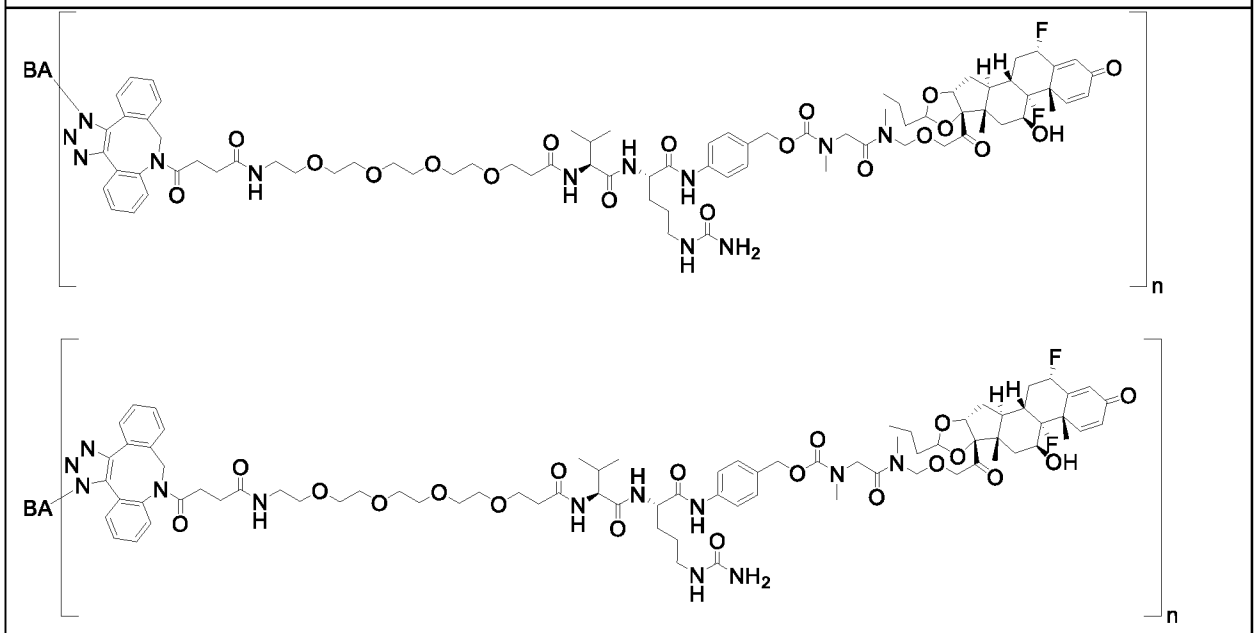
53. An antibody-drug conjugate according to one of the following formulas:



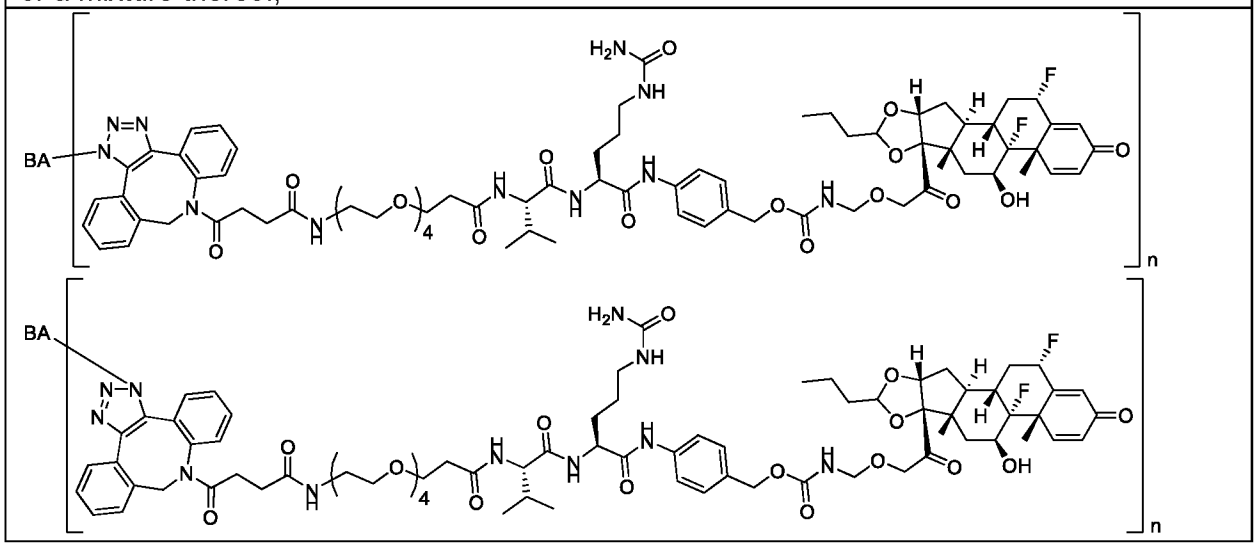
or a mixture thereof;



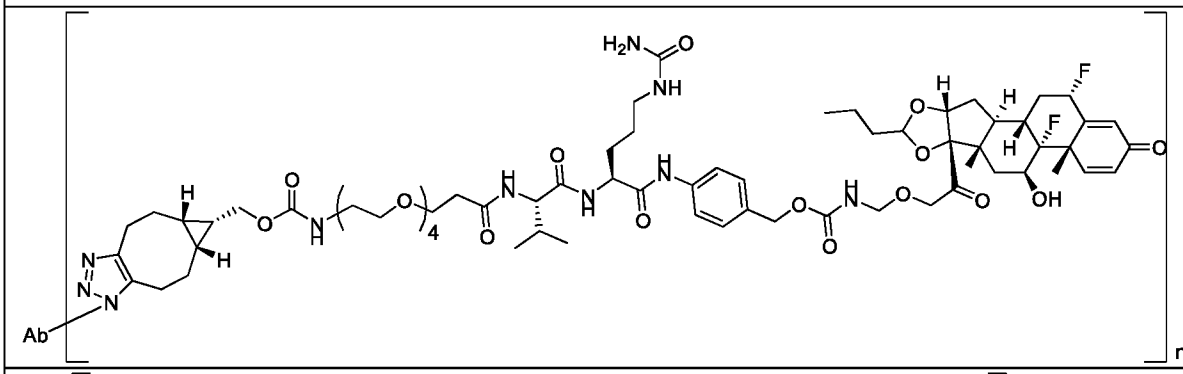


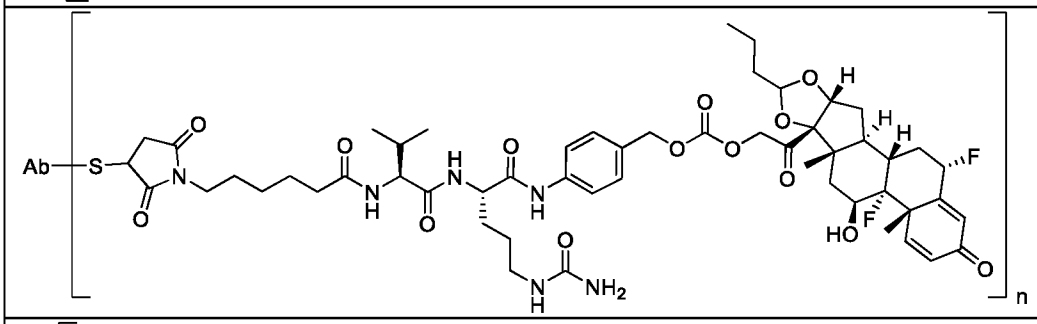


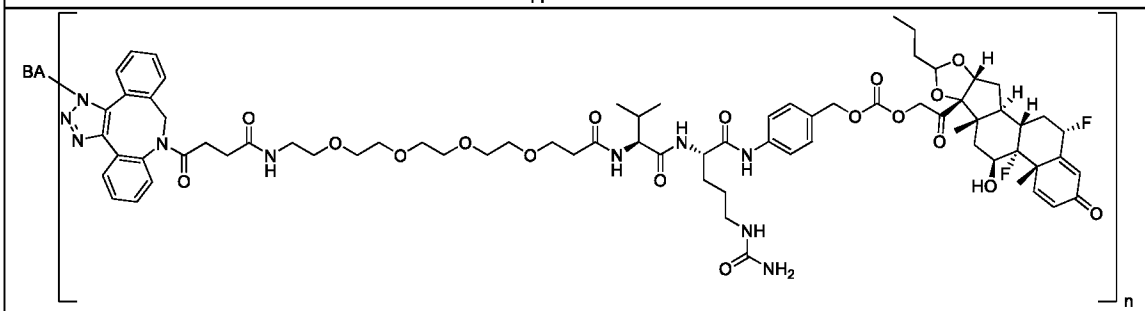
or a mixture thereof,

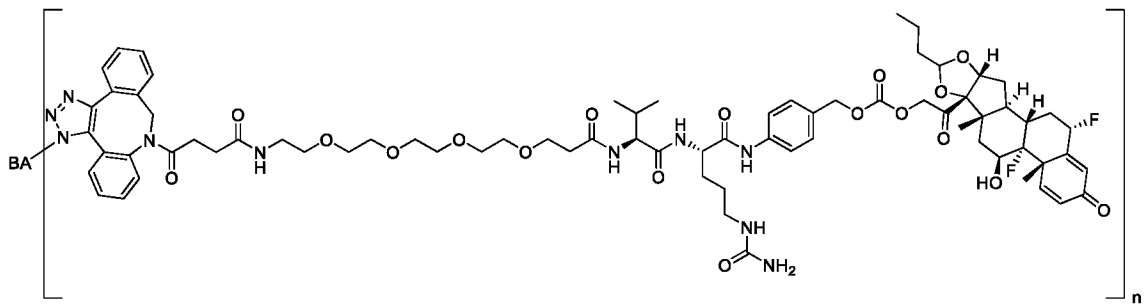


or a mixture thereof

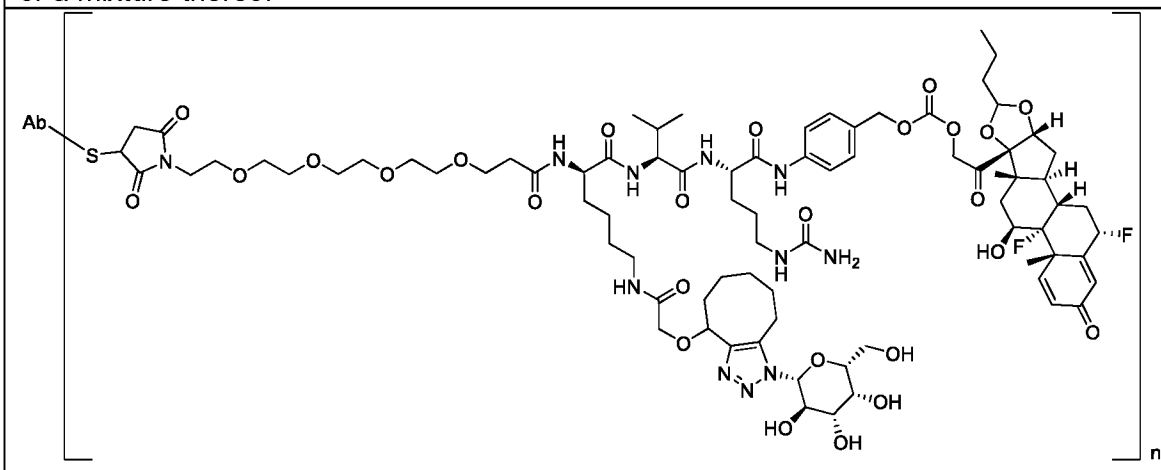


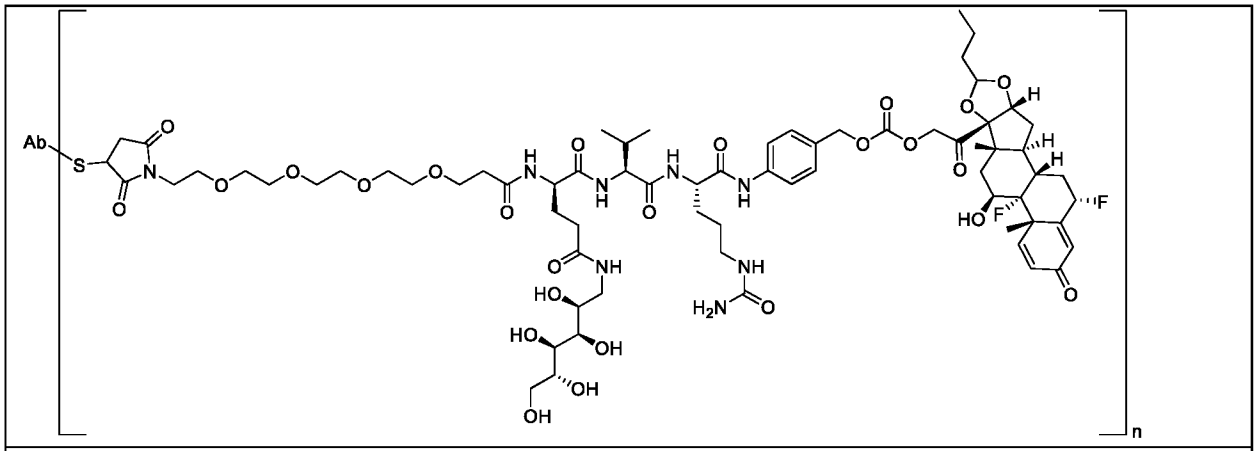


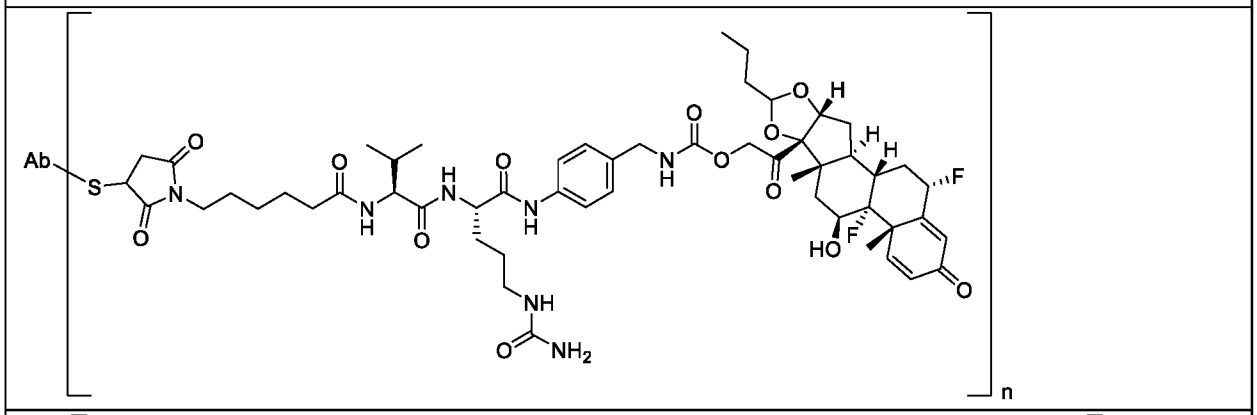


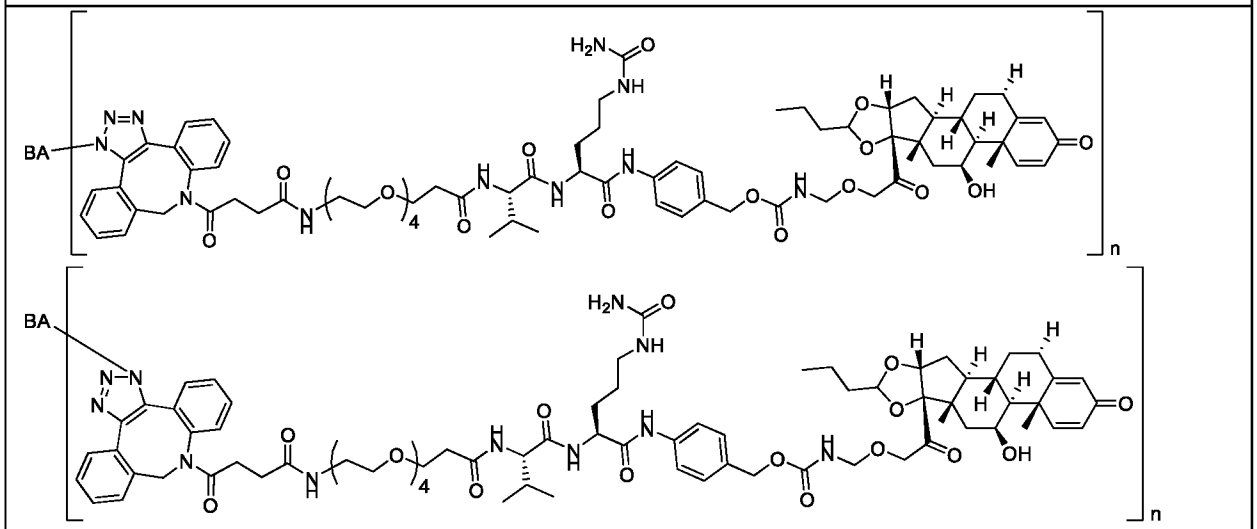


or a mixture thereof

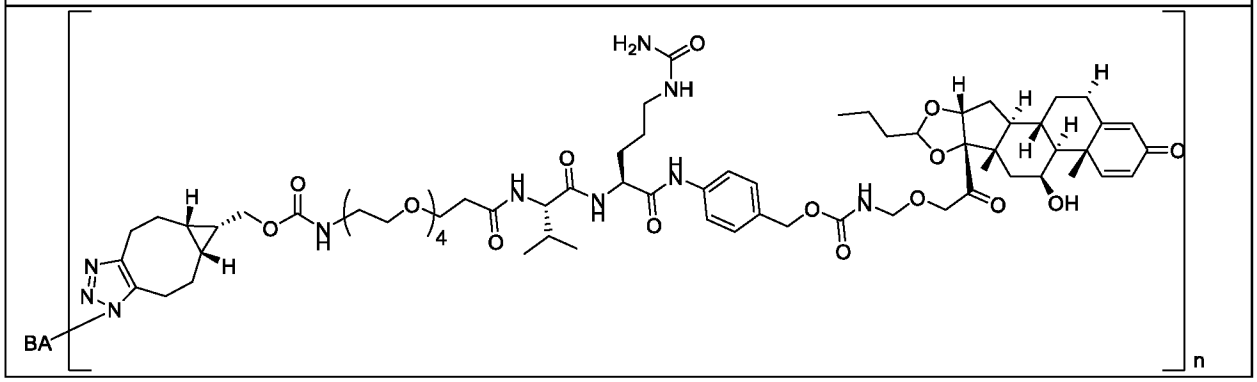






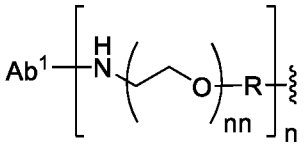


or a mixture thereof



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

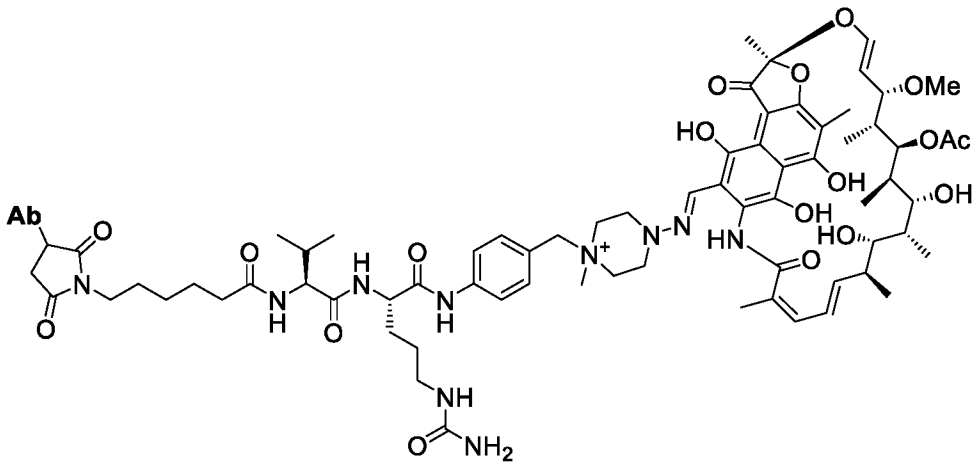
each **Ab** is an anti-MSR1 antibody, or an antigen binding fragment thereof;

each **BA** is  where **Ab**^{1} is an anti-MSR1 antibody, or an antigen-binding fragnment thereof; **R** is C_{2-4}-alkylene; and **nn** is an integer selected from 2 to 4, inclusive.

and

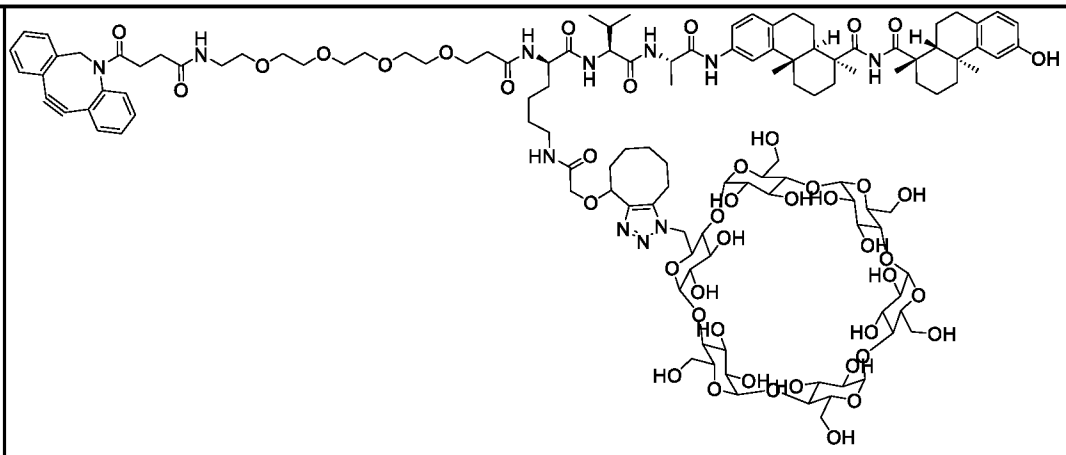
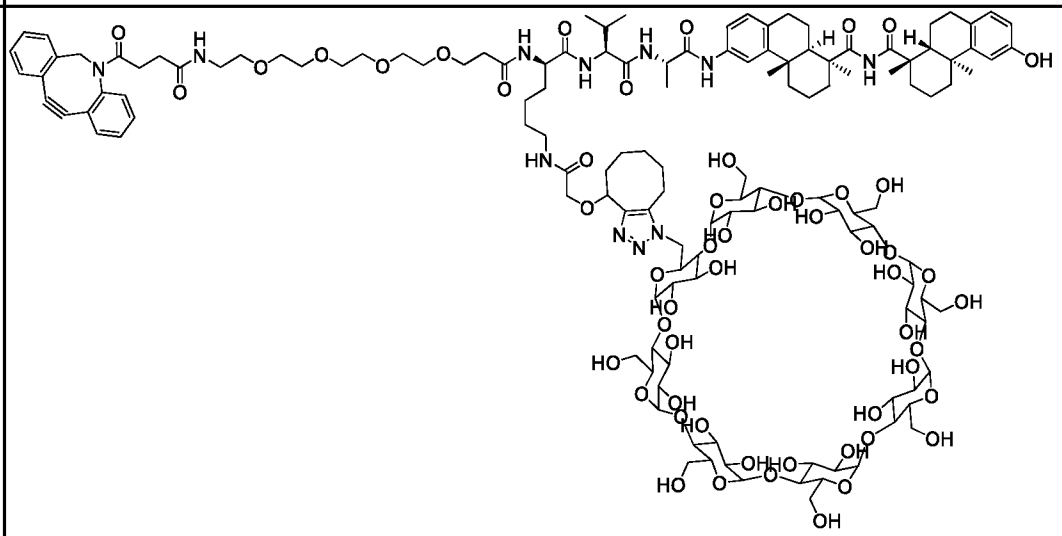
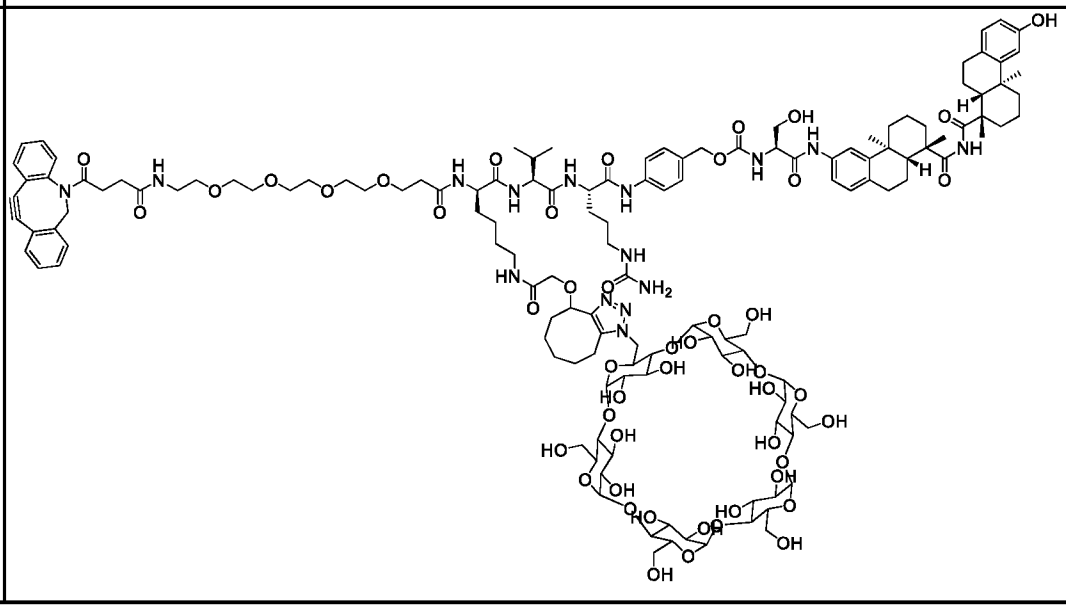
each **n** is an integer from 1 to 4.

