MULTIVALENT LINKERS FOR HIGH LOADING ANTIBODY-DRUG CONJUGATES, AND COMPOSITIONS AND METHODS THEREOF

Priority Claims and Related Applications

[0001] This application claims the benefit of priority to PCT Int'l Application No. PCT/CN2024/083437, filed March 25, 2024, the entire content of which is incorporated herein by reference for all purposes.

Technical Field of the Invention

[0002] The invention generally relates to novel compounds and therapeutic uses thereof. More particularly, the invention provides novel multivalent linkers with increased drug loading capabilities, antibody-drug conjugates thereof, and pharmaceutical compositions and methods of treatment for certain diseases or conditions.

Background of the Invention

[0003] Antibody-drug conjugates (ADCs), a fast-growing class of drugs designed as targeted therapy with long plasma half-lives, represent a promising therapeutic modality in cancer treatment. To date, thirteen ADCs have received market approval by the US FDA. ADCs are an important class of highly potent biopharmaceutical drugs designed as a targeted therapy for the treatment of people with cancer or other diseases. Unlike the nonspecific killing properties of chemotherapy, ADCs are designed to target and kill the cancer cells only and spare healthy cells. The focus of ADC development has increasingly been placed on reduction of systemic toxicity while increasing the therapeutic benefit. (Ducry, *et al.* **2010** *Bioconjugate Chemistry* 21 (1): 5–13; Kovtun, *et al.* **2006** *Cancer Research* 66 (6): 3214–21; Kovtun, *et al.* **2007** *Cancer Letters* 255 (2): 232–40; Dijoseph, *et al.* **2004** *Blood* 103(5):1807–14; Mullard **2013** *Nature Reviews Drug Discovery* 12 (5): 329–32.)

[0004] Synthesis of ADCs is achieved typically by coupling the therapeutic agent to the antibody using amino or thiol specific linkers on the antibody surface. The preferred drug-to-

antibody ratio (DAR) for ADCs tend to be in the range of 2 to 8 drugs/antibody. With improved *in vitro* potency, high DARs are highly desirable due to their increased potency especially for biological targets that have low expression level. Conventional conjugation methods, however, are not suited for making ADCs with high DARs, as such methods are frequently prone to aggregation, poor solubility, and/or instability that led to a poor therapeutic window. (Sun, *et al.* **2005** *Bioconjugate Chemistry* 16, 1282-90; Hamblett, *et al.* **2004** *Cancer Research* 10, 7063-70.)

[0005] Besides novel technologies that allow high DARs, another pressing need is to develop ADCs with dual cytotoxic agents based on distinct MOAs with synergistic effects. Additionally, hydrophilic linkers with low molecular weight (CMC friendly) would benefit ADCs with improved properties of drug-likeness, such as reduced aggregation, improved solubility, improved ADC plasma clearance and pharmacokinetics, reduced immunogenicity, and improved therapeutic window.

[0006] There is an ongoing and urgent need for novel linker strategies and conjugation methodologies that improve drug loading of ADCs while addressing the many shortcomings of existing linkers.

Summary of the Invention

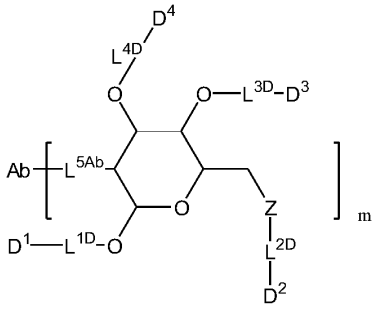
[0007] The invention is based in part on the novel carbohydrate-based multivalent linkers disclosed herein that are capable of increased drug loading, for example, up to 8 units of therapeutic agents per linker unit. In addition to providing the ability to increase and fine-tune drug loadings for ADCs (*e.g.*, with DARs ranging from 4 to 32), these novel linkers are also designed to allow loading of two or more different therapeutic agents at different DARs in a given ADC.

[0008] The invention additionally provides pharmaceutical compositions and methods of therapeutic treatment for certain diseases or conditions using ADCs built on the multivalent linker platform disclosed herein. Furthermore, the invention provides methods of making such multivalent linkers, linker-drug conjugates, and ADCs, as well as methods for modifying or fine-tuning DARs thereof.

[0009] These novel linkers are hydrophilic by design and can help improve properties of ADCs to be more suitable drug, for example with reduced aggregation, improved solubility,

improved ADC plasma clearance and pharmacokinetics, improved efficacy, reduced immunogenicity, and finally improved therapeutic windows.

[0010] In one aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**I**):



(**I**)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups;

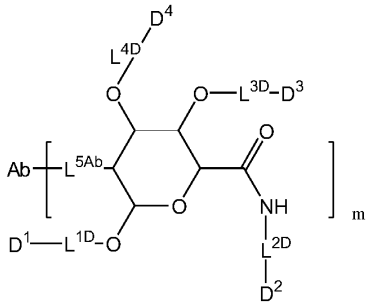
Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents L^{2D}_A and L^{2D}_B, each is independently a hydrophilic group covalently bound to said N; and

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

[0011] In another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**I'**):



(I')

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

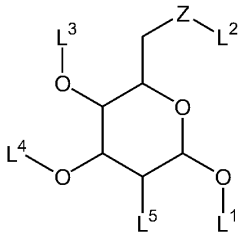
L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

[0012] In yet another aspect, the invention generally relates to a compound having the structural formula (**VI**):



(VI)

wherein

each of L^1, L^2, L^3 and L^4 is independently selected from: H, C_{1-3} alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

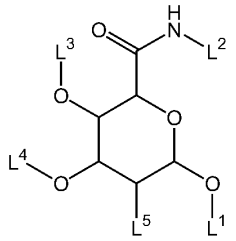
L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

Z is O or N, wherein when Z is N, L^2 represents L^2_A and L^2_B, each is independently a hydrophilic group covalently bound to said N.

wherein

0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} optionally comprises a hydrophilic group.

[0013] In yet another aspect, the invention generally relates to a compound having the structural formula (**VI'**):



(VI')

wherein

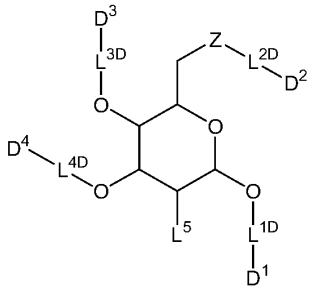
each of L^1, L^2, L^3 and L^4 is independently selected from: H, C_{1-3} alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

wherein

0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a hydrophilic group.

[0014] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**VIII**):



(VIII)

wherein

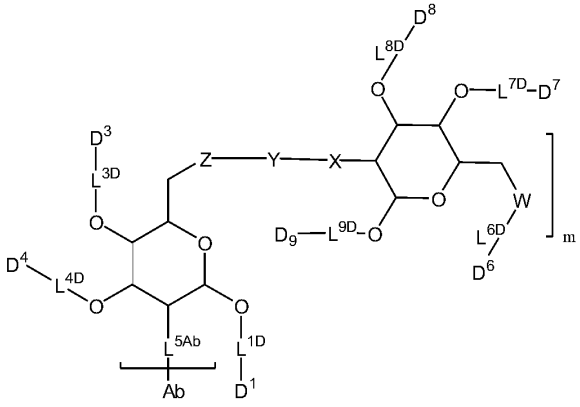
each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N.

[0015] In yet another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**X**):



(XII)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

each of X and Z is independently a triazole group;

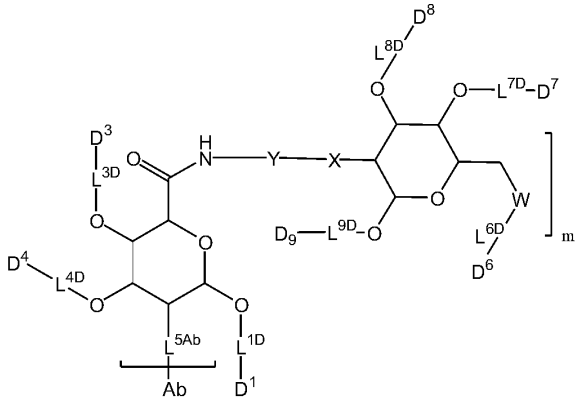
Y is a hydrophilic spacer group;

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is optionally H, a C_{1-3} alkyl or a hydrophilic group.

[0016] In yet another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**XII'**):



(XII')

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

X is a linking group;

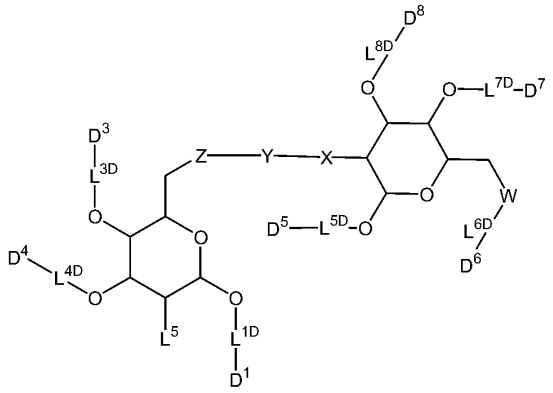
Y is a hydrophilic spacer group;

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

[0017] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**XIII**):



(XIII)

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents L^{6D}_A and L^{6D}_B, each is independently a hydrophilic group covalently bound to said N;

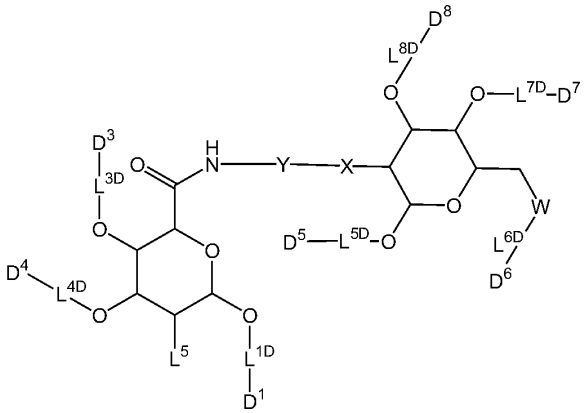
each of X and Z is independently a linking group; and

Y is a hydrophilic spacer group,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

[0018] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**XIII'**):



(XIII')

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents L^{6D}_A and L^{6D}_B, each is independently a hydrophilic group covalently bound to said N;

X is a linking group; and

Y is a hydrophilic spacer group,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

[0019] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising an antibody-drug conjugate disclosed herein.

[0020] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of the invention.

[0021] In yet another aspect, the invention generally relates to a method for treating, reducing, or preventing a disease or disorder, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[0022] In yet another aspect, the invention generally relates to a method for modulating immune response, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[0023] In yet another aspect, the invention generally relates to use of an antibody-drug conjugate disclosed herein for treating or reducing a disease or disorder.

[0024] In yet another aspect, the invention generally relates to use of an antibody-drug conjugate disclosed herein, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating or reducing a disease or disorder.

[0025] In yet another aspect, the invention generally relates to a method for making a compound, a linker-drug conjugate or an antibody-drug conjugate disclosed herein.

Brief Description of the Drawings

[0026] **FIG. 1** shows exemplary HIC and SEC-HPLC chromatogram of ADC-10.

[0027] **FIG. 2** shows exemplary HIC and SEC-HPLC chromatogram of ADC-11.

[0028] **FIG. 3** shows exemplary HIC and SEC-HPLC chromatogram of ADC-3.

[0029] **FIG. 4** shows exemplary HIC and SEC-HPLC chromatogram of ADC-12.

[0030] **FIG. 5** shows exemplary HIC and SEC-HPLC chromatogram of ADC-13.

[0031] **FIG. 6** shows exemplary HIC and SEC-HPLC chromatogram of ADC-14.

[0032] **FIG. 7** shows exemplary HIC and SEC-HPLC chromatogram of ADC-17

[0033] **FIG. 8** shows exemplary HIC and SEC-HPLC chromatogram of ADC-18.

[0034] **FIG. 9** shows exemplary HIC and SEC-HPLC chromatogram of ADC-19.

[0035] **FIG. 10** shows exemplary HIC and SEC-HPLC chromatogram of ADC-20.

[0036] **FIG. 11** shows exemplary HIC and SEC-HPLC chromatogram of ADC-22.

[0037] **FIG. 12** shows exemplary HIC and SEC-HPLC chromatogram of ADC-40.

[0038] **FIG. 13** shows exemplary HIC and SEC-HPLC chromatogram of ADC-41.

[0039] **FIG. 14** shows exemplary HIC and SEC-HPLC chromatogram of ADC-43.

[0040] **FIG. 15** shows exemplary HIC and SEC-HPLC chromatogram of ADC-44.

[0041] **FIG. 16** shows exemplary HIC and SEC-HPLC chromatogram of ADC-45.

[0042] **FIG. 17** shows exemplary HIC and SEC-HPLC chromatogram of ADC-46.

[0043] **FIG. 18** shows exemplary HIC and SEC-HPLC chromatogram of ADC-48.

[0044] **FIG. 19** shows exemplary HIC and SEC-HPLC chromatogram of ADC-49.

[0045] **FIG. 20** shows exemplary HIC and SEC-HPLC chromatogram of ADC-50.

[0046] **FIG. 21** shows exemplary HIC and SEC-HPLC chromatogram of ADC-52.

[0047] **FIG. 22** shows exemplary HIC and SEC-HPLC chromatogram of ADC-53.

[0048] **FIG. 23** shows exemplary HIC and SEC-HPLC chromatogram of ADC-54

[0049] **FIG. 24** shows exemplary HIC and SEC-HPLC chromatogram of ADC-55.

[0050] **FIG. 25** shows exemplary HIC and SEC-HPLC chromatogram of ADC-56.

[0051] **FIG. 26** shows exemplary Mouse PK profile of ADC-7.

[0052] **FIG. 27** shows exemplary Mouse PK profile of ADC-12.

[0053] **FIG. 28** shows exemplary Mouse PK profile of ADC-13.

[0054] **FIG. 29** shows exemplary Mouse PK profile of ADC-16.

[0055] **FIG. 30** shows exemplary Mouse PK profile of ADC-17.

Definitions

[0056] 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. General principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0057] As used herein, "at least" a specific value is understood to be that value and all values greater than that value.

[0058] The term "comprising", when used to define compositions and methods, is intended to mean that the compositions and methods include the recited elements, but do not exclude other elements. The term "consisting essentially of", when used to define compositions and methods, shall mean that the compositions and methods include the recited elements and exclude other elements of any essential significance to the compositions and methods. For example, "consisting essentially of" refers to administration of the pharmacologically active agents expressly recited and excludes pharmacologically active agents not expressly recited. The term consisting essentially of does not exclude pharmacologically inactive or inert agents, e.g., pharmaceutically acceptable excipients, carriers or diluents. The term "consisting of", when used to define compositions and methods, shall mean excluding trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.

[0059] Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

[0060] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

[0061] If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic methods well known in the art, and subsequent recovery of the pure enantiomers.

[0062] Solvates and polymorphs of the compounds of the invention are also contemplated herein. Solvates of the compounds of the present invention include, for example, hydrates.

[0063] Definitions of specific functional groups and chemical terms are described in more detail below. When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example, "C_{1-6} alkyl" is intended to encompass, C_1, C_2, C_3, C_4, C_5, C_6, C_{1-6}, C_{1-5}, C_{1-4}, C_{1-3}, C_{1-2}, C_{2-6}, C_{2-5}, C_{2-4}, C_{2-3}, C_{3-6}, C_{3-5}, C_{3-4}, C_{4-6}, C_{4-5}, and C_{5-6} alkyl.

[0064] As used herein, the term "alkyl" refers to a straight, branched or cyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to ten carbon atoms (*e.g.*, C_{1-10} alkyl). Whenever it appears herein, a numerical range such as "1 to 10" refers to each integer in the given range; *e.g.*, "1 to 10 carbon atoms" means that the alkyl group can consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, *etc.*, up to and including 10 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated. In some embodiments, “alkyl” can be a C_{1-6} alkyl group. In some embodiments, alkyl groups have 1 to 10, 1 to 8, 1 to 6, or 1 to 3 carbon atoms.

[0065] Representative saturated straight chain alkyls include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, and -n-hexyl; while saturated branched alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, and the like. The alkyl is attached to the parent molecule by a single bond.

[0066] Unless stated otherwise in the specification, an alkyl group is optionally substituted by one or more of substituents which independently include: acyl, alkyl, alkenyl, alkynyl, alkoxy, alkylaryl, cycloalkyl, aralkyl, aryl, aryloxy, amino, amido, amidino, imino, azide, carbonate, carbamate, carbonyl, heteroalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, hydroxy, cyano, halo (F, Cl, Br, I), haloalkoxy, haloalkyl, ester, ether, mercapto, thio, alkylthio, arylthio, thiocarbonyl, nitro, oxo, phosphate, phosphonate, phosphinate, silyl, sulfinyl, sulfonyl, sulfonamidyl, sulfoxyl, sulfonate, urea, $-Si(R^a)_3$, $-OR^a$, $-SR^a$, $-OC(O)-R^a$, $-N(R^a)_2$, $-C(O)R_a$, $-C(O)OR^a$, $-OC(O)N(R^a)_2$, $-C(O)N(R^a)_2$, $-N(R^a)C(O)OR^a$, $-N(R^a)C(O)R^a$, $-N(R^a)C(O)N(R^a)_2$, $-N(R^a)C(NR^a)N(R^a)_2$, $-N(R^a)S(O)_tN(R^a)_2$ (where t is 1 or 2), $-P(=O)(R^a)(R^a)$, or $-O-P(=O)(OR^a)_2$ where each R^a is independently hydrogen, alkyl, haloalkyl, carbocyclyl, carbocyclylalkyl, aryl,

aralkyl, heterocycloalkyl, heterocycloalkylalkyl, heteroaryl or heteroarylalkyl, and each of these moieties can be optionally substituted as defined herein. In a non-limiting embodiment, a substituted alkyl can be selected from fluoromethyl, difluoromethyl, trifluoromethyl, 2-fluoroethyl, 3-fluoropropyl, hydroxymethyl, 2-hydroxyethyl, 3-hydroxypropyl, benzyl, and phenethyl.

[0067] As used herein, the term “heteroatom” means one or more of oxygen, sulfur, nitrogen, or phosphorus (including, any oxidized form of nitrogen, sulfur, or phosphorus; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[0068] As used herein, the term “halogen” refers to fluorine (F), chlorine (Cl), bromine (Br), or iodine (I). As used herein, the term "halide" or "halo", means fluoro, chloro, bromo or iodo. The terms "haloalkyl," "haloalkenyl," "haloalkynyl" and "haloalkoxy" include alkyl, alkenyl, alkynyl and alkoxy structures that are substituted with one or more halo groups or with combinations thereof. For example, the terms "fluoroalkyl" and "fluoroalkoxy" include haloalkyl and haloalkoxy groups, respectively, in which the halo is fluorine, such as, but not limited to, trifluoromethyl, difluoromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like. Each of the alkyl, alkenyl, alkynyl and alkoxy groups are as defined herein and can be optionally further substituted as defined herein.

[0069] As used herein, the terms "aromatic" or "aryl" refer to a radical with 6 to 14 ring atoms (*e.g.*, C_{6-14} aromatic or C_{6-14} aryl) that has at least one ring having a conjugated pi electron system which is carbocyclic (*e.g.*, phenyl, fluorenyl, and naphthyl). In some embodiments, the aryl is a C_{6-10} aryl group. For example, bivalent radicals formed from substituted benzene derivatives and having the free valences at ring atoms are named as substituted phenylene radicals. In other embodiments, bivalent radicals derived from univalent polycyclic hydrocarbon radicals whose names end in"-yl" by removal of one hydrogen atom from the carbon atom with the free valence are named by adding "-idene" to the name of the corresponding univalent radical, *e.g.*, a naphthyl group with two points of attachment is termed naphthylidene. Whenever it appears herein, a numerical range such as "6 to 14 aryl" refers to each integer in the given range; *e.g.*, "6 to 14 ring atoms" means that the aryl group can consist of 6 ring atoms, 7 ring atoms, *etc.*, up to and including 14 ring atoms. The term includes monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of ring atoms) groups. Polycyclic aryl groups

include bicycles, tricycles, tetracycles, and the like. In a multi-ring group, only one ring is required to be aromatic, so groups such as indanyl are encompassed by the aryl definition. Non-limiting examples of aryl groups include phenyl, phenalenyl, naphthalenyl, tetrahydronaphthyl, phenanthrenyl, anthracenyl, fluorenyl, indolyl, indanyl, and the like. Unless stated otherwise in the specification, an aryl moiety can be optionally substituted by one or more substituents which independently include: acyl, alkyl, alkenyl, alkynyl, alkoxy, alkylaryl, cycloalkyl, aralkyl, aryl, aryloxy, amino, amido, amidino, imino, azide, carbonate, carbamate, carbonyl, heteroalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, hydroxy, cyano, halo, haloalkoxy, haloalkyl, ester, ether, mercapto, thio, alkylthio, arylthio, thiocarbonyl, nitro, oxo, phosphate, phosphonate, phosphinate, silyl, sulfinyl, sulfonyl, sulfonamidyl, sulfoxyl, sulfonate, urea, $-Si(R^a)_3$, $-OR^a$, $-SR^a$, $-OC(O)-R^a$, $-N(R^a)_2$, $-C(O)R_a$, $-C(O)OR^a$, $-OC(O)N(R^a)_2$, $-C(O)N(R^a)_2$, $-N(R^a)C(O)OR^a$, $-N(R^a)C(O)R^a$, $-N(R^a)C(O)N(R^a)_2$, $-N(R^a)C(NR^a)N(R^a)_2$, $-N(R^a)S(O)_tN(R^a)_2$ (where t is 1 or 2), $-P(=O)(R^a)(R^a)$, or $-O-P(=O)(OR^a)_2$ where each R^a is independently hydrogen, alkyl, haloalkyl, carbocyclyl, carbocyclylalkyl, aryl, aralkyl, heterocycloalkyl, heterocycloalkylalkyl, heteroaryl or heteroarylalkyl, and each of these moieties can be optionally substituted as defined herein.

[0070] As used herein, the terms "TLR7 and/or TLR8 ligand," "ligand for TLR7 and/or TLR8," and "TLR7 and/or signaling agonist" refer to a molecule, other than a compound disclosed herein, that interacts directly or indirectly with TLR7 and/or TLR8 through a TLR7 and/ or TLR8 domain other than a TLR8 domain, and induces TLR7- and/or TLR8-mediated signaling. In certain embodiments, a TLR7 and/or TLR8 ligand is a natural ligand, *i.e.*, a TLR7 and/or TLR8 ligand that is found in nature. In certain embodiments, a TLR7 and/or TLR8 ligand refers to a molecule other than a natural ligand of TLR7 and/or TLR8, *e.g.*, a molecule prepared by human activity.

[0071] As used herein, the term “modulator” is defined as a compound that binds to and /or activates or inhibits the target with measurable affinity, or directly or indirectly affecting the normal regulation of the receptor activity. In certain embodiments, a modulator has an EC_{50} and/or binding constant of less about 50 μM, less than about 1 μM, less than about 500 nM, less than about 100 nM, or less than about 10 nM.

[0072] As used herein, the term "agonist" refers to a compound that, in combination with a receptor (*e.g.*, a TLR), can produce a cellular response. An agonist may be a ligand that directly binds to the receptor. Alternatively, an agonist may combine with a receptor indirectly by, for

example, (a) forming a complex with another molecule that directly binds to the receptor, or (b) otherwise resulting in the modification of another compound so that the other compound directly binds to the receptor. An agonist may be referred to as an agonist of a particular TLR (*e.g.*, a TLR7 and/or TLR8 agonist).

[0073] As used herein, the term "antagonist" refers to a compound that competes with an agonist or inverse agonist for binding to a receptor, thereby blocking the action of an agonist or inverse agonist on the receptor. However, an antagonist has no effect on constitutive receptor activity. More specifically, an antagonist is a compound that inhibits the activity of TLR7 or TLR8 at the TLR7 or TLR8 receptor, respectively.

[0074] As used herein, the term "inhibit" refers to any measurable reduction of biological activity. Thus, as used herein, "inhibit" or "inhibition" may be referred to as a percentage of a normal level of activity.

[0075] As used herein, the term “antigen” as used herein is meant any substance that causes the immune system to produce antibodies or specific cell-mediated immune responses against it. A disease associated antigen is any substance that is associated with any disease that causes the immune system to produce antibodies or a specific-cell mediated response against it. An antigen is capable of being recognized by the immune system and/or being capable of inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T-lymphocytes. An antigen can have one or more epitopes (B- and/or T-cell epitopes). An antigen will preferably react, typically in a highly selective manner, with its corresponding antibody or TCR and not with the multitude of other antibodies or TCRs which may be evoked by other antigens. Antigens as used herein may also be mixtures of several individual antigens.

[0076] As used herein, the term “antibody” refers to molecules that are capable of binding an epitope or antigenic determinant. The term is meant to include whole antibodies and antigen-binding fragments thereof. The term encompasses a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, single chain Fv ("scFv"), diabody, linear antibody, bispecific antibody, multi-specific antibody, chimeric antibody, humanized antibody, human antibody, or fusion protein comprising the antigen-binding portion of an antibody. Antibodies can be from any animal origin. Preferably, the antibodies are mammalian, *e.g.*, human, murine, rabbit, goat, guinea pig, camel, horse and the like, or other suitable animals. Antibodies may recognize polypeptide or polynucleotide antigens. The term includes active

fragments, including for example, an antigen binding fragment of an immunoglobulin, a variable and/or constant region of a heavy chain, a variable and/or constant region of a light chain, a complementarity determining region (cdr), and a framework region. The terms include polyclonal and monoclonal antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, chimeric antibodies, hybrid antibody molecules, $F(ab)_2$ and F(ab) fragments; Fv molecules (for example, noncovalent heterodimers), dimeric and trimeric antibody fragment constructs; minibodies, humanized antibody molecules, and any functional fragments obtained from such molecules, wherein such fragments retain specific binding.

[0077] As used herein, the term "antigen binding fragment" refers to one or more portions of an antibody that retain the ability to specifically interact with, e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution, an epitope of an antigen.

[0078] Examples of binding fragments include, but are not limited to, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), Fab fragments, F(ab') fragments, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and C_H1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al. **1989** *Nature* 341:544- 546,), which consists of a V_H domain; and an isolated complementarity determining region (CDR), or other epitope-binding fragments of an antibody.

[0079] Additionally, the two domains of the Fv fragment, V_L and V_H can be joined using recombinant methods by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules. (known as single chain Fv ("scFv"); see, e.g., Bird et al., **1988** *Science* 242:423-426; and Huston et al. **1988** *Proc. Natl. Acad. Sci.* 85:5879-5883.) Such single chain antibodies are also intended to be encompassed within the term "antigen binding fragment." These antigen binding fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0080] Antigen binding fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv. (See, e.g., Hollinger and Hudson, **2005** *Nature Biotechnology* 23:1 126-1136.) Antigen binding fragments can be grafted into scaffolds based on polypeptides such as fibronectin type

III (Fn3). (See, e.g., U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies.) Antigen binding fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (V_H-C_H1-V_H-C_H1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. (Zapata et al., **1995** *Protein Eng.* 8:1057-1062; U.S. Pat. No. 5,641 ,870.)

[0081] As used herein, the term "bispecific antibody" or "bispecific" refers to an antibody, typically a monoclonal antibody, having binding specificities for at least two different antigenic epitopes. The epitopes can be from the same antigen or from two different antigens. Methods for making bispecific antibodies are known in the art. For example, bispecific antibodies can be produced recombinantly using the co-expression of two immunoglobulin heavy chain/light chain pairs. Alternatively, bispecific antibodies can be prepared using chemical linkage. Bispecific antibodies include bispecific antibody fragments. (See, e.g., Milstein et al. **1983** *Nature* 305:537-39; Brennan et al. **1985** *Science* 229:81; Hollinger et al. **1994** *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-48; Gruber et al. **1994** *J. Immunol.* 152:5368-74.)

[0082] As used herein, the term "chimeric antibody" or "chimeric" refers to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they specifically bind the target antigen and/or exhibit the desired biological activity.

[0083] As used herein, the term "human antibody" refers to antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik et al. **2000** *J. Mol. Biol.* 296:57-86). Human antibodies may include amino acid residues not encoded by human sequences, e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a substitution to promote stability or manufacturing.

[0084] As used herein, the term "humanized antibody" refers to antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, humanized antibodies comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. (See e.g., Cabilly U.S. Pat. No. 4,816,567; Queen et al. **1989** *Proc. Nat'l Acad. Sci. USA* 86:10029-10033; ANTIBODY ENGINEERING: A PRACTICAL APPROACH, Oxford University Press 1996.)

[0085] As used herein, the term "monoclonal antibody", refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. "Monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by various methods known in the art, including the hybridoma method first described by Kohler et al. **1975** *Nature* 256: 495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). "Monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al. **1991** *Nature* 352: 624-628 and Marks et al. **1991** *J. Mol. Biol.* 222: 581-597, for example. These monoclonal antibodies will usually bind with at least a Kd of about 1 μM, more usually at least about 300 nM, typically at least about 30 nM, preferably at least about 10 nM.

[0086] As used herein, the term "cleavable" linker refers to a linker or linker component that connects two moieties by covalent connections, but breaks down to sever the covalent connection between the moieties under physiologically relevant conditions. Typically, a cleavable linker is severed *in vivo* more rapidly in an intracellular environment than when

outside a cell, causing release of a payload to preferentially occur inside the targeted cell. Cleavage may be enzymatic or non-enzymatic. A payload is typically released from an antibody without degrading the antibody. Cleavage may leave some portion of a linker or linker component attached to the payload, or it may release the payload without any residual part or component of the linker (i.e., traceless release).

[0087] As used herein, the term "non-cleavable" linker refers to a linker or linker component that is not especially susceptible to breaking down under physiological conditions, i.e., it is at least as stable as the antibody or antigen binding fragment portion of the immunoconjugate. Such linkers are sometimes referred to as "stable," meaning they are sufficiently resistant to degradation to keep the payload connected to the antigen binding moiety until the antigen binding moiety is itself at least partially degraded. In such a case, the degradation of Ab precedes cleavage of the linker i*n vivo*. Degradation of the antibody portion of an immunoconjugate having a stable or non-cleavable linker may leave some or all of the linker, and one or more amino acid groups from an antibody, attached to the payload or drug moiety that is delivered *in vivo*.

[0088] As used herein, the terms "disease", "condition" or "disorder" are used interchangeably herein and refer to a pathological condition, for example, one that can be identified by symptoms or other identifying factors as diverging from a healthy or a normal state. The term "disease" includes disorders, syndromes, conditions, and injuries. Diseases include, but are not limited to, proliferative, inflammatory, immune, metabolic, infectious, and ischemic diseases.

[0089] As used herein, the term "in need of" a treatment refers to a subject that would benefit biologically, medically or in quality of life from such a treatment.

[0090] As used herein, the term "effective amount" of an active agent refers to an amount sufficient to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound of the invention may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the disease being treated, the mode of administration, and the patient.

[0091] As used herein, the terms "treatment" or "treating" a disease or disorder refers to a method of reducing, delaying or ameliorating such a condition before or after it has occurred. Treatment may be directed at one or more effects or symptoms of a disease and/or the underlying

pathology. The treatment can be any reduction and can be, but is not limited to, the complete ablation of the disease or the symptoms of the disease. As compared with an equivalent untreated control, such reduction or degree of prevention is at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, or 100% as measured by any standard technique.

[0092] As used herein, the terms “prevent”, “preventing”, or “prevention” refer to a method for precluding, delaying, averting, or stopping the onset, incidence, severity, or recurrence of a disease or condition. For example, a method is considered to be a prevention if there is a reduction or delay in onset, incidence, severity, or recurrence of a disease or condition or one or more symptoms thereof in a subject susceptible to the disease or condition as compared to a subject not receiving the method. The disclosed method is also considered to be a prevention if there is a reduction or delay in onset, incidence, severity, or recurrence of osteoporosis or one or more symptoms of a disease or condition in a subject susceptible to the disease or condition after receiving the method as compared to the subject's progression prior to receiving treatment. Thus, the reduction or delay in onset, incidence, severity, or recurrence of osteoporosis can be about a 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between.

[0093] As used herein, a "pharmaceutically acceptable form" of a disclosed compound includes, but is not limited to, pharmaceutically acceptable salts, esters, hydrates, solvates, polymorphs, isomers, prodrugs, and isotopically labeled derivatives thereof. In one embodiment, a "pharmaceutically acceptable form" includes, but is not limited to, pharmaceutically acceptable salts, esters, prodrugs and isotopically labeled derivatives thereof. In some embodiments, a "pharmaceutically acceptable form" includes, but is not limited to, pharmaceutically acceptable isomers and stereoisomers, prodrugs and isotopically labeled derivatives thereof.

[0094] In certain embodiments, the pharmaceutically acceptable form is a pharmaceutically acceptable salt. As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in J. *Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an

amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchioric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, lactic acid, trifluoracetic 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.

[0095] The salts can be prepared *in situ* during the isolation and purification of the disclosed compounds, or separately, such as by reacting the free base or free acid of a parent compound with a suitable base or acid, respectively. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines, including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt can be chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

[0096] In certain embodiments, the pharmaceutically acceptable form is a "solvate" (*e.g.*, a hydrate). As used herein, the term "solvate" refers to compounds that further include a stoichiometric or non-stoichiometric amount of solvent bound by non-covalent intermolecular forces. The solvate can be of a disclosed compound or a pharmaceutically acceptable salt thereof. Where the solvent is water, the solvate is a "hydrate". Pharmaceutically acceptable solvates and hydrates are complexes that, for example, can include 1 to about 100, or 1 to about 10, or 1 to about 2, about 3 or about 4, solvent or water molecules. It will be understood that the term "compound" as used herein encompasses the compound and solvates of the compound, as well as mixtures thereof.

[0097] In certain embodiments, the pharmaceutically acceptable form is a prodrug. As used herein, the term "prodrug" (or "pro-drug") refers to compounds that are transformed *in vivo* to yield a disclosed compound or a pharmaceutically acceptable form of the compound. A prodrug can be inactive when administered to a subject, but is converted *in vivo* to an active compound, for example, by hydrolysis (*e.g.*, hydrolysis in blood). In certain cases, a prodrug has improved physical and/or delivery properties over the parent compound. Prodrugs can increase the bioavailability of the compound when administered to a subject (*e.g.*, by permitting enhanced absorption into the blood following oral administration) or which enhance delivery to a biological compartment of interest (*e.g.*, the brain or lymphatic system) relative to the parent compound. Exemplary prodrugs include derivatives of a disclosed compound with enhanced aqueous solubility or active transport through the gut membrane, relative to the parent compound.

[0098] The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, *e.g.*, Bundgard, H., *Design of Prodrugs* (1985), pp. 7- 9, 21-24 (Elsevier, Amsterdam). A discussion of prodrugs is provided in Higuchi, T., et al., "Pro-drugs as Novel Delivery Systems," *A.C.S. Symposium Series,* Vol. 14, and in *Bioreversible Carriers in Drug Design,* ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated in full by reference herein. Exemplary advantages of a prodrug can include, but are not limited to, its physical properties, such as enhanced water solubility for parenteral administration at physiological pH compared to the parent compound, or it can enhance absorption from the digestive tract, or it can enhance drug stability for long-term storage.

[0099] Prodrugs commonly known in the art include well-known acid derivatives, such as, for example, esters prepared by reaction of the parent acids with a suitable alcohol, amides prepared by reaction of the parent acid compound with an amine, basic groups reacted to form an acylated base derivative, *etc*. Of course, other prodrug derivatives may be combined with other features disclosed herein to enhance bioavailability. As such, those of skill in the art will appreciate that certain of the presently disclosed compounds having free amino, arnido, hydroxy or carboxylic groups can be converted into prodrugs. Prodrugs include compounds having an amino acid residue, or a polypeptide chain of two or more (*e.g.*, two, three or four) amino acid residues which are covalently joined through peptide bonds to free amino, hydroxy or carboxylic acid groups of the presently disclosed compounds. The amino acid residues include the 20 naturally occurring amino acids commonly designated by three letter symbols and also include 4-hydroxyproline, hydroxylysine, demosine, isodemosine, 3-methylhistidine, norvalin, beta-alanine, gamma-aminobutyric acid, citrulline homocysteine, homoserine, ornithine and methionine sulfone. Prodrugs also include compounds having a carbonate, carbamate, amide or alkyl ester moiety covalently bonded to any of the above substituents disclosed herein.

[00100] As used herein, the term “pharmaceutically acceptable” excipient, carrier, or diluent refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers

and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[00101] As used herein, the terms "isolated" or "purified" refer to a material that is substantially or essentially free from components that normally accompany it in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high-performance liquid chromatography.

[00102] As used herein, the term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[00103] As used herein, the term "low dosage" refers to at least 5% less (*e.g.*, at least 10%, 20%, 50%, 80%, 90%, or even 95%) than the lowest standard recommended dosage of a particular compound formulated for a given route of administration for treatment of any human disease or condition. For example, a low dosage of an agent that is formulated for administration by inhalation will differ from a low dosage of the same agent formulated for oral administration.

[00104] As used herein, the term "high dosage" is meant at least 5% (*e.g.*, at least 10%, 20%, 50%, 100%, 200%, or even 300%) more than the highest standard recommended dosage of a particular compound for treatment of any human disease or condition.

[00105] Isotopically-labeled compounds are also within the scope of the present disclosure. As used herein, an "isotopically-labeled compound" or "isotope derivative" refers to a presently disclosed compound including pharmaceutical salts and prodrugs thereof, each as described herein, in which one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds presently disclosed include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively.

[00106] By isotopically-labeling the presently disclosed compounds, the compounds may be useful in drug and/or substrate tissue distribution assays. Tritiated (^{3}H) and carbon-14 (^{14}C) labeled compounds are particularly preferred for their ease of preparation and detectability.

Further, substitution with heavier isotopes such as deuterium (^{2}H) can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds presently disclosed, including pharmaceutical salts, esters, and prodrugs thereof, can be prepared by any means known in the art. Benefits may also be obtained from replacement of normally abundant ^{12}C with ^{13}C. (See, WO 2007/005643, WO 2007/005644, WO 2007/016361, and WO 2007/016431.)

[00107] For example, deuterium (^{2}H) can be incorporated into a compound disclosed herein for the purpose in order to manipulate the oxidative metabolism of the compound by way of the primary kinetic isotope effect. The primary kinetic isotope effect is a change of the rate for a chemical reaction that results from exchange of isotopic nuclei, which in turn is caused by the change in ground state energies necessary for covalent bond formation after this isotopic exchange. Exchange of a heavier isotope usually results in a lowering of the ground state energy for a chemical bond and thus causes a reduction in the rate in rate-limiting bond breakage. If the bond breakage occurs in or in the vicinity of a saddle-point region along the coordinate of a multi-product reaction, the product distribution ratios can be altered substantially. For explanation: if deuterium is bonded to a carbon atom at a non-exchangeable position, rate differences of k_M/k_D = 2-7 are typical. If this rate difference is successfully applied to a compound disclosed herein that is susceptible to oxidation, the profile of this compound in vivo can be drastically modified and result in improved pharmacokinetic properties.

[00108] When discovering and developing therapeutic agents, the person skilled in the art is able to optimize pharmacokinetic parameters while retaining desirable in vitro properties. It is reasonable to assume that many compounds with poor pharmacokinetic profiles are susceptible to oxidative metabolism. In vitro liver microsomal assays currently available provide valuable information on the course of oxidative metabolism of this type, which in turn permits the rational design of deuterated compounds of those disclosed herein with improved stability through resistance to such oxidative metabolism. Significant improvements in the pharmacokinetic profiles of compounds disclosed herein are thereby obtained, and can be expressed quantitatively in terms of increases in the in vivo half-life (t/2), concen-tra-tion at maximum therapeutic effect (C_{max}), area under the dose response curve (AUC), and F; and in terms of reduced clearance, dose and materials costs.

[00109] The following is intended to illustrate the above: a compound which has multiple potential sites of attack for oxidative metabolism, for example benzylic hydrogen atoms and hydrogen atoms bonded to a nitrogen atom, is prepared as a series of analogues in which various combinations of hydrogen atoms are replaced by deuterium atoms, so that some, most or all of these hydrogen atoms have been replaced by deuterium atoms. Half-life determinations enable favorable and accurate determination of the extent of the extent to which the improvement in resistance to oxidative metabolism has improved. In this way, it is determined that the half-life of the parent compound can be extended by up to 100% as the result of deuterium-hydrogen exchange of this type.

[00110] Deuterium-hydrogen exchange in a compound disclosed herein can also be used to achieve a favorable modification of the metabolite spectrum of the starting compound in order to diminish or eliminate undesired toxic metabolites. For example, if a toxic metabolite arises through oxidative carbon-hydrogen (C-H) bond cleavage, it can reasonably be assumed that the deuterated analogue will greatly diminish or eliminate production of the unwanted metabolite, even if the particular oxidation is not a rate-determining step. Further information on the state of the art with respect to deuterium-hydrogen exchange may be found, for example in Hanzlik *et al.*, J. Org. Chem. **55**, 3992-3997, 1990, Reider *et al.*, J. Org. Chem. **52**, 3326-3334, 1987, Foster, Adv. Drug Res. **14**, 1-40, 1985, Gillette et al, Biochemistry **33**(10) 2927-2937, 1994, and Jarman *et al.* Carcinogenesis **16**(4), 683-688, 1993.

[00111] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 95% ("substantially pure"), which is then used or formulated as described herein. In certain embodiments, the compounds of the present invention are more than 99% pure.

[00112] Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject).

[00113] The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The

recitation of an embodiment for a variable herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

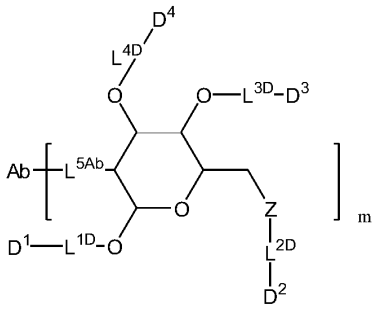
Detailed Description of the Invention

[00114] The invention provides novel multivalent linkers that allow improved drug loading and fine-tune drug of DAR and multiplexed loading of two or more distinct bioactive agents in an ADC. The invention additionally provides pharmaceutical compositions and methods of therapeutic treatment for certain diseases or conditions using ADCs built on the multivalent linker platform disclosed herein. The invention further provides methods of making such multivalent linkers, linker-drug conjugates, and ADCs, as well as methods for modifying or fine-tuning DARs thereof.

[00115] By way of an example to illustrate the invention, 2-azido-2-deoxy-D-glucose was utilized as a scaffold, the 1-, 3-, 4-, 6-OH's were derivatized and each arm attached to a spacer. At the other end of the spacer, a drug (payload) was linked. For example, SN-38 was used as payload as a proof of concept. At a middle point of each spacer, a polyethylene glycol (PEG) (n=0 to 24) was branched out by a trivalent atom such as nitrogen. The branching points can be varied depending on the spacer design and chemistry. The PEG group is introduced to mask the hydrophobic payloads and increase solubility. From an azido group attached to the 2-azido-2-deoxy-D-glucose, a click chemistry was performed to attach a functionalized arm with variable length, where at the end of the arm, a maleimide group was placed for further conjugation chemistry to an antibody. Depending on the number of linkers an antibody is attached to and the number of drug molecules per linker unit, an ADC can be fine-tuned to have 4 to 32 drugs per antibody.

[00116] In addition to improving drug loadings, the linkers of the invention are hydrophilic by design and can help improve ADC properties such that they exhibit reduced aggregation, improved solubility, improved ADC plasma clearance and pharmacokinetics, improved efficacy, reduced immunogenicity, and finally improved therapeutic windows.

[00117] In one aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**I**):



(I)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups;

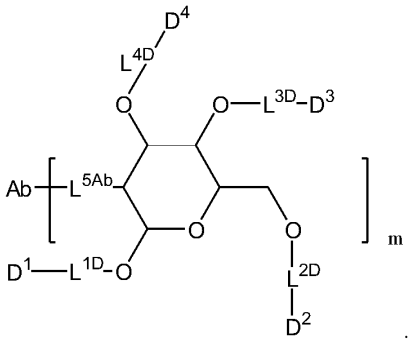
Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N; and

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

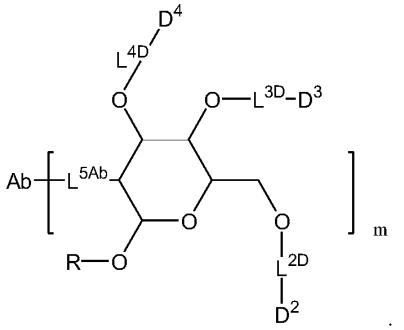
[00118] In certain embodiments of (**I**), Z is O and the antibody-drug conjugate has the structural formula (**I^A**):



(**I**^{A})

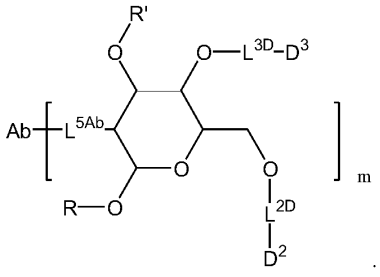
[00119] In certain embodiments of (**I**) and (**I**^{A}), none of L^{1D}-D^{1}, L^{2D}-D^{2}, L^{3D}-D^{3} and L^{4D}-D^{4} is optionally H or a C_{1-3} alkyl.

[00120] In certain embodiments of (**I**) and (**I**^{A}), L^{1D}-D^{1} is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**I**^{B}):



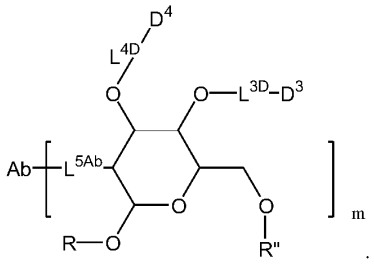
(**I**^{B})

[00121] In certain embodiments of (**I**^{B}), L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I**^{C}):



(**I**^{C})

[00122] In certain embodiments of (**I^{B}**), wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**I^{D}**):



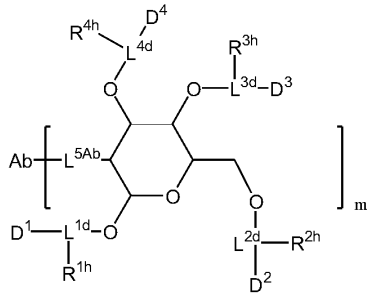
(**I^{D}**)

[00123] In certain embodiments of (**I**)-(**I^{D}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

[00124] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00125] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

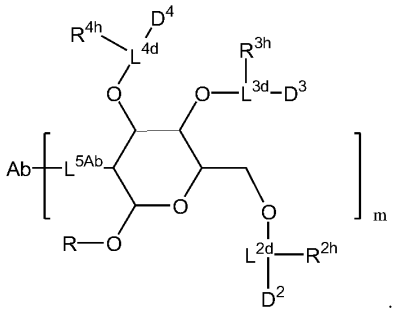
[00126] In certain embodiments of (**I**)-(**I^{A}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**II**):



(**II**)

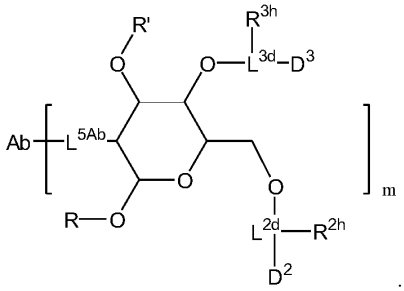
wherein L^{1d}, L^{2d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{2D}, L^{3D} and L^{4D}, respectively.

[00127] In certain embodiments of (**II**), L^{1D}-D^{1} is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**II^{A}**):



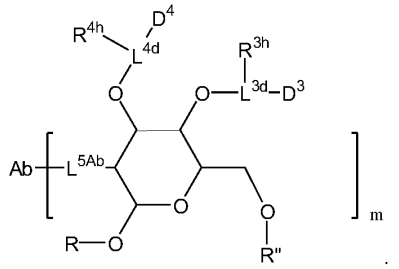
(**II^{A}**)

[00128] In certain embodiments of (**II^{A}**), L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**II^{B}**):



(**II^{B}**)

[00129] In certain embodiments of (**II^{A}**), L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**II^{C}**):

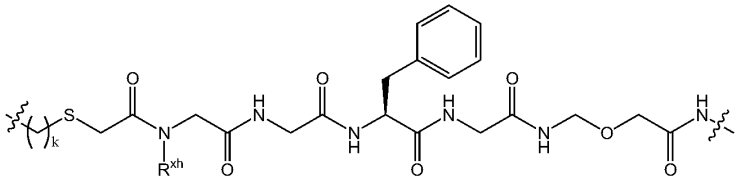


(**II^{C}**)

[00130] In certain embodiments of (**II**)-(**II^{C}**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups wherein each of which comprises *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00131] In certain embodiments of (**II**)-(**II^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00132] In certain embodiments of (**II**)-(**II^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} is independently:



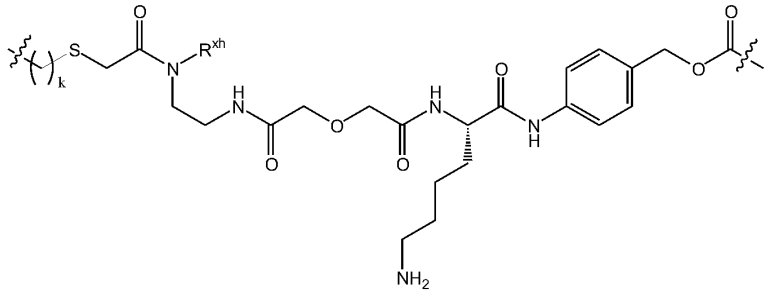
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00133] In certain embodiments of (**II**)-(**II^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00134] In certain embodiments of (**II**)-(**II^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

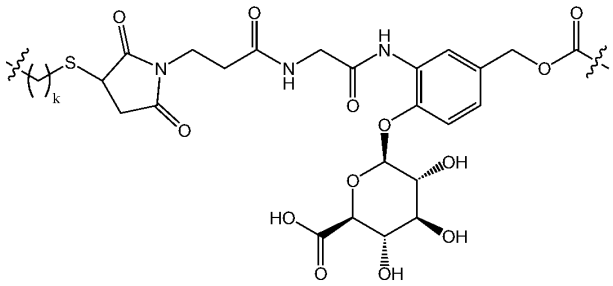


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

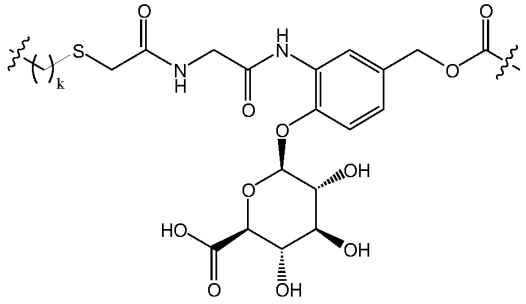
x is 1, 2, 3 or 4.

[00135] In certain embodiments of (**I**)-(**I^D**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



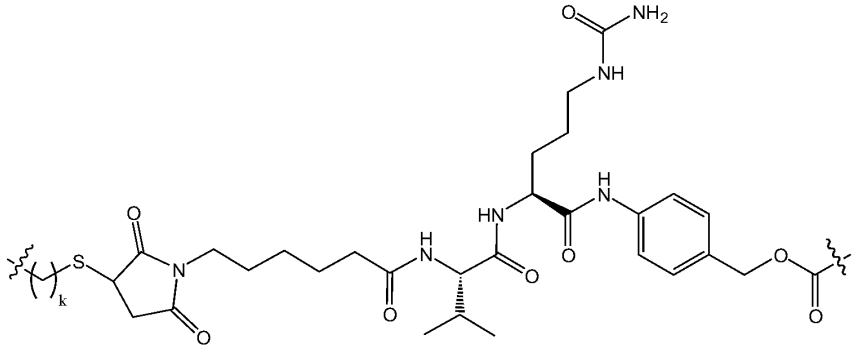
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00136] In certain embodiments of (**I**)-(**I^{D}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

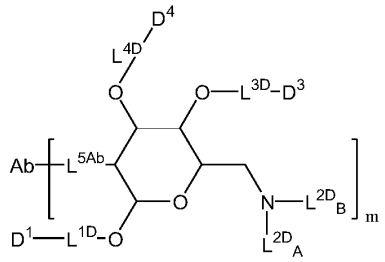
[00137] In certain embodiments of (**I**)-(**I^{D}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00138] In certain embodiments of (**I**)-(**II^{C}**), each of D^1, D^2, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{2d}, L^{3d} and L^{4d} via a linkage selected from carbonate linkage, a carbamate linkage, and an amide linkage.

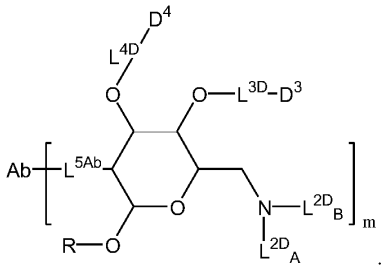
[00139] In certain embodiments of (**I**), Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, having the structural formula (**III**):



(**III**)

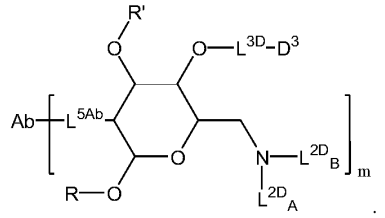
[00140] In certain embodiments of (**I**) and (**III**), none of L^{1D}-D^1, L^{3D}-D^3 and L^{4D}-D^4 is optionally H or a C_{1-3} alkyl.

[00141] In certain embodiments of (**I**) and (**III**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**III^A**):



(**III^A**)

[00142] In certain embodiments of (**III^A**), L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I^B**):



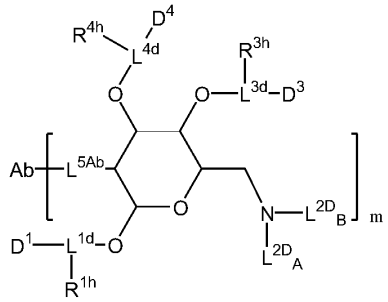
(**III^B**)

[00143] In certain embodiments of (**III**)- (**III^B**), each of L^{1D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

[00144] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00145] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

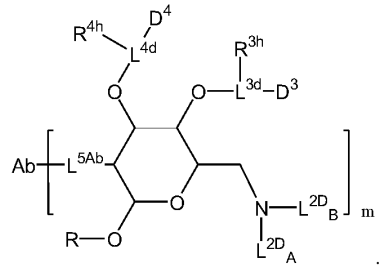
[00146] In certain embodiments of (**I**) and (**III**), each of L^{1D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IV**):



(**IV**)

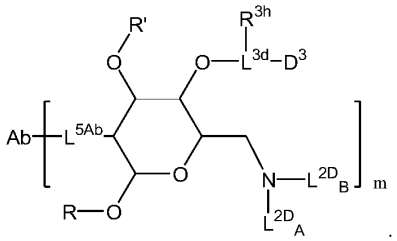
wherein L^{1d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{3D} and L^{4D}, respectively.

[00147] In certain embodiments of (**IV**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**IV^A**):



(**IV^A**)

[00148] In certain embodiments of (**IV^A**), L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**IV^B**):

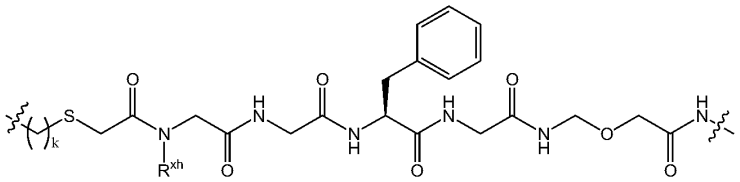


(**IV^B**)

[00149] In certain embodiments of (**IV**)-(**IV^B**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00150] In certain embodiments of (**IV**)-(**IV^B**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00151] In certain embodiments of (**IV**)-(**IV^B**), one or more of L^{1d}, L^{3d} and L^{4d} is independently:



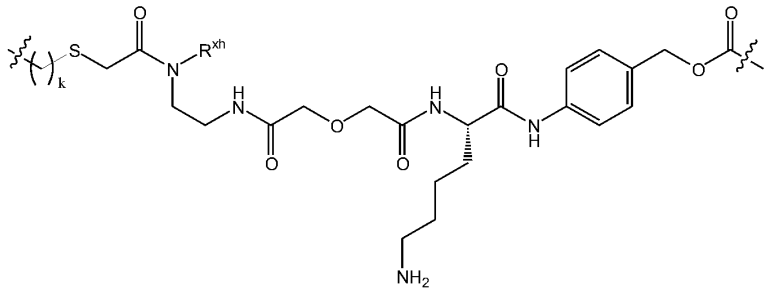
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00152] In certain embodiments of (**IV**)-(**IV^B**), one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00153] In certain embodiments of (**IV**)-(**IV^B**), one or more of L^{1d}, L^{3d} and L^{4d} independently is:

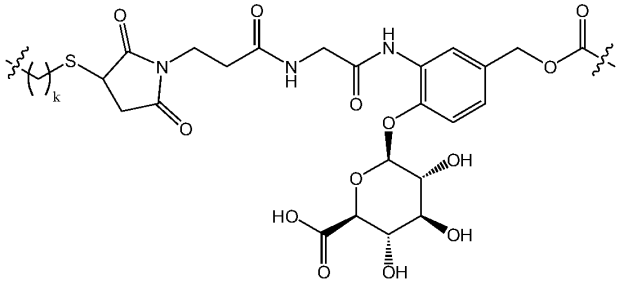


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

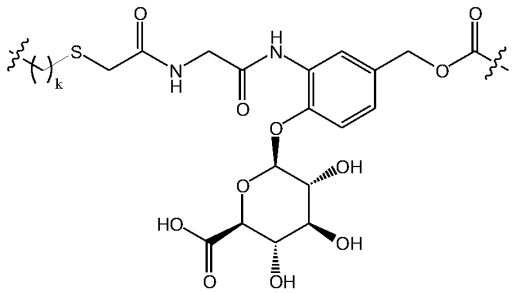
x is 1, 2, 3 or 4.

[00154] In certain embodiments of (**III**)-(**III^{B}**), one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



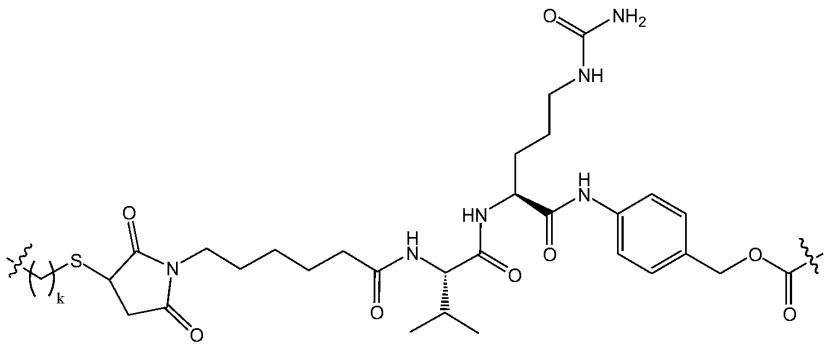
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00155] In certain embodiments of (**III**)-(**III^{B}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

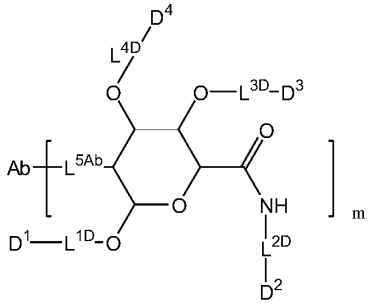
[00156] In certain embodiments of (**III**)-(**III^{B}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00157] In certain embodiments of (**III**)-(**IV^{B}**), each of D^1, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{3d} and L^{4d} via a linkage selected from a carbonate linkage, a carbamate linkage, and an amide linkage.

[00158] In another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**I'**):



(**I'**)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

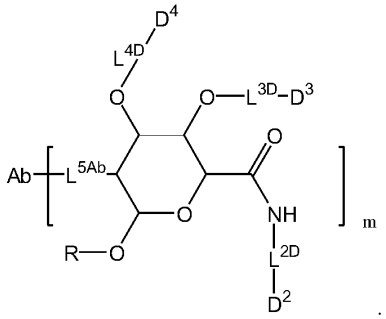
m is an integer selected from 1-8 (*e.g.*, 1, 2, 3, 4, 5, 6, 7 or 8),

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

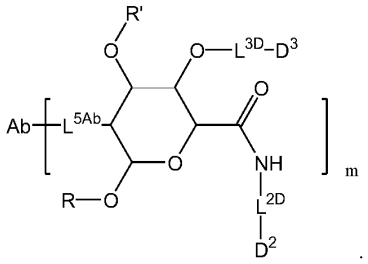
[00159] In certain embodiments of (**I'**), none of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H or a C_{1-3} alkyl.

[00160] In certain embodiments of (**I'**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**I'^{B}**):



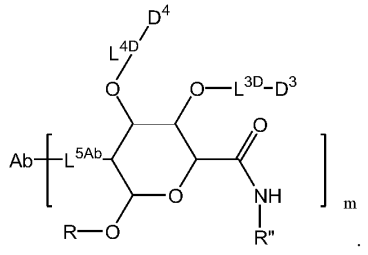
(**I'^{B}**)

[00161] In certain embodiments of (**I'^{B}**), L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I'^{C}**):



(**I'^{C}**)

[00162] In certain embodiments of (**I'^{B}**), L^{2D}-D^2 is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**I'^{D}**):



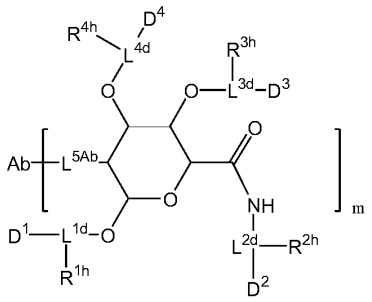
(**I'^{D}**)

[00163] In certain embodiments of (**I'**)-(**I'^{D}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

[00164] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00165] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

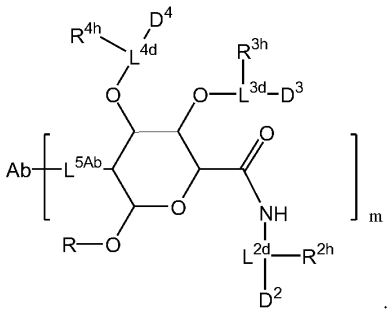
[00166] In certain embodiments of (**I'**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**II'**):



(**II'**)

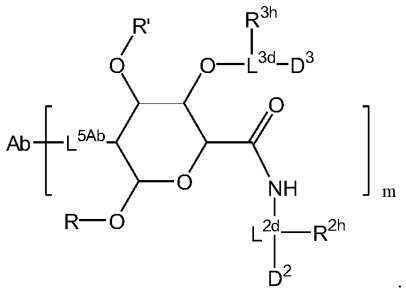
wherein L^{1d}, L^{2d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{2D}, L^{3D} and L^{4D}, respectively.

[00167] In certain embodiments of (**II'**), L^{1D}-D^{1} is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**II'^{A}**):



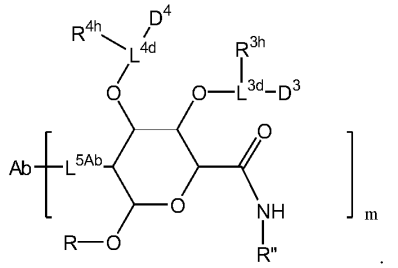
(**II'^{A}**)

[00168] In certain embodiments of (**II'^{A}**), L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**II'^{B}**):



(**II'^{B}**)

[00169] In certain embodiments of (**II'^{A}**), L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**II'^{C}**):

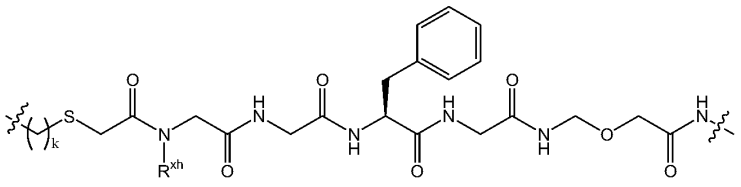


(**II'^{C}**)

[00170] In certain embodiments of (**II'**)-(**II'^{C}**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00171] In certain embodiments of (**II'**)-(**II'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00172] In certain embodiments of (**II'**)-(**II'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} is independently:



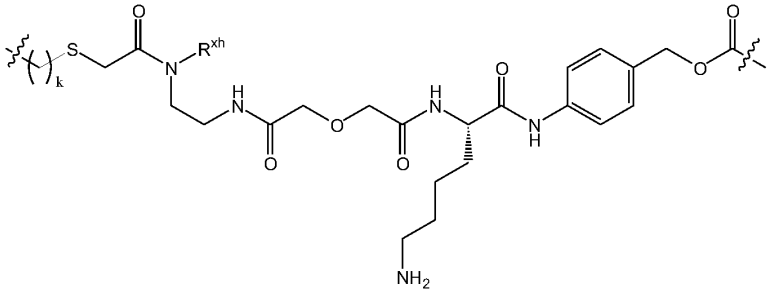
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00173] In certain embodiments of (**II'**)-(**II'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00174] In certain embodiments of (**II'**)-(**II'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

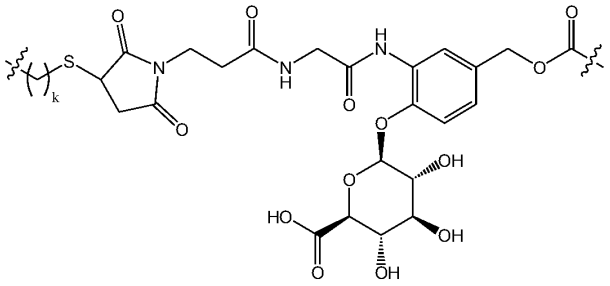


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

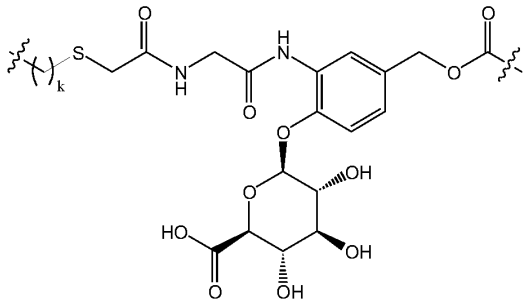
x is 1, 2, 3 or 4.

[00175] In certain embodiments of (**I'**)-(**I'^{D}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



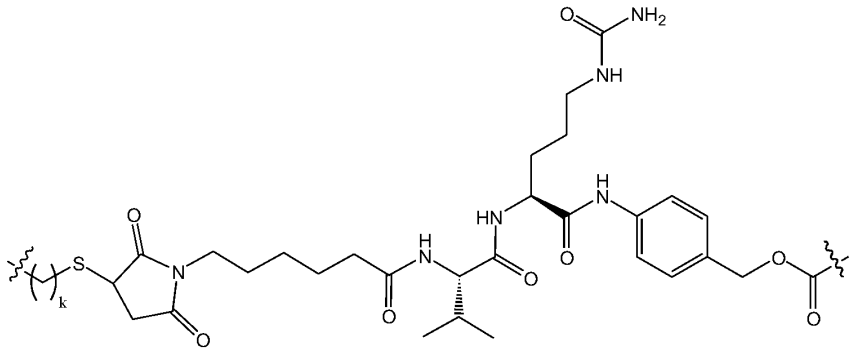
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00176] In certain embodiments of (**I'**)-(**I'^{D}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00177] In certain embodiments of (**I'**)-(**I'^{D}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

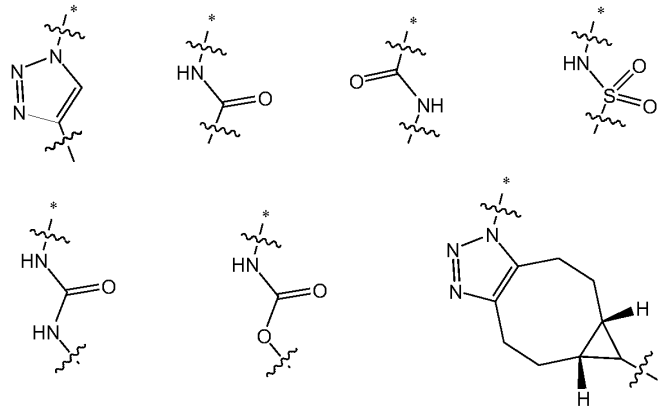
[00178] In certain embodiments of (**I'**)-(**II'^{C}**), each of D^1, D^2, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{2d}, L^{3d} and L^{4d} via a linkage selected from carbonate linkage, a carbamate linkage, and an amide linkage.

[00179] In certain embodiments of (**II**)-(**II^{C}**), (**IV**)-(**IV^{B}**) and (**II'**)-(**II'^{C}**), R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are the same.

[00180] In certain embodiments of (**II**)-(**II^{C}**), (**IV**)-(**IV^{B}**) and (**II'**)-(**II'^{C}**), R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are not the same.

[00181] In certain embodiments of (**II**)-(**II^{C}**), (**IV**)-(**IV^{B}**) and (**II'**)-(**II'^{C}**), one or more of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, is independently $(CH_2\text{-}CH_2O)_pCH_3$, wherein *p* is an integer selected from about 4 to about 12.

[00182] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), L^{5Ab} comprises a group selected from:

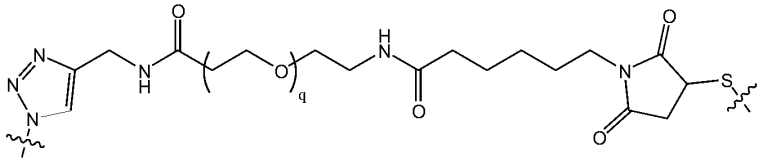


wherein * represents the attachment point to the central sugar ring of Formula (**I**).

[00183] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), L^{5Ab} is covalently attached to Ab via a thioether linkage. In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), the thioether linkage comprises maleimide thioether.

[00184] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), L^{5Ab} comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups.

[00185] In certain embodiments, L^{5Ab} is:



wherein q is an integer selected from 1 to 6.

[00186] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), D^1, D^2, D^3 and D^4, if present, are the same.

[00187] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), D^1, D^2, D^3 and D^4, if present, are not the same.

[00188] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), D^1, D^2, D^3 and D^4, if present, represent two distinct drug moieties.

[00189] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are the same.

[00190] In certain embodiments of (**I**)-(**IV^{B}**) and (**I'**)-(**II'^{C}**), R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are not the same.

[00191] In certain embodiments of (**I**)-(**IV^B**) and (**I'**)-(**II'^{C}**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, is independently $(CH_2\text{-}CH_2O)_pCH_3$, wherein *p* is an integer selected from about 4 to about 12.

[00192] In certain embodiments of (**I**)-(**IV^B**) and (**I'**)-(**II'^{C}**), Ab is an antibody.

[00193] In certain embodiments, Ab is a monoclonal antibody.

[00194] In certain embodiments, Ab is a chimeric antibody.

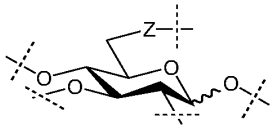
[00195] In certain embodiments, Ab is a humanized antibody.

[00196] In certain embodiments, Ab is an antibody fragment.

[00197] In certain embodiments, Ab is a peptide.

[00198] In certain embodiments of (**I**)-(**IV^B**) and (**I'**)-(**II'^{C}**), each of D^1, D^2, D^3 and D^4, if present, is independently selected from: chemotherapeutic agents and toxins.

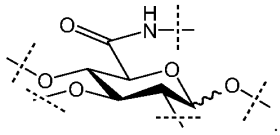
[00199] In certain embodiments of (**I**)-(**IV^B**), the antibody-drug conjugate has the stereochemistry shown in Formula (**V**):



(**V**)

wherein Z is O or N.

[00200] In certain embodiments of (**I'**)-(**II'^{C}**), the antibody-drug conjugate has the stereochemistry shown in Formula (**V'**):



(**V'**)

[00201] Non-limiting examples of antibody-drug conjugates are provided below:

Table A

ADC #	Antibody	Linker Payload
ADC-1	TrastuzumAb	1
ADC-2	Rituximab	1

ADC-3	TrastuzumAb	CL2A-SN38
ADC-4	TrastuzumAb	2
ADC-5	Rituximab	2
ADC-6	SacituzumAb	2
ADC-7	TrastuzumAb	3
ADC-8	SacituzumAb	3
ADC-9	TrastuzumAb	4
ADC-10	TrastuzumAb	7
ADC-11	TrastuzumAb	6
ADC-3	TrastuzumAb	CL2A-SN38
ADC-12	TrastuzumAb	10
ADC-13	TrastuzumAb	11
ADC-14	TrastuzumAb	MC-VC-PAB-MMAE
ADC-15	TrastuzumAb	12
ADC-16	TrastuzumAb	13
ADC-17	TrastuzumAb	15
ADC-18	TrastuzumAb	16
ADC-19	TrastuzumAb	17
ADC-20	TrastuzumAb	mc-GGFG-Dxd
ADC-21	TrastuzumAb	18
ADC-22	Human IgG1 kappa Isotype	15

ADC-23	TrastuzumAb	21
ADC-24	Human IgG1 kappa Isotype	21

[00202] In yet another aspect, the invention generally relates to a method for making an antibody-drug conjugate of (**I**)-(**IV**^{B}) and (**I'**)-(**II'**^{C}).

[00203] In another aspect, the invention generally relates to a pharmaceutical composition comprising an antibody-drug conjugate disclosed herein.

[00204] In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce autoimmune disease, or a related disease or disorder. In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce graft rejection, or a related disease or disorder. In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce an allergy, or a related disease or disorder. In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce immunodeficiency, or a related disease or disorder. In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce infection, sepsis, or a related disease or disorder. In certain embodiments, the pharmaceutical composition is effective to treat, prevent, or reduce cancer, or a related disease or disorder.

[00205] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of the invention.

[00206] In yet another aspect, the invention generally relates to a method for treating, reducing, or preventing a disease or disorder, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

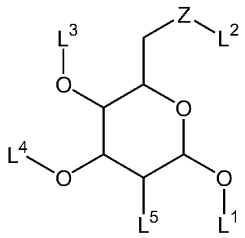
[00207] In yet another aspect, the invention generally relates to a method for modulating immune response, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[00208] In yet another aspect, the invention generally relates to use of an antibody-drug conjugate disclosed herein for treating or reducing a disease or disorder.

[00209] In yet another aspect, the invention generally relates to use of an antibody-drug conjugate disclosed herein, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating or reducing a disease or disorder.

[00210] In certain embodiments, the disease or disorder is selected from autoimmune diseases, graft rejection, allergies, immunodeficiency, infection, sepsis, cancer, or a related disease or disorder thereof. In certain embodiments, the use is to treat or reduce an autoimmune disease, or a related disease or disorder. In certain embodiments, the use is to treat or reduce graft rejection, or a related disease or disorder. In certain embodiments, the use is to treat or reduce allergy, or a related disease or disorder. In certain embodiments, the use is to treat or reduce immunodeficiency, or a related disease or disorder. In certain embodiments, the use is to treat or reduce infection and/or sepsis, or a related disease or disorder. In certain embodiments, the use is to treat or reduce cancer, or a related disease or disorder. In certain embodiments, the use is as a vaccine adjuvant.

[00211] In yet another aspect, the invention generally relates to a compound having the structural formula (**VI**):



(**VI**)

wherein

each of L^1, L^2, L^3 and L^4 is independently selected from: H, $C_{1\text{-}3}$ alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

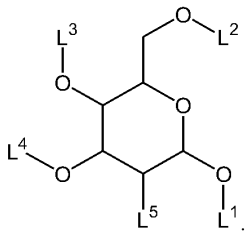
L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

Z is O or N, wherein when Z is N, L^2 represents L^2_A and L^2_B, each is independently a hydrophilic group covalently bound to said N.

wherein

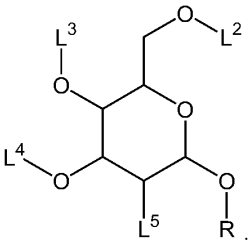
0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} optionally comprises a hydrophilic group.

[00212] In certain embodiments of (**VI**), Z is O, having the structural formula (**VI^A**):



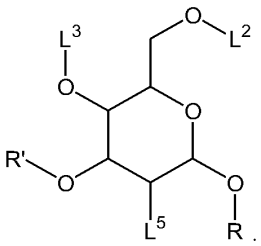
(**VI^{A}**)

[00213] In certain embodiments of (**VI**) and (**VI^{A}**), L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural formula (**VI^{B}**):



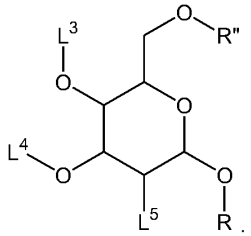
(**VI^{B}**)

[00214] In certain embodiments of (**VI^{B}**), L^4 is R', wherein R' is H or C_{1-3} alkyl, , having the structural formula (**VI^{C}**):



(**VI^{C}**)

[00215] In certain embodiments of (**VI^{B}**), L^2 is R", wherein R" is H or C_{1-3} alkyl, having the structural formula (**VI^{D}**):



(**VI^{D}**)

[00216] In certain embodiments of (**VI**)-(**VI^{D}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

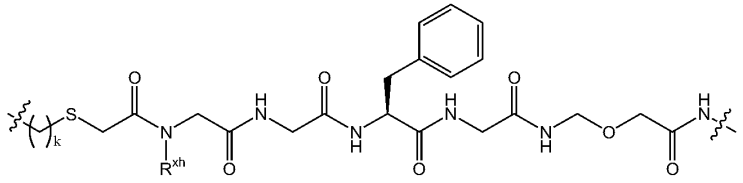
[00217] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00218] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

[00219] In certain embodiments of (**VI**)-(**VI^{D}**), L^4 is N_3.

[00220] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker.

[00221] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprise:



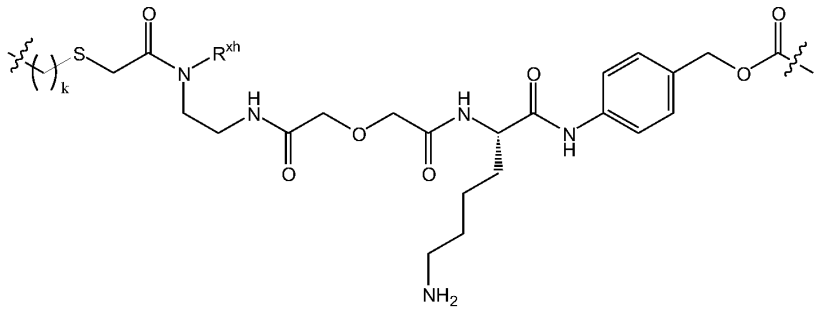
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00222] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprises a lysine unit.

[00223] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4 independently comprises:

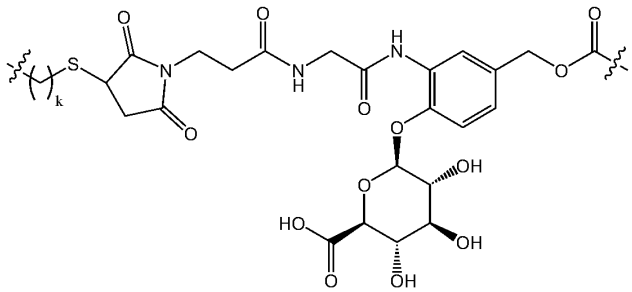


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

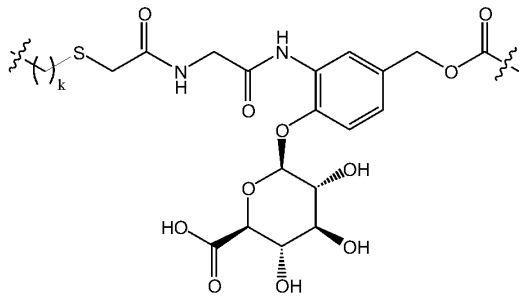
x is 1, 2, 3 or 4.

[00224] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



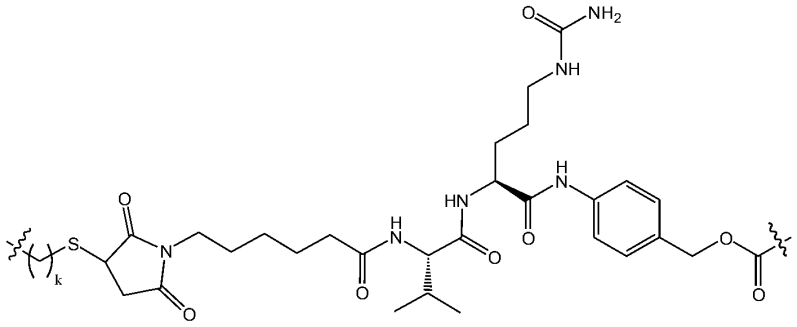
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00225] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



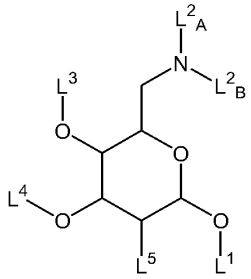
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00226] In certain embodiments of (**VI**)-(**VI^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



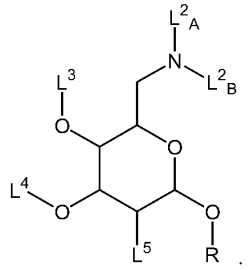
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00227] In certain embodiments of (**VI**), Z is N, D^2 is absent and L^2 represents L^2_A and L^2_B, having the structural formula (**VII**):



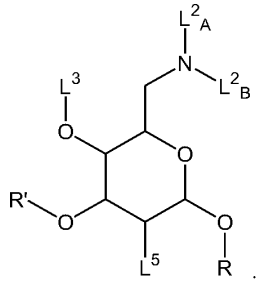
(**VII**)

[00228] In certain embodiments of (**VII**), L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural formula (**VII^A**):



(**VII^A**)

[00229] In certain embodiments of (**VII^A**), L^4 is R', wherein R' is H or C_{1-3} alkyl, having the structural formula (**VII^B**):



(**VII^B**)

[00230] In certain embodiments of (**VII**)-(**VII^B**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

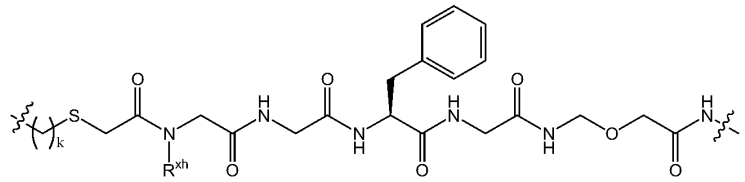
[00231] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00232] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

[00233] In certain embodiments of (**VII**)-(**VII^{B}**), L^4 is N_3.

[00234] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker.

[00235] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4 if present independently comprise:



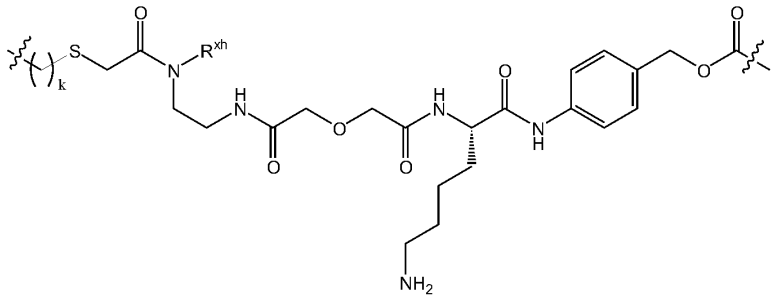
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00236] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4 if present independently comprises a lysine unit.

[00237] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4 independently comprises:

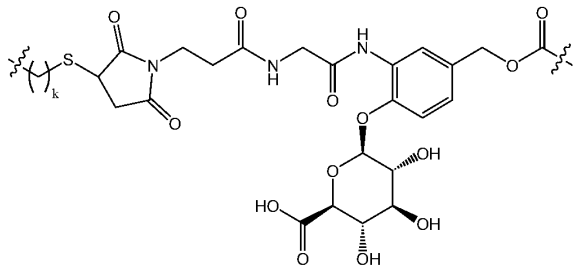


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

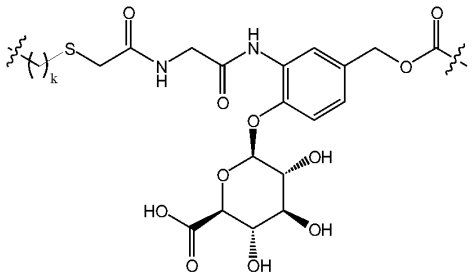
x is 1, 2, 3 or 4.

[00238] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4, if present, comprises:



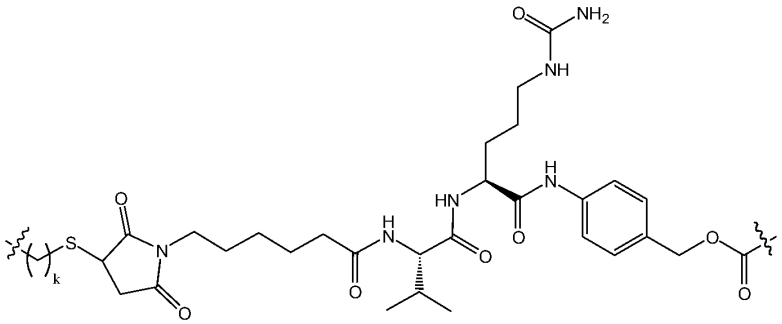
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00239] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

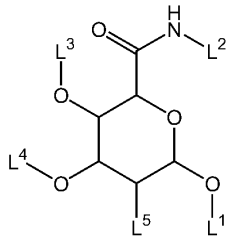
[00240] In certain embodiments of (**VII**)-(**VII^{B}**), one or more of L^1, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00241] In certain embodiments of (**VII**)-(**VII^{B}**), if present each of L^1, L^2, L^3 and L^4 comprises a functional group capable of forming a carbonate linkage, a carbamate linkage, or an amide linkage.

[00242] In yet another aspect, the invention generally relates to a compound having the structural formula (**VI'**):



(**VI'**)

wherein

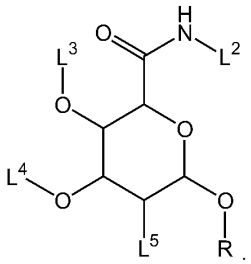
each of L^1, L^2, L^3 and L^4 is independently selected from: H, C_{1-3} alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

wherein

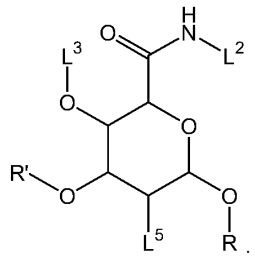
0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a hydrophilic group.

[00243] In certain embodiments of (**VI'**), L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural formula (**VI'^{B}**):



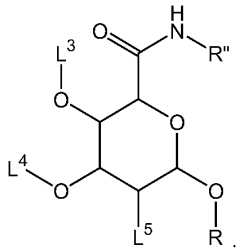
(**VI'^{B}**)

[00244] In certain embodiments of (**VI'^{B}**), L^4 is R', wherein R' is H or C_{1-3} alkyl, , having the structural formula (**VI'^{C}**):



(**VI'^{C}**)

[00245] In certain embodiments of (**VI'^{B}**), L^2 is R", wherein R" is H or C_{1-3} alkyl, having the structural formula (**VI'^{D}**):



(**VI'^{D}**)

[00246] In certain embodiments of (**VI'**)-(**VI'^{D}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

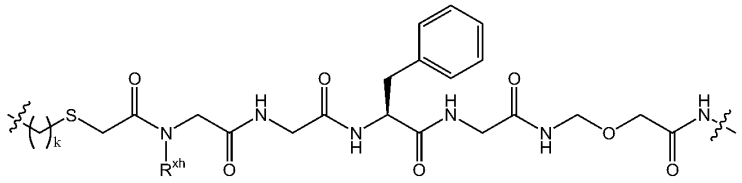
[00247] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00248] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

[00249] In certain embodiments of (**VI'**)-(**VI'^{D}**), L^4 comprises N_3

[00250] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker

[00251] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprise:



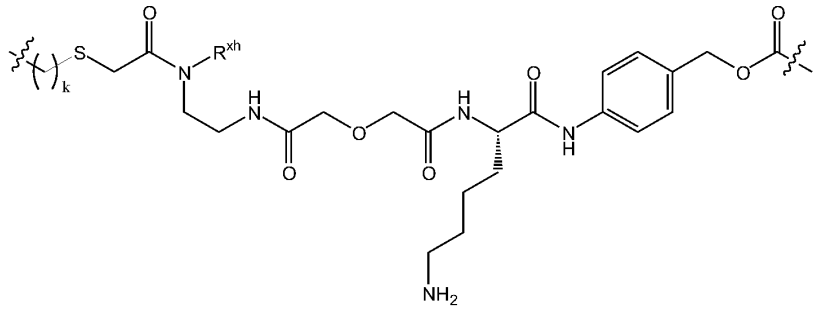
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00252] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4 if present independently comprises a lysine unit.