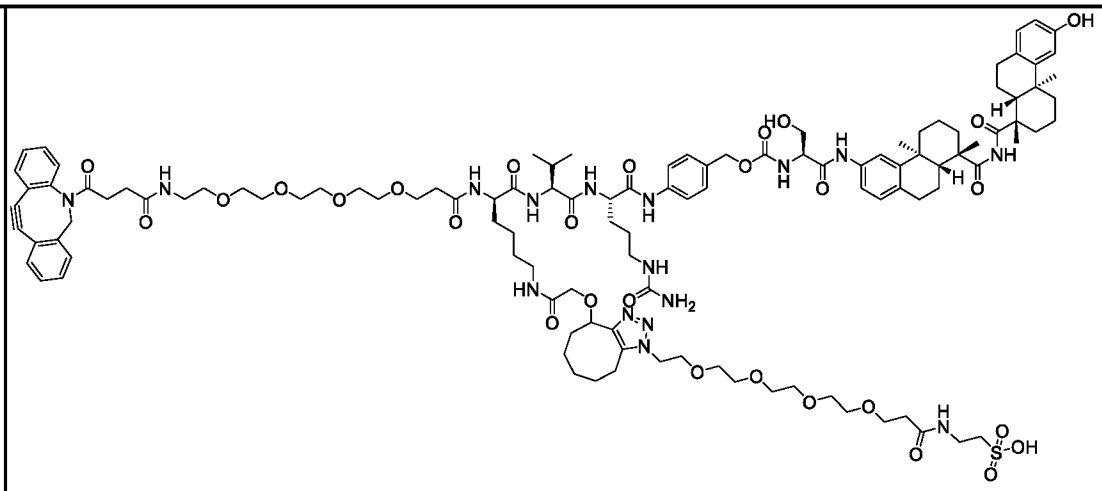
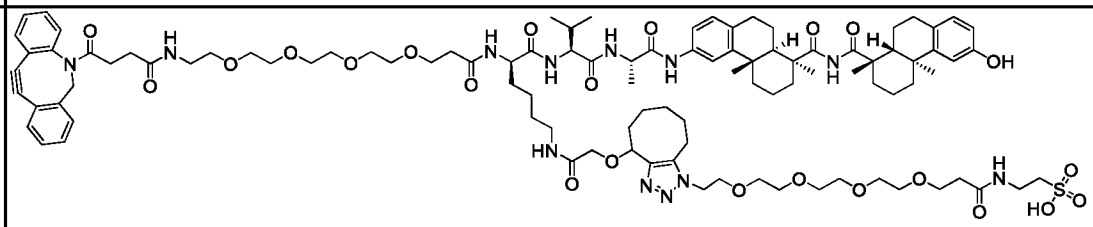
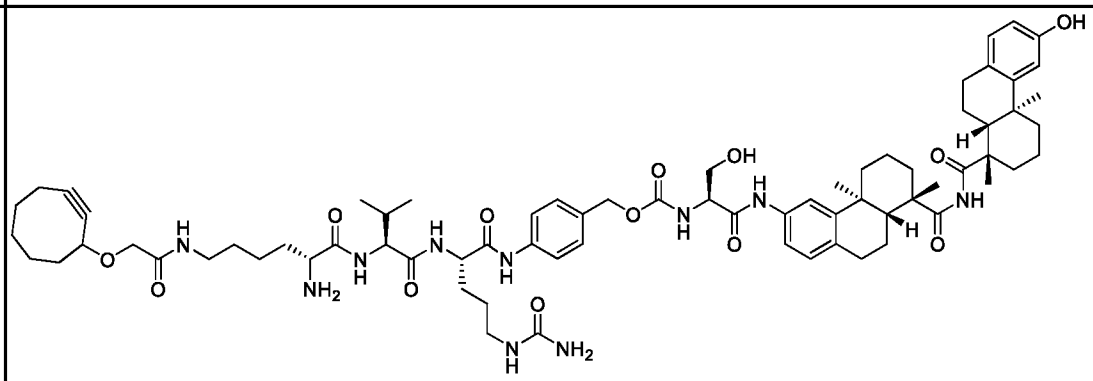
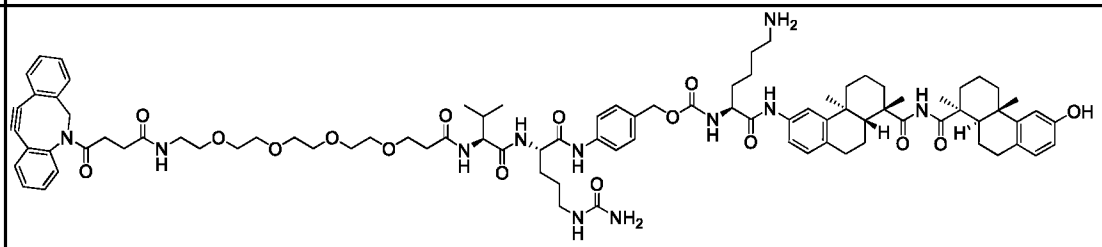
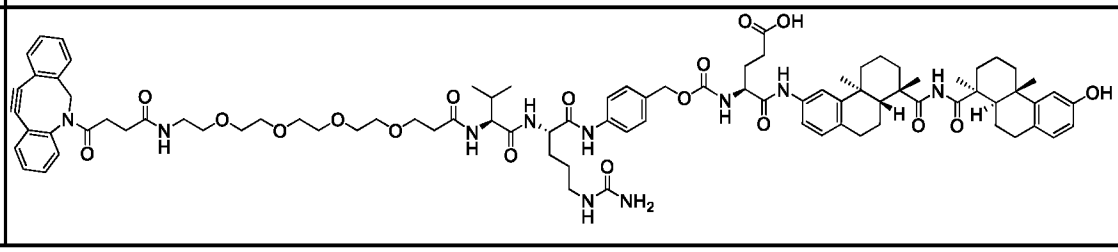
54. An antibody-drug conjugate of any one of claims 9-10 and 24, according to Formula (**7004**):

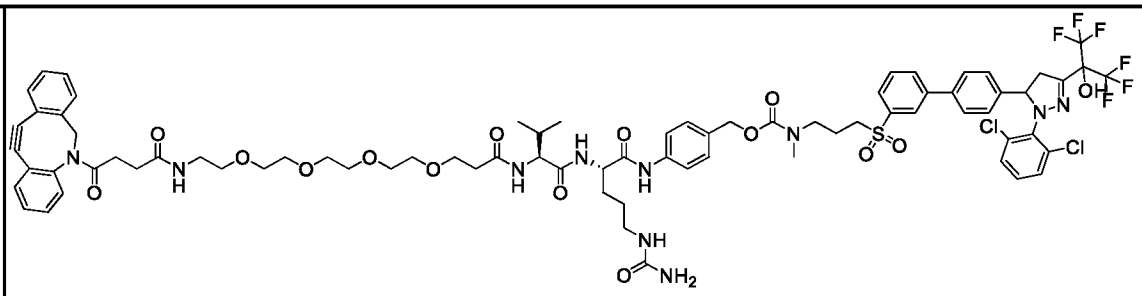
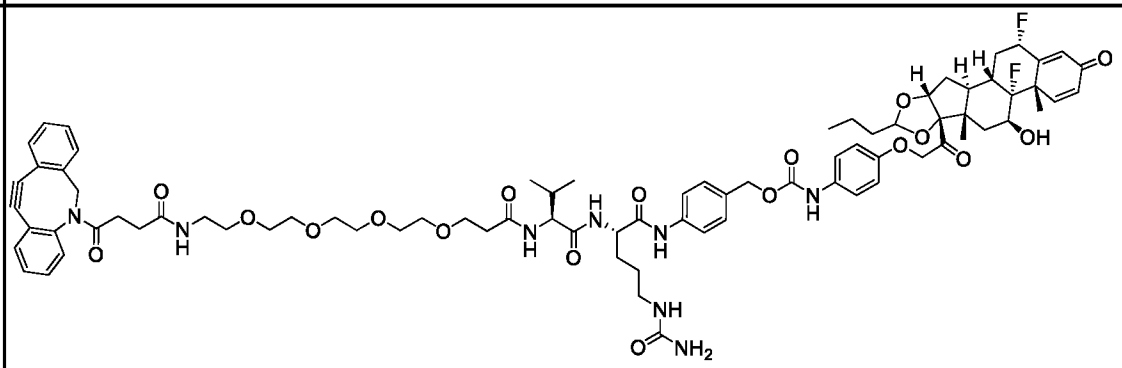
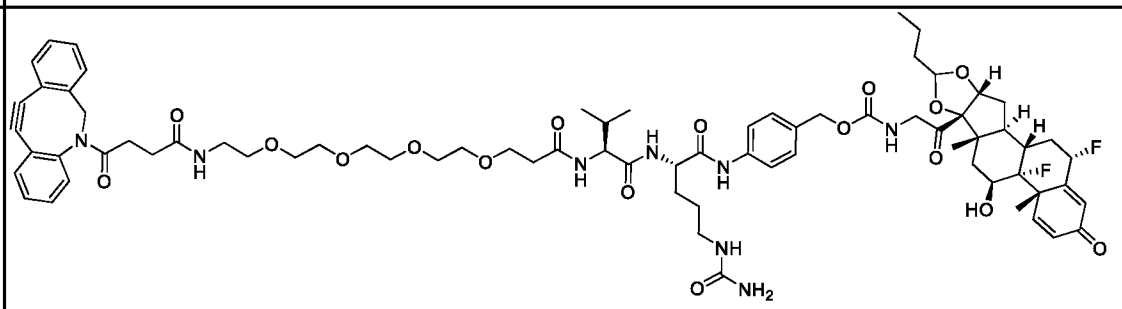
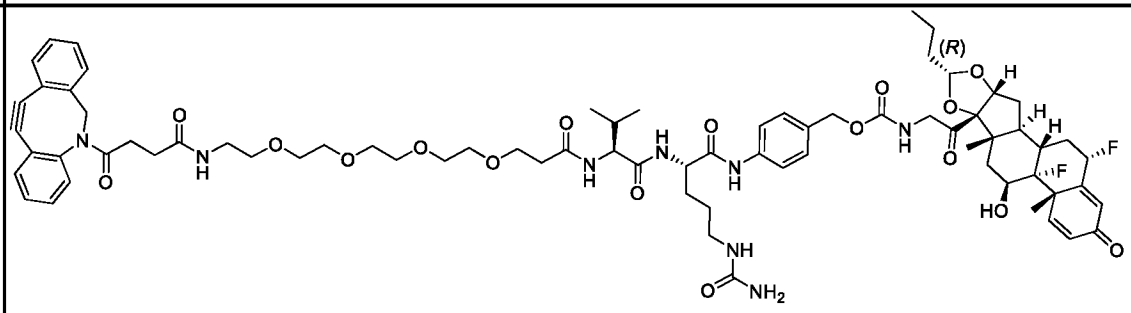
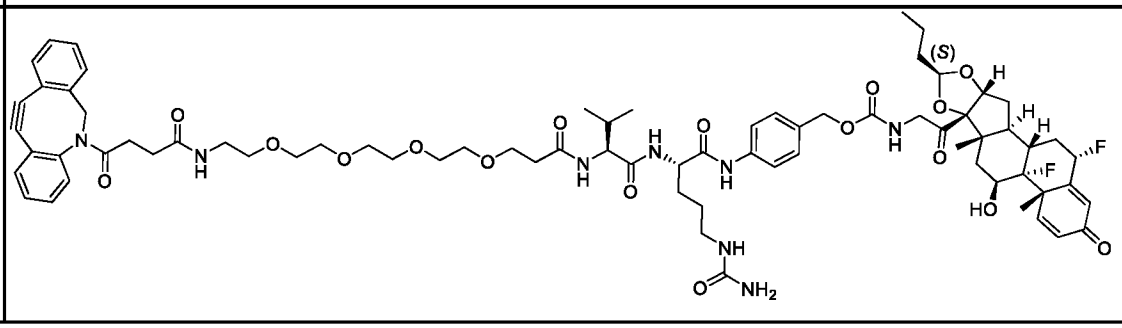


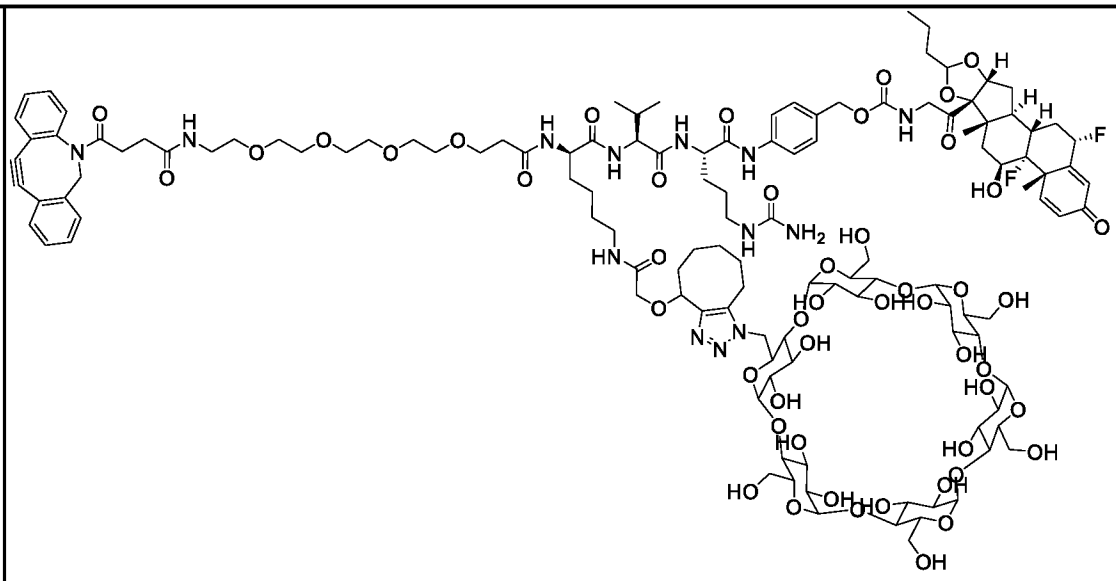
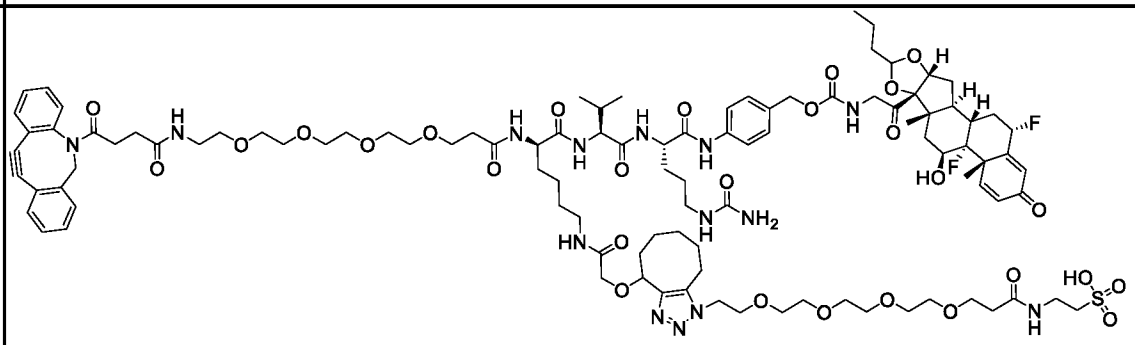
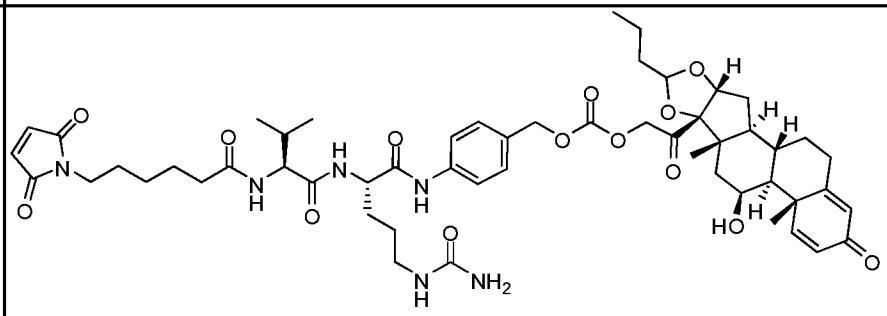
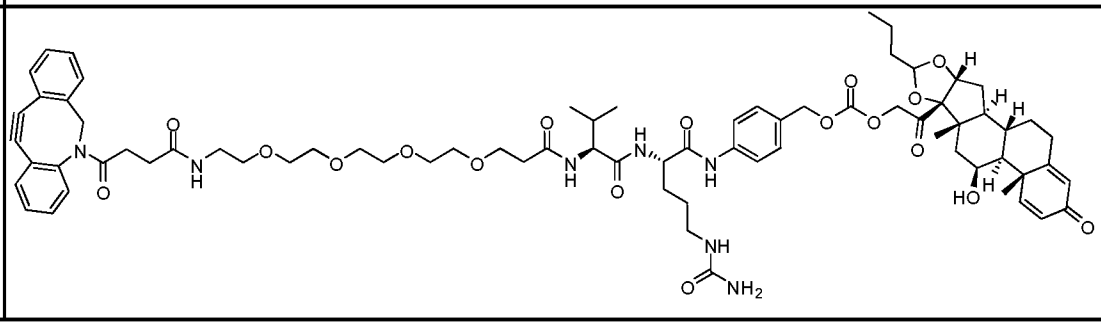
Formula (**7004**).

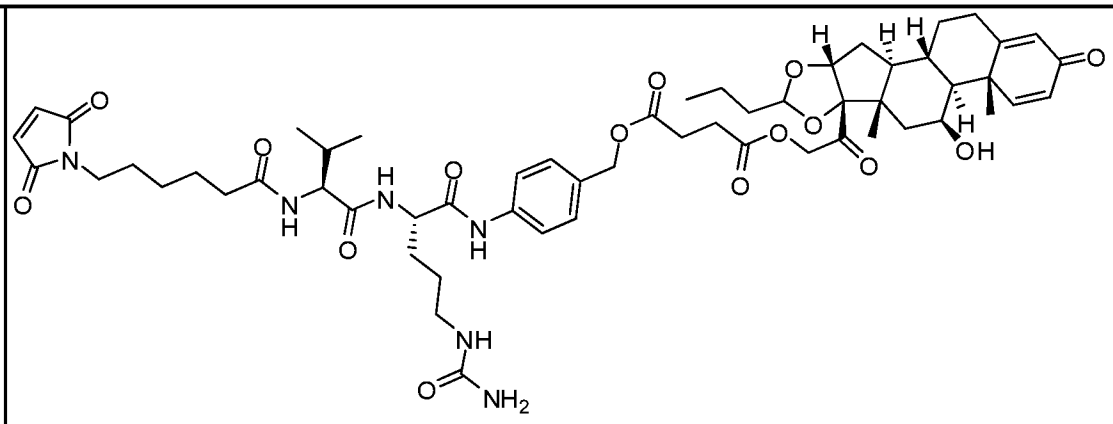
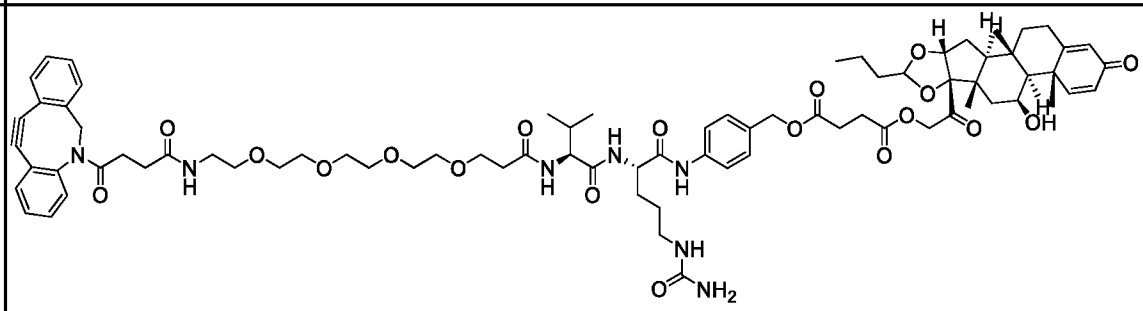
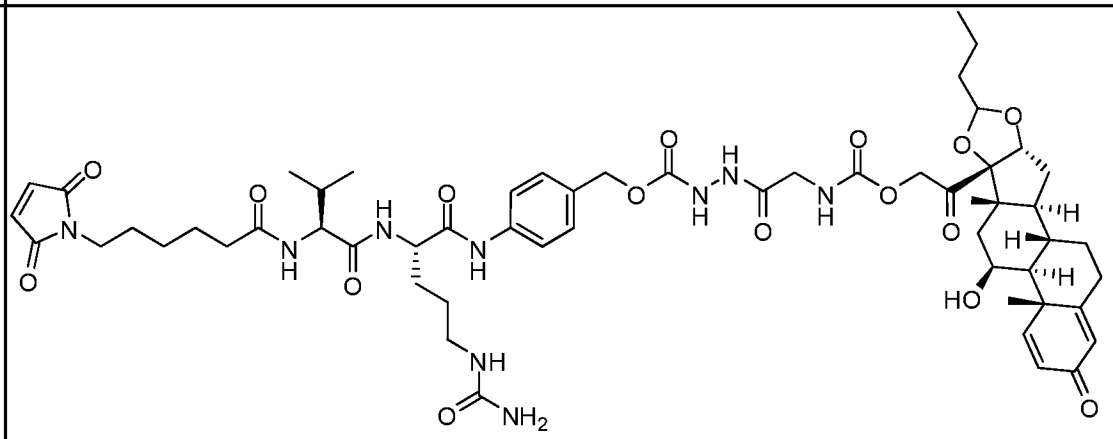
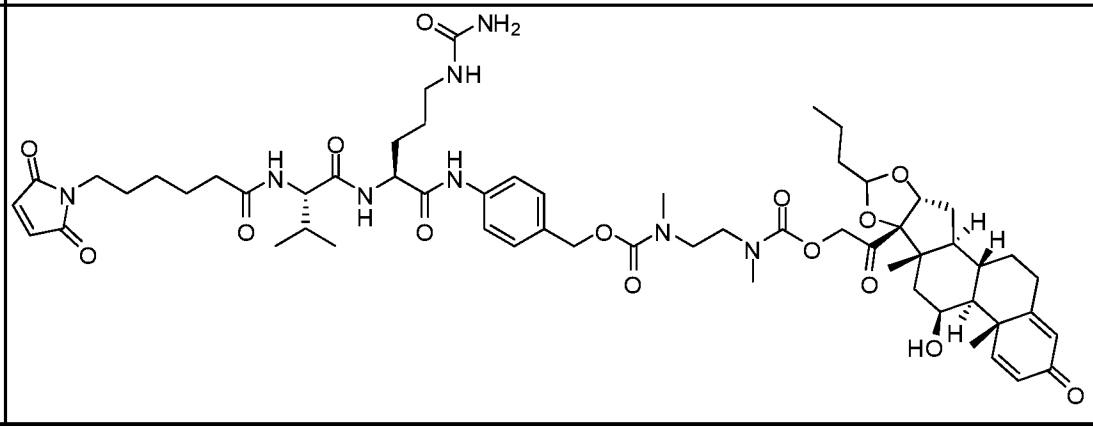
55. An antibody drug conjugate prepared by conjugating an anti-MSR1 antibody or a PEG-modified anti-MSR1 antibody, or an antigen binding fragment thereof, with a linker payload, or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, selected from the group consisting of:

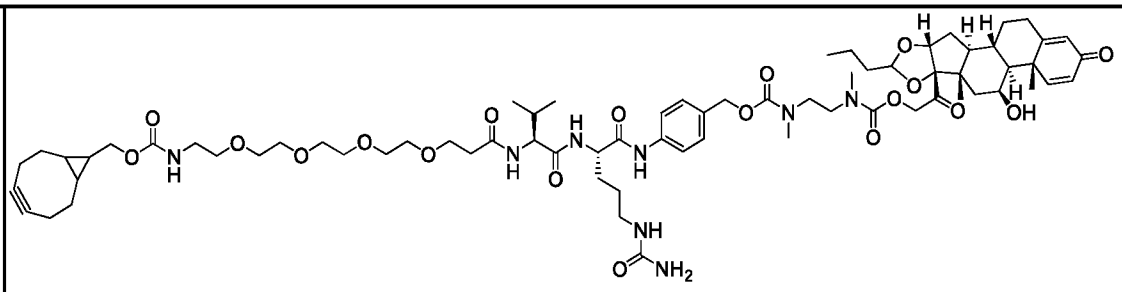
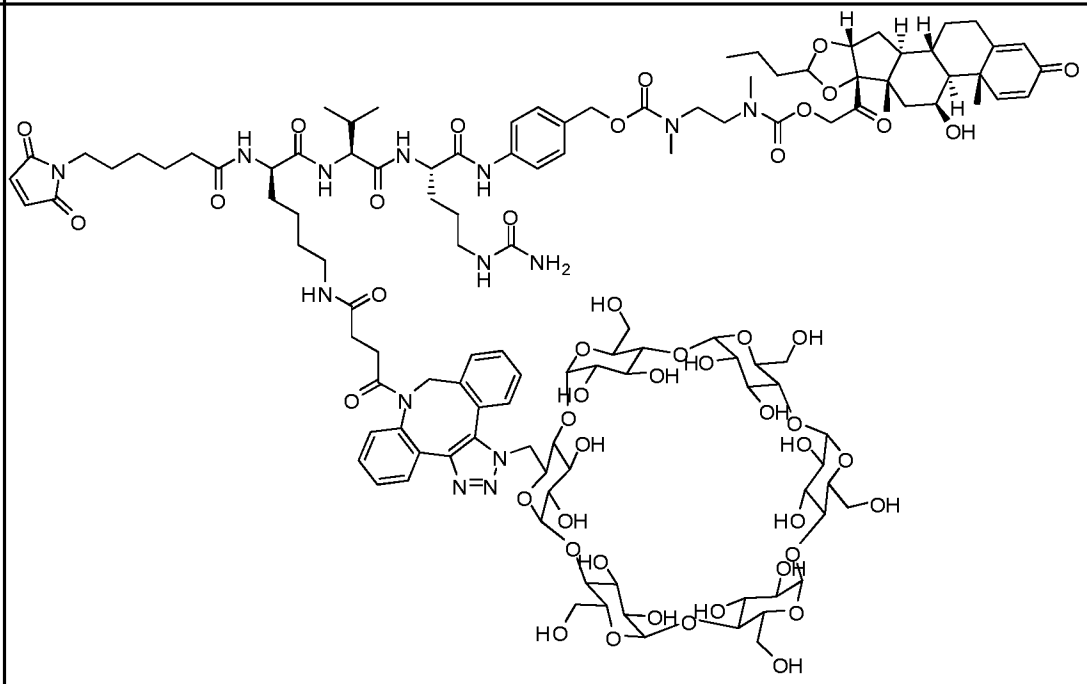
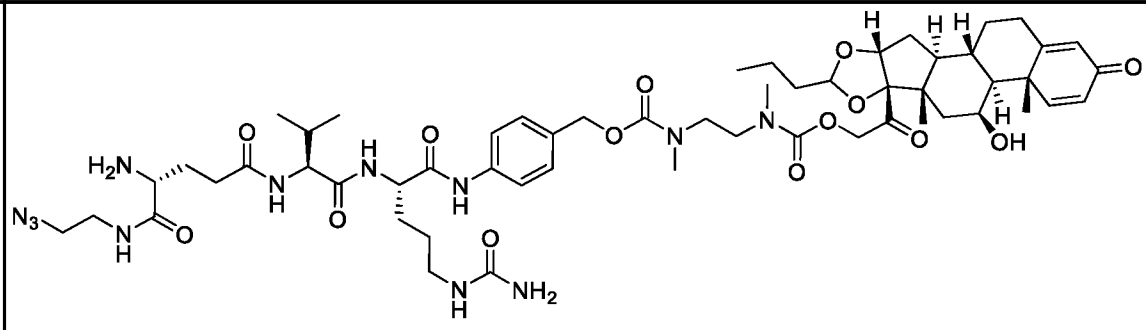
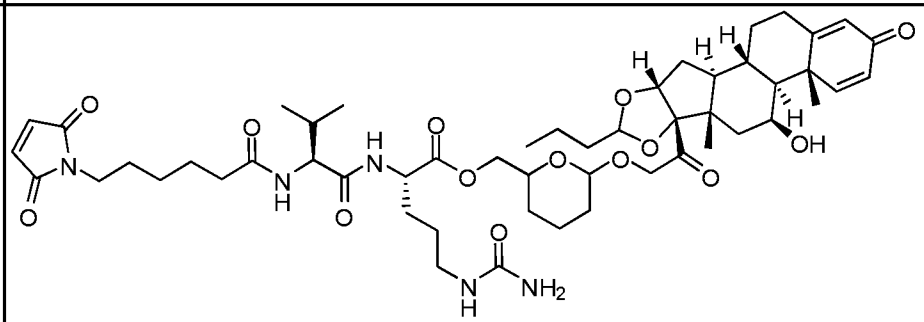
LP1	
LP2	
LP5	

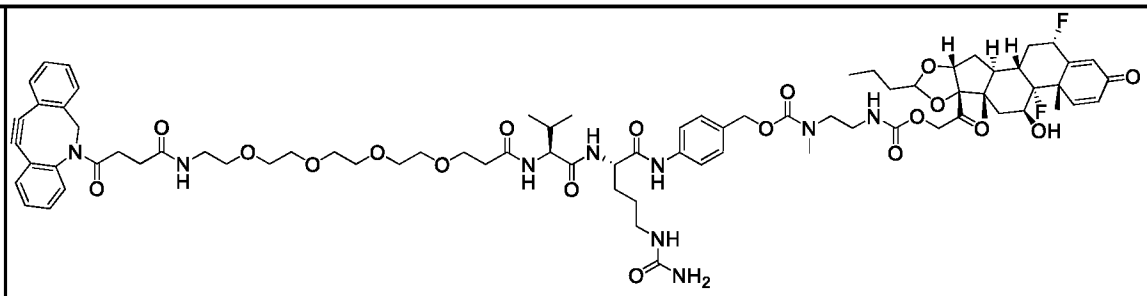
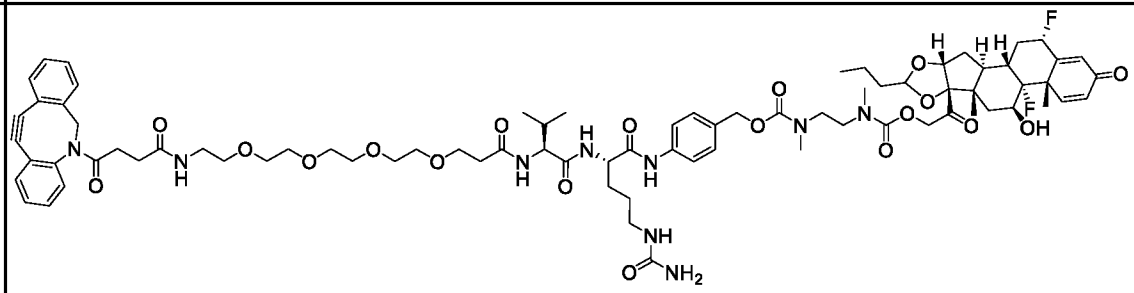
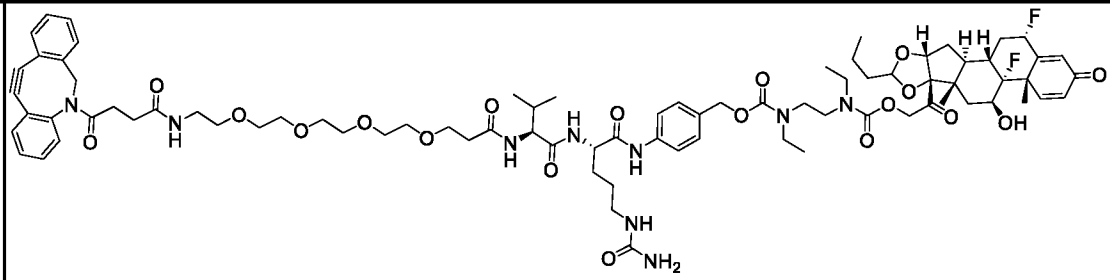
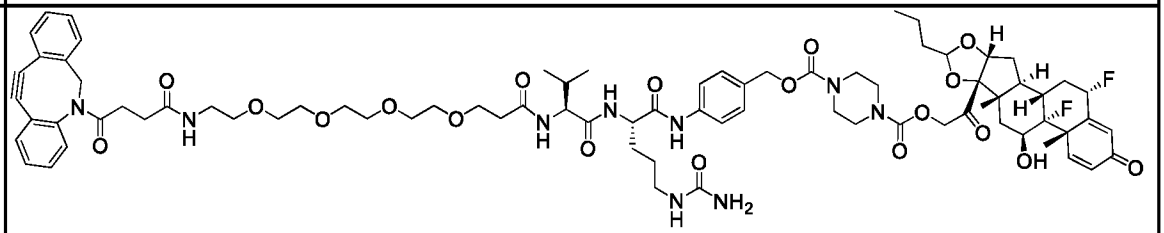
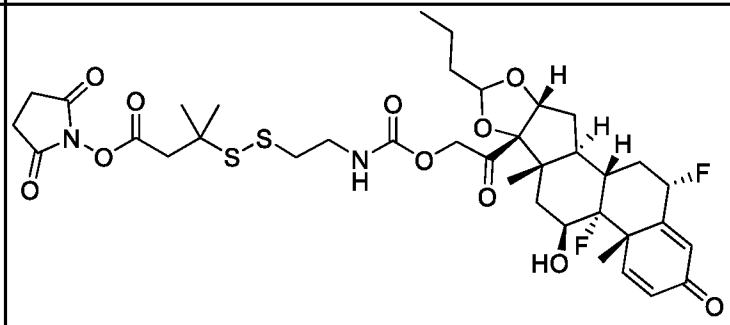
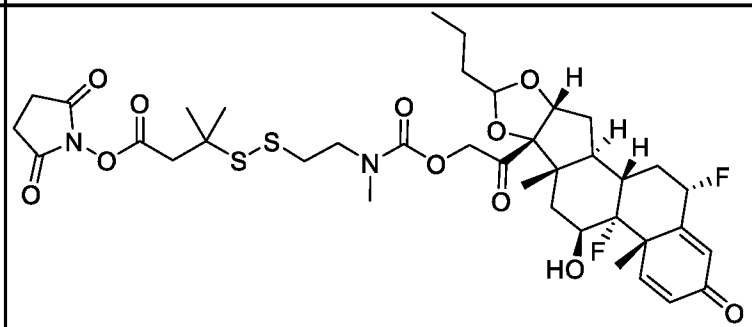
LP6	
LP18	
LP4	
LP11	
LP9	

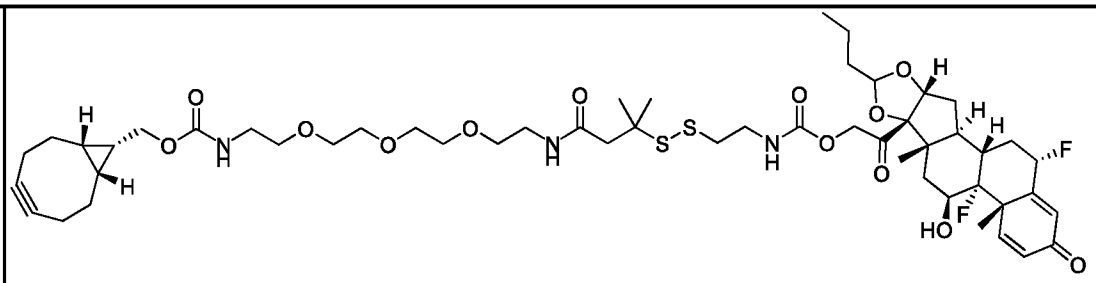
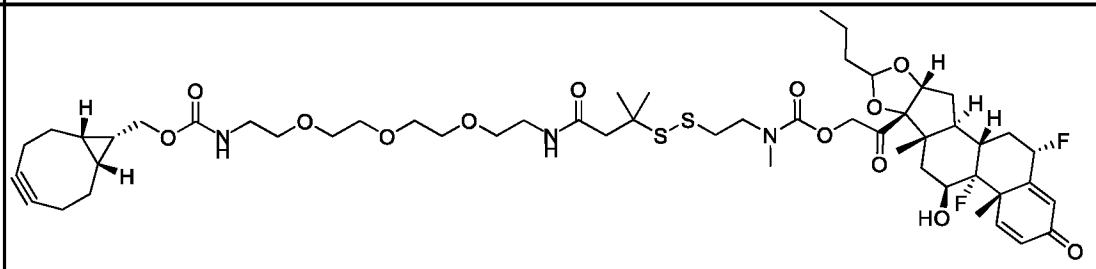
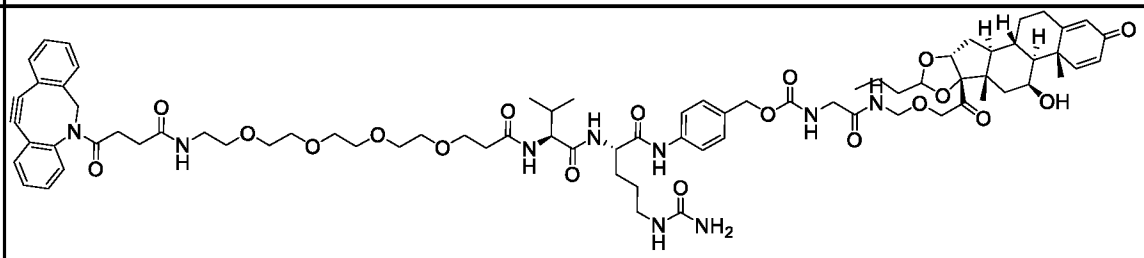
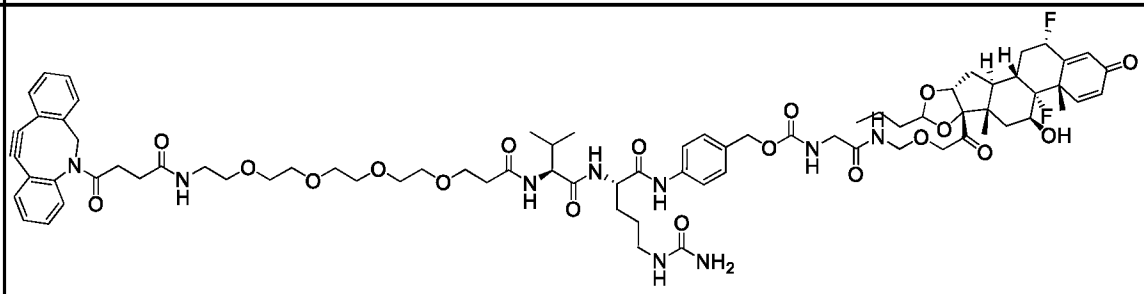
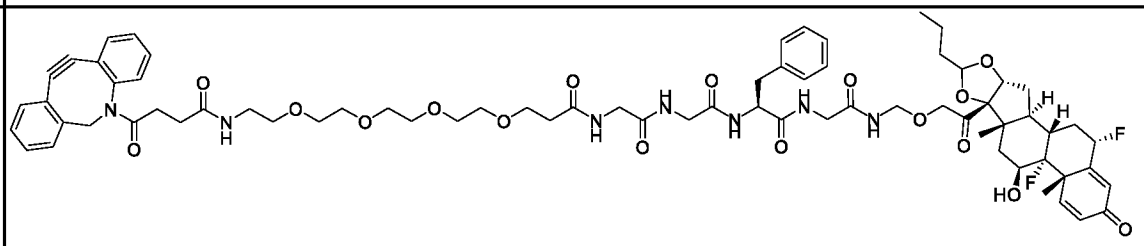
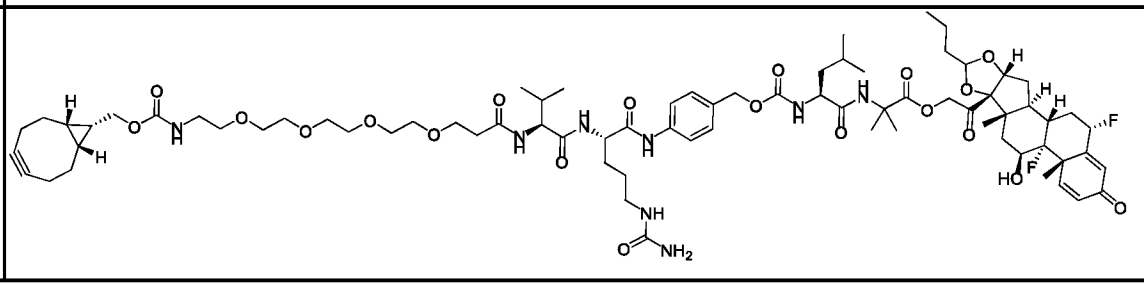
LP12	
LP3	
LP13	
	
	

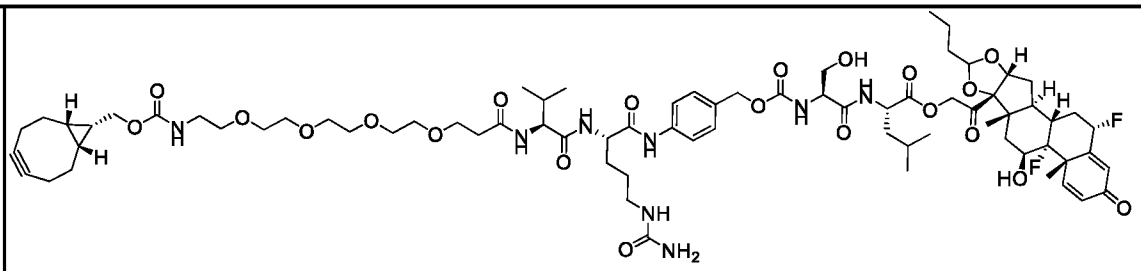
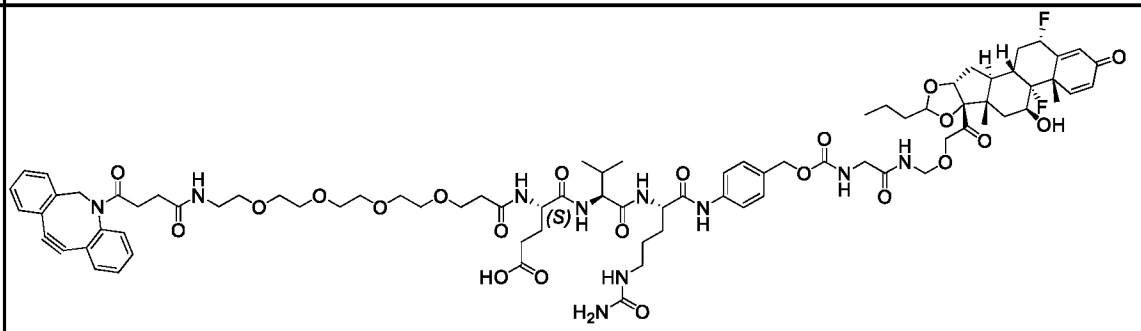
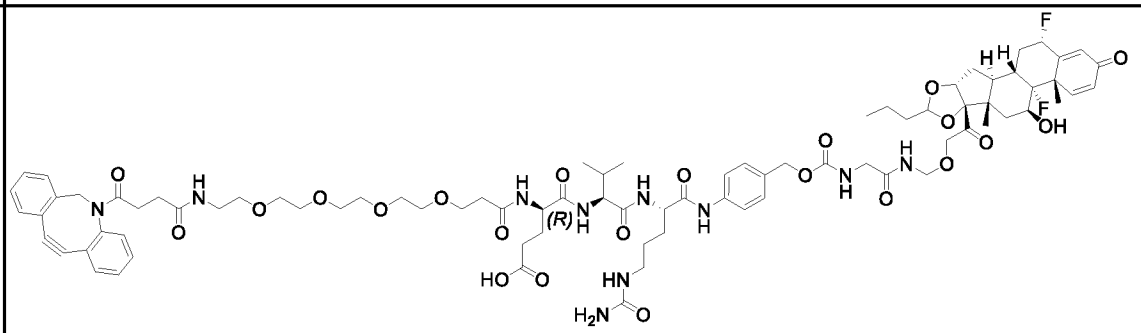
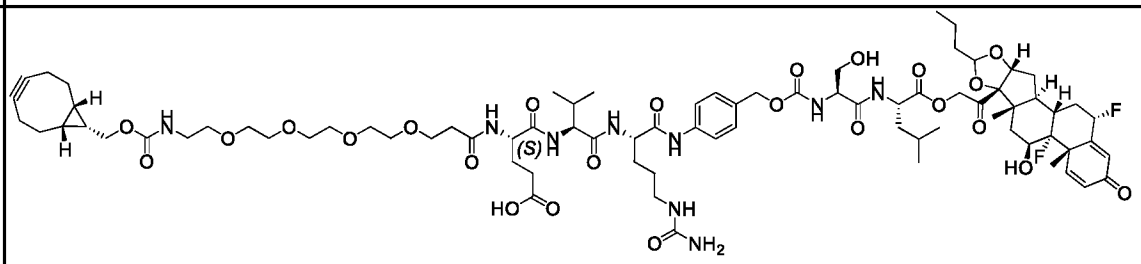
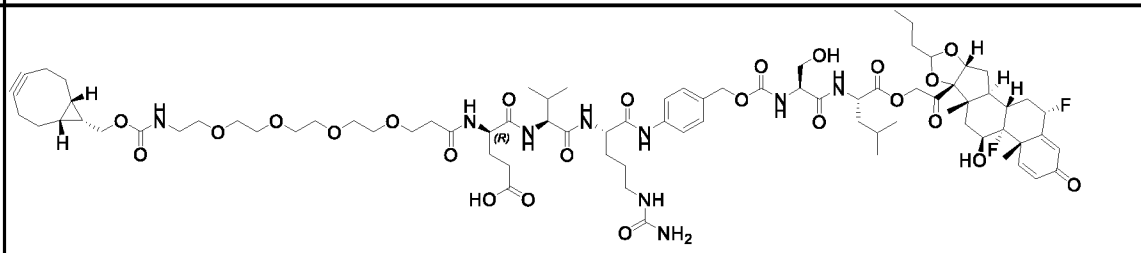
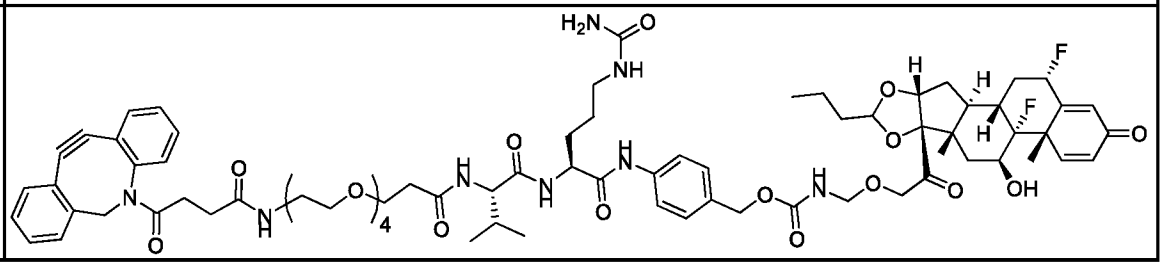
LP14	
LP15	
2a	
2b	

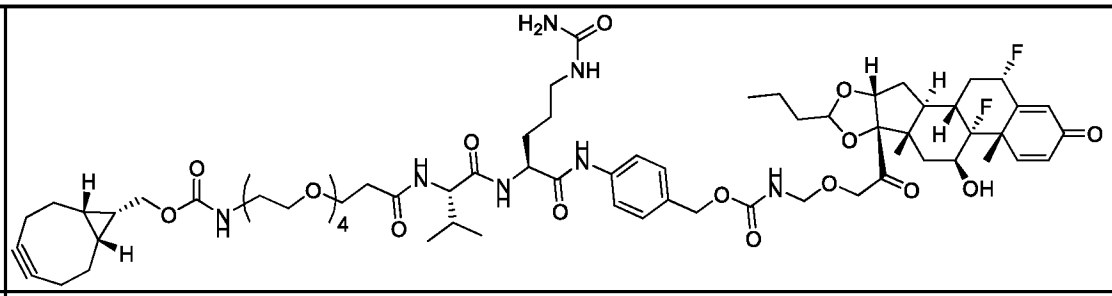
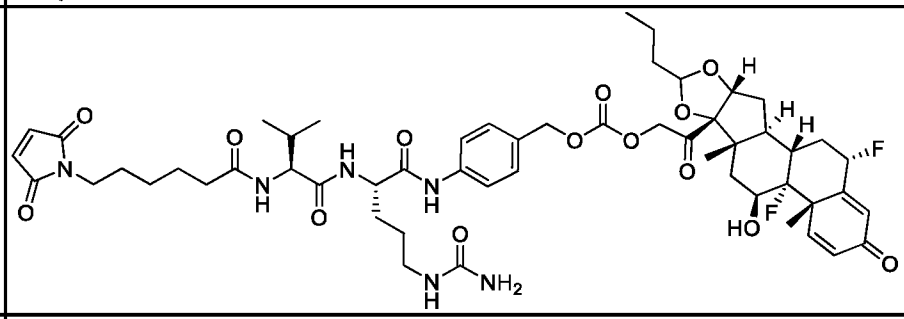
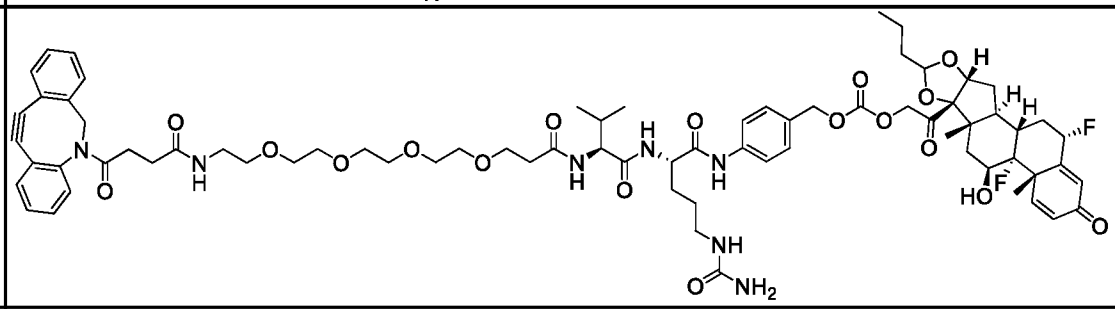
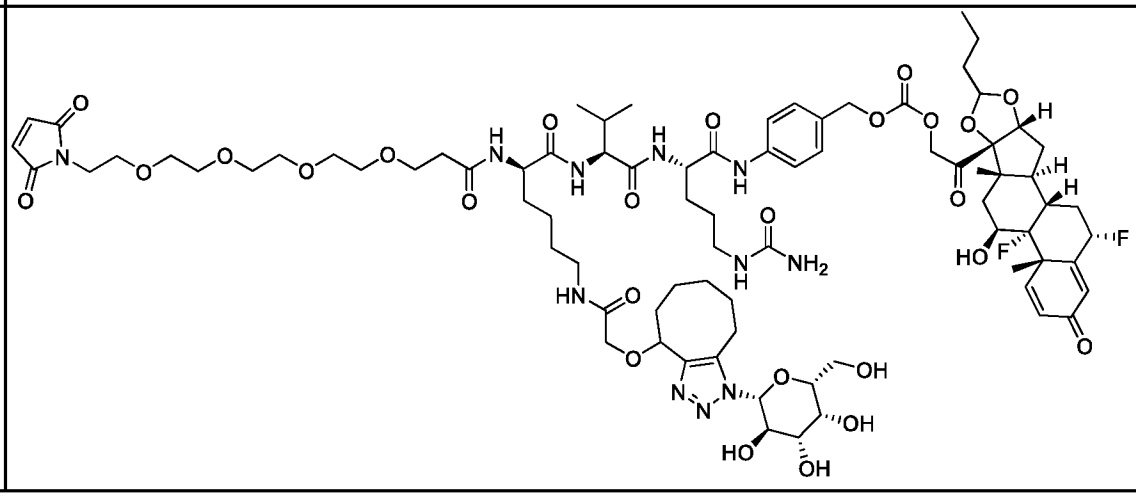
2f	
2g	
2j	
2k	

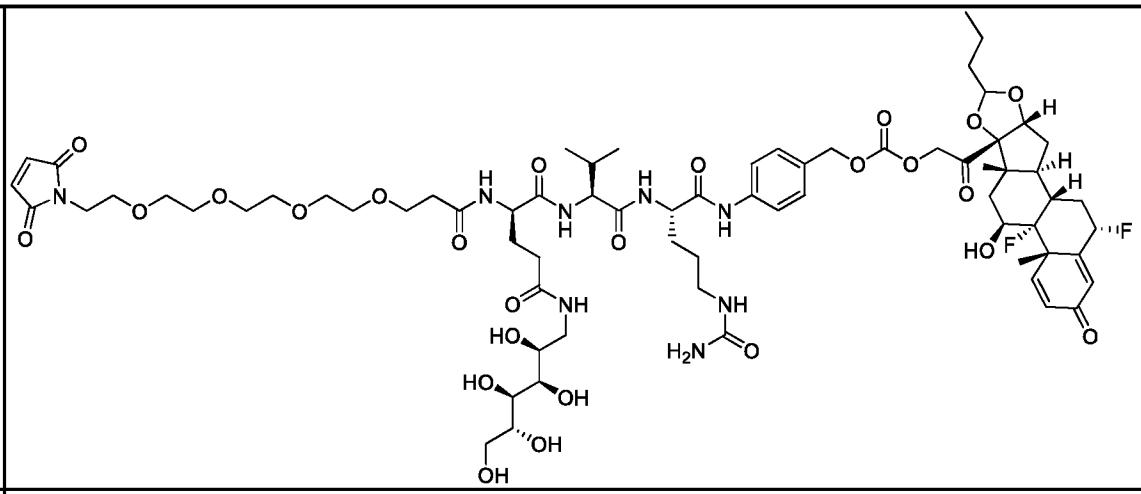
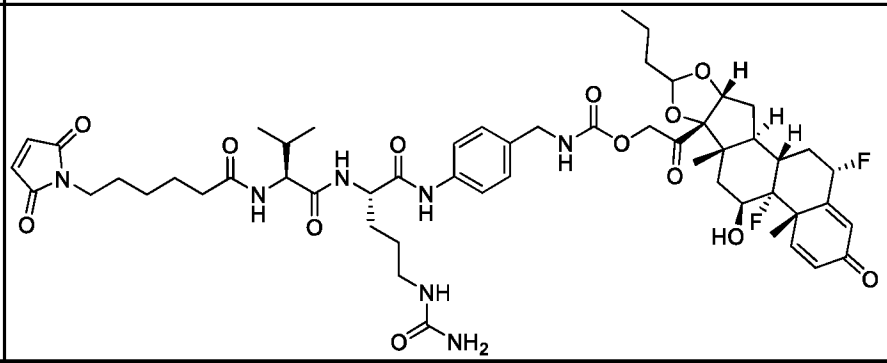
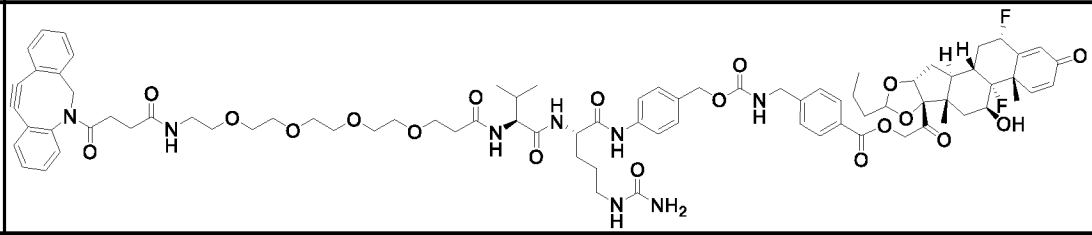
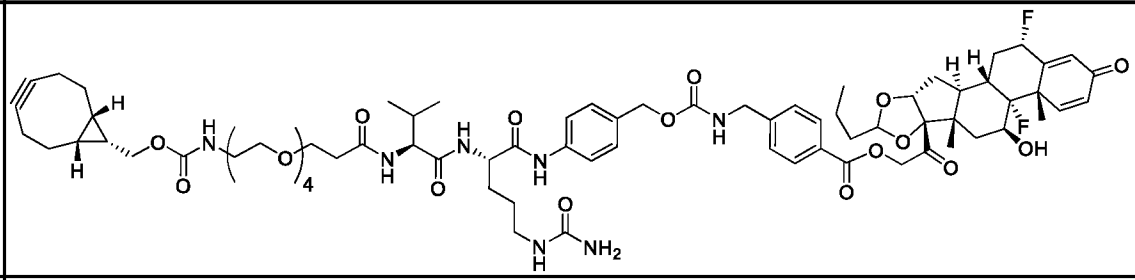
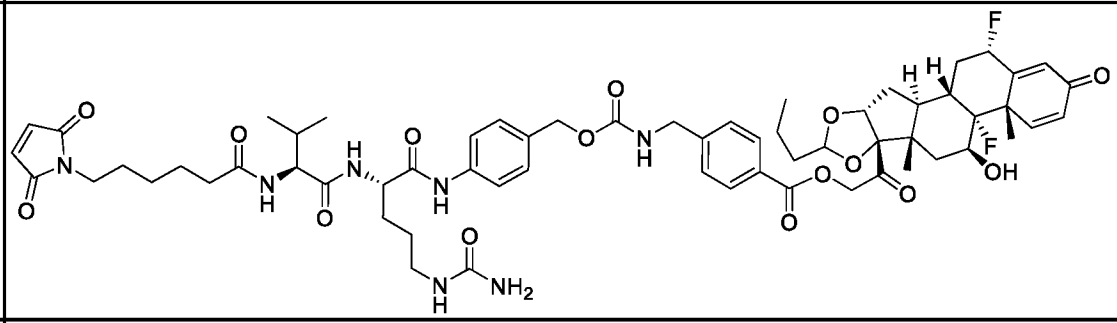
2l	
2m	
2n	 or a mixture thereof
2q	

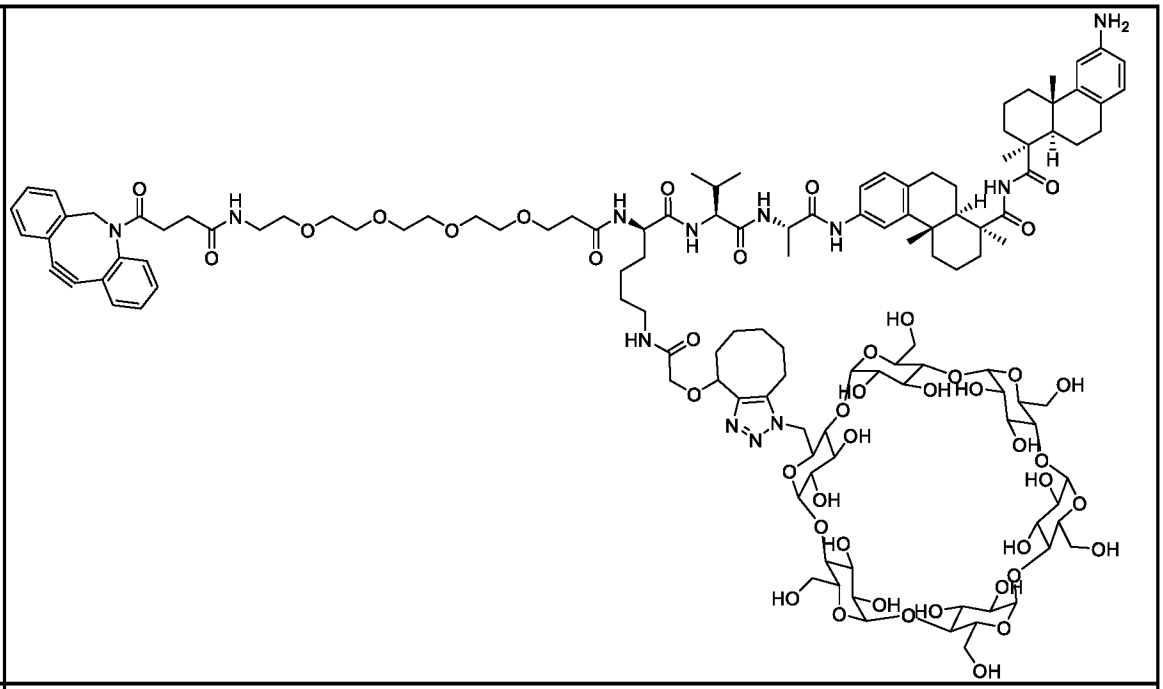
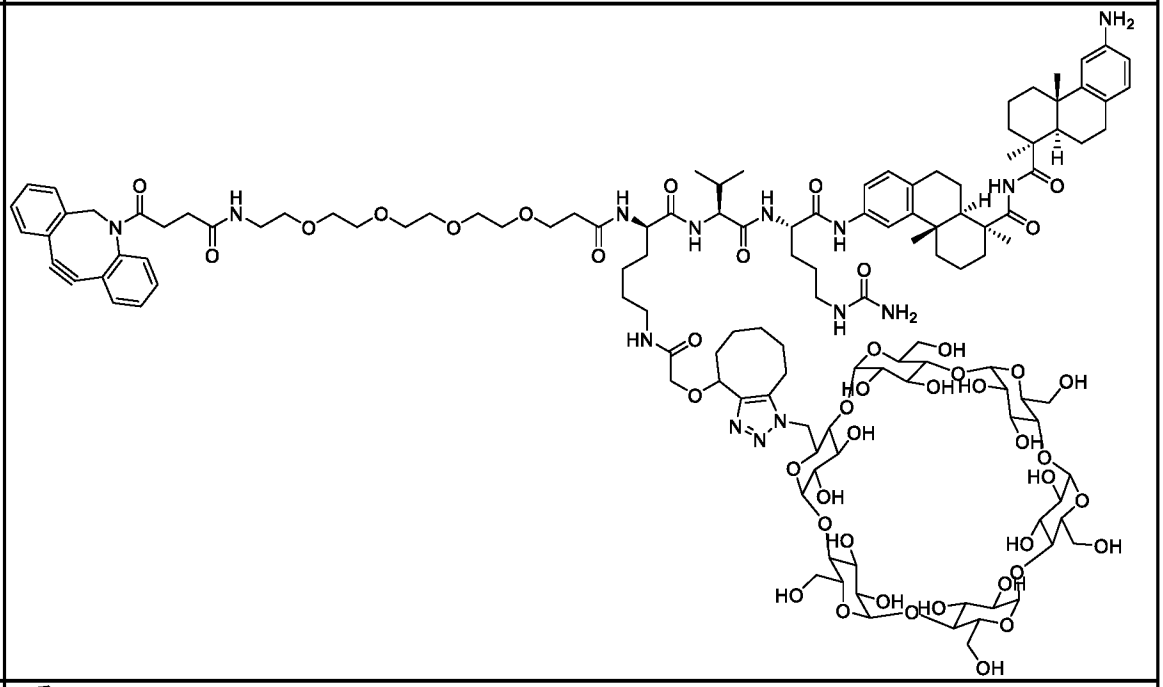
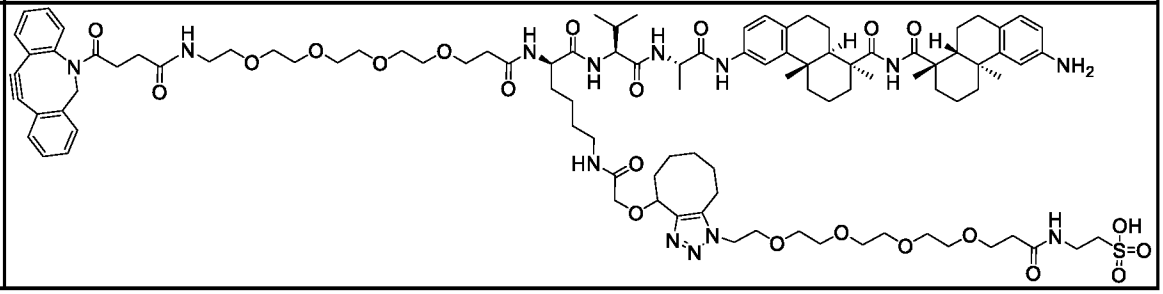
LP1A	
LP2A	
LP3A	
LP4A	
LP5A	
LP6A	