[00253] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4 independently comprises:

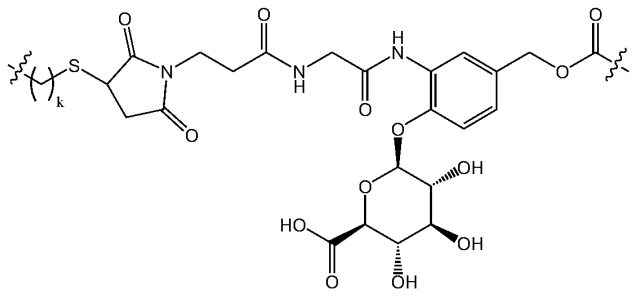


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

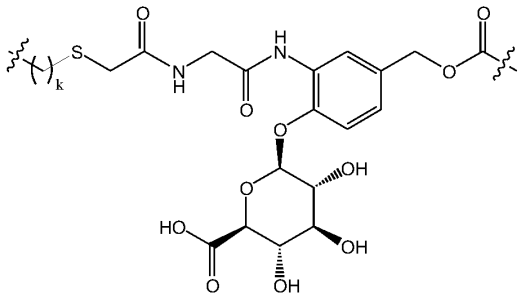
x is 1, 2, 3 or 4.

[00254] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



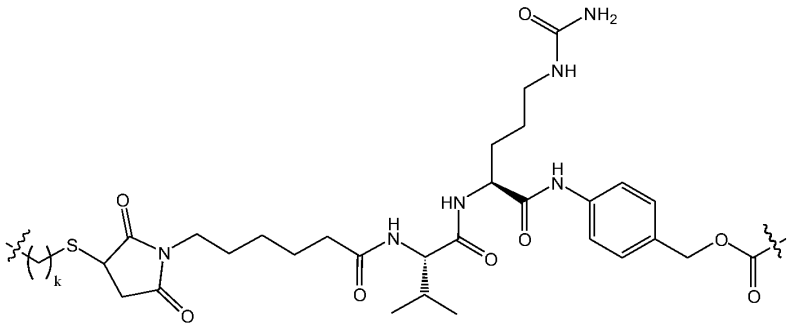
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00255] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



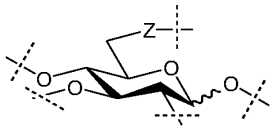
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00256] In certain embodiments of (**VI'**)-(**VI'^{D}**), one or more of L^1, L^2, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

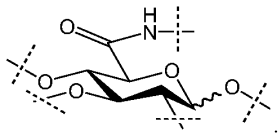
[00257] In certain embodiments of (**VI**)-(**VII^{B}**), compound of any one of claims 116-146, having the stereochemistry shown in Formula (**V**):



(**V**)

wherein Z is O or N.

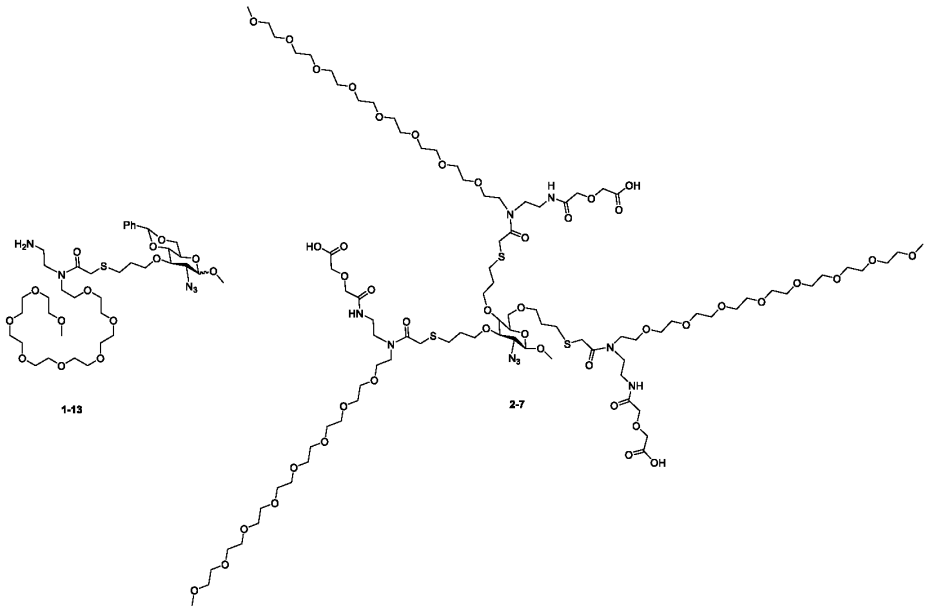
[00258] In certain embodiments of (**VI'**)-(**VI'^{D}**), the has compound the stereochemistry shown in Formula (**V'**):

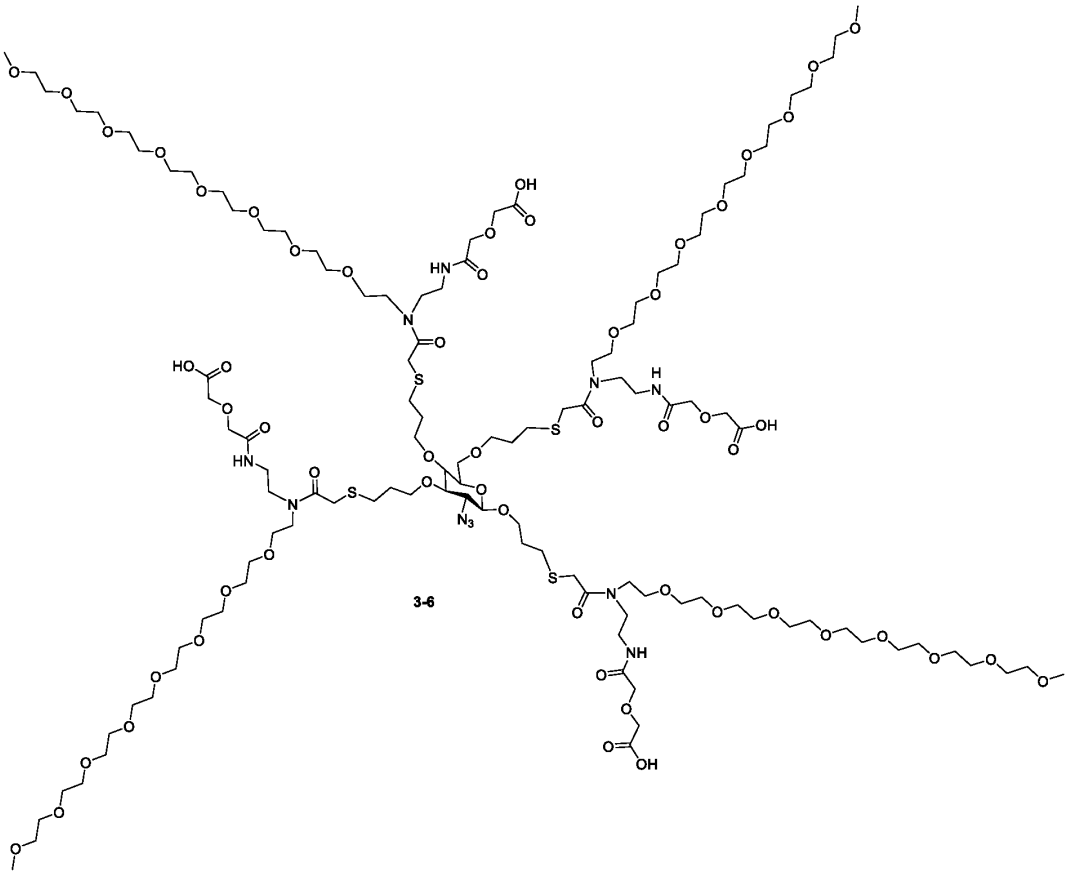


(V')

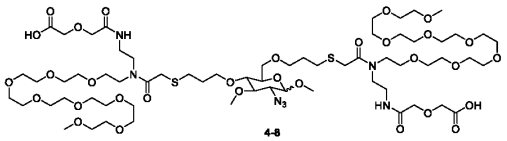
[00259] Non-limiting examples of compounds include:

Table B

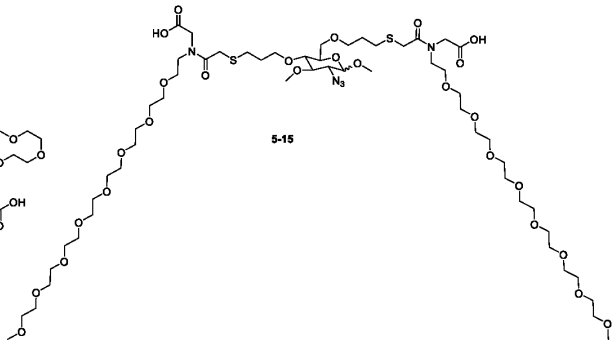




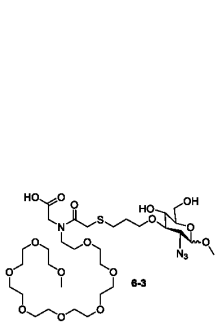
3-6



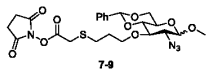
4-8



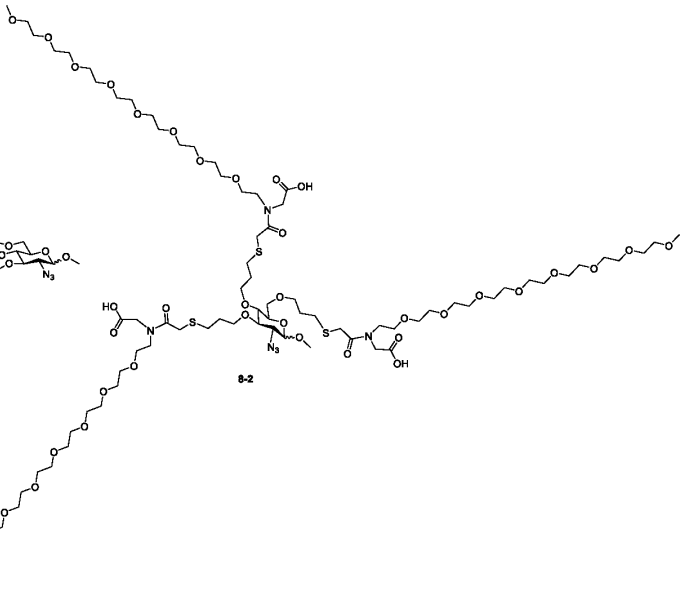
5-15



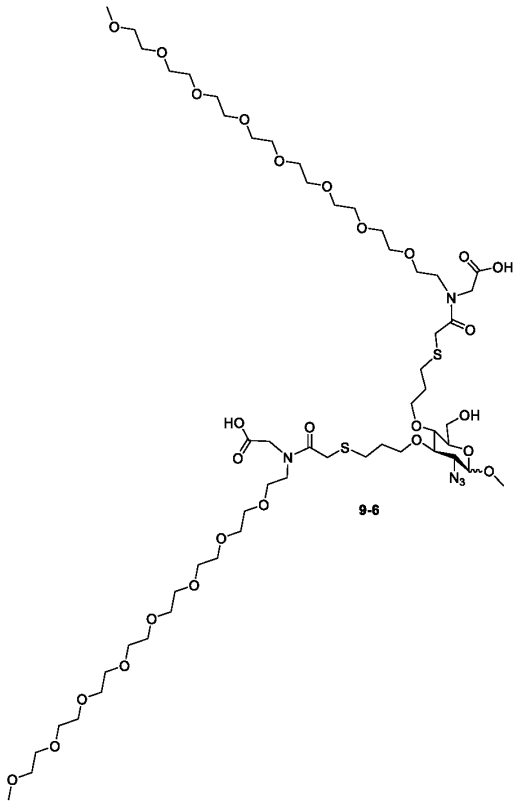
6-3



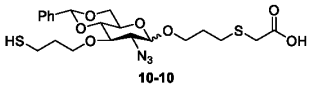
7-9



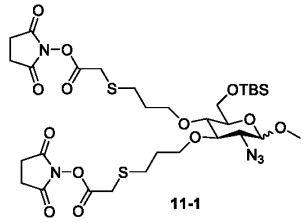
8-2



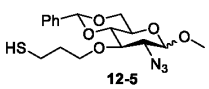
9-6



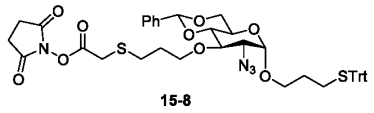
10-10



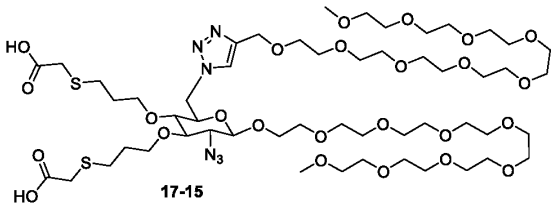
11-1



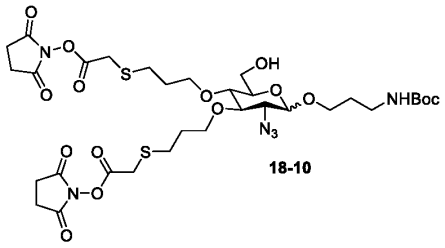
12-5



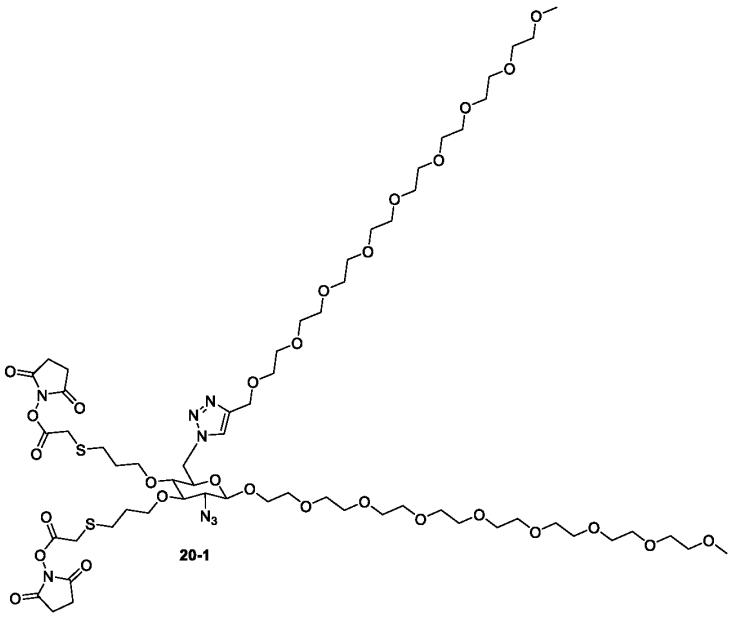
15-8



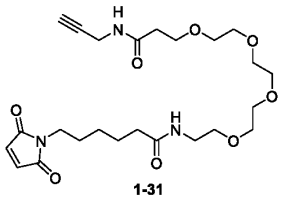
17-15



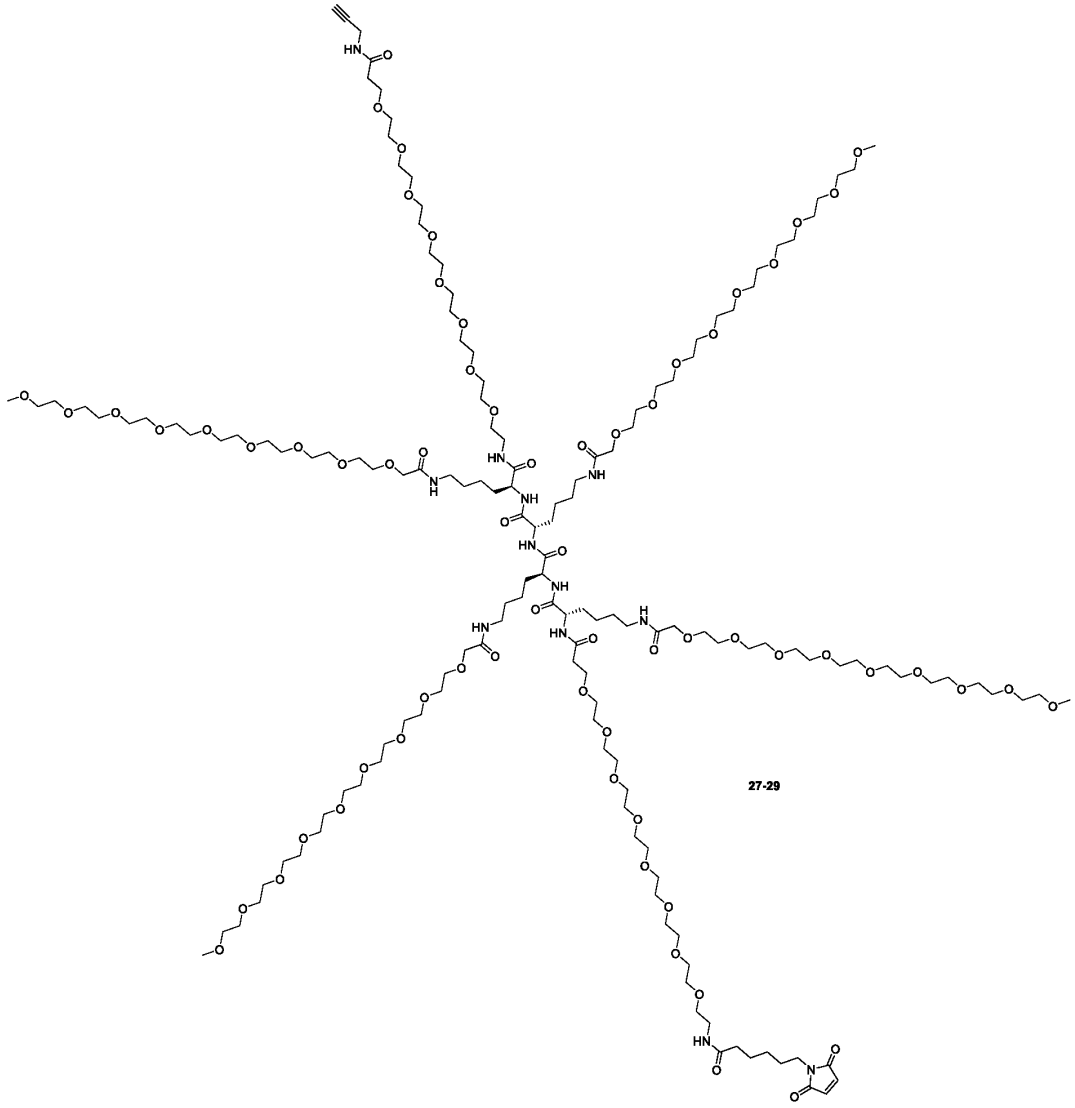
18-10



20-1

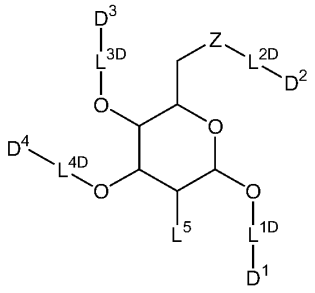


1-31



[00260] In yet another aspect, the invention generally relates to a method for making a compound of any (**VI**)-(**VII^{B}**).

[00261] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**VIII**):



(VIII)

wherein

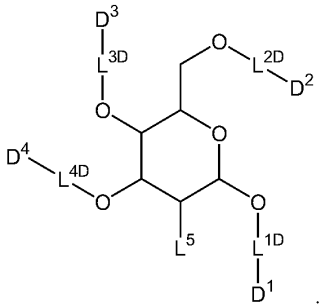
each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

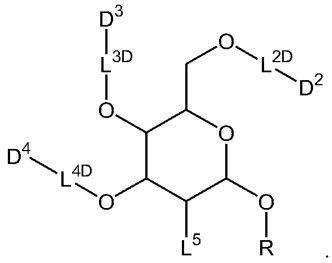
Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N.

[00262] In certain embodiments of **(VIII)**, Z is O, having the structural formula **($VIII^A$)**:



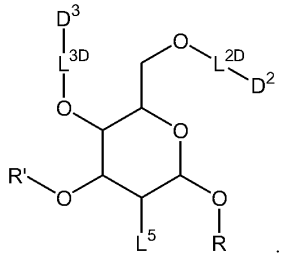
($VIII^A$)

[00263] In certain embodiments of **(VIII)** or **($VIII^A$)**, L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula **($VIII^B$)**:



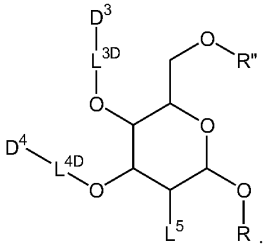
(**VIII^{B}**)

[00264] In certain embodiments of (**VIII^{B}**), L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**VIII^{C}**):



(**VIII^{C}**)

[00265] In certain embodiments of (**VIII^{B}**), wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**VIII^{D}**):



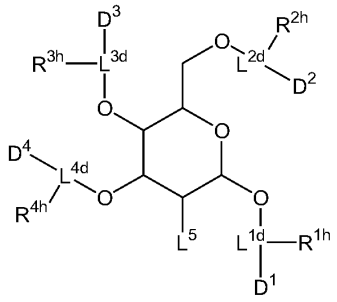
(**VIII^{D}**)

[00266] In certain embodiments of (**VIII**)-(**VIII^{D}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

[00267] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

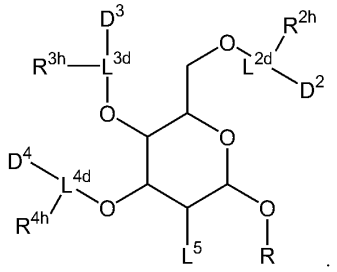
[00268] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

[00269] In certain embodiments of (**VIII**) or (**VIII^{A}**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IX**):



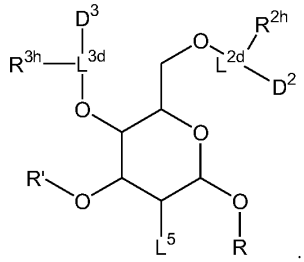
(**IX**)

[00270] In certain embodiments of (**IX**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**IX^A**):



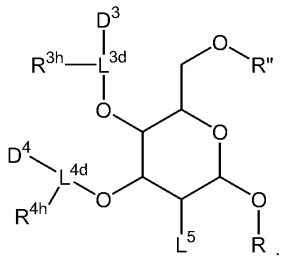
(**IX^A**)

[00271] In certain embodiments of (**IX^A**), L^{4D}-D^4 is R', R' is H or a C_{1-3} alkyl, having the structural formula (**IX^B**):



(**IX^B**)

[00272] In certain embodiments of (**IX^A**), L^{2D}-D^2 is R", R" is H or a C_{1-3} alkyl, having the structural formula (**IX^C**):

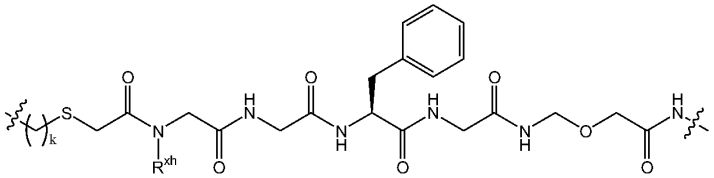


(**IX^C**)

[00273] In certain embodiments of (**IX**)-(**IX^C**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

[00274] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00275] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} if present independently is:



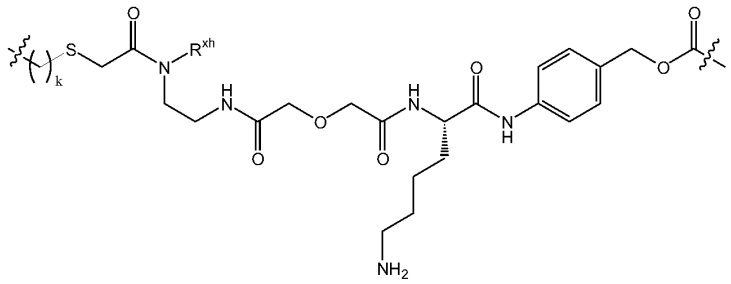
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00276] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00277] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

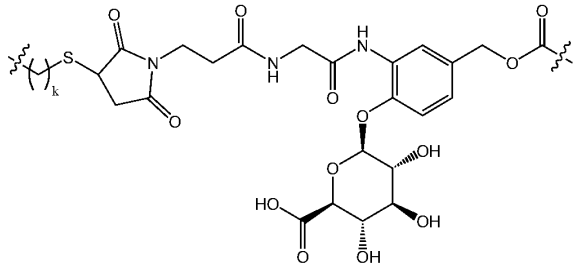


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

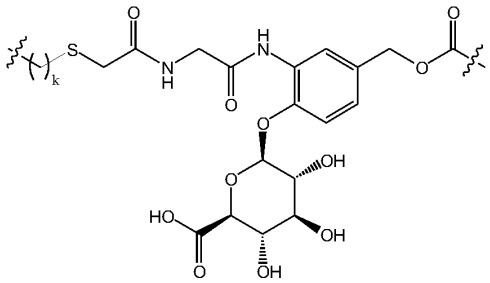
x is 1, 2, 3 or 4.

[00278] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



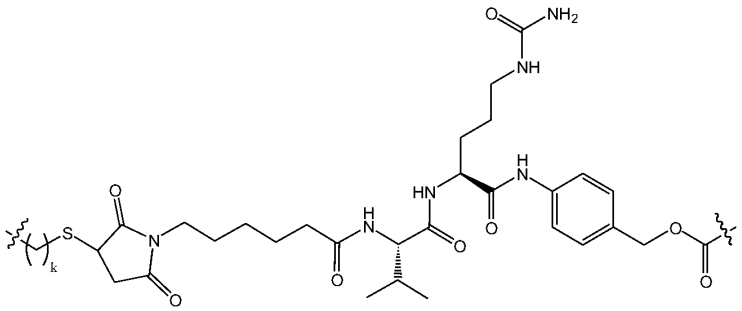
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00279] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



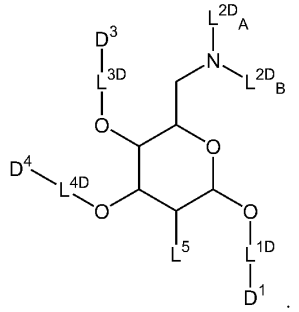
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00280] In certain embodiments of (**IX**)-(**IX^C**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



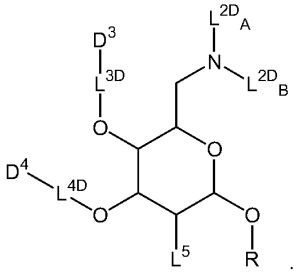
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00281] In certain embodiments of (**VIII**), Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N, having the structural formula (**X**):



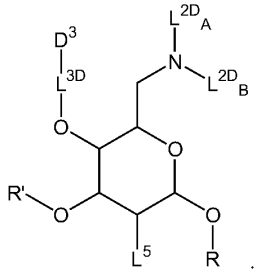
(**X**)

[00282] In certain embodiments of (**X**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula ($\mathbf{X^A}$):



($\mathbf{X^A}$)

[00283] In certain embodiments of ($\mathbf{X^A}$), L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula ($\mathbf{X^B}$):



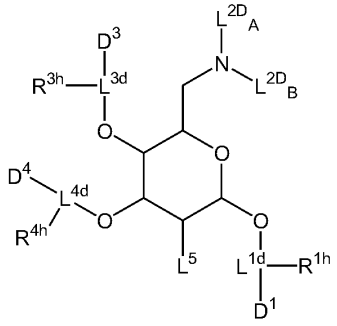
($\mathbf{X^B}$)

[00284] In certain embodiments of (**X**)-($\mathbf{X^B}$), wherein each of L^{1D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

[00285] In certain embodiments, the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

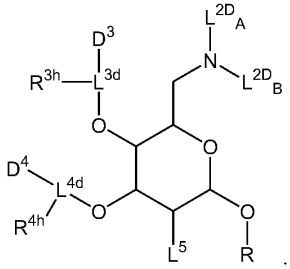
[00286] In certain embodiments, the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6 (*e.g.*, 2, 3, 4, 5 or 6).

[00287] In certain embodiments of (**X**), each of L^{1D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{3h} or R^{4h}, having the structural formula (**XI**):



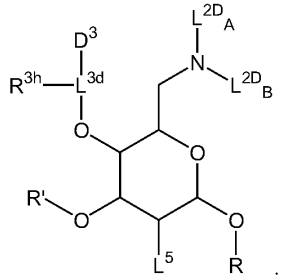
(**XI**)

[00288] In certain embodiments of (**XI**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**XI^A**):



(**XI^A**)

[00289] In certain embodiments of (**XI^A**), L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**XI^B**):

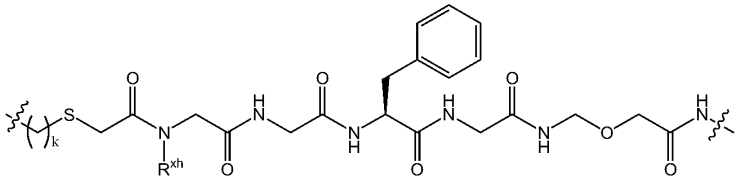


(**XI^B**)

[00290] In certain embodiments of (**XI**)-(**XI^B**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00291] In certain embodiments of (**XI**)-(**XI^B**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00292] In certain embodiments of (**XI**)-(**XI^B**), one or more of L^{1d}, L^{3d} and L^{4d} if present independently is:



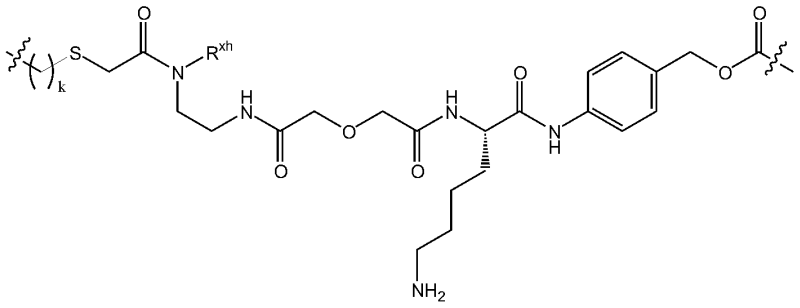
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00293] In certain embodiments of (**XI**)-(**XI^B**), one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00294] In certain embodiments of (**XI**)-(**XI^B**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

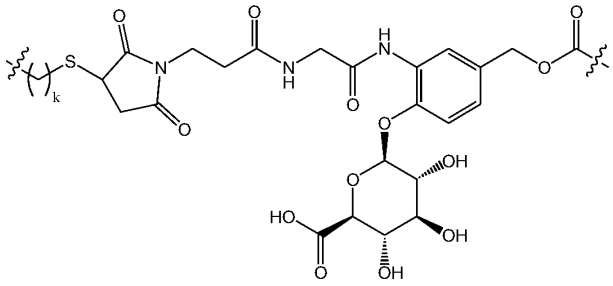


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

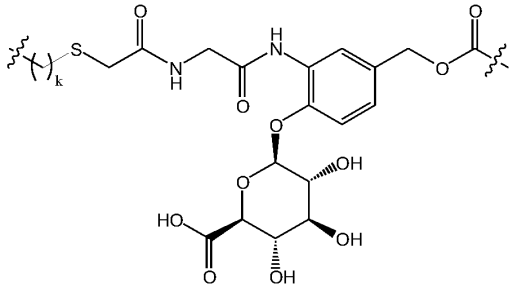
x is 1, 2, 3 or 4.

[00295] In certain embodiments of (**XI**)-(**XI^{B}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



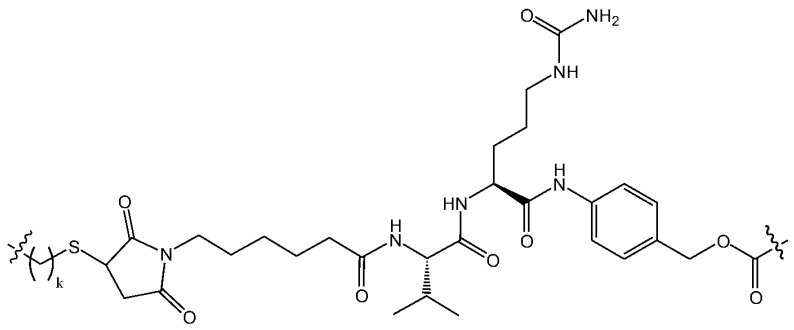
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00296] In certain embodiments of (**XI**)-(**XI^{B}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

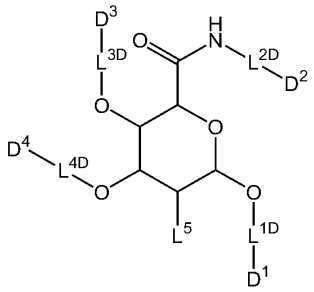
[00297] In certain embodiments of (**XI**)-(**XI^{B}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00298] In certain embodiments of (**XI**)-(**XI^{B}**), if present each of D^1, D^2, D^3 and D^4 is covalently attached respectively to L^{1d}, L^{2d}, L^{3d} and L^{4d} via a carbonate linkage, a carbamate linkage, or an amide linkage.

[00299] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**VIII'**):



(**VIII'**)

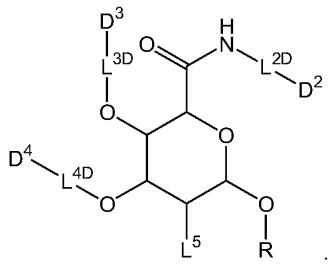
wherein

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group; and

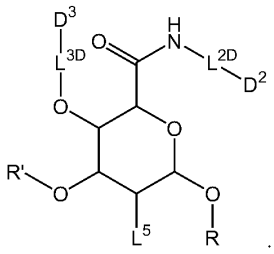
L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group.

[00300] In certain embodiments of (**VIII'**), L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{B}**):



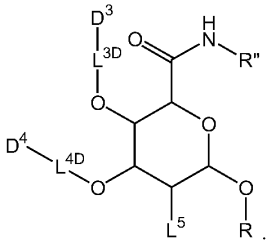
(**VIII'^{B}**)

[00301] In certain embodiments of (**VIII'^{B}**), L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{C}**):



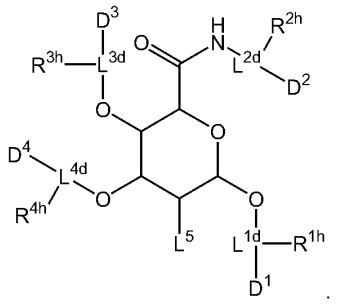
(**VIII'^{C}**)

[00302] In certain embodiments of (**VIII'^{B}**), L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{D}**):



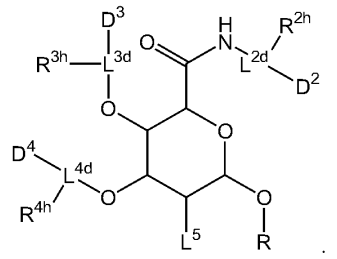
(**VIII'^{D}**)

[00303] In certain embodiments of (**VIII'**), each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IX'**):



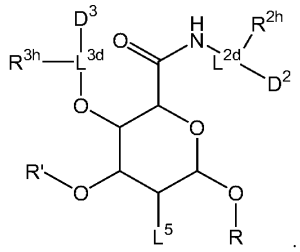
(IX')

[00304] In certain embodiments of **(IX')**, L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula **(IX'^{A})**:



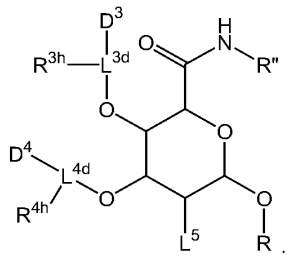
(IX'^{A})

[00305] In certain embodiments of **(IX'^{A})**, L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula **(IX'^{B})**:



(IX'^{B})

[00306] In certain embodiments of **(IX'^{B})**, L^{2D}-D^2 is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula **(IX'^{C})**:

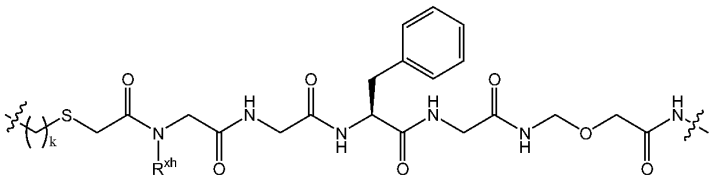


(**IX'^{C}**)

[00307] In certain embodiments of (**IX'**)-(**IX'^{C}**), each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00308] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

[00309] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} if present independently is:



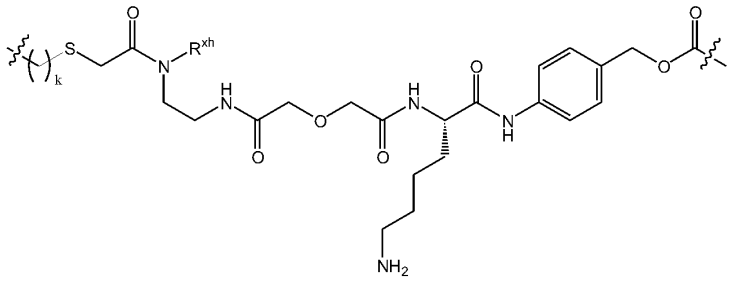
wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

x is 1, 2, 3 or 4.

[00310] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

[00311] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

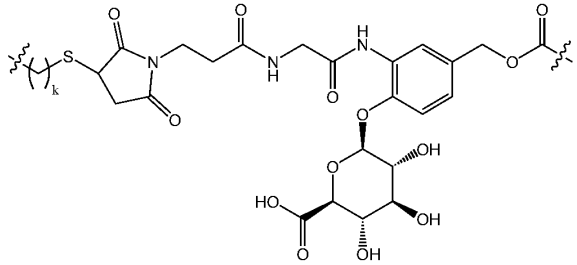


wherein

k is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4); and

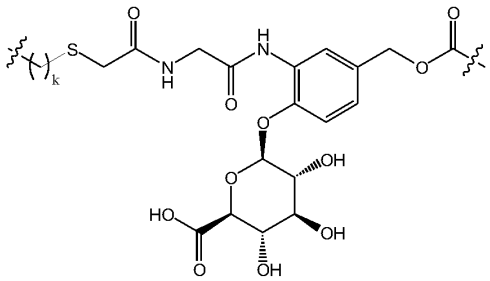
x is 1, 2, 3 or 4.

[00312] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



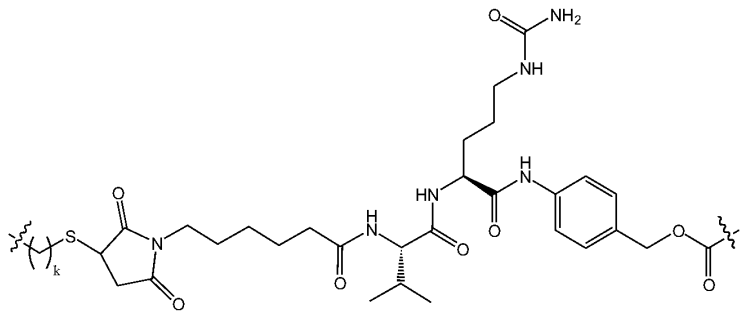
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00313] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



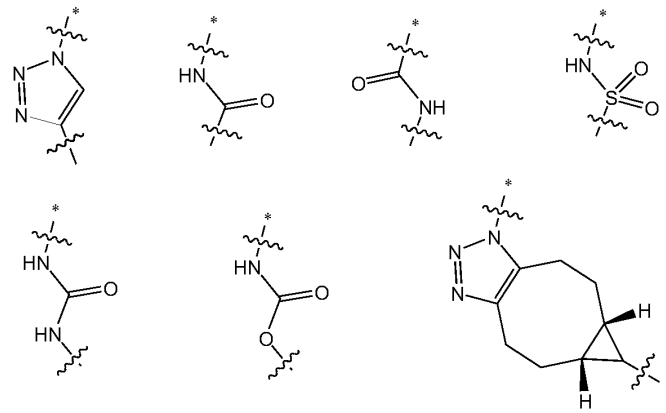
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00314] In certain embodiments of (**IX'**)-(**IX'^{C}**), one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



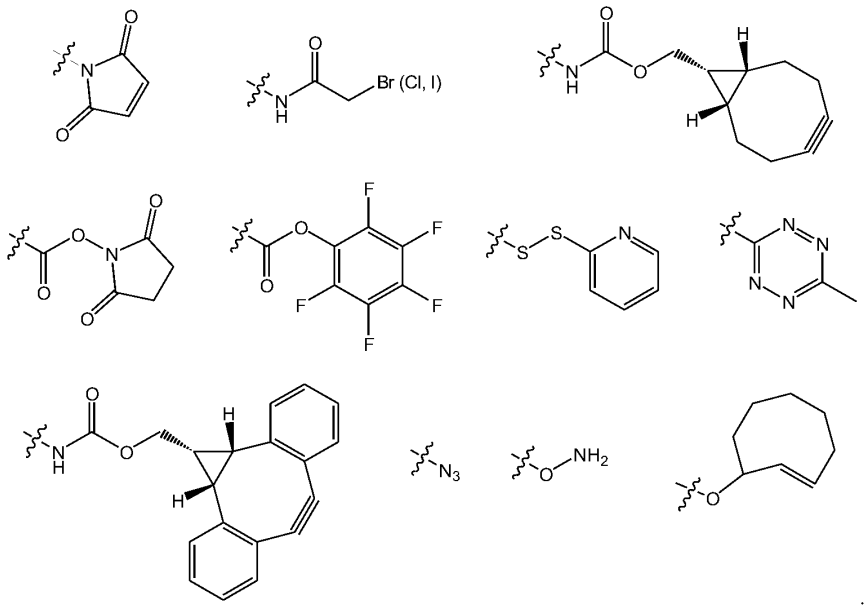
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00315] In certain embodiments of (**VIII**)-(**XI^{B}**) and (**VIII'**)-(**IX'^{C}**), L^5 comprises a group selected from:



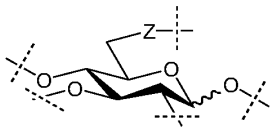
wherein * represents the attachment point to the central sugar ring of Formula (**I**).

[00316] In certain embodiments of (**VIII**)-(**XI^{B}**) and (**VIII'**)-(**IX'^{C}**), L^5 comprises a group selected from:



[00317] In certain embodiments of (**VIII**)-(**XI^{B}**) and (**VIII'**)-(**IX'^{C}**), L^5 comprises 1-4 hydrophilic groups.

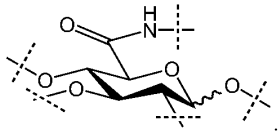
[00318] In certain embodiments of (**VIII**)-(**XI^{B}**), the compound has the stereochemistry shown in Formula (**V**):



(**V**)

wherein Z is O or N.

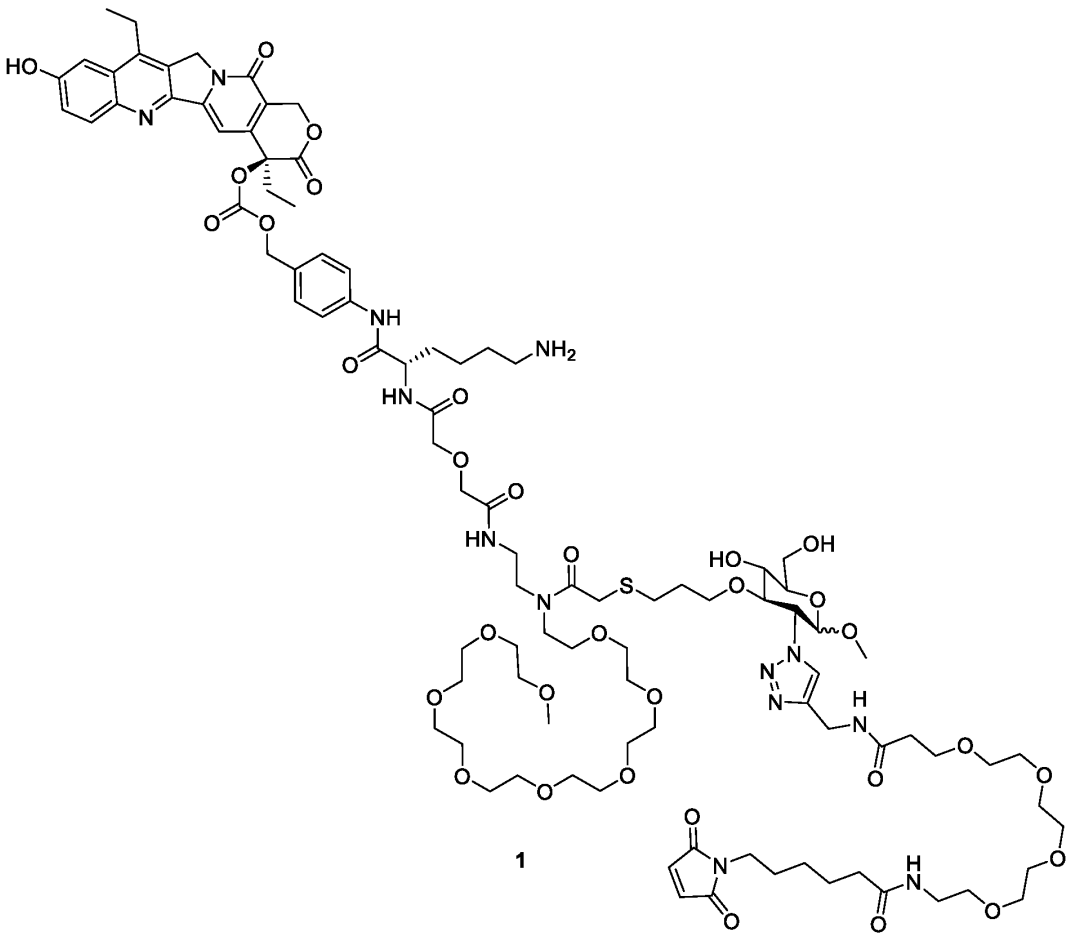
[00319] In certain embodiments of (**VIII'**)-(**IX'^{C}**), the compound has the stereochemistry shown in Formula (**V'**):



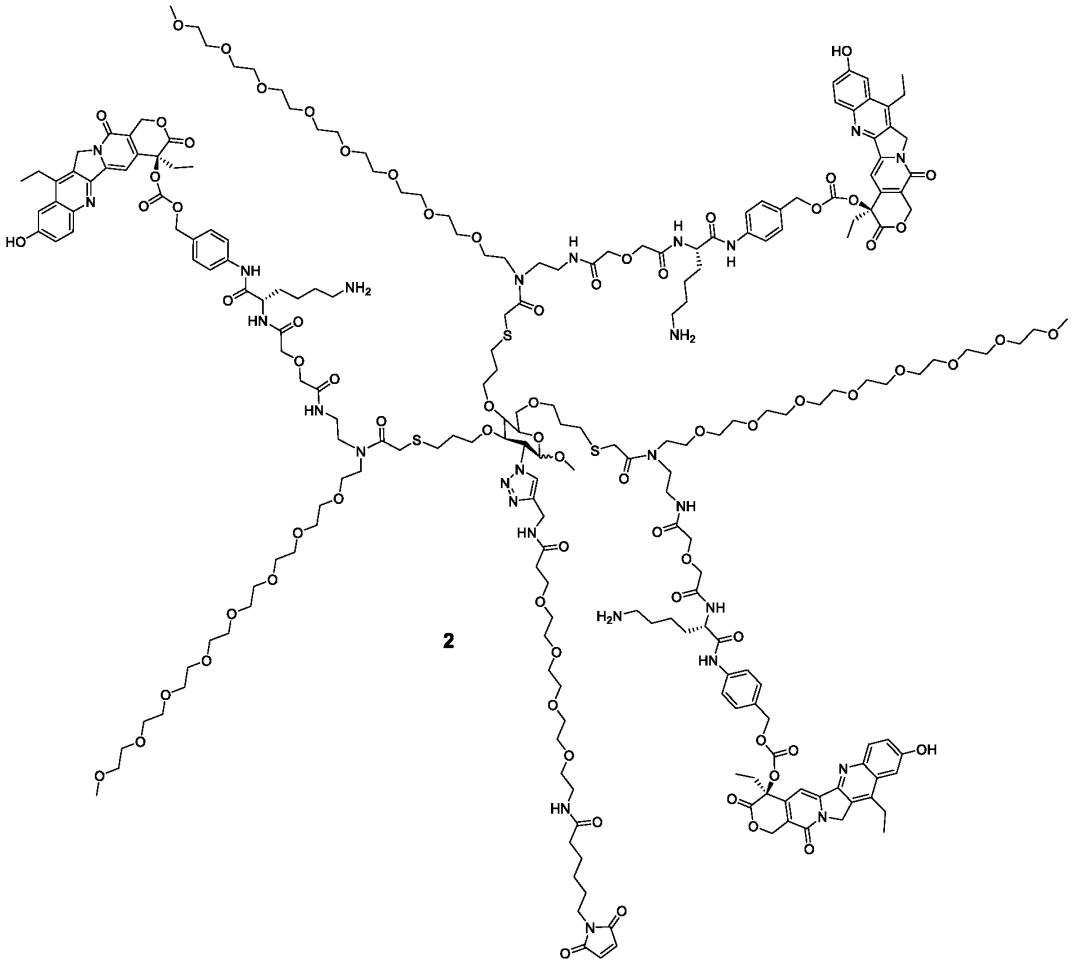
(**V'**)

[00320] Non-limiting examples of linker-drug conjugates include:

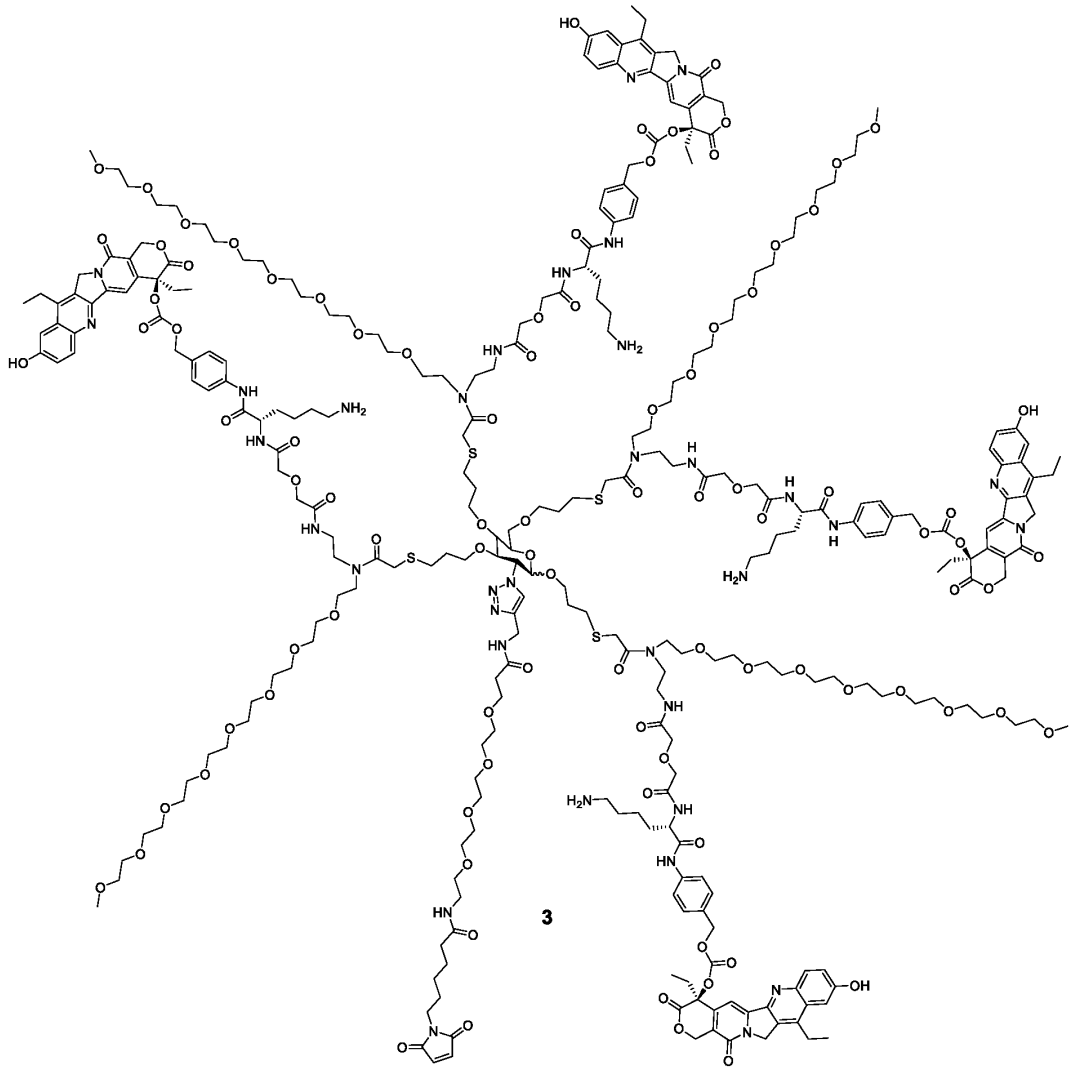
Table C



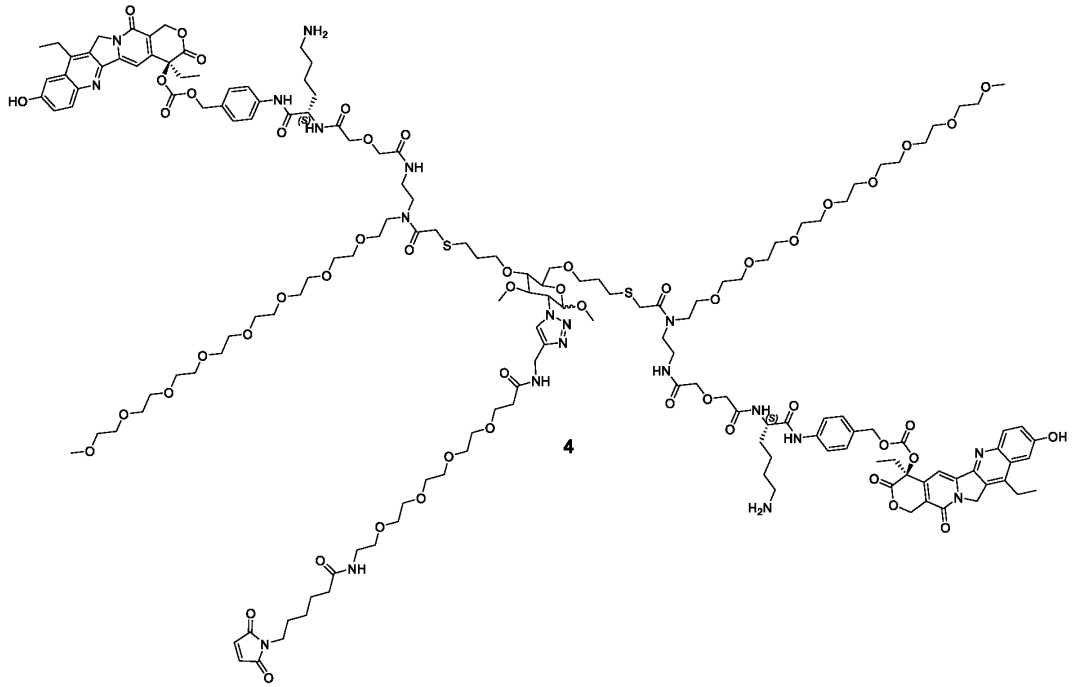
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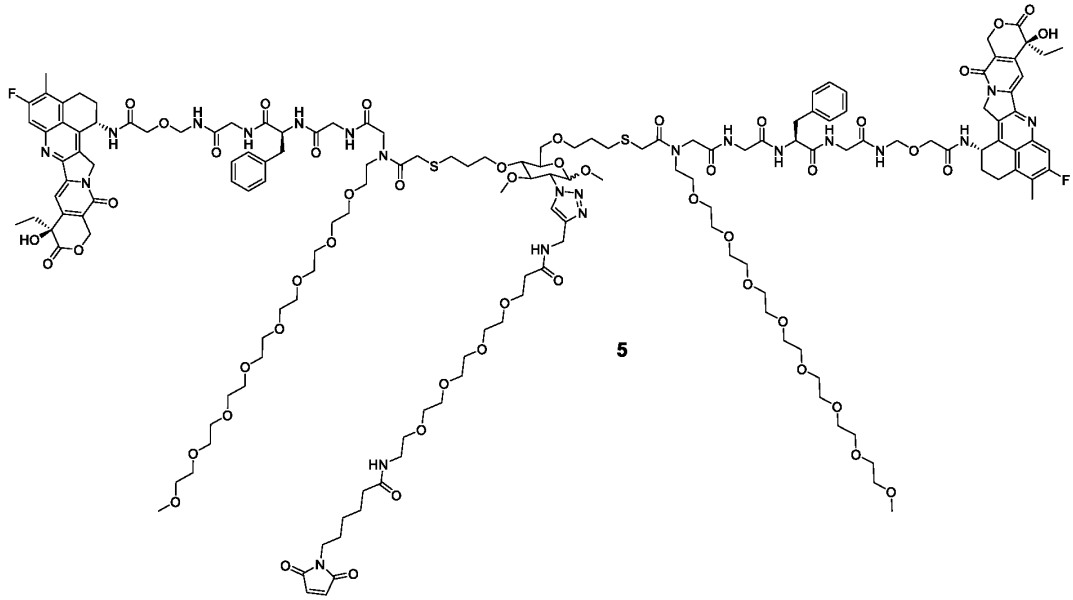
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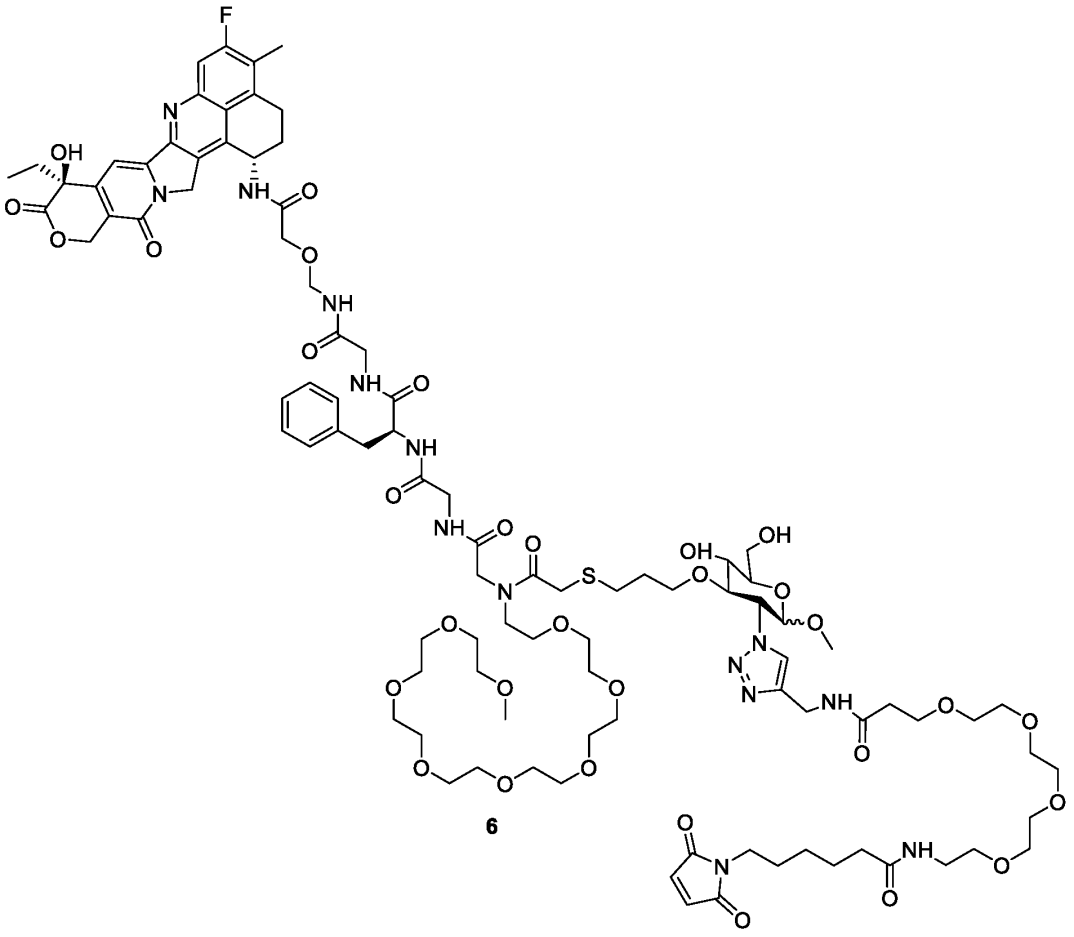
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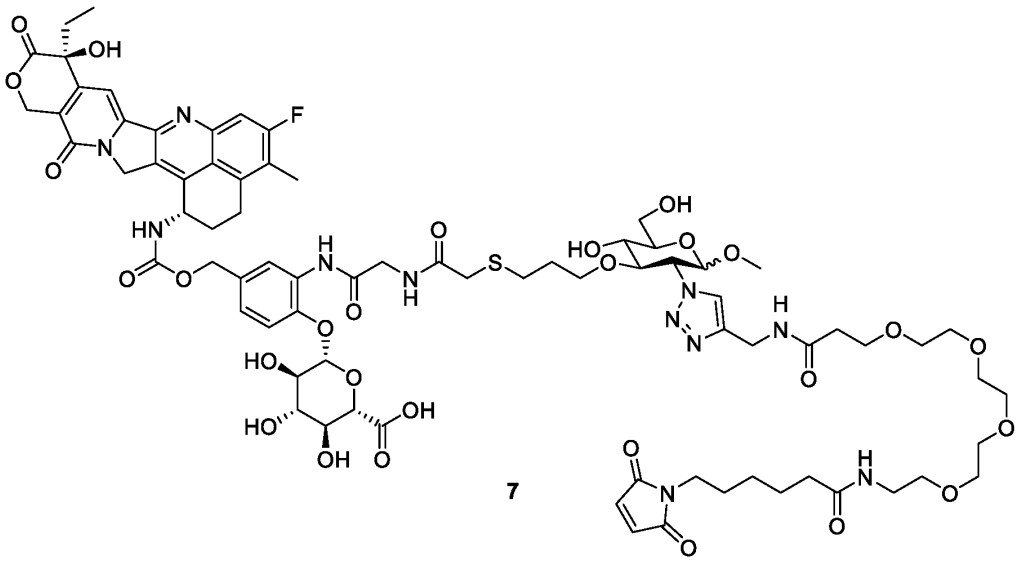
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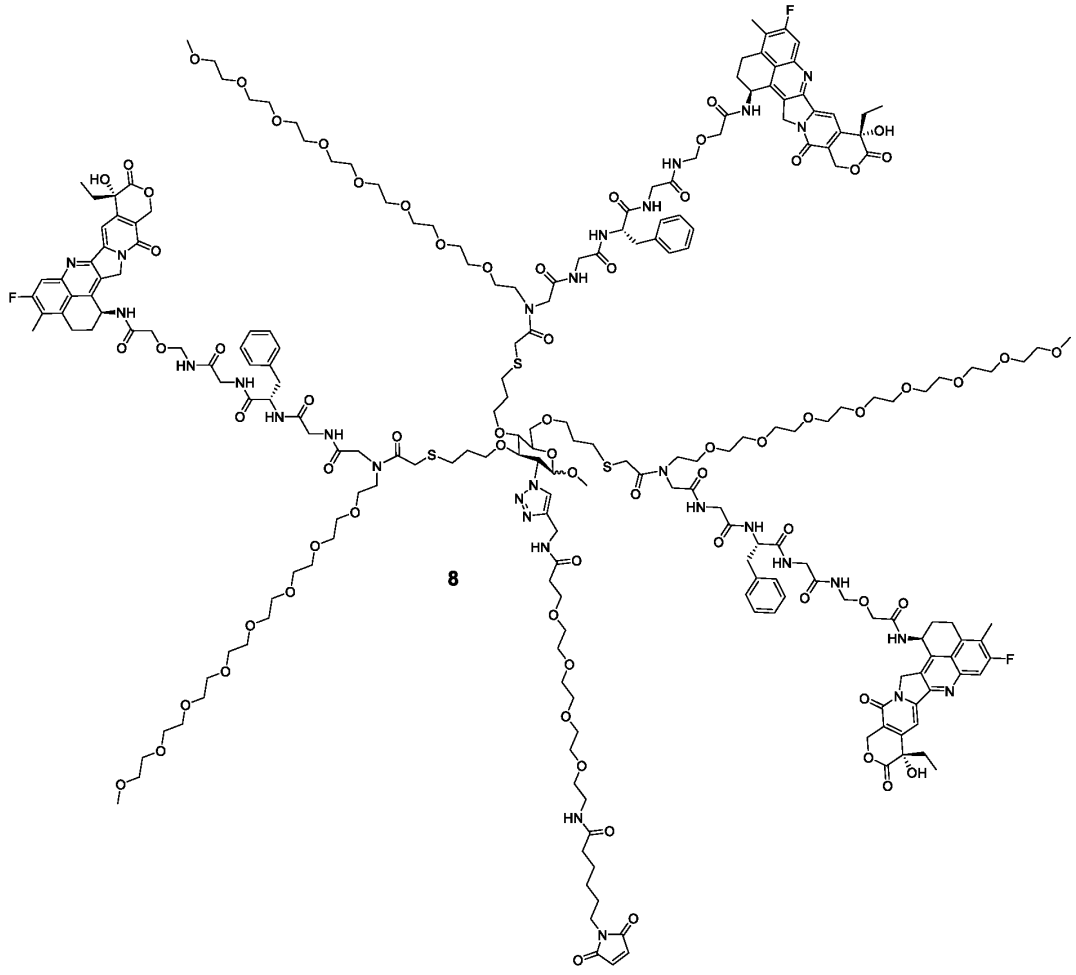
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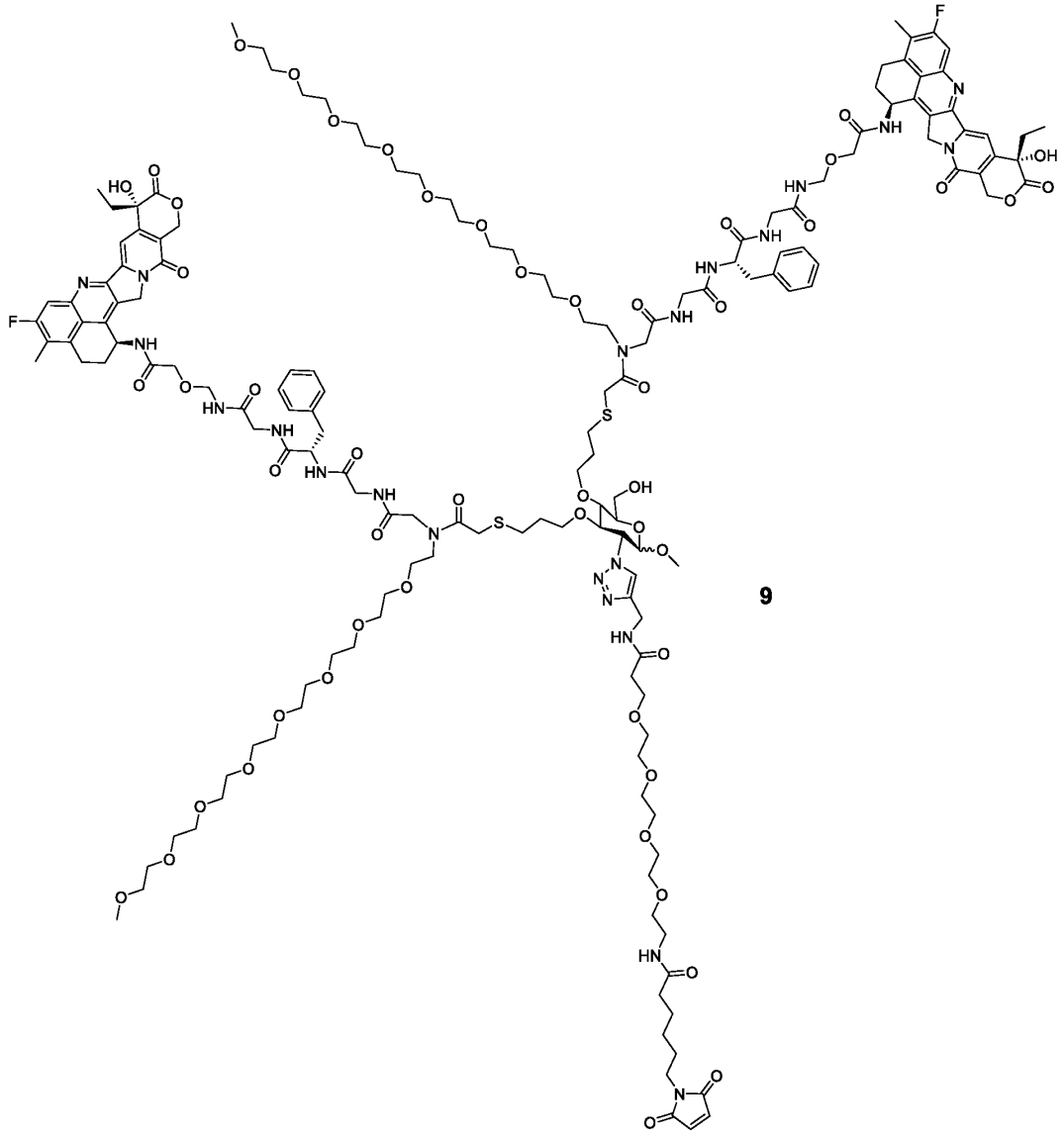
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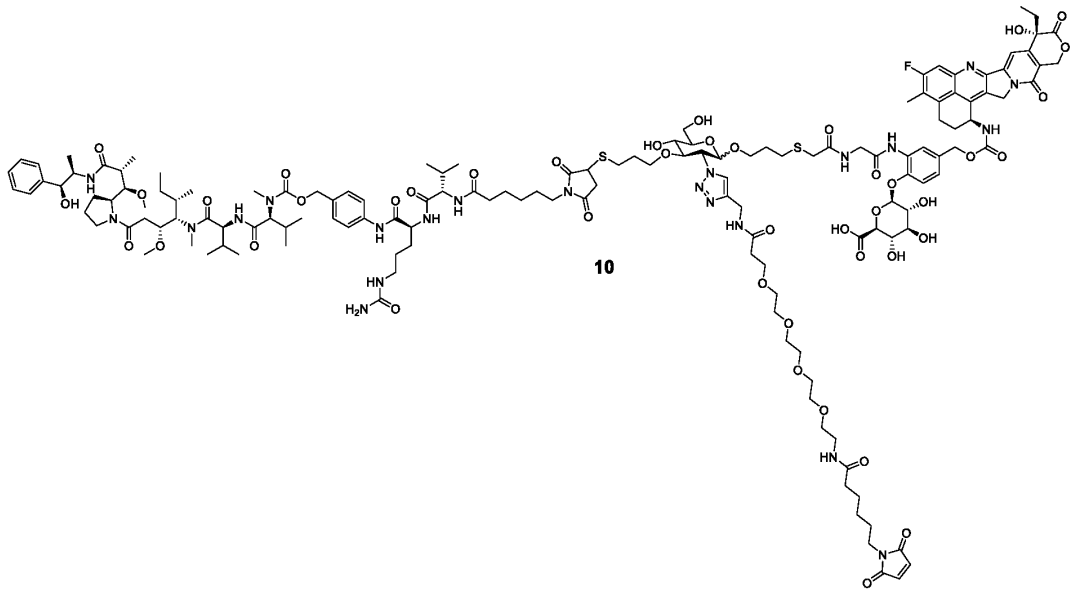
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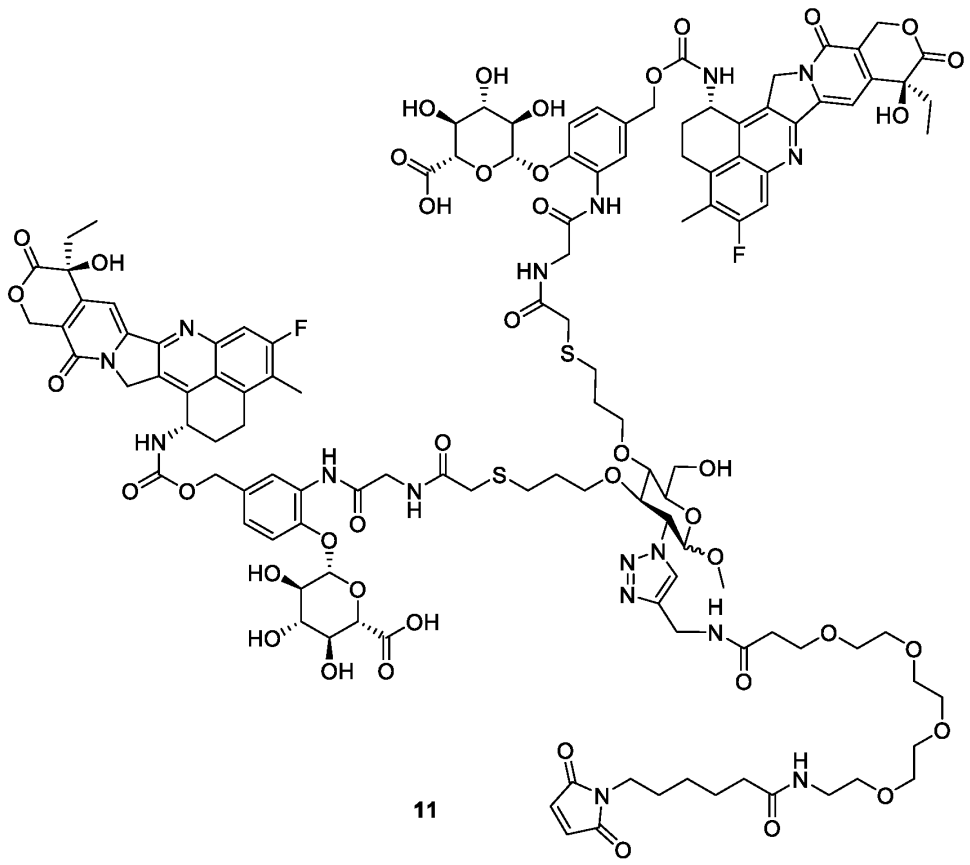
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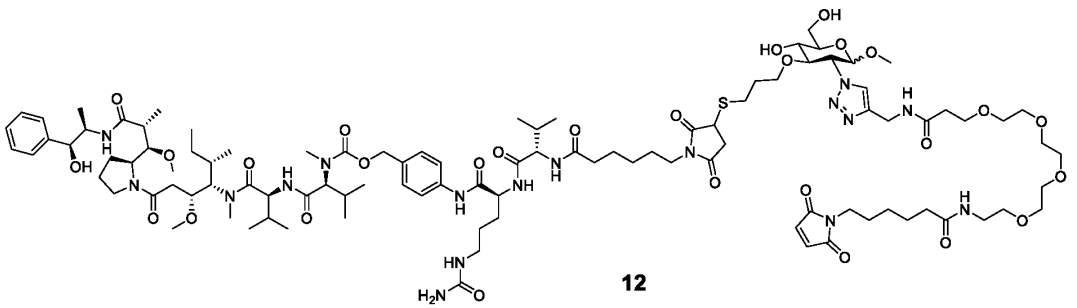
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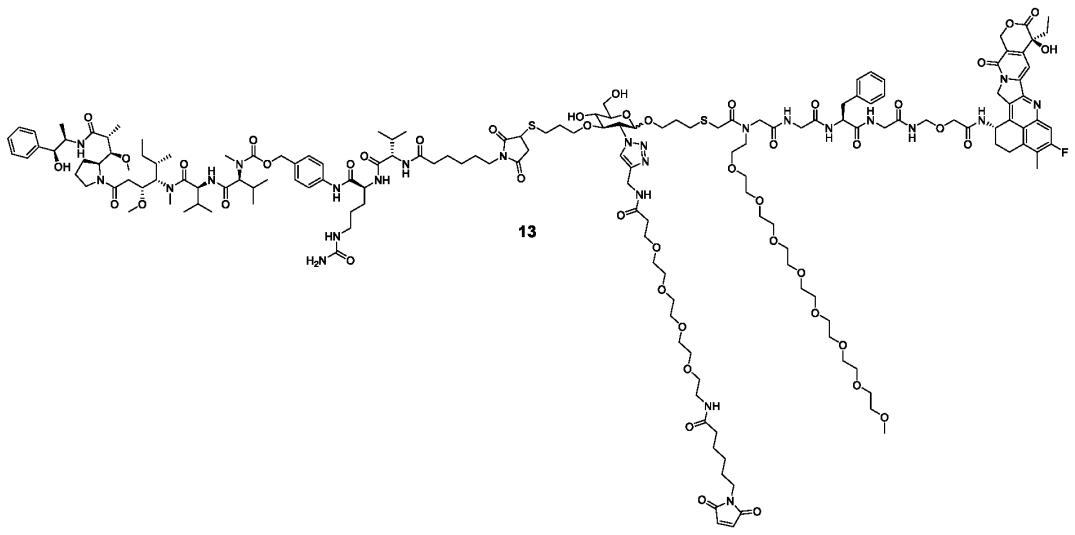
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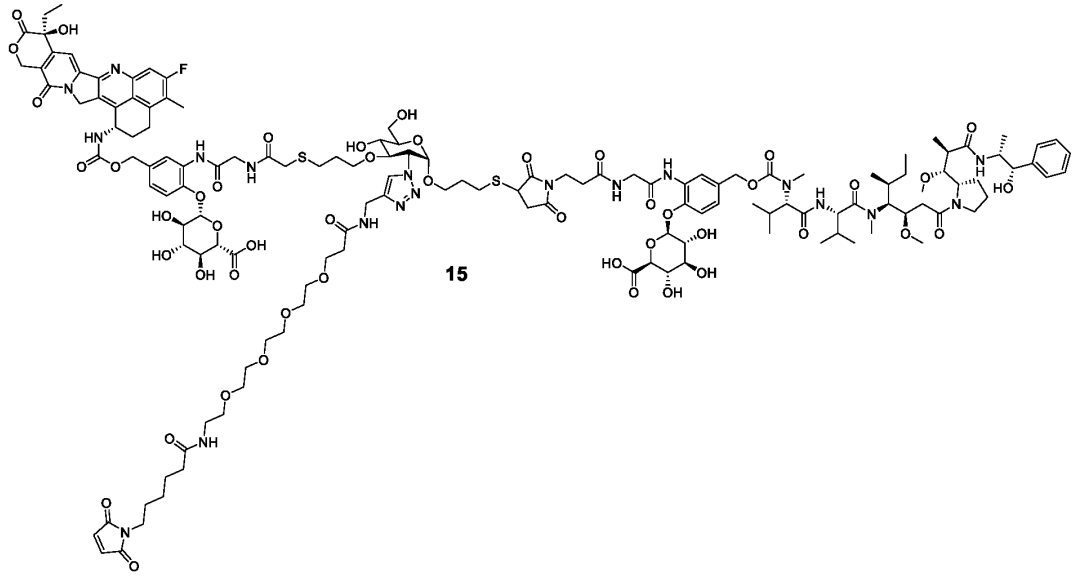
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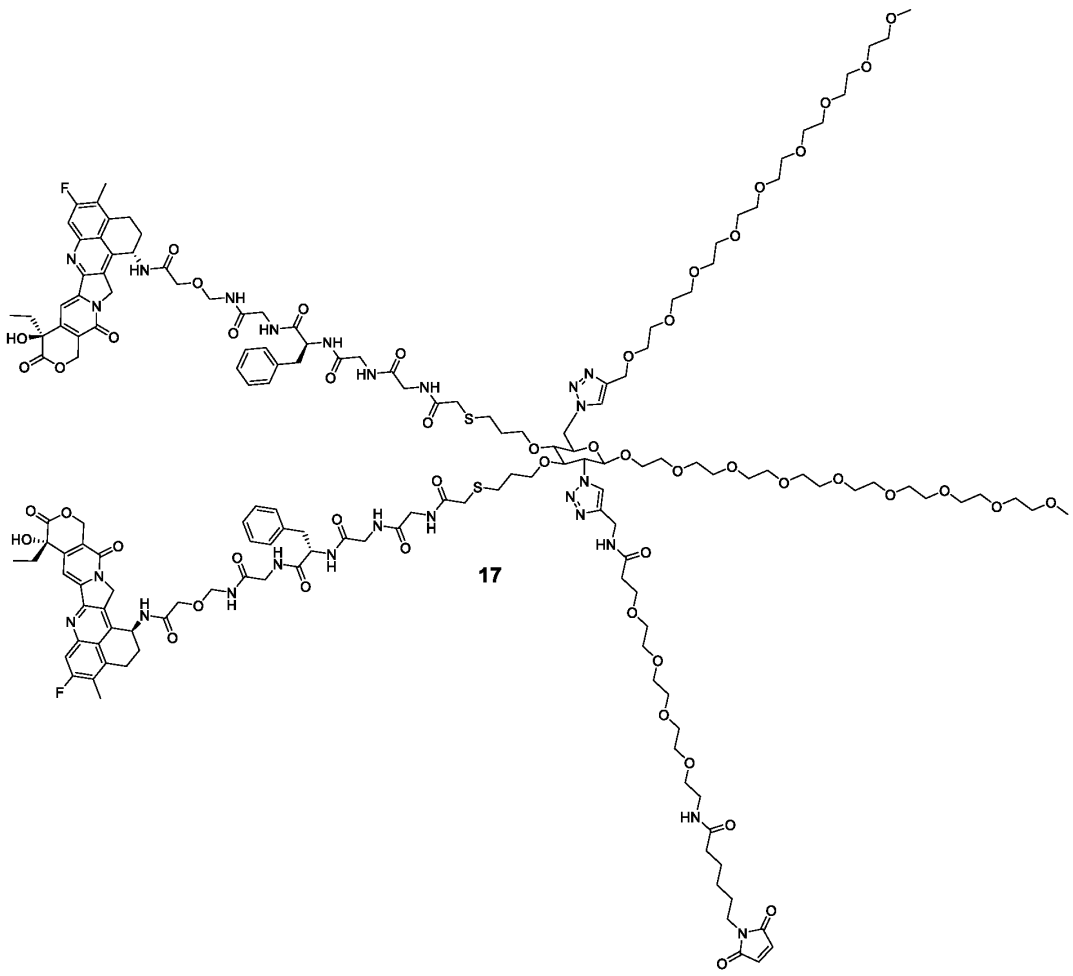
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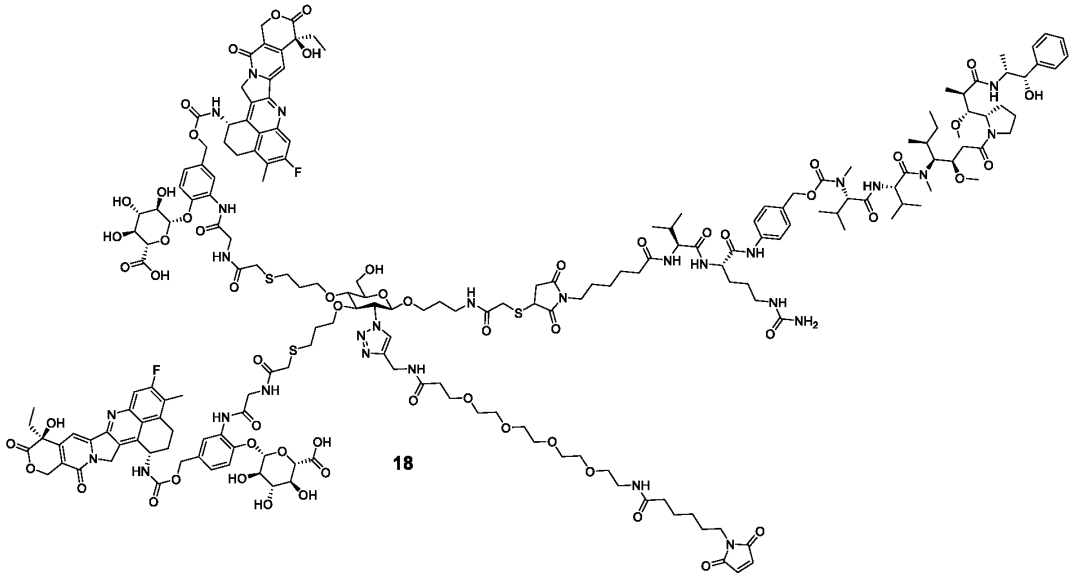
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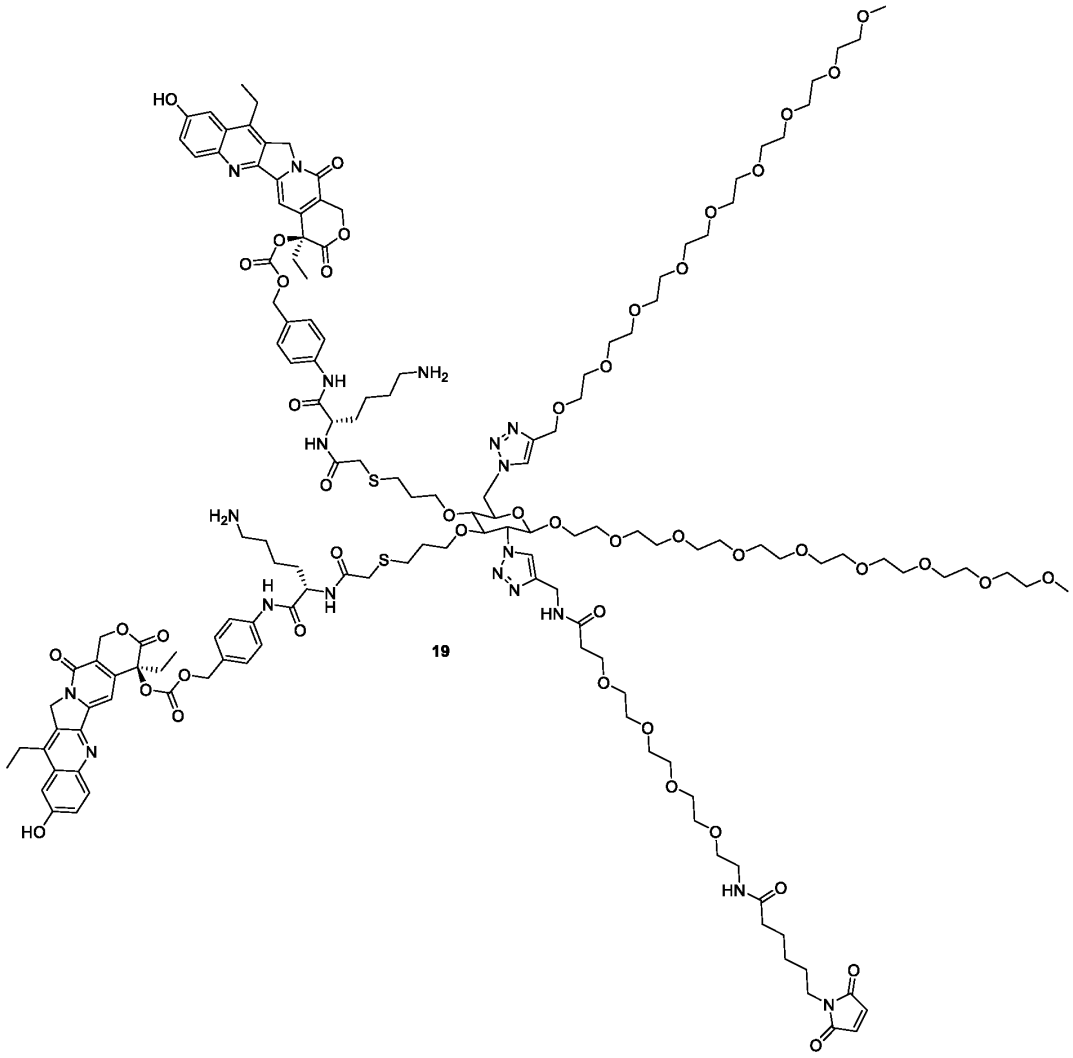
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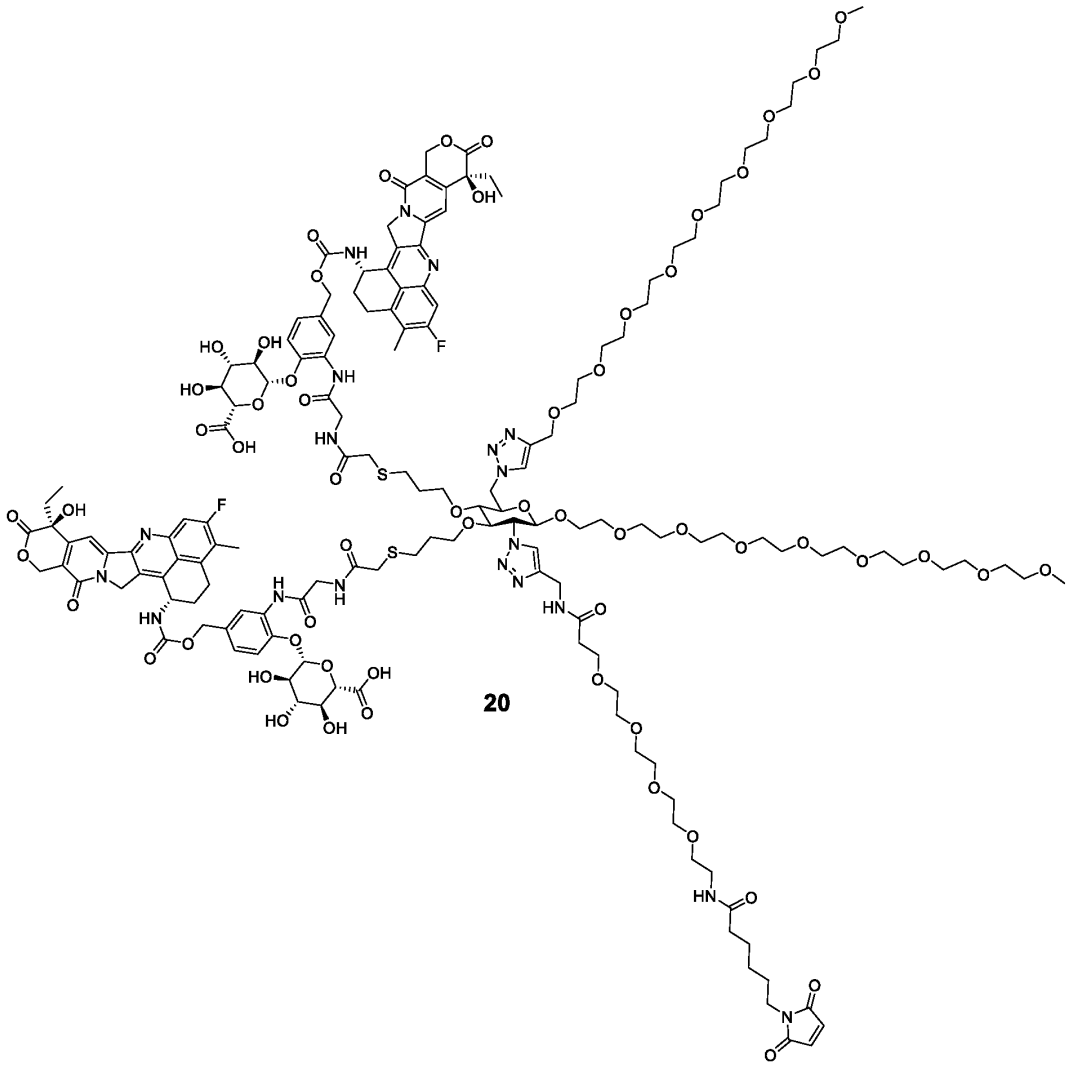
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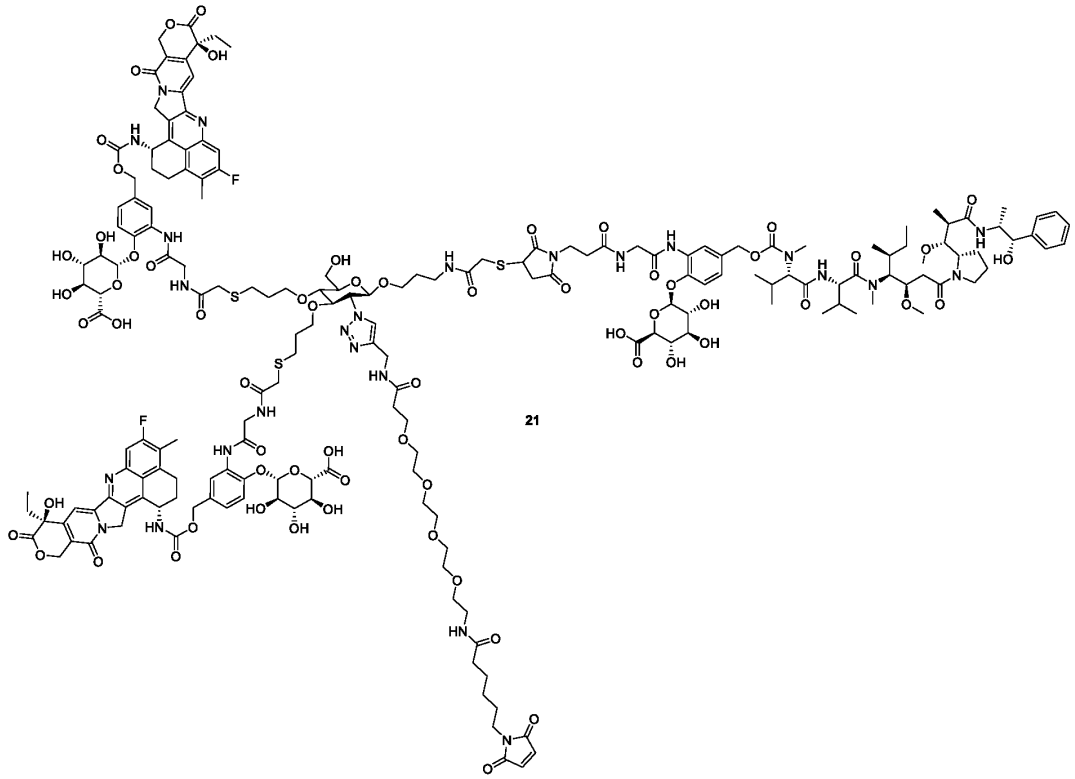
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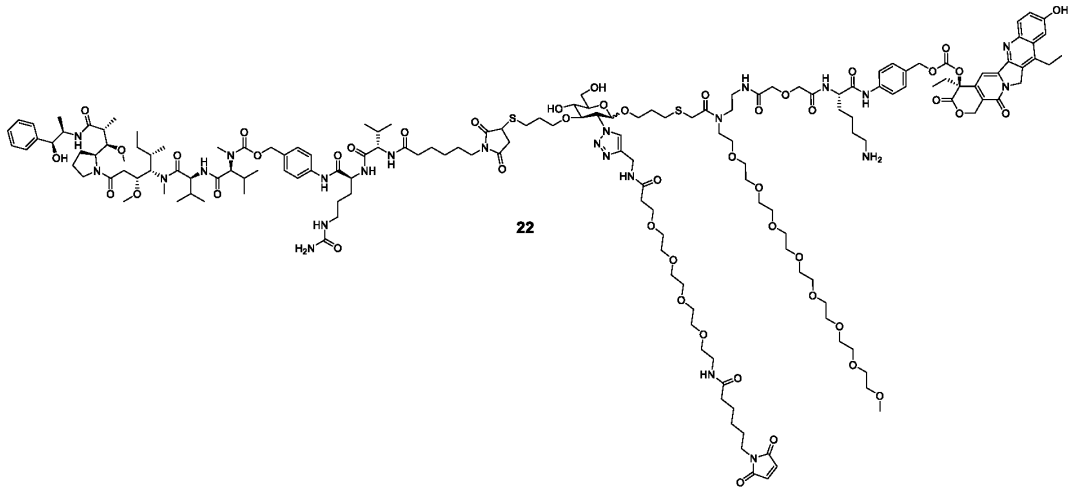
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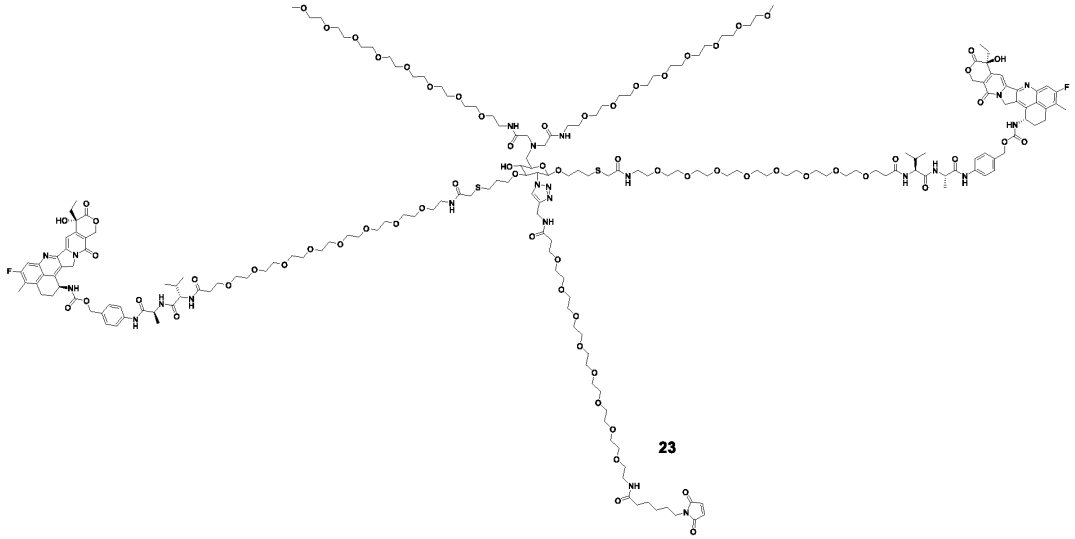
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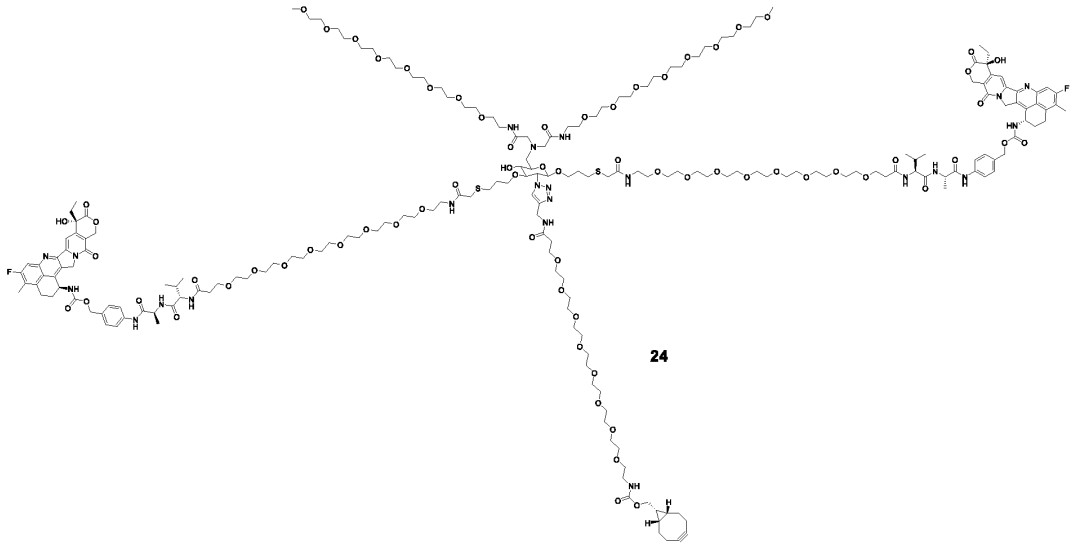
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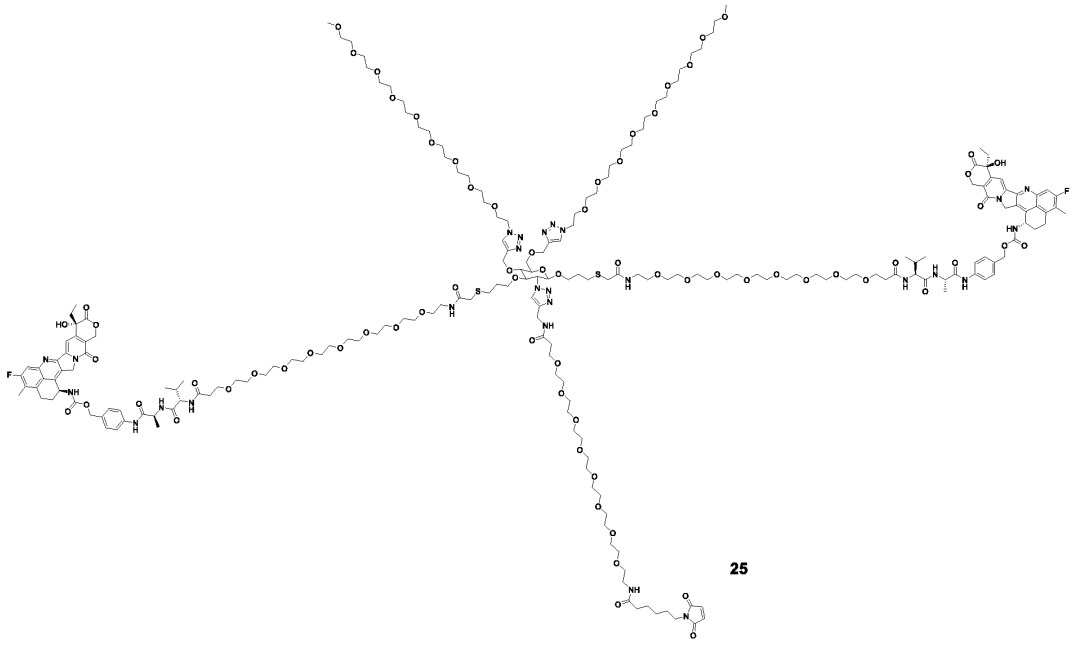
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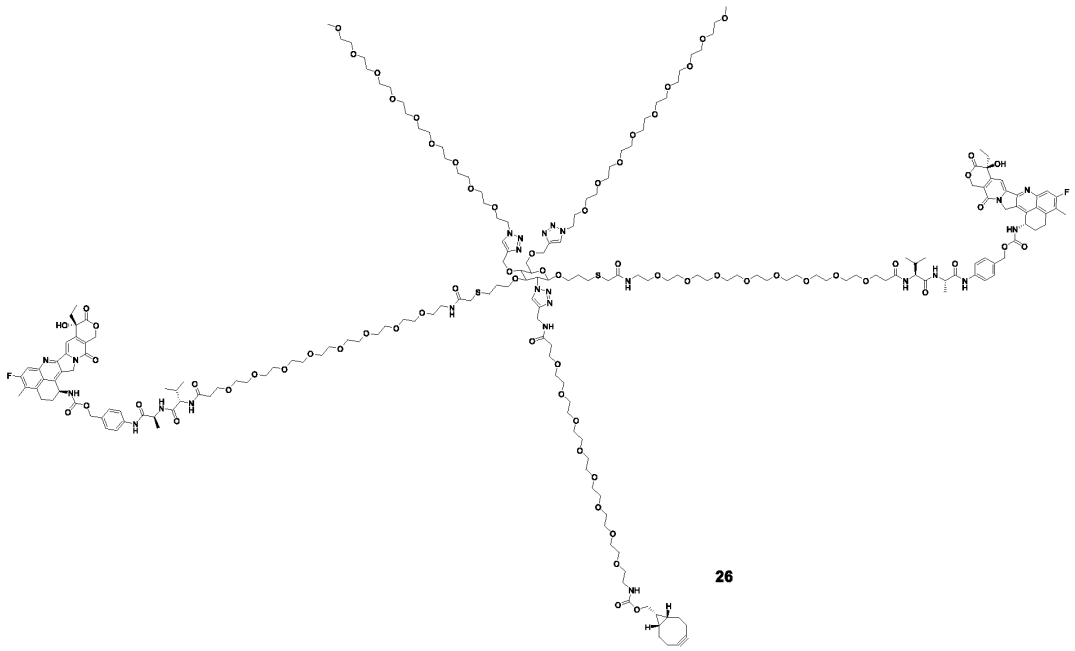
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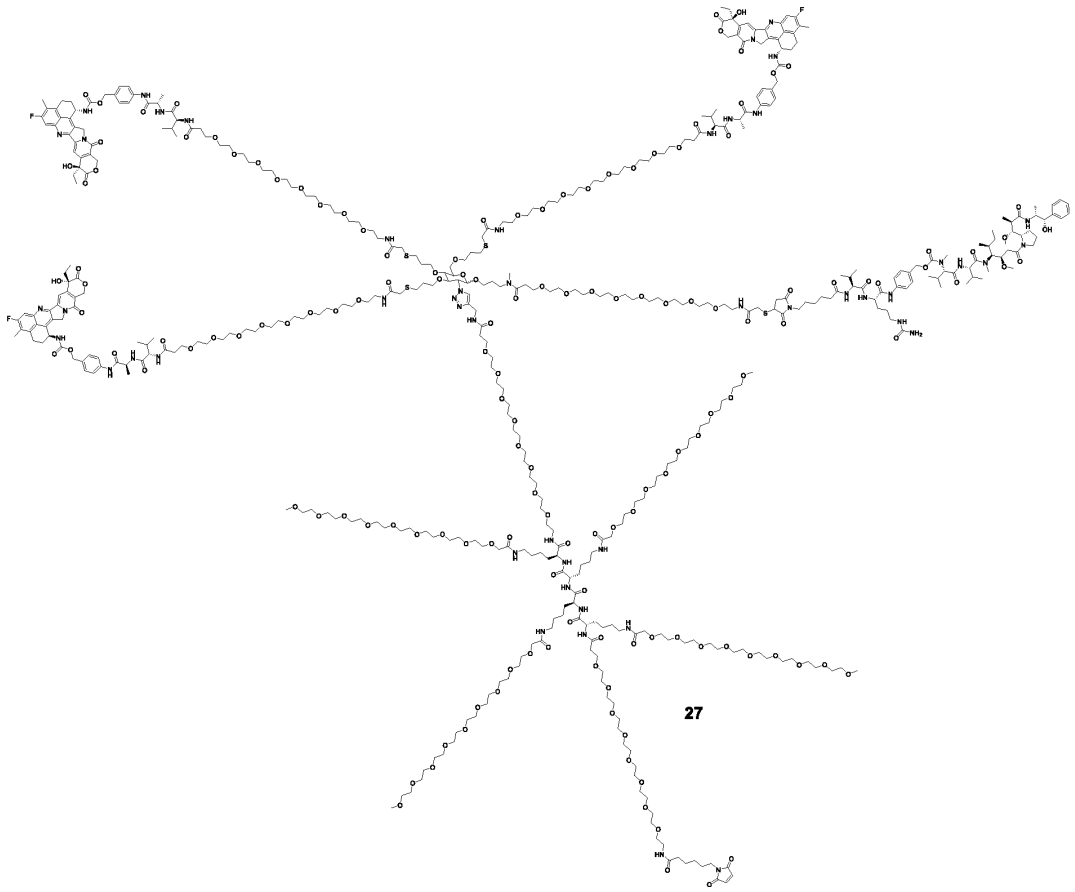
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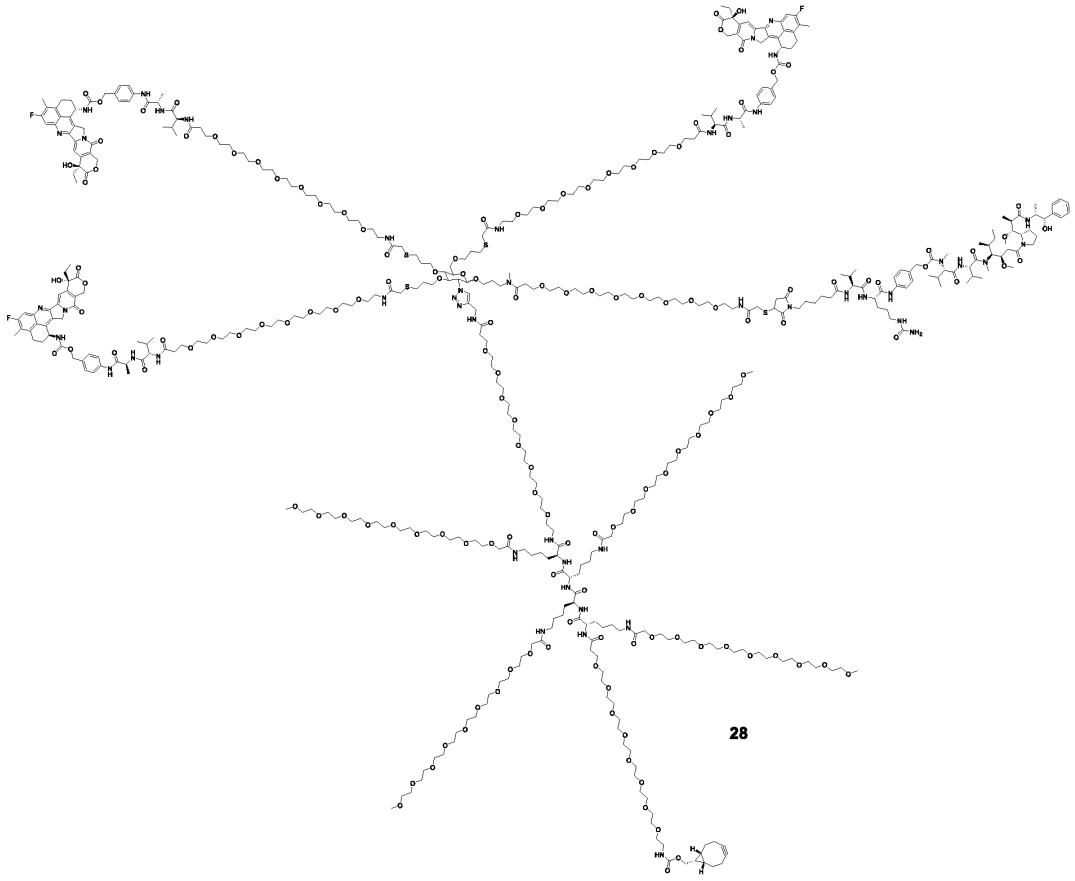
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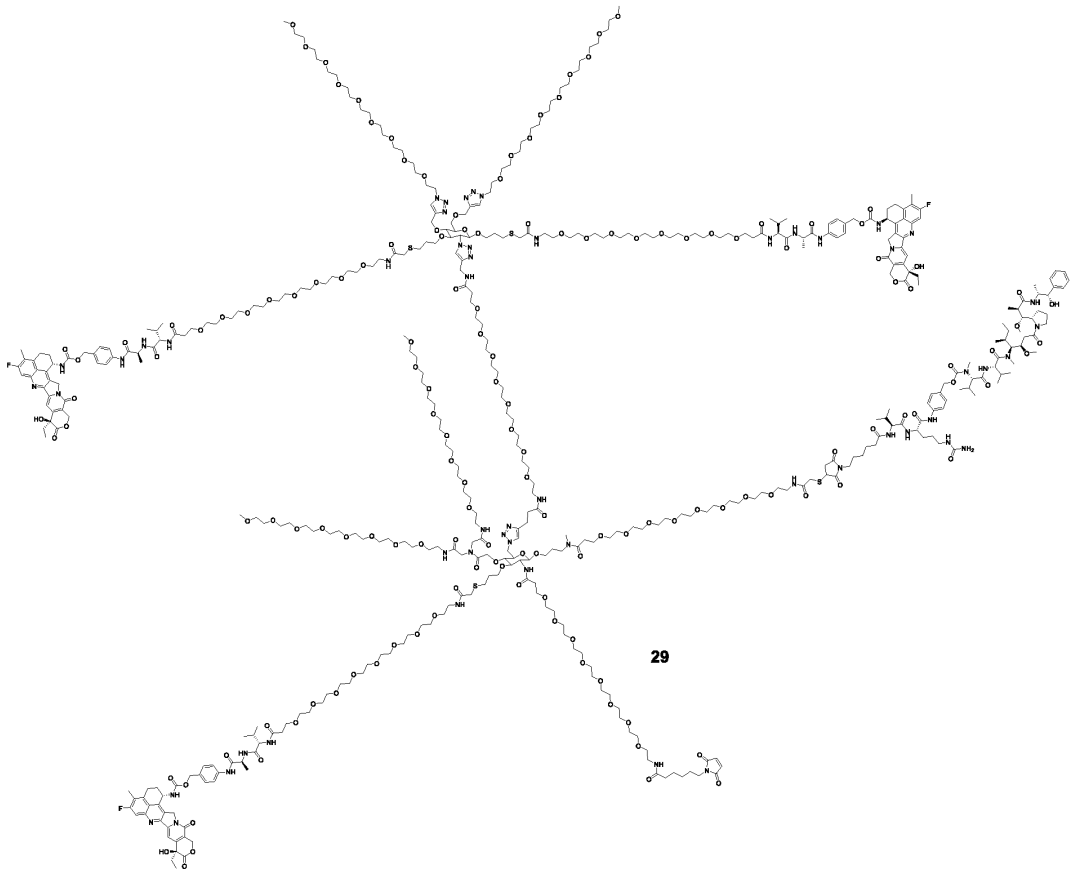
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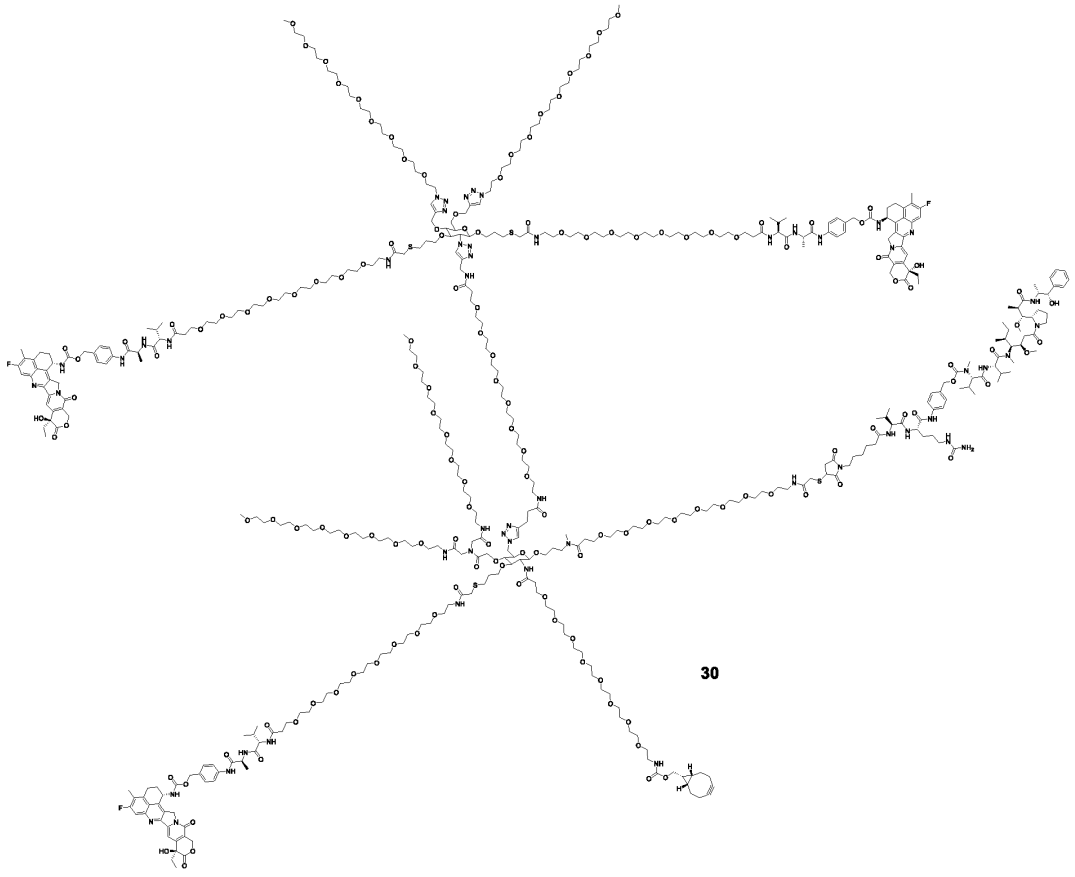
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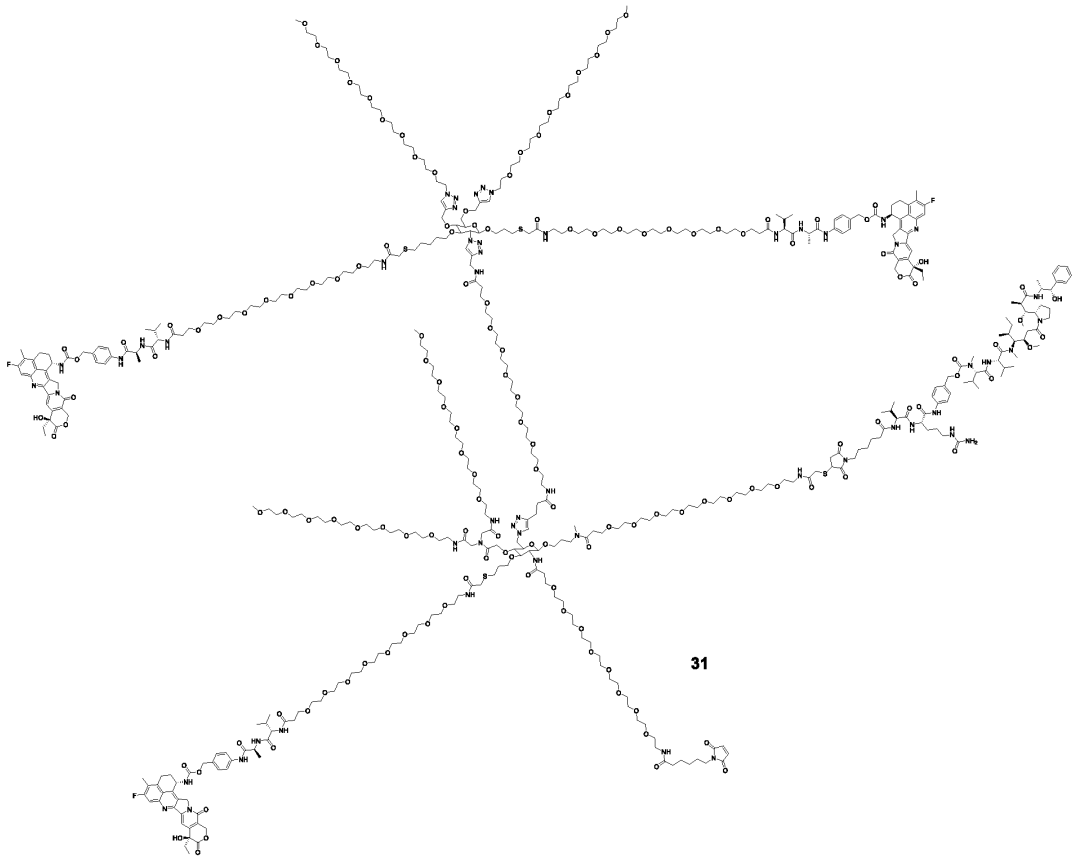
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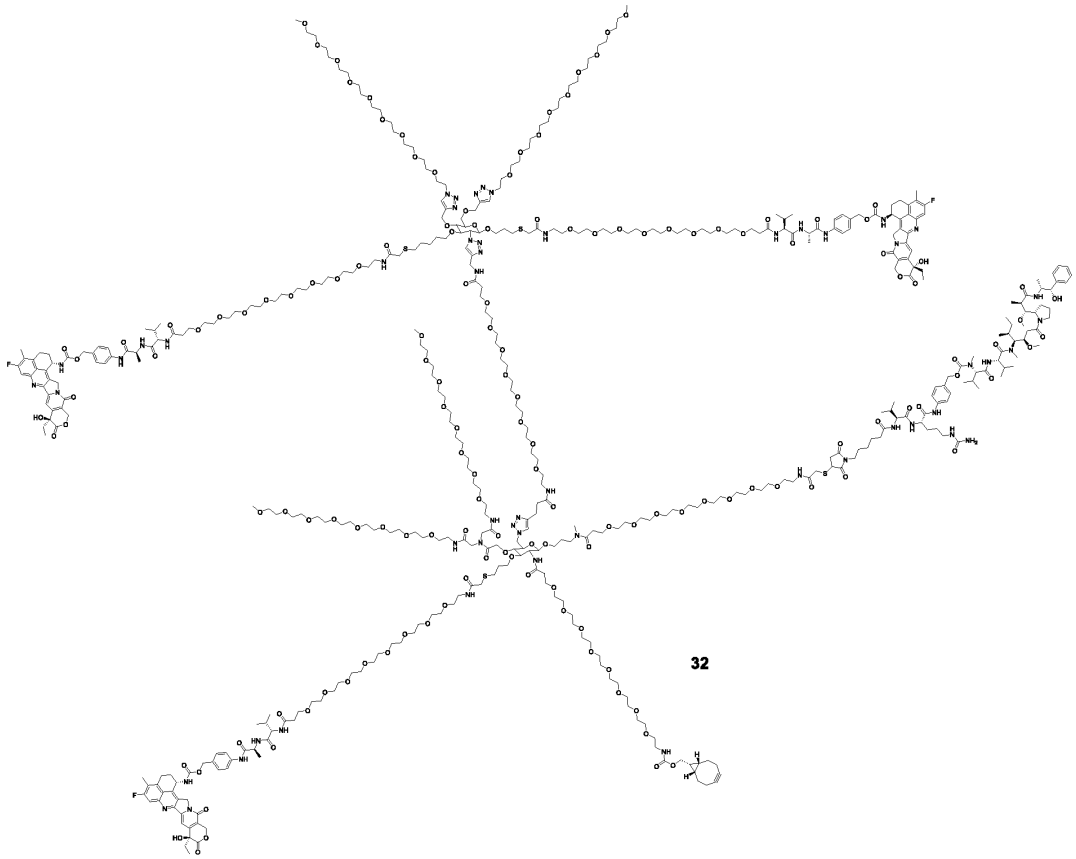
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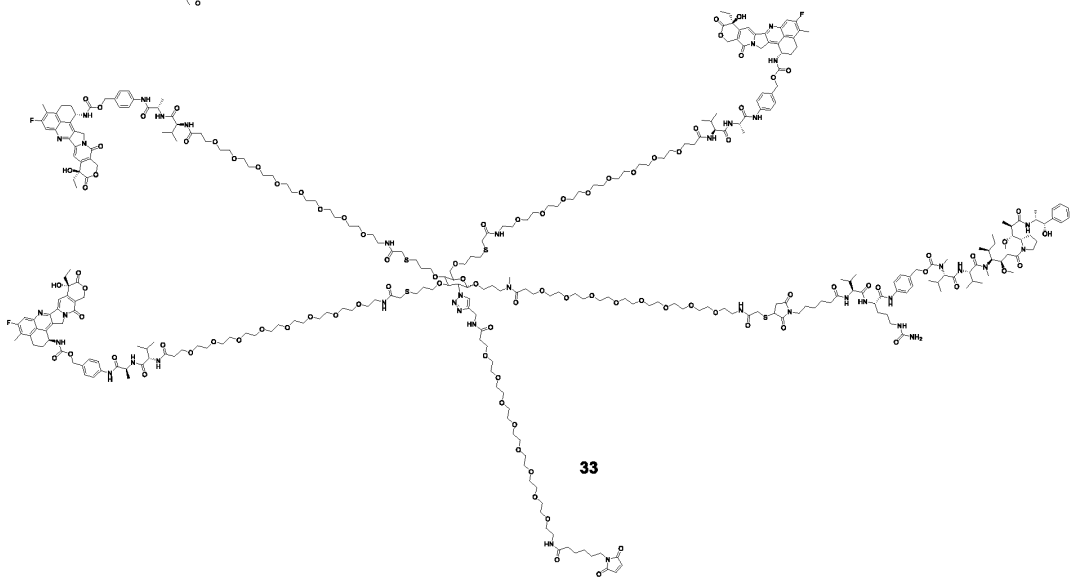
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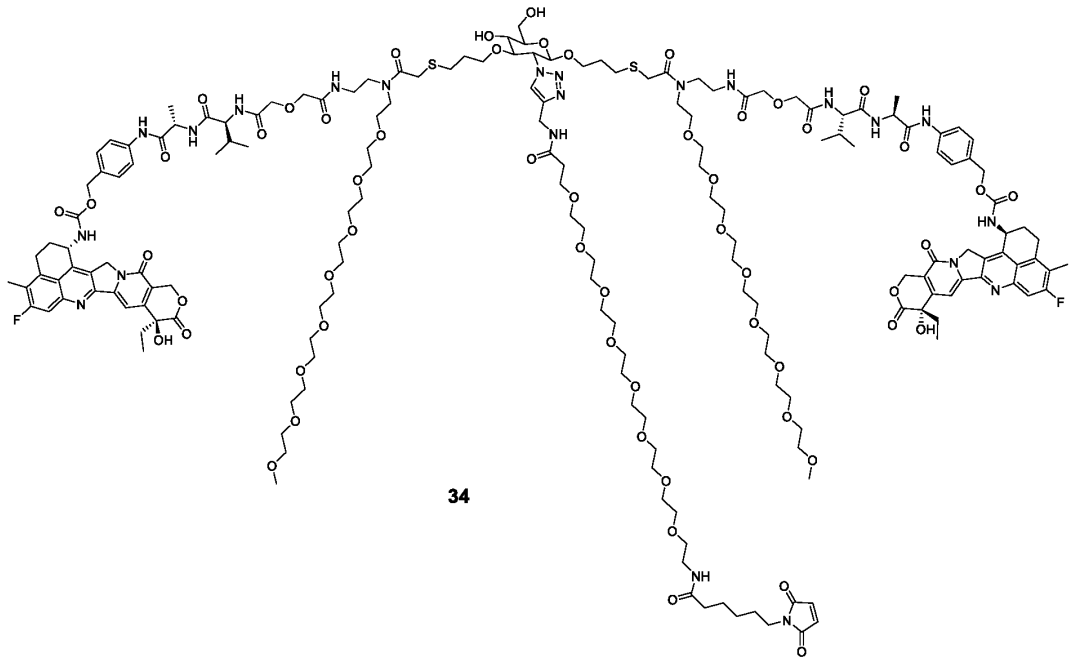
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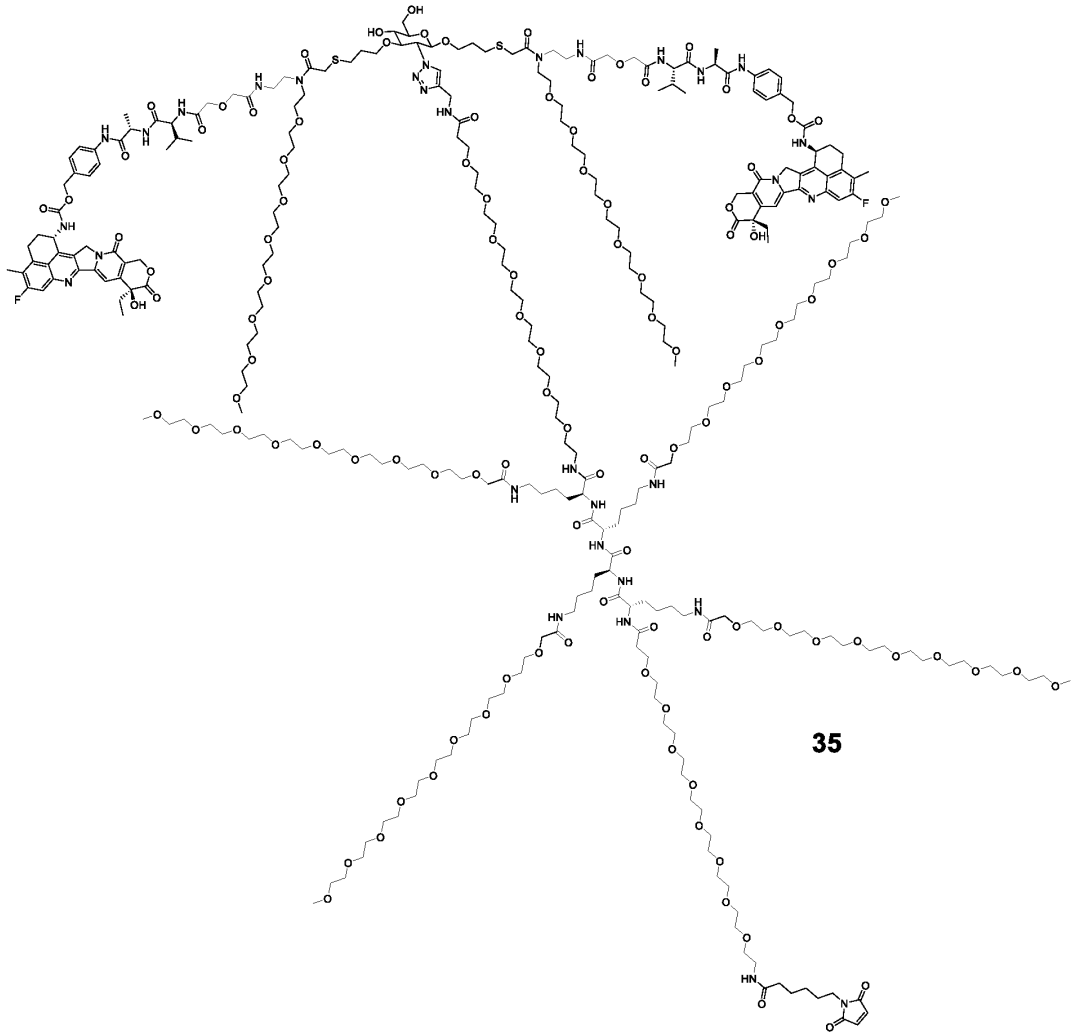
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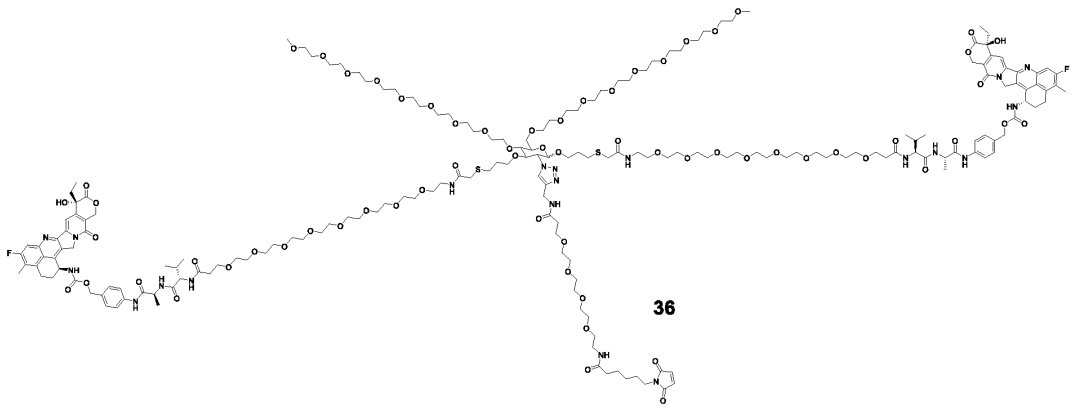
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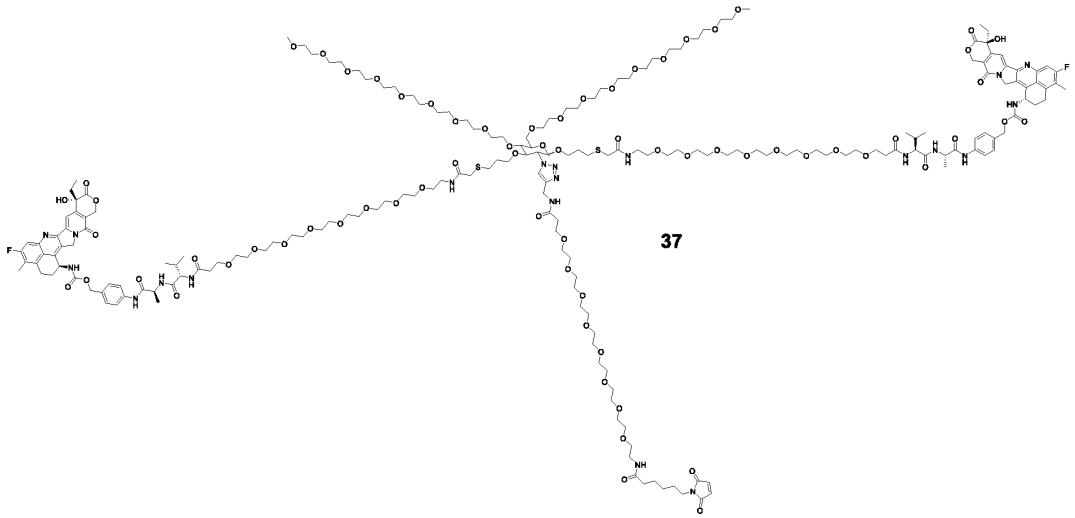
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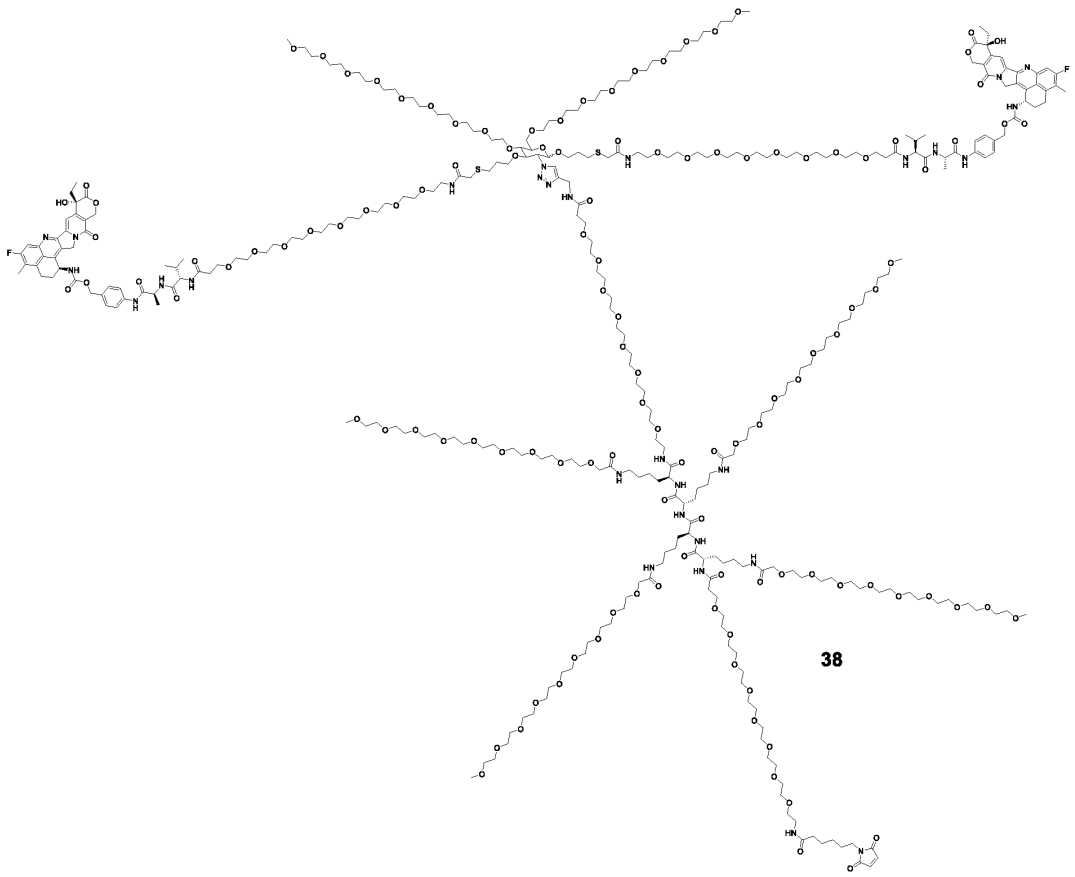
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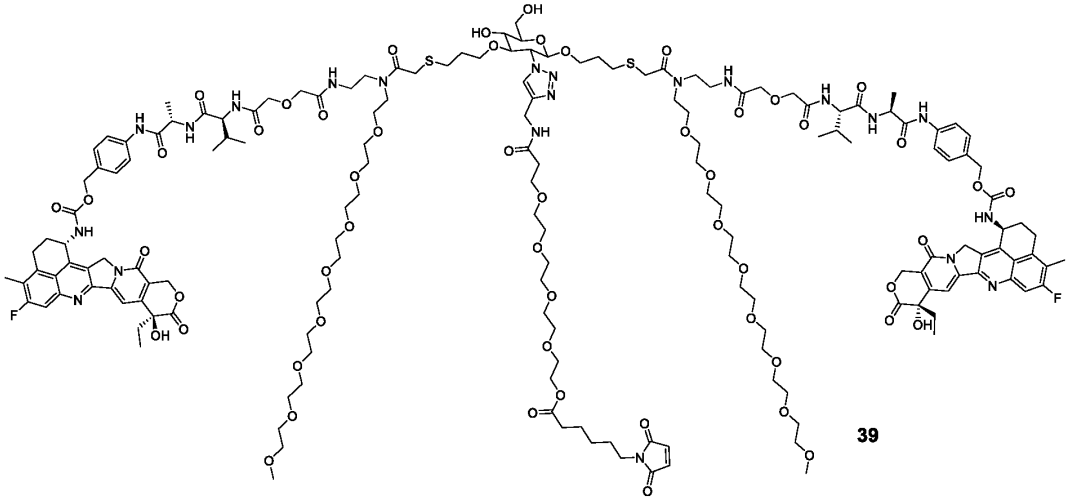
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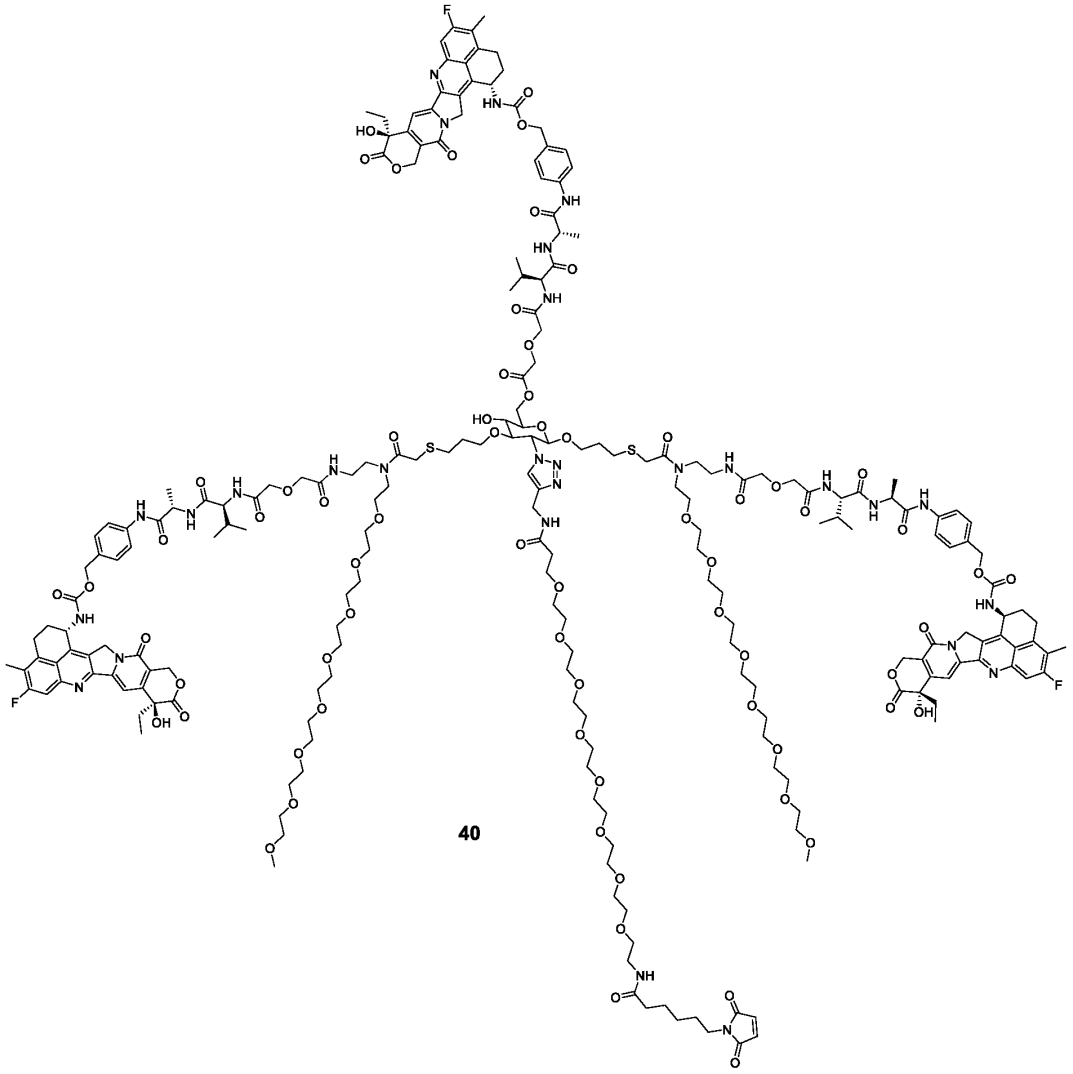
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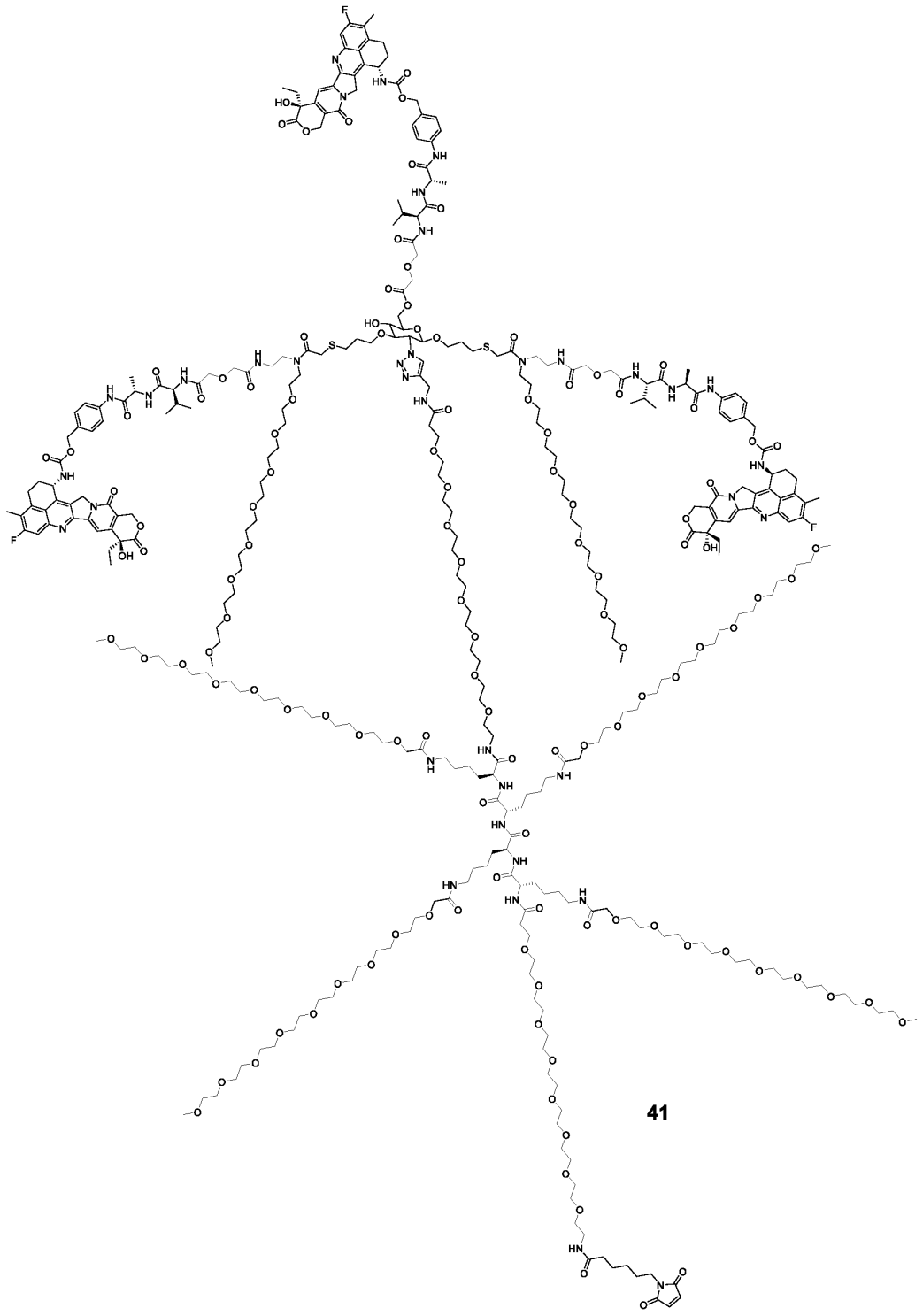
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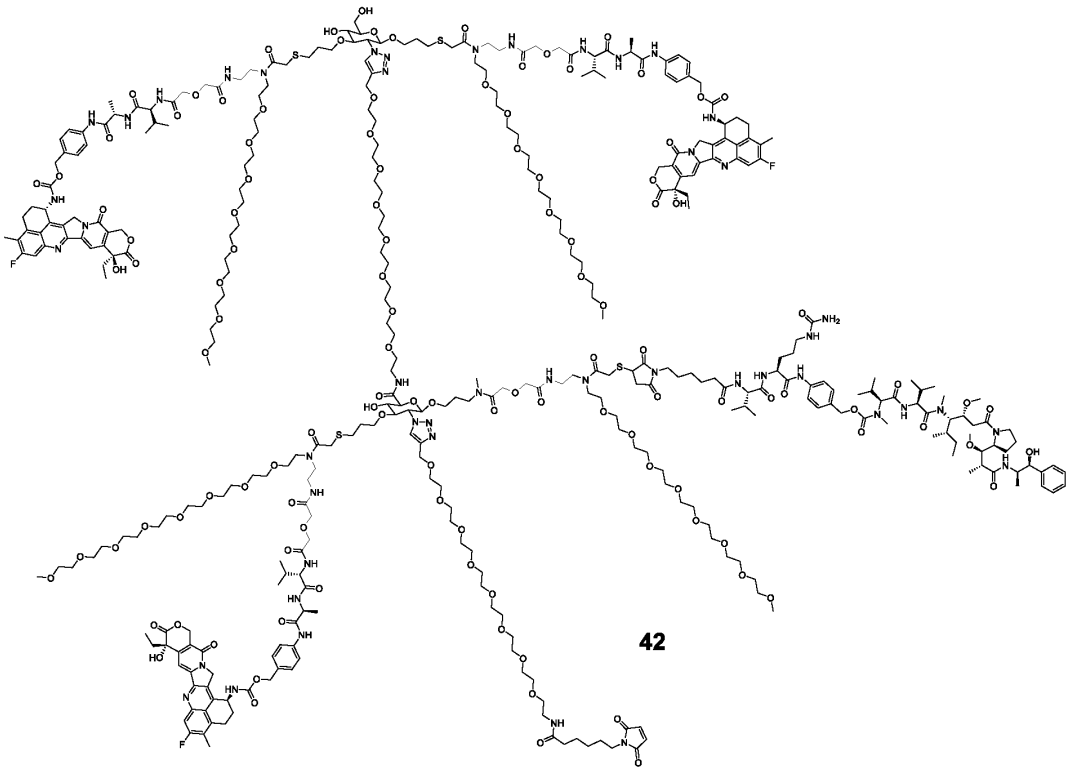
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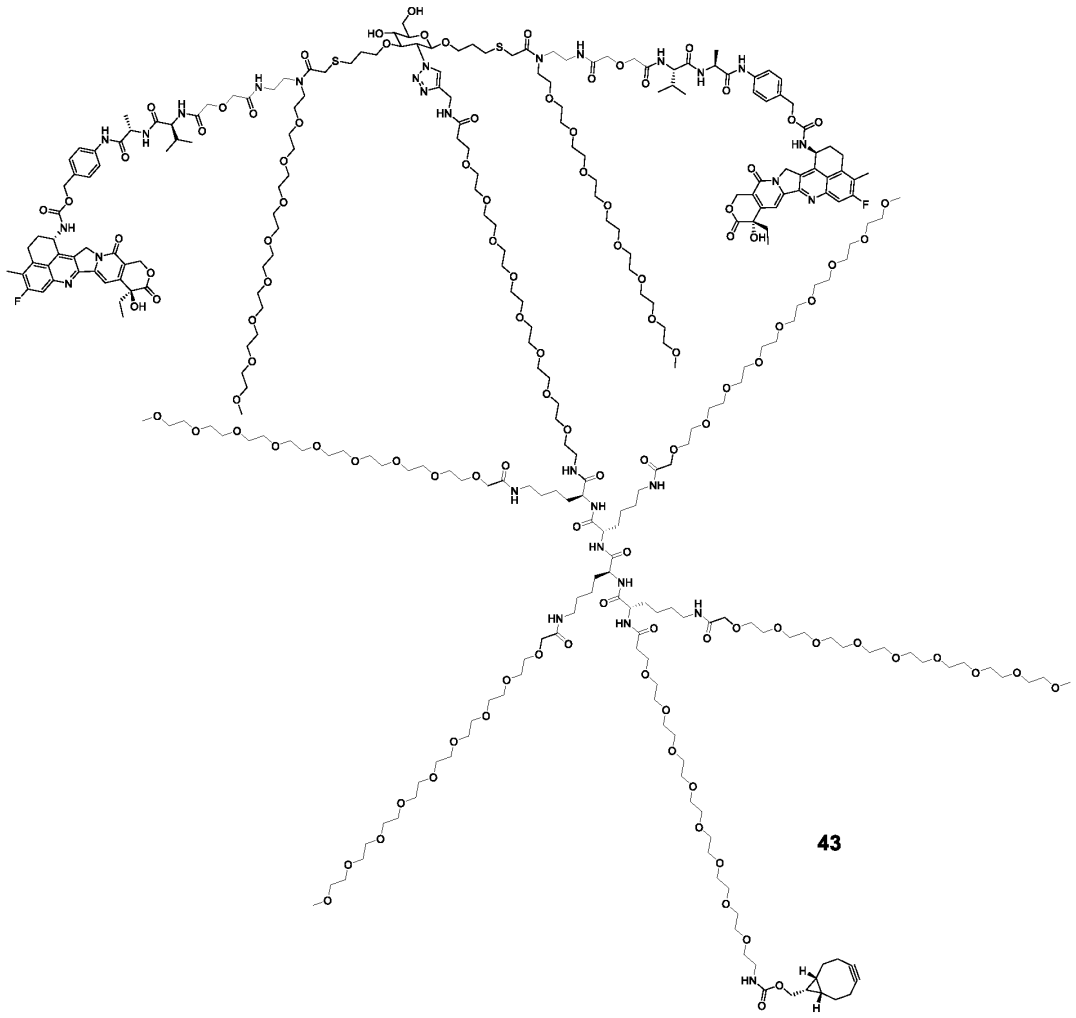
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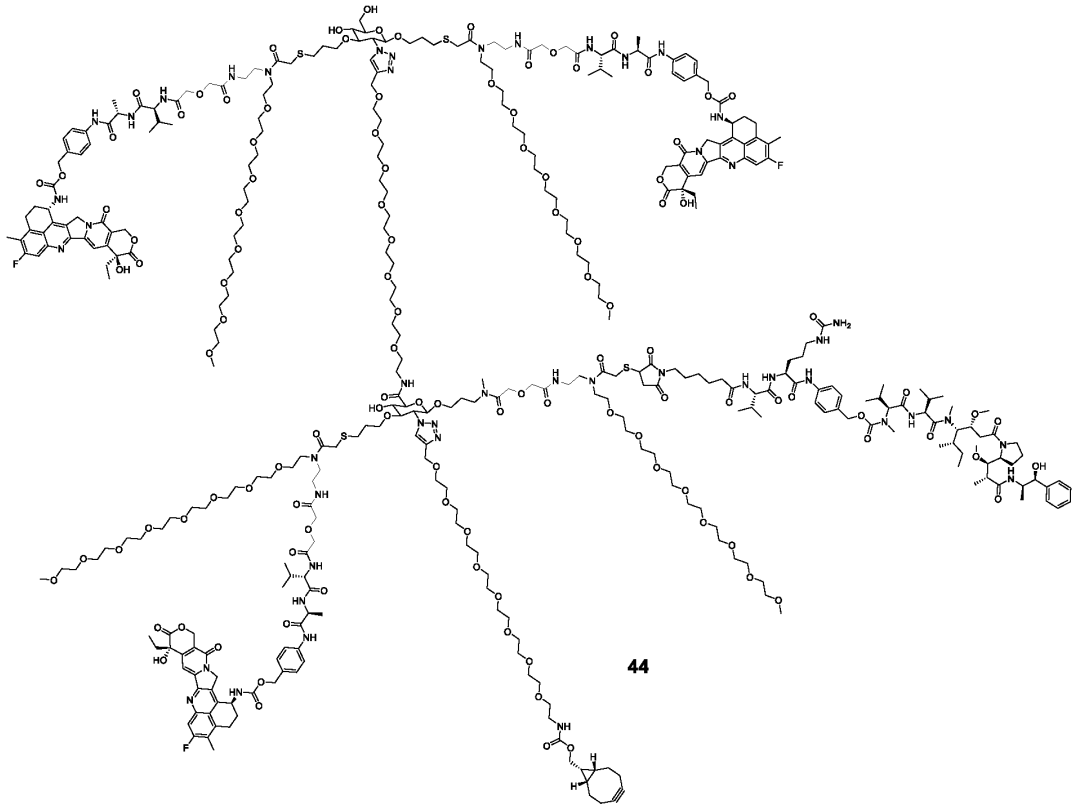
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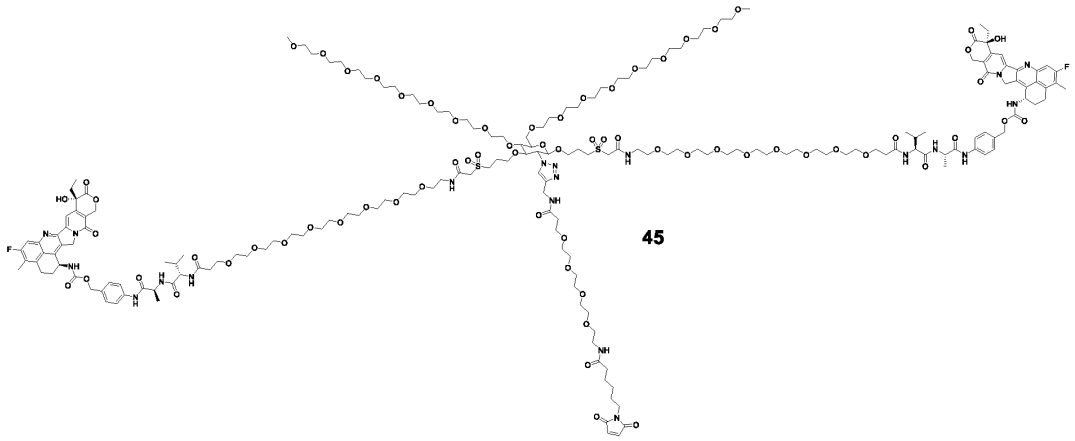
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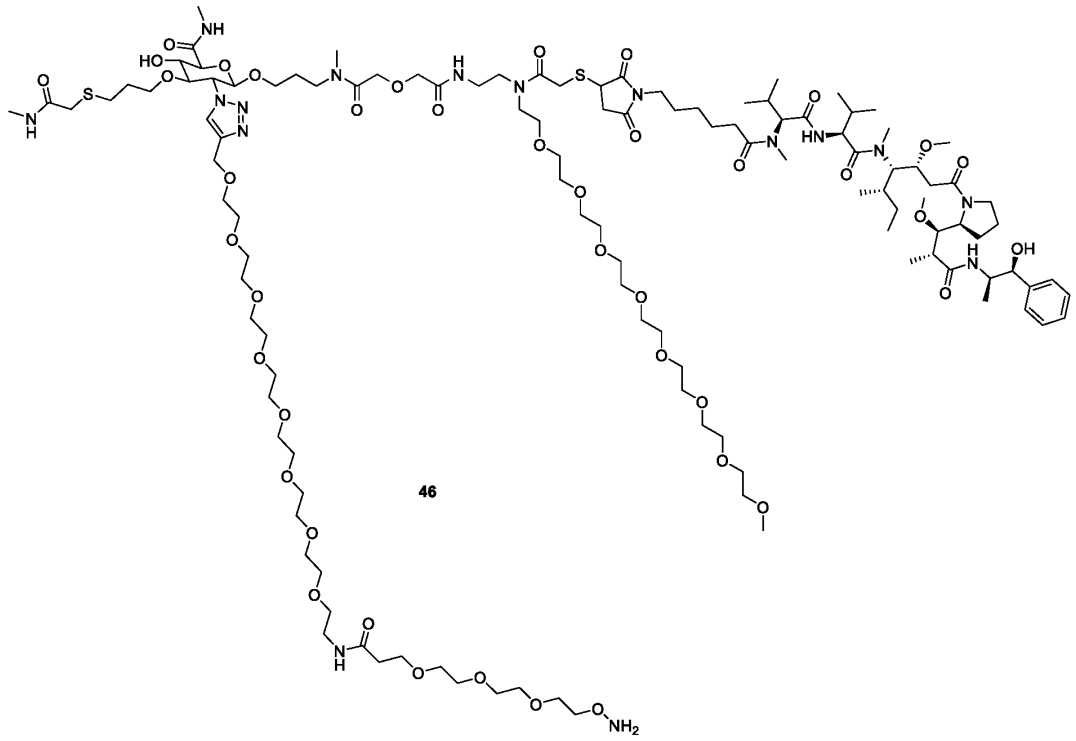
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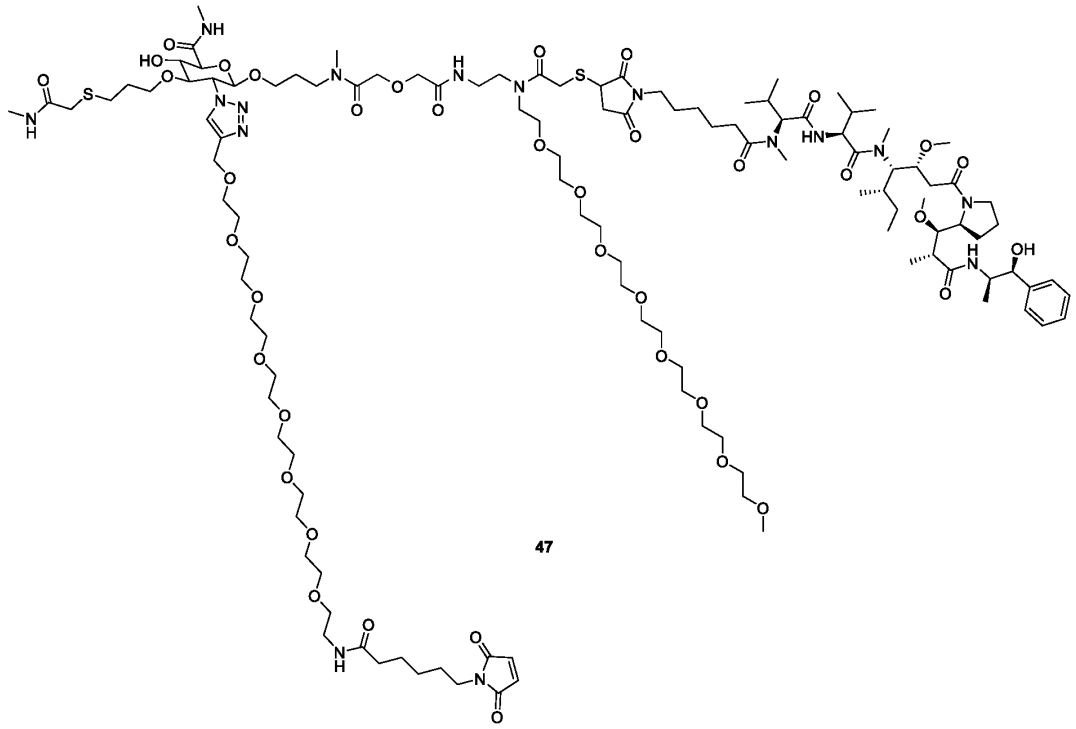
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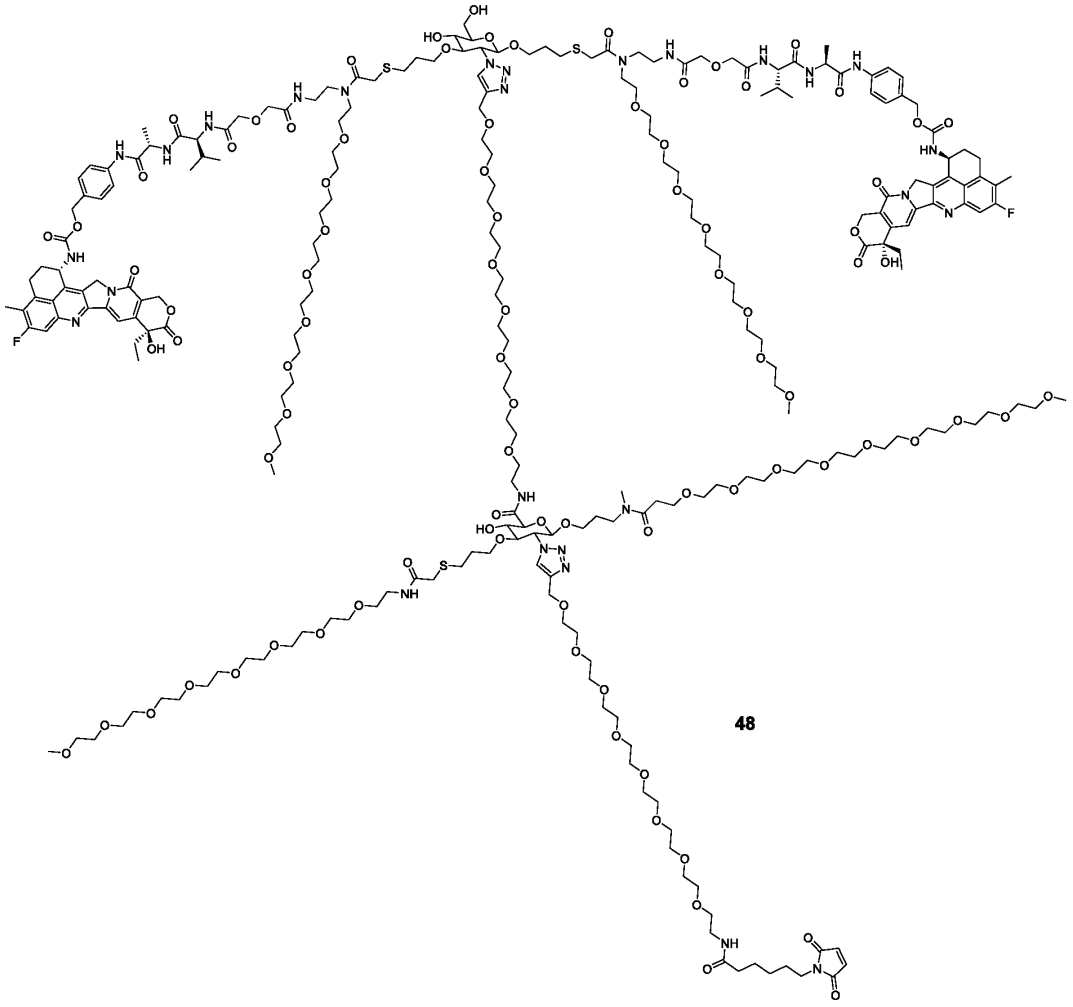
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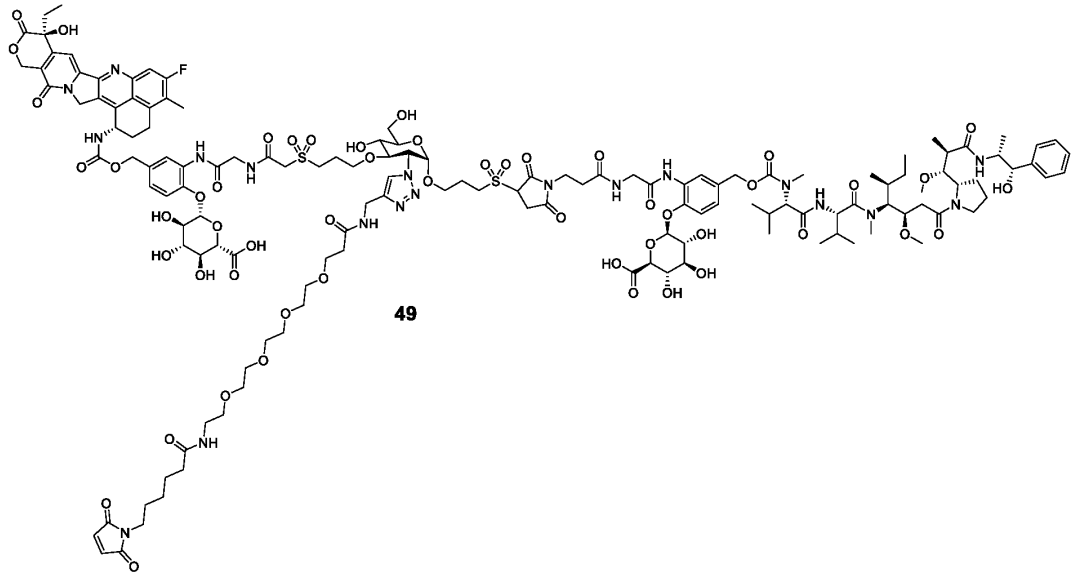
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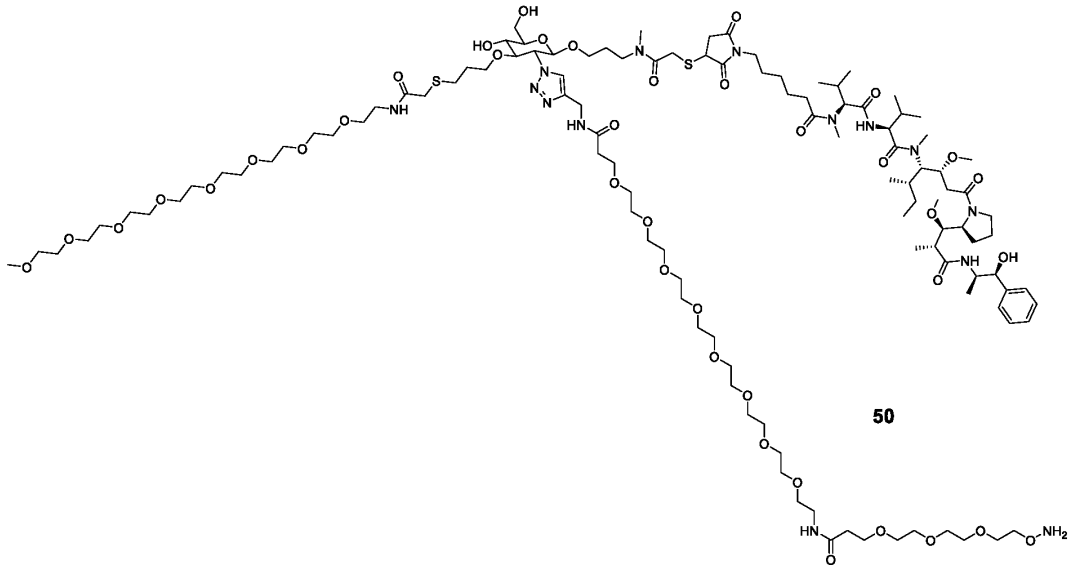
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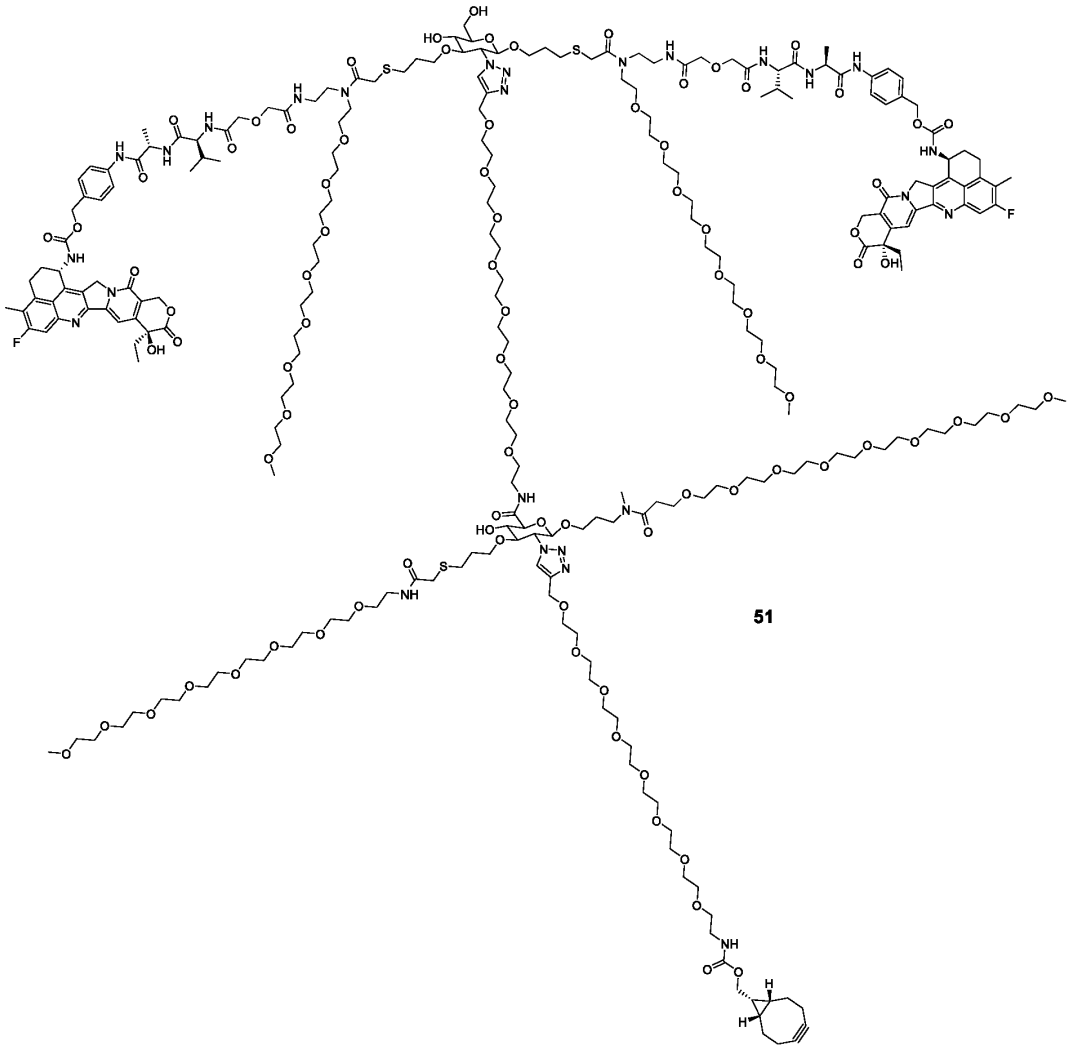
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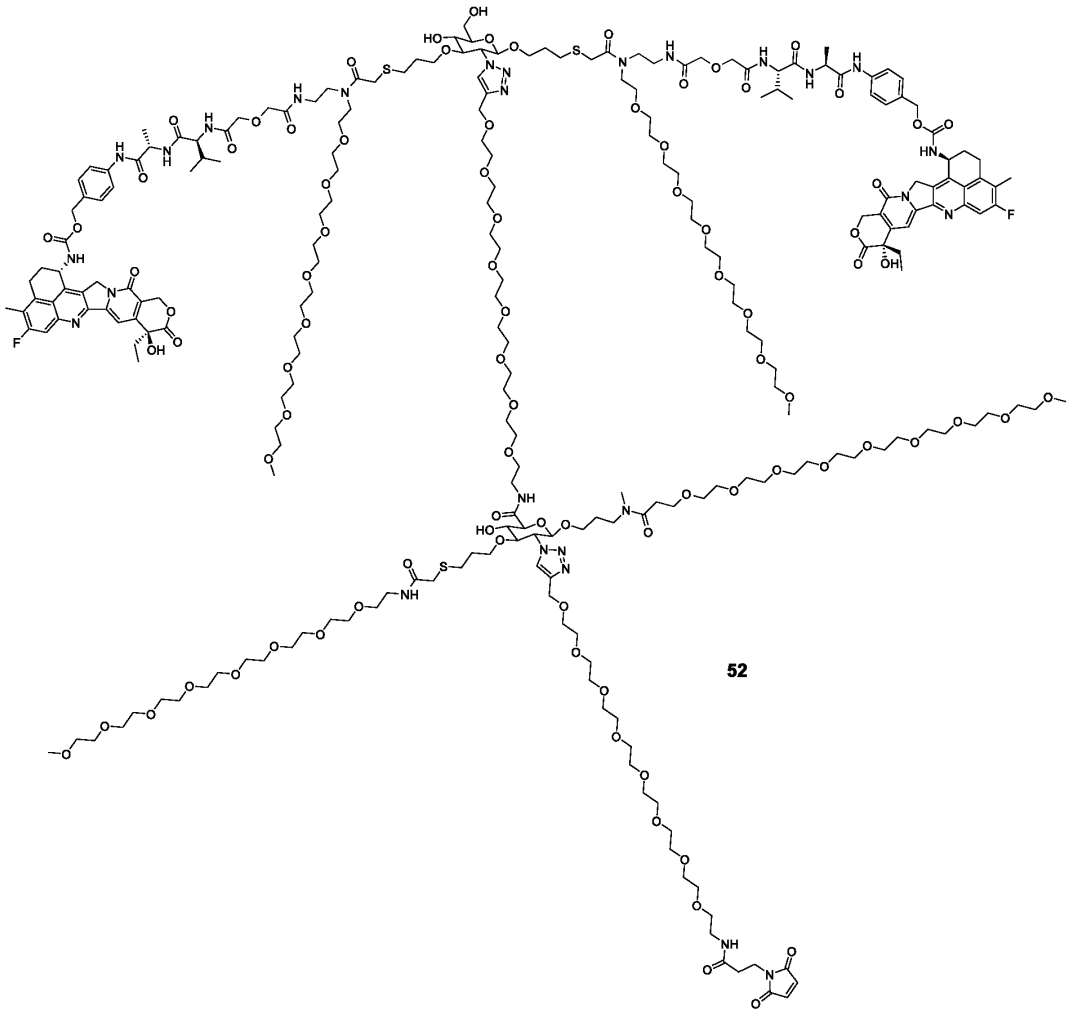
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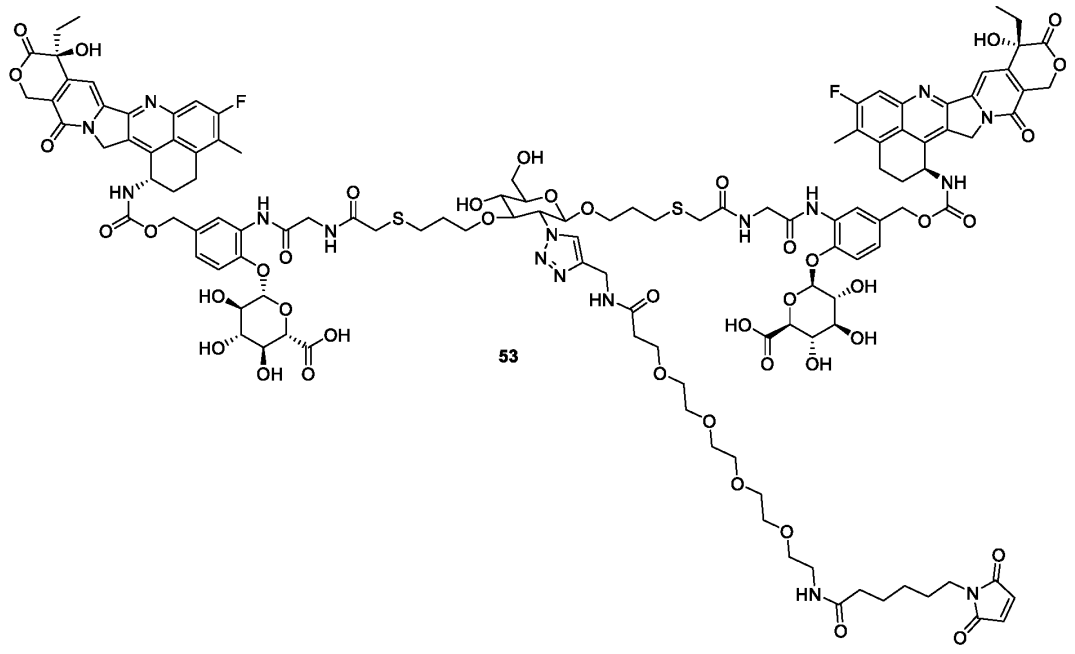
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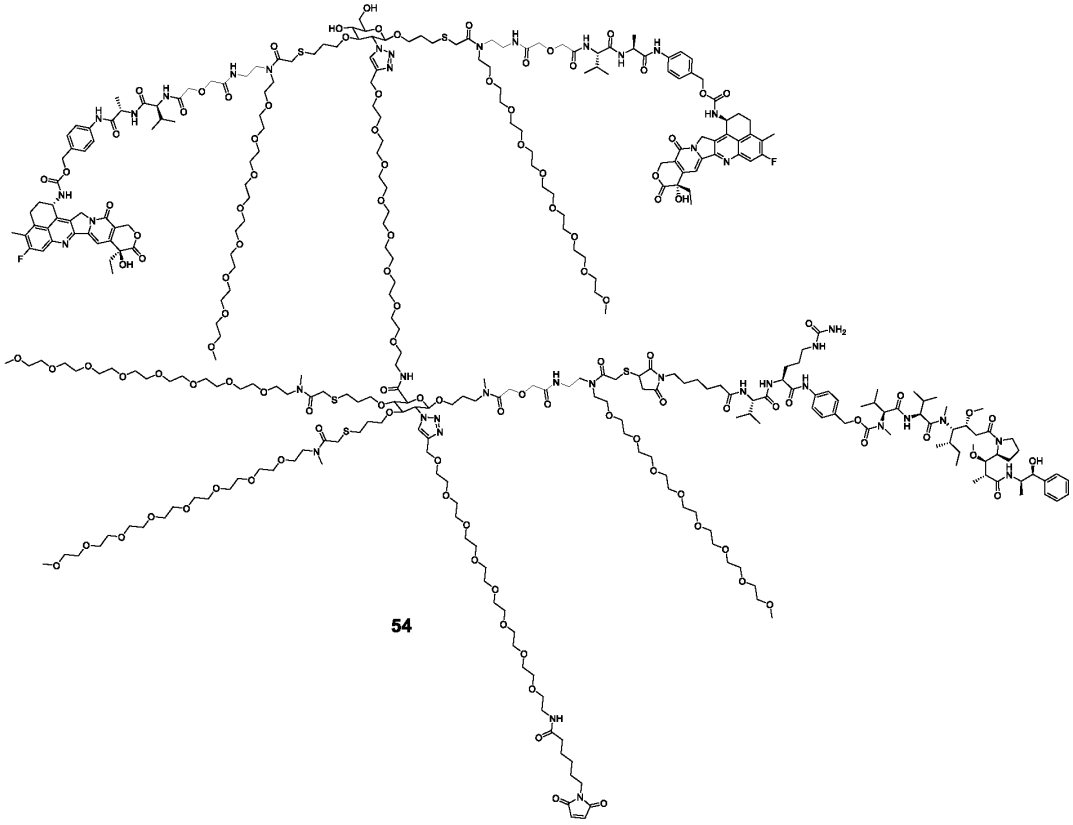