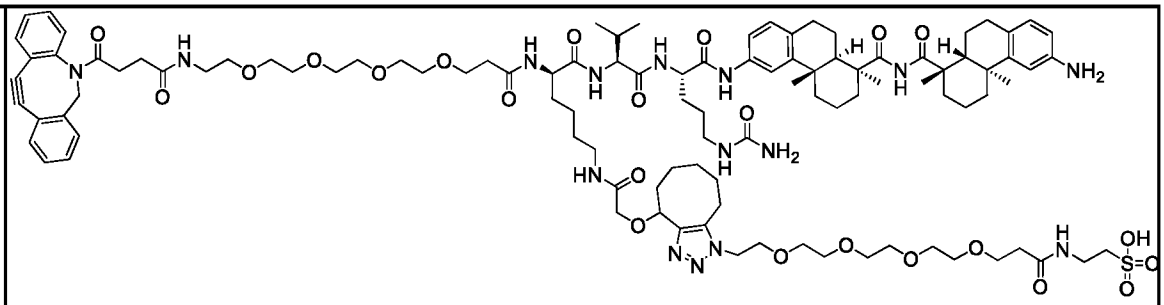
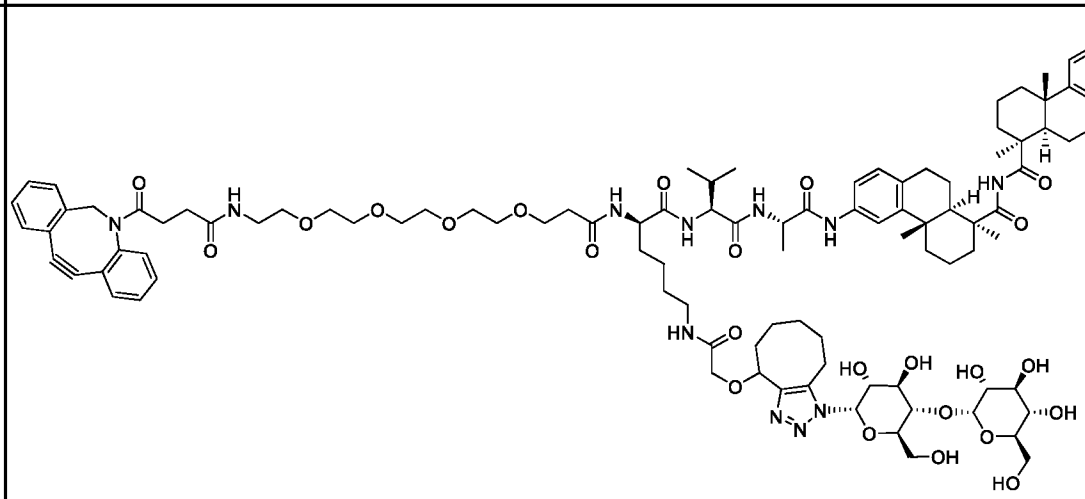
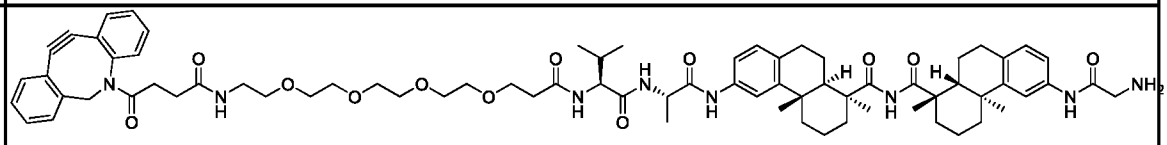
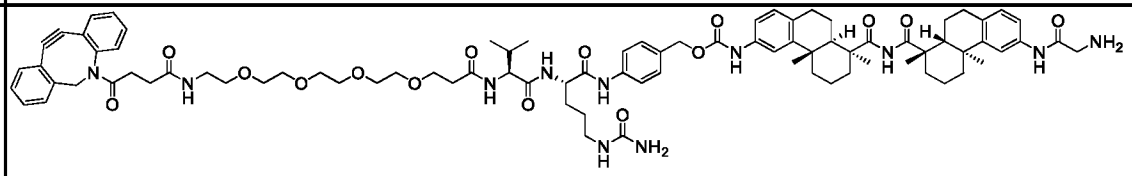
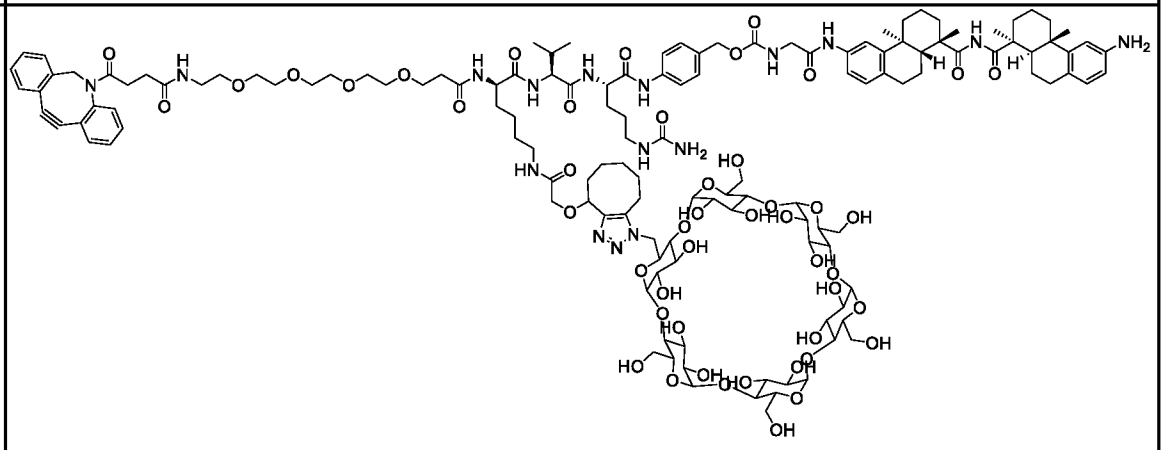
LP7A	
LP8A	
LP9A	
LP10 A	
LP11 A	
LP12 A	

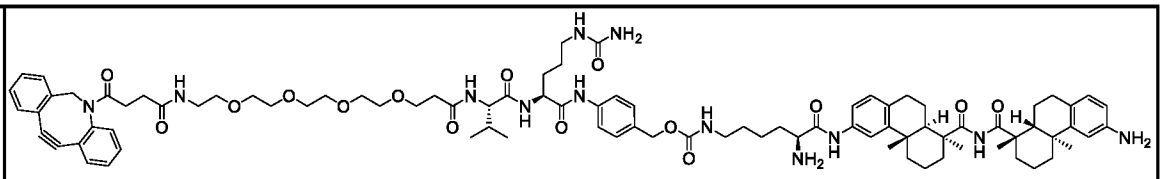
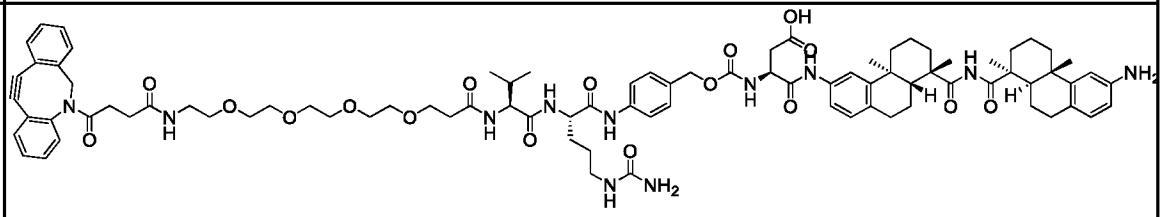
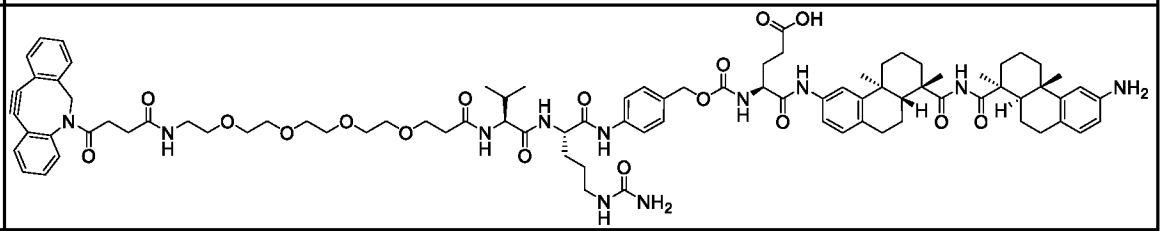
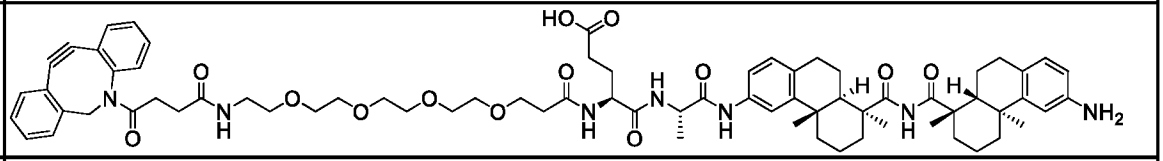
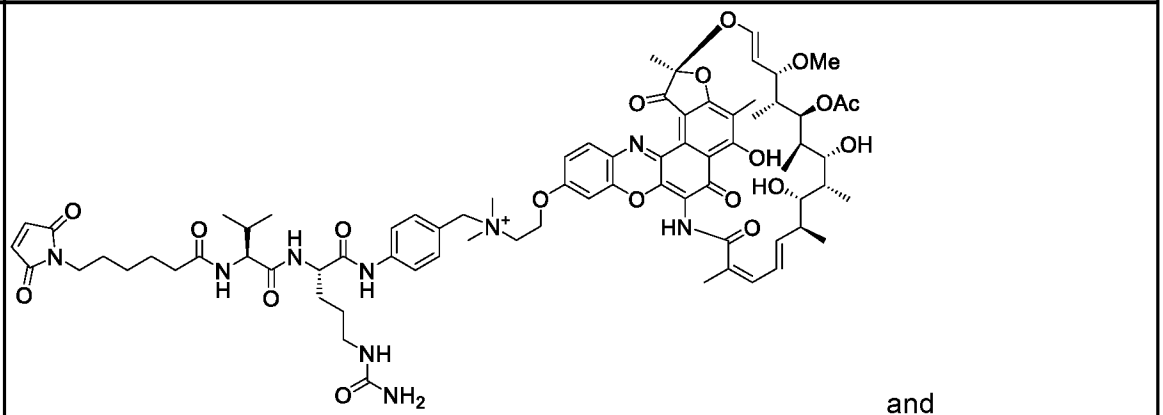
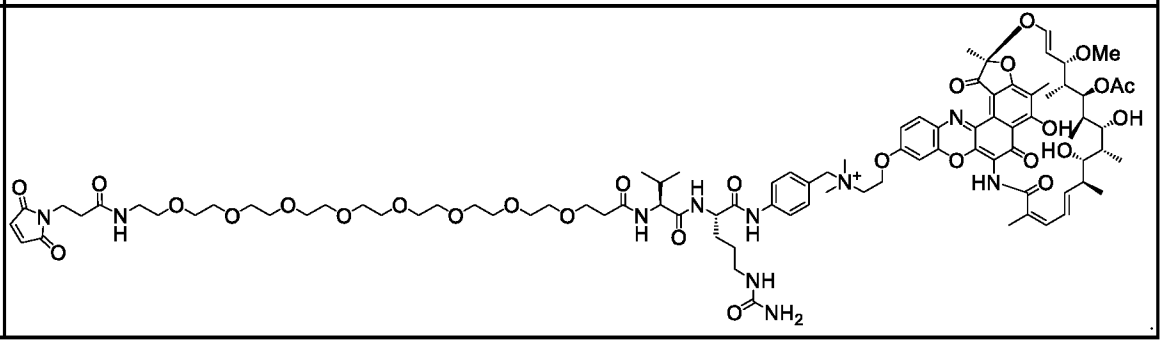
LP13 A	
LP18 A	
LP19 A	
LP20 A	
LP21 A	
LP22 A	

LP23A	
LP24A	
LP25A	
LP26A	

LP27A	
LP28A	
LP29A	
LP30A	
LP31A	

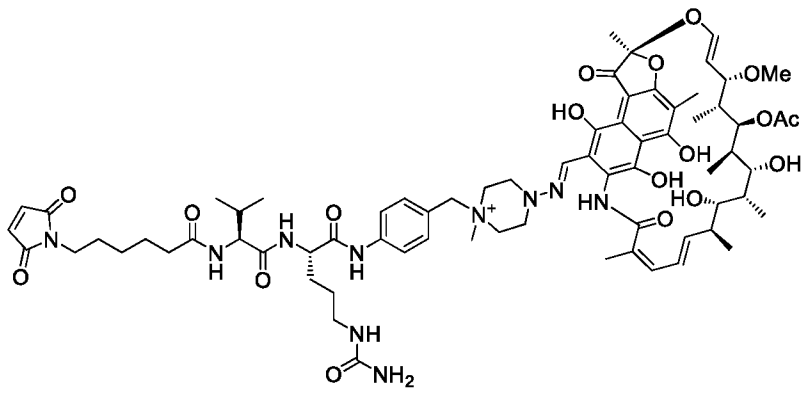
LP1B	
LP2B	
LP3B	

LP4B	
LP5B	
LP6B	
LP7B	
LP8B	

LP9B	
LP10 B	
LP11 B	
LP12 B	
R20	 and
R25	

56. An antibody-drug conjugate of claim 9, prepared by contacting an anti-MSR1 antibody or a PEG-modified anti-MSR1 antibody, or antigen-binding fragment thereof, with a linker

payload, or a stereoisomeric form thereof, or a regioisomer thereof, according to Formula (**D**):



Formula (**D**).

under conditions suitable for forming a bond between anti-MSR1 antibody, or antigen-binding fragment thereof.

57. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 8, and a pharmaceutically acceptable carrier.

58. A pharmaceutical composition comprising the antibody-drug conjugate of any one of claims 9 to 54, and a pharmaceutically acceptable carrier.

59. A method of treating a proliferative disease, a metabolic disease, inflammation, a neurodegenerative disease, or disease, disorder, or condition associated with glucocorticoid receptor signaling, in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate of any one of claims 1-23, 25-34, 51-53, or a pharmaceutical composition of any one of claims 47-48.

60. The method of claim 59, wherein the method is for treating atherosclerosis.

61. The method of claim 59, wherein the disease, disorder, or condition associated with glucocorticoid receptor signaling is an inflammatory disease, disorder, or condition.

62. The method of claim 59, wherein the method is for treating arthritis.

63. The method of claim 59, wherein side effects associated with administration of the unconjugated steroid payload of said compound are reduced.

64. The method of claim 59, wherein the method metabolic disease is dyslipidemia.

65. A method of preventing or treating bacterial infection in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate of any one of claims 1-21, 24, 35-50, and 54, or a pharmaceutical composition of any one of claims 57-58.

66. The method of claim 65, wherein the bacterium is a gram positive bacterium, pencillin-resistant bacterium *Staphylococcus aureus*, methiciliin resistant *Staphylococcus aureus* (MRSA), methicillin susceptible *Staphylococcus aureus* (MSSA), or vancomycin-resistant *Staphylococcus aureus* (VRSA), or multi-drug resistant *Mycobacterium tuberculosis*.

67. The method of claim 65, wherein the antibody-drug conjugate of any one of claims 1-21, 24, 35-50, and 54, or a pharmaceutical composition of any one of claims 57-58, is administered in combination with vancomycin.

68. A method of preventing or treating cellulitis, bacteremia, dermonecrosis, eyelid infection, eye infection, neonatal conjunctivitis, osteomyelitis, impetigo, boils, scalded skin syndrome, food poisoning, pneumonia, surgical infection, urinary tract infection, burn infection, meningitis, endocarditis, septicemia, toxic shock syndrome, septic arthritis, mastitis, infection associated with a prosthetic joint, infection associated with a catheter, or infection associated with an implant, in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate of any one of claims 11-21, 24, 35-50, and 54,or a pharmaceutical composition of any one of claims 57-58.

69. A method of preventing or treating an intracellular bacterial infection in a subject comprising administering to the subject an effective treatment amount of an antibody-drug conjugate of any one of claims 11-21, 24, 35-50, and 54, or a pharmaceutical composition of any one of claims 57-58.

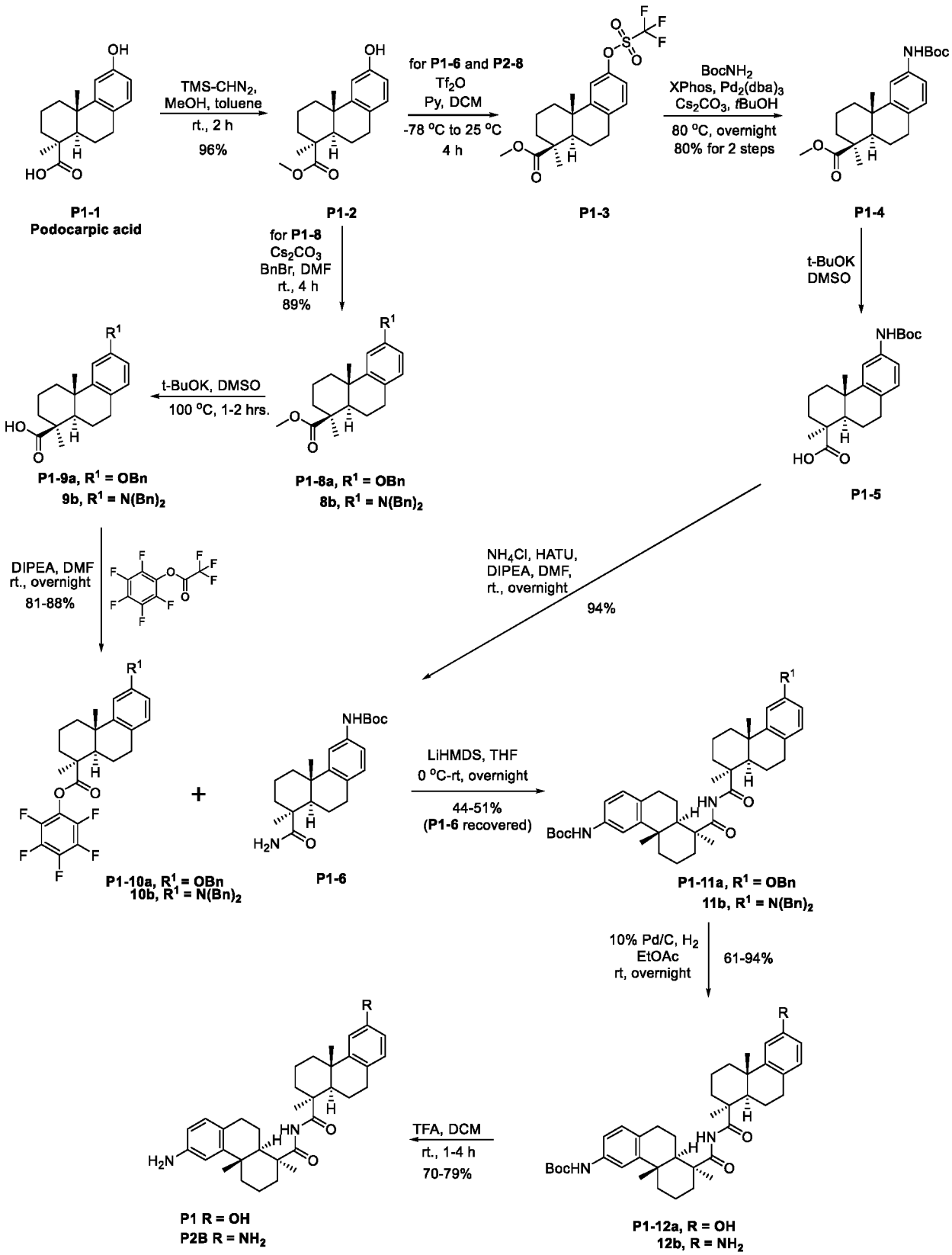

TMS-CHN2, MeOH, toluene
rt., 2 h
96%
for P1-6 and P2-8
Tf2O
Py, DCM
-78 °C to 25 °C
4 h
BocNH2
XPhos, Pd2(dba)3
Cs2CO3, tBuOH
80 °C, overnight
80% for 2 steps
P1-1
Podocarpic acid
P1-2
P1-3
P1-4
for P1-8
Cs2CO3
BnBr, DMF
rt., 4 h
89%
t-BuOK
DMSO
t-BuOK, DMSO
100 °C, 1-2 hrs.
P1-9a, R1 = OBn
9b, R1 = N(Bn)2
P1-8a, R1 = OBn
8b, R1 = N(Bn)2
P1-5
DIPEA, DMF
rt., overnight
81-88%
NH4Cl, HATU,
DIPEA, DMF,
rt., overnight
94%
P1-10a, R1 = OBn
10b, R1 = N(Bn)2
P1-6
LiHMDS, THF
0 °C-rt, overnight
44-51%
(P1-6 recovered)
P1-11a, R1 = OBn
11b, R1 = N(Bn)2
10% Pd/C, H2
EtOAc
rt, overnight
61-94%
TFA, DCM
rt., 1-4 h
70-79%
P1 R = OH
P2B R = NH2
P1-12a, R = OH
12b, R = NH2

FIG. 1

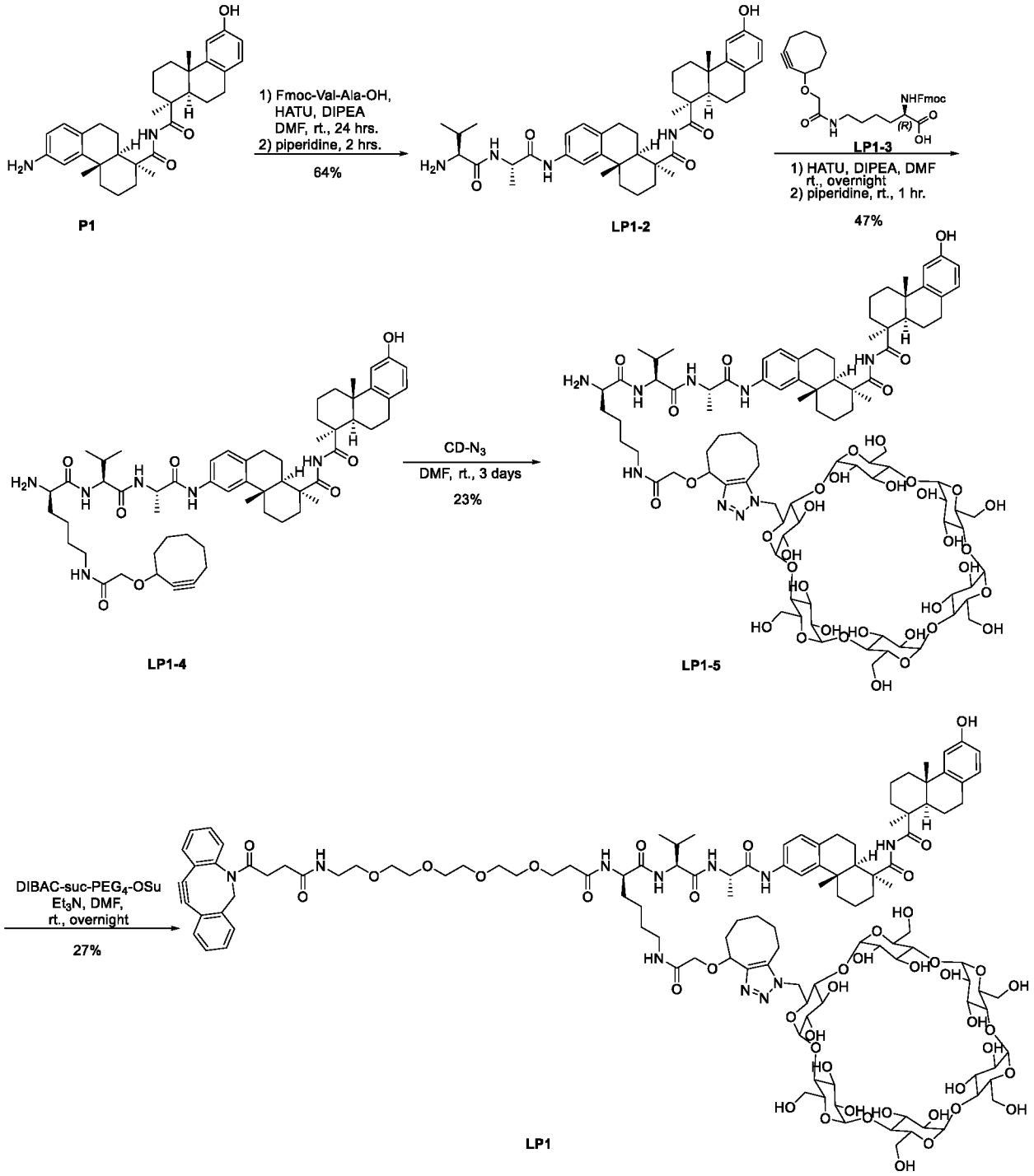

1) Fmoc-Val-Ala-OH, HATU, DIPEA DMF, rt., 24 hrs. 2) piperidine, 2 hrs.
64%
LP1-3
1) HATU, DIPEA, DMF rt., overnight 2) piperidine, rt., 1 hr.
47%
CD-N3
DMF, rt., 3 days
23%
DIBAC-suc-PEG4-OSu
Et3N, DMF,
rt., overnight
27%
P1
LP1-2
LP1-4
LP1-5
LP1

FIG. 2

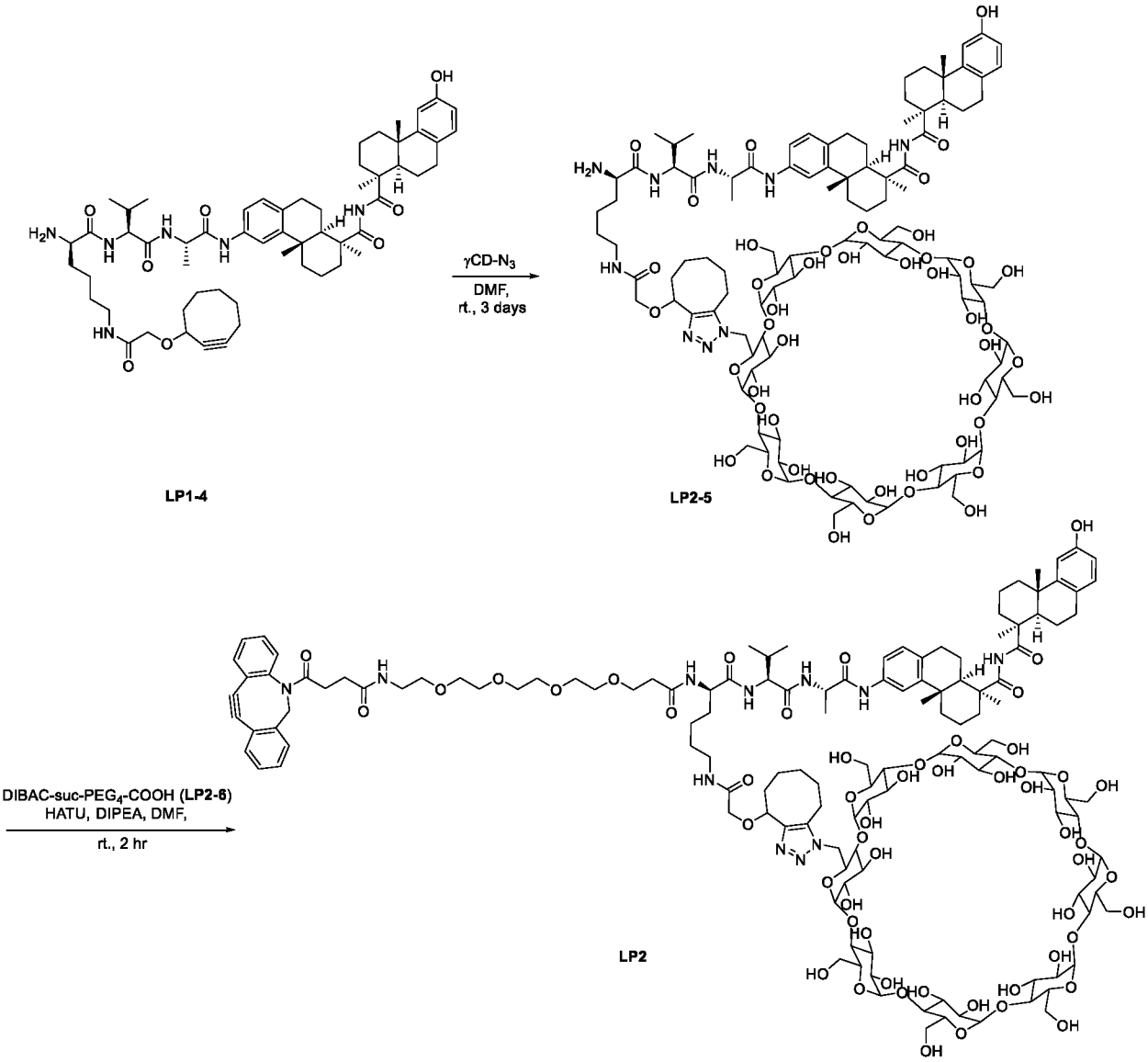

γCD-N3
DMF,
rt., 3 days
LP1-4
LP2-5
DIBAC-suc-PEG4-COOH (LP2-6)
HATU, DIPEA, DMF,
rt., 2 hr
LP2

FIG. 3

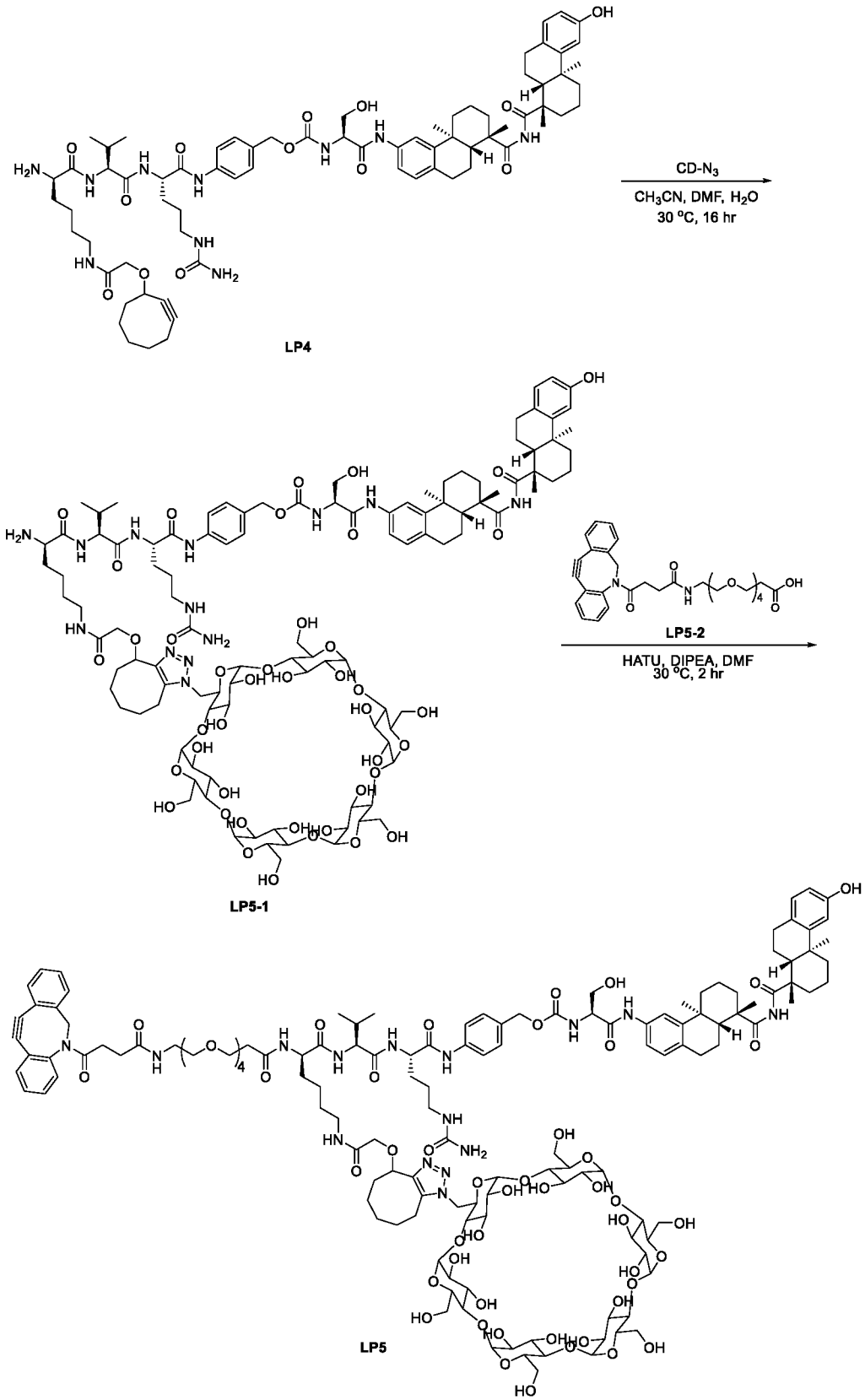

CD-N3
CH3CN, DMF, H2O
30 °C, 16 hr
LP4
LP5-2
HATU, DIPEA, DMF
30 °C, 2 hr
LP5-1
LP5

FIG. 4

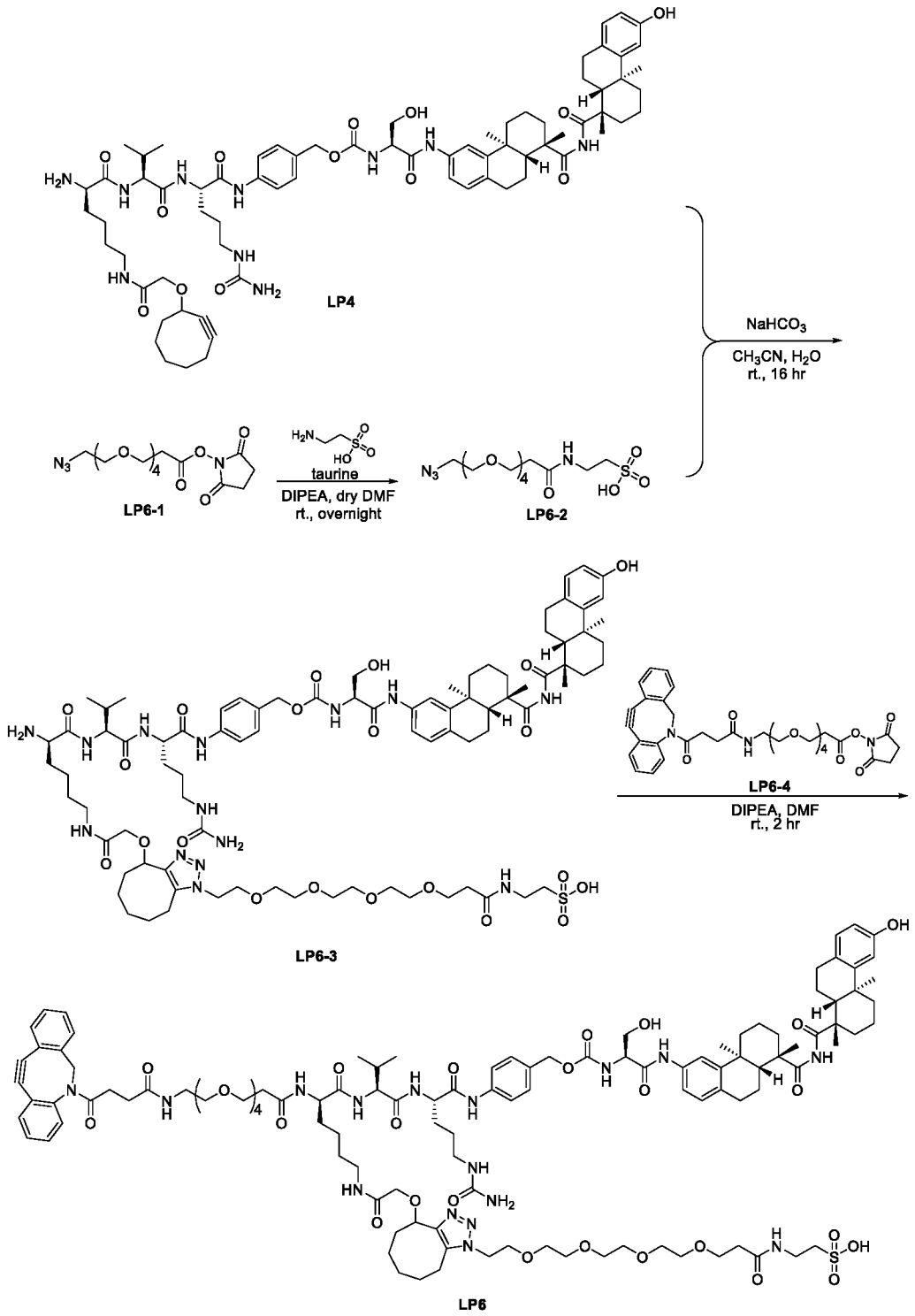

LP4
NaHCO3
CH3CN, H2O
rt., 16 hr
LP6-1
taurine
DIPEA, dry DMF
rt., overnight
LP6-2
LP6-4
DIPEA, DMF
rt., 2 hr
LP6-3
LP6

FIG. 5

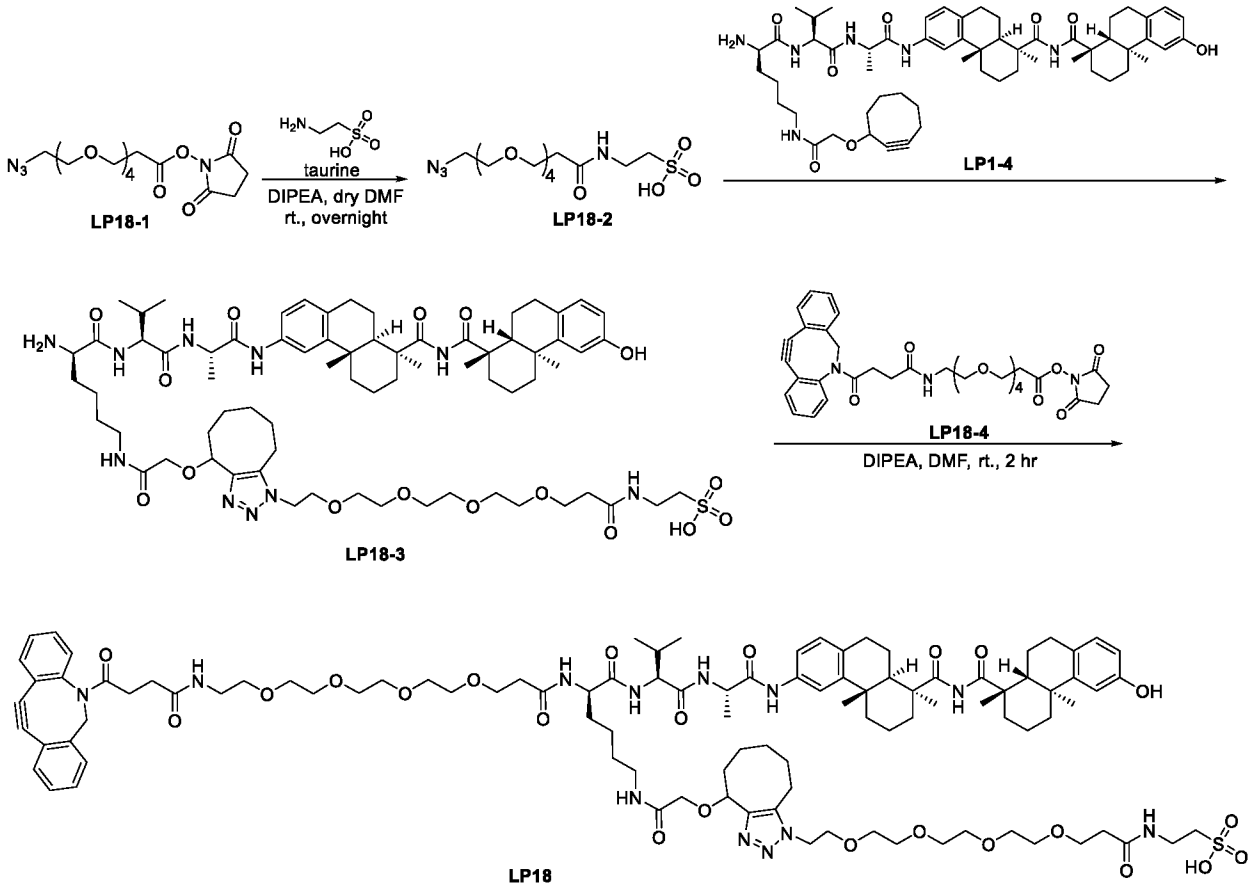

LP18-1
taurine
DIPEA, dry DMF
rt., overnight
LP18-2
LP1-4
LP18-4
DIPEA, DMF, rt., 2 hr
LP18-3
LP18

FIG. 6

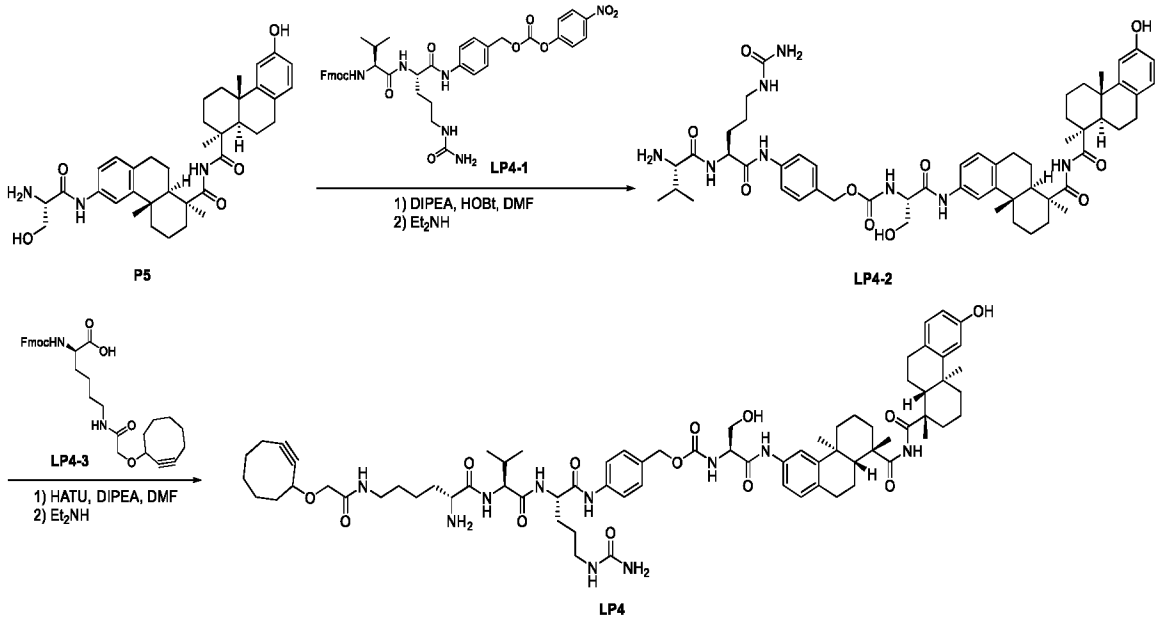

P5
LP4-1
1) DIPEA, HOBt, DMF
2) Et2NH
LP4-2
LP4-3
1) HATU, DIPEA, DMF
2) Et2NH
LP4

FIG. 7

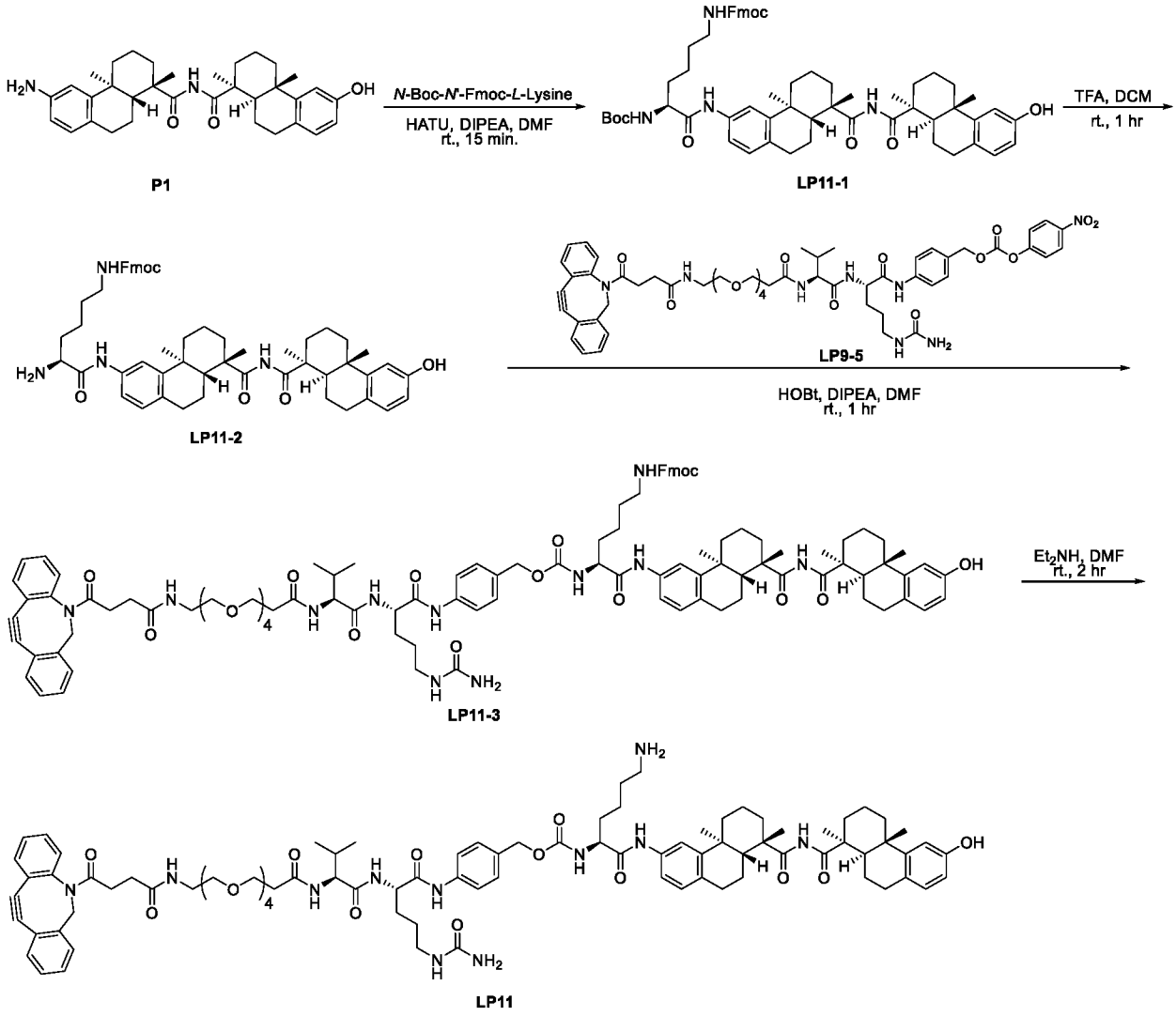

P1
N-Boc-N'-Fmoc-L-Lysine
HATU, DIPEA, DMF
rt., 15 min.
LP11-1
TFA, DCM
rt., 1 hr
LP11-2
LP9-5
HOBt, DIPEA, DMF
rt., 1 hr
LP11-3
Et2NH, DMF
rt., 2 hr
LP11

FIG. 8

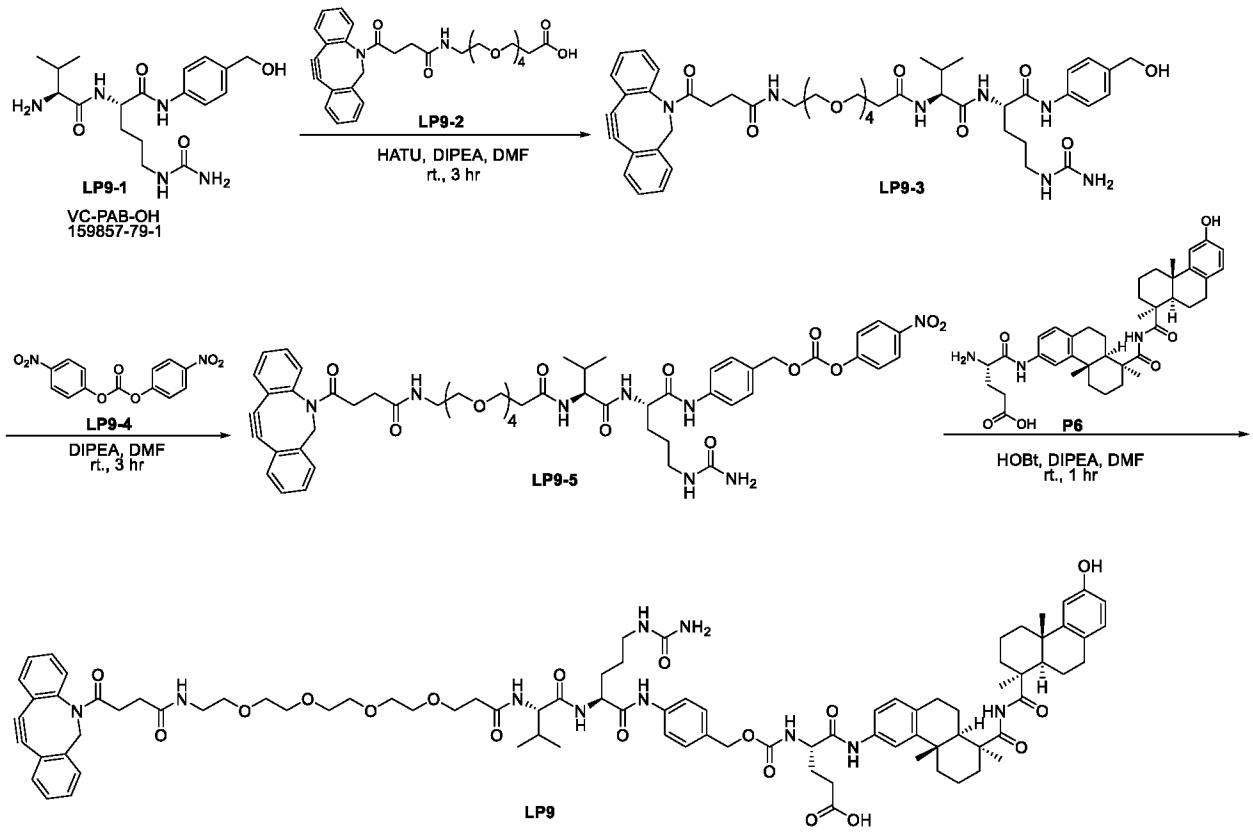

LP9-1
VC-PAB-OH
159857-79-1
LP9-2
HATU, DIPEA, DMF
rt., 3 hr
LP9-3
LP9-4
DIPEA, DMF
rt., 3 hr
LP9-5
P6
HOBt, DIPEA, DMF
rt., 1 hr
LP9

FIG. 9

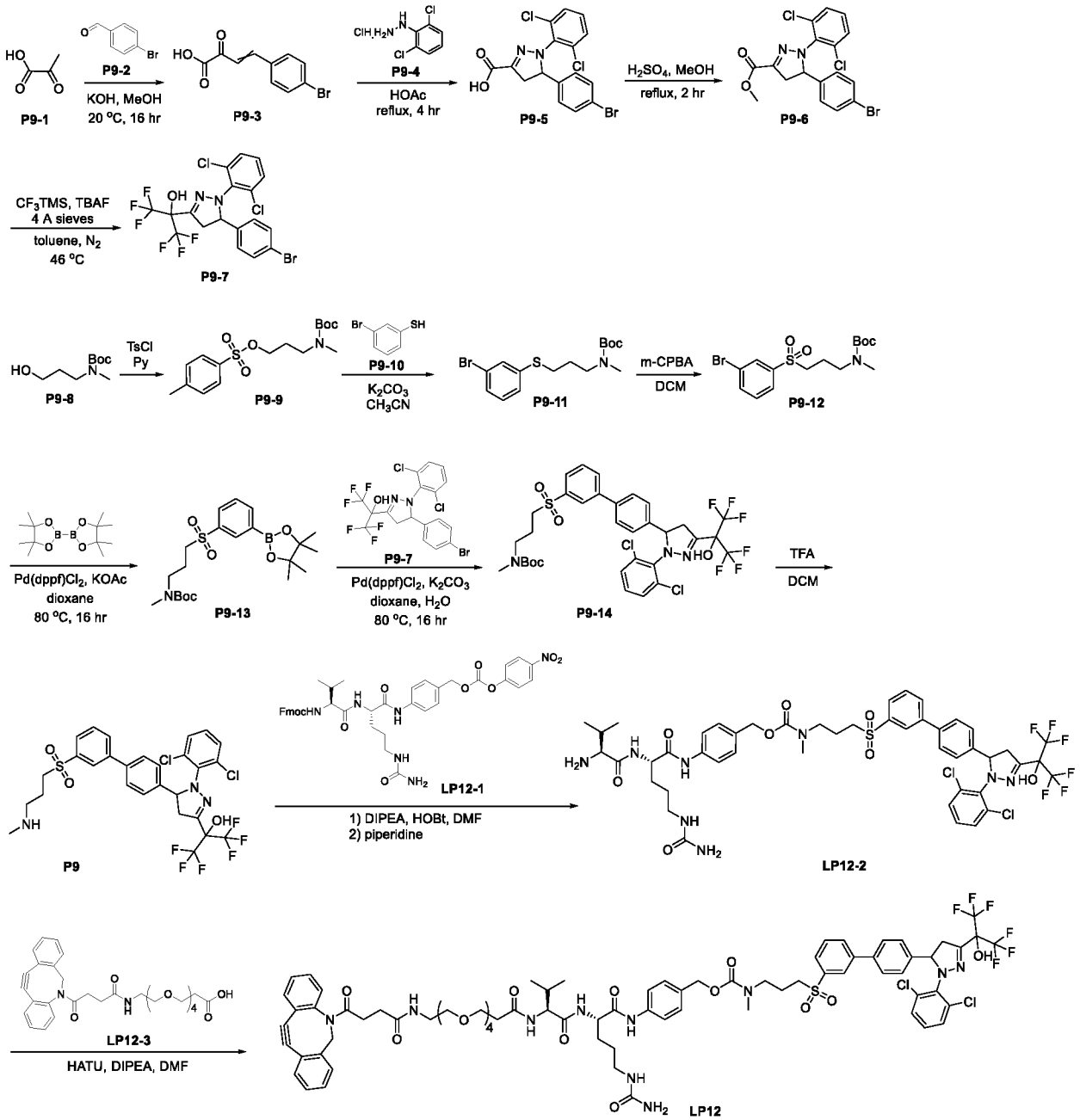

P9-1
P9-2
KOH, MeOH
20 °C, 16 hr
P9-3
P9-4
HOAc
reflux, 4 hr
P9-5
H2SO4, MeOH
reflux, 2 hr
P9-6
CF3TMS, TBAF
4 A sieves
toluene, N2
46 °C
P9-7
P9-8
TsCl
Py
P9-9
P9-10
K2CO3
CH3CN
P9-11
m-CPBA
DCM
P9-12
Pd(dppf)Cl2, KOAc
dioxane
80 °C, 16 hr
P9-13
P9-7
Pd(dppf)Cl2, K2CO3
dioxane, H2O
80 °C, 16 hr
P9-14
TFA
DCM
P9
LP12-1
1) DIPEA, HOBt, DMF
2) piperidine
LP12-2
LP12-3
HATU, DIPEA, DMF
LP12

FIG. 10

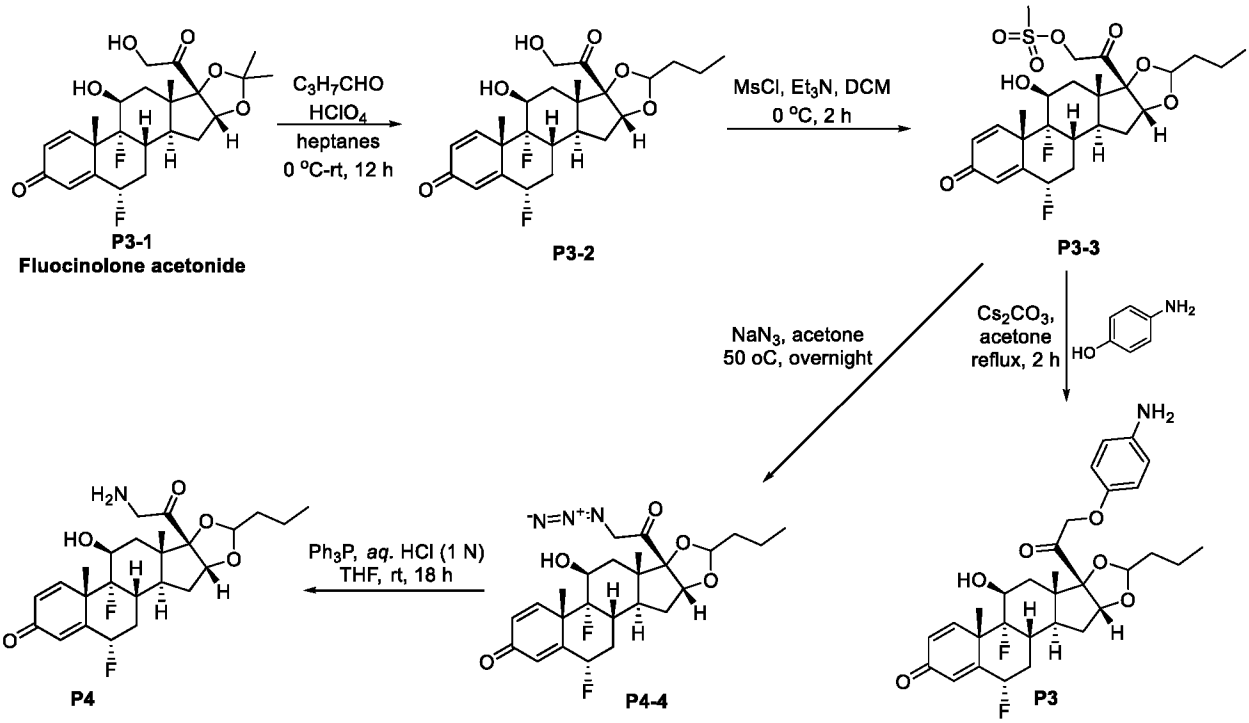

P3-1
Fluocinolone acetonide
C3H7CHO
HClO4
heptanes
0 °C-rt, 12 h
P3-2
MsCl, Et3N, DCM
0 °C, 2 h
P3-3
NaN3, acetone
50 oC, overnight
Cs2CO3,
acetone
reflux, 2 h
P3
P4-4
Ph3P, aq. HCl (1 N)
THF, rt, 18 h
P4