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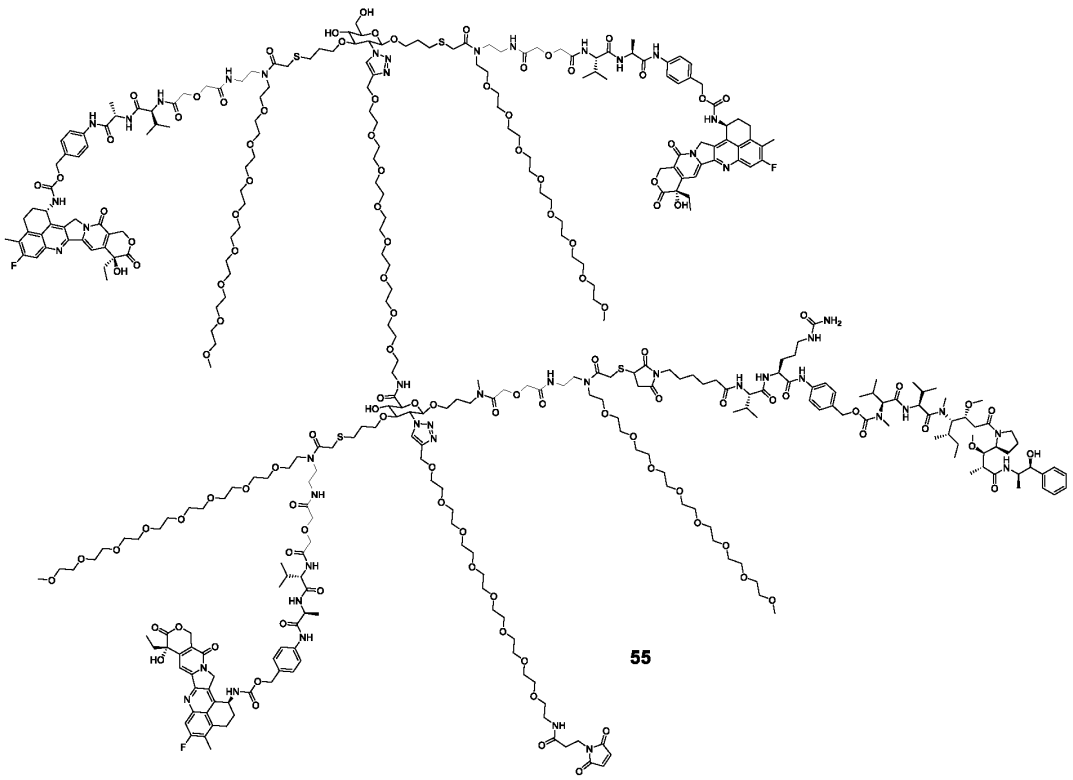
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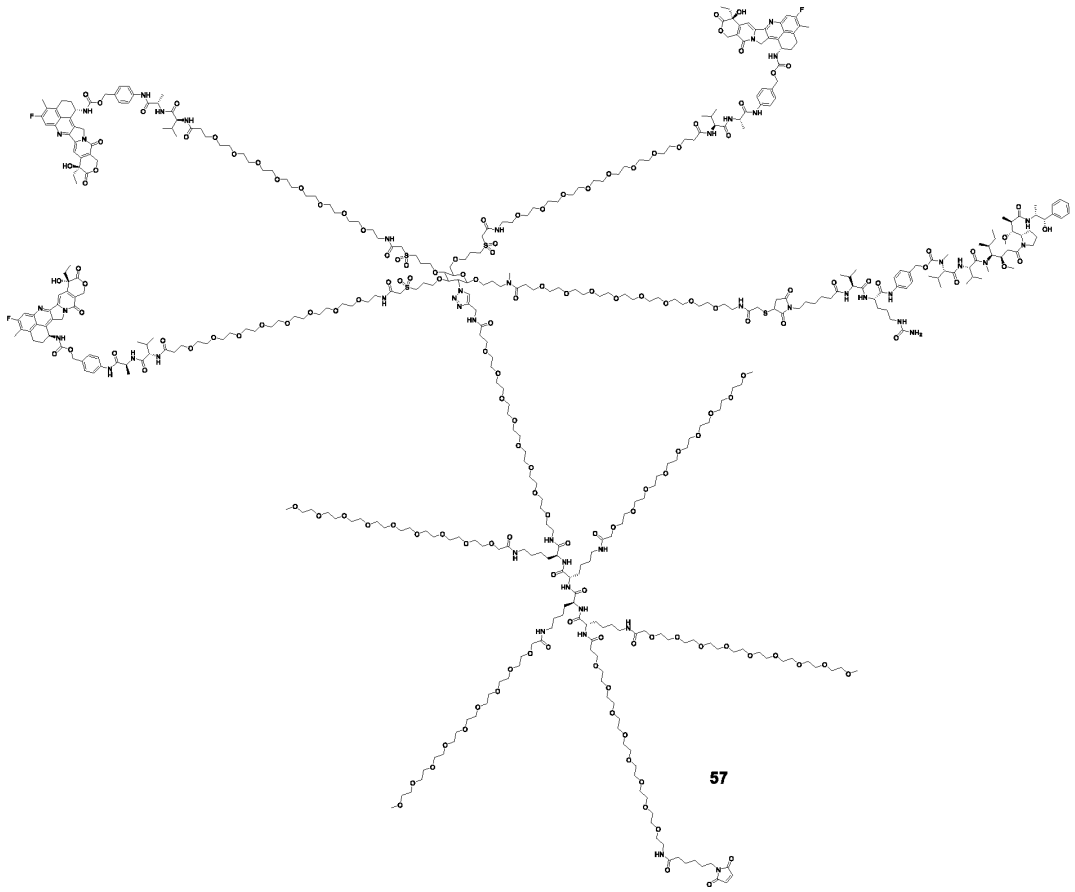
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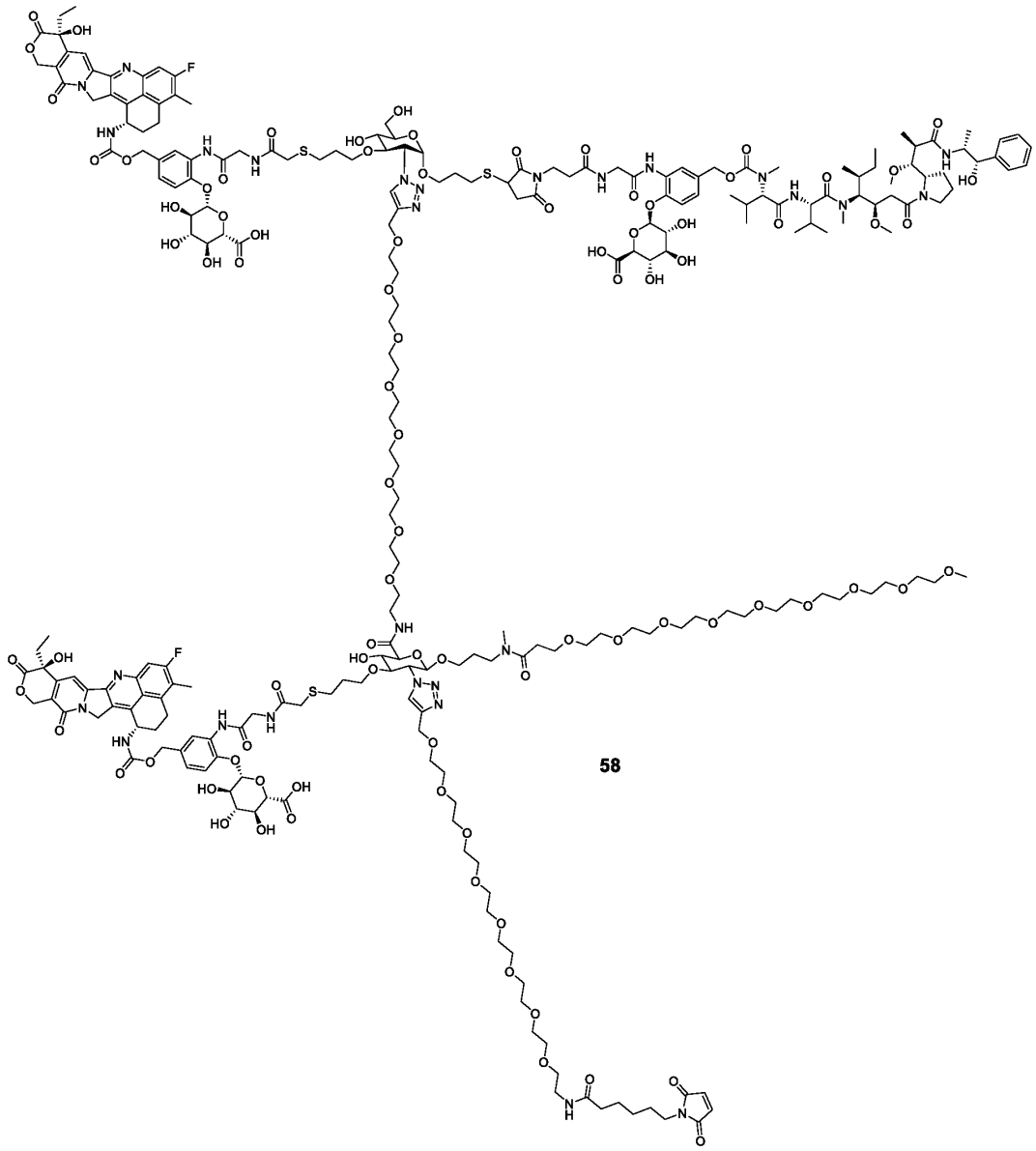
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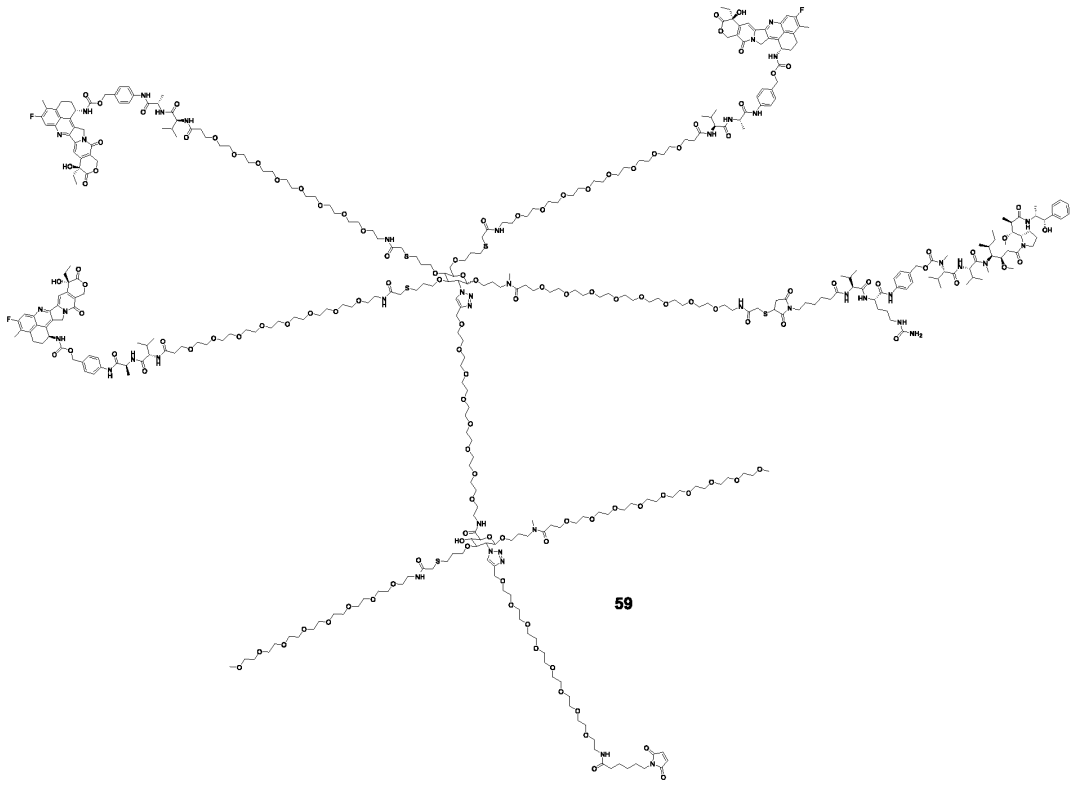
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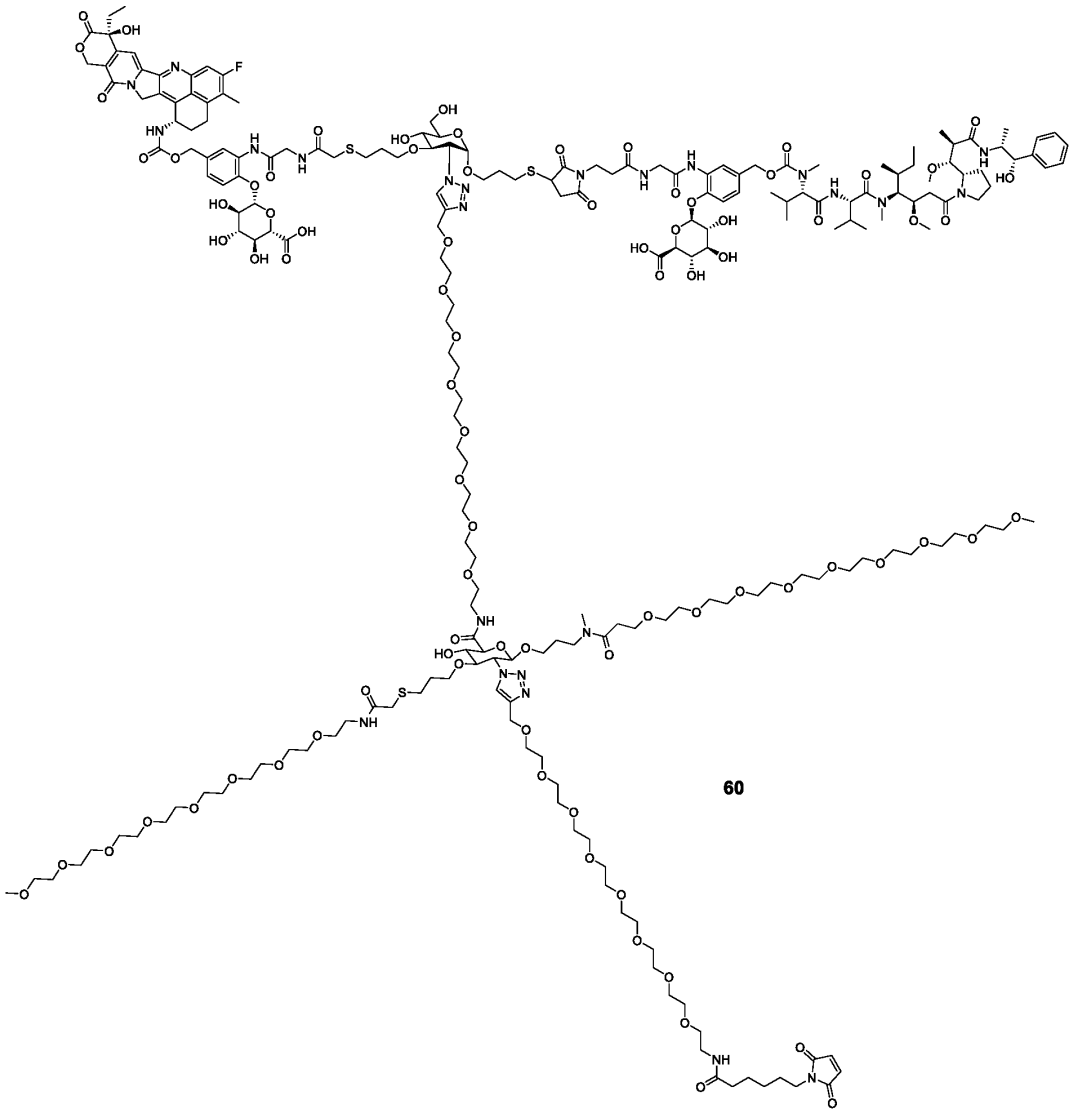
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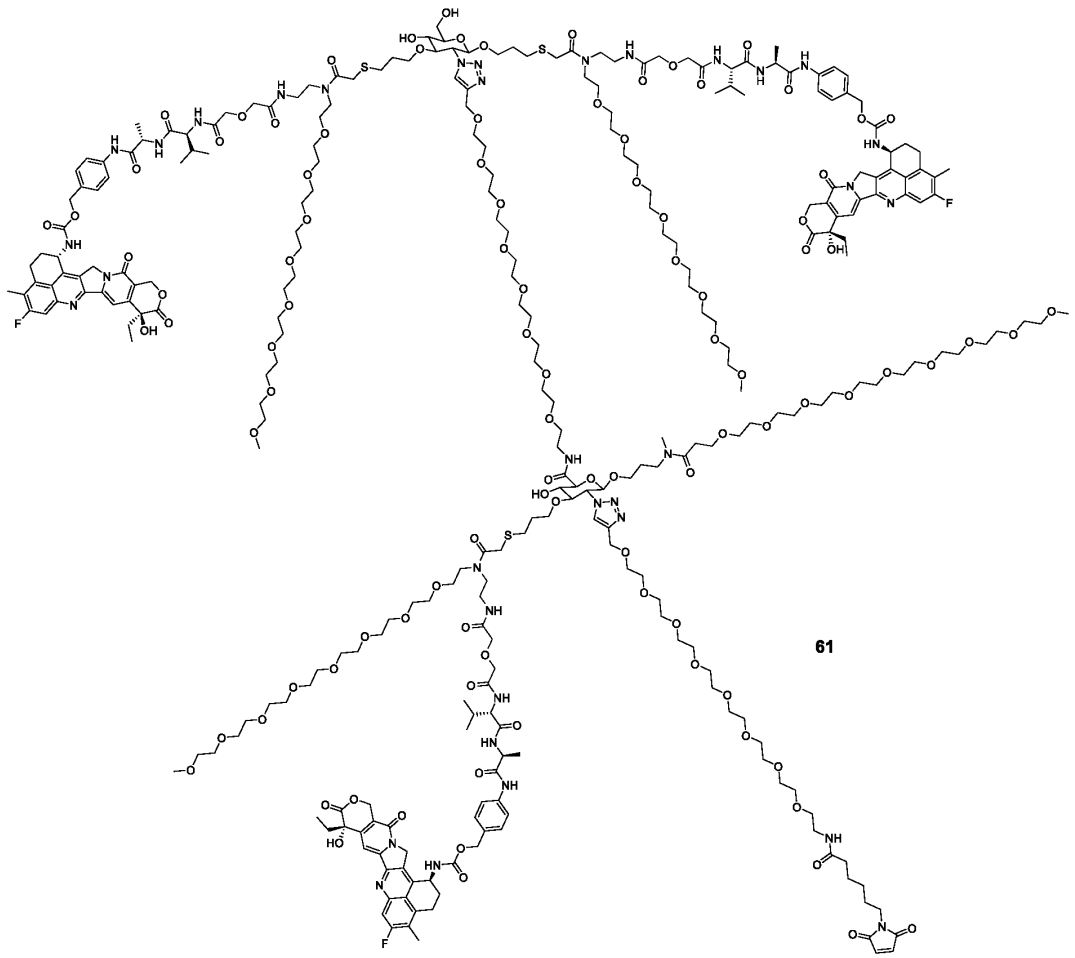
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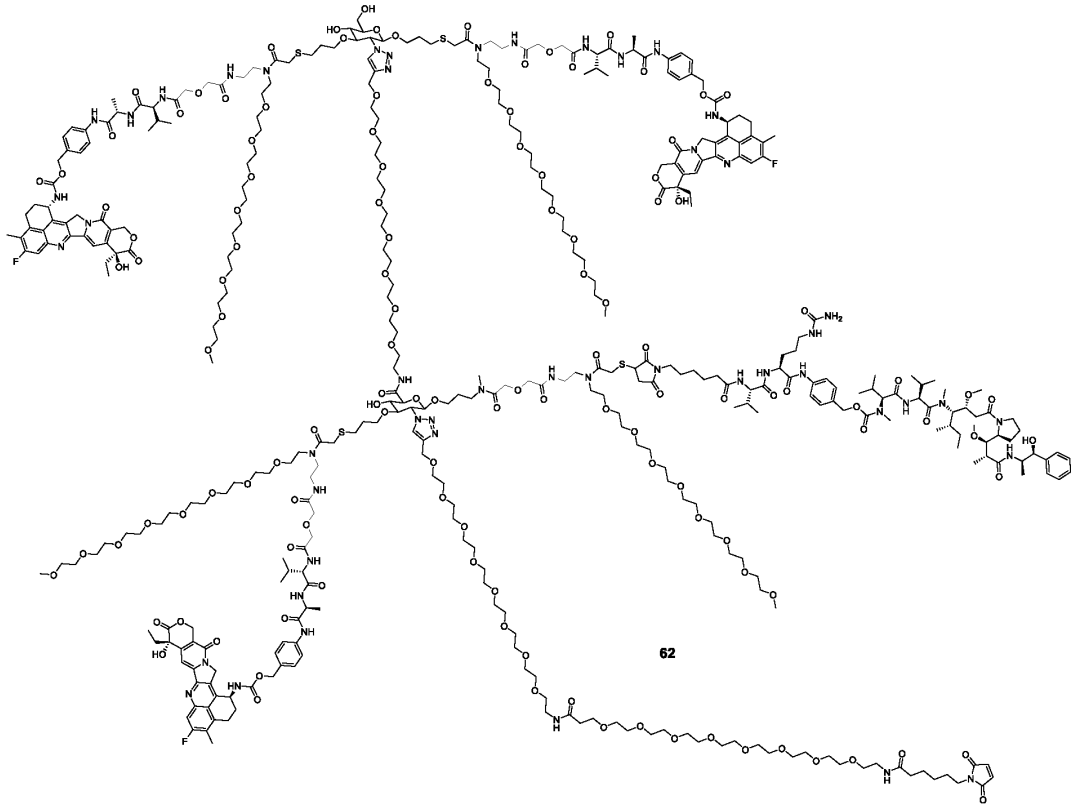
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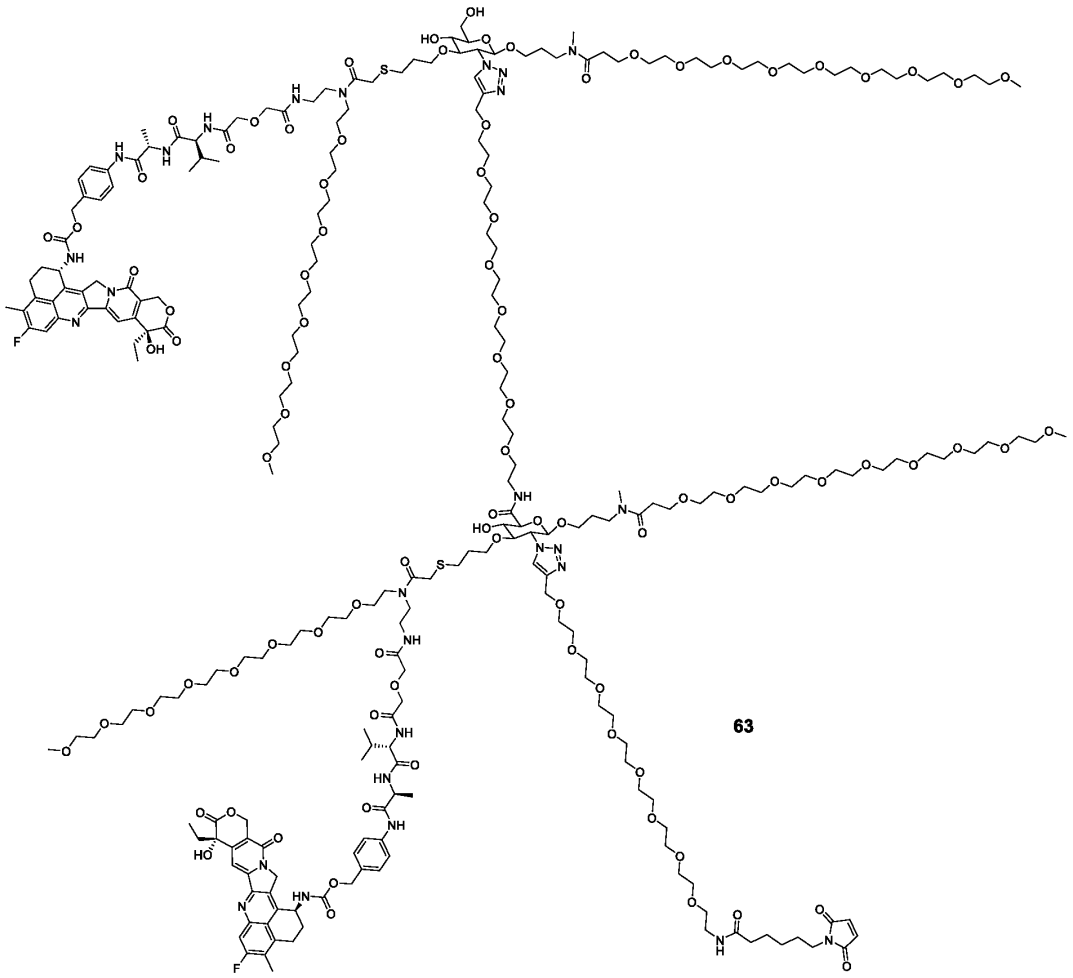
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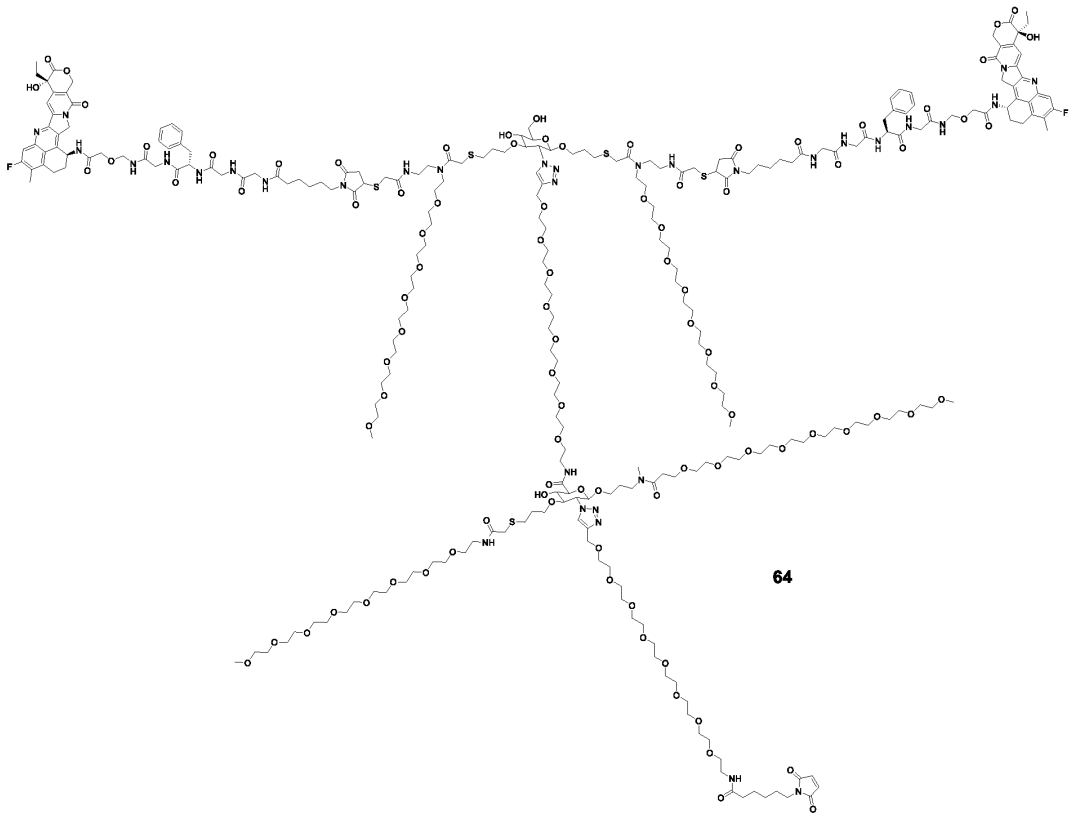
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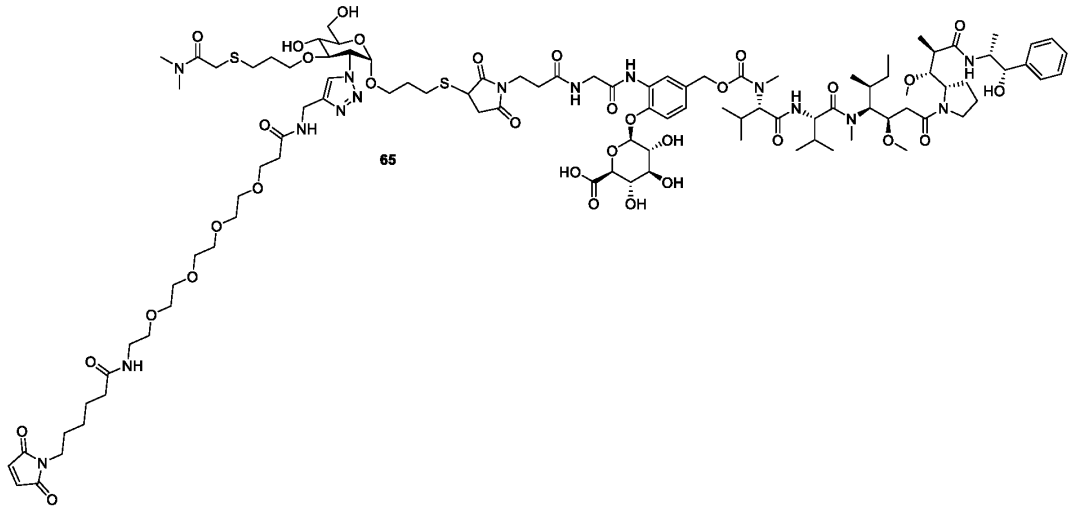
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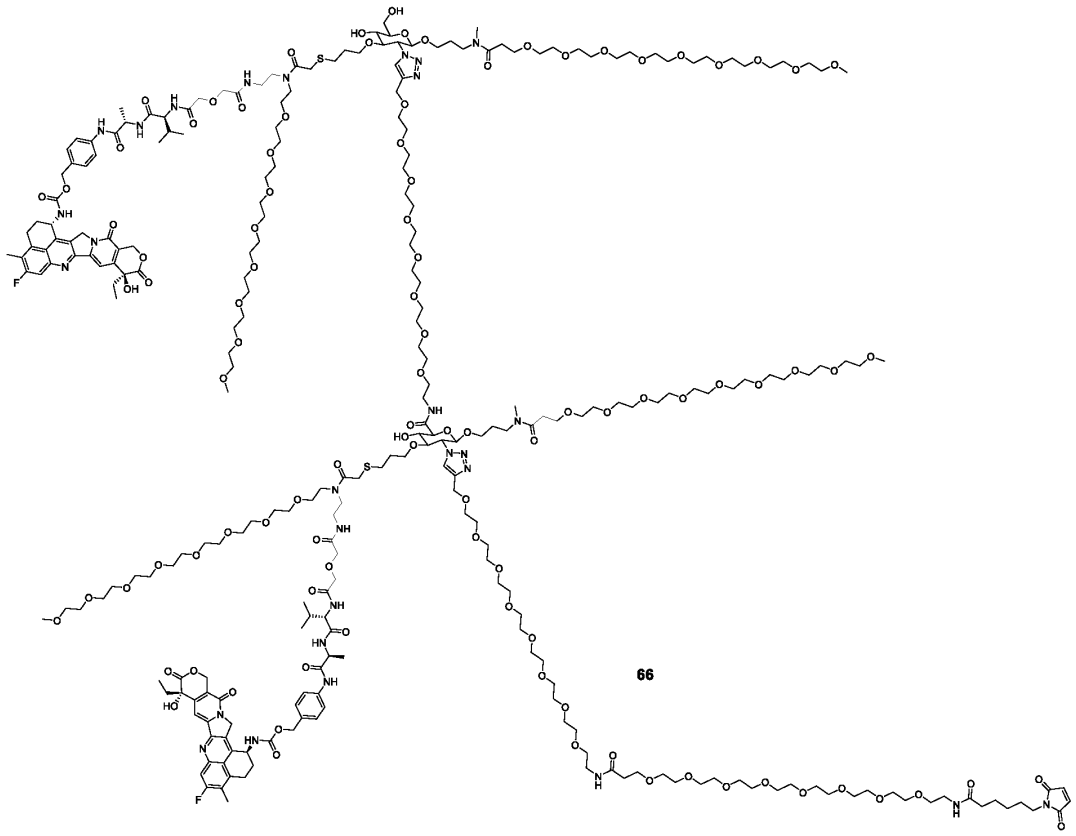
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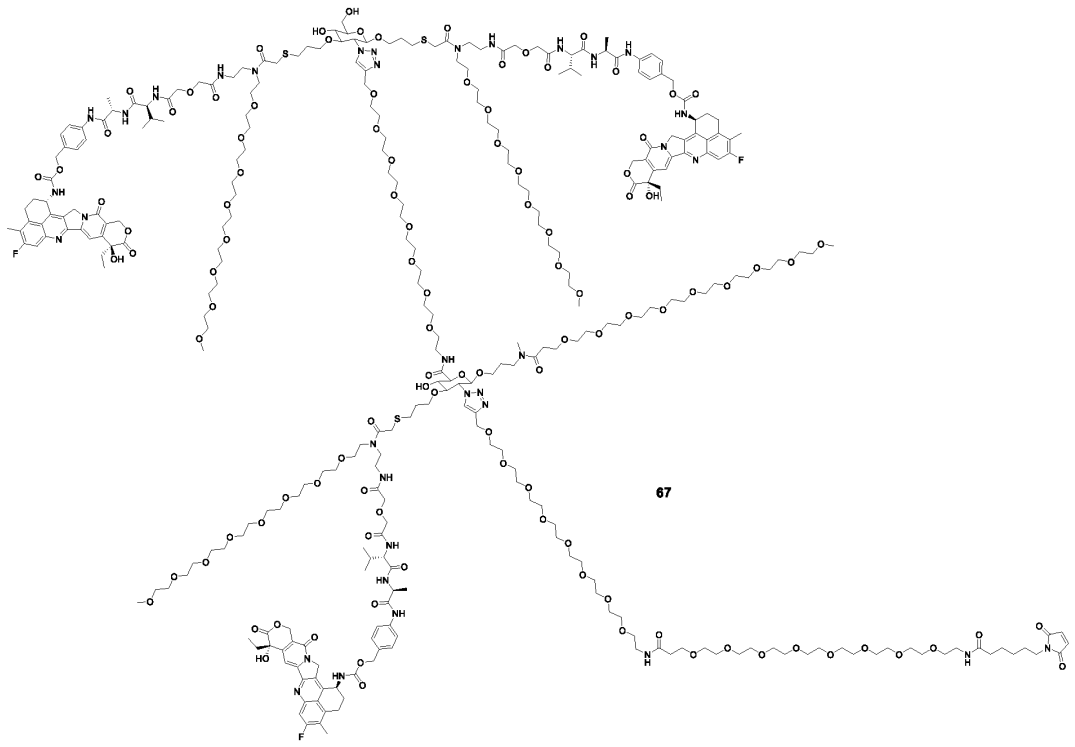
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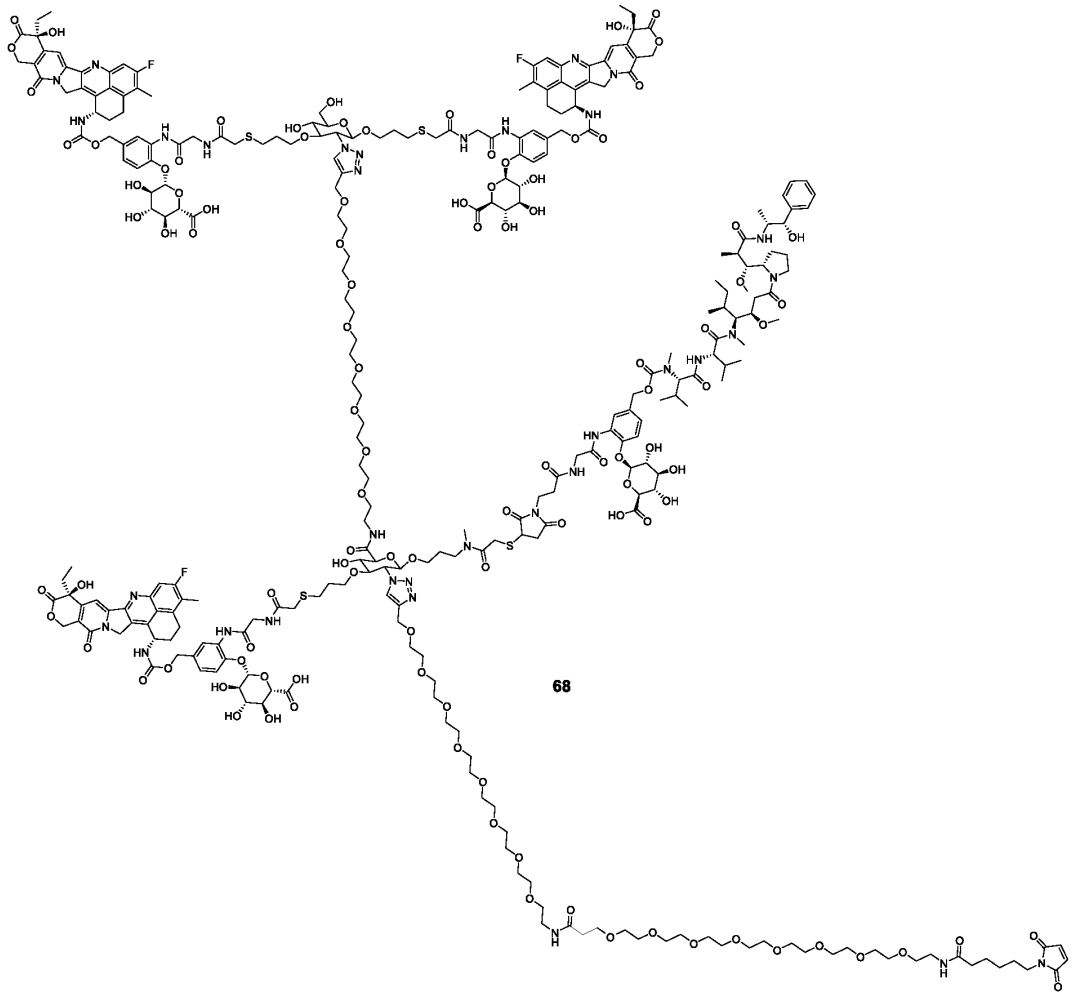
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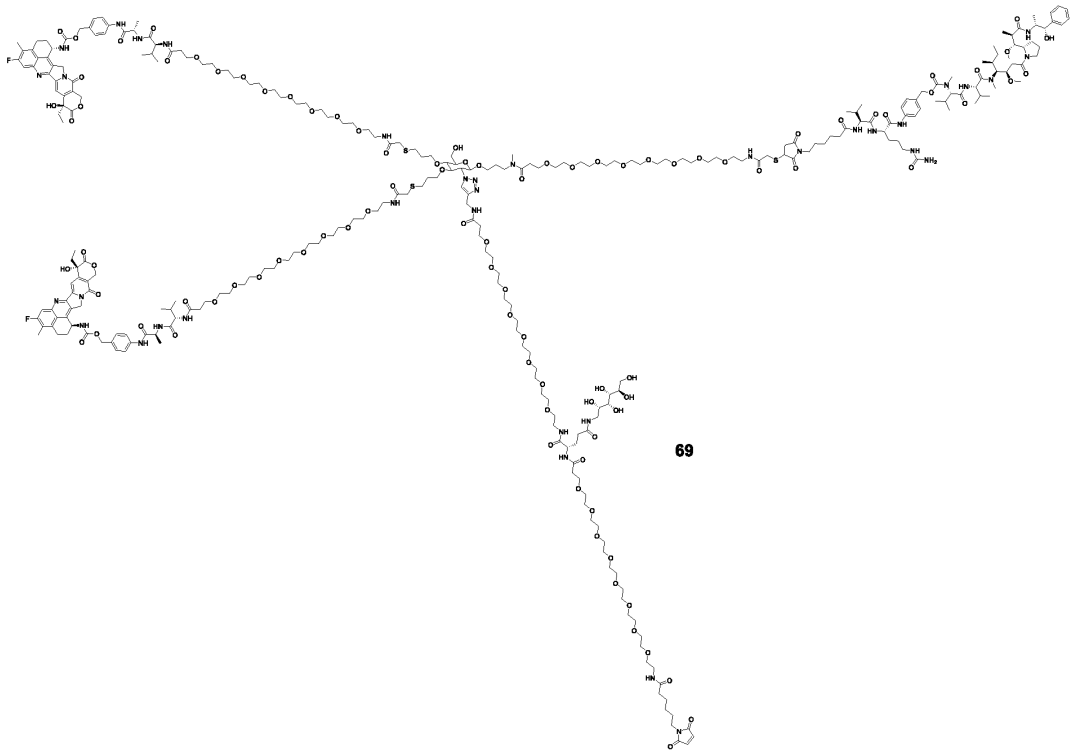
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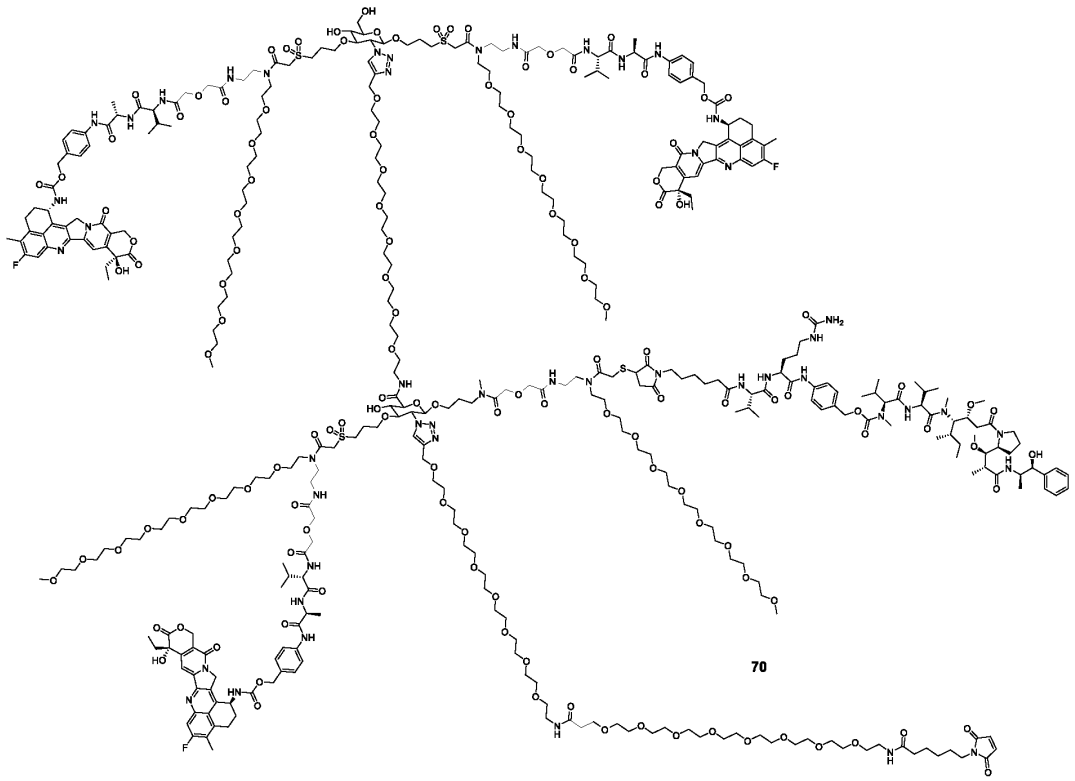
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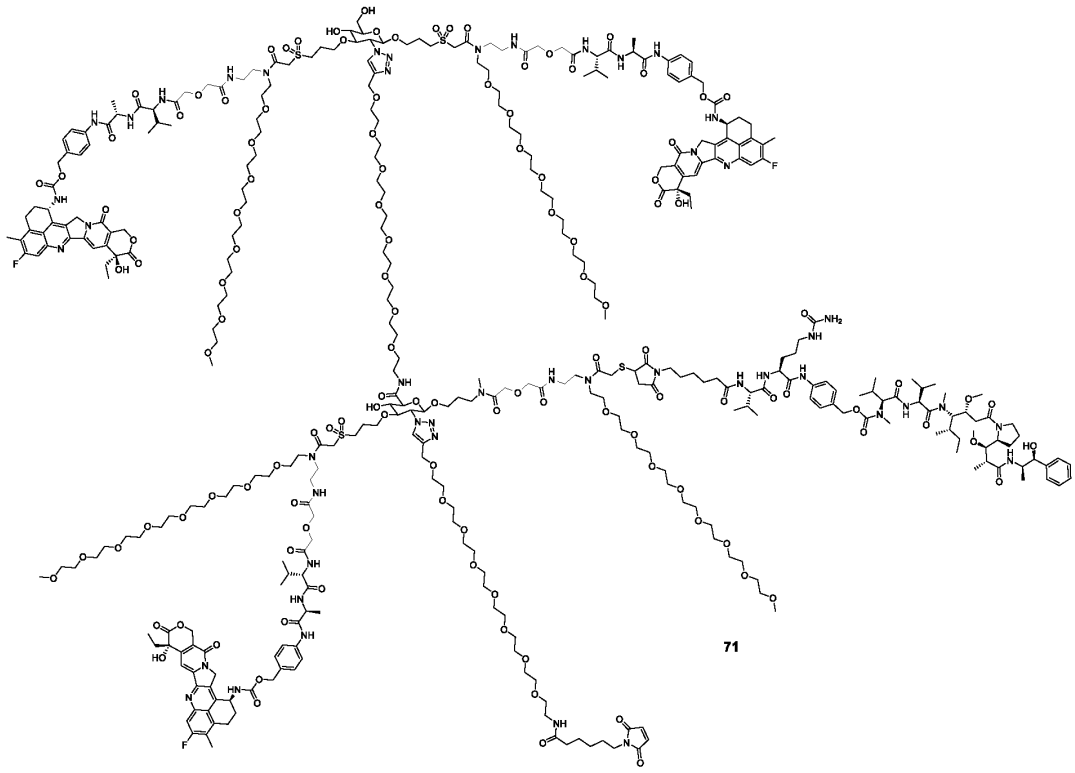
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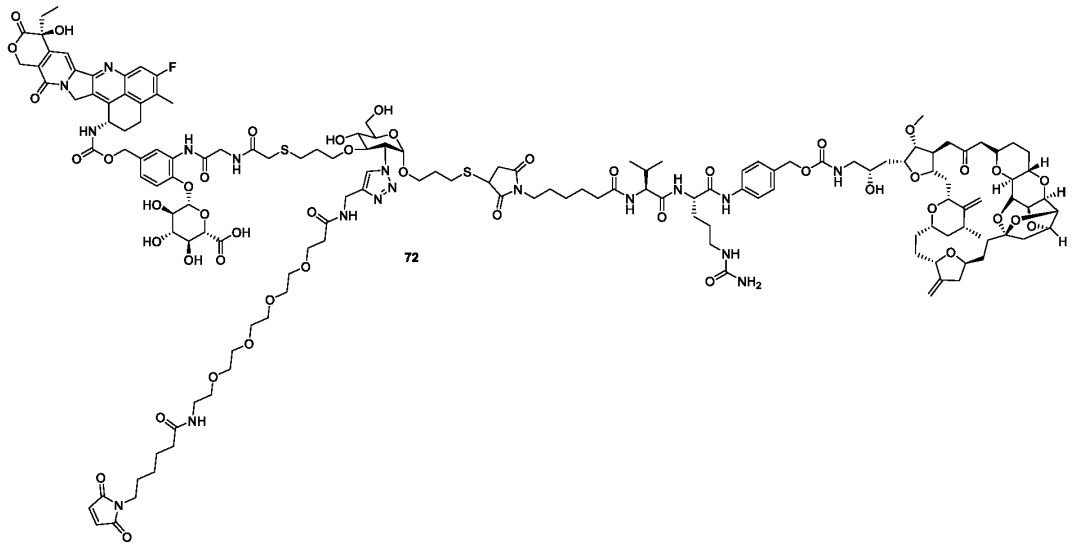
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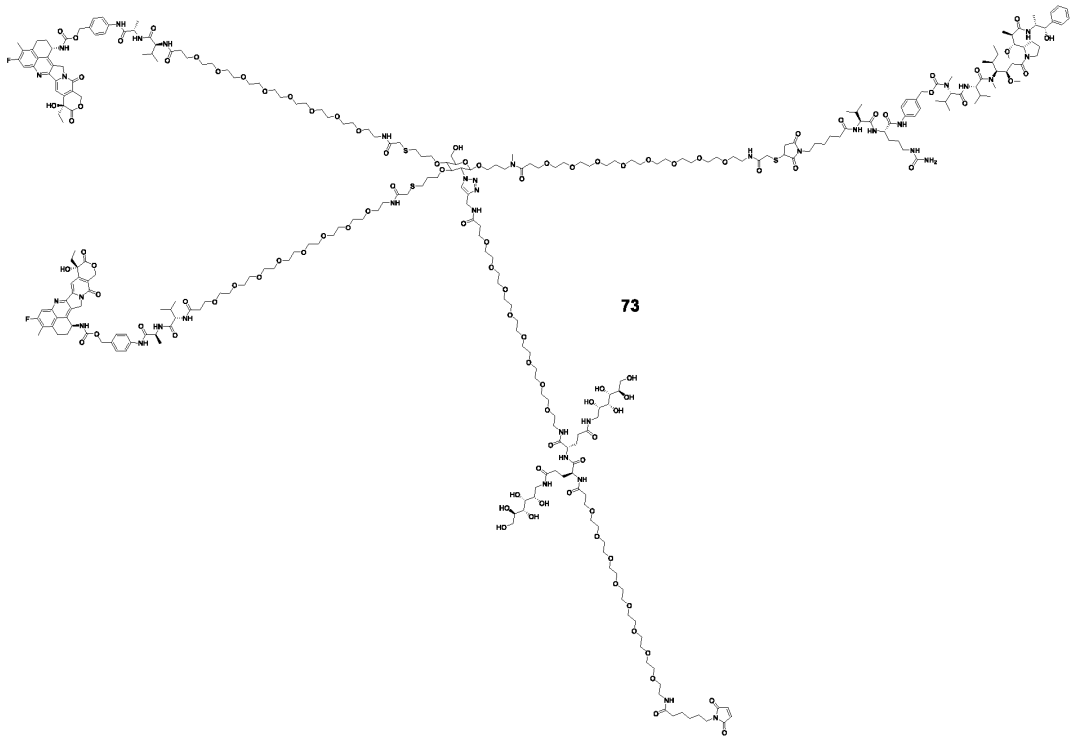
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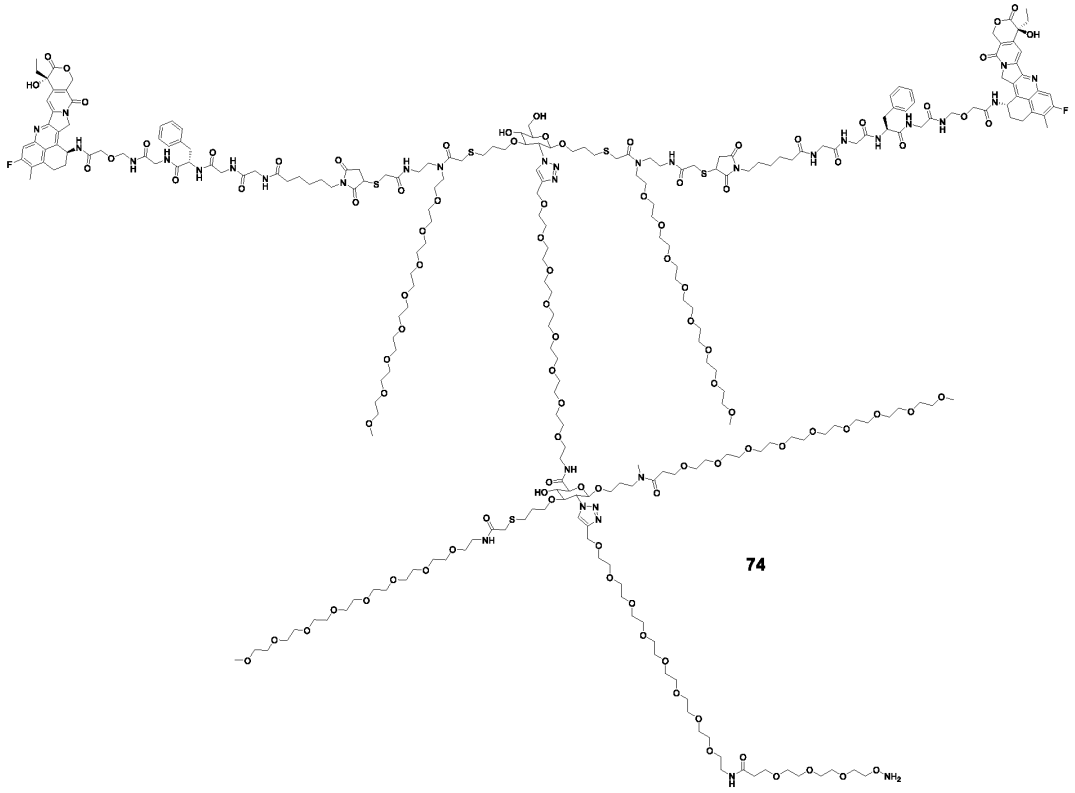
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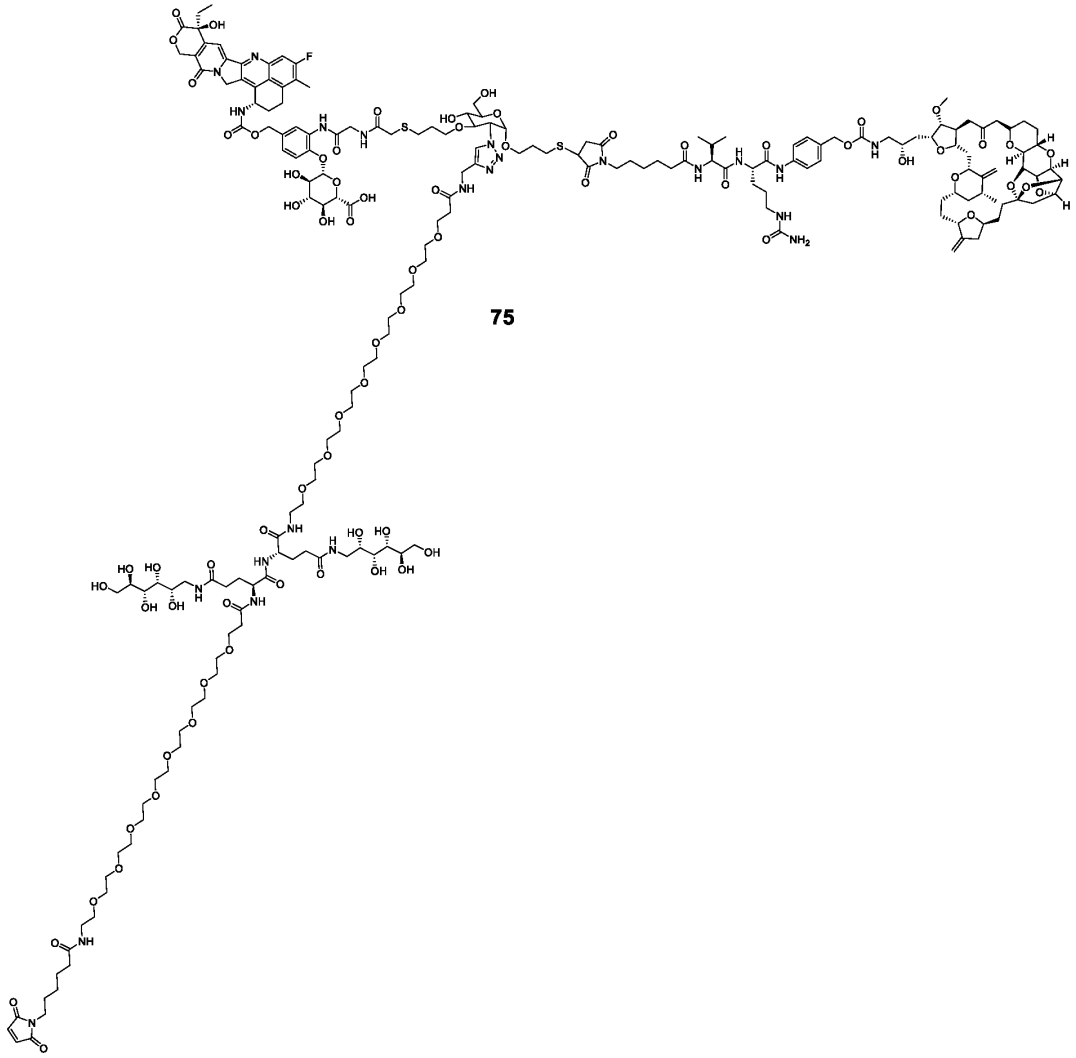
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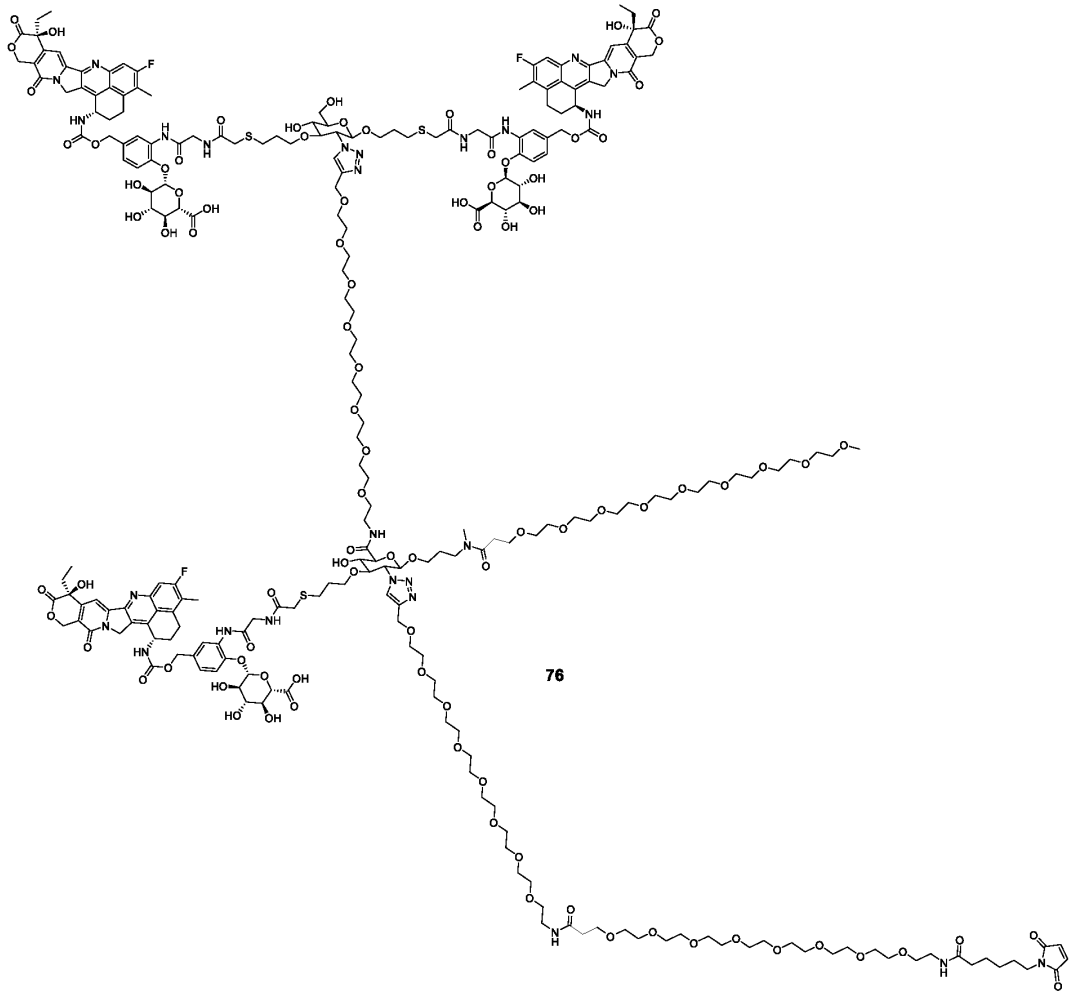
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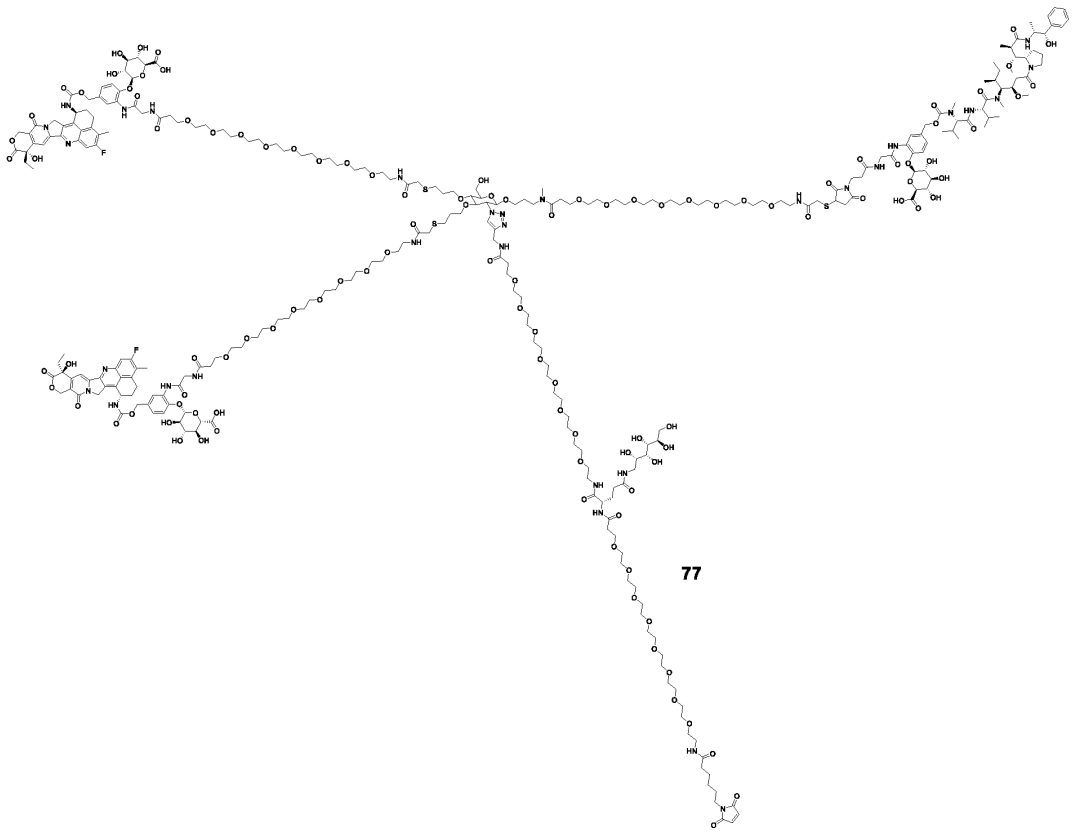
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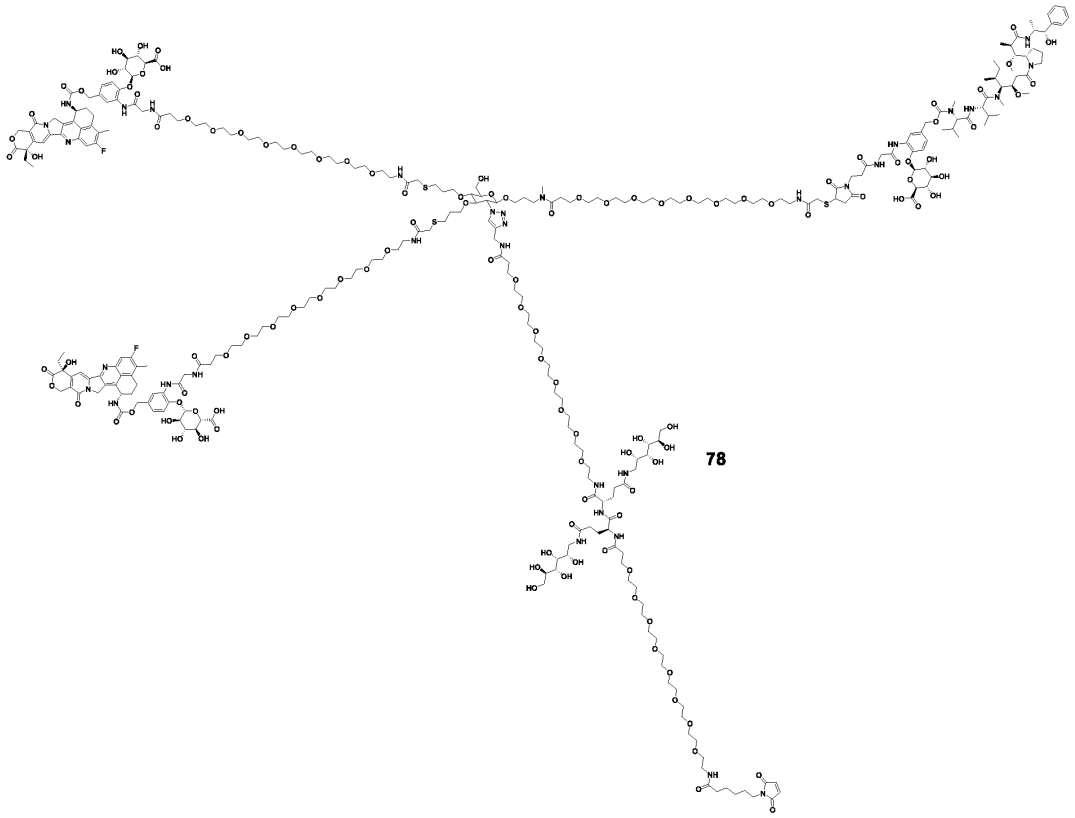
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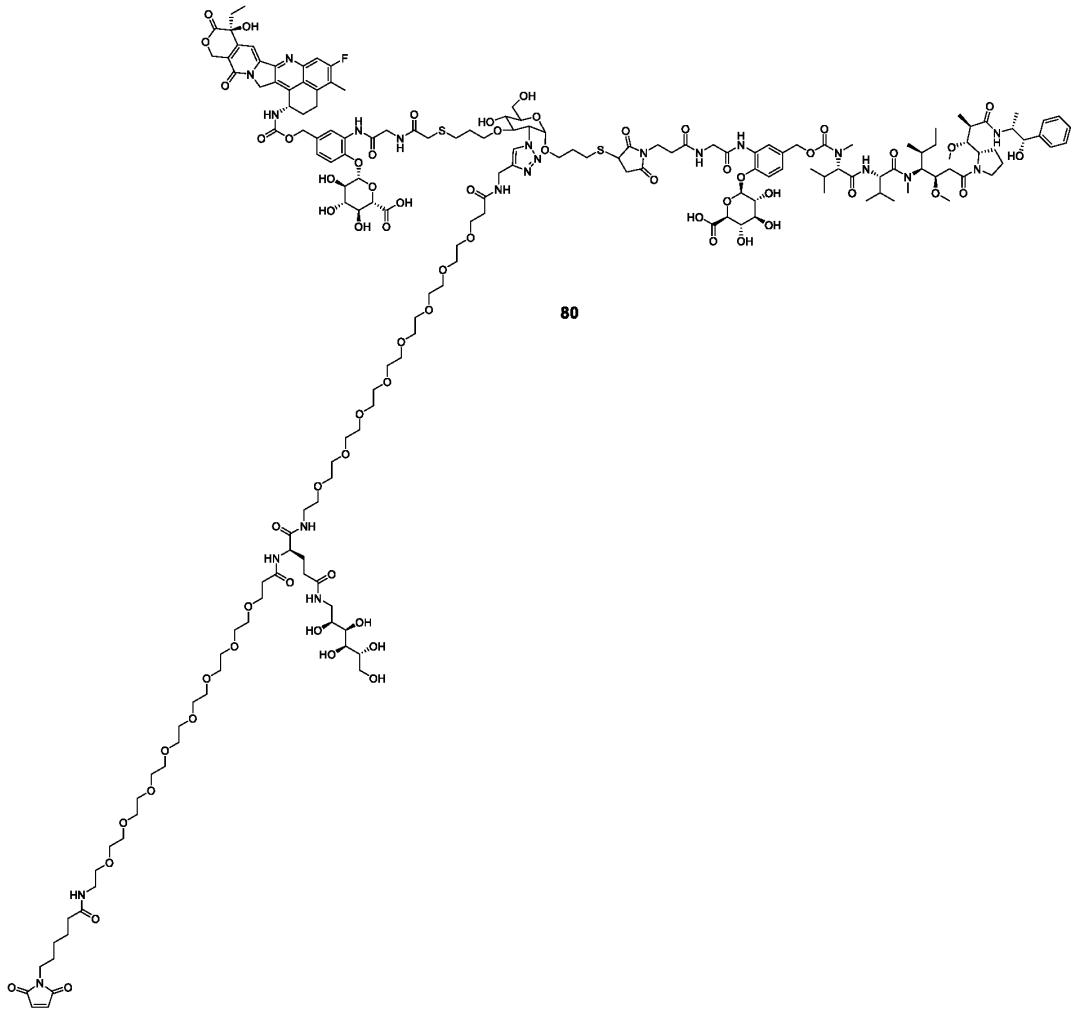
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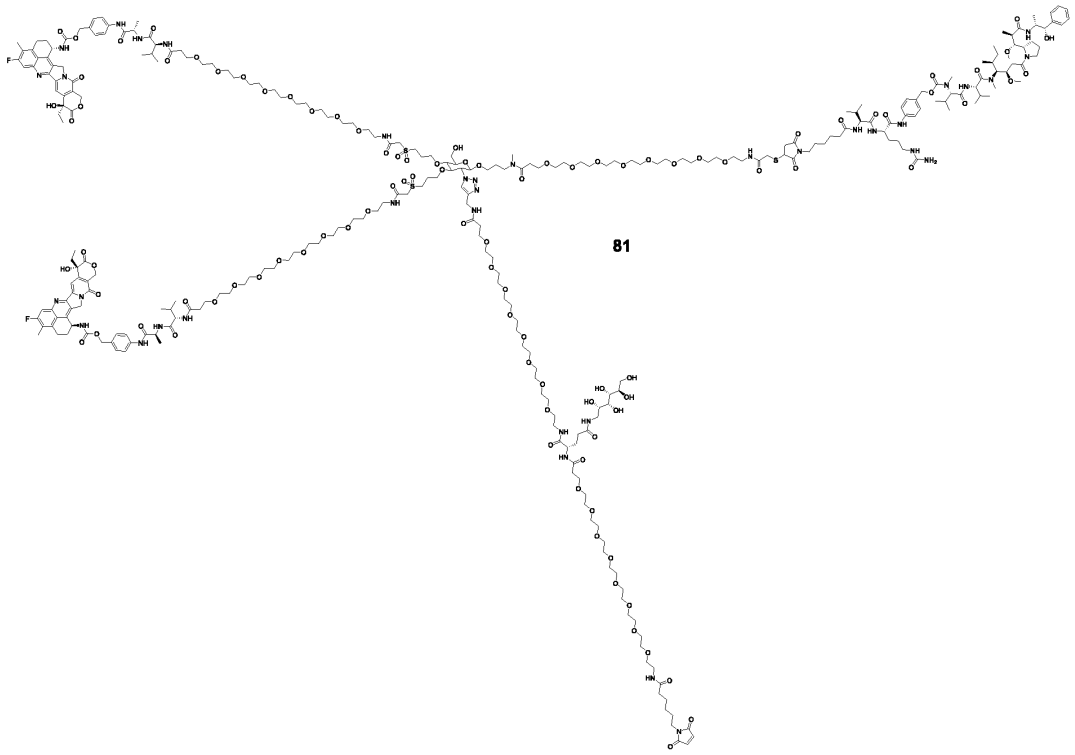
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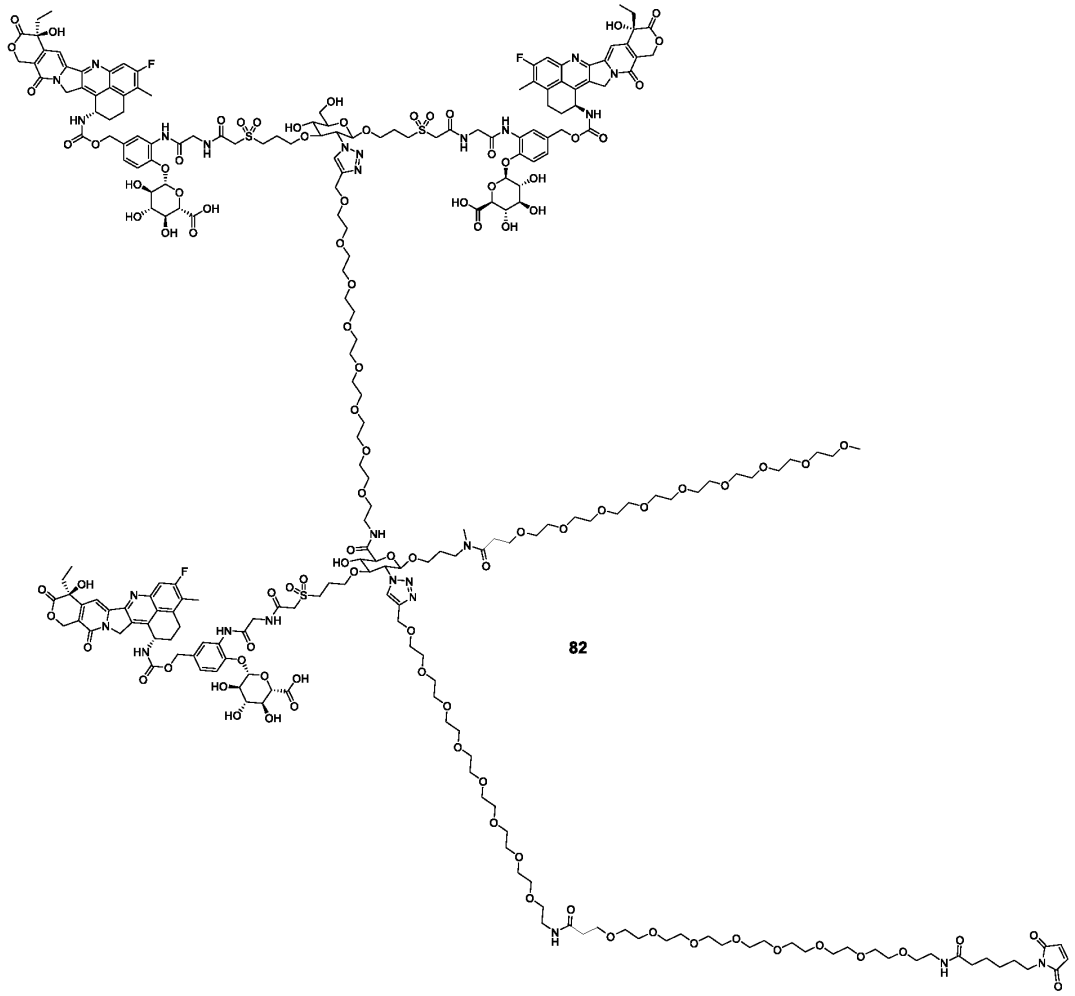
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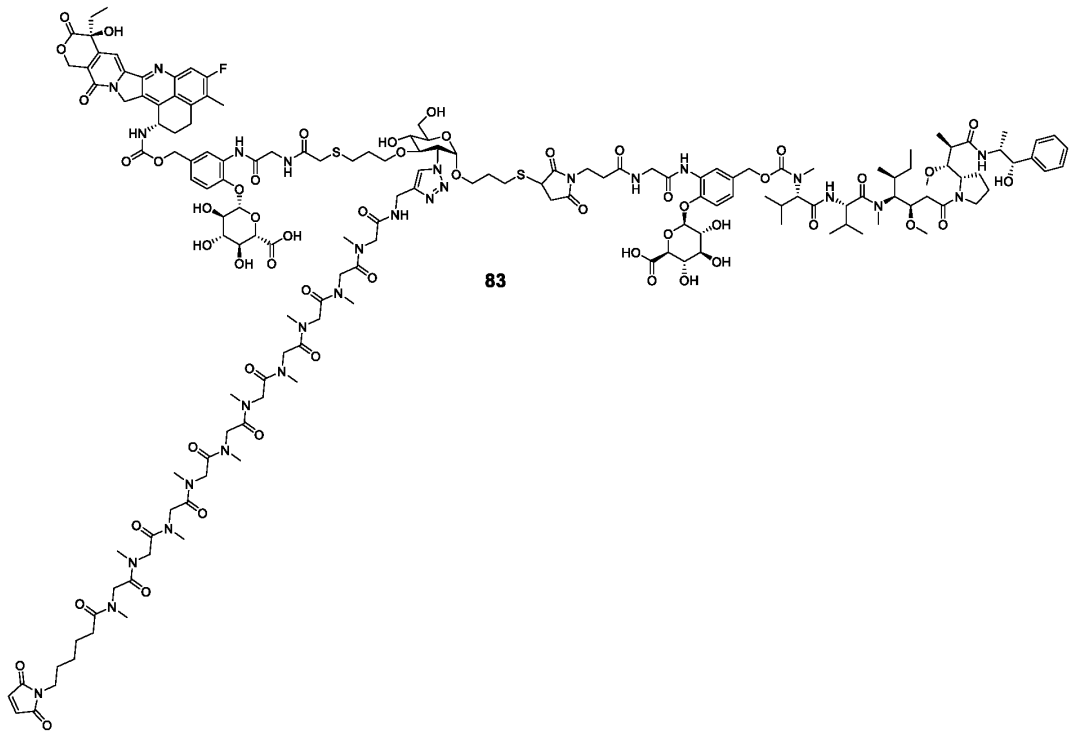
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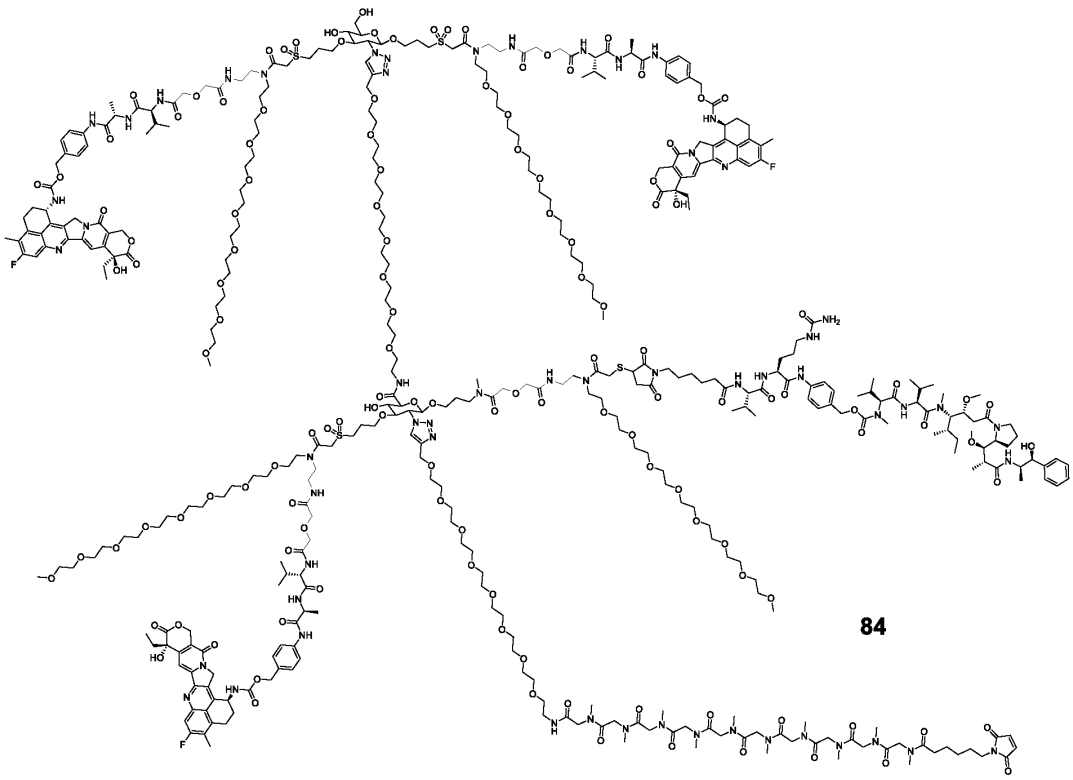
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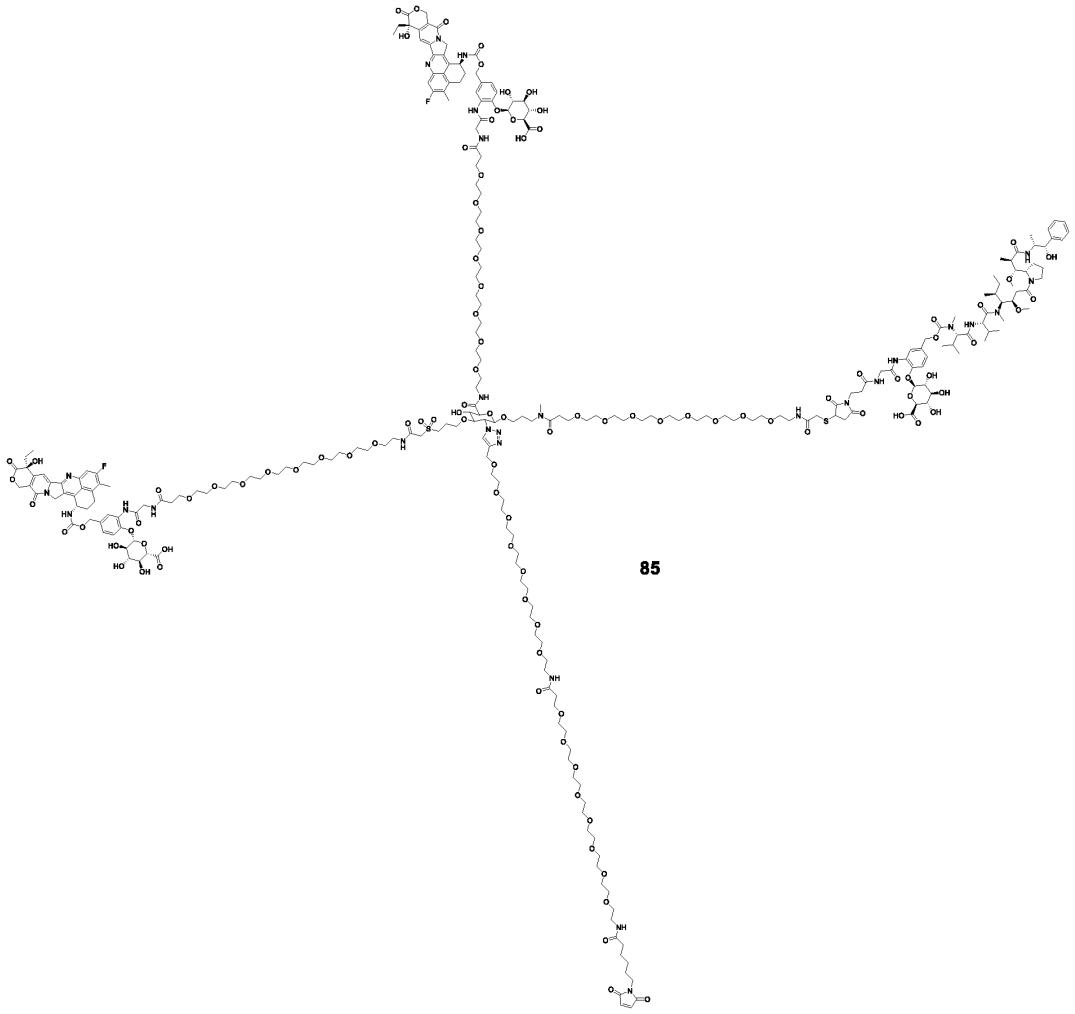
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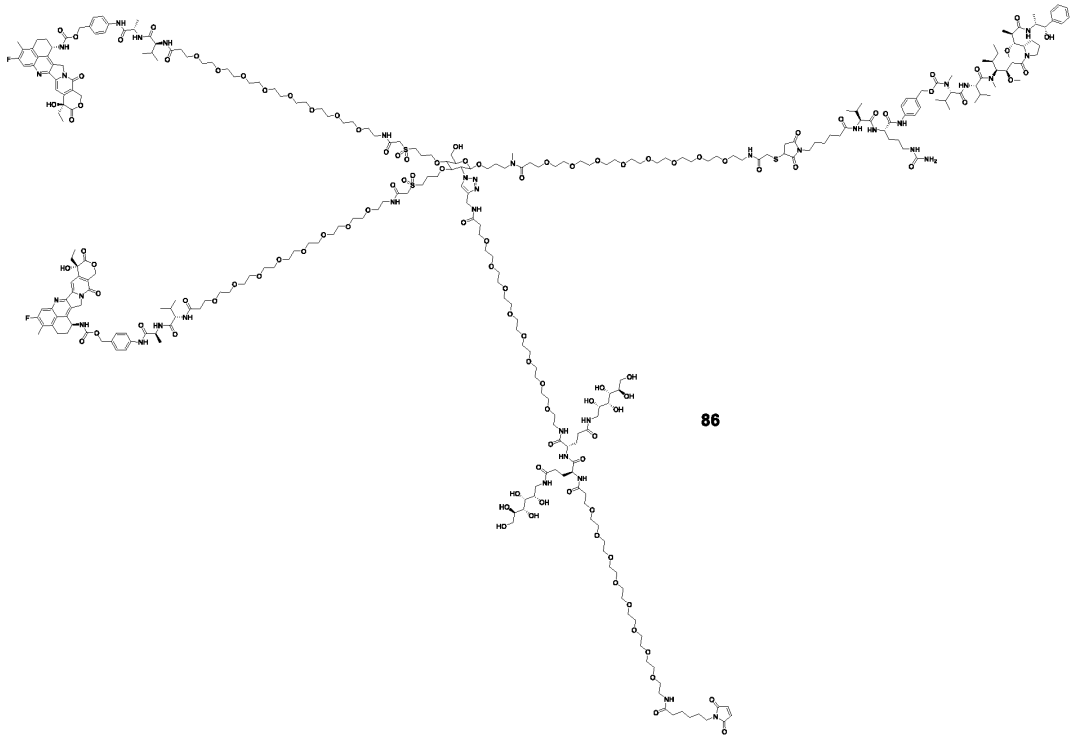
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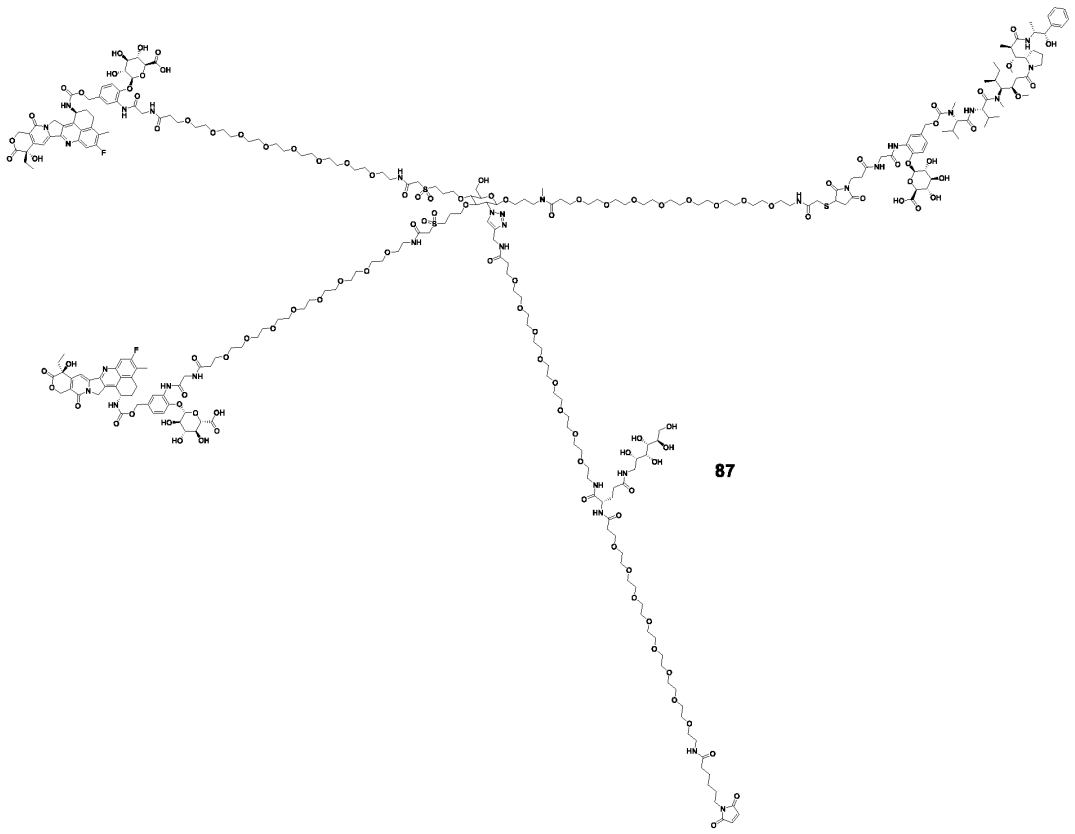
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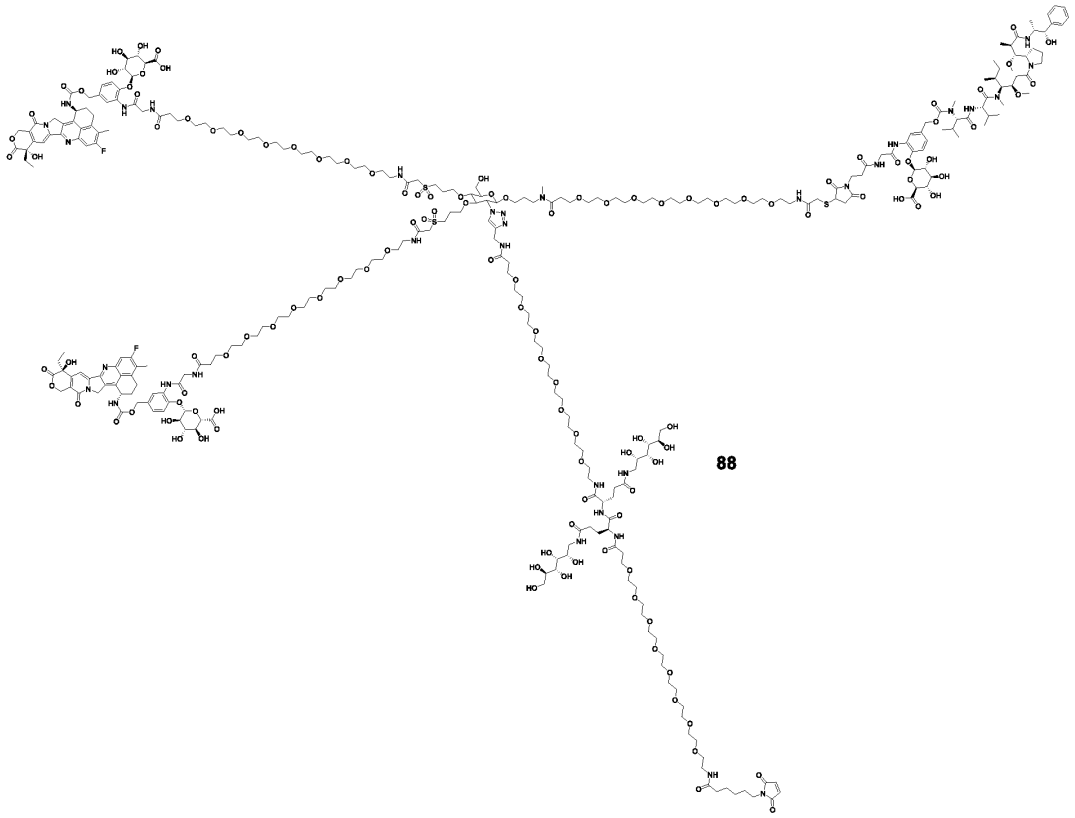
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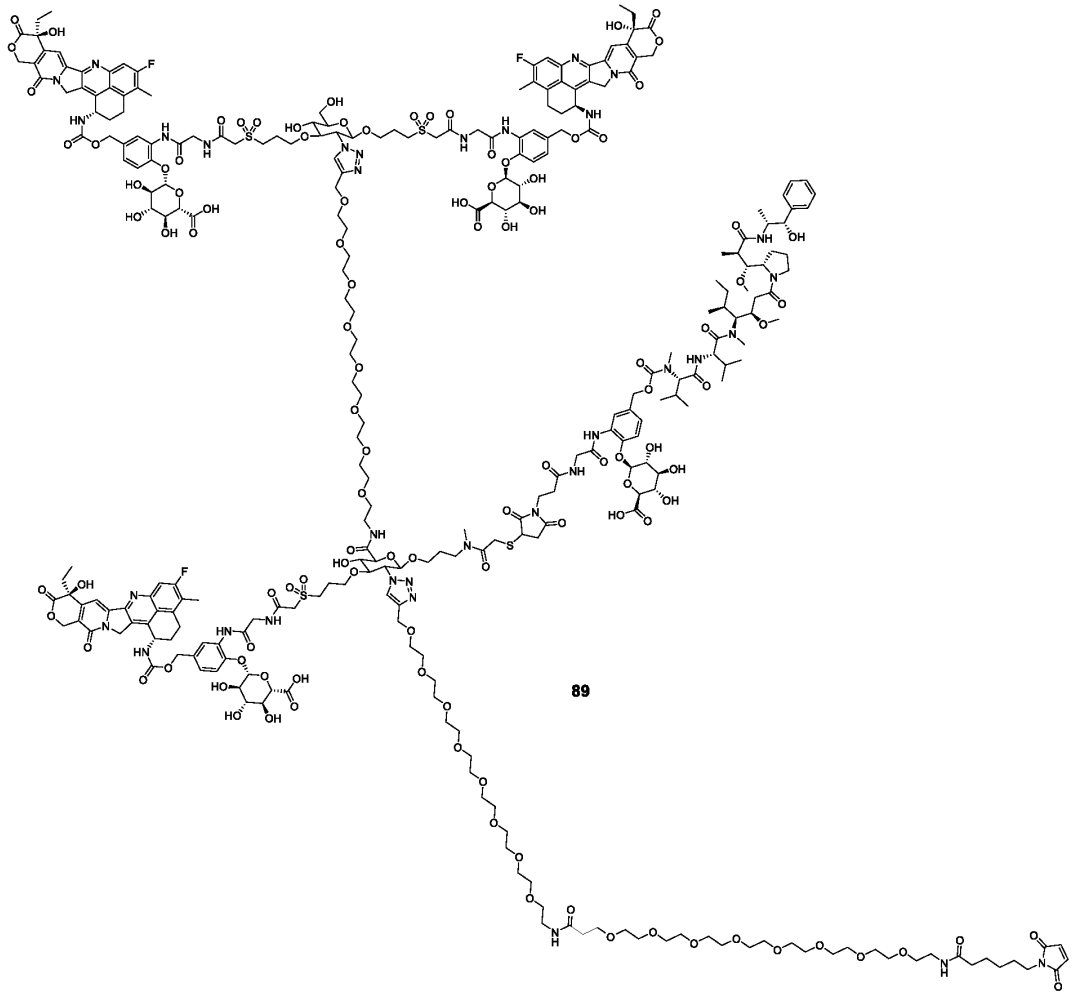
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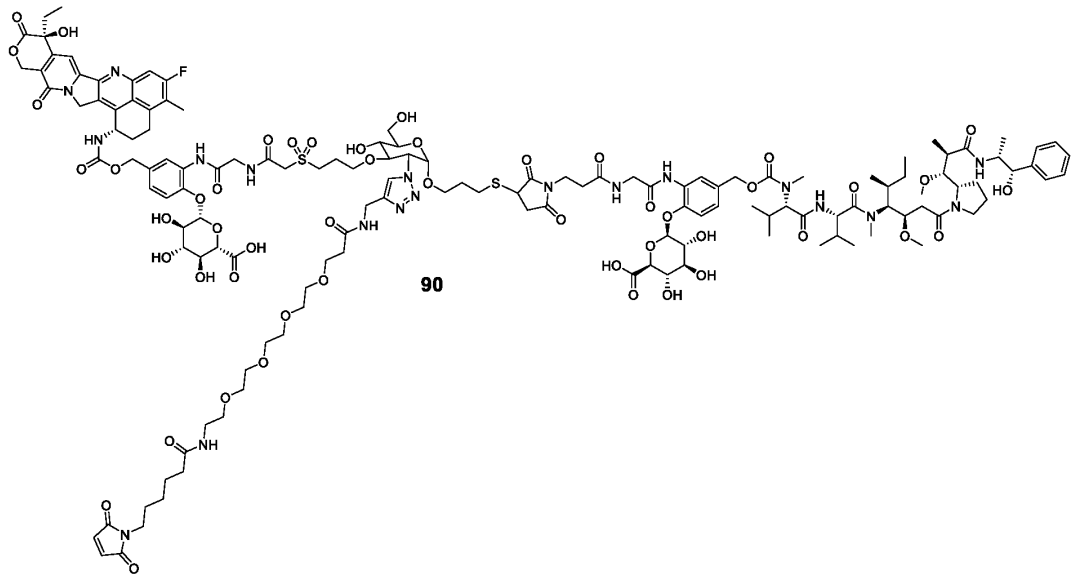
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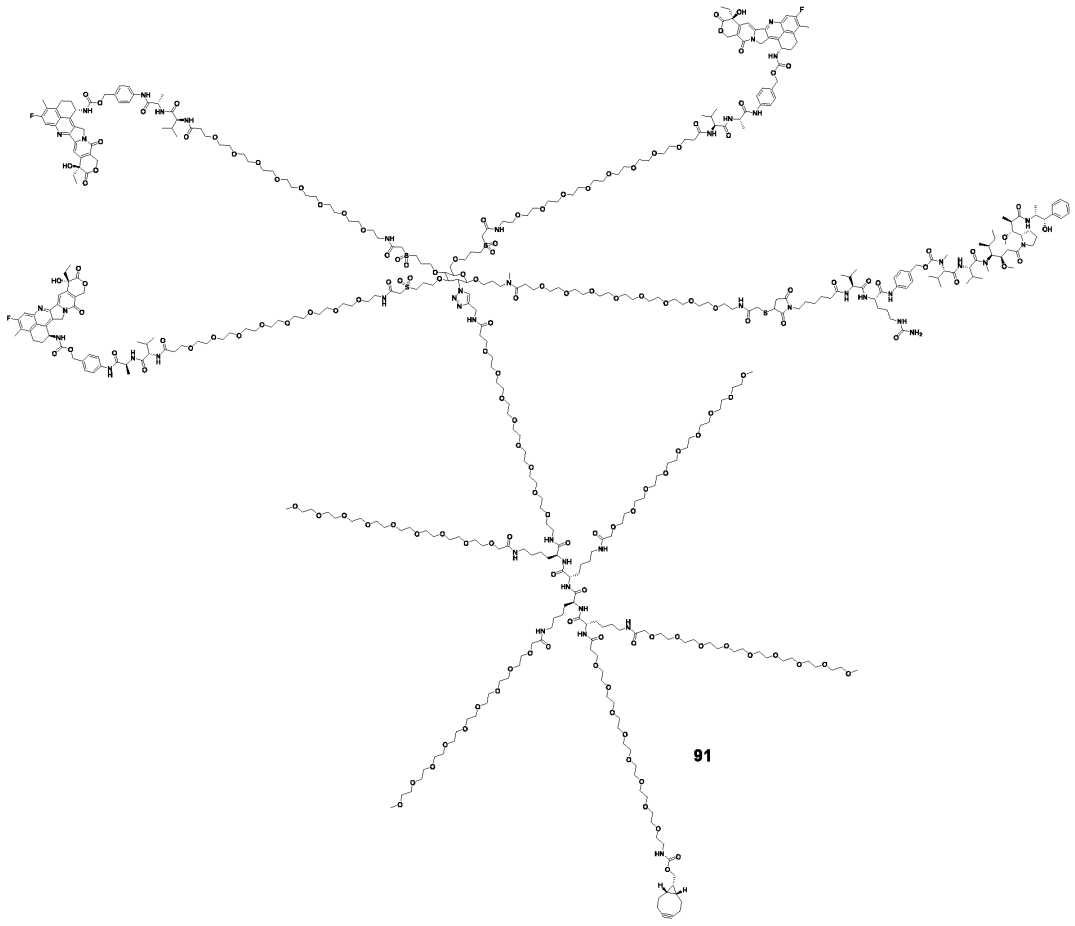
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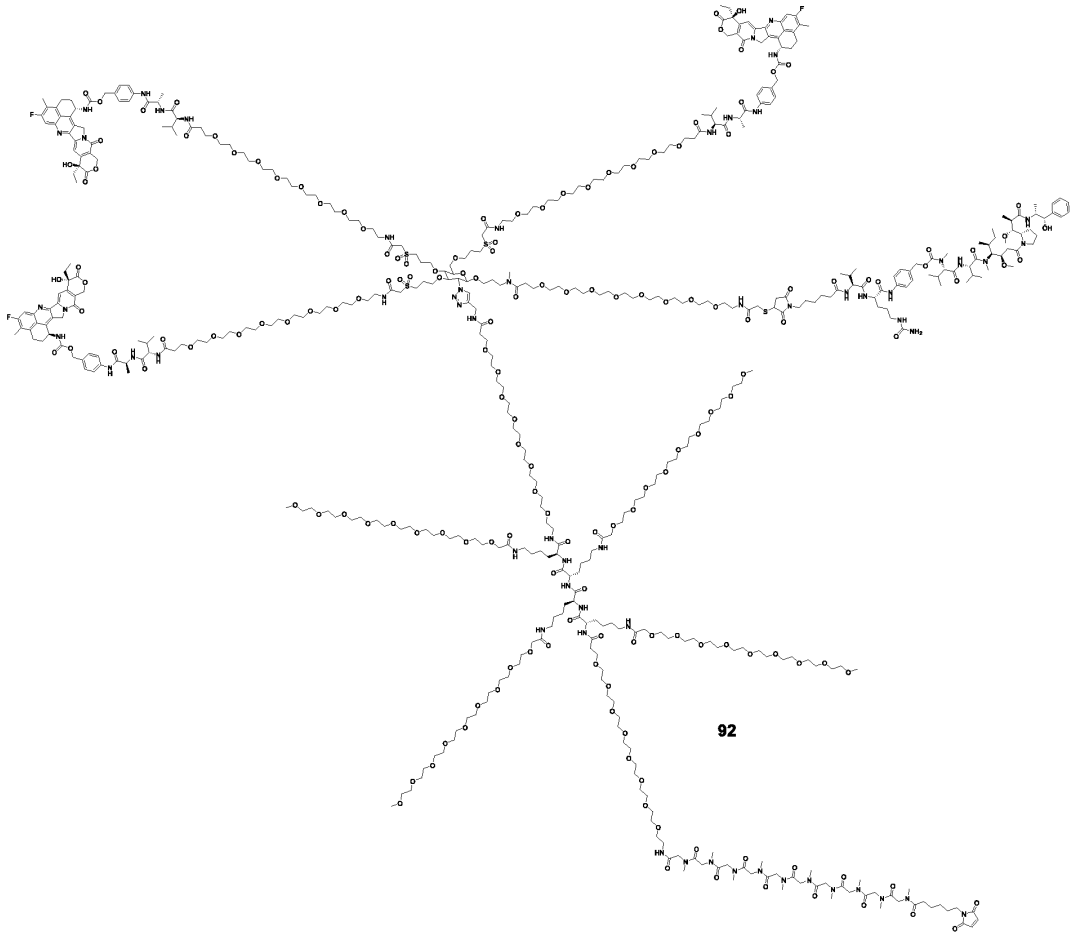
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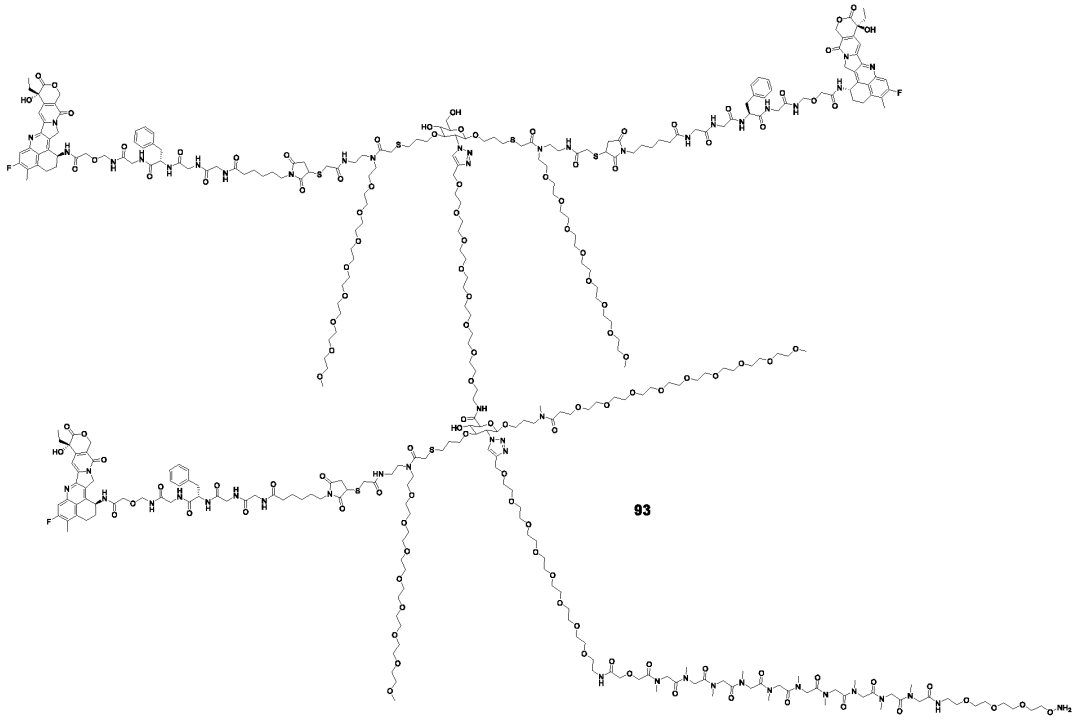
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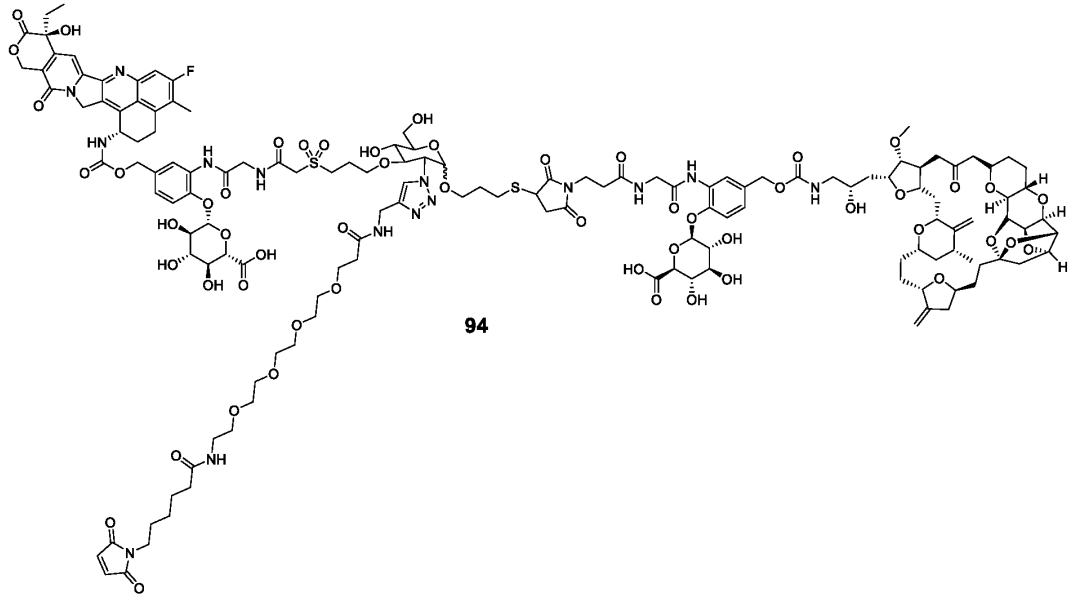
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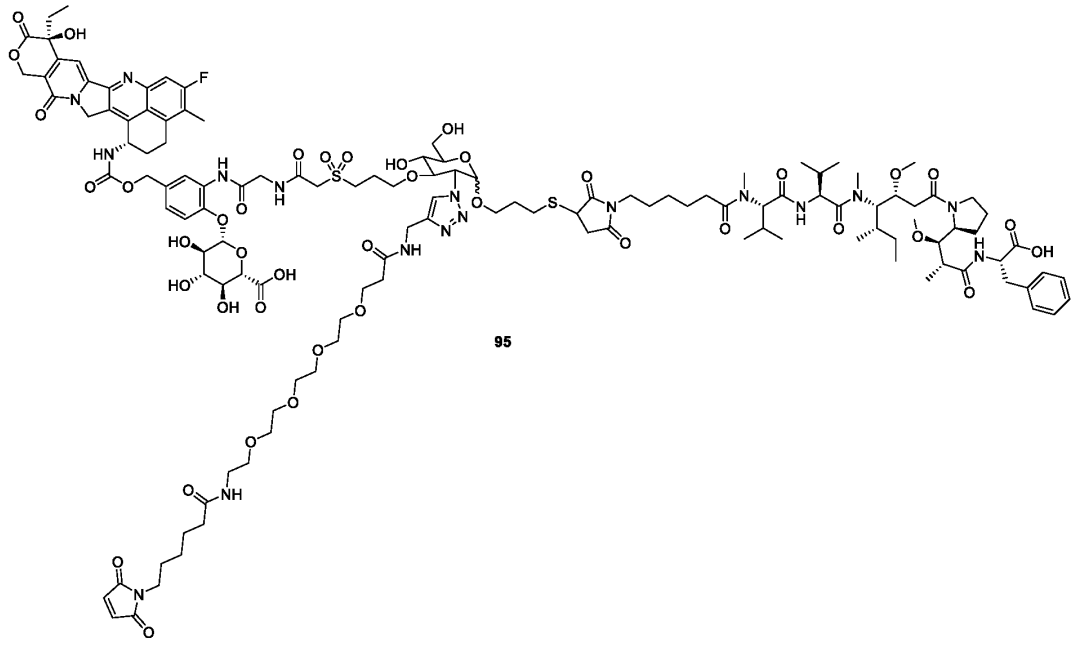
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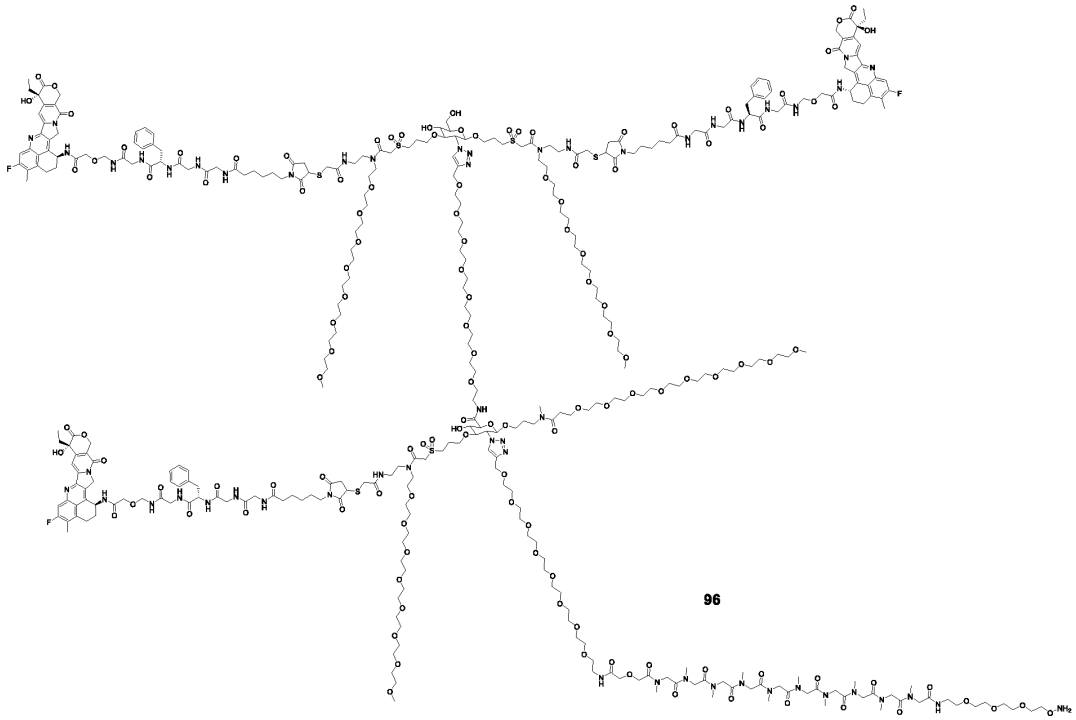
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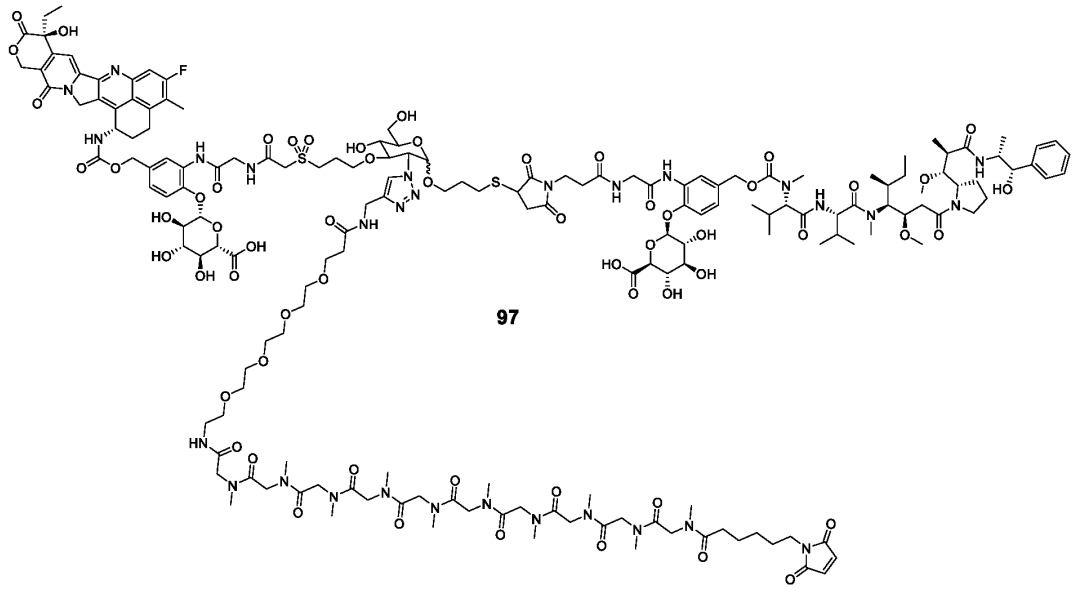
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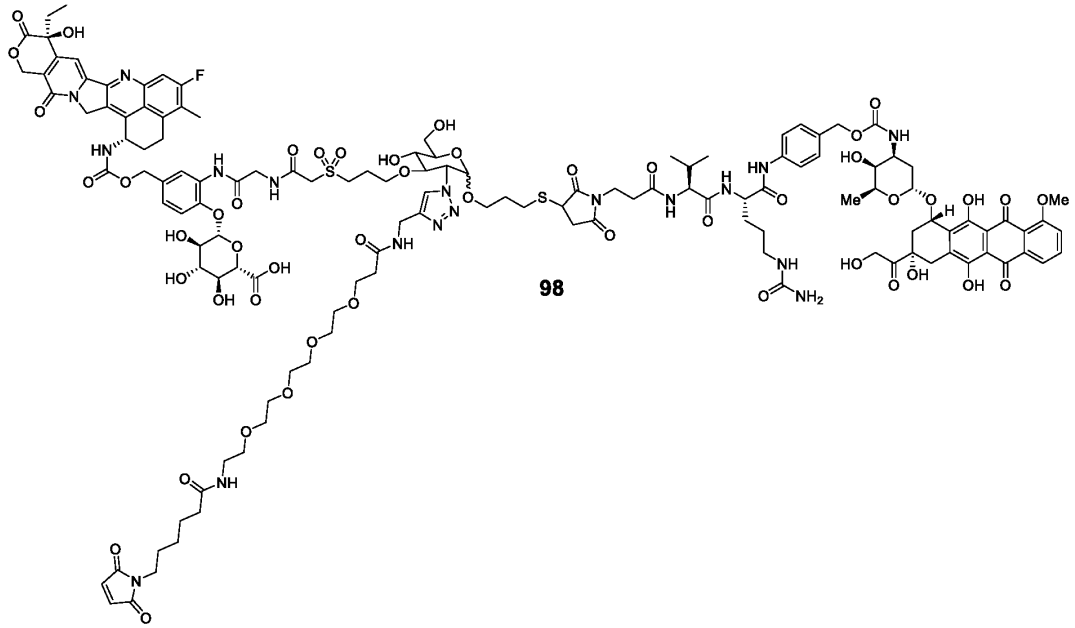
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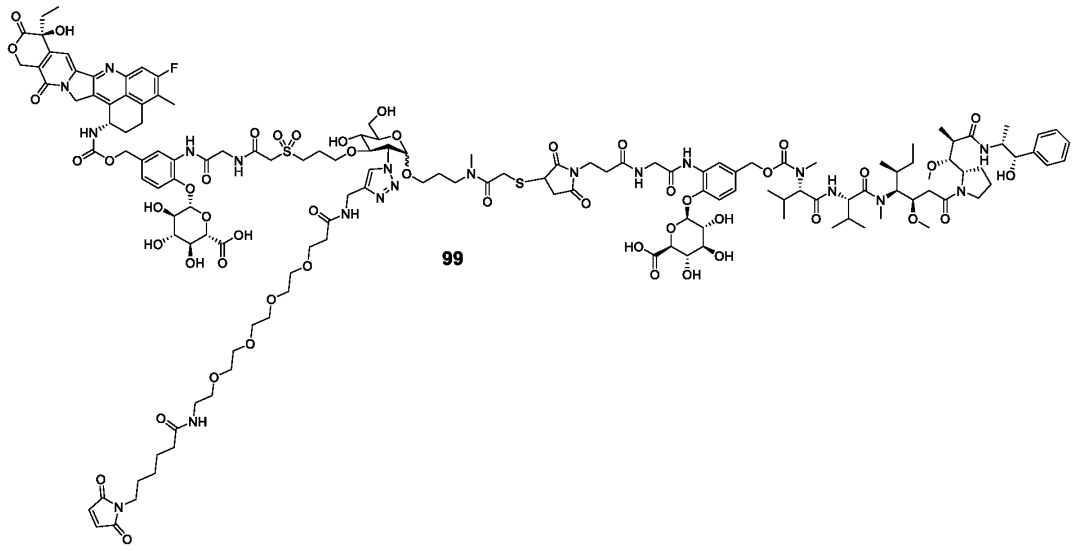
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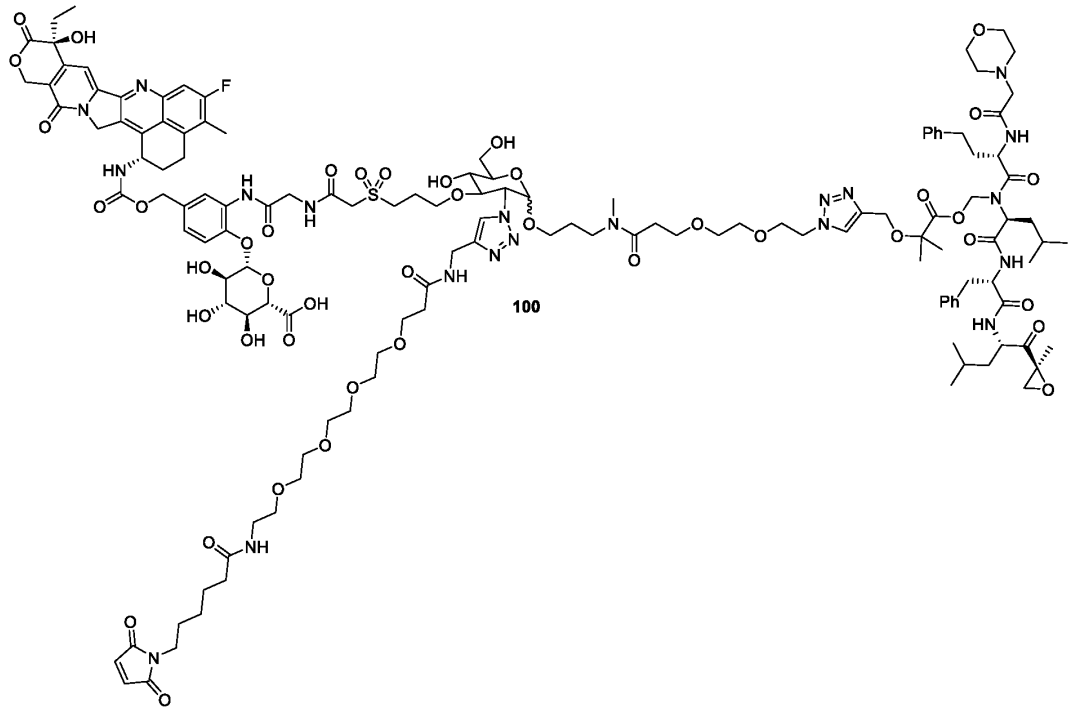
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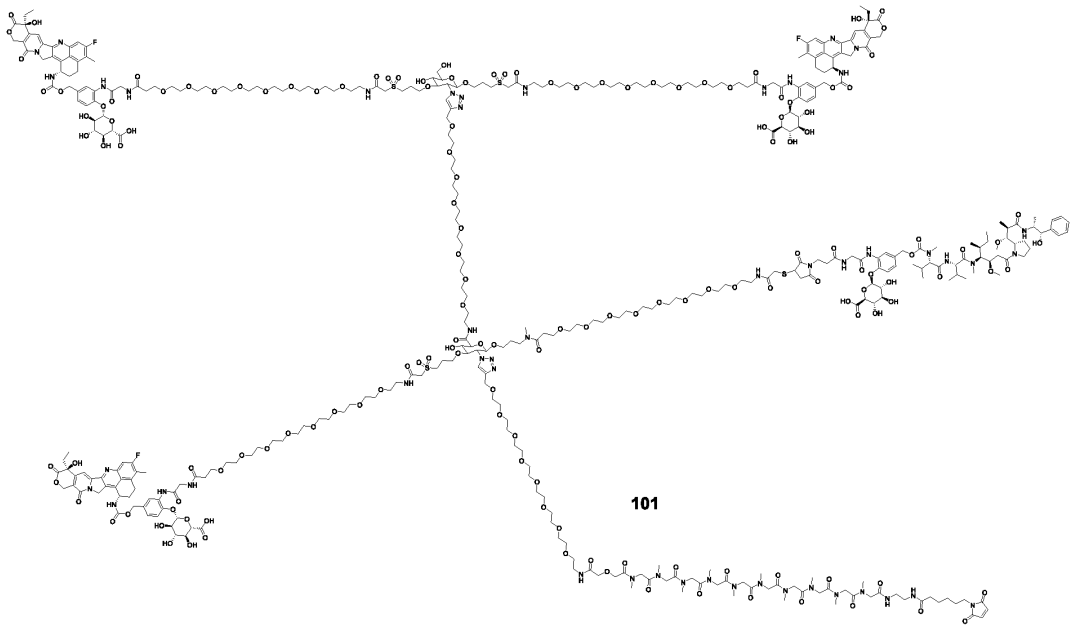
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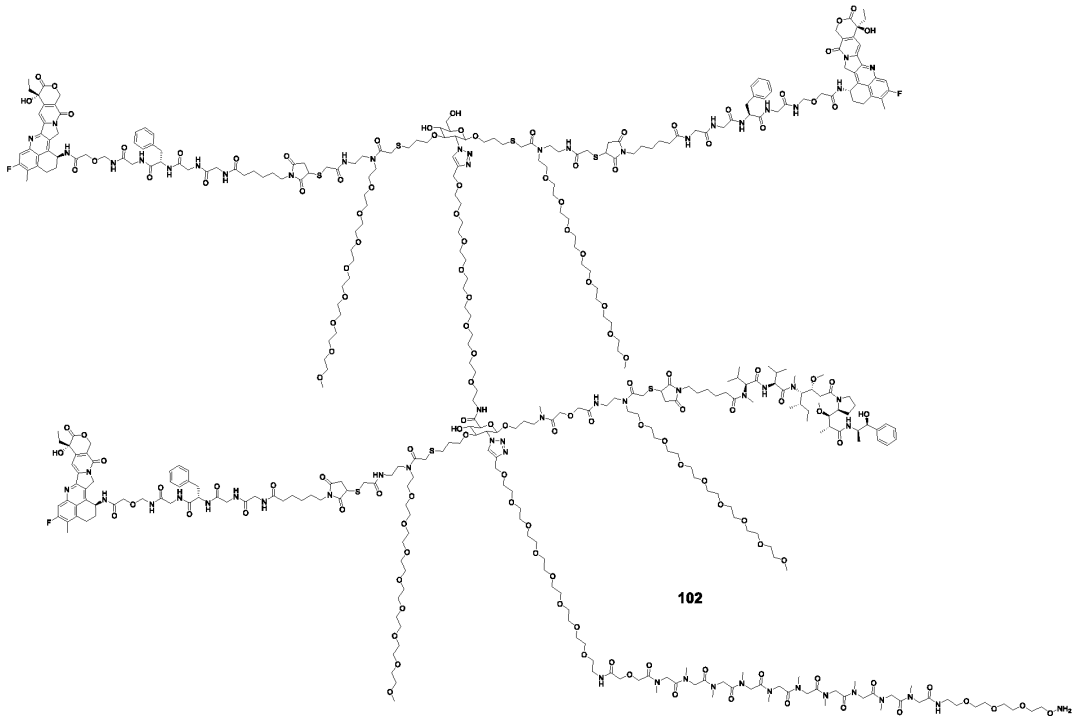
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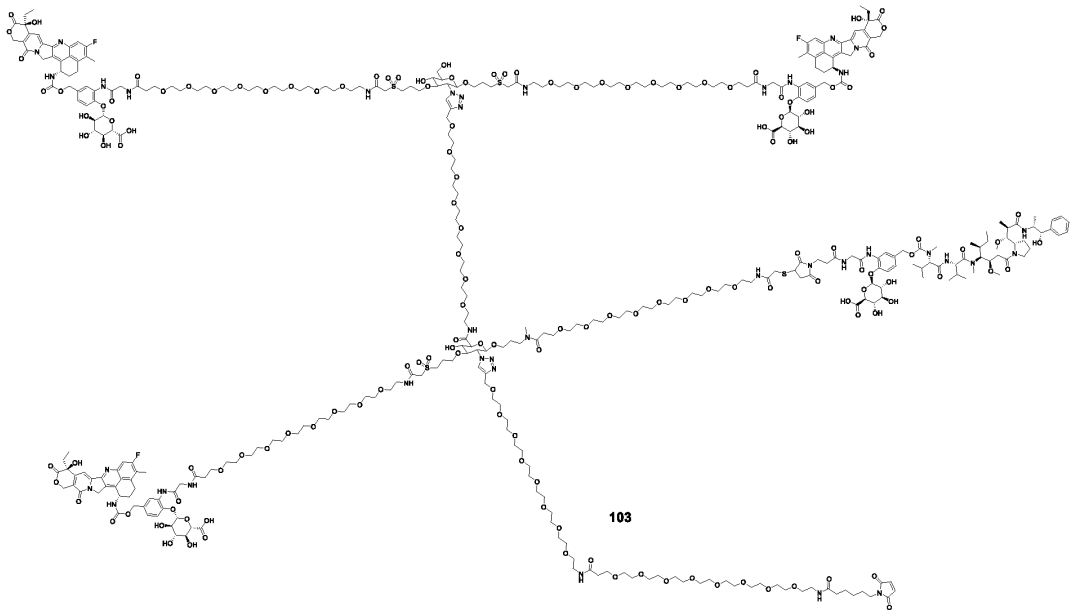
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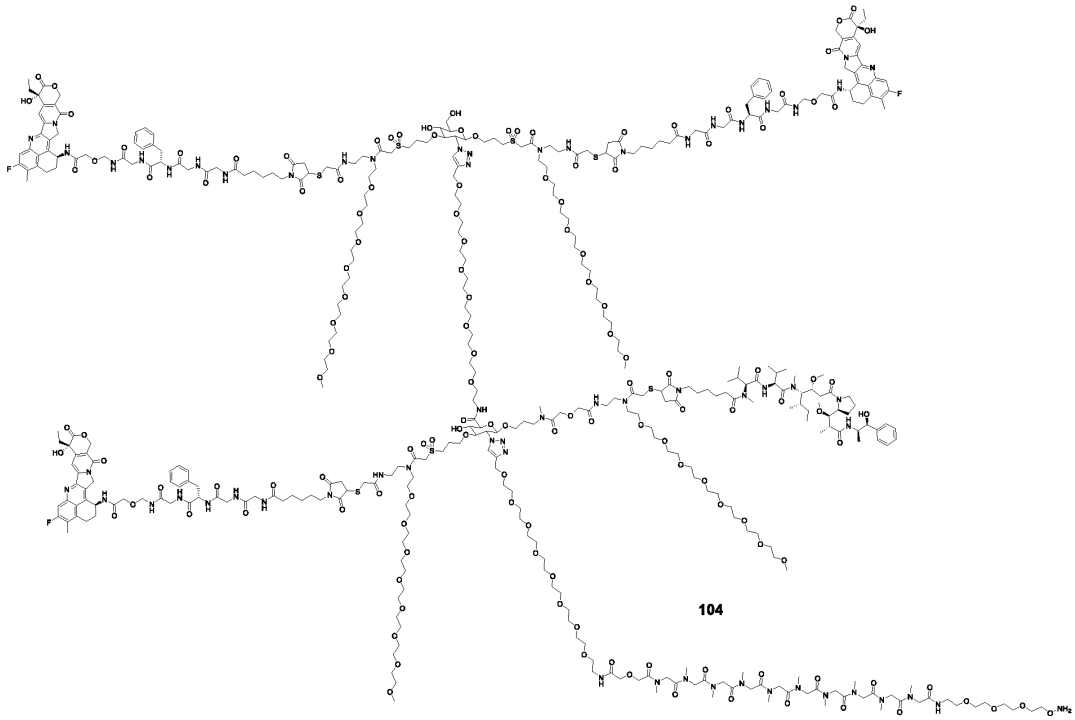
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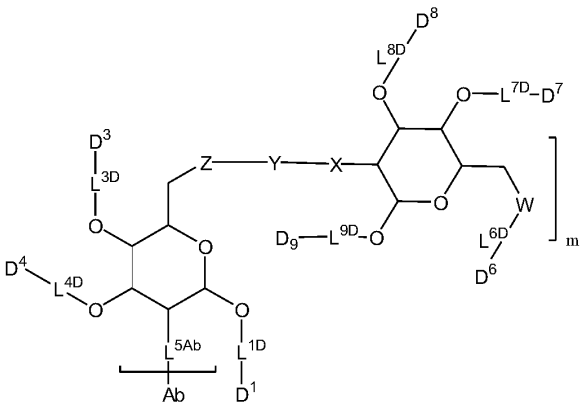


103



104

[00321] In yet another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**XII**):



(XII)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups;

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

each of X and Z is independently a linking group;

Y is a hydrophilic spacer group; and

m is an integer selected from 1-8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

[00322] In certain embodiments of (**XII**), W is O.

[00323] In certain embodiments of (**XII**), W is N.

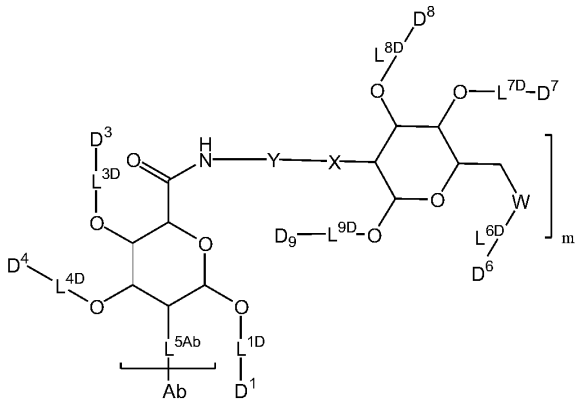
[00324] In certain embodiments of (**XII**), each of X and Z is independently comprises a triazolyl group.

[00325] In certain embodiments of (**XII**), Y comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00326] In another aspect, the invention generally relates to a pharmaceutical composition comprising an antibody-drug conjugate of formula (**XII**).

[00327] In yet another aspect, the invention generally relates to a method for treating, reducing, or preventing a disease or disorder, comprising administering to a subject in need thereof an antibody-drug conjugate of formula (**XII**).

[00328] In yet another aspect, the invention generally relates to an antibody-drug conjugate having the structural formula (**XII'**):



(XII')

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

X is a linking group;

Y is a hydrophilic spacer group;

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

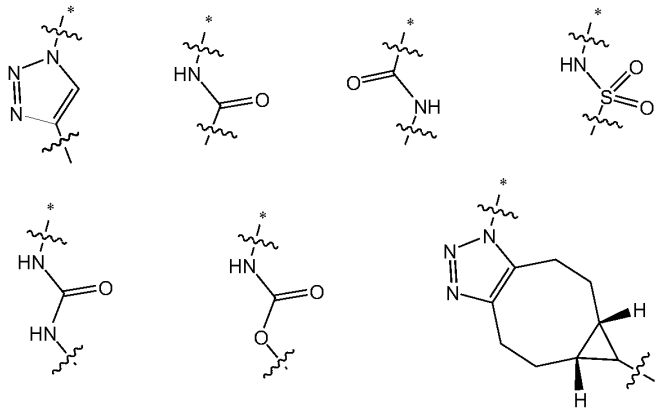
[00329] In certain embodiments of (**XII'**), W is O.

[00330] In certain embodiments of (**XII'**), W is N.

[00331] In certain embodiments of (**XII'**), each of X and Z is independently comprises a triazolyl group.

[00332] In certain embodiments of (**XII'**), Y comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00333] In certain embodiments of (**XII**)-(**XII'**), L^{5Ab} comprises a group selected from:



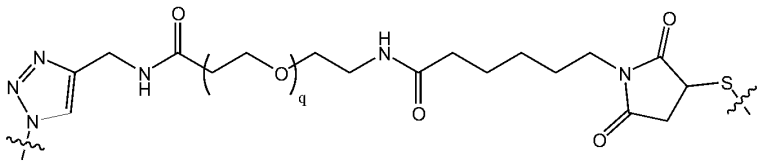
wherein * represents the attachment point to the central sugar ring of Formula (**XII**) or (**XII'**).

[00334] In certain embodiments of (**XII**)-(**XII'**), L^{5Ab} is covalently attached to the Ab via a thioether linkage.

[00335] In certain embodiments of (**XII**)-(**XII'**), L^{5Ab} comprises 1-4 hydrophilic groups.

[00336] In certain embodiments of (**XII**)-(**XII'**), the thioether linkage comprises maleimide thioether.

[00337] In certain embodiments of (**XII**)-(**XII'**), L^{5Ab} is:



wherein q is an integer selected from 1 to 6.

[00338] In certain embodiments of (**XII**)-(**XII'**), each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, is independently selected from chemotherapeutic agents and toxins.

[00339] In certain embodiments of (**XII**)-(**XII'**), D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, are the same.

[00340] In certain embodiments of (**XII**)-(**XII'**), D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, are not the same.

[00341] In certain embodiments of (**XII**)-(**XII'**), D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, represent two distinct drug moieties.

[00342] In certain embodiments of (**XII**)-(**XII'**), Ab is an antibody.

[00343] In certain embodiments of (**XII**)-(**XII'**), Ab is a monoclonal antibody.

[00344] In certain embodiments of (**XII**)-(**XII'**), Ab is a chimeric antibody.

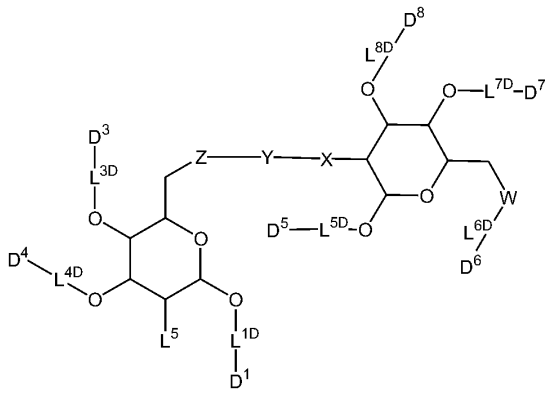
[00345] In certain embodiments of (**XII**)-(**XII'**), Ab is a humanized antibody.

[00346] In certain embodiments of (**XII**)-(**XII'**), Ab is an antibody fragment.

[00347] In certain embodiments of (**XII**)-(**XII'**), Ab is a peptide.

[00348] In certain embodiments of (**XII**)-(**XII'**), Ab binds to a receptor selected from: HER2, HER3, CD7, CD19, CD20, CD22, CD25, CD27, CD30, CD33, CD37, CD38, CD46, CD70, CD71, CD74, CD79b, CD123, CD138, CD142, CD166, CD205, CD228, CCR2, CA6, p-Cadherin, CEA, CEACAM5, C4.4a, DLL3, EGFR, EGFRVIII, ENPP3, PSMA, SLITRK6, STEAP1, TROP2, 5T4, SSEA4, GloboH, Gb5, STn, Tn, B7H3, BCMA, MUC1, cMet, ROR1 MSLN, FRa, CLDN18.2, CLDN6, EphA2, EphrinA, FLOR1, FGFR2, GCC, cKIT, LIV1, LY6E, MSLN, MUC16, NaPi2b, Nectin4, gpNMB, Axl and PTK7.

[00349] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**XIII**):



(XIII)

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

each of X and Z is independently a linking group; and

Y is a hydrophilic spacer group,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

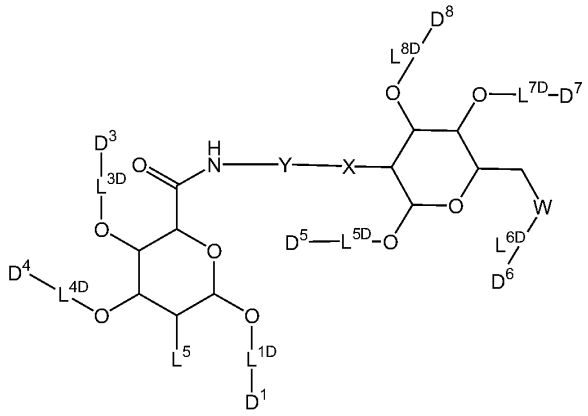
[00350] In certain embodiments of (**XIII**), W is O.

[00351] In certain embodiments of (**XIII**), W is Z.

[00352] In certain embodiments of (**XIII**), each of X and Z is independently comprises a triazolyl group.

[00353] In certain embodiments of (**XIII**), Y comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00354] In yet another aspect, the invention generally relates to a linker-drug conjugate having the structural formula (**XIII'**):



(XIII')

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

X is a linking group; and

Y is a hydrophilic spacer group,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

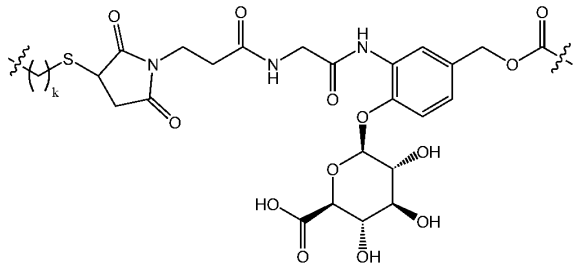
[00355] In certain embodiments of (**XIII'**), W is O.

[00356] In certain embodiments of (**XIII'**), W is Z.

[00357] In certain embodiments of (**XIII'**), each of X and Z is independently comprises a triazolyl group.

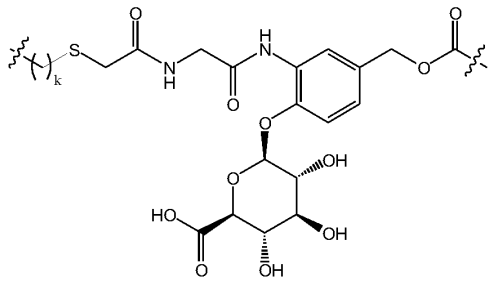
[00358] In certain embodiments of (**XIII'**), Y comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16 (*e.g.*, about 2 to about 6, about 6 to about 12, about 12 to about 16).

[00359] In certain embodiments of (**XIII**)-(**XIII'**), one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



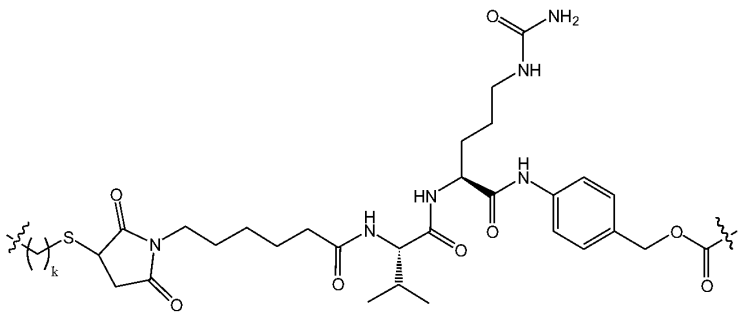
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00360] In certain embodiments of (**XIII**)-(**XIII'**), one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



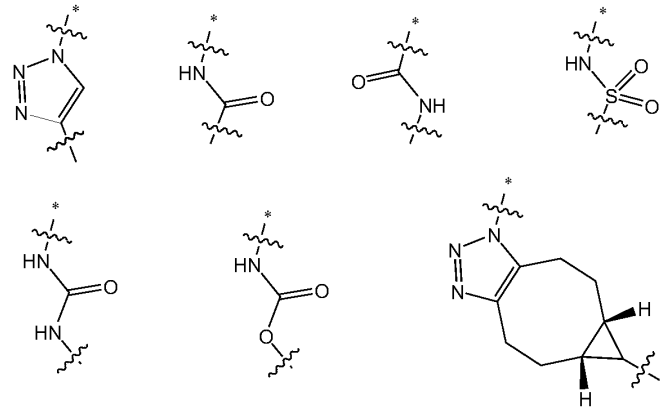
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00361] In certain embodiments of (**XIII**)-(**XIII'**), one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



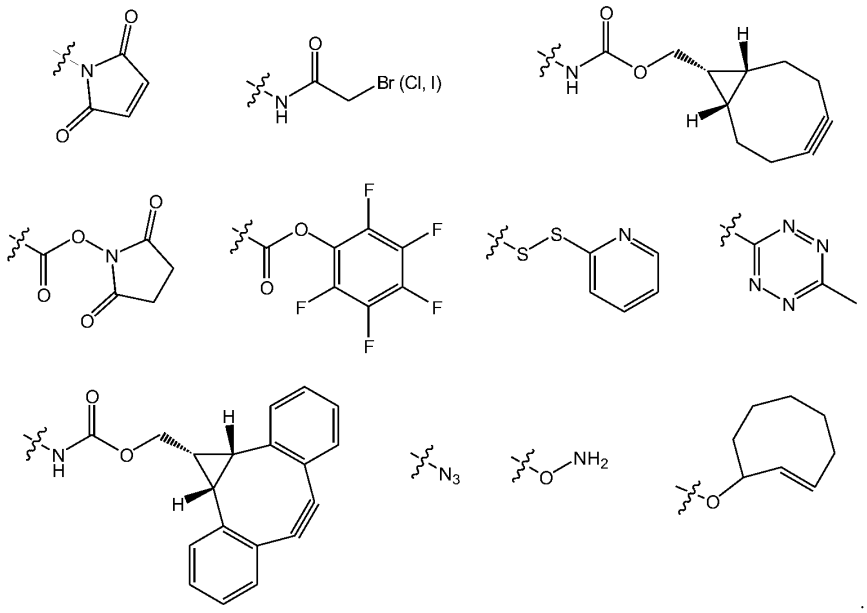
wherein *k* is an integer selected from 1 and 4 (*e.g.*, 1, 2, 3 or 4).

[00362] In certain embodiments of (**XIII**)-(**XIII'**), L^5 comprises a group selected from:



wherein * represents the attachment point to the central sugar ring of Formula (**XIII**) or (**XIII'**).

[00363] In certain embodiments of (**XIII**)-(**XIII'**), L^5 comprises a group selected from:



[00364] In certain embodiments of (**XIII**)-(**XIII'**), L^5 comprises 1-4 hydrophilic groups.

[00365] In yet another aspect, the invention generally relates to a method for making a antibody-drug conjugate or a linker-drug conjugate of (**XII**)-(**XIII'**) disclosed herein.

[00366] The term "drug" or "drug moiety" (D), as used herein, refers to a biologically active agent and can be selected from chemotherapeutic agents and toxins. Each "drug", "drug moiety" or "D" maybe independently selected from:

(a) “drug” of particular interest in this invention are the camptothecin derivatives and metabolites (SN-38, Deruxtecan (Dxd), Exatecan, MMAE, *etc*). Others includes diphtheria toxin, botulium toxin, tetanus toxin, dysentery toxin, cholera toxin, amanitin, o,-amanitin, amanitin derivatives, pyrrolobenzodiazepine, pyrrolobenzodiazepine derivatives, tetrodotoxin, brevetoxin, ciguatoxin, ricin, AM toxin, tubulysin, geldanamycin, maytansinoid, calicheamicin, daunomycin, doxorubicin, methotrexate, vindesine, SG2285, dolastatin, a dolastatin analog, auristatin (such as monomethylauristatin E and monomethylauristatin F), cryptophycin, camptothecin, rhizoxin, a rhizoxin derivative, CC-1065, a CC-1065 analogue or derivative, duocarmycin, an enediyne antibiotic, esperamicin, epothilone, azonafide, aplidine, a toxoid, or a combination of any of the foregoing;

(b) erlotinib, bortezomib, fulvestrant, sutent, letrozole, imatinib mesylate, PTK787/ZK 222584, oxaliplatin, 5-fluorouracil, leucovorin, rapamycin, lapatinib, lonafarnib, sorafenib, gefitinib, AG1478, AG1571, thiotepa, cyclophosphamide, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, ethylenimine, altretamine, triethylenemelamine, trietylenephosphoramide, riethiylenethiophosphoramide, trimethylolomelamine, bullatacin, bullatacinone, amptothecin, topotecan, bryostatin, callystatin, CC-1065, adozelesin, carzelesin, bizelesin, cryptophycin 1, cryptophycin 8,dolastatin, duocarmycin, KW-2189, CB1-TM1, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimnustine, calicheamicin, calicheamicin gamma 1, calicheamicin omega 1, dynemicin, dynemicin A, clodronate, esperamicin, neocarzinostatin chromophore, aclacinomysins, actinomycin, antrmycin, azaserine, bleomycins, cactinomycin, carabicin, carninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubucin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolinodoxorubucin, liposomal doxorubicin, deoxydoxorubicin, epirubicin, esorubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, 5-fluorouracil, denopterin, methotrexate, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thiguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine,

doxifluridine, enocitabine, floxuridine, calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone, aminoglutethimide, mitotane, trilostane, folinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansine, ansamitocins, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, 2-ethylhydrazide, procarbazine, polysaccharide-k, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2', 2"-trichlorotriethylamine, T-2 toxin, verracurin A, roridin A, and anguidine, urethane,vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside, cyclophosphamide, thiotepa, paclitaxel, albumin-engineered nanoparticle formulation of paclitaxel, doxetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, carboplatin, vinblastine, platinum, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, CPT-11, topoisomerase inhibitor RFS 2000, difluoromethylornithine, retinoic acid, capecitabine, or pharmaceutically acceptable salts, solvates or acids of any of the foregoing;

(c) an affinity ligand, wherein the affinity ligand is a substrate, an inhibitor, a stimulating agent, a neurotransmitter, a radioisotope, or a combination of any of the foregoing;

(d) a radioactive label, "P, "S, a fluorescent dye, an electron dense reagent, an enzyme, biotin, streptavidin, dioxigenin, a hapten, an immunogenic protein, a nucleic acid molecule with a sequence complementary to a target, or a combination of any of the foregoing;

(e) an immunomodulatory compound, an immune-stimulating compound, such as Toll-like receptor (TLR) agonist, Stimulator of interferon genes (STING) agonists, an anti-cancer agent, an anti-viral agent, an anti-bacterial agent, an anti-fungal agent, and an anti-parasitic agent, or a combination of any of the foregoing;

(f) tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, or toremifene;

(g) 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, letrozole, or astrozole;

(h) flutamide, nilutamide, bicalutamide, leuprolide, goserelin, or troxacitabine;

(i) an aromatase inhibitor;

(j) a protein kinase inhibitor;

(k) a lipid kinase inhibitor;

(l) an antisense oligonucleotide;

(m) a ribozyme;

(n) a vaccine; and

(o) an anti-angiogenic agent;

(p) CDK4/6 inhibitors, ERK inhibitors, BCL2 inhibitors, CDK7 inhibitors, CDK9 inhibitors, FAK inhibitors;

(q) rapamycin, SF1670, 3-methyladenine, CHIR-99021, LY294002, MELK-8a, AZ20, everolimus, GSK2334470, BX-912, BGT226, SB216763, metformin, ETP-46464, CP-466722, BX517, AS-605240, AZD6482, TGX-221, HS-173, PKI-402, GSK1059615, WYE-354, PP121, dihydromyricetin, torkinib, salidroside, temsirolimus, 3BDO, TDZD-8, cromolyn, kenpaullone, indirubin-3'-monoxime, wortmannin, honokiol, quercetin, MHY1485, AICAR, AZD2858, dorsomorphin, perifosine, TG100-115, BX795, GNE-317, VO-Ohpic, AR-A014418, SB415286, IM-12, bikinin, A-769662, GSK690693, phenformin, WZ4003, chitosan oligosaccharide, 7-methoxyisoflavone, flufenamic acid, guggulsterone, triciribine, isobavachalcone, deguelin, A-674563, miltefosine, recilisib, 1-deoxynojirimycin, artemisinin, SU6656, JQ-1 carboxylic acid, birabresib, Cl-amidine, acetaminophen, A-485, TPOP146, trichostatin A, AT9283, 5-Azacytidine, JIB-04, GSK-J4, BIX01294, gandotinib, PF-03814735, SNS-314, ZM-447439, NU6140, RG108, SGI-1027, 6-thioguanine, zebularine, hinokitiol, IOX1, romidepsin, resveratrol, PMA, XAV-939, decitabine, UNC0642, selisistat, corin, valproic acid, curcumin, 3-deazaneplanocin A, GSK484, C646, daminozide, procaine, GSK-J2, SGC0946, amodiaquine, EPZ020411, UNC1999, AZ505, NSC663284, MM-102, A-366, SGC707, PFI-2, UNC0379, cucurbitacin I, FLLL32, XL019, atractylenolide I, LFM-A13, resatorvid, IKK16, ascomycin, rimiducid, verteporfin, tacrolimus, SB203580, MG-132, silvestrol, bafilomycin A1, deferoxamine, U0126, cisplatin, forskolin, fulvestrant, indomethacin, venetoclax, MRT67307, CCCP, brefeldin A, spautin-1, nutlin-3a, pifithrin-α, mycro 3, Z-VAD-FMK, erastin, GDP366, CID755673, disulfiram, trabectedin, SP600125, jaceosidin, AZD5582, ferrostatin-1, NADPH,

BV6, embelin, necrostatin-1, GSK872, raltitrexed, MX69, GSK583, tipiracil, emricasan, eribulin, pyridostatin, PD0166285, NMS-873, GSK2656157, CCT020312, RS-1, RI-1, CHR-6494, BIBR1532, 2OH-BNPP1, monastrol, rocaglamide, 4E1RCat, pemetrexed, retinoic acid, rosiglitazone, GW9662, tanespimycin, STF-083010, 4μ8C, Kira8, temozolomide, geldanamycin, BAY117082, MPI-0479605, IPA-3, troglitazone, GW843682X, AZ3146, fingolimod, NVP-2, streptozocin, teprenone, PJ34, GW6471, seliciclib, nocodazole, GRL0617, PR-619, G-5555, isoliquiritigenin, AP-III-a4, RO-28-1675, conduritol B epoxide, DMOG, GSK864, eplerenone, diphenyleneiodonium, shikonin, cyclosporin A, GSK650394, 5-iodotubercidin, U-73122, pluriSIn1, pentostatin, clozapine N-oxide, indisulam, 3-bromopyruvic acid, GSK2837808A, SR-18292, GSK2795039, acetylcysteine, RSL3, dutasteride, ibudilast, WWL70, biochanin A, talfirastide, bortezomib, doxycycline, ZLN005, finasteride, NIM811, bucladesine, mitoquinone, LY2183240, MLN-4760, (E)-daporinad, leupeptin, E-64, GW4064, atazanavir, acetazolamide, 2-deoxy-D-glucose, methoxy-PMS, lonidamine, FX-11, tipifarnib, nelfinavir, rotenone, fexaramine, Bay607550, ilomastat, L-ascorbic acid, tipranavir, talabostat, (R)-GNE-140, Mito-TEMPO, PX-478, GNF-7, trastuzumab, PCI29732, piceatannol, BAY613606, GW2580, Ki20227, 2-D08, SGI-7079, cetuximab, bevacizumab, semaxinib, A83-01, NVP-BHG712, NVP-TAE226, AST487, PHA-665752, dihexa, ANA-12, K-252a, motesanib, ISCK03, AGL-2263, kaempferitrin, ibrutinib-biotin, SU5402, PD173074, cediranib, Y15, staurosporine, H-89, bosutinib, AG490, heparan, BI-4020, genistein, SU11274, X-376, TCS359, picropodophyllin, BMS-536924, AZ-23, amitriptyline, GNF-5837, tyrphostin AG879, harmine, AZ191, tBID, ID-8, AG1024, GSK1904529A, PQ401, PP2, AG-1478, NSC228155, mubritinib, pelitinib, butein, PD168393, ADU-S100, quiflapon, L-NAME, MCC950, CH-223191, H-151, ST2825, BMS-1, vadimezan, pirfenidone, maraviroc, celecoxib, aspirin, clemastine, famotidine, astemizole, osthole, pitolisant, cimetidine, ebastine, cetirizine, motixafortide, NBI-74330, UNBS5162, ginsenoside Rb1, nigericin, chloroquine, ibuprofen, tapinarof, limaprost, INCB3344, SB225002, H2DCFDA, trolox, berberine chloride, TPEN, lycopene, zinc protoporphyrin, tempol, α-vitamin E, N-acetylcysteine amide, berberine, rhein, diphenhydramine, AMD3465, ALX404C, Y-27632,

WAY316606, GANT61, IWR-1, purmorphamine, IWP-2, chroman1, DAPT, JK184, LY-411575, cyclopamine, itraconazole, curculigoside, FM-381, 8-Bromo-cAMP, fasudil, HA-100, E2012, L-685458, γ-Secretase-IN-1, semagacestat, ripasudil, D4476, Y-33075, IMR-1, tangeretin, narciclasine, GSK429286A, SR-3677, alantolactone, stattic, fludarabine, niclosamide, AS1517499, C188-9, napabucasin, BP-1-102, nifuroxazide, ellagic acid, PF-670462, RSV604, ST-193, mizoribine, lopinavir, ivermectin, ribavirin, puromycin aminonucleoside, galidesivir, caspofungin, tunicamycin, OSS_128167, entecavir, alisporivir, tenofovir, bay414109, EIDD-1931, ganciclovir, oseltamivir acid, ceftaroline fosamil, maribavir, geneticin, rupintrivir, brequinar, α-mangostin, vancomycin, hygromycin B, merimepodib, suramin, nystatin, lamivudine, delamanid, emtricitabine, efavirenz, dalbavancin, salinomycin, amphotericin B, favipiravir, tigecycline, metronidazole, squalamine, kanamycin, ritonavir, azithromycin, ampicillin, gentamicin, iclaprim, trimetrexate, auranofin, neomycin, aprotinin, sutezolid, anisomycin, PIM-447, AZD1208, hispidulin, TCSPIM-11, quercetagetin, SGD-1882, SG2057, SG3199, tomaymycin DM, AcBut-N-Ac-γ-calicheamicin, polyketomycin, duocarmycin TM, duocarmycin MB, DC1SMe, DC1, DC41SMe, DC41, piericidin A, hemiasterlin, TH1338, mensacarcin, DM4, PF-06380101, DRF-1042, Top1 inhibitor1, N-Me-L-Ala-maytansinol, azonafide-PEABA, DMEA-PNU-159682, PNU-159682 carboxylic acid, dimethyl-SGD-1882, Luisol A, duocarmycin GA, (+)-CBI-CDPI1, (+)-CBI-CDPI2, tubulysin, glycyl-Exatecan-d5, seco-duocarmycin SA, (S)-seco-duocarmycin SA, DC0-NH_2, thailanstatin A, DM3, seco-duocarmycin TM, Daun02, tubulysin IM-1, tubulysin IM-2, tubulysin IM-3, agrochelin, diacetyl agrochelin, 17-GMB-APA-GA, mytoxin B, muscotoxin A, glucocorticoid receptor agonist-1, 7-aminomethyl-10-methyl-11-fluoro camptothecin, 7-MAD-MDCPT, Gly-7-MAD-MDCPT, seco-DUBA, corixetan, hygrolidin, glucopiericidin A, 17-AEP-GA, aminohexylgeldanamycin, PROTAC BRD4 degrader-5-CO-PEG3-N3, DC10SMe, DC4SMe, DC44SMe, DC4, budesonide, aniline-mpb-amino-C3-PBD, py-MPB-amino-C3-PBD, exatecan-d5, (1S,9R)-exatecan, (1R,9R)-exatecan, gly-gly-phe-gly-NH-O-CO-exatecan, AGD-0182, SC209, hemiasterlin derivative-1, duostatin5, (R)-methotrexate-d3, methotrexate-d3, (4-NH_2)-exatecan, (R)-(4-NH_2)-exatecan, (4-

NH_2)-exatecan-d5, debotansine, HDP30.1699, SJG-136, dexamethasone-d3-1, INX-SM-6, INX-SM-56, rezetecán, TAM470, T01-1, tubulin polymerization-IN-38, L2H2-6OTD, dideoxy-amanitin, PBD dimer-2, glucocorticoid receptor agonist-2, gly-cyclopropane-exatecan, S-Me-DM4, NH_2-bicyclo[1.1.1]pentane-7-MAD-MDCPT, maytansinoid B, STING agonist-18, exatecan-amide-cyclopropanol, anticancer agent81, MMAE-d8, decyclohexanamine-exatecan, Benzyl DC-81, Dox-btn2, MMAF-d8, dolastatin10, MMAD, auristatin E, auristatin F, taltobulin, E104, MMAF-OtBu, NMS-P528, 7-hydroxyethyl carbamate-(10Me-11F-camptothecin), $10NH_2$-11F-camptothecin, 7ethanol-$10NH_2$-11F-camptothecin, cyclopropaneacetamide-exatecan, (αR)-cyclopropaneacetamide-exatecan, (1-OH)-exatecan, NH_2-methylpropanamide-Exatecan, 7-hydroxymethyl-10,11-MDCPT, Akt/ROCK-IN-1, exatecan-methylacetamide-OH, FL118-14-propanol, Eg5-IN-2, ansamitocin P-3, mycobacterium tuberculosis-IN-2, exatecan analogue1, O-Me eribulin, mal-exatecan, tubulysin A, AXC-666, glucocorticoid receptor agonist-3, glucocorticoid receptor agonist-4, TAM557, exatecan-amide-CH_2-O-CH_2-CH_2-OH, exatecan-amide-bicyclo[1.1.1]pentan-1-ol, exatecan-amide-bicyclo[1.1.1]pentan-1-ylmethanol, (5-Cl)-exatecan, NMT-IN-7, PBD-monoamide, BTK degrader-1, MYX1715, N-MeVal-Val-Dil-Dap-2-(pyridin-3-yl)ethanamine, glucocorticoid receptor agonist-5, camptothecin-d5, PNU-159682, 7-Methoxy-9-methylfuro[2,3-b]-quinoline-4,5,8(9H)-trione, Duocarmycin MA, maytansinol, RNA polymerase II-IN-2, N-acetyl-calicheamicin, mertansine, rebeccamycin, isofistularin-3, sandramycin, 10-deacetyl-7-xylosyl paclitaxel, TAM558, (rac)-exatecan, (R)-exatecan, 7-Epi10-desacetyl paclitaxel, Dap-NE, MMAF-OMe, Dov-Val-Dil-OH, Ac-exatecan, Ac-exatecan-d5, fluticasone, Cy3.5 maleimide, tubulysin E, tubulysin C, tubulysin D, tubulysin F, tubulysin G, tubulysin H, tubulysin I, tubulysin M, dolastatin15, suicin65, Bac5(1-25), MMAF-methyl ester, daunorubicin-13C,d3, budesonide-d6, DeBoc-cabazitaxel, bevonescein, CTCE-9908, DS-437, topotecan, belotecan, lurtotecan, gimatecan, rubitecan, diflomotecan, silatecan, karenitecin, BNP1350, cositecan, AR-67, ST1481, DX-8951f, CKD-602, TLC388, IDN-5174, olaparib, talazoparib, veliparib, pamiparib, fluzoparib, CEP-9722, E7016, INO-1001, AZD2461, 3-AB, BSI-201, GPI-21016, MP-124, UPF-1069, AZD5305, berzosertib, camonsertib, AZD6738,

BAY-1895344, M4344, ART-0380, RP-3500, ATRN-119, AZD0156, VE-821, VE-822, NU6027, CCT245737, VX-803, M1774, GDC-0575, prexasertib, MK-8776, AZD7762, GDC-0425, PF-00477736, SRA737, LY2603618, AR323, AR678, S1181, BML-277, TAS-116, adavosertib, ZN-c3, IMP7068, Debio 0123, SY-4835, LY3405105, CGM097, GSK-2830371, nedisertib, VX-984, NU7441, CC-115, CC-122, CC-223, CC-292, CC-486, AZD7648, M9831, MSC2490484A, KU-60648, NU7026, IC87361, Novobiocin, CRT-0044876, NCT-501, PolQi1, PolQi2, mitoxantrone, teniposide, amsacrine, idarubicin, valrubicin, pixantrone, NK314, HU-331, MST-16, D3CLP, TAS-103, ICRF-193, sobuzoxane, ellipticine, F11782, XK469, S63845, AMG-176, AZD5991, MIK665, S64315, AMG-397, A-1210477, UMI-77, VU661013, AZD4320, AZD0466, ABBV-467, PRT1419, PRT2527, DS-3032b, AMG-511, S65487, navitoclax, ABT-737, S55746, APG-1252, APG-2575, BGB-11417, LP-118, BM-1074, PNT2258, AT-101, obatoclax, sabutoclax, TW-37, ABT-751, A-1331852, A-1155463, cytarabine, capecitabine, cladribine, nelarabine, hydroxyurea, azacitidine, clofarabine, tegafur, carmofur, idoxuridine, vidarabine, actinomycin D, rifabutin, rifapentine, rifaximin, triptolide, flavopiridol, DRB18, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, CX-5461, CX-3543, BMH-21, BMH-22, BMH-23, LDC000067, LY2857785, THZ1, THZ2, THZ531, alpelisib, idelalisib, copanlisib, duvelisib, sirolimus, dactolisib, gedatolisib, voxtalisib, PQR309, GDC-0941, buparlisib, PF-04691502, PIK-75, PIK-90, PIK-93, GSK2126458, GDC-0980, BEZ235, osimertinib, afatinib, lapatinib, dacomitinib, neratinib, rociletinib, nazartinib, poziotinib, mobocertinib, almonertinib, lazertinib, brigatinib, olmutinib, TAK-788, AZD3759, CI-1033, PD153035, hydroxychloroquine, Lys05, SAR405, PIK-III, ULK-101, SBI-0206965, NSC185058, DC-LC3in-D5, ROC-325, AR-12, WP1130, TC-DAPK6, VPS34-IN1, autophinib, MJO445, RNF5 agonist 1, S130, LV-320, Atg4B-IN-2, vacuolin-1, LCL521, glycochenodeoxycholic acid, sinbaglustat, AP-6, lysosomal P-gp targeted agent 1, WX8, conodurine, N-desethyl sunitinib, ARL 17477, DC661, concanamycin A, defactinib, PF-562271, GSK2256098, BI-853520, VS-4718, CEP-37440, TAE226, PF-573228, INK1007, BMS-936559, TQB2450, HLX20, SHR-1316, AK106, IBI308, JS003, BAT1306, LY3300054, MSB2311, CX-

072, M7824, CA-170, GS-4224, BGB-A333, ONO-4059, PRN1371, PRN694, PRN1126, PRN473, BAY-1834942, resiquimod, imiquimod, GS-9620, MEDI9197, NKTR-262, GSK2245035, DSP-0509, IMO-4200, TMX-202, 3M-052, VTX-2337, CL075, CL097, gardiquimod, loxoribine, ABBV-CLS-484, MK-1454, SB-11285, GSK3745417, IACS-8803, IACS-8779, E7766, SNX281, TAK-676, SR-8278, SR-8993, enzalutamide, abiraterone, flutamide, apalutamide, darolutamide, seviteronel, TAK-700, ODM-201, galeterone, VT-464, TOK-001, ORM-15341, AZD3514, EPI-506, SHR3680, proxalutamide, BMS-641988, CC-90009, CC-885, CC-90011, vincristine, vinblastine, cabazitaxel, vinorelbine, colchicine, combretastatin A-4, plinabulin, T138067, vorinostat, panobinostat, belinostat, tazemetostat, entinostat, mocetinostat, abexinostat, resminostat, givinostat, CUDC-907, guadecitabine, CM-272, OTX015, CPI-0610, WEHI-539, BH3I-1, BH3I-2, palbociclib, ribociclib, abemaciclib, trilaciclib, lerociclib, SHR6390, BPI-16350, PF-06873600, FCN-437c, CS-3002, TQB3616, SY-5609, G1T38, AMG-925, AZD4573, trametinib, cobimetinib, selumetinib, binimetinib, PD0325901, refametinib, pimasertib, TAK-733, AZD8330, RO4987655, GDC-0623, RO5126766, CH5126766, E6201, WX-554, CI-1040, PD318088, SL-327, PD184352, ulixertinib, MK-8353, GDC-0994, LY3214996, LTT462, CC-90003, KO-947, ASTX029, GSK126, GSK343, CPI-1205, CPI-0209, DS-3201, PF-06821497, SHR2554, MAK683, sotorasib, adagrasib, BI-2852, ARS-1620, MRTX1133, ARS-853, ARS-3248, LY3499446, JNJ-74699157, BI-3406, BI1701963, VS-6766, DCC-3116, GDC-6036, GDC-0895, SOS1 agonist-1, GSK3326595, JNJ-64619178, PF-06939999, EPZ015666, PRT543, PRT811, TAK-659, LLY-283, AZD5153, I-BET762, I-BET151, PLX51107, ABBV-075, GSK525762, INCB054329, BAY1238097, FT-1101, ZEN-3694, RVX-208, BI-894999, olinone, AG-270, AGI-24512, MAT2A-IN-1, GDC-0879, SB590885, L-779450, RAF265, encorafenib, naporafenib, dabrafenib, B-Raf inhibitor 1, RAF709, PLX8394, LY3009120, belvarafenib, avutometinib, TAK-632, PLX-4720, agerafenib, regorafenib, donafenib, sorafenib, BAY-734506, vemurafenib, volasertib, GSK461364, HMN-214, ON-01910, rigosertib, TAK-960, NMS-1286937, CFI-400945, poloxin, MLN0905, CYC140, R1530, RO-3306, DAP81, SBE13, TKM-080301, BAY-

1000394314, dinaciclib, roscovitine, SNS-032, AT-7519, milciclib, PHA-793887, RGB-286638, TG02, AZD5438, CYC065, SY-1365, voruciclib314, GSK3368715, MS023, TC-E5003, PF-9366, C7280948, SGC2085, DC_PRMt1-1, AMI-408, AMI-1, TNO155, RMC-4630, SHP099, RLY-1971, JAB-3068, JAB-3312, BBP-398, PF-07284892, ERAS-601, ERAS-3490, belzutifan, PT2385, PT2977, PT2567, PT2399, IOX2, IOX4, IOX5, IOX7, BAY-87-2243, LW6, KC7F2, IDF-11774, vismodegib, sonidegib, glasdegib, taladegib, LY2940680, LEQ506, BMS-833923, TAK-441, IPI-926, saridegib, posaconazole, PF-5274857, antagonist SANT-1, SANT-2, PCLX-001, NMT1-IN-1, NMT2-IN-1, IMP-1088, DDD85646, atuzabrutinib, or pharmaceutically acceptable salts, solvates of any of the foregoing.

[00367] The term "linker", as used herein, refers to is a molecule with two or more reactive termini or a chemical moiety or group serving as a linkage between two or more other groups or chemical moieties. A linker may refer to a molecule (no open valence) that is capable of forming covalent conjugation with two or more other molecules to serve as a covalent linkage therebetween. For example, a linker may react with a drug and/or an antibody to form a linker-drug conjugate, an antibody-linker conjugate, or an antibody-linker-drug conjugate. The term "linker" may also refer to a portion or moiety within a compound (*e.g.*, a bivalent or trivalent group therein) that serves as a covalent linkage within said compound.

[00368] By way of example, the antibody conjugation reactive terminus of a linker molecule typically has a functional group that is capable of conjugation to the antibody through a cysteine thiol or lysine amine group on the antibody. For example, the antibody conjugation reactive terminus may have a thiol-reactive group, such as a double bond (as in maleimide) or a leaving group such as a chloro, bromo, or iodo, or an R-sulfanyl group, or an amine-reactive group such as a carboxyl group.

[00369] By way of example, the drug conjugation reactive terminus of a linker molecule typically has a functional group that is capable of conjugation to the cytotoxin through formation of an amide bond with a basic amino or carboxyl group on the cytotoxin. For example, the drug conjugation reactive terminus may have a carboxyl or basic amine group. Thus, when the term "linker" is used to describe a linkage portion or moiety within a compound, one or both of the reactive termini will be absent (such as the leaving group of the thiol-reactive group) or

incomplete (such as the being only the carbonyl of the carboxylic acid) because of the formation of the bonds between the linker with an antibody and/or a drug.

[00370] The drug to antibody ratio (DAR) or drug loading may be characterized by conventional means such as UV, mass spectroscopy, ELISA assay, HIC, HPLC or electrophoresis. In exemplary embodiments, DAR ranges from about 1 to 32, 1 to 16, 1 to 12, 1 to 8, 1 to 4, 1 to 2, 2 to 32, 4 to 32, 8 to 32, 1 to 4, 4 to 8, 8 to 12, 12 to 16, 16 to 32 or about 1.

[00371] The DAR of an immunoconjugate may be controlled by various methods, including limiting the molar excess of payload-linker intermediate or linker reagent relative to the antibody; limiting the conjugation reaction time or temperature; varying reductive conditions for cysteine thiol modification; and modifying the number and positions of cysteine residues and positions of linker-payload attachments. (See, *e.g.*, WO 2006/034488 A2.)

[00372] To date, numerous unique antigens have been identified and may be potentially used in antibody-based therapy as a target. Several factors are generally considered when selecting an antigen. First, the target antigen should have high expression in the tumor and no or low expression in the healthy cell. An example is the HER2 receptor, which is almost 100-fold higher expressed in the tumor cell compared to the healthy cell. Second, the target antigen should be displayed on the surface of the tumor cell to be available to the circulated monoclonal antibody. In addition, the target antigen should possess internalization properties as it will facilitate the ADC to transport into the cell, which will in turn enhance the efficacy of cytotoxic agent. Though some studies have demonstrated that non-internalized ADC product directed against components of the tumor microenvironment can efficiently detach their drug in the extracellular space and arbitrate a potent therapeutic activity in some cases and that ADCs often induce a strong "bystander effect." (Strohl **2018** *Protein & Cell.* 9(1):86-120; Damelin, *et al.* **2015** *Pharma. Res.* 32(11):3494-507; Diamantis et al. **2016** *British J. Cancer*114(4):362-7; Tipton et al. **2015** *Blood* 125(12):1901-9; Donaghy et al. **2016** *mAbs.* 8(4):659-71; Casi et al. **2015** *Molecular Pharmaceutics* 12(6):1880-4.)

[00373] In general, the antibody should preferably possess target specificity and possess target binding affinity, *e.g.*, a high binding affinity to the tumor cell-surface antigens. Additionally, the antibody should preferably possess good retention, low immunogenicity, low cross-reactivity, and appropriate linkage binding properties. (Peters, *et al.* **2015** *Bioscience Reports* 35(4); Hughes B **2010** *Nature Reviews Drug Discovery* 9(9):665-7.)

[00374] In certain embodiments, Ab is an antibody.

[00375] In certain embodiments, Ab is a monoclonal antibody.

[00376] In certain embodiments, Ab is a chimeric antibody.

[00377] In certain embodiments, Ab is a humanized antibody.

[00378] In certain embodiments, Ab is a bispecific antibody.

[00379] In certain embodiments, Ab is an antibody fragment.

[00380] In certain embodiments, Ab is a Fab fragment.

[00381] Non-limiting examples of antibodies include humanized, chimeric, or human antibody or the antigen binding fragment, which binds to one or more of receptors selected from the group consisting of: HER2, HER3, CD7, CD19, CD20, CD22, CD25, CD27, CD30, CD33, CD37, CD38, CD46, CD70, CD71, CD74, CD79b, CD123, CD138, CD142, CD166, CD205, CD228, CCR2, CA6, p-Cadherin, CEA, CEACAM5, C4.4a, DLL3, EGFR, EGFRVIII, ENPP3, PSMA, SLITRK6, STEAP1, TROP2, 5T4, SSEA4, GloboH, Gb5, STn, Tn, B7H3, BCMA, MUC1, cMet, ROR1 MSLN, FRa, CLDN18.2, CLDN6, EphA2, EphrinA, FLOR1, FGFR2, GCC, cKIT, LIV1, LY6E, MSLN, MUC16, NaPi2b, Nectin4, gpNMB, Axl and PTK7.