FIG. 11

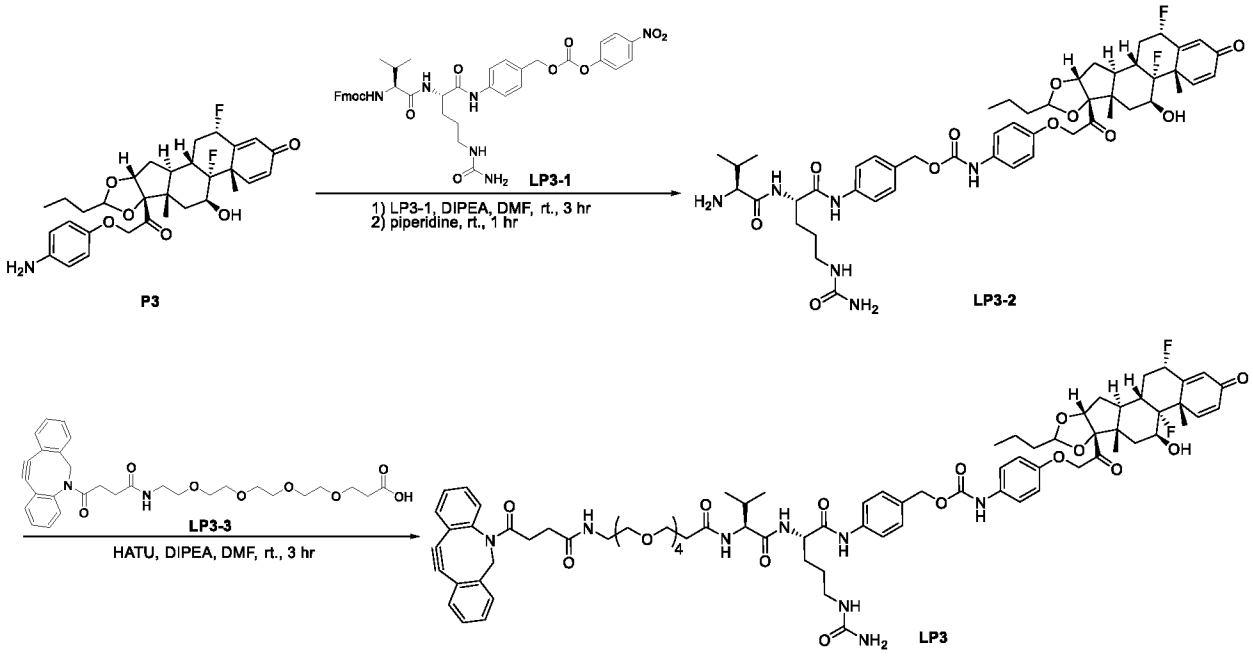

P3
LP3-1
1) LP3-1, DIPEA, DMF, rt., 3 hr
2) piperidine, rt., 1 hr
LP3-2
LP3-3
HATU, DIPEA, DMF, rt., 3 hr
LP3

FIG. 12

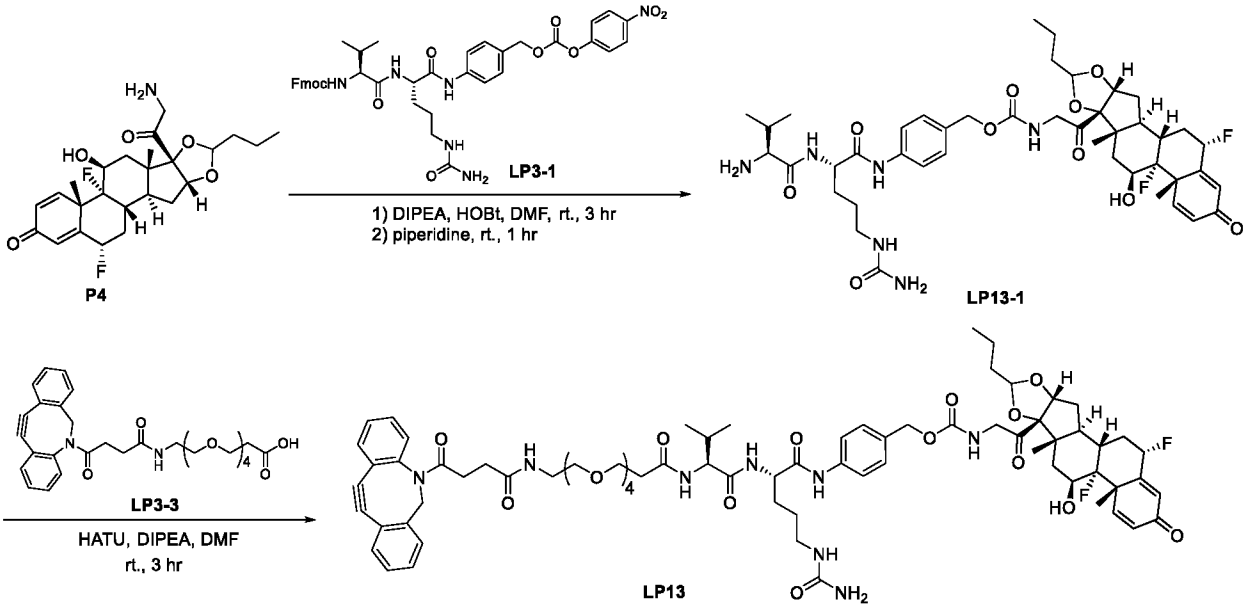

P4
LP3-1
1) DIPEA, HOBt, DMF, rt., 3 hr
2) piperidine, rt., 1 hr
LP13-1
LP3-3
HATU, DIPEA, DMF
rt., 3 hr
LP13

FIG. 13

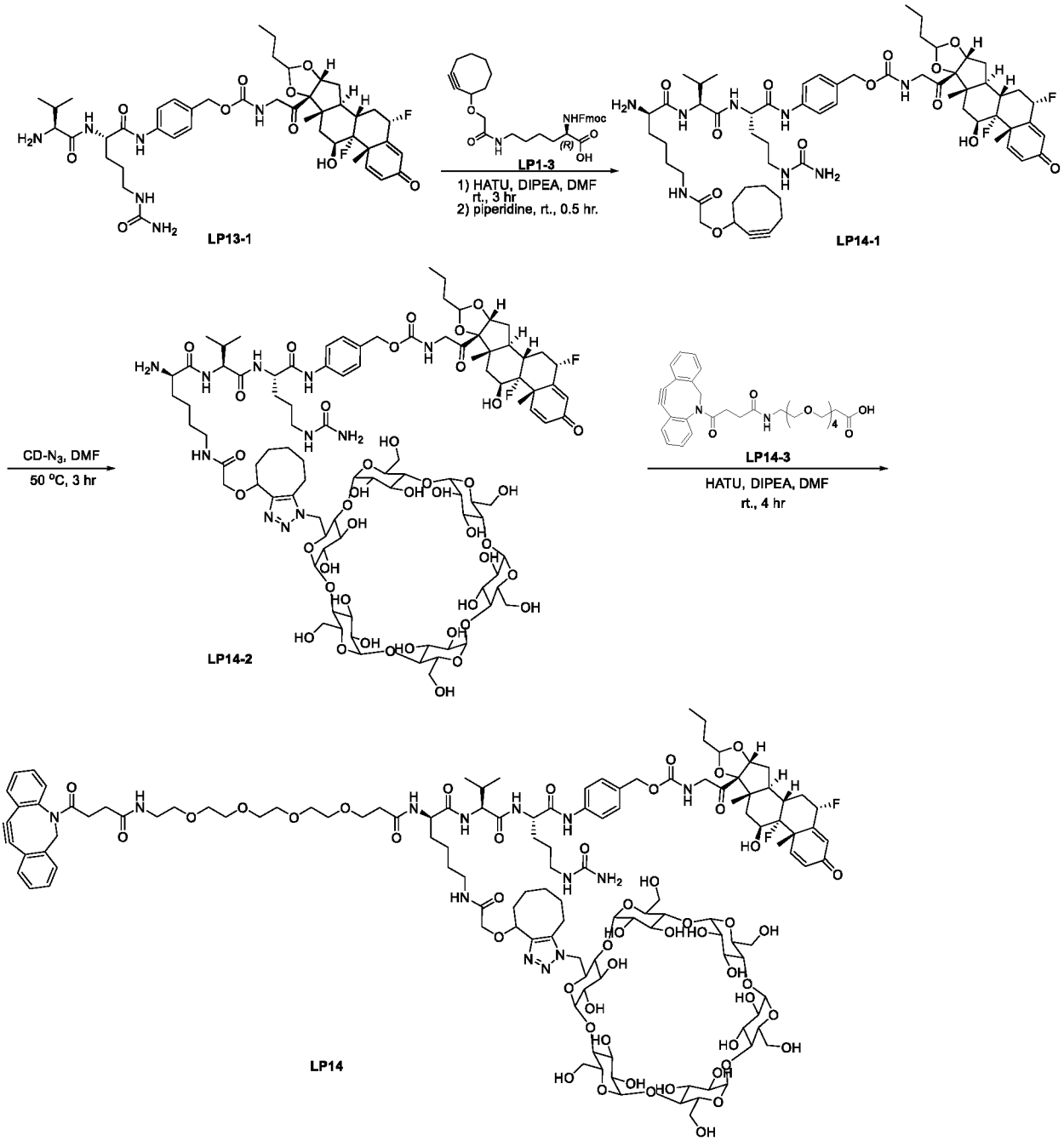

LP13-1
LP1-3
1) HATU, DIPEA, DMF
rt., 3 hr
2) piperidine, rt., 0.5 hr.
LP14-1
CD-N3, DMF
50 °C, 3 hr
LP14-2
LP14-3
HATU, DIPEA, DMF
rt., 4 hr
LP14

FIG. 14

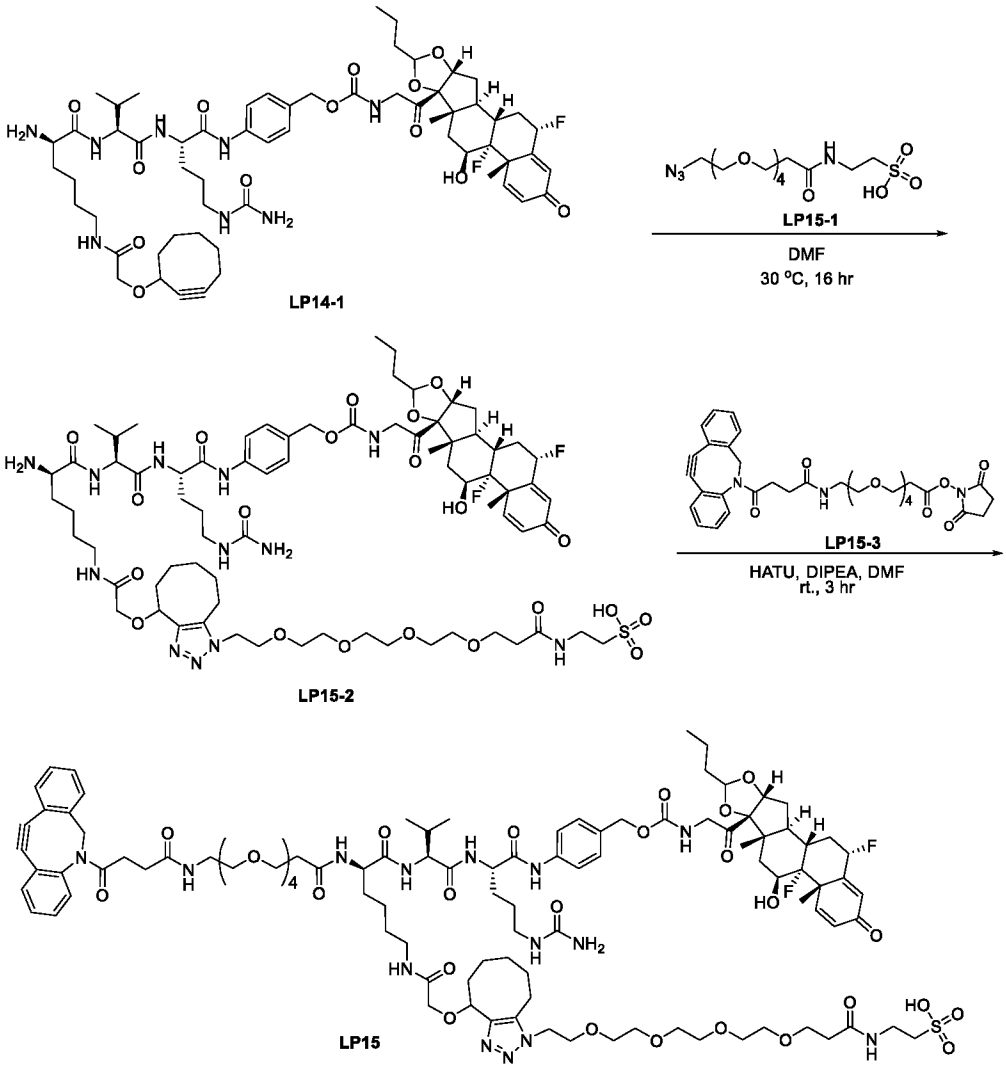

LP14-1
LP15-1
DMF
30 °C, 16 hr
LP15-2
LP15-3
HATU, DIPEA, DMF
rt., 3 hr
LP15

FIG. 15

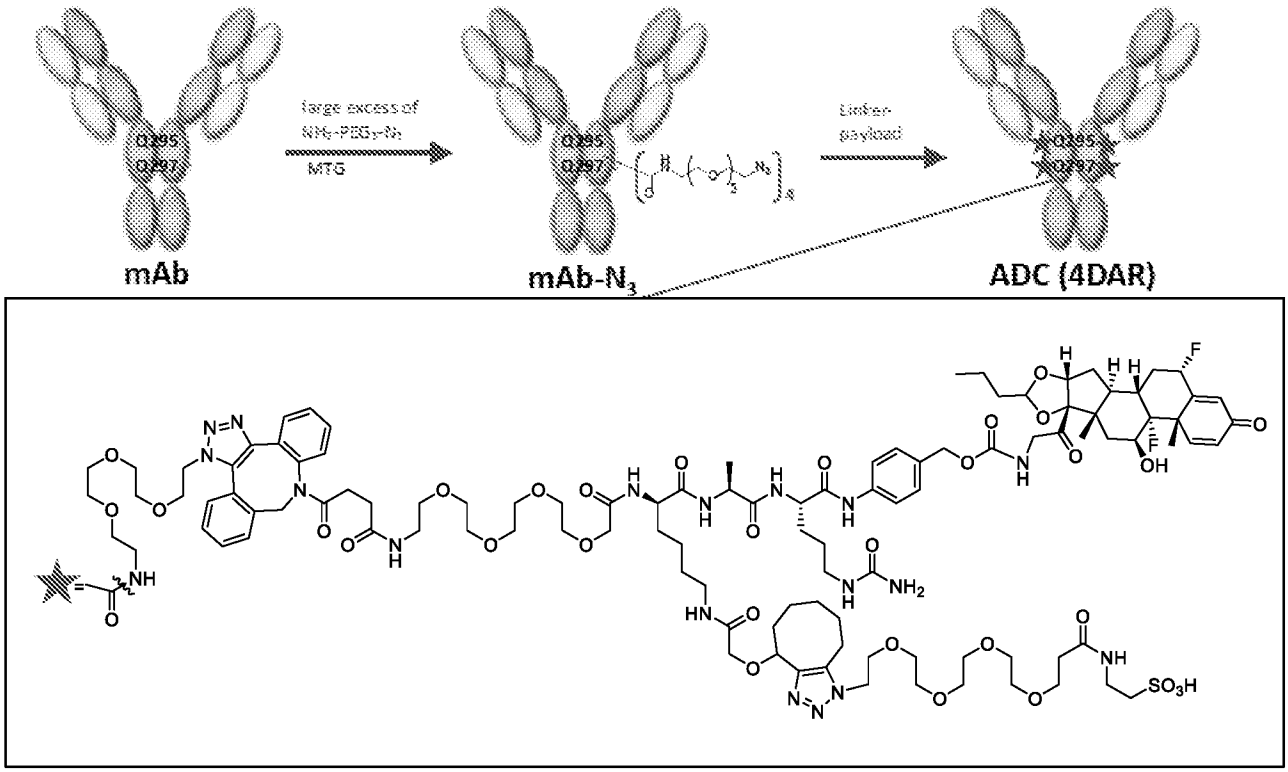


FIG. 16

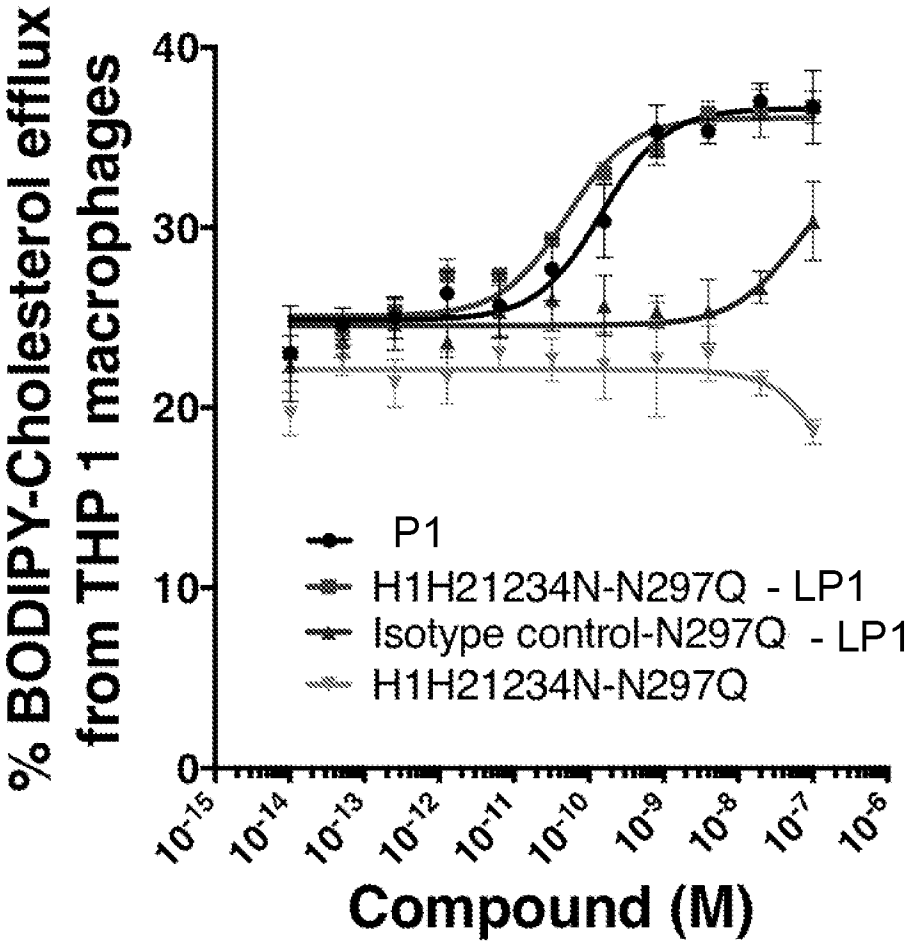

% BODIPY-Cholesterol efflux from THP 1 macrophages
40
30
20
10
0
P1
H1H21234N-N297Q - LP1
Isotype control-N297Q - LP1
H1H21234N-N297Q
10-15
10-14
10-13
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Compound (M)

FIG. 17

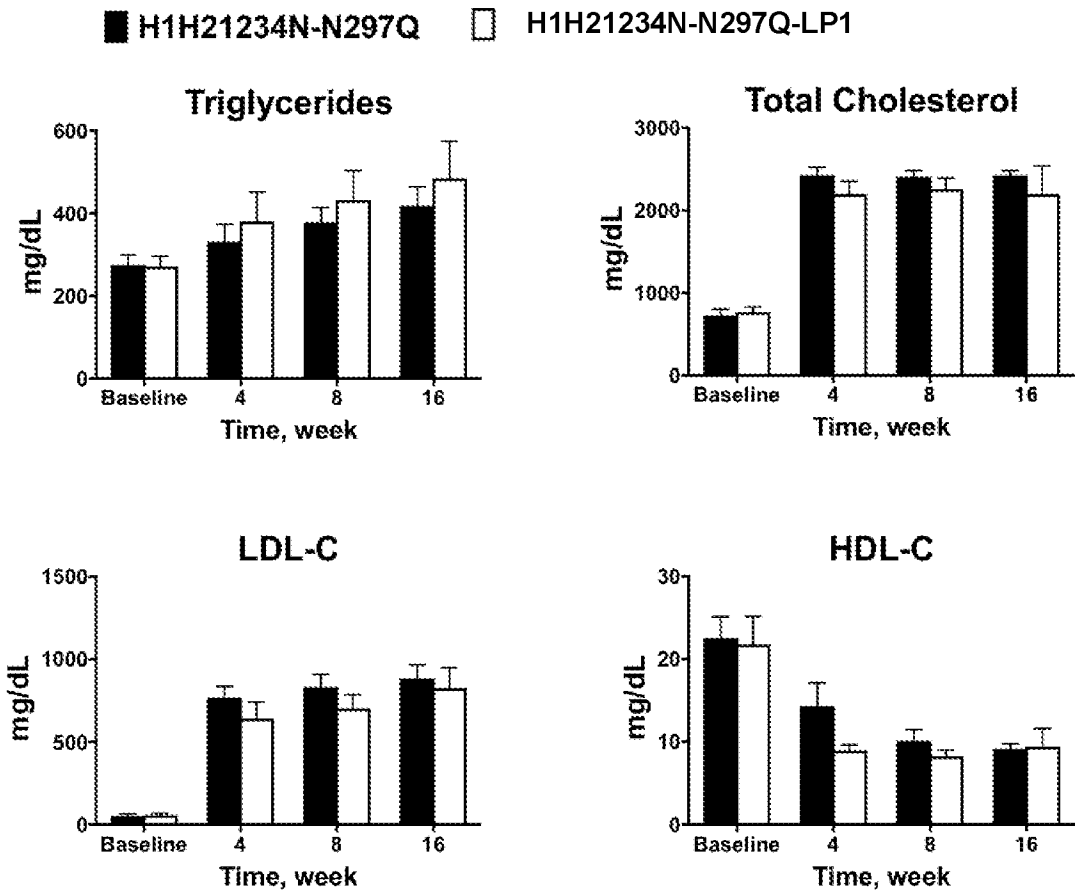
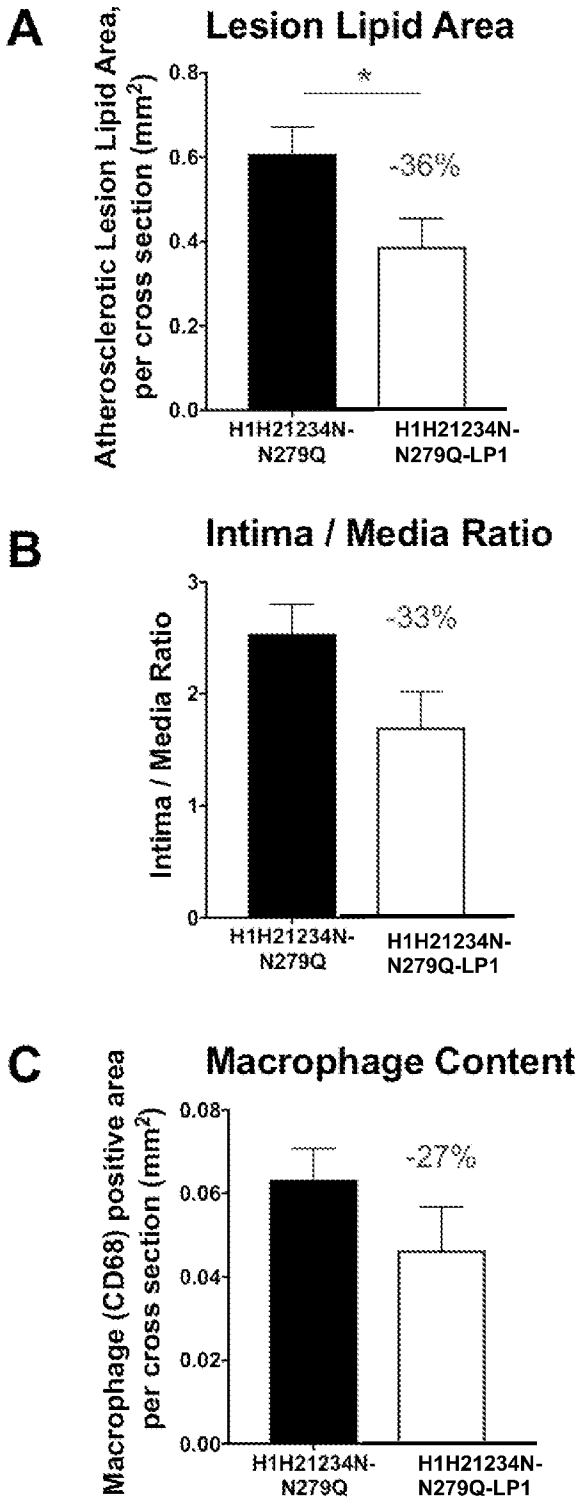

H1H21234N-N297Q
H1H21234N-N297Q-LP1
Triglycerides
mg/dL
600
400
200
0
Baseline
4
8
16
Time, week
Total Cholesterol
mg/dL
3000
2000
1000
0
Baseline
4
8
16
Time, week
LDL-C
mg/dL
1500
1000
500
0
Baseline
4
8
16
Time, week
HDL-C
mg/dL
30
20
10
0
Baseline
4
8
16
Time, week

FIG. 18



Statistical comparison by Welch's t-test, *p<0.05

FIG. 19

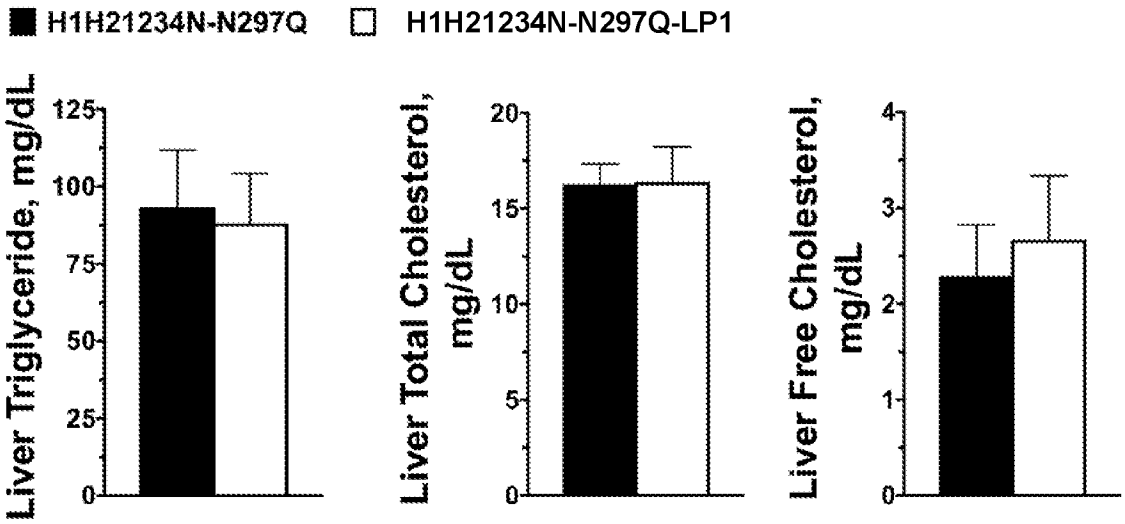

H1H21234N-N297Q
H1H21234N-N297Q-LP1
Liver Triglyceride, mg/dL
125
100
75
50
25
0
Liver Total Cholesterol, mg/dL
20
15
10
5
0
Liver Free Cholesterol, mg/dL
4
3
2
1
0