[00382] Antibodies and antibody fragments useful for the immunoconjugates of the invention include modified or engineered antibodies, such as an antibody modified to introduce a cysteine residue, or other reactive amino acid, including Pel, pyrrolysine, peptide tags, and non-natural amino acids, in place of at least one amino acid of the native sequence, thus providing a reactive site on the antibody or antigen binding fragment for conjugation to a cytotoxic agent.

[00383] Antibodies and antibody fragments can be readily produced by any methods known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, *e.g.*, hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc. (See, *e.g.*, Carvalho et al. **2016** "Production Processes for Monoclonal Antibodies", DOI: 10.5772/64263 (https://www.intechopen.com/chapters/51512); Monoclonal Antibody Production, Committee on Methods of Producing Monoclonal Antibodies, Institute for Laboratory Animal Research, National Research Council, NATIONAL ACADEMY PRESS Washington, DC **1999**; Jakobovits **1998** *Adv. Drug Del. Rev.* 31:33-42; Marks, *et al.* **1991** *J. Mol. Biol.* 222:581; Cole, *et al.* **1985** *Monoclonal Antibodies And Cancer Therapy* 77-

96; Teng, *et al.* **1983** *Proc. Natl. Acad. Sci. USA*. 80:7308-7312; Kozbor, *et al.* **1983** *Immunology Today* 4:72-79; Olsson, *et al.* **1982** *Meth. Enzymol.* 92:3-16; U.S. Pat. No. 6,657,103 B2.)

[00384] The invention provides a method for treating, reducing, or preventing a disease or disorder, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[00385] In yet another aspect, the invention generally relates to a method for modulating immune response, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[00386] In yet another aspect, the invention generally relates to a method for modulating TLR7- and/or TLR8-mediated signaling, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein. In yet another aspect, the invention generally relates to a method for of treating or reducing a condition or disorder treatable by modulation of TLR7- and/or TLR8-mediated cellular activities, comprising administering to a subject in need thereof an antibody-drug conjugate disclosed herein.

[00387] In certain embodiments, the disease or disorder is selected from the group consisting of: autoimmune diseases, graft rejection, allergies, immunodeficiency, infection, sepsis, cancer, and related diseases or disorders. In certain embodiments, the disease or disorder is an autoimmune disease, or a related disease or disorder. In certain embodiments, the disease or disorder is graft rejection, or a related disease or disorder. In certain embodiments, the disease or disorder is allergy, or a related disease or disorder. In certain embodiments, the disease or disorder is immunodeficiency, or a related disease or disorder. In certain embodiments, the disease or disorder is infection and/or sepsis, or a related disease or disorder. In certain embodiments, the disease or disorder is cancer, or a related disease or disorder.

[00388] Cancers of interest for treatment include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric orstomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, oral cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer including, for example, HER2 positive breast cancer, colon cancer,

rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain cancer, head and neck cancers, and associated metastases.

[00389] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds described herein or derivatives thereof are admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (i) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (ii) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (iii) humectants, as for example, glycerol, (iv) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (v) solution retarders, as for example, paraffin, (vi) absorption accelerators, as for example, quaternary ammonium compounds, (vii) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (viii) adsorbents, as for example, kaolin and bentonite, and (ix) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard- filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like. Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others known in the art.

[00390] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers, such as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan, or mixtures of these substances, and the like. Besides such inert

diluents, the composition can also include additional agents, such as wetting, emulsifying, suspending, sweetening, flavoring, or perfuming agents.

[00391] Materials, compositions, and components disclosed herein can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. It is understood that when combinations, subsets, interactions, groups, *etc.* of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including in the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

[00392] Compositions of the present invention are administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intratumoral and intracranial injection or infusion techniques.

[00393] Pharmaceutically acceptable compositions of this invention are orally administered in any orally acceptable dosage form. Exemplary oral dosage forms are capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents are optionally also added.

[00394] Alternatively, pharmaceutically acceptable compositions of this invention are administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[00395] Pharmaceutically acceptable compositions of this invention are also administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[00396] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches are also used.

[00397] For topical applications, provided pharmaceutically acceptable compositions are formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Exemplary carriers for topical administration of compounds of this aremineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, provided pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[00398] Pharmaceutically acceptable compositions of this invention are optionally administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and are prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[00399] Most preferably, pharmaceutically acceptable compositions of this invention are formulated for oral administration. Such formulations may be administered with or without food. In some embodiments, pharmaceutically acceptable compositions of this invention are

administered without food. In other embodiments, pharmaceutically acceptable compositions of this invention are administered with food.

[00400] The amount of compounds of the present invention that are optionally combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, provided compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the compound can be administered to a patient receiving these compositions.

[00401] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a compound of the present invention in the composition will also depend upon the particular compound in the composition.

[00402] The following examples are meant to be illustrative of the practice of the invention and not limiting in any way.

Examples

[00403] As depicted in the Examples below, in certain exemplary embodiments, compounds are prepared according to the following general procedures. It will be appreciated that, although the general methods depict the synthesis of certain compounds of the present invention, the following general methods, and other methods known to one of ordinary skill in the art, can be applied to all compounds and subclasses and species of each of these compounds, as described herein.

Abbreviations

ADC	antibody-drug conjugate
AcOH	acetic acid
aq.	aqueous
ACN	acetonitrile
brine	Saturated aqueous sodium chloride solution
Cbz	benzyloxycarbonyl
Boc	t-butoxycarbonyl
DBU	1,8-diazabicyclo[5. 4.0]undec-7-ene
BAIB	[bis(acetoxy)iodo]benzene

DCM	dichloromethane
DCC	dicyclohexyl carbodiimide
DEA	diethyl amine
DMF	N N-dimethylformamide
DMSO	dimethyl sulfoxide
DIPEA	N,N-diisopropylethylamine
DMTMM	4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride
EDCI	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
EEDQ	2-ethoxyl-1-ethoxycarbonyl-1,2-dihydroquinoline
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	9-fluorenylmethyloxycarbonyl
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
Hex	n-hexane
HIC	hydrophobic interaction chromatography
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
LCMS	liquid chromatograph mass spectrometer
MeOH	methanol
NHS	N-Hydroxy succinimide
OXONE	potassium peroxymonosulfate
PyBOP	Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate
SL	StarLinker
SEC	size exclusion chromatography
THF	tetrahydrofuran
TEA	triethyl amine
TFA	trifluoroacetic acid
Ts	p-toluenesulfonyl
TIPS	triisopropylsilane
TEMPO	2,2,6,6-tetramethylpiperidinyl-1-oxide

General

[00404] ^{1}H NMR was recorded on a Bruker 400 MHz spectrometer, using residual signal of deuterated solvent as internal reference. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (δ = 2.49 ppm for 1H NMR in DMSO-d6). ^{1}H NMR data are reported as

follows: chemical shift (multiplicity, coupling constants, and number of hydrogens). Multiplicity is abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad).

[00405] LCMS-Analysis was performed by Shimadzu LC20A and Shimadzu LCMS2020 under the following conditions:

Method: A: 0.1 % formic acid in H_2O, B: ACN:

Runtime: 6.5 min

Flow Rate: 1.0 mL/min

Gradient: 5-95% B in 4.5 min, wavelength 254 and 215 nM

Mass Scan: 100 - 2000 Da

Column: Waters Sunfire C18, 3.0x50mm, 3.5um, +ve mode

[00406] High performance liquid chromatography (HPLC) method:

Mobile phase A: 0.1 % formic acid in H_2O, B: ACN

Runtime: 20 min

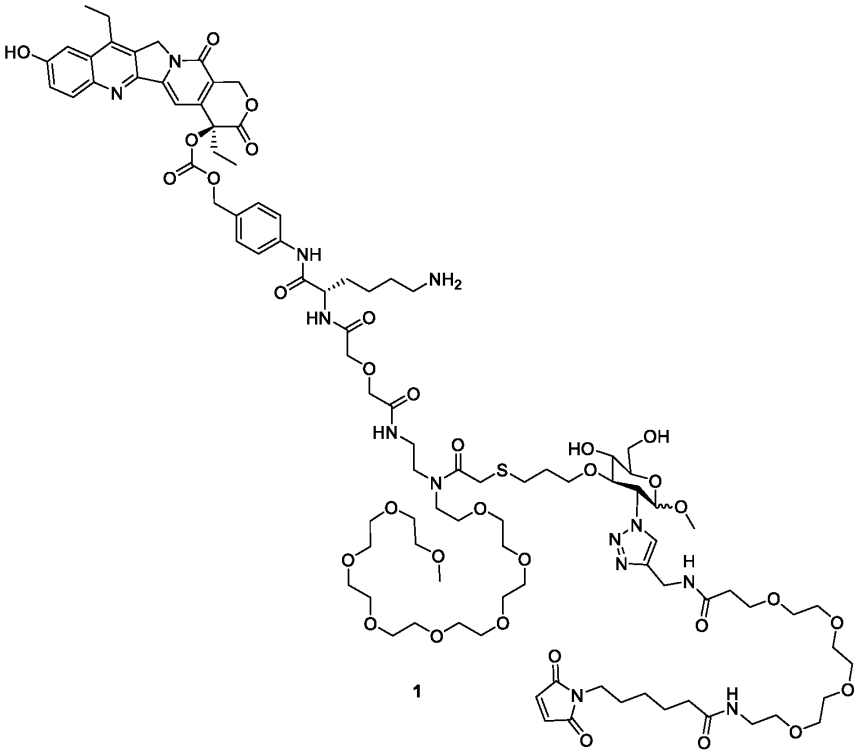
Flow Rate: 1.0 mL/min

Gradient: 20% - 90% B in 6.8 min, 90% B for 8 min, back to 20% B within 0.1 min, 20% B within 5 min. wavelength 254 nM

Column: Shim-pack PREP-ODS (H) kit, 4.6x250mm, 5 um, 300 A

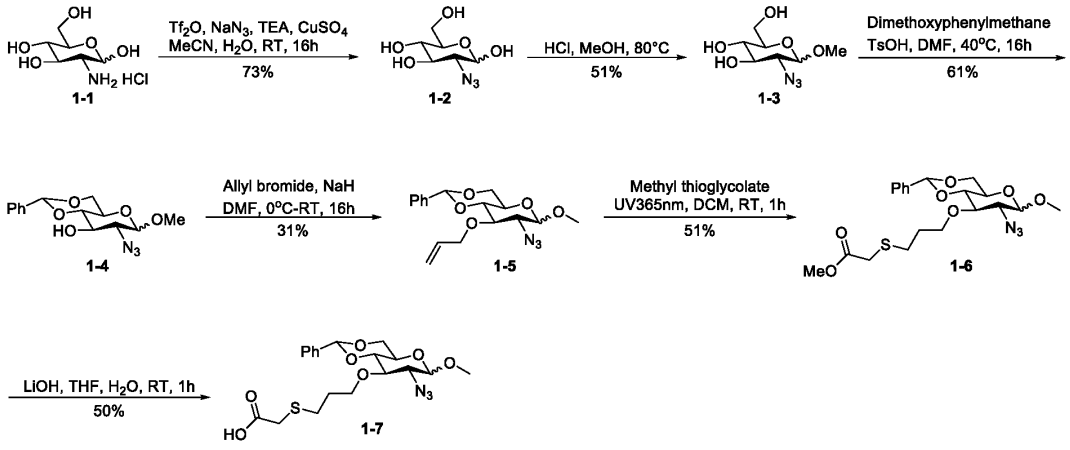
Oven temperature: 40 °C

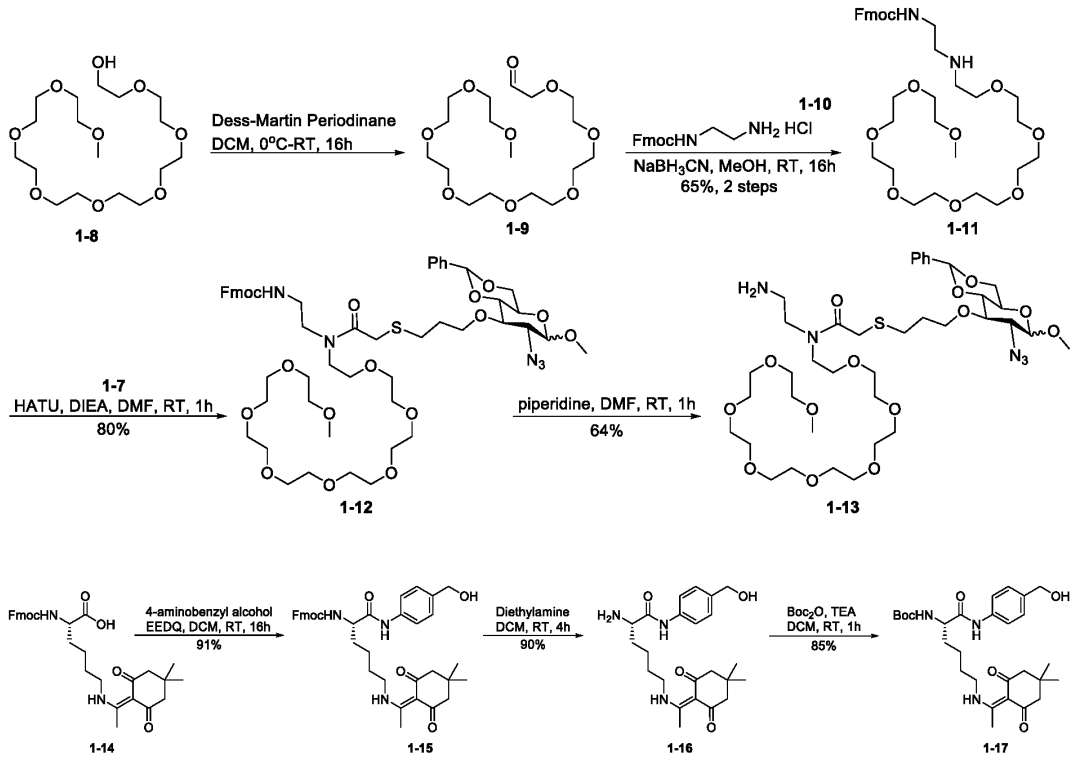
Example 1. Synthesis of 4-((S)-36-(4-aminobutyl)-26-(2-((3-(((3R,4R,5S,6R)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-4-yl)oxy)propyl)thio)acetyl)-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaheptatriacontan-37-amido)benzyl ((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) carbonate (1)

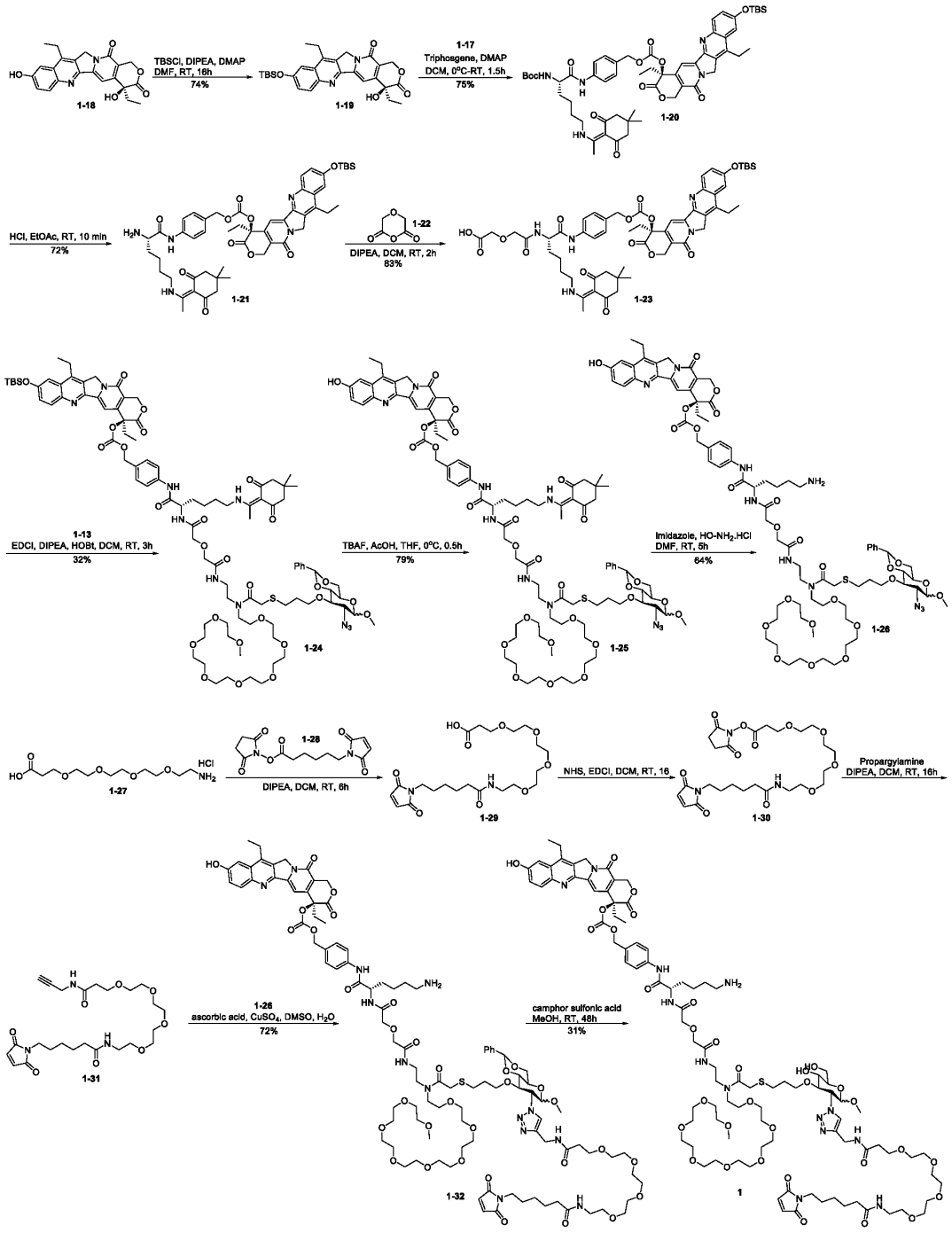


1

Synthetic scheme 1




Dess-Martin Periodinane
DCM, 0°C-RT, 16h
1-10
NaBH3CN, MeOH, RT, 16h
65%, 2 steps
1-7
HATU, DIEA, DMF, RT, 1h
80%
piperidine, DMF, RT, 1h
64%
4-aminobenzyl alcohol
EEDQ, DCM, RT, 16h
91%
Diethylamine
DCM, RT, 4h
90%
Boc2O, TEA
DCM, RT, 1h
85%


TBSCl, DIPEA, DMAP
DMF, RT, 16h
74%
1-17
Triphosgene, DMAP
DCM, 0°C-RT, 1.5h
75%
HCl, EtOAc, RT, 10 min
72%
1-22
DIPEA, DCM, RT, 2h
83%
1-13
EDCI, DIPEA, HOBt, DCM, RT, 3h
32%
TBAF, AcOH, THF, 0°C, 0.5h
79%
Imidazole, HO-NH2.HCl
DMF, RT, 5h
64%
1-28
DIPEA, DCM, RT, 6h
NHS, EDCI, DCM, RT, 16
Propargylamine
DIPEA, DCM, RT, 16h
1-26
ascorbic acid, CuSO4, DMSO, H2O
72%
camphor sulfonic acid
MeOH, RT, 48h
31%

[00407] Step 1: A suspension of sodium azide (4.22 g, 69.91 mmol) in acetonitrile (77 mL) was cooled to 0 °C in an ice-bath. Triflic anhydride (15.7 g, 55.65 mmol) was added to the mixture by a syringe during 0.5 h while stirring. After the reaction was maintained for 2 h at 0 °C, the acetonitrile solution of trifluoromethanesulfonyl azide was filtered and used directly to next reaction. To a stirred solution of compound **1-1** (10 g, 46.37 mmol) in water (50 mL) was added $CuSO_4$ (75 mg, 0.470 mmol) and TEA (9.4 g, 93.07 mmol) at 0 °C. After stirring for 10 min, the acetonitrile solution of trifluoromethanesulfonyl azide was added dropwise. The resulting reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column (eluted with 15% methanol in dichloromethane) to afford the title compound **1-2** (7 g, yield 73%) as a colorless oil.

[00408] Step 2: To a solution of compound **1-2** (5.5 g, 26.81 mmol) in methanol (45 mL) was added concentrated HCl (5 mL) and the reaction mixture was stirred at 90 °C for 20 h. After completion of the reaction, the mixture was concentrated. The residue was re-dissolved in methanol and treated with $NaHCO_3$ until pH was neutral. The resulting mixture was filtered and concentrated. The crude product was purified by silica gel column (eluted with 9% methanol in dichloromethane) to afford the title compound **1-3** (3 g, yield 51%) as a colorless oil.

[00409] Step 3: A solution of compound **1-3** (3 g, 13.68 mmol), p-TsOH (230 mg, 1.33 mmol) and dimethoxyphenylmethane (6.25 g, 41.06 mmol) in DMF (30 mL) was stirred at 40 °C for 16 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column (eluted with 20% ethyl acetate in petroleum ether) to afford the title compound **1-4** (2.6 g, yield 61%) as a colorless oil, which was solidified after overnight.

[00410] Step 4: To a solution of compound **1-4** (3.3 g, 10.74 mmol) in anhydrous DMF (30 mL) at 0 °C was added NaH (60% in mineral oil, 1.28 g, 32.0 mmol) in several portions under nitrogen atmosphere. After the mixture was stirred at 0 °C for 1 h, allyl bromide (2.6 g, 21.49 mmol) was added thereto. The resulting mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was partitioned with ethyl acetate (100 mL) and water (100 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 10% ethyl acetate in petroleum ether) to afford the title compound **1-5** (1.17 g, yield 31%) as a colorless oil. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.51 – 7.45 (m, 2H), 7.42 – 7.35 (m, 3H), 6.04 – 5.89 (m,

1H), 5.57 (s, 1H), 5.30 (dd, J = 17.2, 1.5 Hz, 1H), 5.20 (dd, J = 10.4, 1.3 Hz, 1H), 4.77 (d, J = 3.6 Hz, 0.54H), 4.47 – 4.38 (m, 1H), 4.35 (dd, J = 10.5, 4.9 Hz, 0.44H), 4.31 – 4.22 (m, 2H), 3.96 (t, J = 9.5 Hz, 0.55H), 3.89 (dd, J = 12.7, 5.2 Hz, 0.27H), 3.86 – 3.81 (m, 0.56H), 3.80 – 3.74 (m, 0.79H), 3.64 (td, J = 9.3, 2.7 Hz, 1H), 3.58 (d, J = 0.9 Hz, 1H), 3.53 – 3.47 (m, 0.45H), 3.44 (d, J = 2.6 Hz, 2H), 3.43 – 3.34 (m, 1H).

[00411] Step 5: A solution of compound **1-5** (600 mg, 1.727 mmol) and methyl thioglycolate (1.7 mL, 17.24 mmol) in dichloromethane (6 mL) was irradiated with UV 365 nm at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel (eluted with 15% ethyl acetate in petroleum ether) to afford the title compound **1-6** (400 mg, yield 51%) as a white solid.

[00412] Step 6: A mixture of compound **1-6** (400 mg, 0.882 mmol) and 1 N LiOH solution (1.7 mL, 1.7 mmol) in THF (4 mL) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The aqueous solution was acidized with 1 N HCl to pH = 5 and extracted with ethyl acetate (10 mL x 2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the title compound **1-7** (350 mg, yield 90%) as a white solid, which was used at next step without further purification. LCMS: m/z calculated for $C_{19}H_{25}N_3O_7S$: 439.5; found: 462.15 $[M+Na]^+$.

[00413] Step 7: To a solution of compound **1-8** (1 g, 2.60 mmol) in dichloromethane (20 mL) was added dess-martin periodinane (1.43 g, 3.37 mmol) in several portions at 0 °C. The mixture was warmed to room temperature and stirred for 16 h. After completion of the reaction, the mixture was diluted with petroleum ether (20 mL) and stirred for additional 10 min. the mixture was filtered and concentrated. The residue was re-dissolved in dichloromethane (10 mL) and petroleum ether (10 mL), and the undissolved impurity was filtered. The filtrate was concentrated to afford the title compound **1-9** (800 mg, yield 80%) as a colorless oil, which was used at next step without further purification. LCMS: m/z calculated for $C_{17}H_{34}O_9$: 382.5; found: 405.64 $[M+Na]^+$.

[00414] Step 8: Under nitrogen atmosphere, a solution of compound **1-9** (800 mg, 2.09 mmol) and compound **1-10** (800 mg, 2.51 mmol) in methanol (15 mL) was stirred at room temperature for 2 h. Then the mixture was cooled to 0 °C, and $NaBH_3CN$ (400 mg, 6.36 mmol) was added in three portions. The resulting mixture was warmed to room temperature and stirred for 14 h. After completion of the reaction, the mixture was quenched by addition of saturated $NaHCO_3$ (5 mL)

solution and concentrated. The residue was purified by silica gel column (eluted with 3% methanol in dichloromethane) to afford the title compound **1-11** (1.1 g, yield 80%) as a colorless oil. LCMS: m/z calculated for $C_{34}H_{52}N_2O_{10}$: 648.8; found: 649.63 $[M+H]^+$.

[00415] Step 9: A mixture of compound **1-11** (300 mg, 0.462 mmol), compound **1-7** (245 mg, 0.557 mmol), DIPEA (120 mg, 0.930 mmol) and HATU (263 mg, 0.692 mmol) in anhydrous DMF (3 mL) was stirred at room temperature under nitrogen atmosphere for 4 h. After completion of the reaction, the mixture was partitioned with ethyl acetate (30 mL) and water (30 mL). The aqueous was extracted with ethyl acetate (20 mL) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 3% methanol in ethyl acetate) to afford the title compound **1-12** (400 mg, yield 80%) as a colorless oil. LCMS: m/z calculated for $C_{53}H_{75}N_5O_{16}S$: 1070.3; found: 1070.87 $[M+H]^+$.

[00416] Step 10: A solution of compound **1-12** (400 mg, 0.373 mmol) and piperidine (0.4 mL) in DMF (2 mL) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and directly purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were concentrated to give the title compound **1-13** (trifluoroacetate, 230 mg, yield 64%) as a colorless oil. LCMS: m/z calculated for $C_{38}H_{65}N_5O_{14}S$: 848.0; found: 848.63 $[M+H]^+$.

[00417] Step 11: A solution of compound **1-14** (1 g, 2.13 mmol), 4-aminobenzyl alcohol (340 mg, 2.76 mmol) and EEDQ (930 mg, 3.76 mmol) in dichloromethane (20 mL) was stirred at room temperature in the dark for 16 h. After completion of the reaction, the mixture was diluted with dichloromethane (30 mL), washed with 1 N HCl, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was triturated with petroleum ether for 1 h, filter and dried to give the title compound **1-15** (1.1 g, yield 91%) as a white solid.

[00418] Step 12: A solution of compound **1-15** (1.1 g, 1.72 mmol) and diethylamine (5 mL) in dichloromethane (5 mL) was stirred at room temperature for 4 h. After completion of the reaction, the mixture was concentrated to give the title compound **1-16** (650 mg, yield 90%), which was used at next step without further purification. LCMS: m/z calculated for $C_{38}H_{43}N_3O_6$: 637.8; found: 638.73 $[M+H]^+$.

[00419] Step 13: A mixture of compound **1-16** (650 mg, 1.56 mmol), TEA (320 mg, 3.16 mmol) and Boc_2O (520 mg, 2.38 mmol) in dichloromethane was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **1-17** (650 mg, yield 80%) as white foam. LCMS: m/z calculated for $C_{28}H_{41}N_3O_6$: 515.7; found: 538.26 $[M+Na]^+$.

[00420] Step 14: A mixture of compound **1-18** (500 mg, 1.27 mmol), DIPEA (650 mg, 6.43 mmol), DMAP (15 mg, 0.123 mmol) and t-butyldimethylsilyl chloride (580 mg, 3.85 mmol) in DMF (5 mL) was stirred at room temperature for 16 h. After completion of the reaction, the mixture was partitioned with dichloromethane (50 mL) and water (50 mL). The organic layer was washed with 0.5 N HCl and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **1-19** (480 mg, yield 74%) as a pale-yellow solid. LCMS: m/z calculated for $C_{28}H_{34}N_2O_5Si$: 506.7; found: 507.83 $[M+H]^+$.

[00421] Step 15: Under nitrogen atmosphere, to a solution of compound **1-19** (200 mg, 0.395 mmol) in anhydrous dichloromethane (5 mL) was added triphosgene (58 mg, 0.195 mmol) and DMAP (192 mg, 1.574 mmol) at 0 °C. After the mixture was stirred at 0 °C for 30 min, a solution of compound **1-17**(183 mg, 0.355 mmol) in anhydrous dichloromethane (2 mL) was added thereto. The resulting reaction was warmed to room temperature and stirred for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane (20 mL) and washed with 0.5 N HCl. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (eluted with 80% ethyl acetate in petroleum ether) to afford the title compound **1-20** (280 mg, yield 75%) as a pale-yellow foam. LCMS: m/z calculated for $C_{57}H_{73}N_5O_{12}Si$: 1048.3; found: 1071.51 $[M+Na]^+$.

[00422] Step 16: To a solution of compound **1-20** (230 mg, 0.219 mmol) in ethyl acetate (3 mL) was added dropwise a solution of HCl in ethyl acetate (4 N, 1 mL) at room temperature. After stirring for 10 min, a yellow precipitation was formed completely. The resulting mixture was centrifuged with ethyl acetate for three times and dried in vacuum to afford the title compound **1-21** (hydrochloride, 150 mg, yield 69%) as a yellow solid. LCMS: m/z calculated for $C_{52}H_{65}N_5O_{10}Si$: 948.2; found: 948.78 $[M+H]^+$.

[00423] Step 17: To a solution of compound **1-21** (hydrochloride, 150 mg, 0.158 mmol) and DIPEA (40 mg, 0.310 mmol) in dichloromethane (5 mL) was added compound **1-22** (30 mg, 0.258 mmol) at room temperature. After the reaction was stirred for 2 h, the mixture was diluted with dichloromethane (10 mL) and washed with 0.5 N HCl. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the title compound **1-23** (140 mg, yield 83%) as a yellow solid. LCMS: m/z calculated for $C_{56}H_{69}N_5O_{14}Si$: 1064.3; found: 1065.45 $[M+H]^+$.

[00424] Step 18: A mixture of compound **1-23** (138 mg, 0.129 mmol), compound **1-13** (100 mg, 0.118 mmol), DIPEA (30 mg, 0.232 mmol), HOBt (16 mg, 0.118 mmol) and EDCI (45 mg, 0.235 mmol) in anhydrous dichloromethane (5 mL) was stirred at room temperature for 3 h. After completion of the reaction, the mixture was diluted with dichloromethane (20 mL) and washed with 0.5 N HCl. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by pre-TLC (SiO_2, dichloromethane:methanol = 20:1) to afford the title compound **1-24** (108 mg, yield 48%) as a yellow oil. LCMS: m/z calculated for $C_{94}H_{132}N_{10}O_{27}SSi$: 1894.3; found: 1895.05 $[M+H]^+$.

[00425] Step 19: A mixture of compound **1-24** (108 mg, 0.057 mmol), TBAF (1 M in THF, 0.085 mL) and AcOH (10 mg, 0.167 mmol) in THF (2 mL) was stirred at 0 °C for 0.5 h. After completion of the reaction, the mixture was diluted with dichloromethane (10 mL) and washed with water. The organic was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the title compound **1-25** (82 mg, yield 78%) as a yellow oil, which was used at next step without purification. LCMS: m/z calculated for $C_{88}H_{118}N_{10}O_{27}S$: 1780.0; found: 1781.14 $[M+H]^+$.

[00426] Step 20: A mixture of compound **1-25** (80 mg, 0.045 mmol), hydroxylamine hydrochloride (31 mg, 0.446 mmol) and imidazole (30 mg, 0.440 mmol) in DMF (1 mL) was stirred at room temperature for 5 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **1-26** (trifluoroacetate, 50 mg, yield 64%) as a yellow solid. LCMS: m/z calculated for $C_{78}H_{106}N_{10}O_{25}S$: 1615.8; found: 1616.95 $[M+H]^+$.

[00427] Step 21: To a solution of compound **1-27** (500 mg, 1.66 mmol) and DIPEA (540 mg, 4.18 mmol) in dichloromethane (5 mL) was added compound **1-28** (615 mg, 1.99 mmol) at 0 °C.

The mixture was warmed to room temperature and stirred for 15 h. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **1-29** (730 mg, yield 95%) as a yellow oil. LCMS: m/z calculated for $C_{21}H_{34}N_2O_9$: 458.51; found: 459.90 $[M+H]^+$.

[00428] Step 22: To a solution of compound **1-29** (450 mg, 0.98 mmol) and N-Hydroxysuccinimide (225 mg, 1.96 mmol) in anhydrous dichloromethane (10 mL) was added EDCI (380 mg, 1.99 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was diluted with dichloromethane (10 mL) and washed with cold water (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to afford the title compound **1-30** (580 mg, crude) as a yellow oil. LCMS: m/z calculated for $C_{25}H_{37}N_3O_{11}$: 555.58; found: 556.91 $[M+H]^+$.

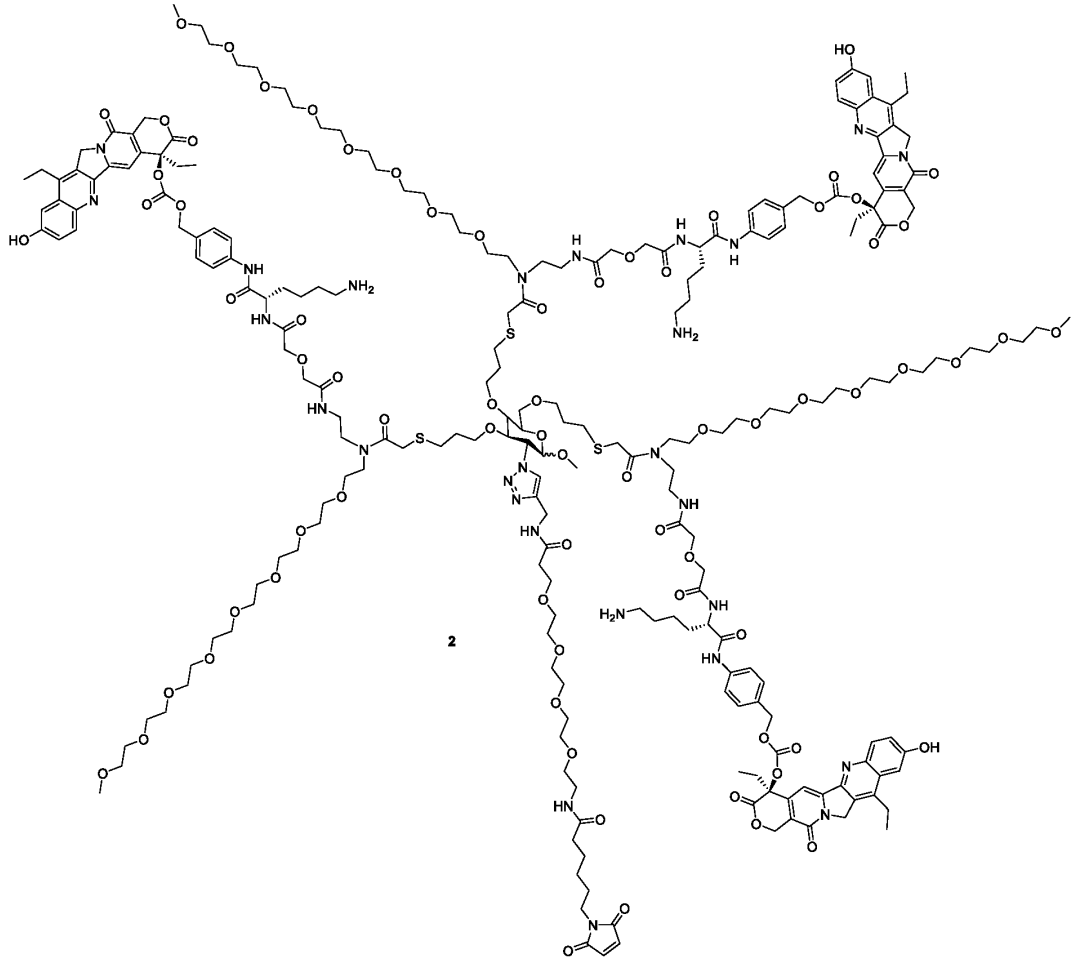
[00429] Step 23: To a solution of propargylamine (70 mg, 1.27 mmol) and DIPEA (222 mg, 1.72 mmol) in dichloromethane (5 mL) was added a solution of compound **1-30** (580 mg, crude) in dichloromethane (5 mL). The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was diluted with dichloromethane (20 mL) and washed with water (15 mL x 2). The organic layer was washed 0.5 N HCl, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (eluted with 9% MeOH in dichloromethane) to afford the title compound **1-31** (335 mg, yield 70% over 2 steps) as an off-white solid. LCMS: m/z calculated for $C_{24}H_{37}N_3O_8$: 495.57; found: 497.09 $[M+H]^+$.

[00430] Step 24: To a solution of compound **1-26** (30 mg, 0.018 mmol) and compound **1-31** (11 mg, 0.022 mmol) in degassed DMSO (1 mL) was added a fresh aqueous solution of ascorbic acid (1 M, 0.162 mL, 0.162 mmol) and a fresh aqueous solution of Cu_2SO_4 (1 M, 0.054 mL, 0.054 mmol). The mixture was purged with nitrogen for three times and stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **1-32** (trifluoroacetate, 28 mg, yield 72%) as a yellow solid. LCMS: m/z calculated for $C_{102}H_{143}N_{13}O_{33}S$: 2111.4; found: 1056.66 $[M+2H]^{2+}$.

[00431] Step 25: A solution of **1-32** (32 mg, 0.015 mmol) and camphor sulfonic acid (21 mg, 0.090 mmol) in methanol (1 mL) was stirred at room temperature for 48 h. After completion of

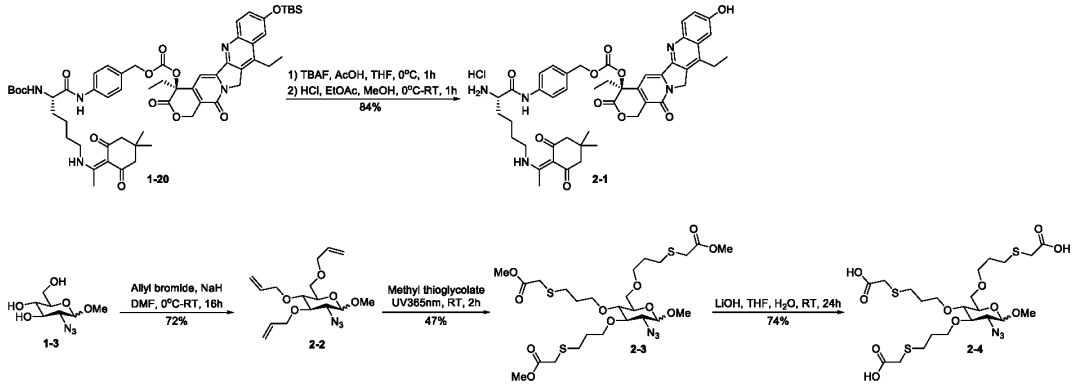
the reaction, the mixture was concentrated. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **1** (trifluoroacetate, 9.6 mg, yield 31%) as a yellow solid. LCMS: m/z calculated for $C_{95}H_{139}N_{13}O_{33}S$: 2023.3; found: 1012.13 $[M+2H]^{2+}$.

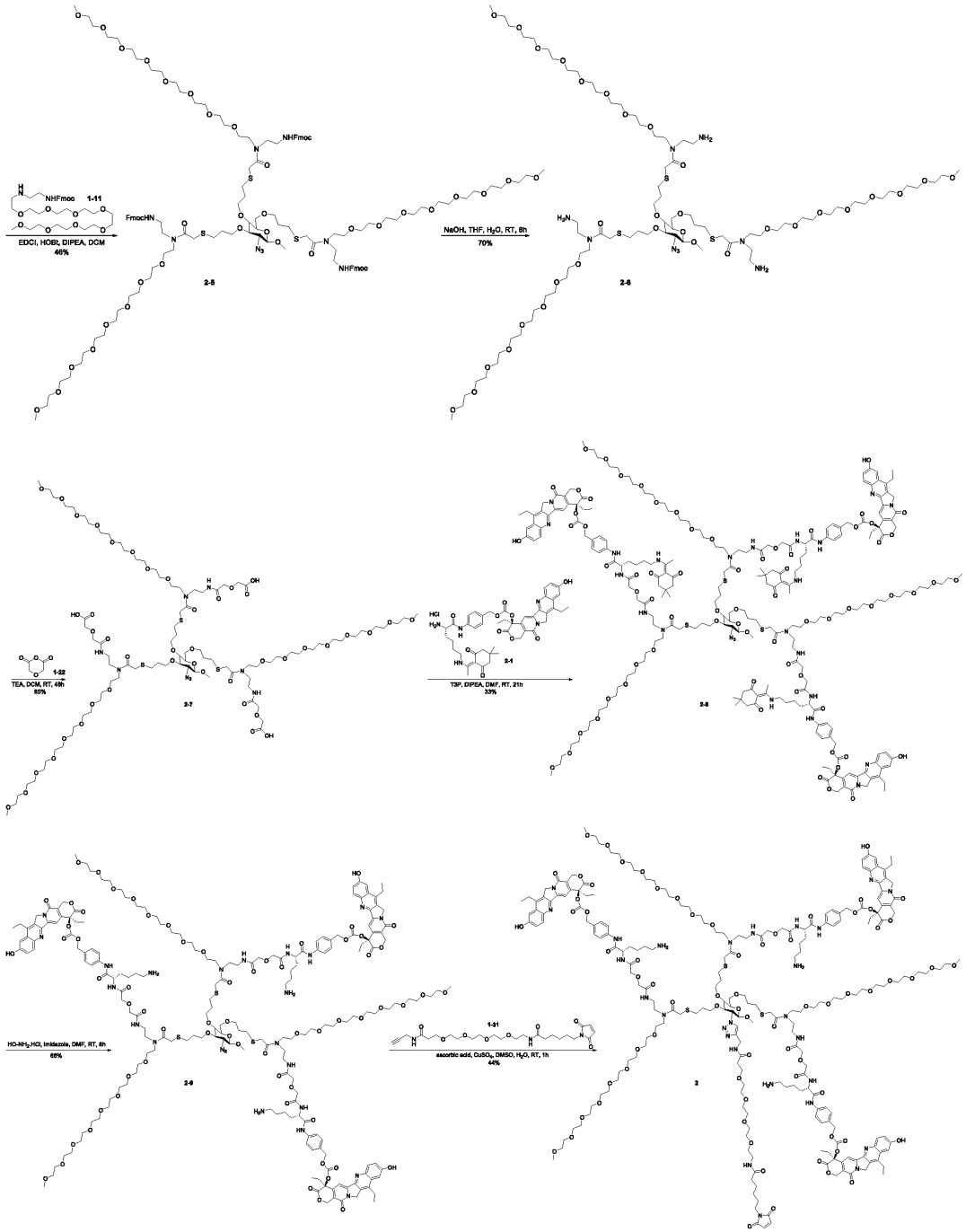
Example 2. Synthesis of 4-((S)-26-(2-((3-(((2R,3S,4R,5R)-3-((26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-2-(26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23,33-nonaoxa-29-thia-26-azatetratriacontan-34-yl)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-6-methoxytetrahydro-2H-pyran-4-yl)oxy)propyl)thio)acetyl)-36-(4-aminobutyl)-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaheptatriacontan-37-amido)benzyl ((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) carbonate (2)



2

Synthetic scheme 2





[00432] Step 1: Under nitrogen atmosphere, TBAF (1 M THF solution, 2.6 mL, 2.6 mmol) was added to a solution of compound **1-20** (1.8 g, 1.717 mmol) and acetic acid (206 mg, 3.43

mmol) in THF (18 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and diluted with dichloromethane (100 mL), washed with water (100 mL x 2) and brine (100 mL x 2). The organic layer was dried by anhydrous Na_2SO_4, filtered and concentrated. The residue was redissolved in dichloromethane (20 mL) and methanol (1 mL). 4 N HCl in ethyl acetate (20 mL) was added and the mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was filtered and the yellow solid was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-1** (hydrochloride, 1.27 g, yield 84%) as a yellow solid. LCMS: m/z calculated for $C_{46}H_{51}N_5O_{10}$: 833.9; found: 856.92 $[M+Na]^+$.

[00433] Step 2: Under nitrogen atmosphere, NaH (60% in mineral oil, 730 mg, 18.25 mmol) was added in portions to a solution of compound **1-3** (800 mg, 3.65 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h, to this was added slowly allyl bromide (2.2 g, 18.18 mmol). The reaction was warmed slowly to room temperature and stirred for additional 16 h. After completion of the reaction, the mixture was quenched by addition of cold water (100 mL). The mixture was extracted with ethyl acetate (50 mL x 2) and washed with brine (100 mL). The organic layer was dried by anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 5% ethyl acetate in petroleum ether) to afford the title compound **2-2** (900 mg, yield 72%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.95 (dddt, *J* = 28.3, 16.8, 11.3, 5.1 Hz, 3H), 5.35 – 5.23 (m, 3H), 5.18 (t, *J* = 10.5 Hz, 3H), 4.76 (d, *J* = 3.5 Hz, 0.5H), 4.37 – 4.24 (m, 3H), 4.16 – 4.05 (m, 2.5H), 4.00 (dd, *J* = 12.7, 5.6 Hz, 1H), 3.78 – 3.60 (m, 3.3H), 3.54 (s, 1H), 3.52 – 3.21 (m, 4.7H).

[00434] Step 3: A mixture of compound **2-2** (900 mg, 2.65 mmol) and methyl thioglycolate (8 g, 75.3 mmol) was irradiated with UV 365 nm at room temperature for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **2-3** (820 mg, yield 47%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.73 (d, *J* = 3.3 Hz, 0.63H), 4.25 (d, *J* = 7.4 Hz, 0.37H), 3.79 (dt, *J* = 8.9, 6.1 Hz, 1H), 3.71 (qd, *J* = 7.3, 5.9, 4.2 Hz, 2H), 3.63 (s, 9H), 3.60 – 3.42 (m, 9H), 3.42 – 3.36 (m, 5H), 3.27 (s, 2H), 3.20 (dq, *J* = 17.3, 8.8, 7.8 Hz, 2H), 2.64 (p, *J* = 6.9, 6.1 Hz, 6H), 1.78 (dt, *J* = 15.9, 7.0 Hz, 6H). LCMS: m/z calculated for $C_{25}H_{43}N_3O_{11}S_3$: 657.8; found: 680.88 $[M+Na]^+$.

[00435] Step 4: 2 N LiOH solution (6 mL, 12 mmol) was added at 0 °C to a solution of compound **2-3** (1 g, 1.52 mmol) in THF (10 mL). The reaction was stirred at room temperature for 24 h. After completion of the reaction, the mixture was concentrated and adjusted pH to 3 with 1 N HCl at 0 °C. The aqueous solution was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-4** (670 mg, yield 71%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.73 (d, J = 3.2 Hz, 0.63H), 4.25 (d, J = 7.7 Hz, 0.37H), 3.83 – 3.75 (m, 1H), 3.74 – 3.67 (m, 2H), 3.62 – 3.41 (m, 9H), 3.40 – 3.32 (m, 3H), 3.27 (s, 3H), 3.20 (s, 2H), 3.16 (dd, J = 14.9, 5.3 Hz, 1H), 2.64 (q, J = 10.7, 9.1 Hz, 6H), 1.79 (dt, J = 13.3, 6.9 Hz, 6H). LCMS: m/z calculated for $C_{22}H_{37}N_3O_{11}S_3$: 615.7; found: 638.52 $[M+Na]^+$.

[00436] Step 5: A solution of compound **2-4** (350 mg, 0.568 mmol), HOBt (460 mg, 3.41 mmol), DIPEA (880 mg, 6.82 mmol) and EDCI (650mg, 3.40 mmol) in dichloromethane (5 mL) was stirred at room temperature under nitrogen atmosphere for 10 min, a solution of compound **1-11** (1.6 g, 2.46 mmol) in dichloromethane (5 mL) was added thereto. The resulting reaction mixture was stirred at room temperature for 24 h. The mixture was diluted with dichloromethane (20 mL) and washed with 0.5 N HCl (20 mL) and water (20 mL). The organic layer was concentrated and the residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-5** (660 mg, yield 46%) as a colorless oil. LCMS: m/z calculated for $C_{124}H_{187}N_9O_{38}S_3$: 2508.1; found: 1278.09 $[M+2Na]^{2+}$.

[00437] Step 6: To a solution of compound **2-5** (400 mg, 0.159 mmol) in THF (4 mL) was added slowly 3 N NaOH solution (0.53 mL, 1.59 mmol) at 0 °C. The mixture was stirred at room temperature for 5 h. After completion of the reaction, the mixture was concentrated and the residue was acidized at 0 °C with 1 N HCl to pH = 3. The aqueous solution was washed with ethyl acetate (10 mL x 2) and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-6** (hydrochloride, 260 mg, yield 83%) as a colorless oil. LCMS: m/z calculated for $C_{79}H_{157}N_9O_{32}S_3$: 1841.3; found: 921.96 $[M+2H]^{2+}$.

[00438] Step 7: To a solution of compound **2-6** (hydrochloride, 260 mg, 0.133 mmol) and TEA (170 mg, 1.68 mmol) in dichloromethane (5 mL) was added diglycolic anhydride **1-22** (98 mg, 0.844 mmol). The reaction was stirred at room temperature for 48 h. After completion of the

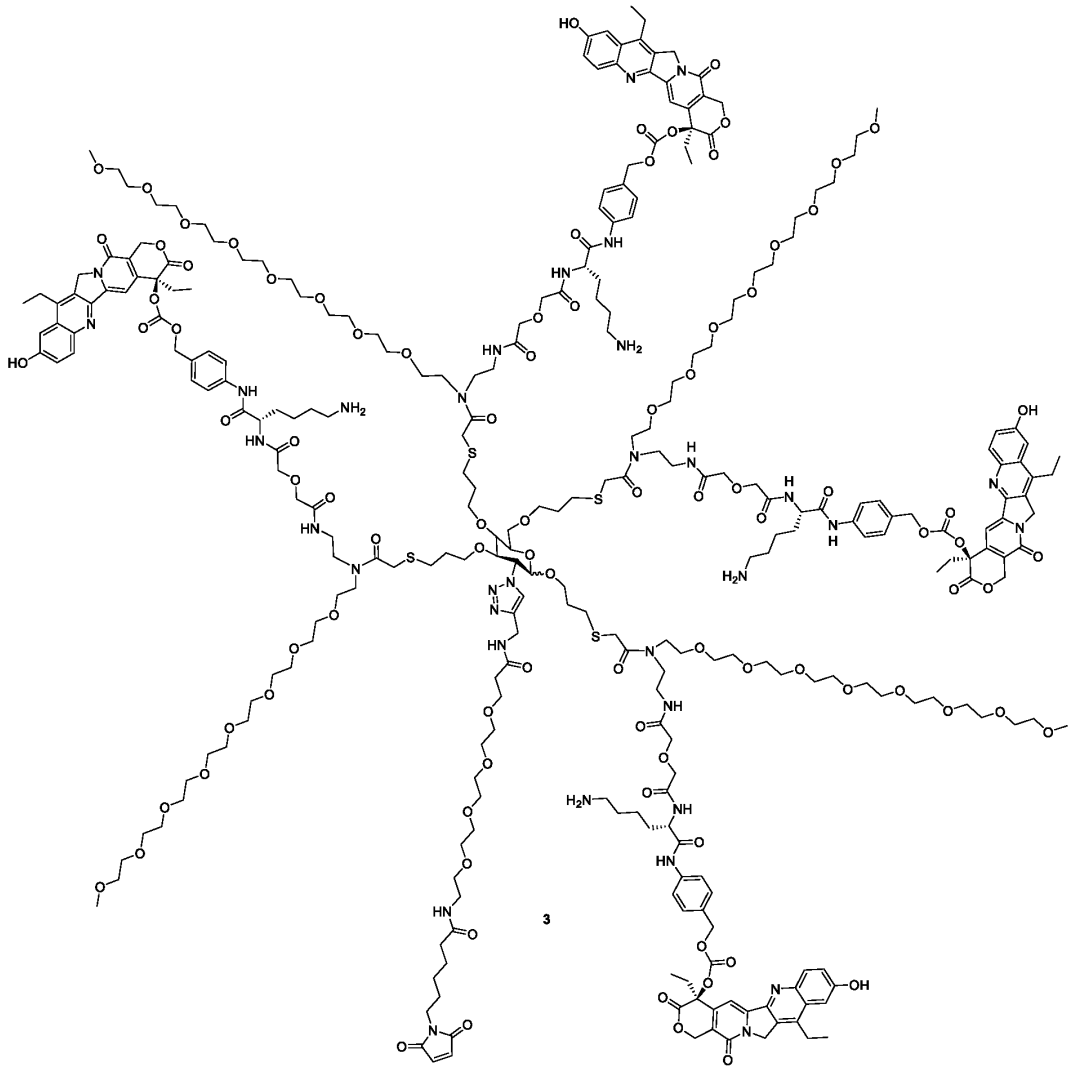
reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-7** (250 mg, yield 85%) as a colorless oil. LCMS: m/z calculated for $C_{91}H_{169}N_9O_{44}S_3$: 2189.6; found: 1096.35 $[M+2H]^{2+}$.

[00439] Step 8: To a solution of compound **2-7** (100 mg, 0.0457 mmol), compound **2-1** (hydrochloride, 180 mg, 0.206 mmol) and DIPEA (94 mg, 0.729 mmol) in DMF (2 mL) was added T3P (50% in DMF solution, 175 mg, 0.275 mmol). The mixture was stirred at room temperature for 14 h, LC-MS showed desired product was formed but not completely converted. DIPEA (45 mg, 0.349 mmol) and T3P (50% in DMF solution, 90 mg, 0.141mmol) were added again. After stirring for additional 8 h, the resulting mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **2-8** (70 mg, yield 33%) as a yellow solid. LCMS: m/z calculated for $C_{229}H_{316}N_{24}O_{71}S_3$: 4637.3; found: 1547.16 $[M+3H]^{3+}$.

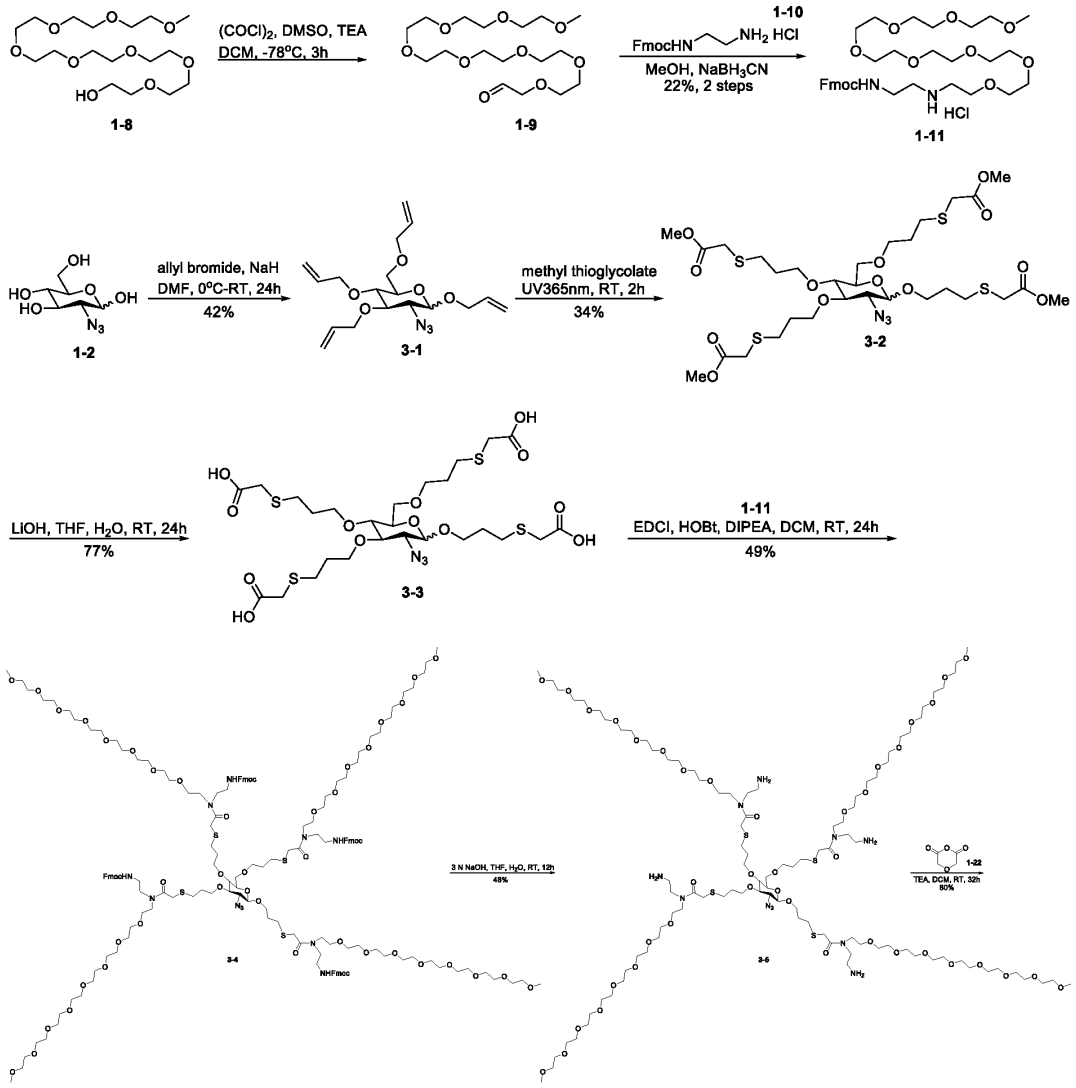
[00440] Step 9: To a solution of compound **2-8** (105 mg, 0.0226 mmol) in DMF (1 mL) was added hydroxylamine hydrochloride (47 mg, 0.676 mmol) and imidazole (46 mg, 0.676 mmol). The mixture was stirred at room temperature for 8 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **2-9** (trifluoroacetate, 67 mg, yield 66%) as a yellow solid. LCMS: m/z calculated for $C_{199}H_{280}N_{24}O_{65}S_3$: 4144.7; found: 1382.85 $[M+3H]^{3+}$.

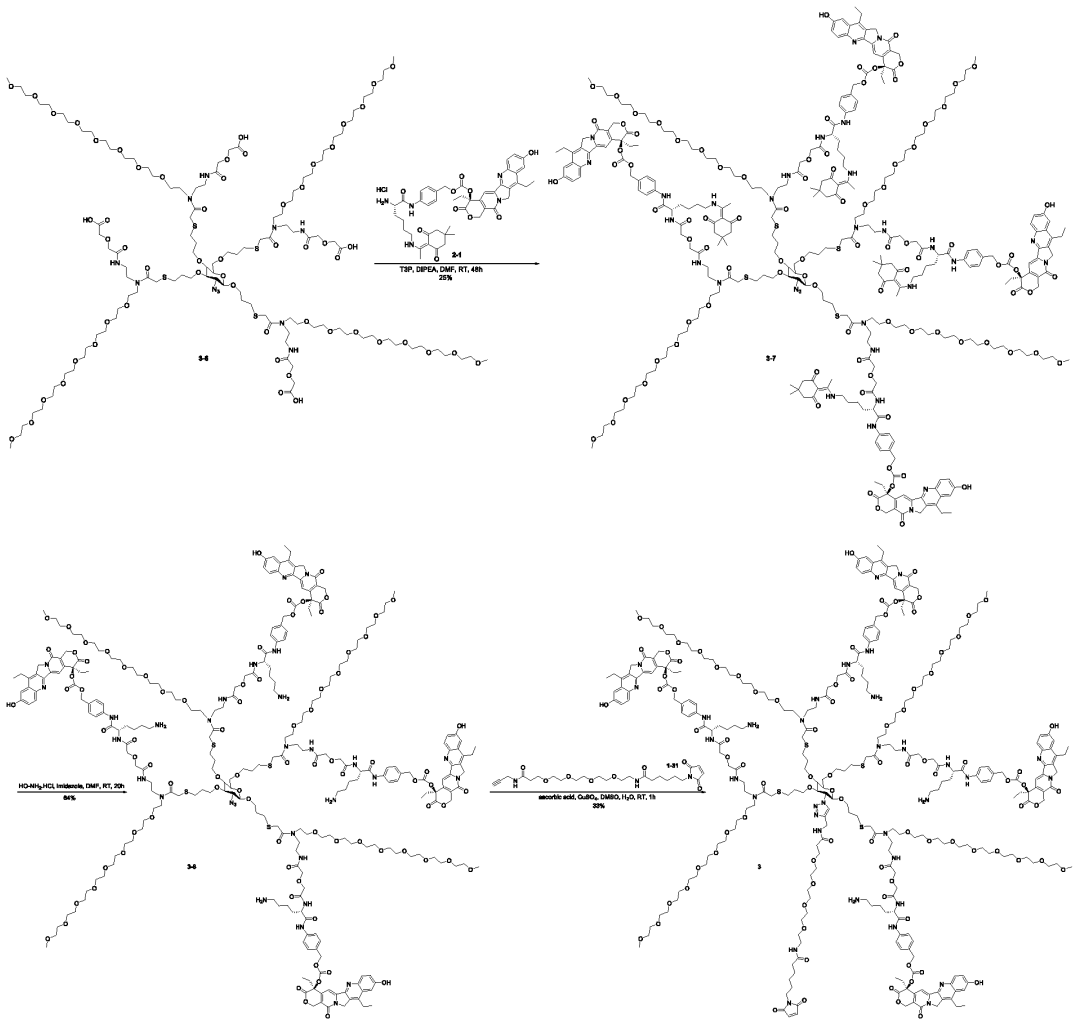
[00441] Step 10: To a solution of compound **2-9** (trifluoroacetate, 53 mg, 0.0118 mmol) and compound **1-31** (13 mg, 0.0262 mmol) in degassed DMSO (2 mL) was added a fresh aqueous solution of ascorbic acid (1 M, 0.106 mL, 0.106 mmol) and a fresh aqueous solution of Cu_2SO_4 (1 M, 0.035 mL, 0.035 mmol). The mixture was purged with nitrogen for three times and stirred at room temperature for 1 h. After completion of the reaction, the resulting mixture was filtered and purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **1** (trifluoroacetate, 26 mg, yield 44%) as a yellow solid. LCMS: m/z calculated for $C_{223}H_{317}N_{27}O_{73}S_3$: 4640.3; found: 1547.98 $[M+3H]^{3+}$.

Example 3. Synthesis of 4-((S)-36-(4-aminobutyl)-26-(2-((3-(((3R,4R,5S,6R)-2,5-bis((26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-6-(26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23,33-nonaoxa-29-thia-26-azatetratriacontan-34-yl)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-4-yl)oxy)propyl)thio)acetyl)-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaheptatriacontan-37-amido)benzyl ((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) carbonate (3)



Synthetic scheme 3


(COCl)2, DMSO, TEA
DCM, -78°C, 3h
1-10
FmocHN NH2 HCl
MeOH, NaBH3CN
22%, 2 steps
1-8
1-9
1-11
allyl bromide, NaH
DMF, 0°C-RT, 24h
42%
methyl thioglycolate
UV365nm, RT, 2h
34%
1-2
3-1
3-2
LiOH, THF, H2O, RT, 24h
77%
1-11
EDCI, HOBt, DIPEA, DCM, RT, 24h
49%
3-3
3 N NaOH, THF, H2O, RT, 12h
48%
1-22
TEA, DCM, RT, 32h
80%
3-4
3-5



[00442] Step 1: Under nitrogen atmosphere, DMSO (1.5 g, 19.20 mmol) was added dropwise to a stirred solution of oxalyl chloride (2 g, 15.76 mmol) in anhydrous dichloromethane (50 mL) at -78 °C. After stirring for 15 min at -78 °C, to this was added dropwise a solution of alcohol **1-8** (3 g, 7.80 mmol) in anhydrous dichloromethane (30 mL). After stirring at -78 °C for 30 min, TEA (3.9 g, 38.61 mmol) was added dropwise. After stirring at that temperature for 1 h, the mixture was warmed slowly to room temperature over 1 h. Aqueous ammonium chloride solution (100 mL) was added thereto. The organic layer was separated, washed with water and

brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product **1-9** (2.46 g) was used at next step without further purification.

[00443] Step 2: Under nitrogen atmosphere, a solution of compound **1-9** (2.46 g, 6.43 mmol) and compound **1-10** (1.6 g, 5.02 mmol) in MeOH (20 mL) was stirred at room temperature for 2 h. Then the mixture was cooled to 0 °C, and $NaBH_3CN$ (1 g, 15.87 mmol) was added in three portions. The resulting mixture was warmed to room temperature and stirred for 15 h. After completion of the reaction, the mixture was concentrated and redissolved in dichloromethane (50 mL). The solution was washed with water and brine. The organic layer was concentrated and the residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **1-11** (hydrochloride, 1.2 g, yield 22% over 2 steps) as a colorless oil. LCMS: m/z calculated for $C_{34}H_{52}N_2O_{10}$: 648.8; found: 649.87 $[M+H]^+$.

[00444] Step 3: Under nitrogen atmosphere, NaH (60% in mineral oil, 1.61 g, 40.25 mmol) was added in portions to a solution of compound **1-2** (1.38 g, 6.73 mmol) in DMF (30 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h, to this was added slowly allyl bromide (4.88 g, 40.33mmol). The reaction was warmed slowly to room temperature and stirred for additional 16 h. After completion of the reaction, the mixture was quenched by addition of cold water (200 mL). The mixture was extracted with ethyl acetate (100 mL x 3) and washed with brine (100 mL x 2). The organic layer was dried by anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 5% ethyl acetate in petroleum ether) to afford the title compound **3-1** (1.05 g, yield 42%) as a colorless oil. ^1H NMR (400 MHz, Chloroform-*d*) δ 5.93 (dddt, *J* = 25.9, 15.5, 10.3, 5.6 Hz, 4H), 5.36 – 5.15 (m, 8H), 4.42 – 4.36 (m, 1H), 4.35 – 4.22 (m, 4H), 4.13 (d, *J* = 5.7 Hz, 1H), 4.10 (dd, *J* = 6.1, 1.4 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.69 (dd, *J* = 10.9, 2.0 Hz, 1H), 3.62 (dd, *J* = 10.9, 4.6 Hz, 1H), 3.44 – 3.28 (m, 3H), 3.23 (t, *J* = 9.3 Hz, 1H).

[00445] Step 4: A mixture of compound **3-1** (1.05 g, 2.87 mmol) and methyl thioglycolate (12 g, 113 mmol) was irradiated with UV 365 nm at room temperature for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **3-2** (780 mg, yield 34%) as a colorless oil. ^1H NMR (400 MHz, DMSO-d_6) δ 4.36 (d, *J* = 7.9 Hz, 1H), 3.83 – 3.78 (m, 1H), 3.71 (tdd, *J* = 9.6, 5.7, 3.3 Hz, 3H), 3.63 (s, 12H), 3.58 – 3.50 (m, 4H), 3.50 – 3.41 (m, 3H),

3.37 (dd, J = 6.0, 3.3 Hz, 6H), 3.26 (td, J = 7.8, 2.9 Hz, 1H), 3.21 – 3.12 (m, 2H), 2.69 – 2.56 (m, 8H), 1.84 – 1.69 (m, 8H). LCMS: m/z calculated for $C_{30}H_{51}N_3O_{13}S_4$: 790.0; found: 813.22 $[M+Na]^+$.

[00446] Step 5: 2 N LiOH solution (7.6 mL, 15.2 mmol) was added at 0 °C to a solution of compound **3-2** (1 g, 1.26 mmol) in THF (10 mL). The reaction was stirred at room temperature for 24 h. After completion of the reaction, the mixture was concentrated and adjusted pH to 3 with 1 N HCl at 0 °C. The aqueous solution was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **3-3** (720 mg, yield 77%) as a colorless oil. LCMS: m/z calculated for $C_{26}H_{43}N_3O_{13}S_4$: 733.9; found: 756.41$[M+Na]^+$.

[00447] Step 6: A solution of compound **3-3** (250 mg, 0.341 mmol), HOBt (370 mg, 2.74 mmol), DIPEA (700 mg, 5.42 mmol) and EDCI (520 mg, 2.72 mmol) in dichloromethane (5 mL) was stirred at room temperature under nitrogen atmosphere for 10 min, a solution of compound **1-11** (hydrochloride, 1.2 g, 1.75 mmol) in dichloromethane (5 mL) was added thereto. The resulting reaction mixture was stirred at room temperature for 24 h. The mixture concentrated and the residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **3-4** (550 mg, yield 49%) as a colorless oil. LCMS: m/z calculated for $C_{162}H_{243}N_{11}O_{49}S_4$: 3257.0; found: 1629.90 $[M+2H]^{2+}$.

[00448] Step 7: To a solution of compound **3-4** (550 mg, 0.169 mmol) in THF (5 mL) was added slowly 3 N NaOH solution (0.67 mL, 2.01 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was concentrated and the residue was acidized at 0 °C with 1 N HCl to pH = 3. The aqueous solution was washed with ethyl acetate (10 mL x 2) and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **3-5** (hydrochloride, 205 mg, yield 48%) as a colorless oil. LCMS: m/z calculated for $C_{102}H_{203}N_{11}O_{41}S_4$: 2368.0; found: 1185.40 $[M+2H]^{2+}$.

[00449] Step 8: To a solution of compound **3-5** (hydrochloride, 86 mg, 0.0342 mmol) and TEA (56 mg, 0.554 mmol) in dichloromethane (3 mL) was added diglycolic anhydride **1-22** (32 mg, 0.275 mmol). The reaction was stirred at room temperature for 32 h. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash

chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **3-6** (78 mg, yield 80%) as a colorless oil. LCMS: m/z calculated for $C_{118}H_{219}N_{11}O_{57}S_4$: 2832.3; found: 1417.70 $[M+2H]^{2+}$.

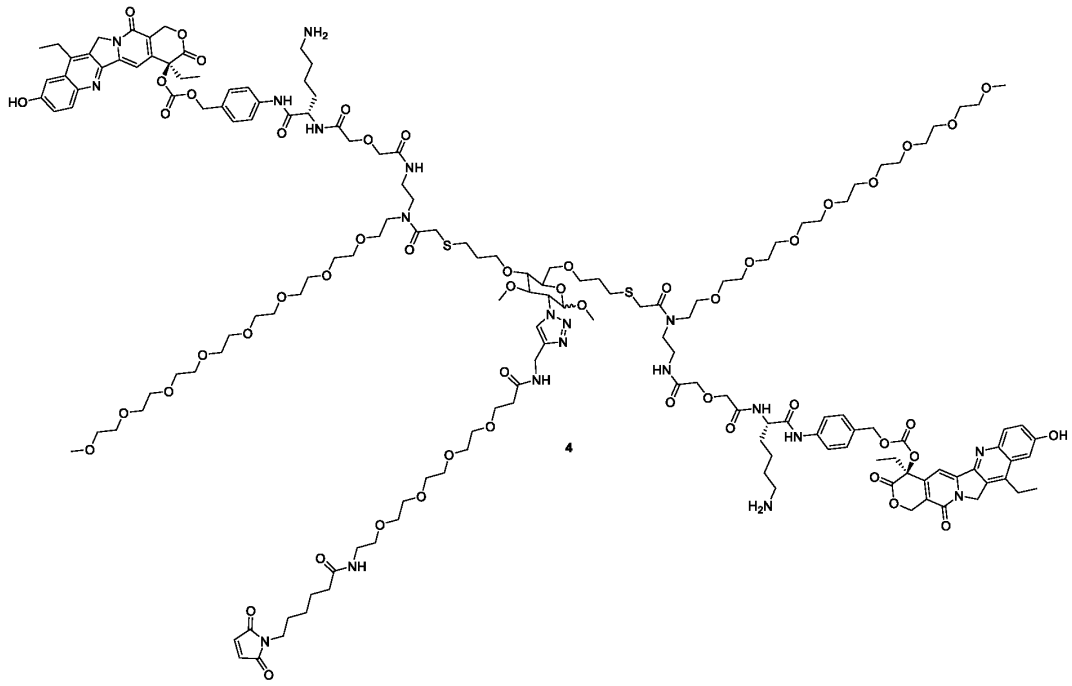
[00450] Step 9: To a solution of compound **3-6** (90mg, 0.0318 mmol), compound **2-1** (hydrochloride, 190 mg, 0.218 mmol) and DIPEA (98mg, 0.760 mmol) in DMF (2 mL) was added T3P (50% in DMF solution, 200 mg, 0.314 mmol). The mixture was stirred at room temperature for 24 h, LC-MS showed desired product was formed but not completely converted. Compound **2-1** (hydrochloride, 190 mg, 0.218 mmol), DIPEA (98mg, 0.760 mmol) and T3P (50% in DMF solution, 200 mg, 0.314 mmol) were added again. After stirring for additional 24 h at 45 °C, the resulting mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **3-7** (50 mg, yield 25%) as a yellow solid. LCMS: m/z calculated for $C_{302}H_{415}N_{31}O_{93}S_4$: 6096.0; found: 1525.43 $[M+4H]^{4+}$.

[00451] Step 10: To a solution of compound **3-7** (80 mg, 0.0131 mmol) in DMF (1 mL) was added hydroxylamine hydrochloride (36 mg, 0.518 mmol) and imidazole (36 mg, 0.529mmol). The mixture was stirred at room temperature for 20 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **3-8** (trifluoroacetate, 50 mg, yield 64%) as a yellow solid. LCMS: m/z calculated for $C_{262}H_{367}N_{31}O_{85}S_4$: 5439.2; found: 1361.10 $[M+4H]^{4+}$.

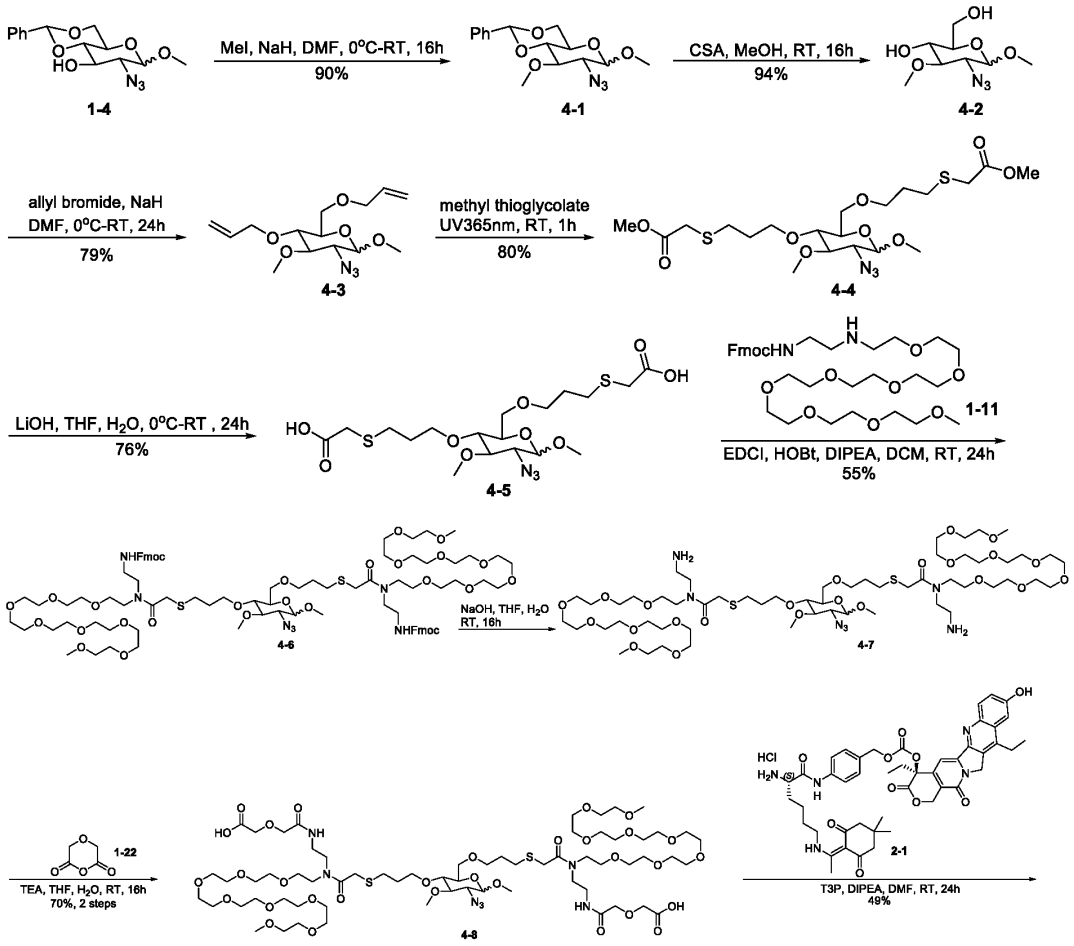
[00452] Step 11: To a solution of compound **3-8** (trifluoroacetate, 80 mg, 0.0136 mmol) and compound **1-31** (20 mg, 0.0404 mmol) in degassed DMSO (3 mL) was added a fresh aqueous solution of ascorbic acid (1 M, 0.122 mL, 0.122 mmol) and a fresh aqueous solution of Cu_2SO_4 (1 M, 0.041 mL, 0.041 mmol). The mixture was purged with nitrogen for three times and stirred at room temperature for 1 h. After completion of the reaction, the resulting mixture was filtered and purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **3** (trifluoroacetate, 29 mg, yield 33%) as a yellow solid. LCMS: m/z calculated for $C_{286}H_{404}N_{34}O_{93}S_4$: 5934.8; found: 1485.28 $[M+4H]^{4+}$.

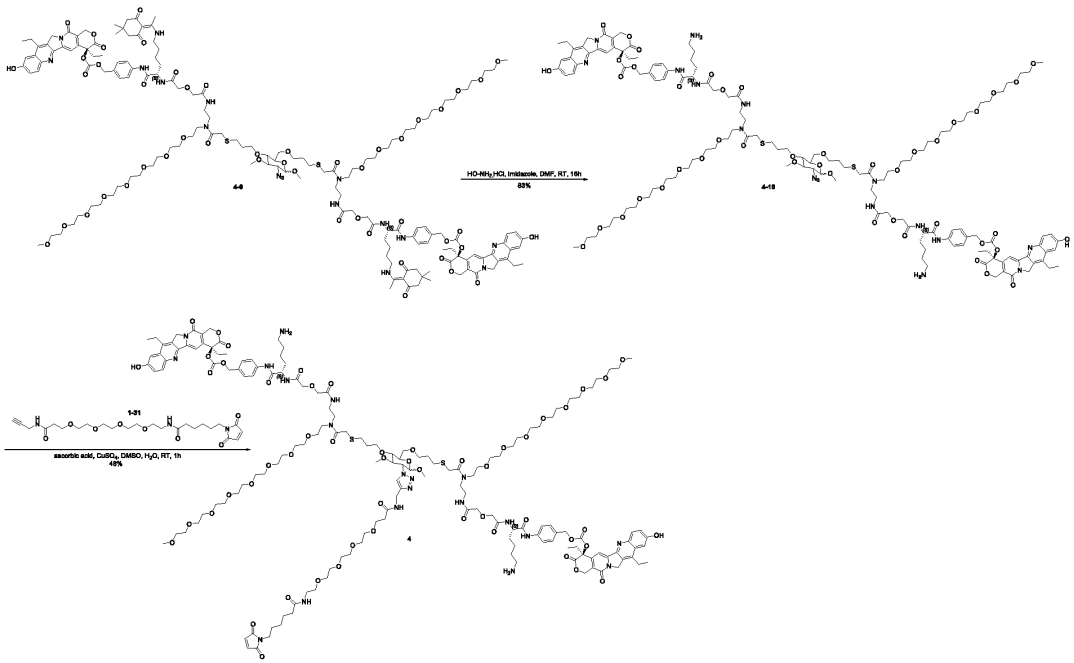
Example 4. Synthesis of 4-((S)-26-(2-((3-(((2R,3S,4R,5R)-3-((26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-

pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-4,6-dimethoxytetrahydro-2H-pyran-2-yl)methoxy)propyl)thio)acetyl)-36-(4-aminobutyl)-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaheptatriacontan-37-amido)benzyl ((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) carbonate (4)



Synthetic scheme 4


MeI, NaH, DMF, 0°C-RT, 16h
90%
CSA, MeOH, RT, 16h
94%
1-4
4-1
4-2
allyl bromide, NaH
DMF, 0°C-RT, 24h
79%
methyl thioglycolate
UV365nm, RT, 1h
80%
4-3
4-4
LiOH, THF, H2O, 0°C-RT , 24h
76%
1-11
EDCI, HOBt, DIPEA, DCM, RT, 24h
55%
4-5
NaOH, THF, H2O
RT, 16h
4-6
4-7
1-22
TEA, THF, H2O, RT, 16h
70%, 2 steps
4-8
T3P, DIPEA, DMF, RT, 24h
49%
2-1



[00453] Step 1: Under nitrogen, to a solution of compound **1-4** (1.8 g, 5.85 mmol) in DMF (15 mL) at 0 °C was added NaH (60% in mineral oil, 586 mg, 14.65 mmol) in several portions. After stirring for 30 min at 0 °C, MeI (1.66 g, 11.69 mmol) was added thereto. The mixture was warmed to room temperature and stirred for 16 h. After completion of the reaction, the mixture was quenched by adding NH_4Cl solution and extracted with ethyl acetate (100 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 10% ethyl acetate in Petroleum ether) to afford the title compound **4-1** (1.7 g, yield 90%) as a colorless oil.

[00454] Step 2: To a solution of compound **4-1** (2.49 g, 7.72 mmol) in MeOH (20 mL) was added D-(+)-10-Camphorsulfonic Acid (5.39 g, 23.2 mmol) at room temperature. The reaction mixture was stirred for 16 h. After completion of the reaction, the resulting mixture was concentrated. The residue was purified by silica gel column (eluted with 25% ethyl acetate in Petroleum ether) to afford the title compound **4-2** (1.7 g, yield 94%) as a colorless oil.

[00455] Step 3: Under nitrogen, to a solution of compound **4-2** (2.4 g, 10.29 mmol) in DMF (30 mL) at 0 °C was added NaH (60% in mineral oil, 1.64 g, 41.0 mmol) in several portions. After stirring for 1 h at 0 °C, allyl bromide (3.73 g, 30.83 mmol) was added dropwise thereto. The mixture was warmed to room temperature and stirred for 24 h. After completion of the

reaction, the mixture was quenched by adding water and extracted with ethyl acetate (100 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 5% ethyl acetate in Petroleum ether) to afford the title compound **4-3** (2.55 g, yield 79%) as a colorless oil.

[00456] Step 4: A mixture of compound **4-3** (1.9 g, 6.06 mmol) and methyl thioglycolate (6 mL, 67.1 mmol) was irradiated with UV 365 nm at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under high vacuum. The residue was purified by silica gel (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **4-4** (2.7 g, yield 80%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.73 (d, *J* = 3.5 Hz, 0.65H), 4.09 (d, *J* = 8.0 Hz, 0.35H), 3.83 (s, 1H), 3.73 (s, 6H), 3.67 (t, *J* = 5.2 Hz, 1H), 3.65 – 3.55 (m, 8H), 3.53 (s, 2H), 3.38 (s, 2H), 3.28 (s, 1H), 3.26 (d, *J* = 2.6 Hz, 1H), 3.23 (d, *J* = 2.9 Hz, 4H), 2.77 – 2.68 (m, 4H), 1.90 (dp, *J* = 21.0, 7.5, 6.9 Hz, 4H).

[00457] Step 5: A mixture of compound **4-4** (1.0 g, 1.90 mmol) and 1 M LiOH solution (7.6 mL, 7.6 mmol) in THF (10 mL) was stirred at room temperature for 24 h. After completion of the reaction, the mixture was concentrated. The aqueous solution was acidized with 1 N HCl to pH = 5 and filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were concentrated to give the title compound **4-5** (720 mg, yield 76%) as a pale-yellow oil. LCMS: m/z calculated for $C_{18}H_{31}N_3O_9S_2$: 497.58; found: 520.82 $[M+Na]^+$.

[00458] Step 6: A solution of compound **4-5** (350 mg, 0.703 mmol), HOBt (190 mg, 1.41 mmol), DIPEA (362 mg, 2.80 mmol) and EDCI (537 mg, 2.81 mmol) in anhydrous dichloromethane (5 mL) was stirred at room temperature for 10 min. To this was added a solution of compound **1-11** (hydrochloride, 1.49 g, 2.17 mmol) and DIPEA (260 mg, 2.01 mmol) in anhydrous dichloromethane (5 mL). The resulting mixture was stirred at room temperature for 24 h. After completion of the reaction, the mixture was diluted with dichloromethane (40 mL) and washed with water and brine. The organic layers were concentrated and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were concentrated to give the title compound **4-6** (680 mg, yield 55%) as a pale-yellow oil. LCMS: m/z calculated for $C_{86}H_{131}N_7O_{27}S_2$: 1759.14; found: 1760.25 $[M+H]^+$.

[00459] Step 7: To a solution of compound **4-6** (350 mg, 0.199 mol) in THF (4 mL) was added aqueous NaOH solution (2 N, 0.4 mL, 0.4 mmol) at room temperature. The mixture was stirred for 16 h. After completion of the reaction, the mixture was concentrated to get rid of the THF solvents. The aqueous residue was acidified with 1 N HCl to pH = 2, and washed with dichloromethane (10 mL x 2). The left aqueous solution contain compound **4-7** was used at next step without further purification. LCMS: m/z calculated for $C_{56}H_{111}N_7O_{23}S_2$: 1314.65; found: 1315.68 $[M+H]^+$.

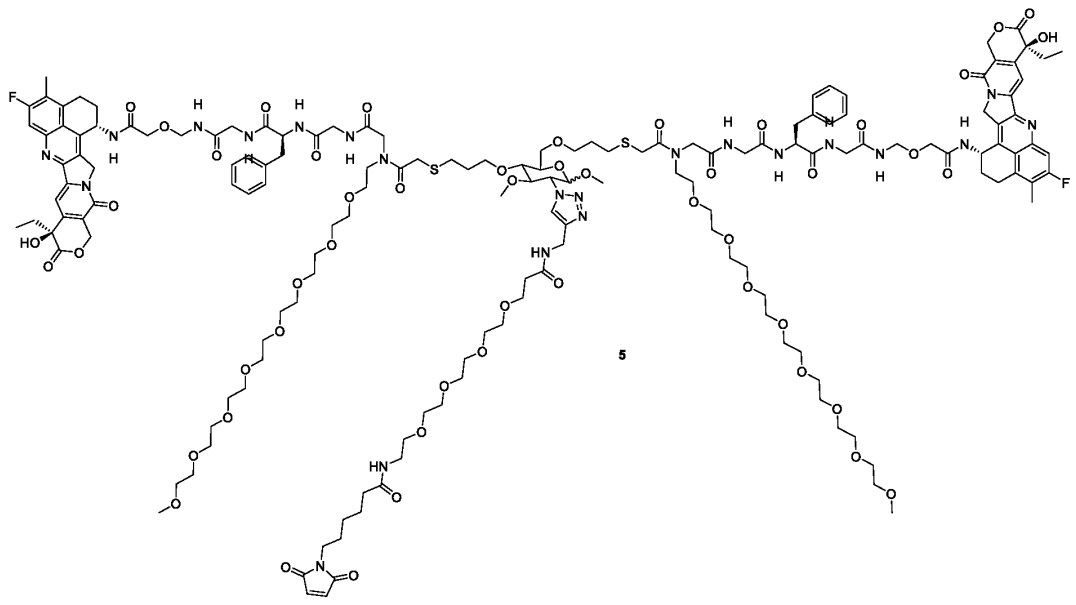
[00460] Step 8: The aqueous solution contain compound **4-7** was treated with THF and adjusted pH = 8 with TEA. To this was added compound **1-22** (100 mg, 0.862 mmol). The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were concentrated to give the title compound **4-8** (218 mg, yield 70%, 2 steps) as a colorless oil. LCMS: m/z calculated for $C_{64}H_{119}N_7O_{31}S_2$: 1546.79; found: 1547.98 $[M+H]^+$.