FIG. 20

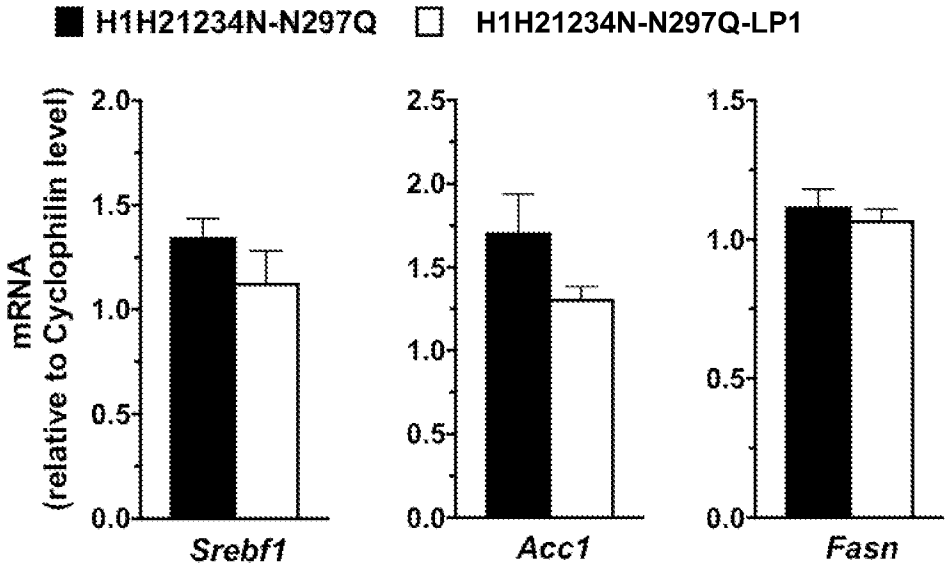
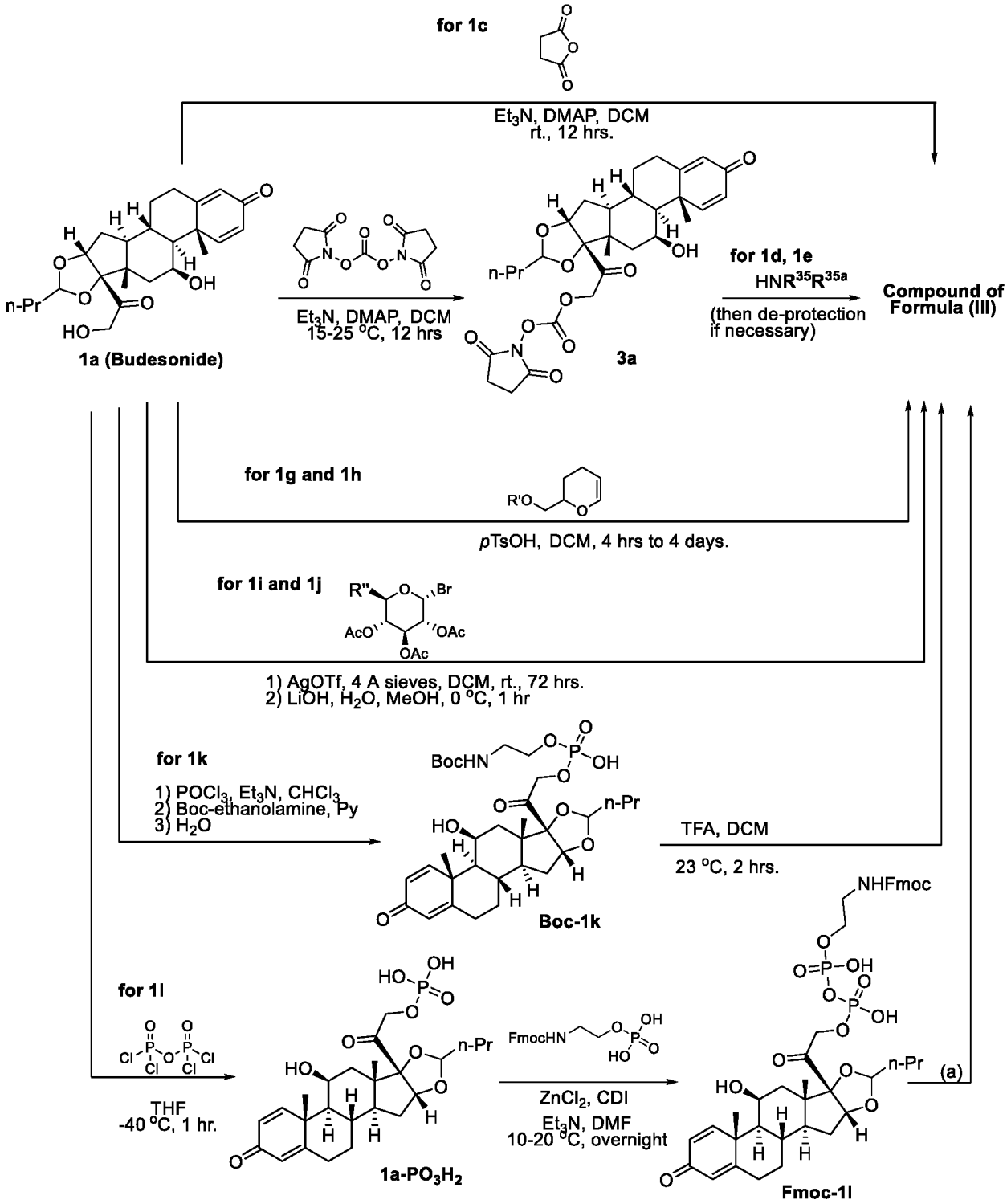

H1H21234N-N297Q
H1H21234N-N297Q-LP1
mRNA
(relative to Cyclophilin level)
2.0
1.5
1.0
0.5
0.0
Srebf1
2.5
2.0
1.5
1.0
0.5
0.0
Acc1
1.5
1.0
0.5
0.0
Fasn

FIG. 21

Scheme 1. Synthesis of Spacer-Budesonide 1c-1e, 1g-1i, and 1j-1l



(a) piperidine, DCM,10-20 °C, 16 hrs.

Where the Compound of Formula (III) comprises **1c, 1d, 1e, 1g, 1h, 1i, 1j, 1k, 1l**

FIG. 22

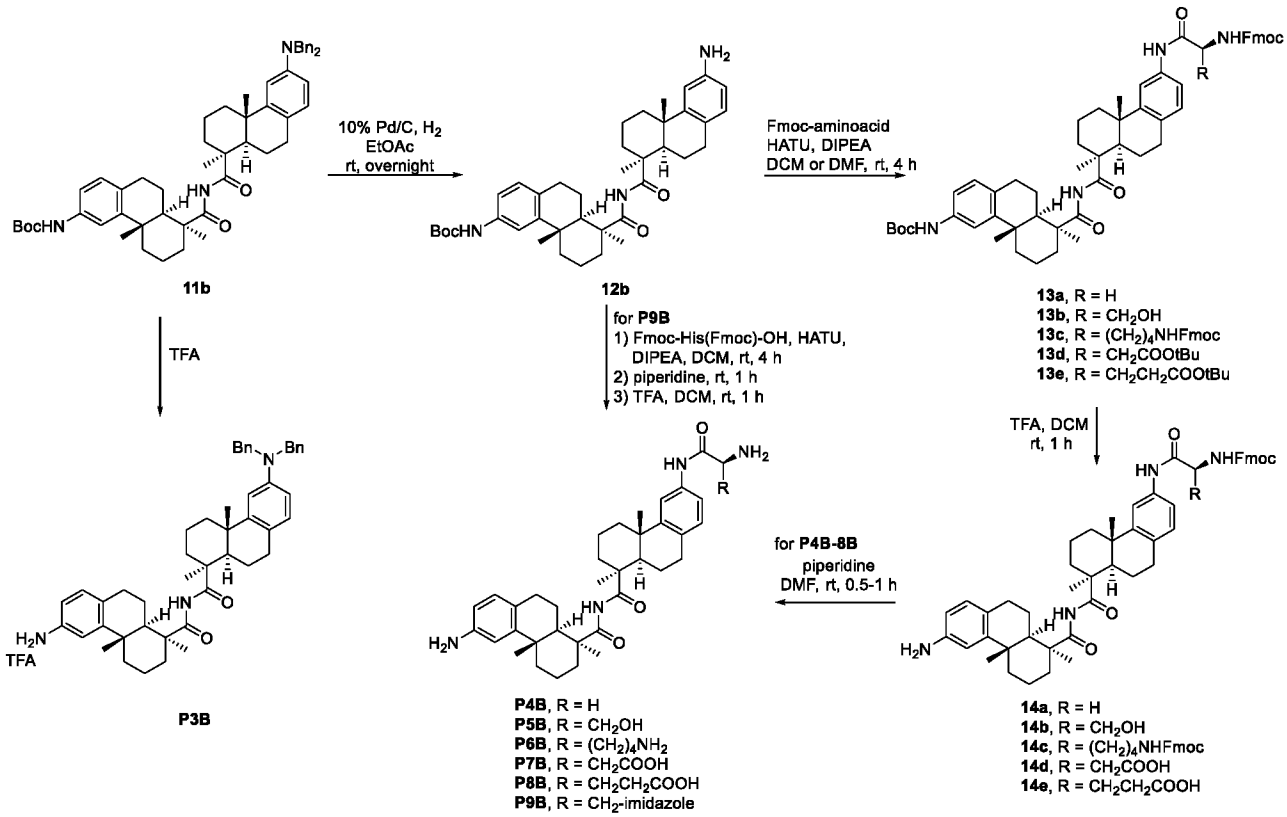
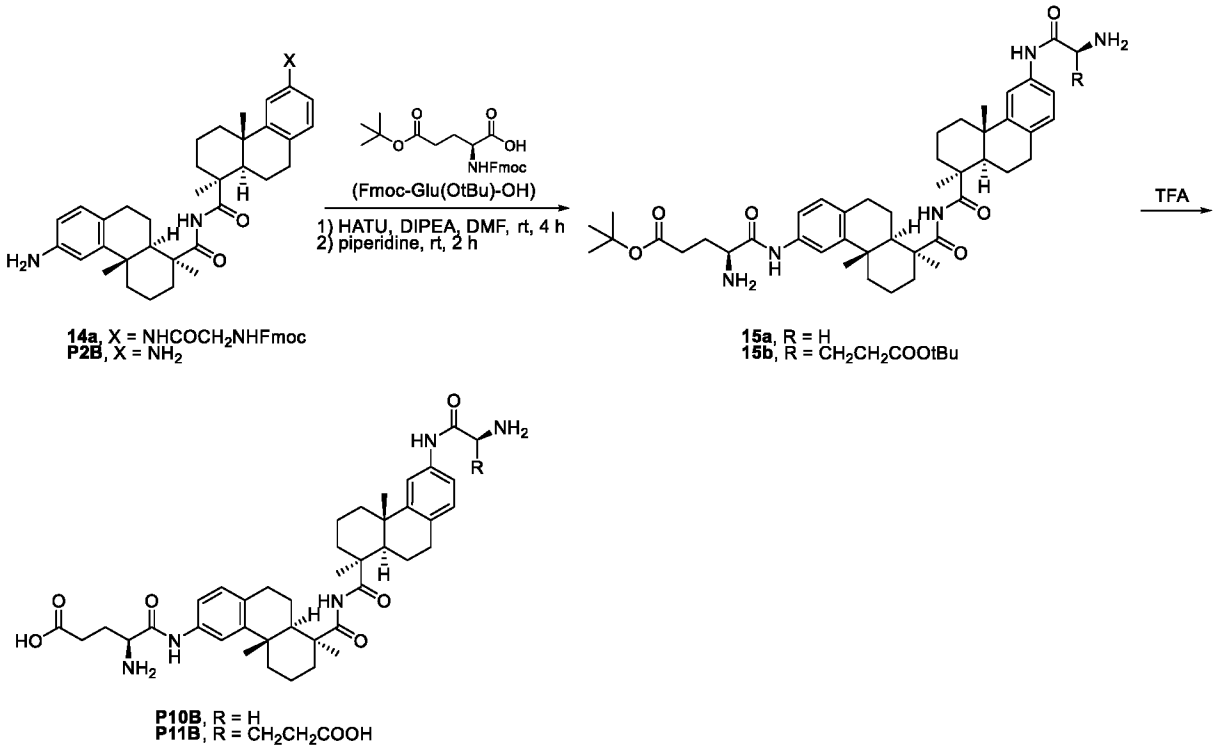


FIG. 23 Synthesis of Payloads P3B-P9B

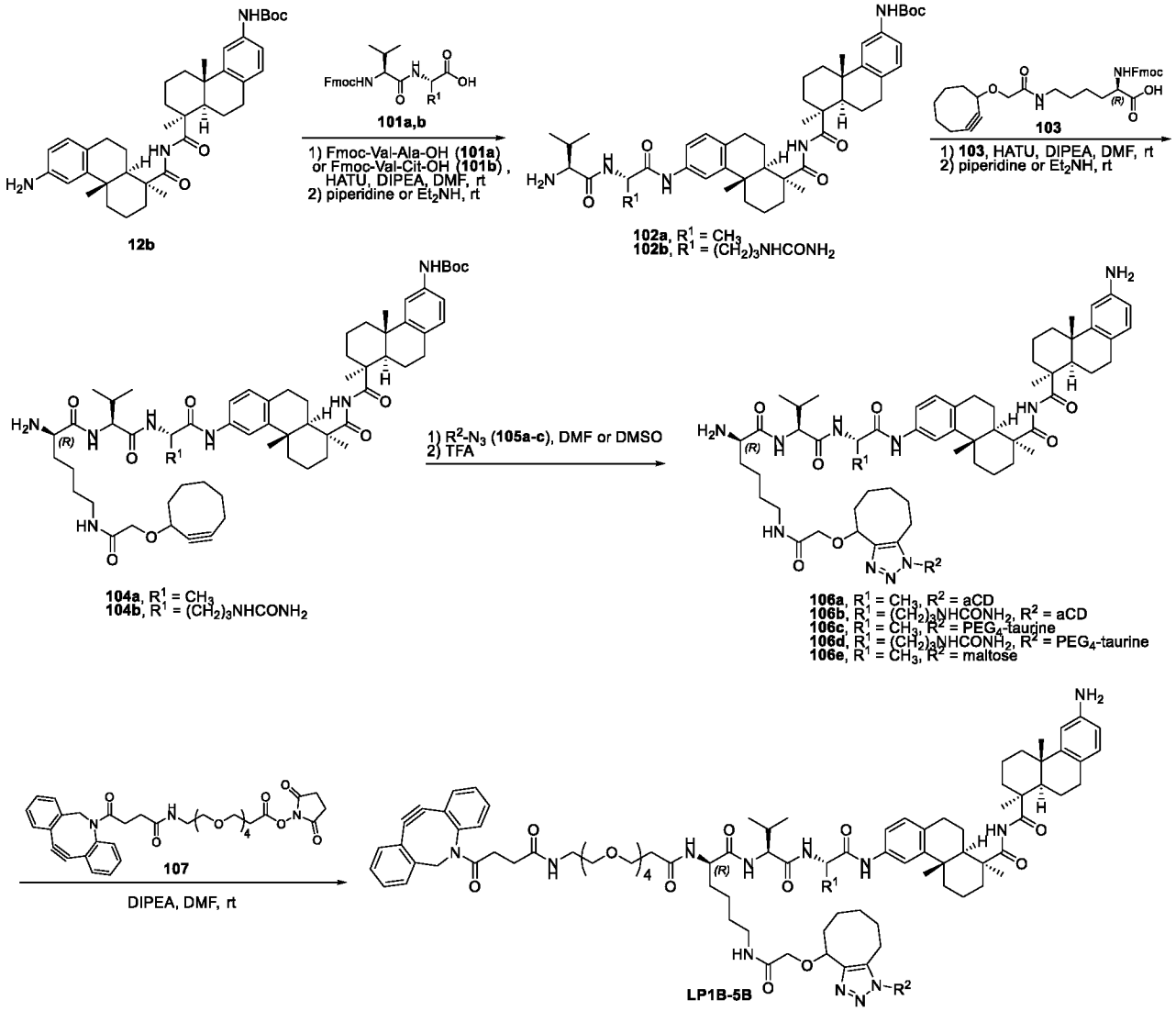


14a, X = $NHCOCH_2NHFmoc$
P2B, X = NH_2

15a, R = H
15b, R = $CH_2CH_2COOtBu$

P10B, R = H
P11B, R = CH_2CH_2COOH

FIG. 24 Synthesis of Payloads P10B and P11B



Cpd#	R^1	R^2
LP1B	CH_3	αCD
LP2B	$(CH_2)_3NHCONH_2$	αCD
LP3B	CH_3	PEG_4-taurine
LP4B	$(CH_2)_3NHCONH_2$	PEG_4-taurine
LP5B	CH_3	maltose

FIG. 25 General Synthesis of Linker and LP1B-5B

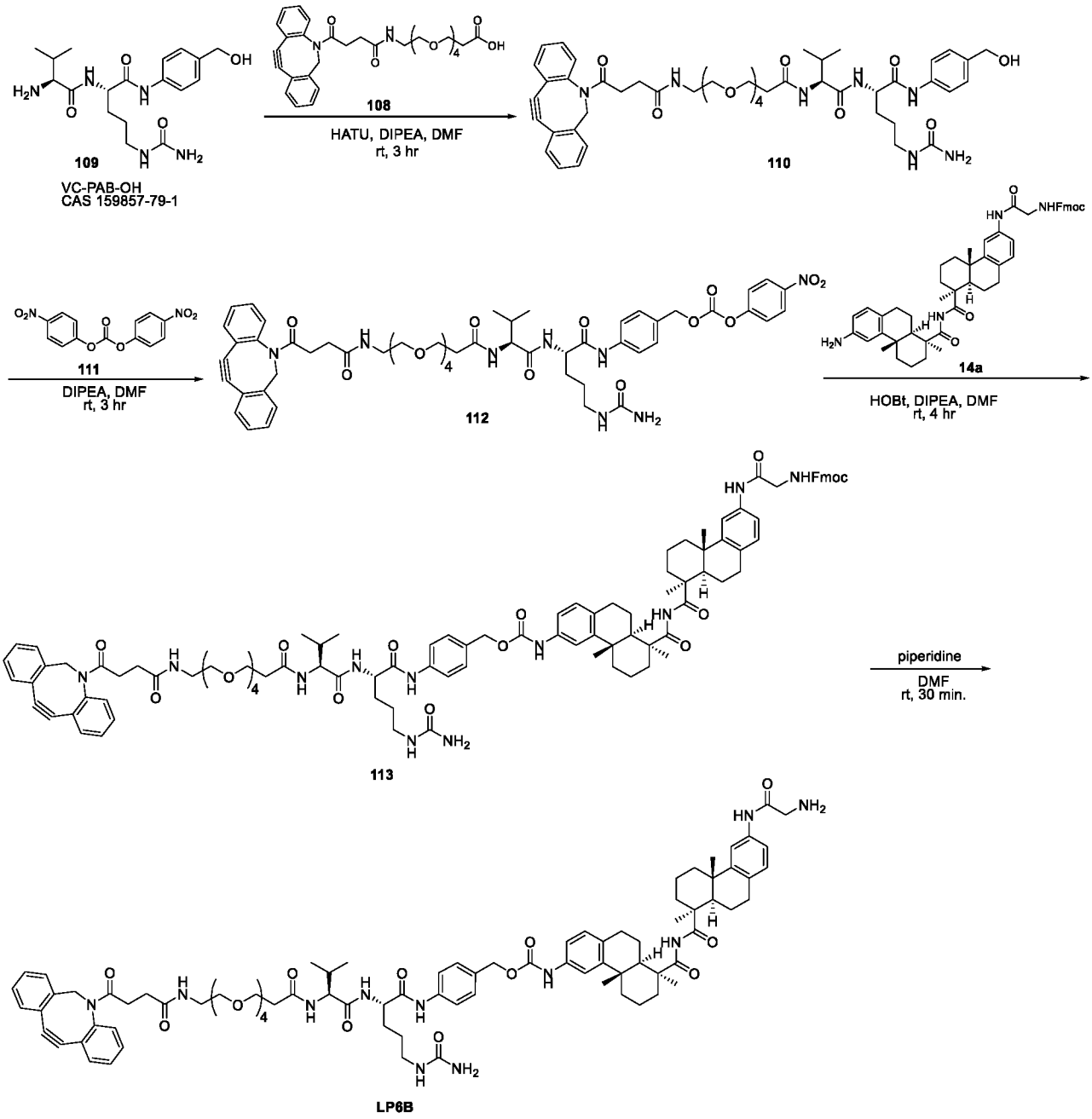


FIG. 26 Synthesis of LP6B

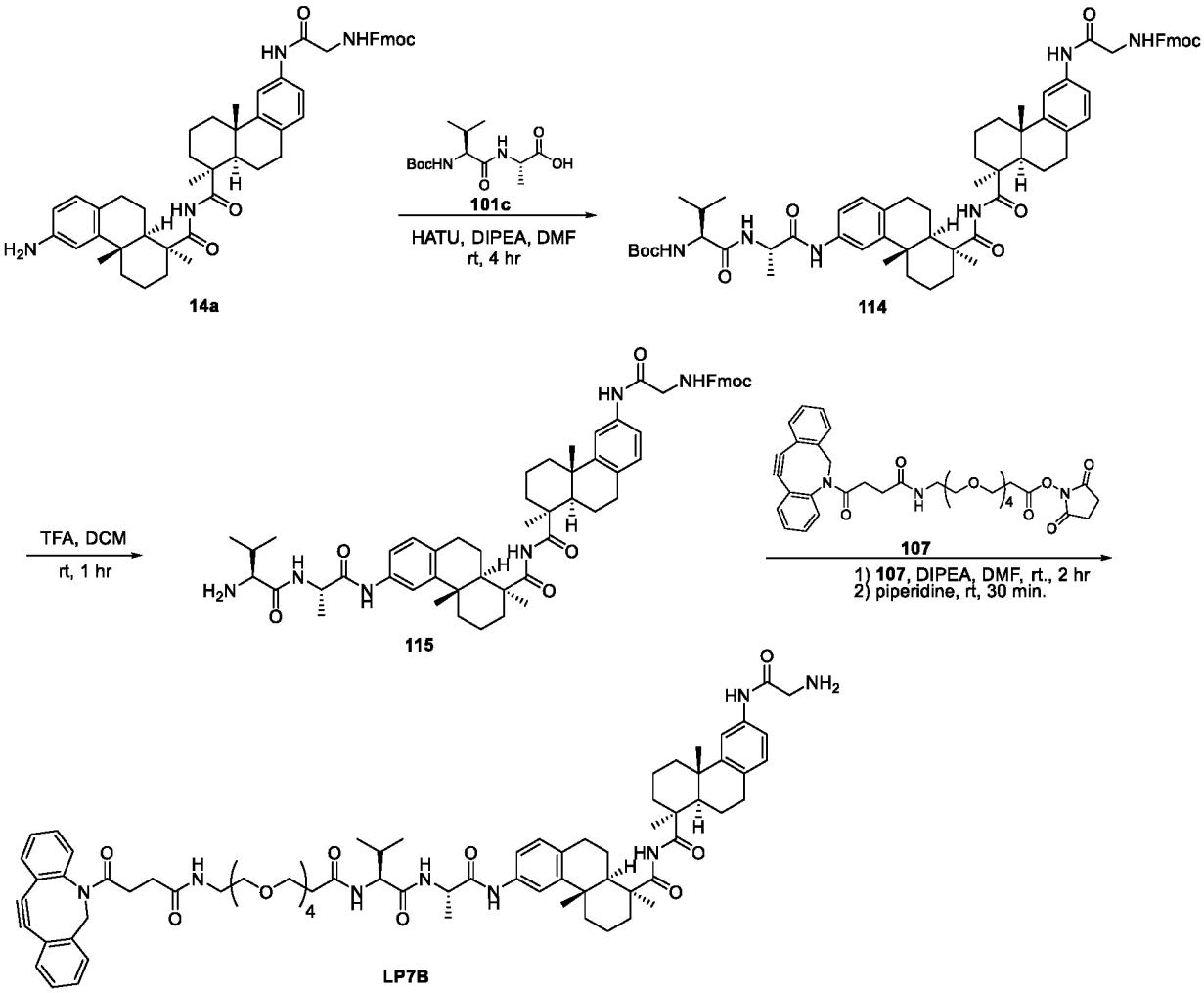


FIG. 27 Synthesis of LP7B

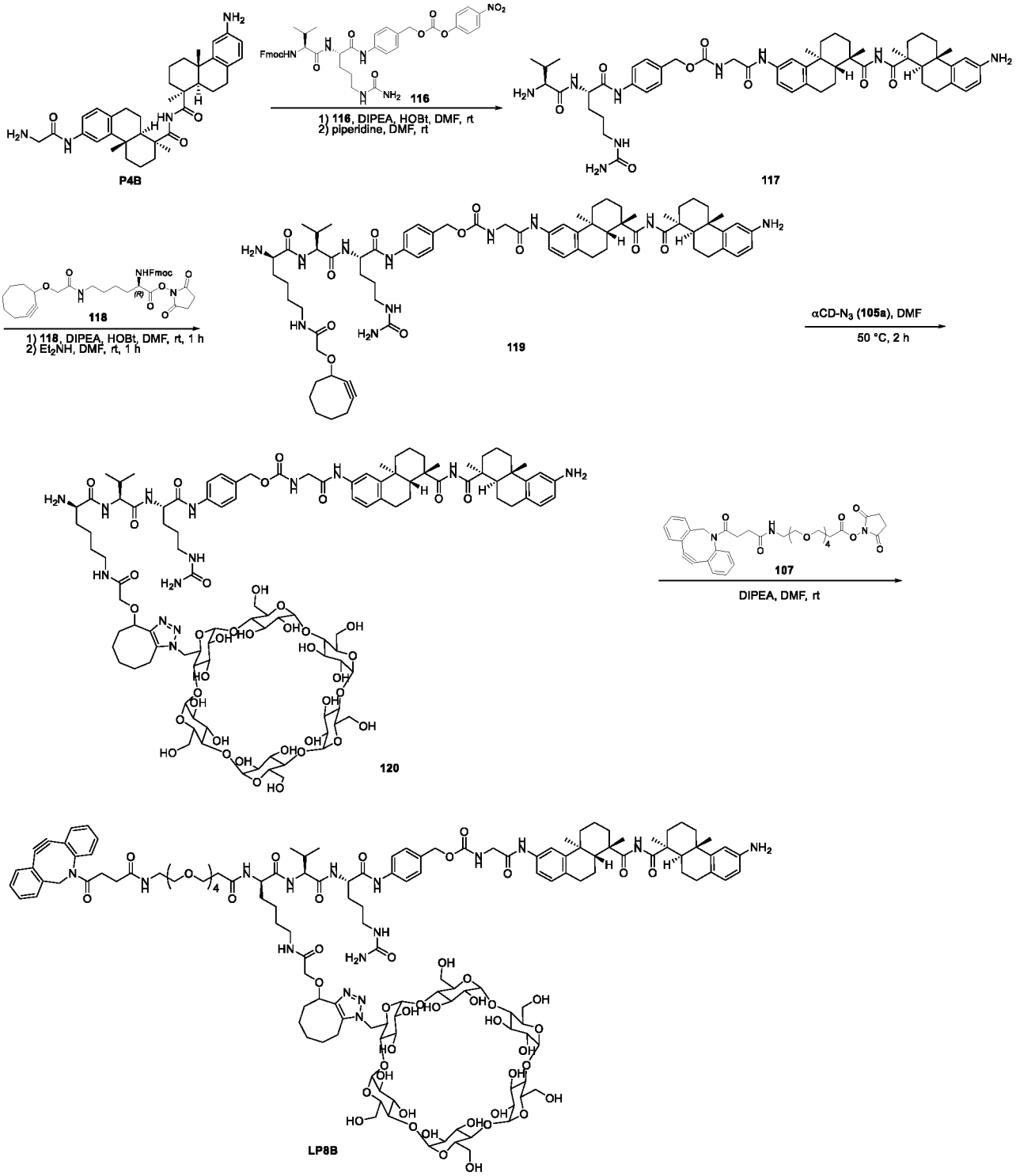


FIG. 28 Synthesis of LP8B

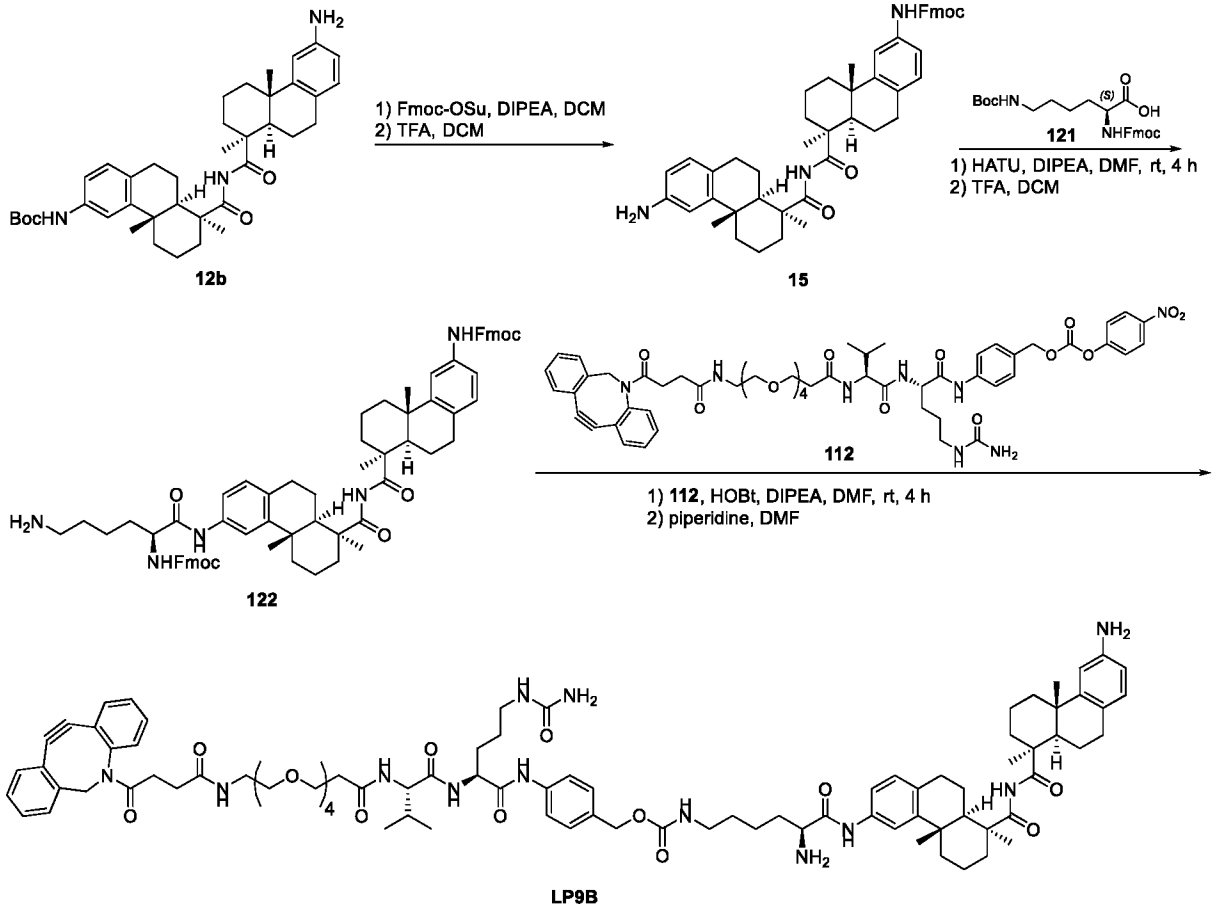


FIG. 29 Synthesis of LP9B

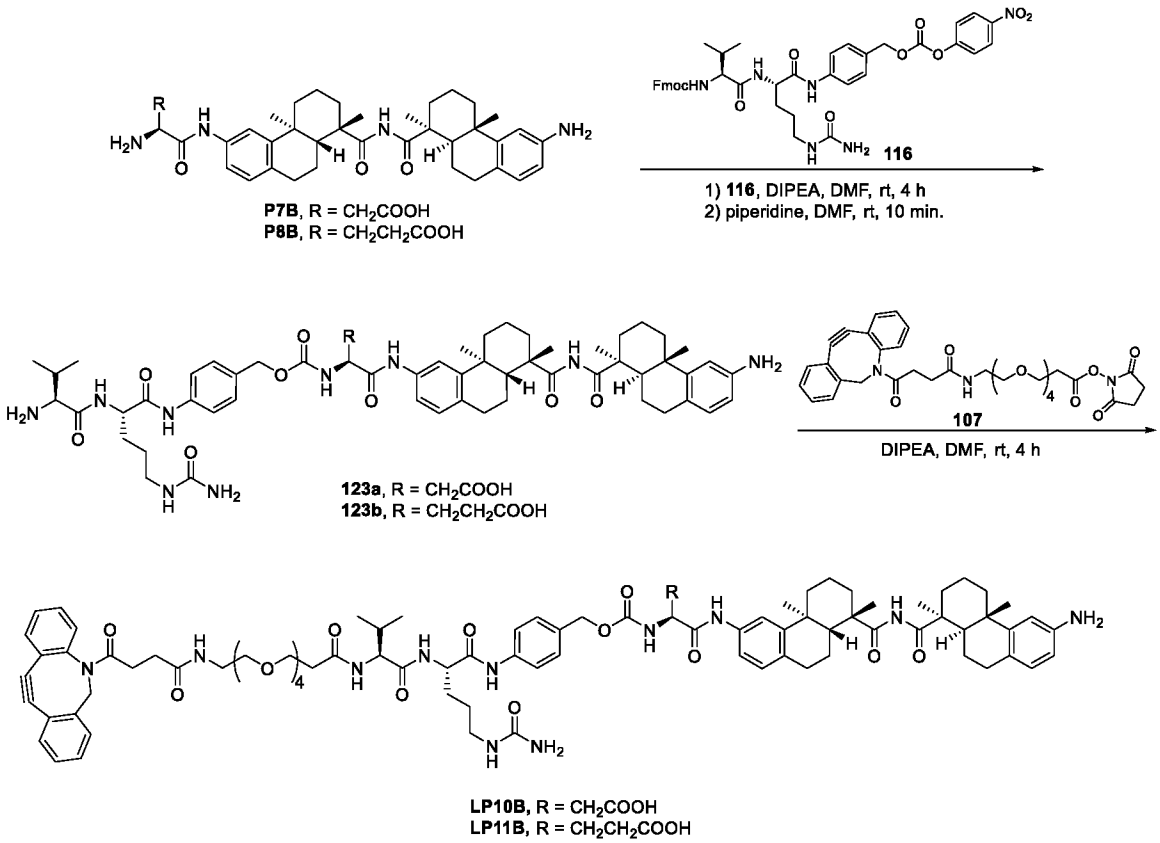


FIG. 30 Synthesis of LP10B and LP11B

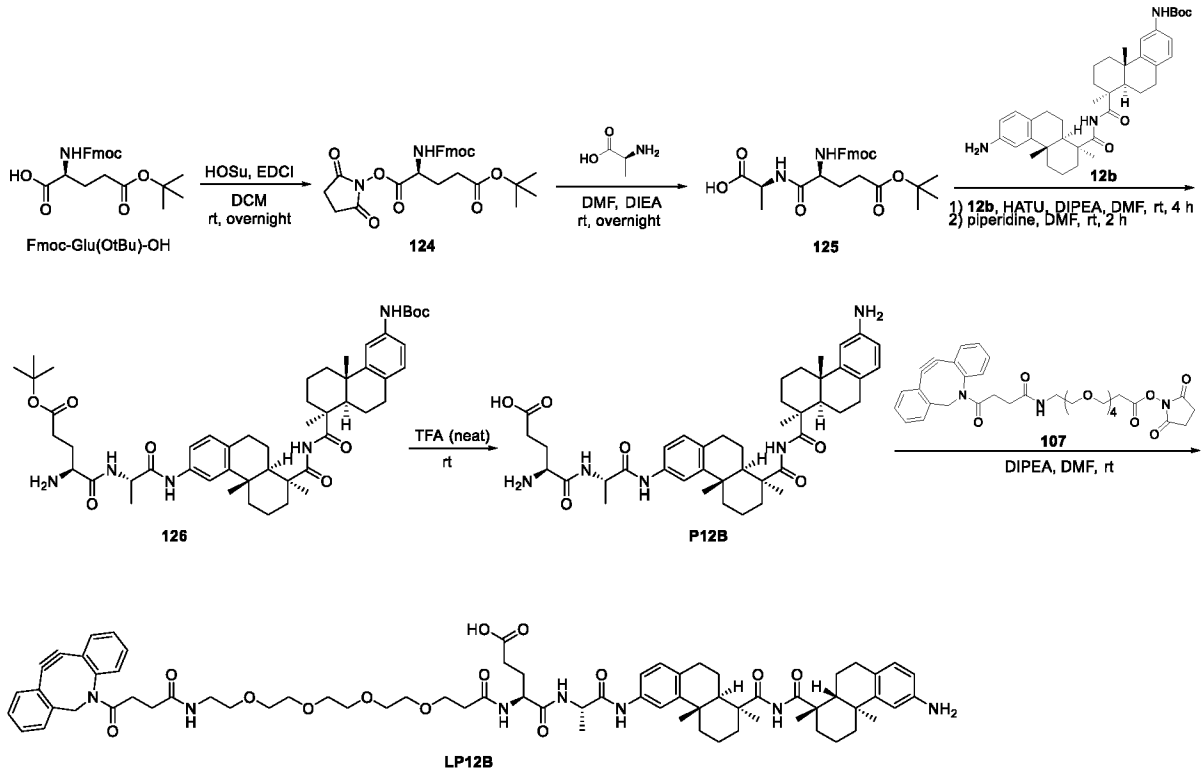


FIG. 31 Synthesis of P12B and LP12B

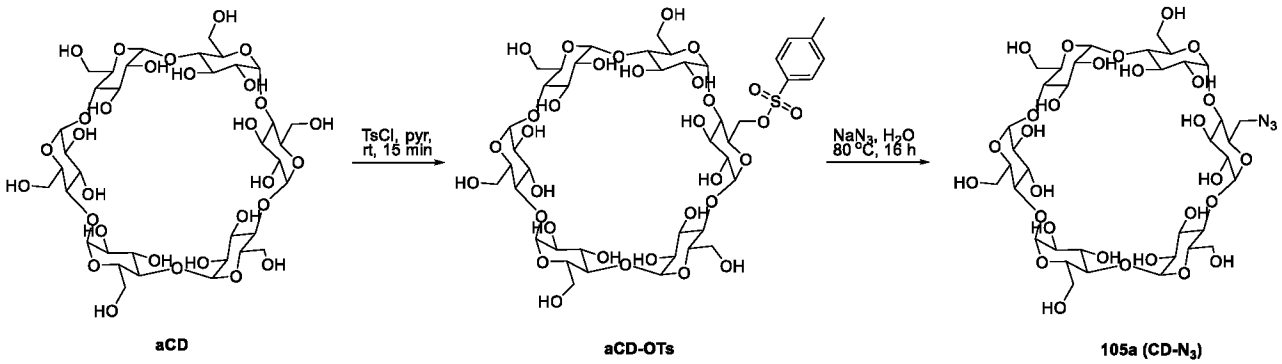


FIG. 32 Synthesis of Azide 105a

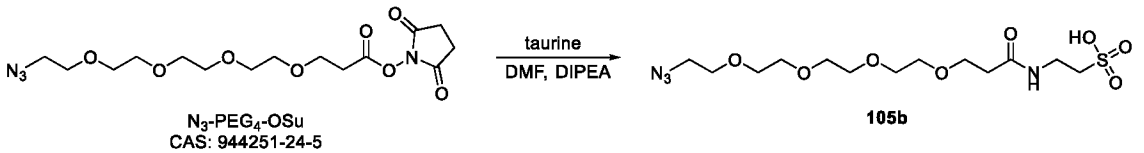


FIG. 33 Synthesis of Azide 105b

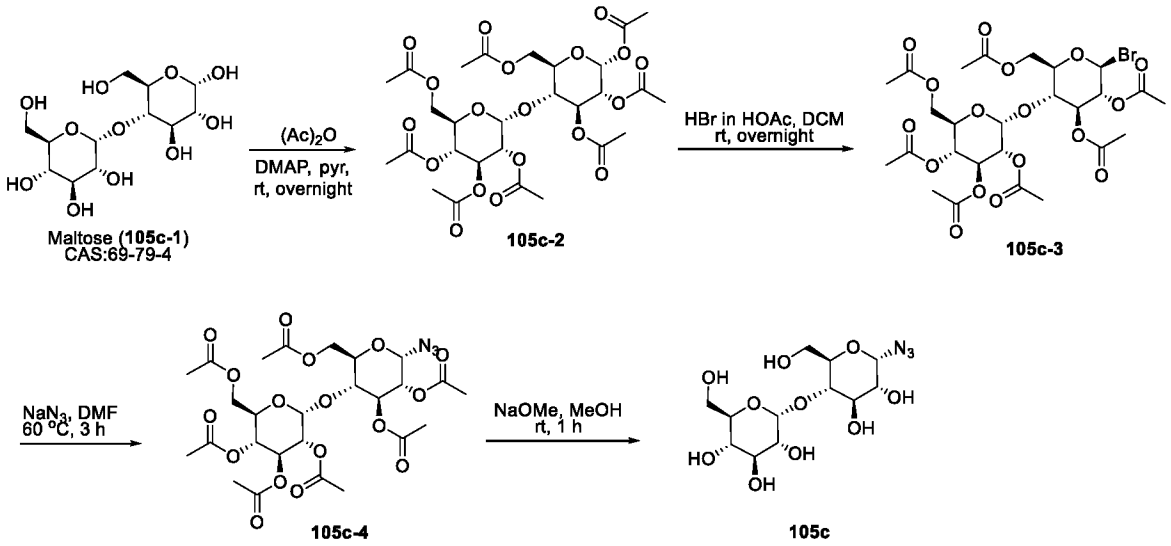


FIG. 34 Synthesis of intermediate 105c

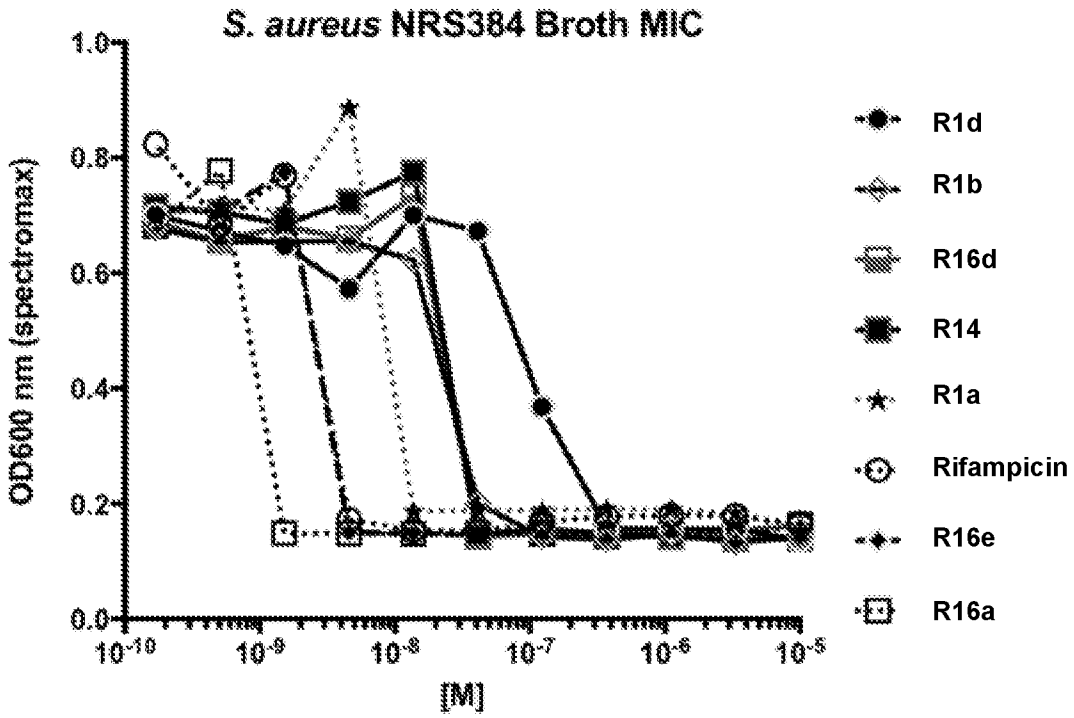

S. aureus NRS384 Broth MIC
OD600 nm (spectromax)
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
10-6
10-5
[M]
R1d
R1b
R16d
R14
R1a
Rifampicin
R16e
R16a

FIG. 35

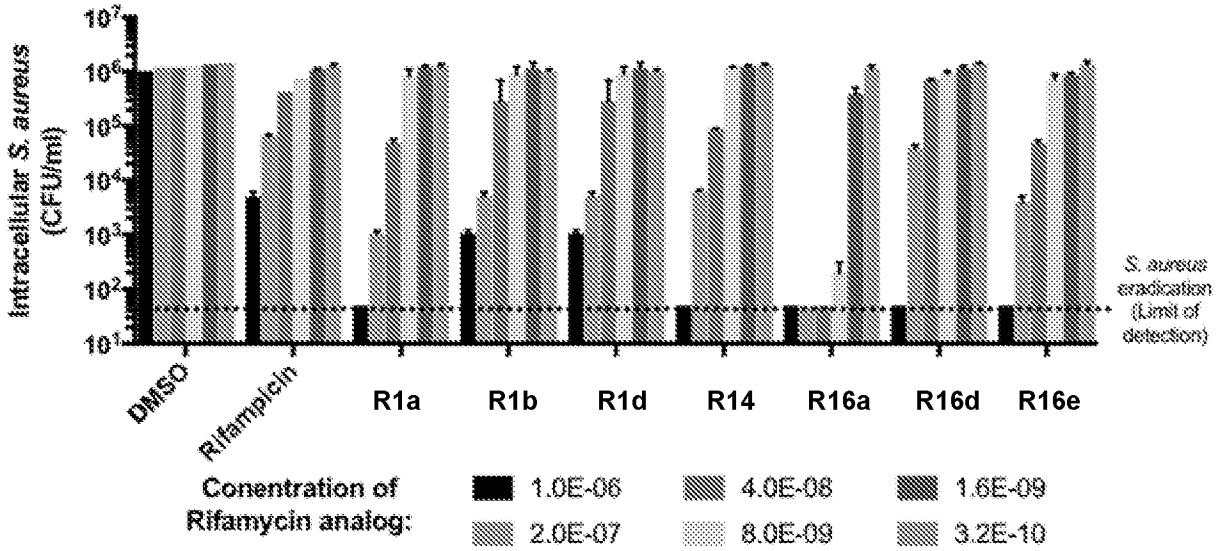

Intracellular S. aureus (CFU/ml)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
S. aureus eradication (Limit of detection)
DMSO
Rifampicin
R1a
R1b
R1d
R14
R16a
R16d
R16e
Conentration of Rifamycin analog:
1.0E-06
2.0E-07
4.0E-08
8.0E-09
1.6E-09
3.2E-10

FIG. 36

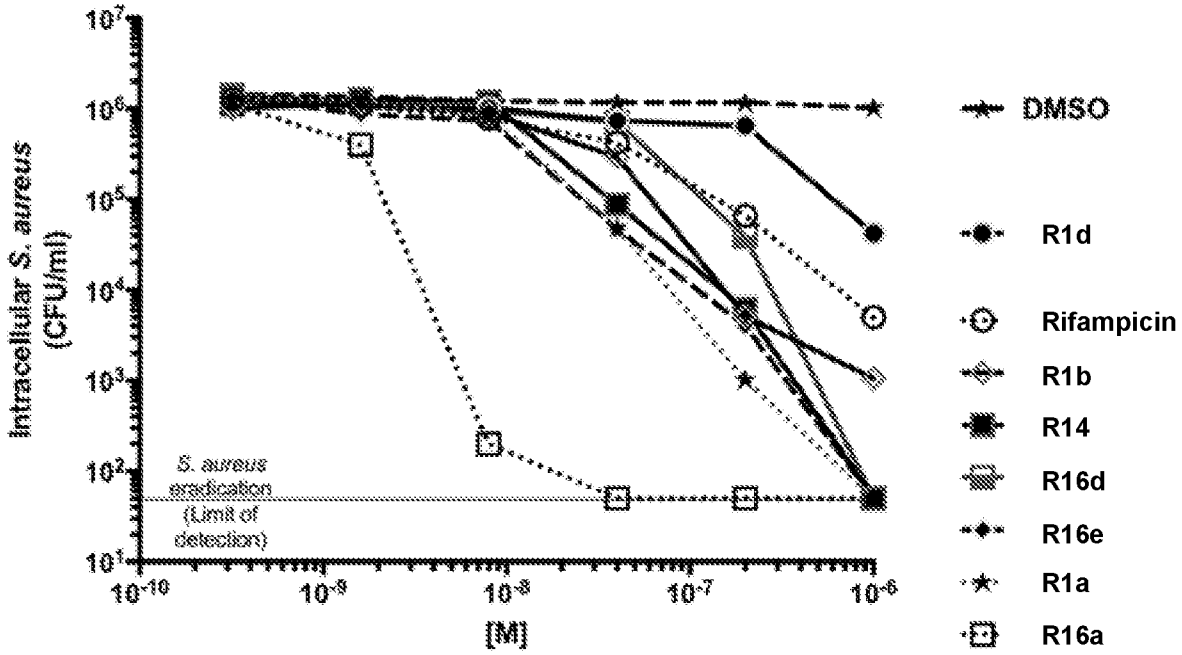

Intracellular S. aureus (CFU/ml)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
S. aureus eradication (Limit of detection)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
[M]
DMSO
R1d
Rifampicin
R1b
R14
R16d
R16e
R1a
R16a

FIG. 37

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/031383

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K47/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CZERKIES MACIEJ ET AL: "An interplay between scavenger receptor A and CD14 during activation of J774 cells by high concentrations of LPS", IMMUNOBIOLOGY, URBAN UND FISCHER VERLAG, DE, vol. 218, no. 10, 12 April 2013 (2013-04-12), pages 1217-1226, XP028686779, ISSN: 0171-2985, DOI: 10.1016/J.IMBIO.2013.04.005 page 1218, left-hand column, paragraph 4 discussion; page 1221, left-hand column, paragraph 2 - right-hand column, paragraph 1; figure 4 ----- -/--	1-8,57

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2019	17/10/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Annette

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/103389 A1 (UNIV CORNELL [US]; LATOV NORMAN [US]; LEE GRACE [US]) 25 August 2011 (2011-08-25)	1-8,57
Y	paragraph [0006] - paragraph [0012] paragraphs [0016], [0019], [0021] - [0025], [0028] - [0071]; claims 1-22; examples 1-3	9-40,51, 54,56, 58-69
X	WO 2010/010119 A1 (ABLYNX NV [BE]; SARAIVA MARIA JOAO [PT] ET AL.) 28 January 2010 (2010-01-28)	1-8,57
Y	the whole document; in particular SEQ ID NOs: 327-333, page 18, line 5-page 21, line 20; page 128, lines 25-33; example 3	9-40,51, 54,56, 58-69
Y	WO 2005/119266 A1 (UNIV LEIDEN [NL]; BIESSEN ERIK ANNA LEONARDUS [NL] ET AL.) 15 December 2005 (2005-12-15) page 3, line 27 - page 5, line 12 page 6, line 23 - page 7, line 12 page 19, line 3 - line 24 page 25, line 19 - page 27, line 3; claims 13-27	9-40,51, 54,56, 58-69
A	REYNA K. V. LIM ET AL: "Targeted Delivery of LXR Agonist Using a Site-Specific Antibody-Drug Conjugate", BIOCONJUGATE CHEMISTRY, vol. 26, no. 11, 18 November 2015 (2015-11-18), pages 2216-2222, XP055495447, US ISSN: 1043-1802, DOI: 10.1021/acs.bioconjchem.5b00203 the whole document, in particular page 2217, right-hand column, fourth paragraph - page 2220, right-hand column, first paragraph; figure 2	9-40,51, 54,56, 58-69
A	SOPHIE M. LEHAR ET AL: "Novel antibody-antibiotic conjugate eliminates intracellular S. aureus", NATURE, vol. 527, no. 7578, 4 November 2015 (2015-11-04), pages 323-328, XP055260158, London ISSN: 0028-0836, DOI: 10.1038/nature16057 cited in the application the whole document, in particular page 2, left-hand column, last paragraph - page 3, right-hand column, first paragraph; figure 2	9-40,51, 54,56, 58-69

-/--

2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/031383

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ALAIN BECK ET AL: "Strategies and challenges for the next generation of antibody-drug conjugates", NATURE REVIEWS. DRUG DISCOVERY, vol. 16, no. 5, 17 March 2017 (2017-03-17) , pages 315-337, XP055552084, GB ISSN: 1474-1776, DOI: 10.1038/nrd.2016.268 the whole document	9-40,51, 54,56, 58-69
E	WO 2019/136487 A2 (REGENERON PHARMA [US]) 11 July 2019 (2019-07-11) the whole document, in particular paragraph 171, SEQ ID NOs 2, 10	1-40,51, 54,56-69
X,P	WO 2018/213077 A1 (REGENERON PHARMA [US]) 22 November 2018 (2018-11-22) the whole document, in particular examples 74, 75	1-40,51, 54,56-69
E	WO 2019/094395 A2 (REGENERON PHARMA [US]) 16 May 2019 (2019-05-16) the whole document, in particular examples 28 and 31	1-40,51, 54,56-69

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/031383

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-6(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-40, 51, 54, 56-69(all partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-40, 51, 54, 56-69(all partially)

An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:
(i) binds human MSR1 at 37°C with a Kd of less than about 1.4 nM as measured by surface plasmon resonance;
(ii) binds human MSR1 at 37°C with a VA of greater than about 4 minutes as measured by surface plasmon resonance;
(iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC50 of less than about 10 nM as measured by ligand uptake assay;
(iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay;
(v) exhibits binding of human MSR1 and internalization of the bound complex of more than about 10% as assayed by binding in THP-1 cells;
(vi) binds to cell surface expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC50 of less than 3.5 nM; and
wherein the antibody or antigen-binding fragment comprises a HCDR1 comprising SEQ ID NO: 4; a HCDR2 comprising SEQ ID NO: 6; a HCDR3 comprising SEQ ID NO: 8; a LCDR1 comprising SEQ ID NO: 12; a LCDR2 comprising SEQ ID NO: 14; and a LCDR3 comprising SEQ ID NO: 16; and related subject-matter.

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2. claims: 1-40, 51, 54, 56-69(all partially)

An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:
(i) binds human MSR1 at 37°C with a Kd of less than about 1.4 nM as measured by surface plasmon resonance;
(ii) binds human MSR1 at 37°C with a VA of greater than about 4 minutes as measured by surface plasmon resonance;
(iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC50 of less than about 10 nM as measured by ligand uptake assay;
(iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay;
(v) exhibits binding of human MSR1 and internalization of the bound complex of more than about 10% as assayed by binding in THP-1 cells;
(vi) binds to cell surface expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC50 of less than

3.5 nM; and
wherein the antibody or antigen-binding fragment comprises a HCDR1 comprising SEQ ID NO: 36; a HCDR2 comprising SEQ ID NO: 38; a HCDR3 comprising SEQ ID NO: 40; a LCDR1 comprising SEQ ID NO: 44; a LCDR2 comprising SEQ ID NO: 46; and a LCDR3 comprising SEQ ID NO: 48; and related subject-matter.

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3. claims: 1-40, 51, 54, 56-69(all partially)

An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:
(i) binds human MSR1 at 37°C with a Kd of less than about 1.4 nM as measured by surface plasmon resonance;
(ii) binds human MSR1 at 37°C with a VA of greater than about 4 minutes as measured by surface plasmon resonance;
(iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC50 of less than about 10 nM as measured by ligand uptake assay;
(iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay;
(v) exhibits binding of human MSR1 and internalization of the bound complex of more than about 10% as assayed by binding in THP-1 cells;
(vi) binds to cell surface expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC50 of less than 3.5 nM; and
wherein the antibody or antigen-binding fragment comprises a HCDR1 comprising SEQ ID NO: 52; a HCDR2 comprising SEQ ID NO: 54; a HCDR3 comprising SEQ ID NO: 56; a LCDR1 comprising SEQ ID NO: 60; a LCDR2 comprising SEQ ID NO: 62; and a LCDR3 comprising SEQ ID NO: 64; and related subject-matter.

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4. claims: 1-40, 51, 54, 56-69(all partially)

An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:
(i) binds human MSR1 at 37°C with a Kd of less than about 1.4 nM as measured by surface plasmon resonance;
(ii) binds human MSR1 at 37°C with a VA of greater than about 4 minutes as measured by surface plasmon resonance;
(iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC50 of less than about 10 nM as measured by ligand uptake assay;
(iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay;
(v) exhibits binding of human MSR1 and internalization of

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the bound complex of more than about 10% as assayed by binding in THP-1 cells;
(vi) binds to cell surface expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC50 of less than 3.5 nM; and
wherein the antibody or antigen-binding fragment comprises a HCDR1 comprising SEQ ID NO: 92; a HCDR2 comprising SEQ ID NO: 94; a HCDR3 comprising SEQ ID NO: 96; a LCDR1 comprising SEQ ID NO: 100; a LCDR2 comprising SEQ ID NO: 102; and a LCDR3 comprising SEQ ID NO: 104; and related subject-matter.

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5. claims: 1-40, 51, 54, 56-69(all partially)

An isolated antibody or antigen-binding fragment thereof that binds macrophage scavenger receptor 1 (MSR1), wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:
(i) binds human MSR1 at 37°C with a Kd of less than about 1.4 nM as measured by surface plasmon resonance;
(ii) binds human MSR1 at 37°C with a VA of greater than about 4 minutes as measured by surface plasmon resonance;
(iii) exhibits maximum inhibition of modified LDL uptake by human MSR1 of less than about 90% with an IC50 of less than about 10 nM as measured by ligand uptake assay;
(iv) blocks < 50% of modified LDL binding to human MSR1 as measured by competition ELISA assay;
(v) exhibits binding of human MSR1 and internalization of the bound complex of more than about 10% as assayed by binding in THP-1 cells;
(vi) binds to cell surface expressed macrophage scavenger receptor 1 (MSR1) and blocks inhibition of modified LDL uptake by MSR1 by less than 80% with an IC50 of less than 3.5 nM; and
wherein the antibody or antigen-binding fragment comprises a HCDR1 comprising SEQ ID NO: 284; a HCDR2 comprising SEQ ID NO: 286; a HCDR3 comprising SEQ ID NO: 288; a LCDR1 comprising SEQ ID NO: 292; a LCDR2 comprising SEQ ID NO: 294; and a LCDR3 comprising SEQ ID NO: 296; and related subject-matter.

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6. claims: 41-50(completely); 58-69(partially)

An antibody-rifamycin analog conjugate having the structure of Formula (7003) as recited in present claim 41 and related subject-matter.

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7. claims: 52(completely); 58-69(partially)

An antibody-drug conjugate as recited in present claim 52 and related subject-matter.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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8. claims: 53(completely); 58-69(partially)

An antibody-drug conjugate as recited in present claim 53 and related subject-matter.

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9. claim: 55

An antibody-drug conjugate as recited in present claim 55 and related subject-matter.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-6(partially)

Present claims 1-6 relate to an extremely large number of possible antibodies. Support and disclosure in the sense of Article 6 and 5 PCT is to be found however for only a very small proportion of the antibodies claimed, see the antibodies defined by at least all of their six CDR sequences in the right order and according to a clear definition disclosed and supported by the present application in table 4 (amino acid sequences) and table 5 (corresponding nucleic acid sequences).
The
non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 1-6 (PCT Guidelines 9.19 and 9.23).
The search of these claims was restricted to those claimed antibodies which appear to be supported (see sequences of CDRs, VH/VL in tables 4 and 5).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/031383

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