[00461] Step 9: To a solution of compound **4-8** (110 mg, 0.0711), compound **2-11** (hydrochloride, 210 mg, 0.241 mmol) and DIPEA (96 mg, 0.742 mmol) in anhydrous DMF (3 mL) was added dropwise T3P (50% in DMF, 200 mg, 0.314 mmol). The mixture was stirred at room temperature for 24 h. After completion of the reaction, the mixture was quenched by adding water (1 mL) and filtered. The filtrate was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were concentrated to give the title compound **4-9** (trifluoroacetate, 120 mg, yield 49%) as a yellow solid. LCMS: m/z calculated for $C_{156}H_{217}N_{17}O_{49}S_2$: 3178.64; found: 1060.95 $[M+3H]^{3+}$.

[00462] Step 10: To a solution of compound **4-9** (120 mg, 0.0352 mmol) in DMF (2.5 mL) was added imidazole (40 mg, 0.588 mmol) and Hydroxylamine hydrochloride (0.580). The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was filtered and diluted with water (1 mL). The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were concentrated to give the title compound **4-10** (trifluoroacetate, 90 mg, yield 83%) as a yellow solid. LCMS: m/z calculated for $C_{136}H_{193}N_{17}O_{45}S_2$: 2850.23; found: 1426.89 $[M+2H]^{2+}$.

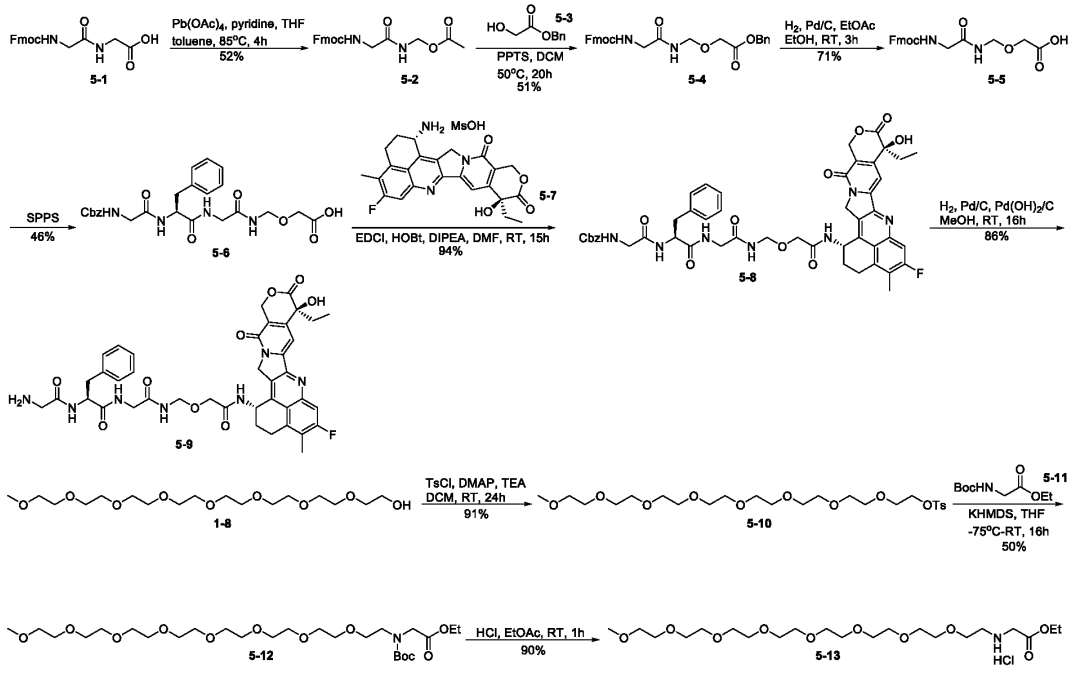
[00463] Step 11: To a solution of compound **4-10** (trifluoroacetate, 90 mg, 0.0292 mmol) and compound **1-31** (24 mg, 0.0484 mmol) in degassed DMSO (3 mL) was added a fresh aqueous solution of ascorbic acid (1 M, 0.285 mL, 0.285 mmol) and a fresh aqueous solution of Cu_2SO_4 (1 M, 0.095 mL, 0.095 mmol). The mixture was purged with nitrogen for three times and stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and diluted with water (1 mL). The resulting mixture was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **4** (trifluoroacetate, 50.3 mg, yield 48%) as a yellow solid. LCMS: m/z calculated for $C_{160}H_{230}N_{20}O_{53}S_2$: 3345.8; found: 1116.87 $[M+3H]^{3+}$.

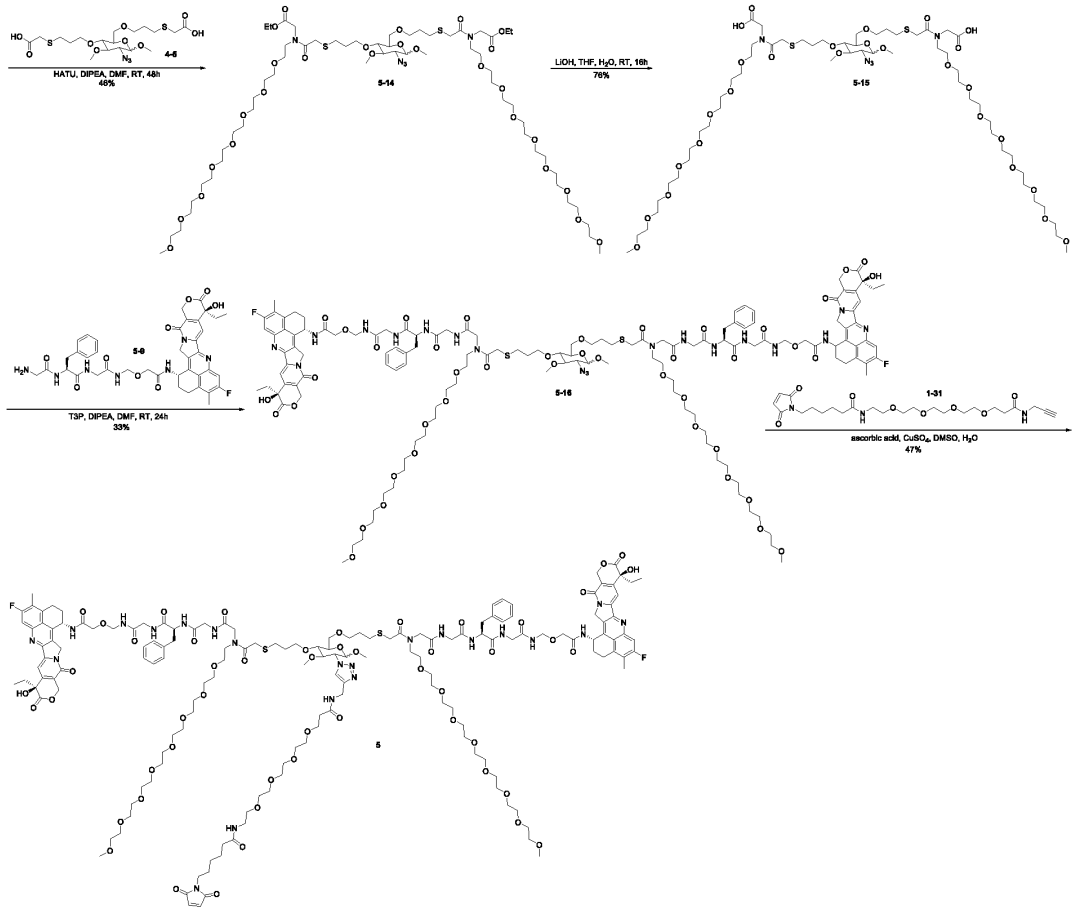
Example 5. Synthesis of N-((1-((3R,4R,5S,6R)-5-((26-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-6-(26-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)-27-oxo-2,5,8,11,14,17,20,23,33-nonaoxa-29-thia-26-azatetratriacontan-34-yl)-2,4-dimethoxytetrahydro-2H-pyran-3-yl)-1H-1,2,3-triazol-4-yl)methyl)-1-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3,6,9,12-tetraoxapentadecan-15-amide (5)



5

Synthetic scheme 5





[00464] Step 1: To a mixture of compound **5-1** (20.0 g, 56.4 mmol) in tetrahydrofuran (400 mL) and toluene (100 mL) was added lead tetraacetate (30.0 g, 69.2 mmol) in several portions at room temperature with stirring. The mixture was stirred at 85 °C for 4 h under nitrogen atmosphere. After completion of the reaction, the mixture was cooled to room temperature and the insoluble white solid was removed by filtration through diatomite. The filtrate was concentrated and the residue was re-dissolved in ethyl acetate (200 mL), washed with water (200 mL) and brine (200 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 45% ethyl acetate in petroleum ether) to afford the title compound **5-2** (10.8 g, yield 52%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (t, J = 7.2 Hz, 1H), 7.88 (d, J = 7.5 Hz,

2H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.56 (t, *J* = 6.3 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 5.08 (d, *J* = 6.9 Hz, 2H), 4.28 (d, *J* = 6.9 Hz, 2H), 4.25 – 4.18 (m, 1H), 3.64 (d, *J* = 6.2 Hz, 2H), 1.98 (s, 3H).

[00465] Step 2: A mixture of compound **5-2** (10.0 g, 27.1 mmol), compound **5-3** (45.1 g, 271 mmol) and PPTS (680 mg, 2.71 mmol) in anhydrous dichloromethane (100 mL) was stirred at 50 °C for 20 h under nitrogen atmosphere. After completion of the reaction, the mixture was cooled and diluted with ethyl acetate (400 mL), washed with water (300 mL) and brine (300 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 60% ethyl acetate in petroleum ether) to afford the title compound **5-4** (6.57 g, yield 51%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (t, *J* = 6.7 Hz, 1H), 7.91 – 7.86 (m, 2H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 5.8 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 7H), 5.13 (s, 2H), 4.62 (d, *J* = 6.6 Hz, 2H), 4.28 (d, *J* = 6.9 Hz, 2H), 4.21 (dd, *J* = 13.3, 5.5 Hz, 1H), 4.14 (s, 2H), 3.62 (d, *J* = 5.9 Hz, 2H).

[00466] Step 3: A mixture of compound **5-4** (6.0 g, 12.6 mmol) and 5w% Pd/C (1.2 g) in ethanol (150 mL) and ethyl acetate (150 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. After completion of the reaction, the mixture was filtered over diatomite. The filtrate was concentrated and the crude product was triturated with ethyl acetate and petroleum ether (v:v = 1:3) for 1 h to afford the title compound **5-5** (3.45 g, yield 71%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (t, *J* = 6.7 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.57 (t, *J* = 6.0 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 4.59 (d, *J* = 6.6 Hz, 2H), 4.28 (d, *J* = 6.9 Hz, 2H), 4.22 (d, *J* = 6.8 Hz, 1H), 3.94 (s, 2H), 3.61 (d, *J* = 5.8 Hz, 2H).

[00467] Step 4: 2-Chorotrityl Chloride resin (0.71 mmol/g, 11.00 g, 7.81 mmol) was swollen in dichloromethane (50 mL) with nitrogen bubbling for 30 min. After removing the dichloromethane, the first amino acid compound **5-5** (3.00 g, 7.80 mmol), dichloromethane (50 mL) and DIPEA (4.03 g, 31.18 mmol) were added. The mixture was bubbled with nitrogen for 2 h, the unreacted Cl group was blocked with MeOH (5 mL) for 30 min. Next, the resin was washed with DMF and dichloromethane. Then Fmoc was removed by 20 % piperidine in DMF for 30 min. The next amino acids were added by using the following coupling condition: For coupling conditions: Fmoc-AA-OH/HBTU/DIPEA (15.60 : 14.82 : 31.20 mmol) in DMF for 30

min. The following amino acids were coupled one by one: Fmoc-Gly-OH, Fmoc-Phe-OH and Cbz-Gly-OH. The final resin was washed with DMF and MeOH. At last, desired linear peptide was cleaved from the resin with 20% hexafluoroisopropanol in dichloromethane for 1 h. The mixture was filtered and the filtrate was concentrated under reduced pressure at 40 °C to give a residue. The crude product was purified by reverse-phase flash chromatography (eluted with acetonitrile in water, neutral condition). The desired components were lyophilized to afford the title compound **5-6** (1.80 g, 46%) as a white solid. LCMS: m/z calculated for $C_{24}H_{28}N_4O_8$: 500.19; found 523.91 $[M+Na]^+$.

[00468] Step 5: To a suspension of compound **5-7** (mesylate, 450 mg, 0.84 mmol), HOBt (135 mg, 1.0 mmol) and DIPEA (390 mg, 3.01 mmol) in anhydrous DMF (5 mL) was added compound **5-6** (500 mg, 1.0 mmol) and EDCI (382 mg, 2.0 mmol). The mixture was stirred at room temperature for 15 h. After completion of the reaction, the mixture was extracted with ethyl acetate (50 mL x 5) and H_2O (50 mL). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue. The crude product was purified by silica gel column (eluted with 9% MeOH in dichloromethane) to afford the title compound **5-8** (730 mg, yield 94%) as a yellow solid. LCMS: m/z calculated for $C_{48}H_{48}FN_7O_{11}$: 917.34; found: 918.60 $[M+H]^+$.

[00469] Step 6: A mixture of compound **5-8** (625 mg, 0.68 mmol), $Pd(OH)_2/C$ (5 w%, 150 mg) and Pd/C (5 w%, 150 mg) in MeOH (30 mL) was stirred at room temperature under hydrogen atmosphere (0.8 MPa) for 16 h. After completion of the reaction, the mixture was filtered through diatomite and concentrated to afford the title compound **5-9** (460 mg, yield 86%) as a yellow solid. LCMS: m/z calculated for $C_{40}H_{42}FN_7O_9$: 783.30; found: 784.76 $[M+H]^+$.

[00470] Step 7: To a solution of compound **1-8** (2.7 g, 7.02 mmol) and DMAP (170 mg, 1.39 mmol) in dichloromethane (30 mL) was added Et_3N (2.84 g, 28.06 mmol) and tosyl chloride (4.69 g, 24.60 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 14 h. After completion of the reaction, the mixture was diluted with dichloromethane (100 mL) and washed with H_2O (100 mL), 1 N HCl (100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue. The crude product was purified by silica gel column (eluted with 6% MeOH in dichloromethane) to afford the title compound **5-10** (3.45 g, yield 91%) as a colorless oil. LCMS: m/z calculated for $C_{24}H_{42}O_{11}S$: 538.24; found: 539.88 $[M+H]^+$.

[00471] Step 8: Under nitrogen atmosphere, a solution of compound **5-11** (1.6 g, 7.87 mmol) in anhydrous THF (6 mL) was stirred and cooled to -75 °C over 10 min. To this was added slowly KHMDS (1 M in THF, 10 mL, 10.0 mmol). The mixture was stirred at -75 °C for 1 h before a solution of compound **5-10** (3.0 g, 5.57 mmol) in anhydrous THF (8 mL) was added dropwise. The resulting mixture was stirred at -75 °C for 4 h and warmed to room temperature with further stirring for 12 h. After completion of the reaction, the mixture was added dropwise to a cold 1 N HCl solution (30 mL) in an ice-bath. The mixture was extracted with ethyl acetate (50 mL x 2) and H_2O (30 mL). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue. The crude product was purified by silica gel column (eluted with 5% MeOH in dichloromethane) to afford the title compound **5-12** (1.6 g, yield 50%) as a colorless oil. LCMS: m/z calculated for $C_{26}H_{51}NO_{12}$: 569.69; found: 593.13 $[M+Na]^+$.

[00472] Step 9: 4 N HCl in ethyl acetate (1.6 mL) was added to a solution of compound **5-12** (1.6 g, 2.81 mmol) in ethyl acetate (3 mL). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated to give the title compound **5-13** (hydrochloride, 1.4 g, yield 98%) as a yellow oil. LCMS: m/z calculated for $C_{21}H_{43}NO_{10}$: 469.57; found: 470.81 $[M+H]^+$.

[00473] Step 10: To a solution of compound **4-5** (460 mg, 0.92 mmol) and DIPEA (716 mg, 5.53 mmol) in anhydrous DMF (5 mL) was added HATU (1.4 g, 3.68 mmol). The mixture was stirred at room temperature for 10 min before a solution of compound **5-13** (1.4 g, 2.75 mmol) and DIPEA (716 mg, 5.53 mmol) in anhydrous DMF (5 mL) was added thereto. The reaction mixture was stirred at room temperature for 12 h, additional HATU (702 mg, 1.85 mmol) and DIPEA (240 mg, 1.86 mmol) was added. The resulting mixture was stirred at room temperature for further 12 h. The mixture was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **5-14** (600 mg, yield 46%) as a light brown oil. LCMS: m/z calculated for $C_{60}H_{113}N_5O_{27}S_2$: 1400.69; found: 1401.92 $[M+H]^+$.

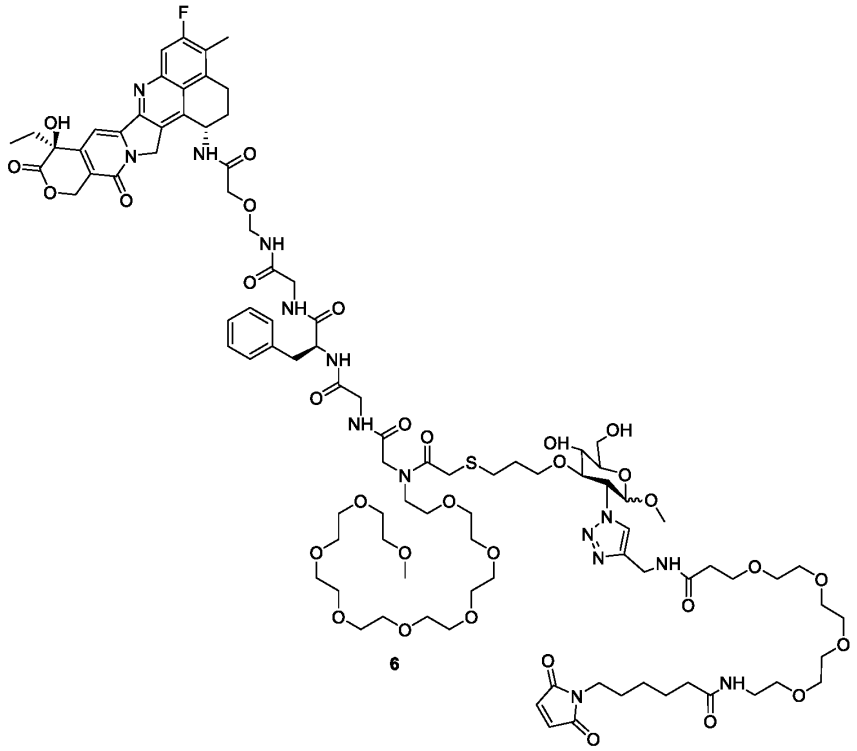
[00474] Step 11: 1 N LiOH (0.86 mL, 0.86 mmol) was added to a solution of compound **5-14** (300 mg, 0.21 mmol) in THF (6 mL) at 0 °C. The reaction mixture was warmed slowly to room temperature and stirred for 18 h. After completion of the reaction, the mixture was concentrated and adjusted to pH = 5. The aqueous solution was purified directly by reverse phase flash

chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **5-15** (220 mg, yield 76%) as a pale-yellow oil. LCMS: m/z calculated for $C_{56}H_{105}N_5O_{27}S_2$: 1344.58; found: 1345.87 $[M+H]^+$.

[00475] Step 12: To a solution of compound **5-15** (70 mg, 0.05 mmol), compound **5-9** (120 mg, 0.15 mmol) in anhydrous DMF (3 mL) was added DIPEA (80 mg, 0.62 mmol) and T3P (50w% in DMF, 200 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the mixture was quenched by addition of H_2O (0.5 mL). The resulting mixture was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **5-16** (57 mg, yield 38%) as a pale-yellow oil. LCMS: m/z calculated for $C_{136}H_{185}F_2N_{19}O_{43}S_2$: 2876.18; found: 1440.00 $[M+2H]^{2+}$.

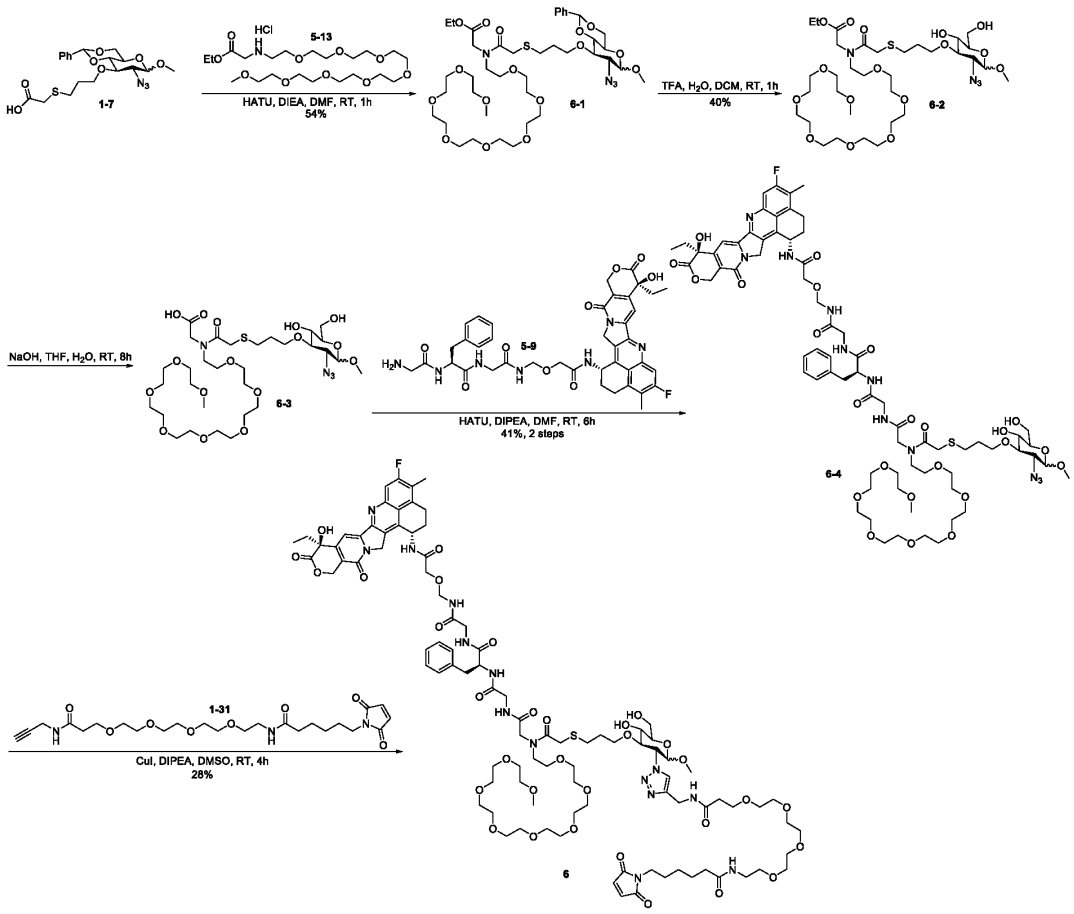
[00476] Step 13: Under nitrogen atmosphere, to a solution of compound **5-16** (57 mg, 0.02 mmol) and compound **1-31** (20 mg, 0.04 mmol) in DMSO (1 mL) was added an aqueous ascorbic acid solution (1 M, 0.18 mL, 0.18 mmol) and an aqueous $CuSO_4$ solution (1 M, 0.06 mL, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **5** (31.6 mg, yield 47%) as a pale-yellow solid. LCMS: m/z calculated for $C_{160}H_{222}F_2N_{22}O_{51}S_2$: 3371.76; found: 1687.39 $[M+2H]^{2+}$.

Example 6. Synthesis of N-((1-((3R,4R,5S,6R)-4-((26-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-5-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-3-yl)-1H-1,2,3-triazol-4-yl)methyl)-1-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3,6,9,12-tetraoxapentadecan-15-amide (6)



6

Synthetic scheme 6



[00477] Step 1: To a solution of compound **1-7** (500 mg, 1.14 mmol) in anhydrous dichloromethane (8 mL) was added HOBt (150 mg, 1.11 mmol) and EDCI (437 mg, 2.28 mmol). After the mixture was stirred at room temperature for 10 min, to this was added a solution of compound **5-13** (800 mg, 1.58 mmol) and DIPEA (440 mg, 3.40 mmol). The reaction mixture was stirred at room temperature for further 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **6-1** (550 mg, yield 54%) as a yellow oil. LCMS: m/z calculated for $C_{40}H_{66}N_4O_{16}S$: 891.04; found: 892.25 $[M+H]^+$.

[00478] Step 2: A mixture of compound **6-1** (550 mg, 0.62 mmol) and 80% aqueous trifluoroacetic acid solution (1.5 mL) in dichloromethane (6 mL) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated to give a residue. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **6-2** (200 mg, yield 40%) as a pale-yellow oil. LCMS: m/z calculated for $C_{33}H_{62}N_4O_{16}S$: 802.93; found: 804.23 $[M+H]^+$.

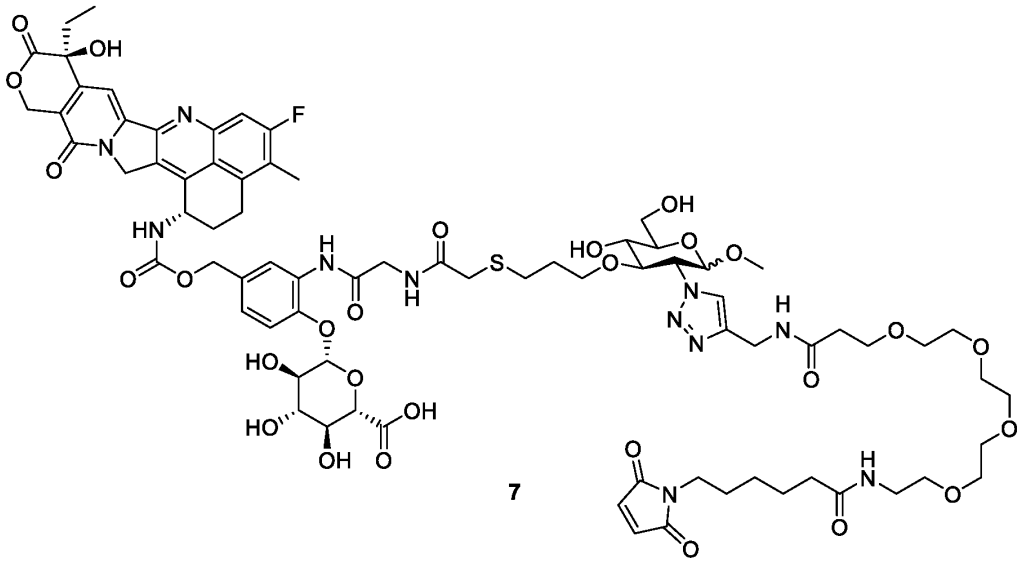
[00479] Step 3: To a solution of compound **6-2** (200 mg, 0.25 mmol) in THF (4 mL) was added 1 N aqueous LiOH solution (0.5 mL, 0.5 mmol). The reaction mixture was stirred at room temperature for 8 h. After completion of the reaction, the mixture was concentrated. The resulting residue was adjusted to pH = 5 with 1 N aqueous HCl solution. The final aqueous solution was filtered and concentrated to afford the title compound **6-3** (200 mg crude) as a pale-yellow oil. LCMS: m/z calculated for $C_{31}H_{58}N_4O_{16}S$: 774.88; found: 776.38 $[M+H]^+$.

[00480] Step 4: To a solution of compound **6-3** (200 mg crude) and DIPEA (88 mg, 0.68 mmol) in anhydrous DMF (5 mL) was added HATU (128 mg, 0.34 mmol). After the mixture was stirred at room temperature for 10 min, to this was added compound **5-9** (265 mg, 0.34 mmol). The reaction mixture was stirred at room temperature for further 6 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **6-4** (160 mg, yield 41% over 2 steps) as a yellow solid. LCMS: m/z calculated for $C_{71}H_{98}FN_{11}O_{24}S$: 1540.68; found: 1541.74 $[M+H]^{2+}$.

[00481] Step 5: Under nitrogen atmosphere, to a solution of compound **6-4** (80 mg, 0.05 mmol) and compound **1-31** (51 mg, 0.10 mmol) in DMSO (1 mL) was added CuI (19 mg, 0.10 mmol) and DIPEA (53 mg, 0.41 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **6** (30 mg, yield 28%) as a white solid. LCMS: m/z calculated for $C_{95}H_{135}FN_{14}O_{32}S$: 2036.25; found: 1019.65 $[M+2H]^{2+}$.

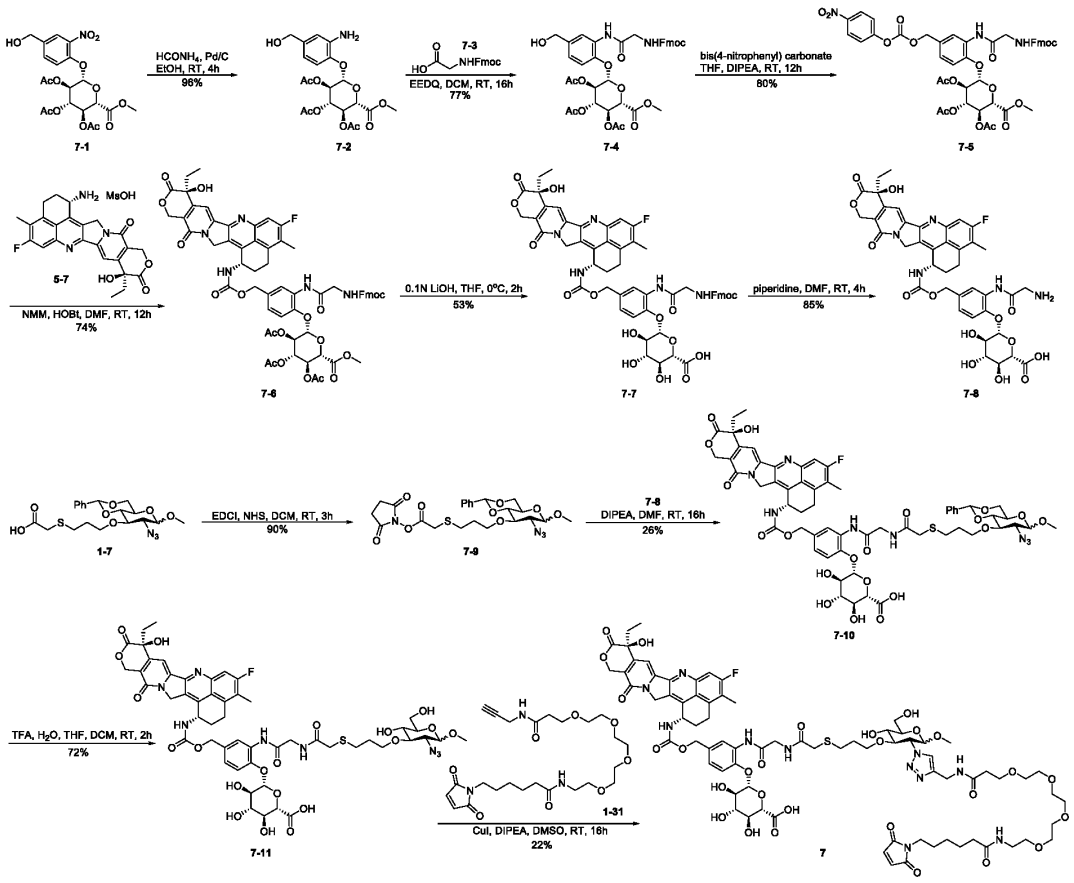
Example 7. Synthesis of (2S,3S,4S,5R,6S)-6-(2-(2-(2-((3-(((3R,4R,5S,6R)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-4-

yl)oxy)propyl)thio)acetamido)acetamido)-4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (7)



7

Synthetic scheme 7



[00482] Step 1: Under nitrogen atmosphere, to a solution of compound **7-1** (10.0 g, 20.60 mmol) in EtOH (200 mL) was added 5w% Pd/C (1.5 g) and ammonium formate (10.4 g, 221.00 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was filtered over diatomite. The filtrate was concentrated to give the title compound **7-2** (9.0 mg, yield 96%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.80 (d, J = 8.2 Hz, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.45 (dd, J = 8.3, 2.1 Hz, 1H), 5.48 (t, J = 9.7 Hz, 1H), 5.39 (d, J = 7.9 Hz, 1H), 5.14 – 5.02 (m, 2H), 4.97 (t, J = 5.7 Hz, 1H), 4.66 (d, J = 10.0 Hz, 1H), 4.58 (s, 2H), 4.31 (d, J = 5.7 Hz, 2H), 3.65 (s, 3H), 2.02 (d, J = 15.8 Hz, 9H). LCMS: m/z calculated for $C_{20}H_{25}NO_{11}$: 455.42; found: 456.88 $[M+H]^+$.

[00483] Step 2: Under nitrogen atmosphere, to a solution of compound **7-2** (9.0 mg, 19.76 mmol) and compound **7-3** (7.75 g, 26.07 mmol) in dichloromethane (150 mL) was added EEDQ

(16.12 g, 65.19 mmol). The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was diluted with dichloromethane (100 mL) and washed with 1 N aqueous HCl solution (200 mL) and H_2O (150 mL x 2). The organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **7-4** (11.2 g, yield 77%) as a white solid. LCMS: m/z calculated for $C_{37}H_{38}N_2O_{14}$: 734.71; found: 736.04 $[M+H]^+$.

[00484] Step 3: Under nitrogen atmosphere, to a solution of compound **7-4** (11.2 g, 15.24 mmol) in anhydrous THF (120 mL) at 0 °C was added DIPEA (8.8 g, 68.09 mmol) and bis(4-nitrophenyl) carbonate (10.2 g, 33.53 mmol). The reaction mixture was warmed slowly to room temperature and stirred for 12 h. After completion of the reaction, the mixture was concentrated. The residue was extracted with H_2O (150 mL) and ethyl acetate (150 mL x 2). The organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (eluted with 40% ethyl acetate in petroleum ether) to afford the title compound **7-5** (11.0 g, yield 80%) as a white solid. LCMS: m/z calculated for $C_{44}H_{41}N_3O_{18}$: 899.82; found: 900.87 $[M+H]^+$.

[00485] Step 4: To a mixture of compound **7-5** (3.06 g, 3.40 mmol) and compound **5-7** (1.64 g, 3.08 mmol) in anhydrous DMF (20 mL) was added HOBt (416 mg, 3.08 mmol) and 4-methylmorpholine (936 mg, 9.25 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was added dropwise to a stirring cold water (100 mL) to form a brown precipitate. The final mixture was stirred at room temperature for 30 min before filtration. The solid was redissolved in dichloromethane (150 mL) and washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (eluted with 4% dichloromethane in MeOH) to afford the title compound **7-6** (2.76 g, yield 74%) as a brown solid. LCMS: m/z calculated for $C_{62}H_{58}FN_5O_{19}$: 1195.37; found: 1196.20$[M+H]^+$.

[00486] Step 5: To a solution of compound **7-6** (1.4 g, 1.17 mmol) in THF (30 mL) was added dropwise 0.1 N aqueous LiOH solution (70 mL, 7.00 mmol) at 0 °C. The addition of LiOH was over 1.5 h and the pH of the reaction mixture was not more than 10. The reaction mixture was stirred at 0 °C for 0.5 h. After completion of the reaction, the mixture was adjusted to pH = 5 with saturated citric acid solution. The resulting mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water,

TFA condition). The desired components were lyophilized to afford the title compound **7-7** (659 mg, yield 53%) as a yellow solid. LCMS: m/z calculated for $C_{55}H_{50}FN_5O_{16}$: 1056.02; found: 1057.25. $[M+H]^+$.

[00487] Step 6: To a solution of compound **7-7** (462 mg, 0.44 mmol) in DMF (7 mL) was added piperidine (112 mg, 1.31 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **7-8** (312 mg, yield 85%) as a yellow solid. LCMS: m/z calculated for $C_{40}H_{40}FN_5O_{14}$: 833.26; found: 834.21 $[M+H]^+$.

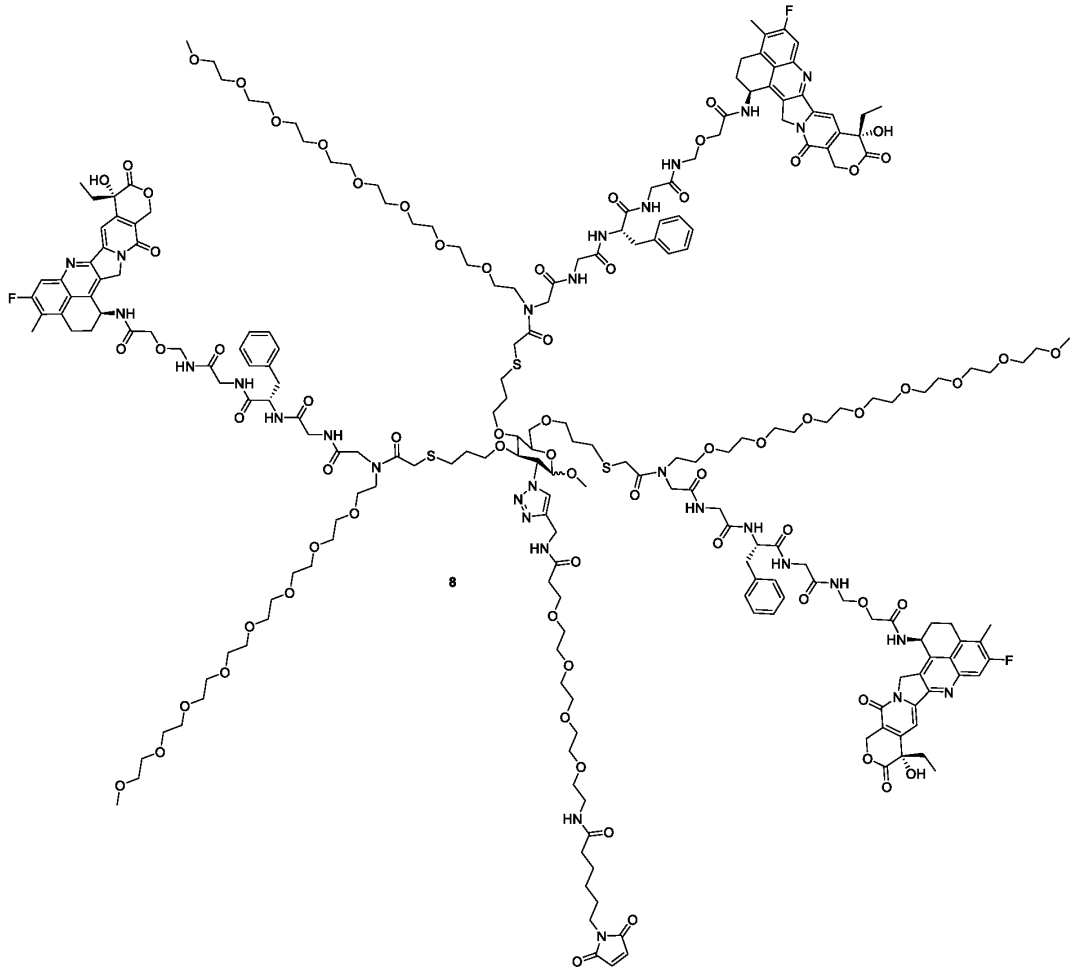
[00488] Step 7: To a solution of compound **1-7** (80 mg, 0.18 mmol) and N-Hydroxysuccinimide (42 mg, 0.36 mmol) in anhydrous dichloromethane (3 mL) was added EDCI (70 mg, 0.36 mmol). The reaction mixture was stirred at room temperature for 3 h. After TLC showed the completion of the reaction, the mixture was diluted with dichloromethane (10 mL) and washed with cold 0.1 N aqueous HCl solution (20 mL) and cold water (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to afford the title compound **1-30** (87 mg, yield 90%) as a yellow oil.

[00489] Step 8: To a solution of compound **7-8** (80 mg, 0.096 mmol) and DIPEA (56 mg, 0.43 mmol) in DMF (2 mL) was added a solution of compound **7-9** (87 mg, 0.16 mmol) in dichloromethane (3 mL). The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **7-10** (32 mg, yield 26%) as a yellow solid. LCMS: m/z calculated for $C_{59}H_{63}FN_8O_{20}S$: 1255.25; found: 1257.57 $[M+H]^+$.

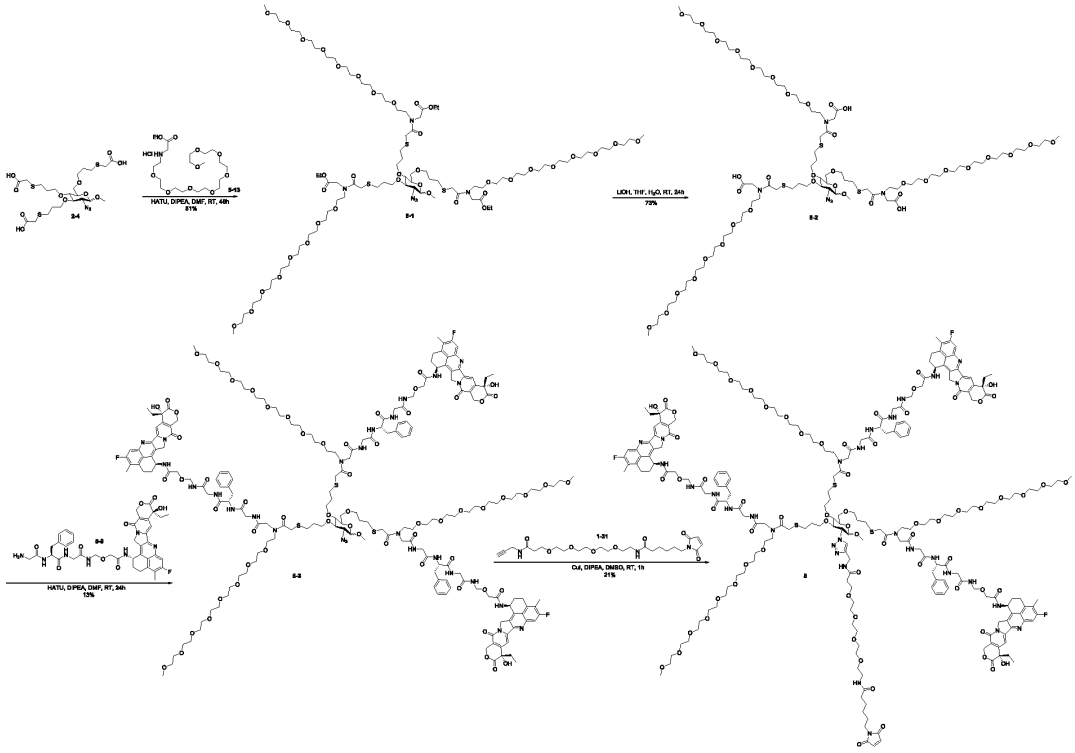
[00490] Step 9: To a solution of compound **7-10** (40 mg, 0.032 mmol) in THF (2 mL) and dichloromethane (1 mL) was added 80% aqueous trifluoroacetic acid solution (1 mL) at room temperature. After the reaction was stirred for 2 h, the mixture was diluted with dichloromethane and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **7-11** (27 mg, yield 72%) as a yellow solid. LCMS: m/z calculated for $C_{52}H_{59}FN_8O_{20}S$: 1167.14; found: 1168.53 $[M+H]^+$.

[00491] Step 10: Under nitrogen atmosphere, to a solution of compound **7-11** (27 mg, 0.023 mmol) and compound **1-31** (25 mg, 0.05 mmol) in DMSO (1 mL) was added CuI (8 mg, 0.04 mmol) and DIPEA (20 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **7** (8.8 mg, yield 22%) as an off-white solid. LCMS: m/z calculated for $C_{76}H_{96}FN_{11}O_{28}S$: 1662.71; found: 1664.06 $[M+H]^+$.

Example 8. Synthesis of (2S,2'S)-2,2'-((26,26'-(2,2'-(((((2R,3S,4R,5R)-2-(26-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)-27-oxo-2,5,8,11,14,17,20,23,33-nonaoxa-29-thia-26-azatetratriacontan-34-yl)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-6-methoxytetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(28-oxo-2,5,8,11,14,17,20,23-octaoxa-26,29-diazahentriacontan-31-oyl))bis(azanediyl))bis(N-(2-(((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)-3-phenylpropanamide) (8)



Synthetic scheme 8



[00492] Step 1: To a solution of compound **2-4** (200 mg, 0.32 mmol), **5-13** (720 mg, 1.42 mmol) and DIPEA (500 mg, 3.87 mmol) in anhydrous DMF (9 mL) was added HATU (740 mg, 1.95 mmol) in 2 portions. The reaction mixture was stirred at room temperature for 2 days. After completion of the reaction, the mixture was diluted with dichloromethane (50 mL) and washed with H_2O (50 mL). The organic layer was filtered and concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **8-1** (325 mg, yield 51%) as a pale-yellow oil. LCMS: m/z calculated for $C_{85}H_{160}N_6O_{38}S_3$: 1970.40 found: 986.86 $[M+2H]^{2+}$.

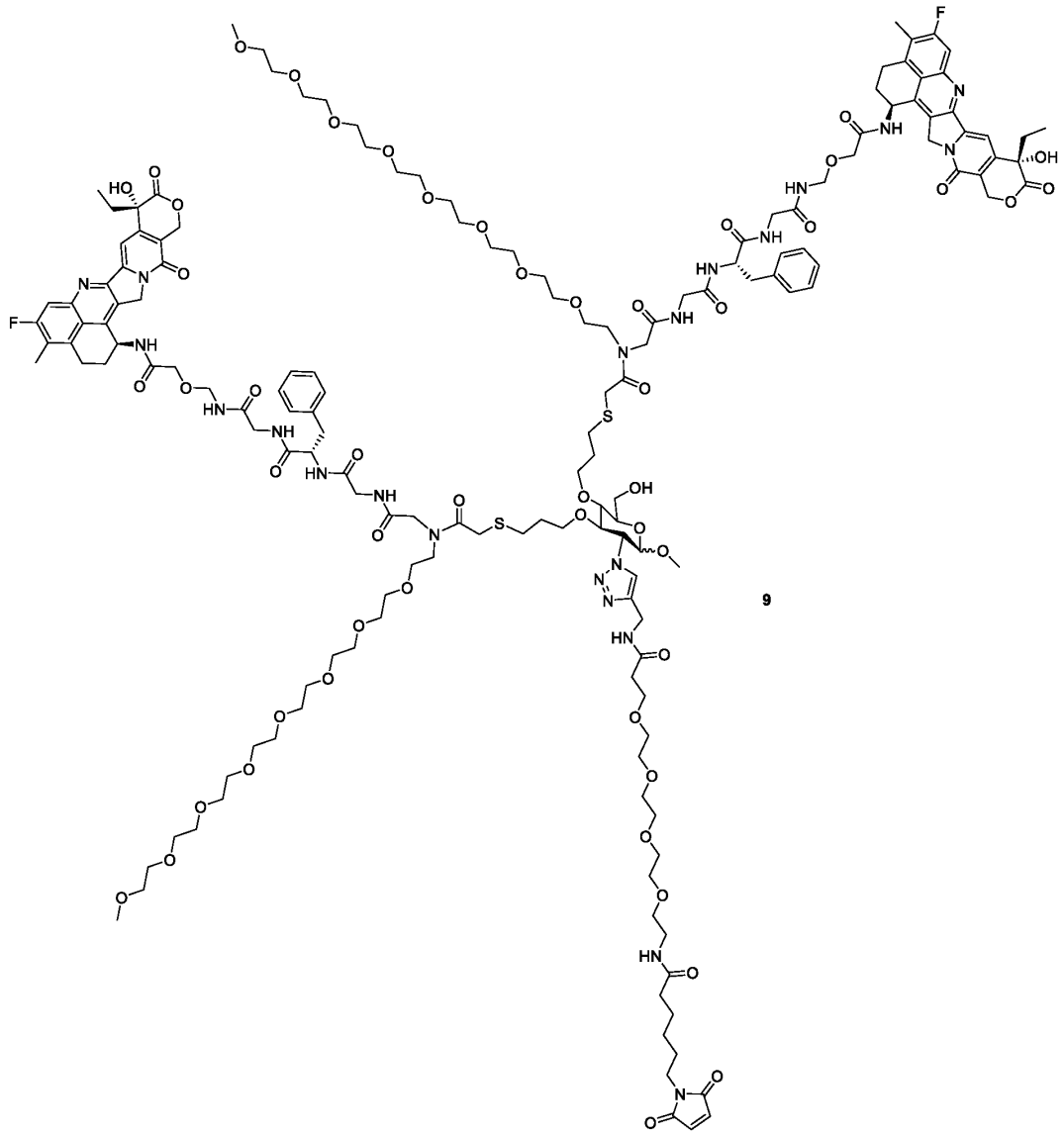
[00493] Step 2: To a solution of compound **8-1** (325 mg, 0.16 mmol) in THF (4 mL) was added 2 N LiOH aqueous solution (0.5 mL, 1 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, the mixture was adjusted to pH = 5 with 1 N HCl and concentrated. The aqueous solution was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **8-3** (228

mg, yield 73%) as a yellow oil. LCMS: m/z calculated for $C_{79}H_{148}N_6O_{38}S_3$: 1886.24; found: 1887.92 $[M+H]^+$.

[00494] Step 3: To a solution of compound **8-2** (100 mg, 0.053 mmol) and DIPEA (42.5 mg, 0.33 mmol) in anhydrous DMF (2 mL) was added HATU (122 mg, 0.32 mmol). After the mixture was stirred at room temperature for 10 min, a solution of compound **5-9** (196 mg, 0.25 mmol) and DIPEA (42.5 mg, 0.33 mmol) in anhydrous DMF (2 mL) was added thereto. The reaction mixture was stirred at room temperature for 24 h and quenched by adding H_2O (0.2 mL). The resulting mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **8-3** (30 mg, yield 13%) as an off-white solid. LCMS: m/z calculated for $C_{199}H_{268}F_3N_{27}O_{62}S_3$: 4183.64; found: 1396.84 $[M+3H]^{3+}$.

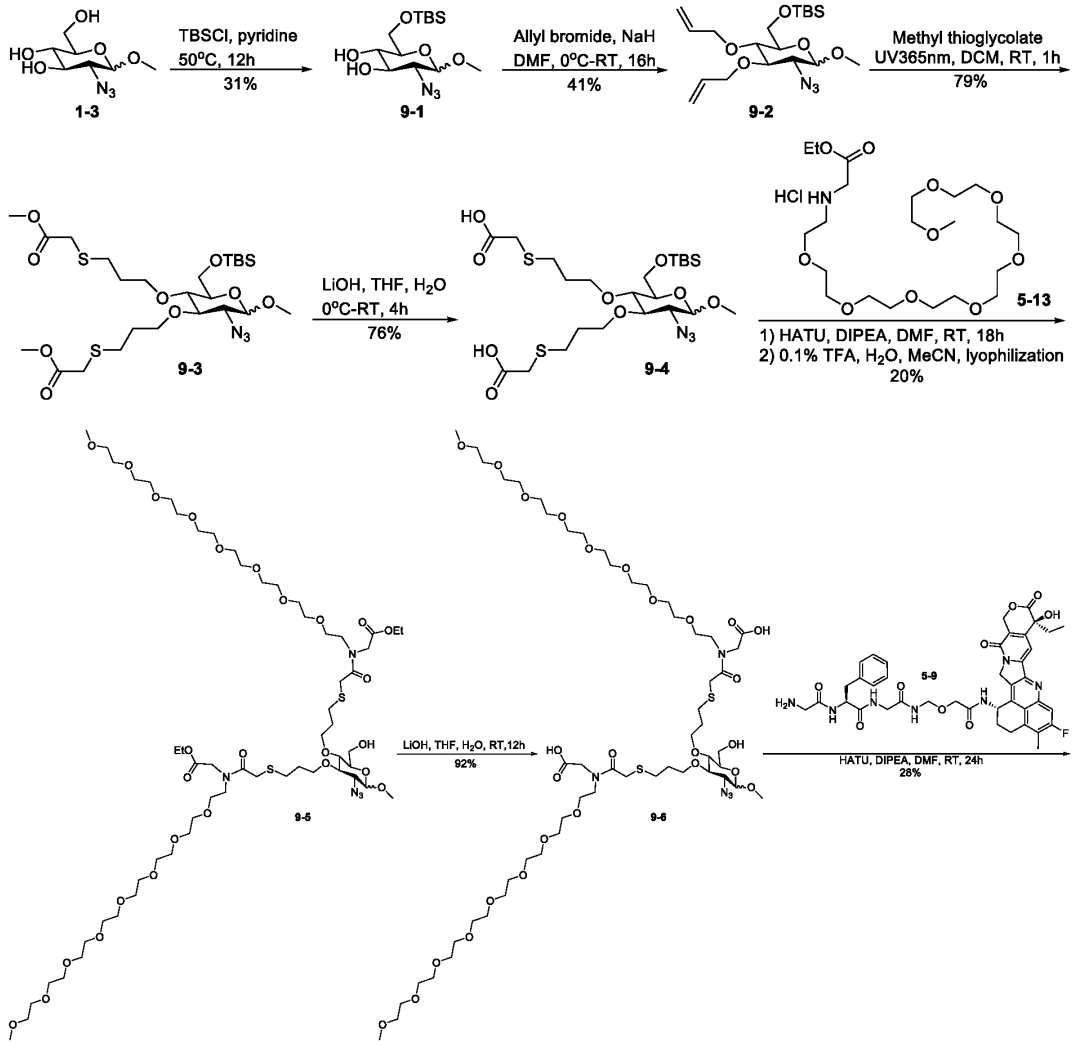
[00495] Step 4: Under nitrogen atmosphere, to a solution of compound **8-3** (30 mg, 0.007 mmol) and compound **1-31** (7.5 mg, 0.015 mmol) in DMSO (1 mL) was added CuI (6 mg, 0.03 mmol) and DIPEA (16 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **8** (7 mg, yield 21%) as an off-white solid. LCMS: m/z calculated for $C_{223}H_{305}F_3N_{30}O_{70}S_3$: 4679.21; found: 1561.91 $[M+3H]^{3+}$.

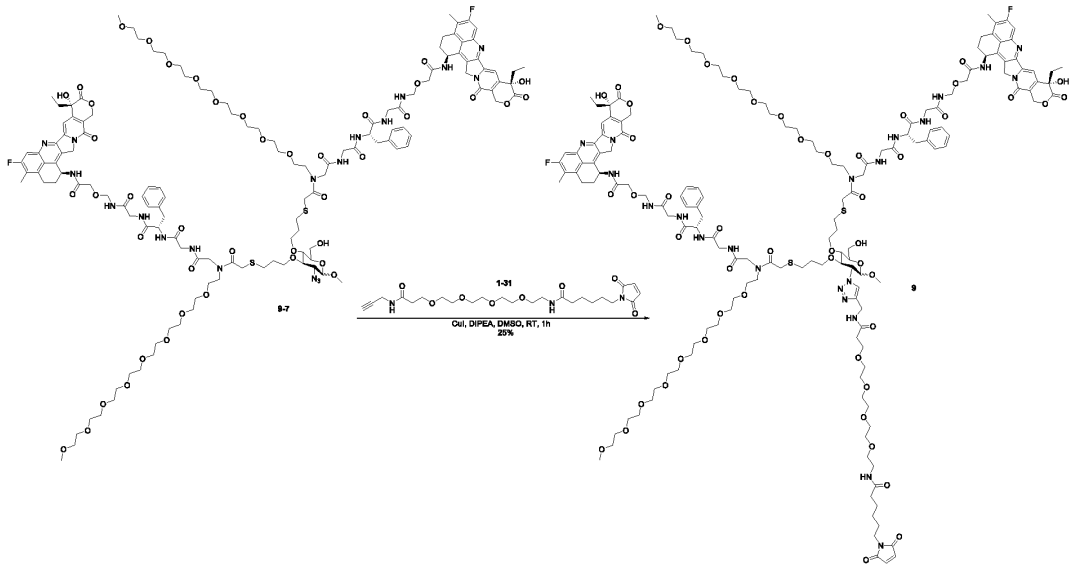
Example 9. Synthesis of (2S,2'S)-2,2'-((26,26'-(2,2'-(((((2R,3S,4R,5R)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(28-oxo-2,5,8,11,14,17,20,23-octaoxa-26,29-diazahentriacontan-31-oyl))bis(azanediyl))bis(N-(2-(((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)-3-phenylpropanamide) (9)



9

Synthetic scheme 9


TBSCl, pyridine
50°C, 12h
31%
Allyl bromide, NaH
DMF, 0°C-RT, 16h
41%
Methyl thioglycolate
UV365nm, DCM, RT, 1h
79%
LiOH, THF, H2O
0°C-RT, 4h
76%
1) HATU, DIPEA, DMF, RT, 18h
2) 0.1% TFA, H2O, MeCN, lyophilization
20%
LiOH, THF, H2O, RT,12h
92%
HATU, DIPEA, DMF, RT, 24h
28%



[00496] Step 1: To a solution of compound **1-3** (10.27 g, 46.85 mmol) in pyridine (50 mL) was added t-butyldimethylsilyl chloride (15.54 g, 103.11 mmol) at 0 °C. The reaction mixture was warmed slowly to room temperature and stirred for 12 h. After completion of the reaction, the mixture was diluted with dichloromethane (200 mL) and washed with 0.5 N HCl (200 mL x 2). The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **9-1** (8.46 g, yield 54%) as a colorless oil. A few compounds of mixed configuration were purified by pre-TLC to afford the single chiral compounds **9-1(α)** and **9-1(β)**, which were used to confirm the structure. **9-1(α)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.76 (d, *J* = 3.5 Hz, 1H), 4.03 – 3.96 (m, 1H), 3.90 (dd, *J* = 10.4, 4.5 Hz, 1H), 3.79 (dd, *J* = 10.4, 5.7 Hz, 1H), 3.66 – 3.53 (m, 2H), 3.42 (s, 3H), 3.33 (s, 1H), 3.23 (dd, *J* = 10.3, 3.5 Hz, 1H), 2.87 (s, 1H), 0.91 (s, 9H), 0.10 (s, 6H). **9-1(β)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.23 (d, *J* = 7.9 Hz, 1H), 3.95 (dd, *J* = 10.4, 4.9 Hz, 1H), 3.83 (dd, *J* = 10.4, 6.3 Hz, 1H), 3.57 (d, *J* = 14.2 Hz, 5H), 3.47 – 3.41 (m, 1H), 3.36 – 3.30 (m, 1H), 3.30 – 3.24 (m, 1H), 2.88 (s, 1H), 0.90 (s, 9H), 0.10 (s, 6H).

[00497] Step 2: To a solution of compound **9-1** (4.23 g, 12.69 mmol) in anhydrous DMF (45 mL) at 0 °C was added NaH (60% in mineral oil, 2.03 g, 50.75 mmol) in several portions under nitrogen atmosphere. After the mixture was stirred at 0 °C for 1 h, allyl bromide (4.61 g, 38.10 mmol) was added thereto. The resulting mixture was stirred at room temperature for 16 h. After

completion of the reaction, the mixture was partitioned with ethyl acetate (100 mL) and water (100 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 15% ethyl acetate in petroleum ether) to afford the title compound **9-2** (2.18 g, yield 41%) as a colorless oil.

[00498] Step 3: A solution of compound **9-2** (2.18 g, 5.27 mmol) and methyl thioglycolate (5.59 g, 52.66 mmol) in dichloromethane (10 mL) was irradiated with UV 365 nm at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel (eluted with 15% ethyl acetate in petroleum ether) to afford the title compound **9-3** (2.62 g, yield 79%) as a colorless oil.

[00499] Step 4: A mixture of compound **9-3** (1.0 g, 1.60 mmol) and 2 N LiOH solution (3.2 mL, 6.4 mmol) in THF (10 mL) was stirred at room temperature for 4 h. After completion of the reaction, the mixture was acidized with AcOH to pH = 5 before concentration. The resulting aqueous solution was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition) to give the title compound **9-4** (730 mg, yield 76%) as a pale-brown oil. LCMS: m/z calculated for $C_{23}H_{43}N_3O_9S_2Si$: 597.81; found: 599.10 $[M+H]^+$.

[00500] Step 5: To a solution of compound **9-4** (330 mg, 0.55 mmol) and DIPEA (568 mg, 4.39 mmol) in anhydrous DMF (4 mL) was added HATU (840 mg, 2.21 mmol) in 2 portions. After the mixture was stirred at room temperature for 10 min, a solution of **5-13** (850 mg, 1.68 mmol) and DIPEA (213 mg, 1.65 mmol) in anhydrous DMF (5 mL) was added thereto. The reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the mixture was diluted with dichloromethane (60 mL) and washed with H_2O (60 mL). The organic layer was filtered and concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition) and the desired components were lyophilized in 0.1% TFA/H_2O to remove the TBS group. The title compound **9-5** (170 mg, yield 20%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{59}H_{111}N_5O_{27}S_2$: 1386.67 found: 1387.92 $[M+H]^+$.

[00501] Step 6: To a solution of compound **9-5** (170 mg, 0.123 mmol) in THF (4 mL) was added 1 N LiOH aqueous solution (0.49 mL, 0.49 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was adjusted to pH = 5 with 1 N HCl and concentrated. The aqueous solution was filtered and the filtrate was

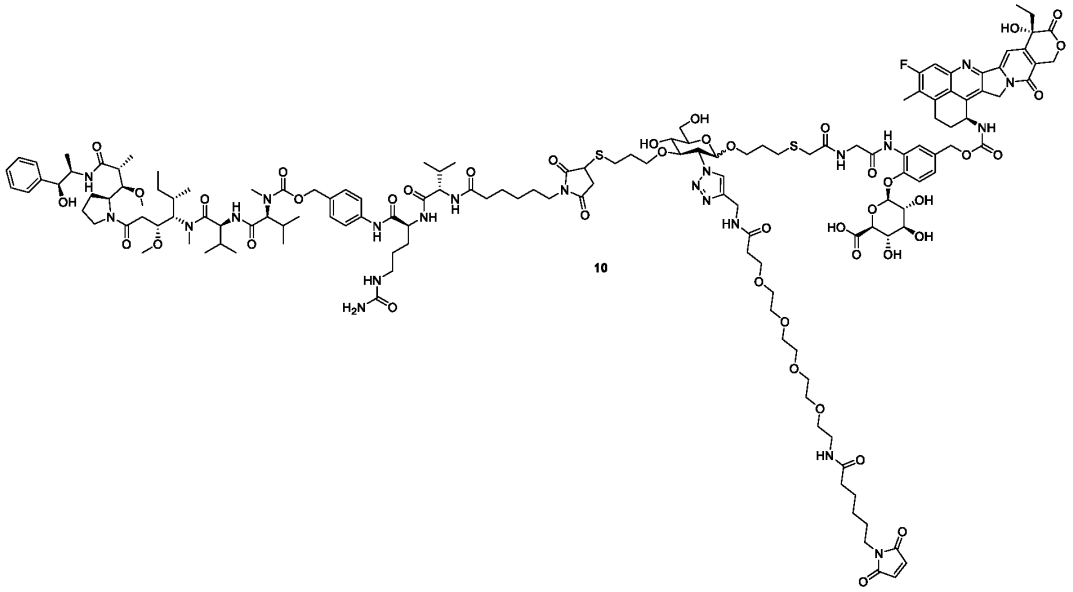
purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **9-6** (152 mg, yield 92%) as a colorless oil. LCMS: m/z calculated for $C_{55}H_{103}N_5O_{27}S_2$: 1330.56; found: 1331.74 $[M+H]^+$.

[00502] Step 7: To a solution of compound **9-6** (60 mg, 0.046 mmol), compound **5-9** (106 mg, 0.135 mmol) and DIPEA (48 mg, 0.37 mmol) in anhydrous DMF (2 mL) was added HATU (68 mg, 0.18 mmol) in 2 portions. The reaction mixture was stirred at room temperature for 24 h and quenched by adding H_2O (0.3 mL). The resulting mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **9-7** (38 mg, yield 28%) as a pale-yellow solid. LCMS: m/z calculated for $C_{135}H_{183}F_2N_{19}O_{43}S_2$: 2862.16; found: 1432.52 $[M+2H]^{2+}$.

[00503] Step 8: Under nitrogen atmosphere, to a solution of compound **9-7** (38 mg, 0.014 mmol) and compound **1-31** (14 mg, 0.028 mmol) in DMSO (2 mL) was added CuI (5 mg, 0.026 mmol) and DIPEA (15 mg, 0.116 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **9** (12 mg, yield 25%) as an off-white solid. LCMS: m/z calculated for $C_{159}H_{220}F_2N_{22}O_{51}S_2$: 3355.47; found: 1119.93 $[M+3H]^{3+}$.

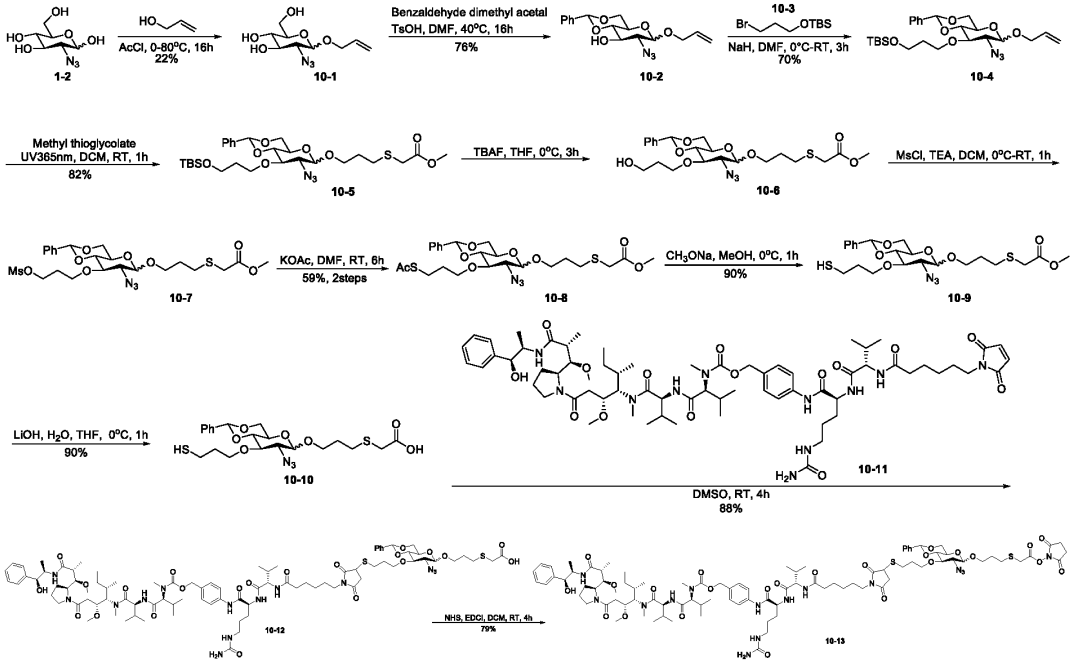
Example 10. Synthesis of (2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-({2-[(1-{[(3R,4R,5S,6R)-3-{4-[24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-2,18-diaza-6,9,12,15-tetraoxatetracos-1-yl]-1,2,3-triazol-1-yl}-5-hydroxy-6-(hydroxymethyl)-4-{[3-({1-[(6S,9S)-1-amino-6-[({4-[(5S,8S,11S,12R)-11-[(2S)-but-2-yl]-12-{2-[(2S)-2-[(3R,4R,7R,8S)-8-hydroxy-4,7-dimethyl-5-oxo-8-phenyl-6-aza-2-oxaoct-3-yl]tetrahydro-1H-pyrrol-1-yl]-2-oxoethyl}-4,10-dimethyl-3,6,9-trioxo-5,8-di(prop-2-yl)-2,13-dioxa-4,7,10-triazatetradec-1-yl]phenyl}amino)carbonyl]-1,8,11-trioxo-9-(prop-2-yl)-2,7,10-triazahexadec-16-yl]-2,5-dioxotetrahydro-1H-pyrrol-3-yl}sulfanyl)propyl]oxy}-3,4,5,6-tetrahydro-2H-pyran-2-yl]oxy}-6,9-dioxo-7-aza-4-thianon-9-yl)amino]-4-{[({[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,12,15-hexahydro-1H-cyclohexa[1,2,3-

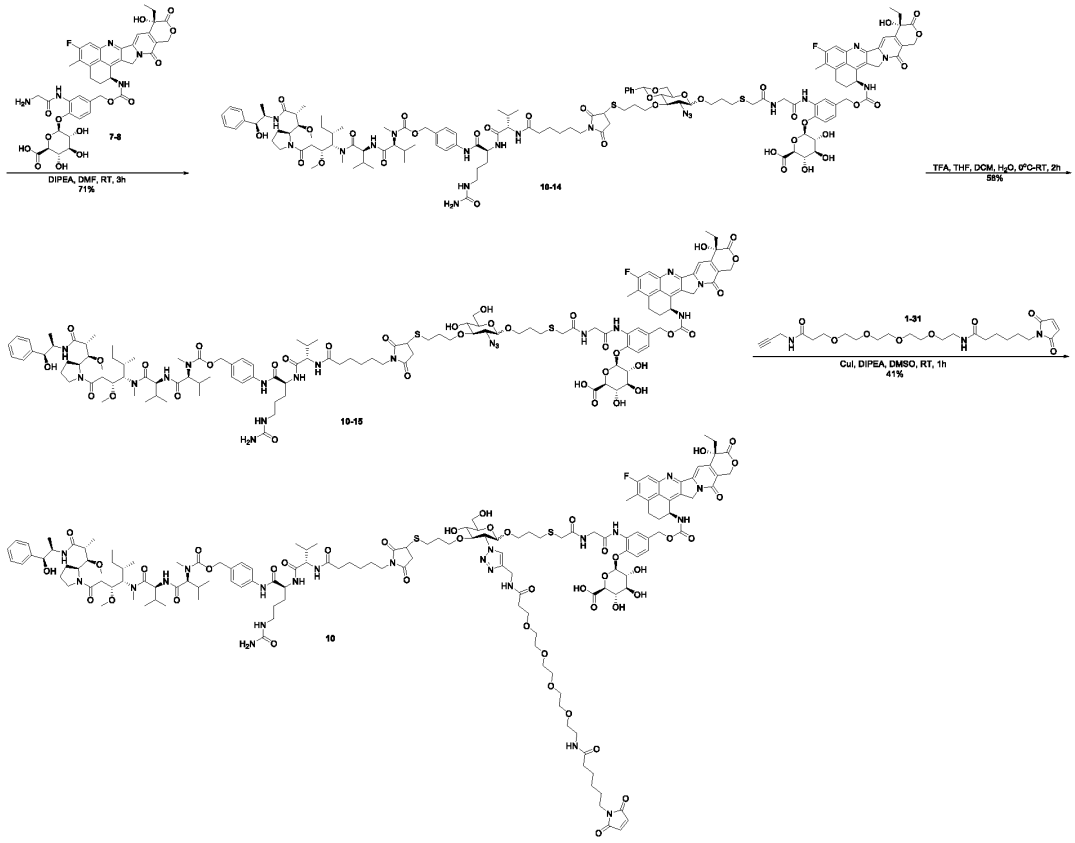
de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}carbonyl)oxy]methyl}phenyl}oxy)tetrahydropyran-2-carboxylic acid (10)



10

Synthetic scheme 10





[00504] Step 1: Under nitrogen atmosphere, to a solution of compound **1-2** (20.0 g, 97.48 mmol) in allyl alcohol (80 mL) was added acetyl chloride (11.48 g, 146.24 mmol) at 0 °C. The reaction mixture was warmed slowly to 70 °C and stirred for 16 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column chromatography (eluted with 80% ethyl acetate in petroleum ether) to afford the title compound **10-1** (5.89 g, yield 24%) as a yellow oil.

[00505] Step 2: A solution of compound **10-1** (5.89 g, 24.02 mmol), p-TsOH (433 mg, 2.52 mmol) and dimethoxyphenylmethane (12.79 g, 84.03 mmol) in acetonitrile (60 mL) was stirred at 40 °C for 16 h. After completion of the reaction, the mixture was concentrated. The residue was extracted with ethyl acetate (100 mL x 2) and H_2O (150 mL). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product

was purified by silica gel column (eluted with 20% ethyl acetate in petroleum ether) to afford the title compound **10-2** (6.11 g, yield 76%) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (dq, *J* = 6.2, 3.1, 2.2 Hz, 2H), 7.38 (td, *J* = 4.2, 3.6, 2.0 Hz, 3H), 5.95 (ddq, *J* = 16.8, 11.4, 5.9 Hz, 1H), 5.54 (d, *J* = 2.9 Hz, 1H), 5.36 (dd, *J* = 17.2, 2.0 Hz, 1H), 5.26 (dt, *J* = 10.4, 1.5 Hz, 1H), 4.99 – 4.38 (m, 1H), 4.37 – 4.21 (m, 2H), 4.21 – 3.86 (m, 1H), 3.81 – 3.64 (m, 2H), 3.58 – 3.50 (m, 2H), 3.46 (q, *J* = 7.5, 7.1 Hz, 1H), 3.43 – 3.27 (m, 1H).

[00506] Step 3: To a solution of compound **10-2** (5.26 g, 15.78 mmol) in anhydrous DMF (55 mL) at 0 °C was added NaH (60% in mineral oil, 1.9 g, 47.5 mmol) in several portions under nitrogen atmosphere. After the mixture was stirred at 0 °C for 1 h, compound **10-3** (6.0g, 23.69 mmol) was added thereto. The resulting mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was partitioned with ethyl acetate (150 mL) and water (150 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 6% ethyl acetate in petroleum ether) to afford the title compound **10-4** (4.98 g, yield 62%) as a colorless oil.

[00507] Step 4: A mixture of compound **10-4** (4.73 g, 9.35 mmol) and methyl thioglycolate (19.85 g, 187.02 mmol) was irradiated with UV 365 nm at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel (eluted with 20% ethyl acetate in petroleum ether) to afford the title compound **10-5** (4.73 g, yield 82%) as a pale-yellow oil.

[00508] Step 5: To a solution of compound **10-5** (4.73 g, 7.73 mmol) in THF (30 mL) was added TBAF (1 M in THF, 23.2 mL, 23.2 mmol) at 0 °C under nitrogen atmosphere. The mixture was stirred at 0 °C for 3 h. After completion of the reaction, the mixture was diluted with ethyl acetate (100 mL) and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 30% ethyl acetate in petroleum ether) to afford the title compound **10-6** (2.52 g, yield 65%) as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 (dt, *J* = 6.8, 3.3 Hz, 2H), 7.37 (dt, *J* = 4.5, 2.4 Hz, 3H), 5.55 (d, *J* = 5.3 Hz, 1H), 4.90 – 4.25 (m, 1H), 4.08 –

3.92 (m, 2H), 3.92 – 3.83 (m, 2H), 3.81 – 3.67 (m, 7H), 3.64 – 3.45 (m, 2H), 3.42 – 3.25 (m, 2H), 3.24 (d, J = 1.8 Hz, 2H), 2.78 (q, J = 7.3 Hz, 2H), 2.33 (t, J = 6.7 Hz, 1H), 1.95 (q, J = 6.7 Hz, 2H), 1.86 – 1.80 (m, 2H).

[00509] Step 6: To a solution of compound **10-6** (2.47 g, 4.96 mmol) and Et_3N (1.01 g, 9.98 mmol) in anhydrous dichloromethane (30 mL) was added slowly MsCl (1.18 g, 10.30 mmol) at 0 °C. The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with ethyl acetate (100 mL) and washed with 0.5 N HCl aqueous solution. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the title compound **10-7** (2.85 g, crude product) as a yellow oil, which was used into the next step without further purification.

[00510] Step 7: To a solution of compound **10-7** (2.85 g, 4.95 mmol) in anhydrous DMF (30 mL) was added potassium thioacetate (1.13 g, 9.89 mmol). The reaction was stirred at room temperature for 6 h. After completion of the reaction, the mixture was extracted with ethyl acetate (50 mL x 2) and H_2O (50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 9% ethyl acetate in petroleum ether) to afford the title compound **10-8** (1.63 g, yield 59% over 2 steps) as a pale-brown oil. LCMS: m/z calculated for $C_{24}H_{33}N_3O_8S_2$: 555.66; found: 578.0 $[M+Na]^+$.

[00511] Step 8: To a solution of compound **10-8** (500 mg, 0.90 mmol) in anhydrous MeOH (4 mL) was added dropwise sodium methoxide (0.5 M in MeOH, 3.6 mL, 1.80 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was acidized with AcOH to pH = 5. The resulting mixture was extracted with ethyl acetate (20 mL x 2) and H_2O (20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to afford the title compound **10-9** (416 mg, yield 90%) as a colorless oil.

[00512] Step 9: Under nitrogen atmosphere, to a solution of compound **10-9** (416 mg, 0.81 mmol) in THF (5 mL) was 1 N LiOH aqueous solution (1.35 mL, 1.35 mmol) at 0 °C. The

reaction mixture was stirred at 0 °C for 2 h. After completion of the reaction, the mixture was acidized with 1 N HCl (1.35 mL). The resulting mixture was concentrated to dryness to afford the title compound **10-10** (434 mg, yield 99%) as a grey-white oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (tt, *J* = 6.5, 2.3 Hz, 2H), 7.41 – 7.34 (m, 3H), 5.55 (d, *J* = 7.5 Hz, 1H), 4.61 (dd, *J* = 207.2, 3.8 Hz, 1H), 4.33 – 4.26 (m, 1H), 4.09 – 3.95 (m, 2H), 3.93 – 3.74 (m, 4H), 3.71 – 3.53 (m, 2H), 3.40 – 3.28 (m, 2H), 3.26 (d, *J* = 6.1 Hz, 2H), 2.85 – 2.77 (m, 2H), 2.67 – 2.59 (m, 2H), 1.97 (dddd, *J* = 10.4, 8.8, 4.6, 1.5 Hz, 2H), 1.92 – 1.83 (m, 2H).

[00513] Step 10: To a solution of compound **10-10** (150 mg, 0.30 mmol) in DMSO (5 mL) was added **10-11** (107 mg, 0.08 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **10-12** (130 mg, yield 88%) as a white solid. LCMS: m/z calculated for $C_{89}H_{134}N_{14}O_{22}S_2$: 1816.25; found: 909.68 $[M+2H]^{2+}$.

[00514] Step 11: To a mixture of compound **10-12** (130 mg, 0.07 mmol) and N-Hydroxysuccinimide (25 mg, 0.22 mmol) in anhydrous dichloromethane (5mL) was added EDCI (28 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 4 h under nitrogen atmosphere. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **10-13** (108 mg, yield 79%) as a white solid. LCMS: m/z calculated for $C_{93}H_{137}N_{15}O_{24}S_2$: 1913.32; found: 957.04 $[M+2H]^{2+}$.

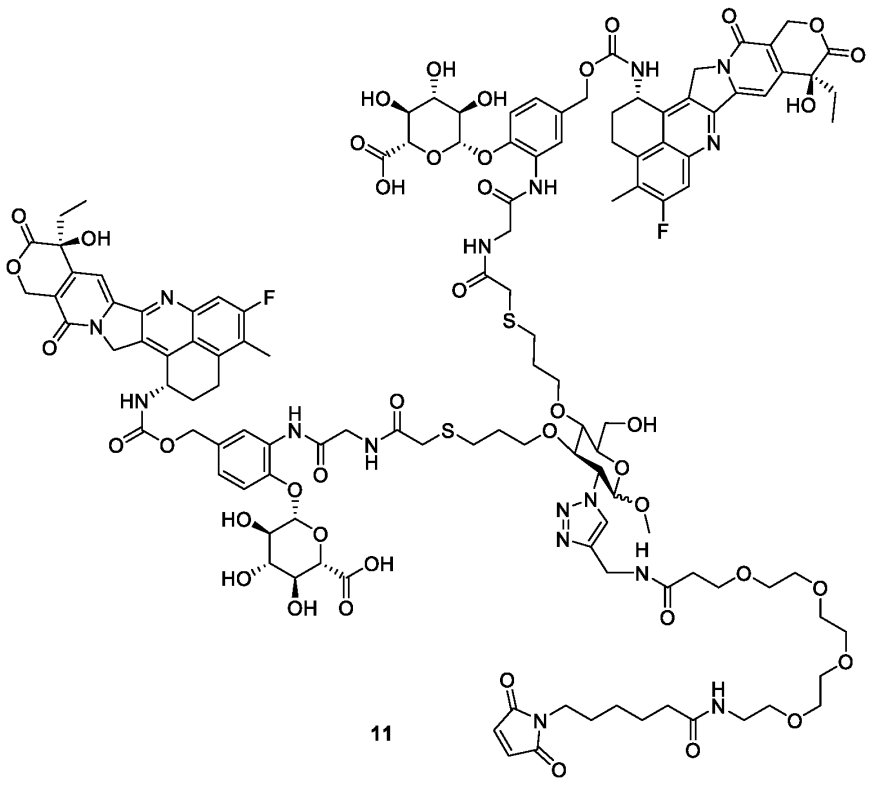
[00515] Step 12: To a solution of compound **7-8** (57 mg, 0.068) and DIPEA (27 mg, 0.21 mmol) in DMF (3.5 mL) was added compound **10-13** (108 mg, 0.056 mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated. The residue was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired

components were lyophilized to afford the title compound **10-14** (106 mg, yield 71%) as a white solid. LCMS: m/z calculated for $C_{129}H_{172}FN_{19}O_{35}S_2$: 2632.01; found: 1316.60 $[M+2H]^{2+}$.

[00516] Step 13: To a solution of compound **10-14** (106 mg, 0.040 mmol) in THF (3 mL) and dichloromethane (1.5 mL) was added 80% aqueous trifluoroacetic acid solution (1.1 mL) at 0 °C. After the reaction was stirred at room temperature for 3 h, additional 80% aqueous trifluoroacetic acid solution (1 mL) was added thereto. The reaction was stirred at room temperature for further 6 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **10-15** (30 mg, yield 29%) as a yellow solid. LCMS: m/z calculated for $C_{122}H_{168}FN_{19}O_{35}S_2$: 2543.90; found: 1273.08 $[M+2H]^{2+}$.

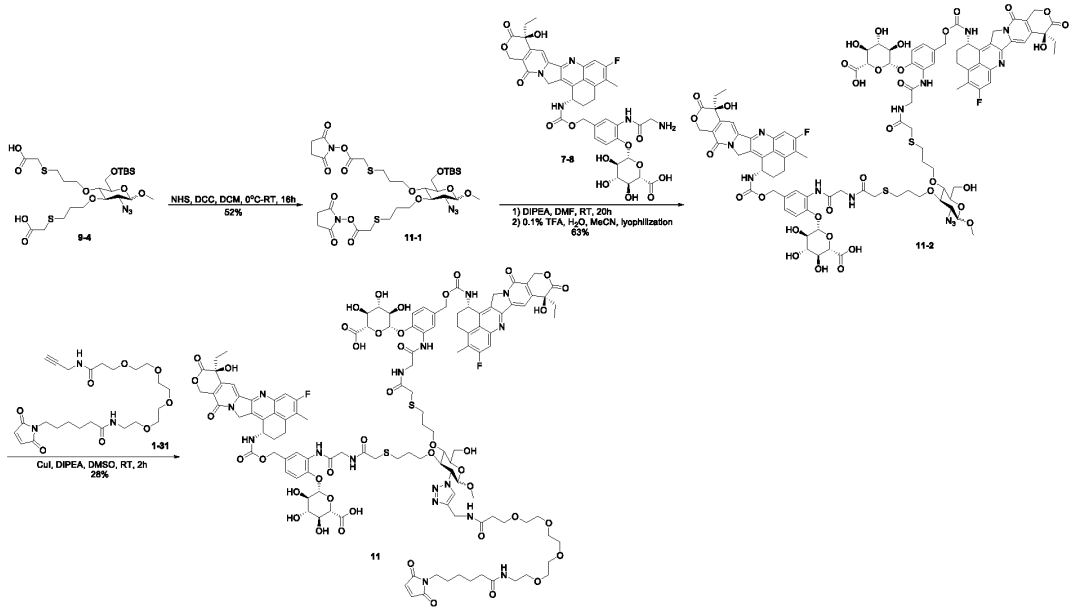
[00517] Step 14: Under nitrogen atmosphere, to a solution of compound **10-15** (30 mg, 0.012 mmol) and compound **1-31** (9 mg, 0.018 mmol) in DMSO (2 mL) was added CuI (5 mg, 0.026 mmol) and DIPEA (13 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **10** (14.8 mg, yield 41%) as a yellow solid. LCMS: m/z calculated for $C_{146}H_{205}FN_{22}O_{43}S_2$: 3039.48; found: 1520.34 $[M+2H]^{2+}$.

Example 11. Synthesis of (2S,2'S,3S,3'S,4S,4'S,5R,5'R,6S,6'S)-6,6'-((((2,2'-((2,2'-(((((2R,3S,4R,5R)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(azanediyl))bis(acetyl))bis(azanediyl))bis(4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)-2,1-phenylene))bis(oxy))bis(3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid) (11)



11

Synthetic scheme 11

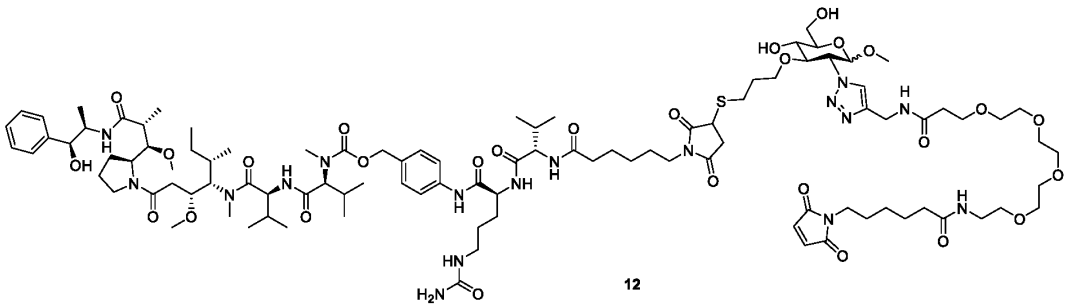


[00518] Step 1: To a mixture of compound **9-4** (200 mg, 0.33 mmol) and N-Hydroxysuccinimide (230 mg, 2.00 mmol) in anhydrous dichloromethane (5 mL) was added DCC (210 mg, 1.02 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 16 h under nitrogen atmosphere. After completion of the reaction, the mixture was filtered and concentrated. The residue was purified by pre-TLC (SiO_2, petroleum ether/ethyl acetate=1:1) to afford the title compound **11-1** (136 mg, yield 52%) as a colorless oil. LCMS: m/z calculated for $C_{31}H_{49}N_5O_{13}S_2Si$: 791.96; found: 793.46 $[M+H]^+$.

[00519] Step 2: To a solution of compound **7-8** (247 mg, 0.30 mmol) and DIPEA (78 mg, 0.60 mmol) in anhydrous DMF (3 mL) was added dropwise a solution of compound **11-1** (80 mg, 0.10 mmol) in anhydrous DMF (1 mL). The reaction mixture was stirred at room temperature for 20 h. After completion of the reaction, the mixture was filtered and purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition) and the desired components were lyophilized in 0.1% TFA/H_2O to remove the TBS group. The title compound **11-2** (135 mg, yield 63%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{97}H_{105}F_2N_{13}O_{35}S_2$: 2115.08 found: 1057.8 $[M+2H]^{2+}$.

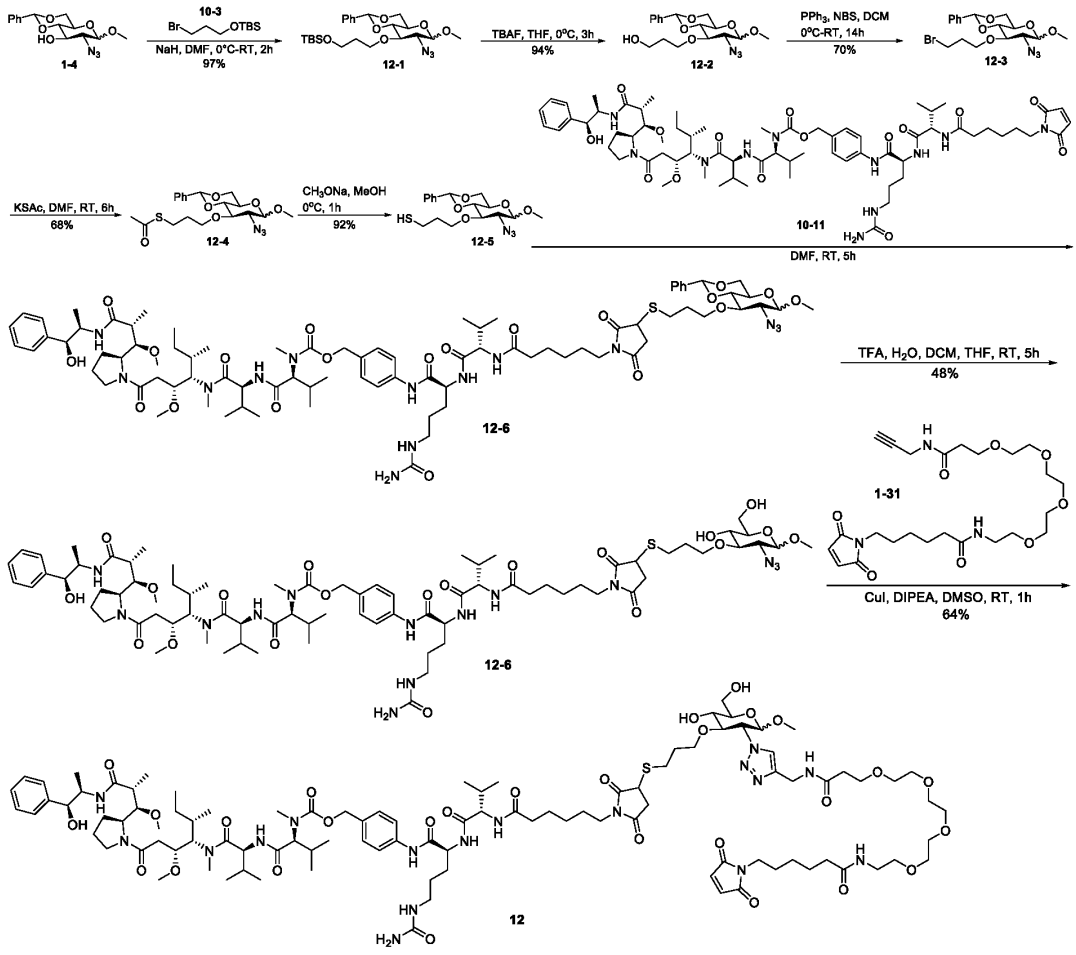
[00520] Step 3: Under nitrogen atmosphere, to a solution of compound **11-2** (30 mg, 0.014 mmol) and compound **1-31** (14 mg, 0.028 mmol) in DMSO (2 mL) was added CuI (6 mg, 0.032 mmol) and DIPEA (15 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **11** (10.6 mg, yield 28%) as a yellow solid. LCMS: m/z calculated for $C_{121}H_{142}F_2N_{16}O_{43}S_2$: 2610.65; found: 1306.24 $[M+2H]^{2+}$.

Example 12. Synthesis of 4-((2S)-2-((2S)-2-(6-(3-((3-(((3R,4R,5S,6R)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-4-yl)oxy)propyl)thio)-2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (12)



12

Synthetic scheme 12



[00521] Step 1: To a solution of compound **1-4** (3.80 g, 12.36 mmol) in anhydrous DMF (30 mL) was added NaH (60% in mineral oil, 1.48 g, 37.00 mmol) in several portions under nitrogen

atmosphere. After the mixture was stirred at 0 °C for 1 h, a solution of compound **10-3** (4.70 g, 15.56 mmol) in anhydrous DMF (5 mL) was added thereto. The resulting mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was partitioned with ethyl acetate (200 mL) and water (300 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column (eluted with 9% ethyl acetate in petroleum ether) to afford the title compound **12-1** (5.78 g, yield 97%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 (dt, *J* = 5.6, 3.4 Hz, 2H), 7.36 (dd, *J* = 5.3, 2.0 Hz, 3H), 5.55 (s, 1H), 4.76 (d, *J* = 3.7 Hz, 2H), 4.01 (dq, *J* = 14.3, 5.5, 4.5 Hz, 1H), 3.86 (d, *J* = 9.3 Hz, 1H), 3.82 – 3.75 (m, 2H), 3.69 (dd, *J* = 7.5, 5.0 Hz, 3H), 3.58 (d, *J* = 6.0 Hz, 2H), 3.43 (s, 2H), 3.41 (s, 1H), 1.81 (q, *J* = 6.1 Hz, 2H), 0.86 (d, *J* = 3.6 Hz, 9H), 0.00 (d, *J* = 3.7 Hz, 6H).

[00522] Step 2: To a solution of compound **12-1** (5.74 g, 11.97 mmol) in THF (60 mL) was added TBAF (1 M in THF, 17.94 mL, 17.94 mmol) at 0 °C under nitrogen atmosphere. The mixture was stirred at 0 °C for 3 h. After completion of the reaction, the mixture was extracted with ethyl acetate (100 mL x 2) and H_2O (100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **12-2** (4.14 g, yield 94%) as a pale-yellow oil.

[00523] Step 3: Under nitrogen atmosphere, to a mixture of compound **12-2** (4.09 g, 11.20 mmol) and triphenylphosphine (3.23 g, 12.31 mmol) in anhydrous dichloromethane (50 mL) was added N-bromosuccinimide (2.19 g, 12.30 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 14 h. After completion of the reaction, the mixture diluted with ethyl acetate (100 mL) and washed with water (100 mL). The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 15% ethyl acetate in petroleum ether) to afford the title compound **12-3** (3.4 g, yield 70%) as a colorless oil.

[00524] Step 4: A mixture of compound **12-3** (990 mg, 2.31 mmol) and potassium thioacetate (528 mg, 4.62 mmol) in anhydrous DMF (7 mL) was stirred at room temperature for 6 h. After completion of the reaction, the mixture was extracted with ethyl acetate (50 mL x 2) and H_2O (70 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography

(eluted with 15% ethyl acetate in petroleum ether) to afford the title compound **12-4** (670 mg, yield 68%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 (ddd, *J* = 6.1, 4.1, 2.0 Hz, 2H), 7.41 – 7.35 (m, 3H), 5.56 (d, *J* = 2.3 Hz, 1H), 4.78 – 4.22 (m, 2H), 3.96 (ddd, *J* = 13.6, 9.7, 6.8 Hz, 1H), 3.90 – 3.80 (m, 1H), 3.79 – 3.70 (m, 2H), 3.63 – 3.56 (m, 2H), 3.43 (s, 2H), 3.42 – 3.30 (m, 2H), 2.97 (td, *J* = 7.1, 3.2 Hz, 2H), 2.26 (d, *J* = 5.0 Hz, 3H), 1.90 – 1.82 (m, 2H).

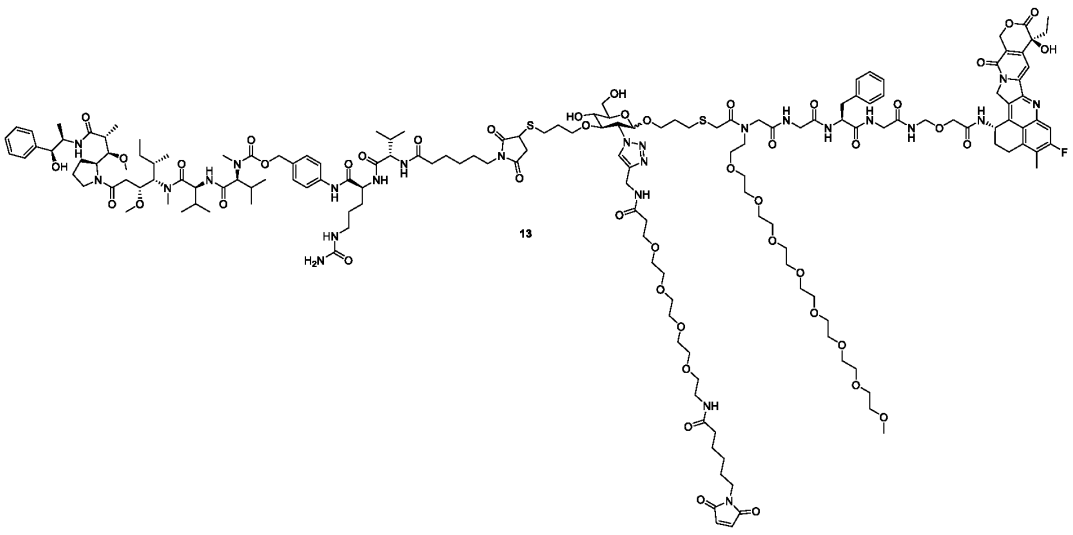
[00525] Step 5: Under nitrogen atmosphere, to a solution of compound **12-4** (300 mg, 0.71 mmol) in anhydrous MeOH (3 mL) was added dropwise sodium methoxide (0.5 M in MeOH, 7 mL, 0.35 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was acidized with AcOH to pH = 5. The resulting mixture was extracted with ethyl acetate (30 mL x 2) and H_2O (30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **12-5** (251 mg, yield 92%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.50 – 7.45 (m, 2H), 7.41 – 7.34 (m, 3H), 5.56 (d, *J* = 2.0 Hz, 1H), 4.80 – 4.24 (m, 2H), 3.99 (ddt, *J* = 12.3, 9.8, 5.9 Hz, 1H), 3.91 – 3.80 (m, 2H), 3.79 – 3.72 (m, 1H), 3.63 – 3.55 (m, 2H), 3.44 (s, 2H), 3.42 – 3.30 (m, 2H), 2.68 – 2.55 (m, 2H), 1.88 (tt, *J* = 6.8, 4.3 Hz, 2H).

[00526] Step 6: Step 10: To a solution of compound **12-5** (52 mg, 0.14 mmol) in DMF (5 mL) was added **10-11** (150 mg, 0.11 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **12-6** (127 mg, yield 68%) as an off-white solid. LCMS: m/z calculated for $C_{85}H_{128}N_{14}O_{20}S$: 1698.10; found: 1699.46 $[M+H]^+$.

[00527] Step 7: To a solution of compound **12-6** (127 mg, 0.075 mmol) in THF (1 mL) and dichloromethane (2 mL) was added 80% aqueous trifluoroacetic acid solution (0.5 mL) at 0 °C. The reaction was stirred at room temperature for 5 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **12-7** (58 mg, yield 48%) as a white solid. LCMS: m/z calculated for $C_{78}H_{124}N_{14}O_{20}S$: 1609.99; found: 1610.34 $[M+H]^+$.

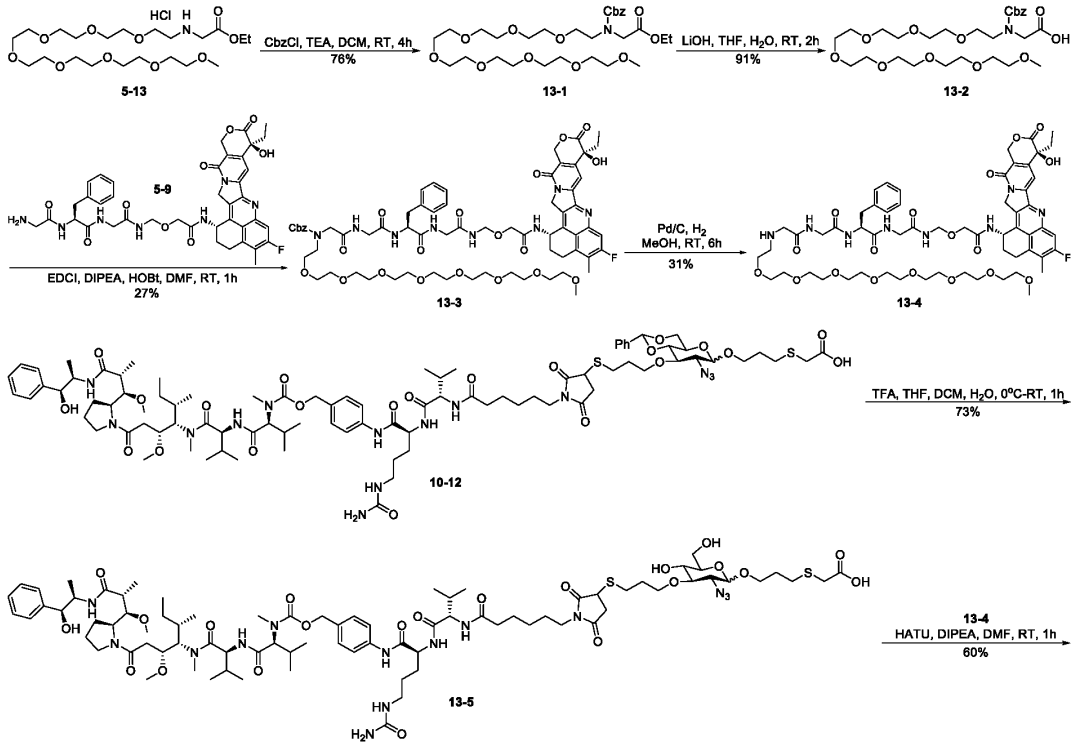
[00528] Step 8: Under nitrogen atmosphere, to a solution of compound **12-7** (50 mg, 0.031 mmol) and compound **1-31** (31 mg, 0.062 mmol) in DMSO (2 mL) was added CuI (6 mg, 0.031 mmol) and DIPEA (16 mg, 0.124 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **12** (41.9 mg, yield 64%) as a white solid. LCMS: m/z calculated for $C_{102}H_{161}N_{17}O_{28}S$: 2105.56; found: 1053.79 $[M+2H]^{2+}$.

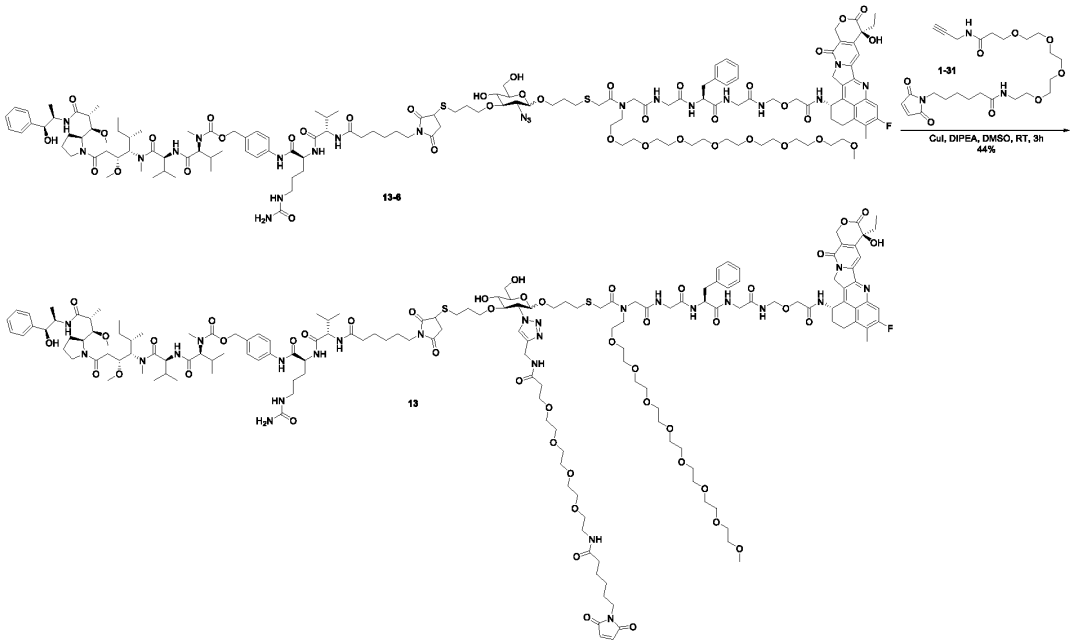
Example 13. Synthesis of 4-((2S)-2-((2S)-2-(6-(3-((3-(((3R,4R,5S,6R)-2-((26-((S)-10-benzyl-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1,6,9,12,15-pentaoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propyl)thio)-2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (13)



13

Synthetic scheme 13





[00529] Step 1: To a solution of compound **5-13** (900 mg, 1.78 mmol) and Et_3N (970 mg, 9.58 mmol) in dichloromethane (20 mL) was added benzyl chloroformate (981 mg, 5.75 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was extracted with ethyl acetate (50 mL x 3) and washed with H_2O (50 mL). The organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 1% methanol in dichloromethane) to afford the title compound **13-1** (816 mg, yield 76%) as a pale-yellow oil. LCMS: m/z calculated for $C_{29}H_{49}NO_{12}$: 603.71; found: 605.00 $[M+H]^+$.

[00530] Step 2: To a solution of compound **13-1** (346 mg, 0.57 mmol) in THF (4 mL) was added aqueous 1 N LiOH solution (1.14 mL, 1.14 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was adjusted to pH = 5 with aqueous 1 N HCl solution. The resulting mixture was concentrated. The residue was purified by silica gel column chromatography (eluted with 9% methanol in dichloromethane) to afford the title compound **13-2** (302 mg, yield 91%) as a yellow oil.

[00531] Step 3: To a solution of compound **13-2** (300 mg, 0.52 mmol) and compound **5-9** (340 mg, 0.43 mmol) in DMF (5 mL) was added HOBt (58 mg, 0.43 mmol), DIPEA (166 mg, 1.28 mmol) and EDCI (165 mg, 0.86 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched by adding H_2O (0.5 mL) and filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **13-3** (158 mg, yield 27%) as a yellow solid. LCMS: m/z calculated for $C_{67}H_{85}FN_8O_{20}$: 1341.45; found: 1342.38 $[M+H]^+$.

[00532] Step 4: Under hydrogen atmosphere, a mixture of compound **13-3** (158 mg, 0.12 mmol) and 5w% Pd/C (50 mg) in anhydrous MeOH (5 mL) was stirred at room temperature for 6 h. After completion of the reaction, the mixture was filtered through diatomite. The filtrate was concentrated to afford the title compound **13-4** (44 mg, yield 31%) as a yellow solid. LCMS: m/z calculated for $C_{59}H_{79}FN_8O_{18}$: 1206.55; found: 1207.42 $[M+H]^+$.

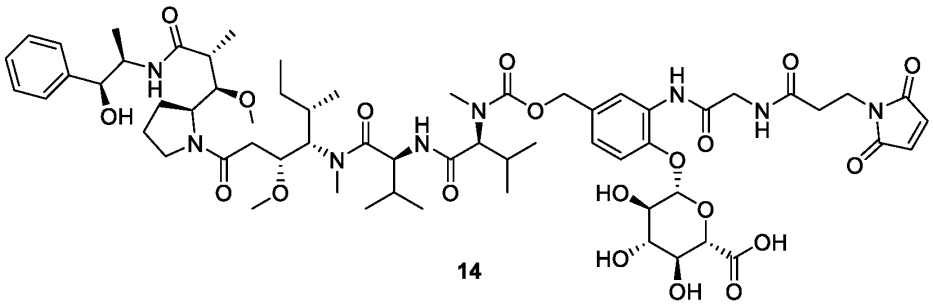
[00533] Step 5: To a solution of compound **10-12** (130 mg, 0.071 mmol) in THF (3 mL) and dichloromethane (1 mL) was added 80% aqueous trifluoroacetic acid solution (1.5 mL) at 0 °C. The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **13-5** (90 mg, yield 73%) as a yellow solid. LCMS: m/z calculated for $C_{82}H_{130}N_{14}O_{22}S_2$: 1728.14; found: 1729.42 $[M+H]^+$.

[00534] Step 6: To a solution of compound **13-5** (90 mg, 0.052 mmol) and compound **13-4** (75 mg, 0.062 mmol) in anhydrous DMF (2 mL) was added DIPEA (17 mg, 0.13 mmol) and HATU (30 mg, 0.079). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **13-6** (91 mg, yield 60%)

as a yellow solid. LCMS: m/z calculated for $C_{141}H_{207}FN_{22}O_{39}S_2$: 2917.44; found: 1460.58 $[M+2H]^{2+}$.

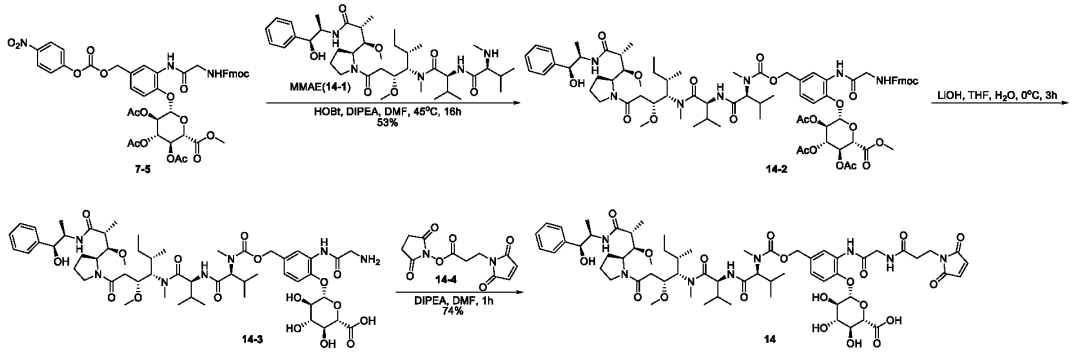
[00535] Step 7: The title compound **13** was prepared in analogy to **Example 7, Step 10** by using compound **13-6** and compound **1-31**. The product (6.5 mg, yield 44%) was obtained as a white solid. LCMS: m/z calculated for $C_{165}H_{244}FN_{25}O_{47}S_2$: 3413.01; found: 1707.47 $[M+2H]^{2+}$.

Example 14. Synthesis of (2S,3S,4S,5R,6S)-6-(4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)-2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)acetamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (14)



14

Synthetic scheme 14



[00536] Step 1: To a mixture of compound **7-5** (5.51 g, 6.12 mmol) and MMAE (**14-1**) (4.00 g, 5.57 mmol) in anhydrous DMF (20 mL) was added HOBt (752 mg, 5.57 mmol) and DIPEA (2.16 g, 16.71 mmol). The reaction mixture was stirred at room temperature for 16 h. After

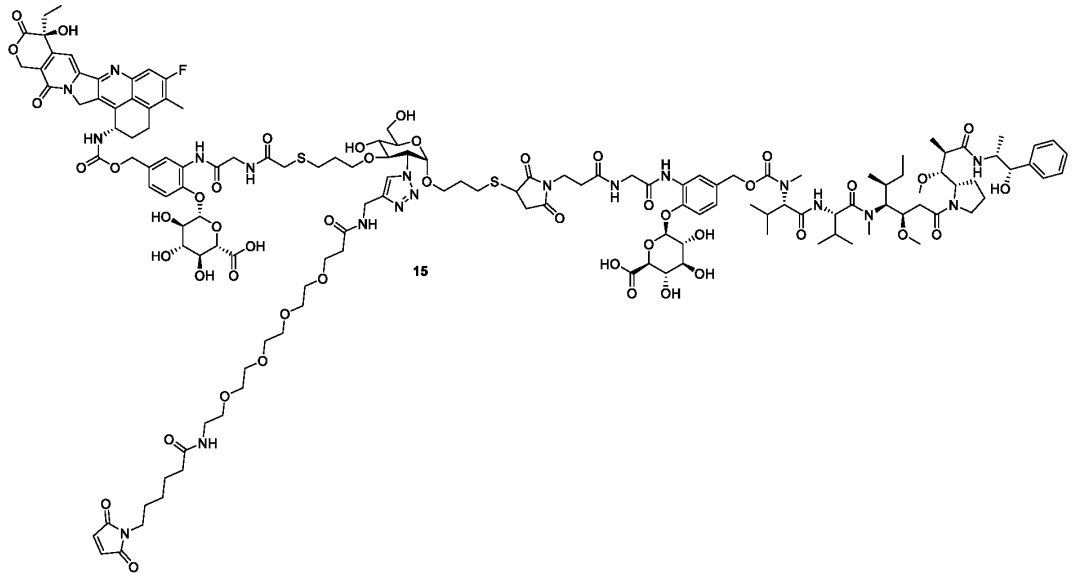
completion of the reaction, the mixture was extracted with ethyl acetate (300 mL) and water (200 mL). The organic layer was washed with brine (100 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **14-2** (6.30 g, yield 76%) as a white solid. LCMS: m/z calculated for $C_{77}H_{103}N_7O_{22}$: 1477.72; found: 1478.48 $[M+H]^+$.

[00537] Step 2: To a solution of compound **14-2** (2.00 g, 1.35 mmol) in THF (100 mL) was added dropwise 0.1 N aqueous LiOH solution (101 mL, 10.10 mmol) at 0 °C. The addition of LiOH was over 5 h and the pH of the reaction mixture was not more than 10. The reaction mixture was stirred at 0 °C for 0.5 h. After completion of the reaction, the mixture was adjusted to pH = 5 with saturated citric acid solution. The resulting mixture was concentrated and lyophilized to afford a white solid. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 47% acetonitrile in water with 0.05% TFA). The desired components were lyophilized to afford the title compound **14-3** (1.20 g, yield 80%) as a white solid. LCMS: m/z calculated for $C_{55}H_{85}N_7O_{17}$: 1115.60; found: 1116.44 $[M+H]^+$.

[00538] Step 3: To a solution of compound **14-3** (1.20 g, 1.08 mmol) in DMF (10 mL) was added DIPEA (347 g, 2.67 mmol) and compound **14-4** (370 mg, 1.29 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with 56% acetonitrile in water with 0.1% TFA). The desired components were lyophilized to afford the title compound **14** (958 mg, yield 70%) as a white solid. LCMS: m/z calculated for $C_{62}H_{90}N_8O_{20}$: 1267.44; found: 1267.42 $[M+H]^+$.

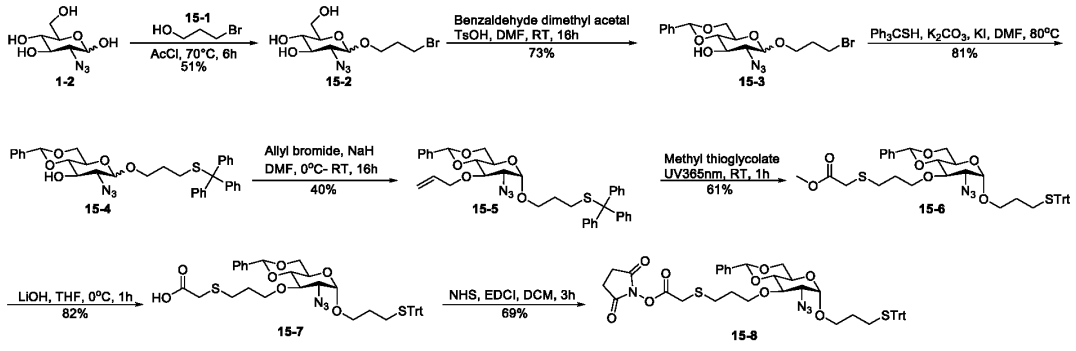
Example 15. Synthesis of (2S,3S,4S,5R,6S)-6-(2-(2-(2-((3-(((2S,3R,4R,5S,6R)-2-(3-((1-(3-((2-((5-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-2-oxoethyl)amino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-

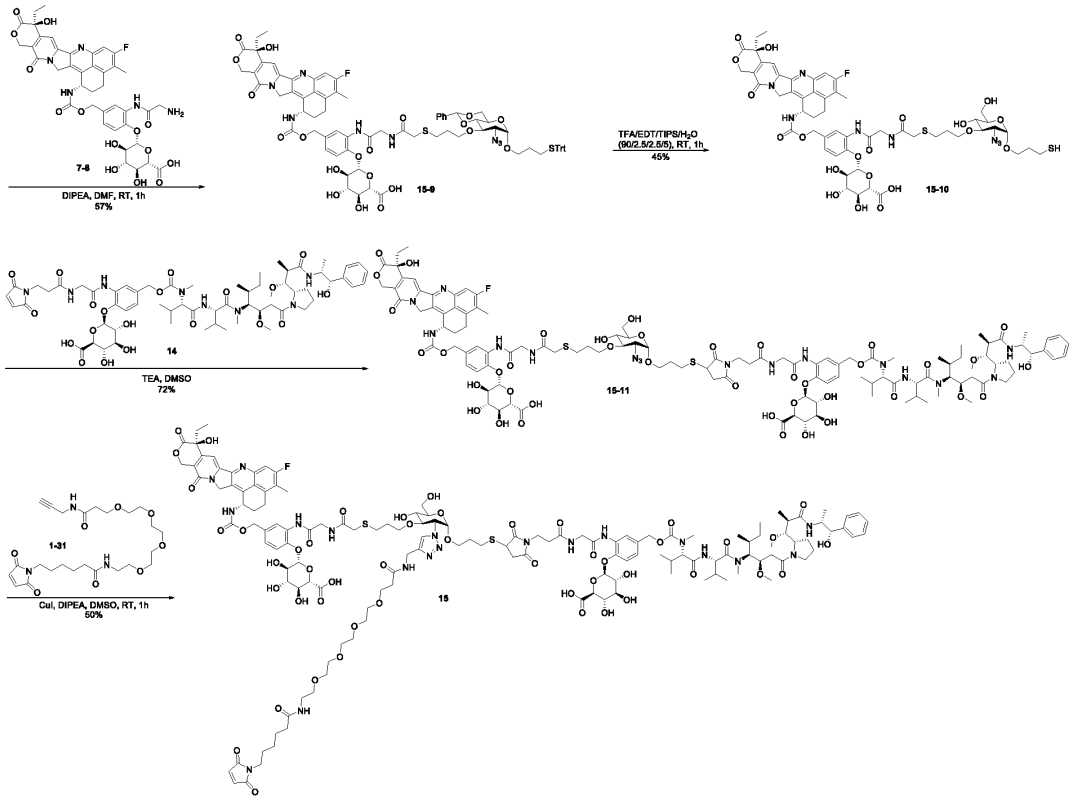
yl)thio)propoxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propyl)thio)acetamido)acetamido)-4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (15)



15

Synthetic scheme 15





[00539] Step 1: The title compound **15-2** was prepared in analogy to **Example 10, Step 1** by using compound **1-2** and 3-bromo-1-propanol (**15-1**). The product (3.76 g, yield 51%) was obtained as a colorless oil.

[00540] Step 2: The title compound **15-3** was prepared in analogy to **Example 10, Step 2** by using compound **15-2** in DMF. The product (1.66 g, yield 73%) was obtained as a colorless oil.

[00541] Step 3: The title compound **15-4** was prepared in analogy to **Example 10, Step 7** by using compound **15-3** and triphenylmethanethiol in DMF. The product (405 mg, yield 81%) was obtained as a colorless oil.

[00542] Step 4: The title compound **15-5** was prepared in analogy to **Example 1, Step 4** by using compound **15-4**. The product (346 mg, yield 40%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (dd, *J* = 6.6, 2.6 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 6H), 7.38 (dd, *J* = 5.1, 1.8 Hz, 3H), 7.31 – 7.26 (m, 6H), 7.20 (t, *J* = 7.3 Hz, 3H), 5.97 (ddt, *J* = 16.3, 10.4,

5.8 Hz, 1H), 5.55 (s, 1H), 5.31 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.19 (dt, *J* = 10.4, 1.5 Hz, 1H), 4.77 (d, *J* = 3.6 Hz, 1H), 4.43 (ddt, *J* = 12.5, 5.8, 1.4 Hz, 1H), 4.24 (ddd, *J* = 14.6, 8.1, 3.1 Hz, 2H), 3.89 (t, *J* = 9.5 Hz, 1H), 3.79 (dt, *J* = 9.6, 5.0 Hz, 1H), 3.73 – 3.58 (m, 3H), 3.46 – 3.38 (m, 1H), 3.27 (dd, *J* = 10.0, 3.6 Hz, 1H), 2.28 (t, *J* = 7.2 Hz, 2H), 1.70 (td, *J* = 6.8, 2.7 Hz, 2H).

[00543] Step 5: The title compound **15-6** was prepared in analogy to **Example 1, Step 5** by using compound **15-5**. The product (400 mg, yield 61%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{41}H_{45}N_3O_7S_2$: 755.95; found: 773.29 $[M+H_2O+H]^+$.

[00544] Step 6: The title compound **15-7** was prepared in analogy to **Example 1, Step 6** by using compound **15-6**. The product (162 mg, yield 82%) was obtained as a white solid. LCMS: m/z calculated for $C_{40}H_{43}N_3O_7S_2$: 741.92; found: 759.25 $[M+H_2O+H]^+$.

[00545] Step 7: The title compound **15-8** was prepared in analogy to **Example 10, Step 11** by using compound **15-7**. The product (400 mg, yield 69%) was obtained as a white solid. LCMS: m/z calculated for $C_{44}H_{46}N4O_9S_2$: 838.27; found: 857.47 $[M+H_2O+H]^+$.

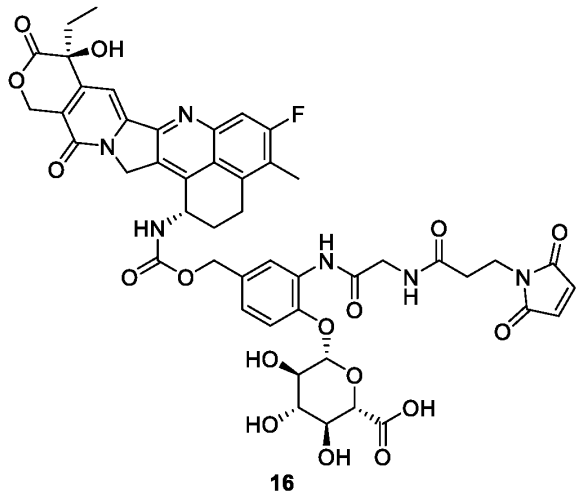
[00546] Step 8: The title compound **15-9** was prepared in analogy to **Example 10, Step 12** by using compound **15-8** and compound **7-8**. The product (148 mg, yield 57%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{80}H_{81}FN_8O_{20}S_2$: 1556.50; found: 1557.44 $[M+H]^+$.

[00547] Step 9: The title compound **15-10** was prepared in analogy to **Example 7, Step 9** by using compound **15-9** in TFA, H_2O, EDT and TIPS. The product (32 mg, yield 45%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{54}H_{63}FN_8O_{20}S_2$: 1226.36; found: 1227.27 $[M+H]^+$.

[00548] Step 10: The title compound **15-11** was prepared in analogy to **Example 10, Step 10** by using compound **15-10** and compound **14**. The product (47 mg, yield 72%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{116}H_{153}FN_{16}O_{40}S_2$: 2492.99; found: 1247.72 $[M+2H]^{2+}$.

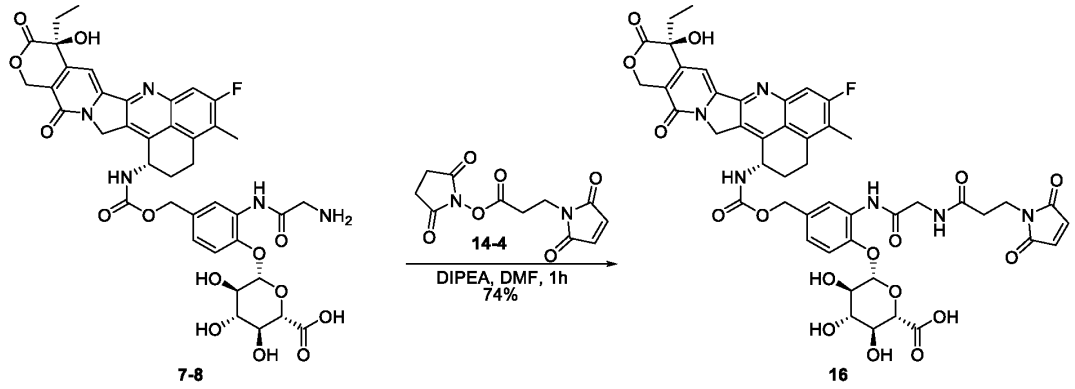
[00549] Step 11: The title compound **15** was prepared in analogy to **Example 10, Step 13** by using compound **15-11** and compound **1-31**. The product (18 mg, yield 50%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{140}H_{190}FN_{19}O_{48}S_2$: 2990.26; found: 1495.48 $[M+2H]^{2+}$.

Example 16. Synthesis of (2S,3S,4S,5R,6S)-6-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)acetamido)-4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (16)



16

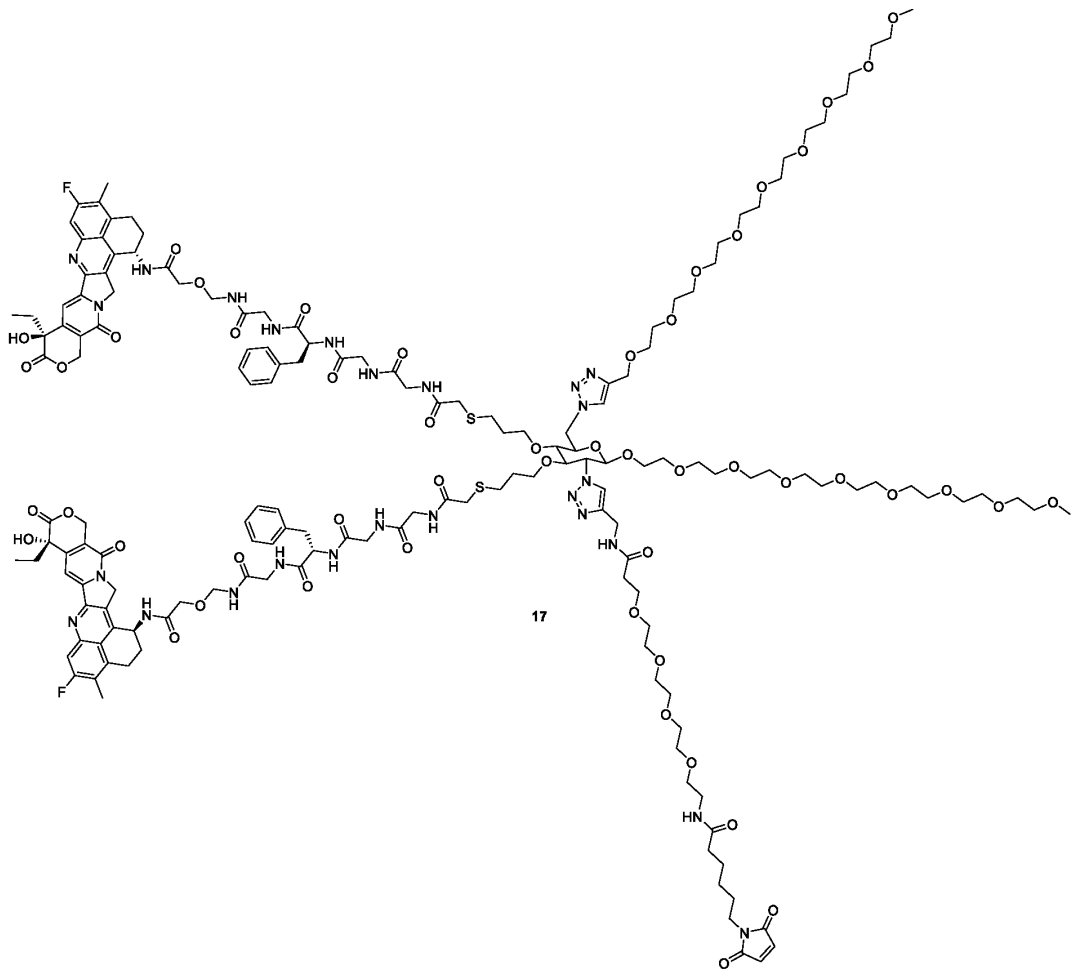
Synthetic scheme 16



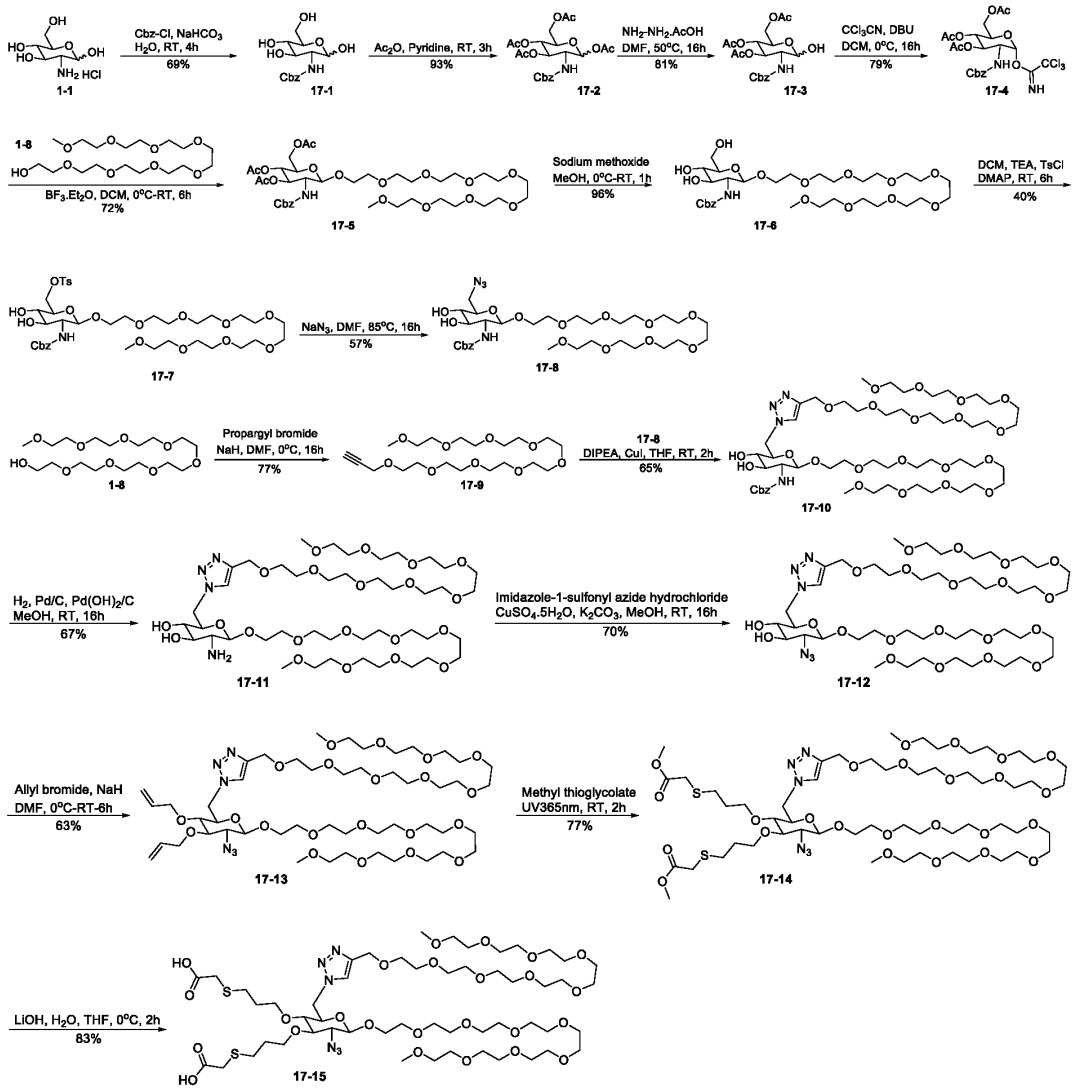
[00550] Step 1: To a solution of compound **7-8** (230 mg, 0.28 mmol) in DMF (3 mL) was added DIPEA (54 mg, 0.41 mmol) and compound **14-4** (88 mg, 0.33 mol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by pre-HPLC (C4 column, eluted with 48% acetonitrile in

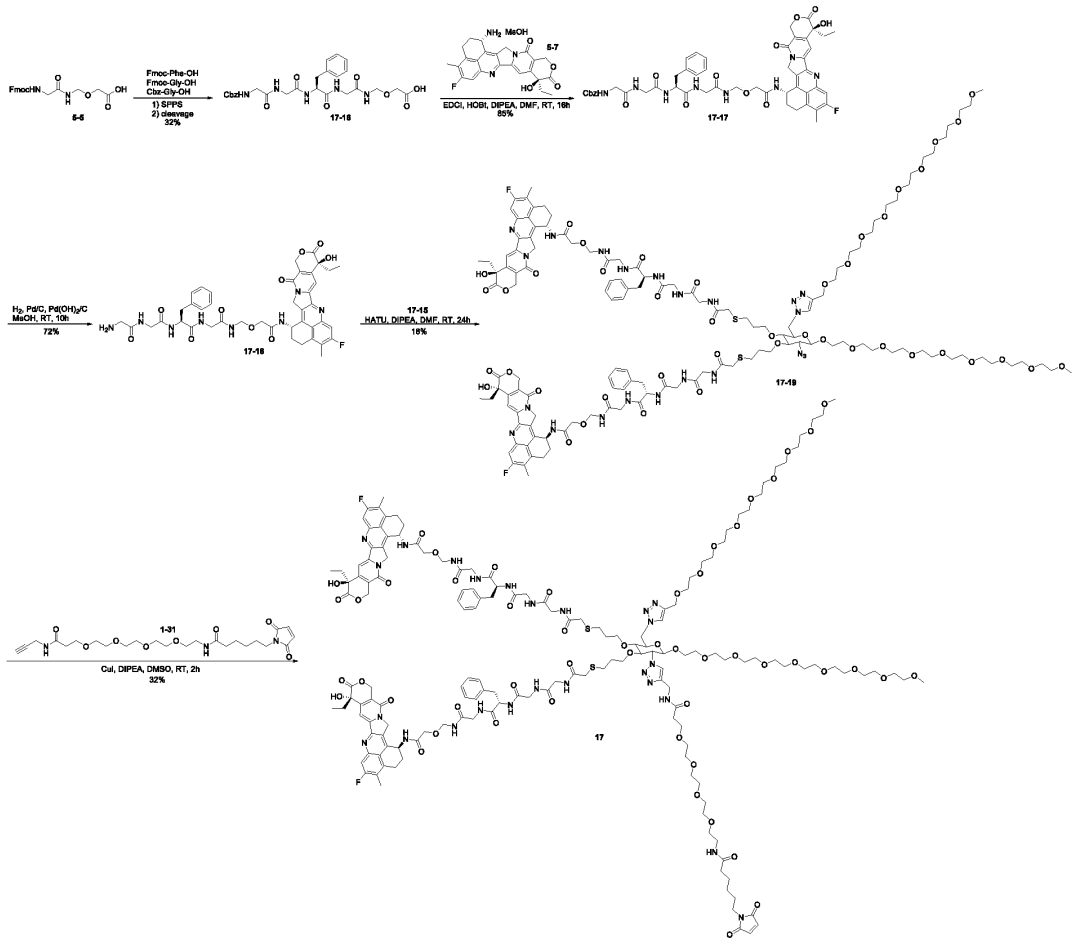
water with 0.1% AcOH). The desired components were lyophilized to afford the title compound **16** (204 mg, yield 74%) as a yellow solid. LCMS: m/z calculated for $C_{47}H_{45}FN_6O_{17}$: 984.90; found: 986.24 $[M+H]^+$.

Example 17. Synthesis of (2S,2'S)-2,2'-((2,2'-((2,2'-((2,2'-(((((2R,3R,4R,5R,6R)-2-((4-(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)-1H-1,2,3-triazol-1-yl)methyl)-6-((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(azanediyl))bis(acetyl))bis(azanediyl))bis(acetyl))bis(azanediyl))bis(N-(2-(((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)-3-phenylpropanamide) (17)



Synthetic scheme 17


Cbz-Cl, NaHCO3
H2O, RT, 4h
69%
Ac2O, Pyridine, RT, 3h
93%
NH2-NH2.AcOH
DMF, 50°C, 16h
81%
CCl3CN, DBU
DCM, 0°C, 16h
79%
BF3.Et2O, DCM, 0°C-RT, 6h
72%
Sodium methoxide
MeOH, 0°C-RT, 1h
96%
DCM, TEA, TsCl
DMAP, RT, 6h
40%
NaN3, DMF, 85°C, 16h
57%
Propargyl bromide
NaH, DMF, 0°C, 16h
77%
17-8
DIPEA, CuI, THF, RT, 2h
65%
H2, Pd/C, Pd(OH)2/C
MeOH, RT, 16h
67%
Imidazole-1-sulfonyl azide hydrochloride
CuSO4.5H2O, K2CO3, MeOH, RT, 16h
70%
Allyl bromide, NaH
DMF, 0°C-RT-6h
63%
Methyl thioglycolate
UV365nm, RT, 2h
77%
LiOH, H2O, THF, 0°C, 2h
83%
1-1
17-1
17-2
17-3
17-4
1-8
17-5
17-6
17-7
17-8
1-8
17-9
17-10
17-11
17-12
17-13
17-14
17-15



[00551] Step 1: To a mixture of compound **1-1** (200 g, 927.515 mmol) in H_2O (2 L) was added sodium bicarbonate (61.6 g, 2782.544 mmol) and stirred at RT for 30 min. Then Benzyl Chloroformate (170 mL, 1205.769 mmol) was slowly added at RT. The mixture was stirred at room temperature for 16h. After completion of the reaction, the mixture was filtered and washed cake with water and dichloromethane to afford the title compound **17-1** (200.8 g, yield 69%) as a white solid. The crude product was directly used for the next step.

[00552] Step 2: To a mixture of compound **17-1** (226 g, 721.33 mmol) in pyridine (1 L) was added acetic anhydride (61.6 g, 3606.651 mmol) at 0°C. The mixture was stirred at room temperature for 16h. After completion of the reaction, the mixture was concentrated and

quenched with hydrochloric acid water (3 L) and extracted with ethyl acetate (4L x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **17-2** (322.5 g, yield 93%) as a colorless gelatinous oil.

[00553] Step 3: To a mixture of compound **17-2** (322 g, 668.813 mmol) in N,N-dimethylformamide (1 L) was added hydrazinium acetate (61.6 g, 668.813 mmol) at 0°C. The mixture was stirred at room temperature for 16h. After completion of the reaction, the mixture was quenched with cold water (5 L) and extracted with ethyl acetate (6L x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **17-3** (237.4 g, yield 81%) as a colorless oil.

[00554] Step 4: Under nitrogen atmosphere, to a mixture of compound **17-3** (136.5 g, 310.063 mmol) in anhydrous dichloromethane (1 L) was slowly added trichloroacetonitrile (93.443 mL, 931.910 mmol) and DBU (2.32 mL, 15.532 mmol) at 0 °C. The mixture was stirred at 0 °C for 16 h. After completion of the reaction, the mixture was poured into water (2L) and extracted with dichloromethane (1.5 L x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **17-4** (10 g, yield 79%) as a yellow oil.

[00555] Step 5: A flask weighted 4A MS (10 g) was flamed over 10 min in vacuum and purged with nitrogen. After cooling slowly to room temperature, to this flask was added a solution of compound **17-4** (10.09 g, 17.31 mmol) and compound **1-8** (8.63 g, 22.45 mmol) in anhydrous dichloromethane (100 mL). The mixture was cooled to 0 °C and boron trifluoride etherate (48% BF_3, 490 mg, 3.46 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h. After completion of the reaction, the mixture was

filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with ethyl acetate) to afford the title compound **17-5** (10.02 g, yield 72%) as a pale-yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 (s, 5H), 5.87 (d, *J* = 5.5 Hz, 1H), 5.10 (s, 3H), 5.04 (t, *J* = 9.5 Hz, 1H), 4.76 (d, *J* = 8.5 Hz, 1H), 4.25 (dd, *J* = 12.3, 4.6 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.88 (d, *J* = 12.5 Hz, 1H), 3.84 – 3.78 (m, 1H), 3.74 (d, *J* = 9.4 Hz, 1H), 3.68 – 3.60 (m, 25H), 3.54 (dd, *J* = 5.9, 3.4 Hz, 5H), 3.37 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H). LCMS: m/z calculated for $C_{37}H_{59}NO_{18}$: 805.87; found: 806.35 $[M+H]^+$.

[00556] Step 6: To a solution of compound **17-5** (10.02 g, 12.43 mmol) in anhydrous methanol (30 mL) was added slowly 0.1 M NaOMe (62 mL methanol solution, 6.2 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column chromatography (eluted with 8% methanol in dichloromethane) to afford the title compound **17-6** (8.12 g, yield 96%) as a pale-yellow oil. LCMS: m/z calculated for $C_{31}H_{53}NO_{15}$: 679.76; found: 698.31 $[M+H_2O+H]^+$.

[00557] Step 7: To a solution of compound **17-6** (2.74 g, 4.03 mmol) in dichloromethane (27 mL) was added TEA (815 mg, 8.07 mmol), DMAP (24 mg, 0.19 mmol) and TsCl (768 mg, 4.02 mmol). The reaction mixture was stirred at room temperature for 6 h. After completion of the reaction, the mixture was concentrated. The residue was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **17-7** (1.37 g, yield 40%) as a colorless oil. LCMS: m/z calculated for $C_{38}H_{59}NO_{17}S$: 833.94; found: 834.31 $[M+H]^+$.

[00558] Step 8: To a solution of compound **17-7** (1.37 g, 1.64 mmol) in DMF (10 mL) was added sodium azide (374 mg, 5.75 mmol). The reaction mixture was stirred at 85 °C for 16 h. After completion of the reaction, the mixture was filtered. The filtrate purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **17-8** (658 mg,

yield 57%) as colorless oil. LCMS: m/z calculated for $C_{31}H_{52}N_4O_{14}$: 704.77; found: 705.31 $[M+H]^+$.

[00559] Step 9: To a solution of compound **1-8** (5.0 g, 13.00 mmol) in anhydrous DMF (25 mL) was added NaH (60% in mineral oil, 780 mg, 19.50 mmol) in several portions at 0 °C. The mixture was stirred at 0 °C before propargyl bromide (2.32 g, 19.50 mmol) was added thereto. The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was quenched by adding water (1 mL). The resulting mixture was partitioned between ethyl acetate (200 mL) and water (100 mL). The organic layer was washed with brine (200 mL), dried over anhydrous sodium sulfate and concentrated. The crude product was purified by silica gel column chromatography (eluted with 5% methanol in dichloromethane) to afford the title compound **17-9** (4.23 g, yield 77%) as a colorless oil.

[00560] Step 10: The title compound **17-10** was prepared in analogy to **Example 10, Step 14** by using compound **17-8** and compound **17-9** in THF instead of DMSO. The product (1.06 g, yield 65%) was obtained as a white solid. LCMS: m/z calculated for $C_{51}H_{90}N_4O_{23}$: 1127.29; found: 1128.46 $[M+H]^+$.

[00561] Step 11: A mixture of compound **17-10** (2.1 g, 1.86 mmol), 5wt% $Pd(OH)_2/C$ (400 mg) and 5wt% Pd/C (400 mg) in methanol (20 mL) was stirred at room temperature under hydrogen atmosphere for 16 h. After completion of the reaction, the mixture was filtered and concentrated to afford the title compound **17-11** (1.24 g, yield 67%) as a colorless oil. The product was used into the next step without further purification.

[00562] Step 12: To a mixture of compound **17-11** (1.1 g, 1.11 mmol), potassium carbonate (328 mg, 2.38 mmol) and copper sulfate pentahydrate (2.9 mg, 0.01 mmol) in methanol (11 mL) was added Imidazole-1-sulfonyl azide hydrochloride (299 mg, 1.42 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 h. After completion of the reaction, the mixture was filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 5% methanol in dichloromethane) to afford the title

compound **17-12** (785 mg, yield 70%) as a pale-yellow oil. LCMS: m/z calculated for $C_{43}H_{82}N_6O_{21}$: 1019.15; found: 1020.48 $[M+H]^+$.

[00563] Step 13: The title compound **17-13** was prepared in analogy to **Example 1, Step 4** by using compound **17-12**. The product (705 mg, yield 63%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{49}H_{90}N_6O_{21}$: 1099.28; found: 1100.33 $[M+H]^+$.

[00564] Step 14: The title compound **17-14** was prepared in analogy to **Example 1, Step 5** by using compound **17-13**. The product (641 mg, yield 77%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{55}H_{102}N_6O_{25}S_2$: 1311.56; found: 1312.12 $[M+H]^+$.

[00565] Step 15: The title compound **17-15** was prepared in analogy to **Example 4, Step 5** by using compound **17-14**. The product (539 mg, yield 83%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{53}H_{98}N_6O_{25}S_2$: 1283.50; found: 1284.13 $[M+H]^+$.

[00566] Step 16: The title compound **17-16** was prepared in analogy to **Example 5, Step 4** by using compound **5-5**, Fmoc-L-Phe-OH, Fmoc-Gly-OH and Cbz-Gly-OH. The product (4.6 g, yield 32%) was obtained as an off-white solid.

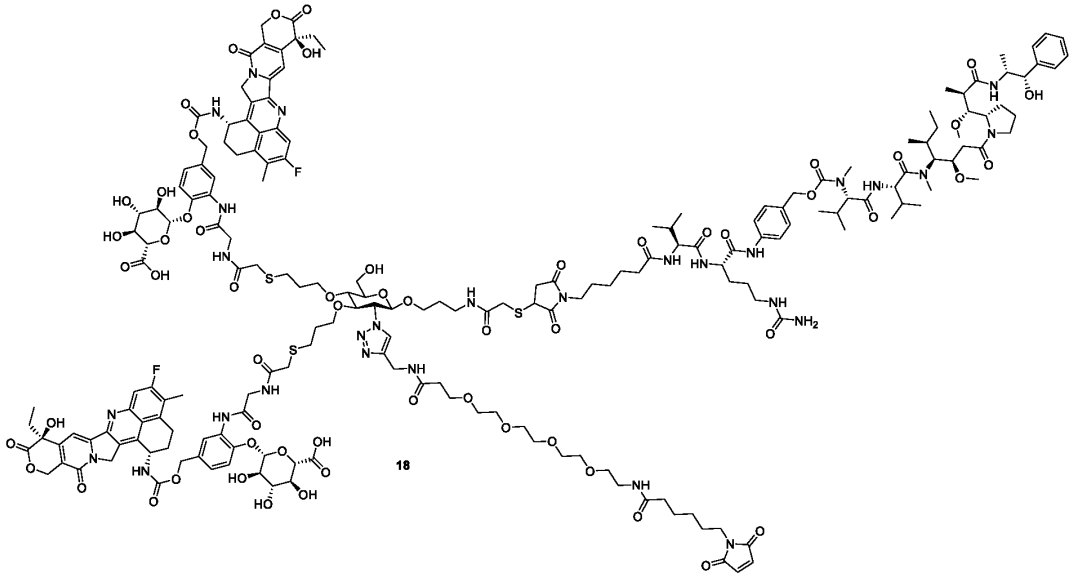
[00567] Step 17: The title compound **17-17** was prepared in analogy to **Example 5, Step 5** by using compound **17-16** and compound **5-7**. The product (854 mg, yield 85%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{50}H_{51}FN_8O_{12}$: 975.00; found: 976.23 $[M+H]^+$.

[00568] Step 18: The title compound **17-18** was prepared in analogy to **Example 5, Step 6** by using compound **17-17**. The product (511 mg, yield 72%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{42}H_{45}FN_8O_{10}$: 840.87; found: 842.17 $[M+H]^+$.

[00569] Step 19: The title compound **17-19** was prepared in analogy to **Example 5, Step 12** by using compound **17-15** and compound **17-18**. The product (511 mg, yield 18%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{137}H_{184}F_2N_{22}O_{43}S_2$: 2929.21; found: 977.2 $[M+3H]^{3+}$.

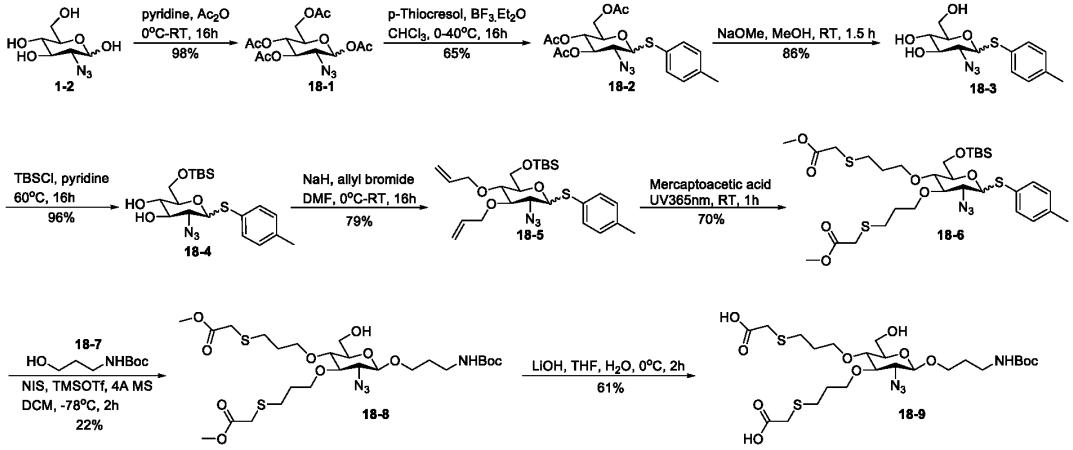
[00570] Step 20: The title compound **17** was prepared in analogy to **Example 7, Step 10** by using compound **17-19** and compound **1-31**. The product (15 mg, yield 32%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{161}H_{221}F_2N_{25}O_{51}S_2$: 3424.78; found: 1142.5 $[M+3H]^{3+}$.

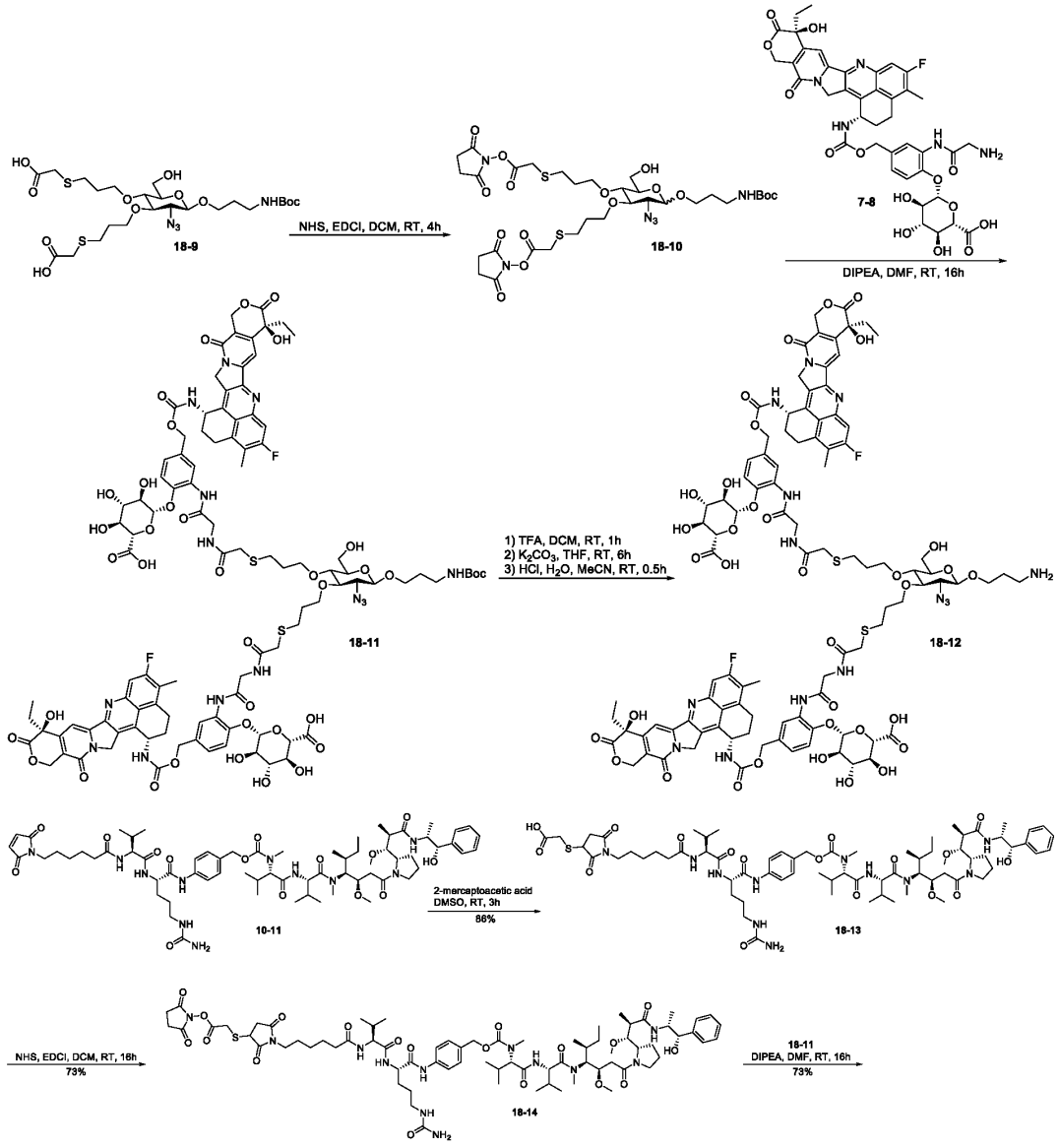
Example 18. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(4*R*,6*R*)-4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)-6-[3-[[2-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[4-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]propoxy]tetrahydropyran-3-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (18)

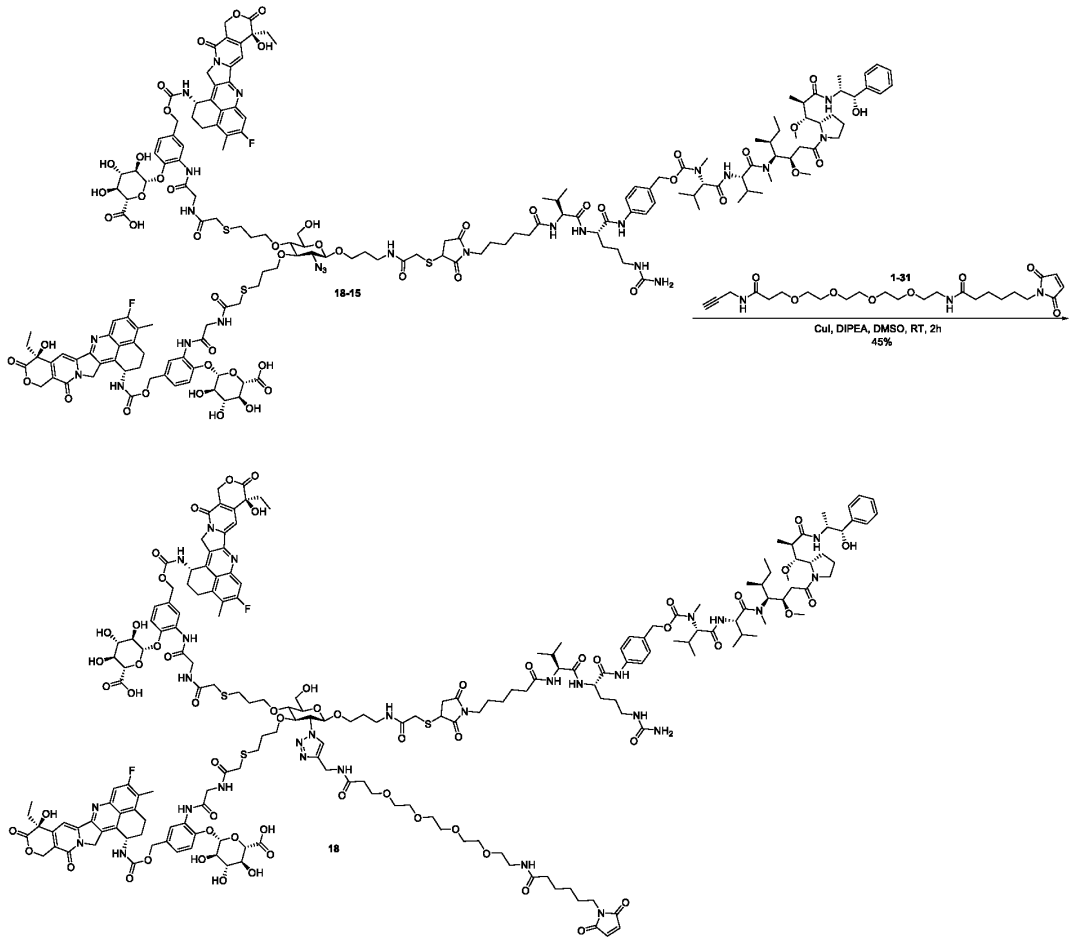


18

Synthetic scheme 18




18-9
NHS, EDCI, DCM, RT, 4h
18-10
7-8
DIPEA, DMF, RT, 16h
18-11
1) TFA, DCM, RT, 1h
2) K2CO3, THF, RT, 6h
3) HCl, H2O, MeCN, RT, 0.5h
18-12
10-11
2-mercaptoacetic acid
DMSO, RT, 3h
86%
18-13
NHS, EDCI, DCM, RT, 16h
73%
18-14
18-11
DIPEA, DMF, RT, 16h
73%



[00571] Step 1: To a mixture of compound **1-2** (30.48 g, 148.56 mmol) in pyridine (300 mL) was added acetic anhydride (139 mL, 1485.60 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was concentrated and quenched with hydrochloric acid water (1 L) and extracted with ethyl acetate (2 L x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **18-1** (53.9 g, yield 98%) as a colorless oil.

[00572] Step 2: Under nitrogen atmosphere, to a mixture of compound **18-1** (30 g, 80.36 mmol) and 4-methylbenzenethiol (40 g, 321.44 mmol) in trichloromethane (250 mL) was slowly added $BF_3.Et_2O$ (19.3 mL, 152.68 mmol) at 0 °C. The mixture was stirred at 0 °C for 16 h. After completion of the reaction, the mixture was concentrated. The crude product was purified silica gel column chromatography (eluted with 14% ethyl acetate in petroleum ether) to afford the title compound **18-2** (23.1 g, yield 65%) as a brown oil.

[00573] Step 3: The title compound **18-3** was prepared in analogy to **Example 17, Step 6**. The product (14.11 g, yield 86%) was obtained as a colorless oil.

[00574] Step 4: Under nitrogen atmosphere, to a mixture of compound **18-3** (14.11 g, 45.32 mmol) in pyridine (100 mL) was slowly added t-butyldimethylchlorosilane (10.93 g, 72.51 mmol) in dichloromethane at 0°C. The mixture was stirred at 60°C for 16h. After completion of the reaction, the mixture was concentrated. The residue was poured into 1N hydrochloric acid (800 mL) and extracted with ethyl acetate (800 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 14% ethyl acetate in petroleum ether) to afford the title compound **18-4** (18.59 g, yield 96%) as a brown oil.

[00575] Step 5: The title compound **18-5** was prepared in analogy to **Example 1, Step 4** by using compound **18-4**. The product (17.45 g, yield 79%) was obtained as a colorless oil.

[00576] Step 6: The title compound **18-6** was prepared in analogy to **Example 1, Step 5** by using compound **18-5**. The product (11.12 g, yield 70%) was obtained as a colorless oil.

[00577] Step 7: The 4A molecular sieve in flask was flamed for 30 minutes under reduced pressure. A solution of compound **18-6** (6.07 g, 8.43 mmol) and compound **18-7** (1.78 g, 10.14 mmol) in anhydrous dichloromethane (60 mL) was added to the flask under nitrogen atmosphere. The mixture was stirred at room temperature for 0.5 h. NIS (2.85 g, 12.679 mmol) was added under nitrogen atmosphere. Then TMSOTf (7.11 mL, 39.311 mmol) was added slowly to the reaction solution at -78°C. The mixture was stirred at -78°C for 1h under nitrogen atmosphere. After completion of the reaction, the mixture was quenched with triethylamine (18.16 mL,

131.04 mmol) at 0°C before filtration and concentration. The crude product was purified silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **18-8** (1.25 g, yield 22%) as a colorless oil. LCMS: m/z calculated for $C_{26}H_{46}N_4O_{11}S_2$: 654.79; found: 655.98 $[M+H]^+$.

[00578] Step 8: The title compound **18-9** was prepared in analogy to **Example 2, Step 4** by using compound **18-8**. The product (288 mg, yield 61%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{24}H_{42}N_4O_{11}S_2$: 626.74; found: 628.05 $[M+H]^+$.

[00579] Step 9: A mixture of compound **18-9** (100 mg, 0.16 mmol) and N-Hydroxy succinimide (74 mg, 0.64 mmol) in anhydrous dichloromethane (5 mL) was added EDCI (92 mg, 0.48 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was diluted with dichloromethane (15 mL) and washed with cold water (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to afford the title compound **18-10** (104 mg, yield 80%) as a colorless oil. The crude product was used into the next step without further purification. LCMS: m/z calculated for $C_{32}H_{48}N_6O_{15}S_2$: 820.88; found: 721.2 $[M\text{-Boc}+H]^+$.

[00580] Step 10: To a solution of compound **7-8** (93 mg, 0.11 mmol) in DMF (3 mL) was added DIPEA (74 mg, 0.57 mmol) and compound **18-10** (104 mg, yield 80%). The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was filtered and the filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **18-11** (80 mg, yield 30%) as a yellow solid. LCMS: m/z calculated for $C_{104}H_{118}F_2N_{14}O_{37}S_2$: 2258.27; found: 1130.1 $[M+2H]^{2+}$.

[00581] Step 11: To a solution of compound **18-11** (70 mg, 0.03 mmol) in dichloromethane (4 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was redissolved in THF (10 mL) and treated with potassium carbonate (83 mg, 0.6 mmol) for 6 h. After deprotection of trifluoroacetyl-group, the mixture was concentrated. The residue was treated with acetonitrile

and adjusted pH = 4 with 1 N HCl. The resulting mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **18-12** (48 mg, yield 75%) as a yellow solid. LCMS: m/z calculated for $C_{99}H_{110}F_2N_{14}O_{35}S_2$: 2158.15; found: 1079.7 $[M+2H]^{2+}$.

[00582] Step 12: A mixture of compound **10-11** (200 mg, 0.15 mmol) and 2-mercaptoacetic acid (18 mg, 0.19 mmol) in DMSO (2 mL) was stirred at room temperature for 3 h. After completion of the reaction, the mixture was filtered. The filtrate was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **18-13** (184 mg, yield 86%) as an off-white solid. LCMS: m/z calculated for $C_{70}H_{109}N_{11}O_{17}S$: 1408.76; found: 1409.5 $[M+H]^+$.

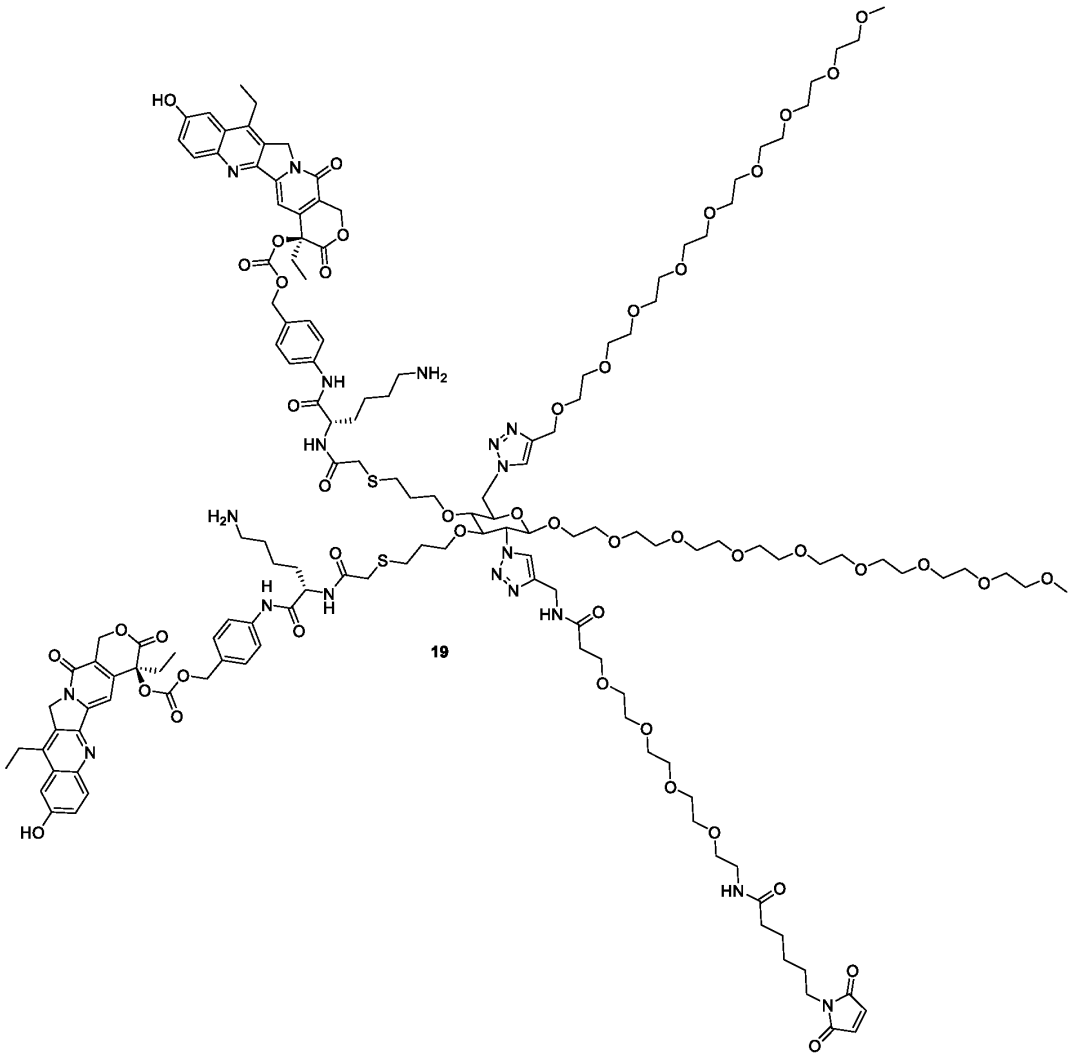
[00583] Step 13: The title compound **18-14** was prepared in analogy to **Example 11, Step 1** by using compound **18-13**. The reaction mixture was concentrated and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **18-14** (70 mg, yield 73%) as an off-white solid. LCMS: m/z calculated for $C_{74}H_{112}N_{12}O_{19}S$: 1505.84; found: 1506.4 $[M+H]^+$.

[00584] Step 14: The title compound **18-15** was prepared in analogy to **Example 10, Step 12** by using compound **18-11** and compound **18-14**. The product (28 mg, yield 73%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{169}H_{217}F_2N_{25}O_{51}S_3$: 3548.90; found: 1183.8 $[M+3H]^{3+}$.

[00585] Step 15: The title compound **18** was prepared in analogy to **Example 7, Step 10** by using compound **18-15** and compound **1-31**. The product (14.5 mg, yield 45%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{193}H_{254}F_2N_{28}O_{59}S_3$: 4044.47; found: 1348.8 $[M+3H]^{3+}$.

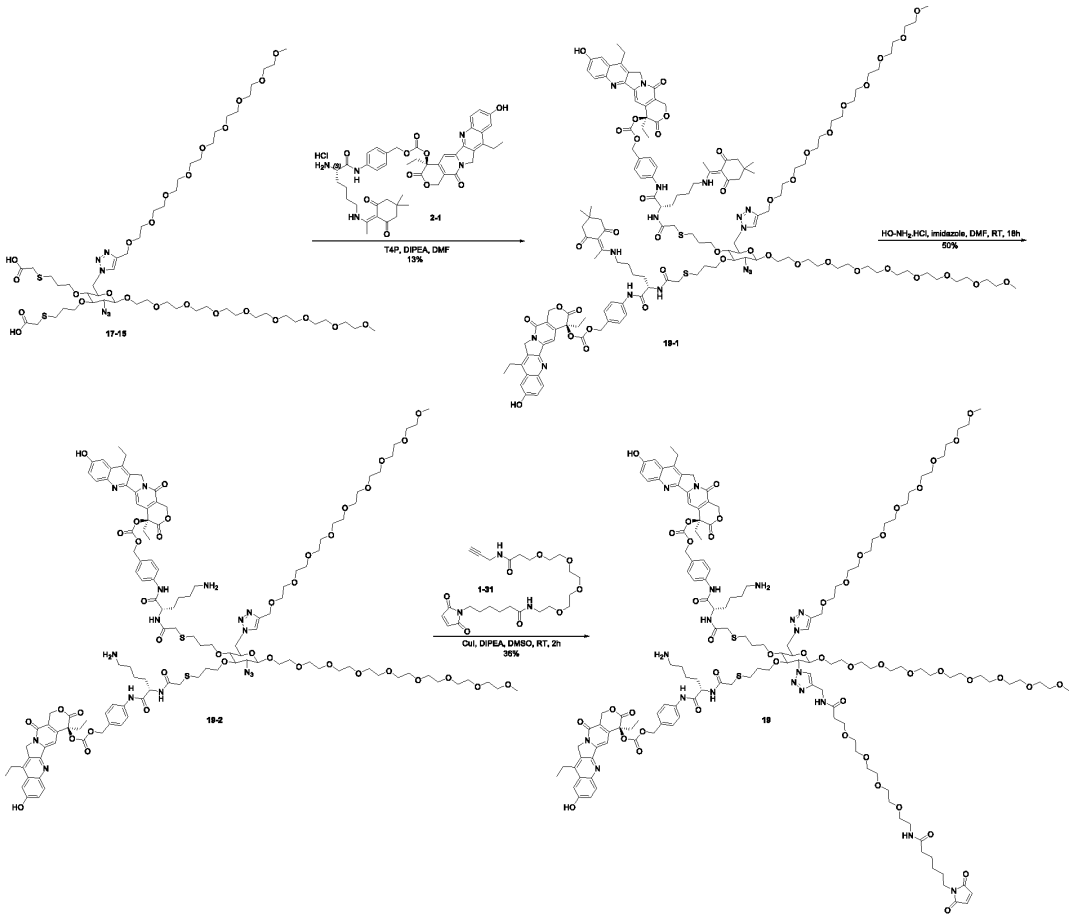
Example 19. Synthesis of ((((2S,2'S)-2,2'-((2,2'-(((((2R,3R,4R,5R,6R)-2-((4-(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)-1H-1,2,3-triazol-1-yl)methyl)-6-

((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(azanediyl))bis(6-aminohexanoyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) bis(carbonate) (19)



19

Synthetic scheme 19

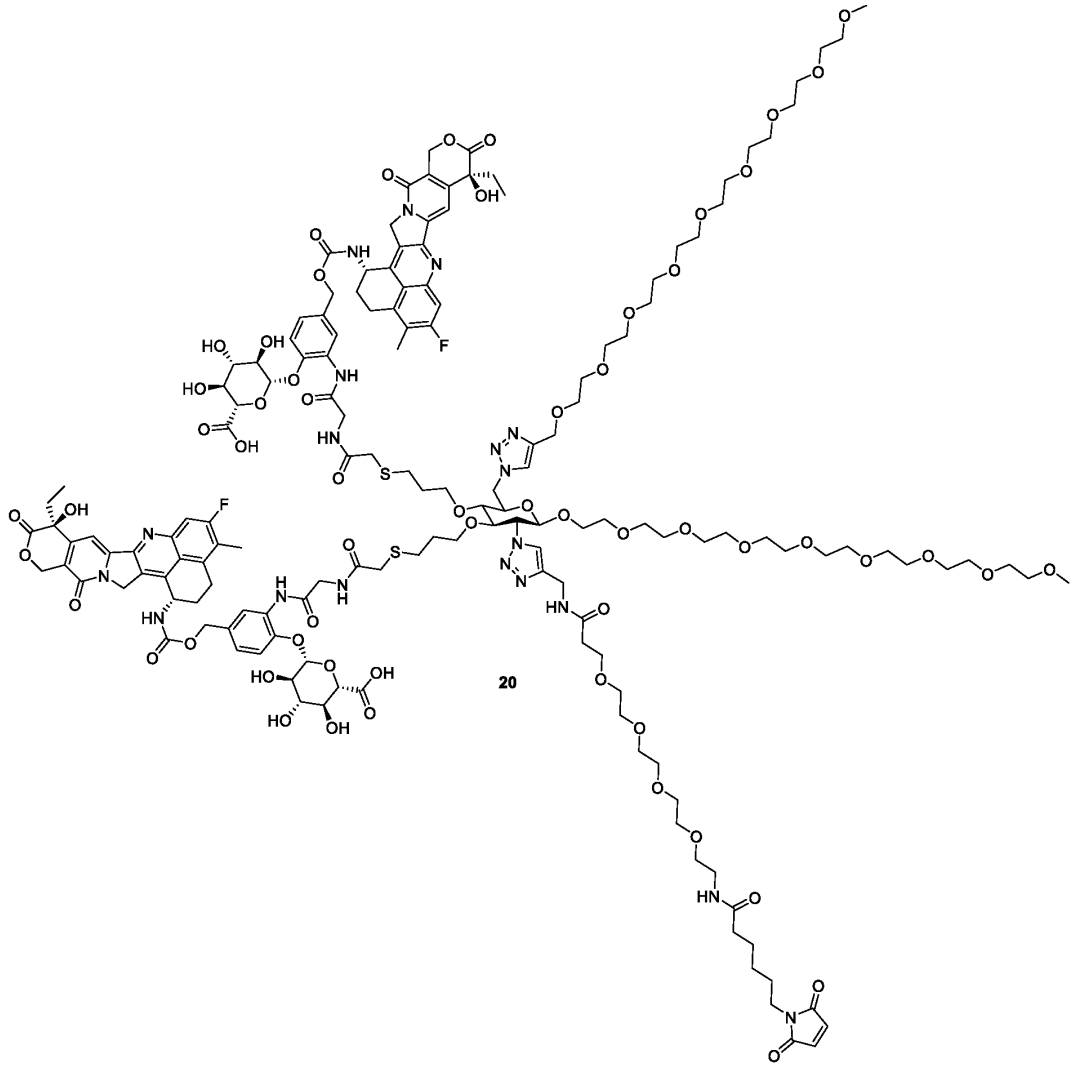


[00586] Step 1: The title compound **19-1** was prepared in analogy to **Example 2, Step 8** by using compound **2-1**, compound **17-15** and T4P instead of T3P. The product (32 mg, yield 13%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{145}H_{196}N_{16}O_{43}S_2$: 2915.35; found: 1458.86 $[M+2H]^{2+}$.

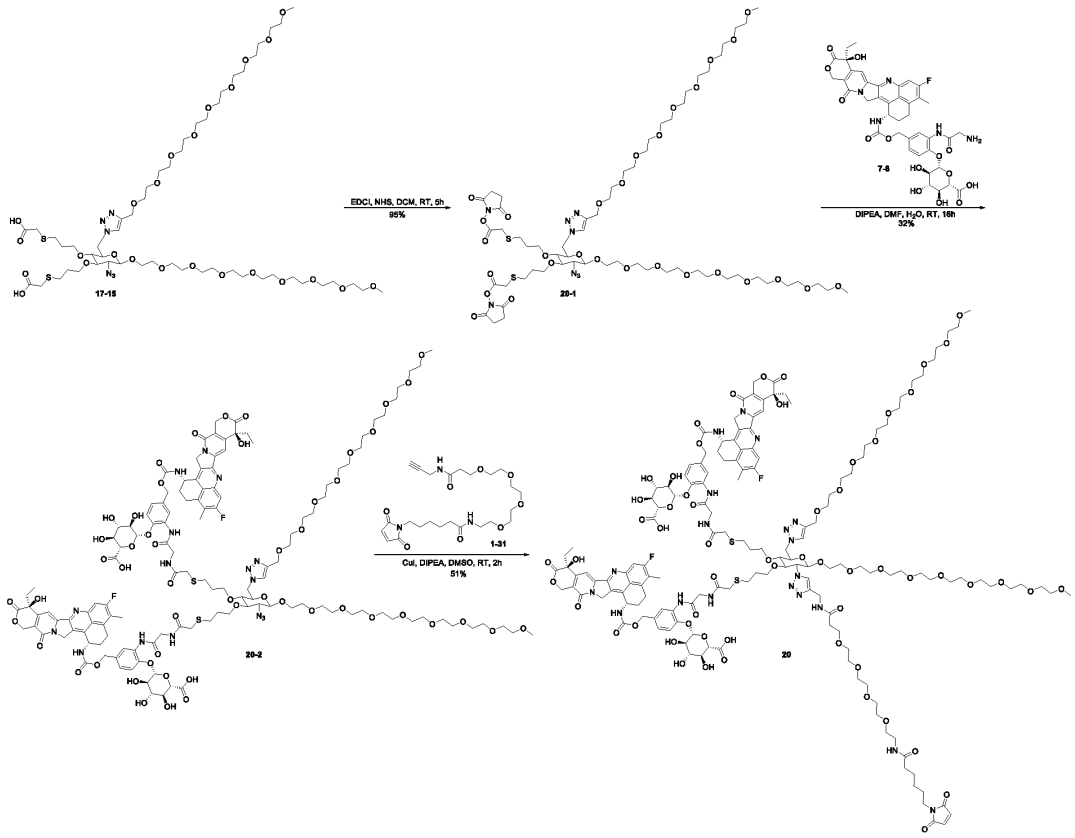
[00587] Step 2: The title compound **19-2** was prepared in analogy to **Example 1, Step 20** by using compound **19-1**. The product (13 mg, yield 50%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{125}H_{172}N_{16}O_{39}S_2$: 2586.94; found: 1295.38 $[M+2H]^{2+}$.

[00588] Step 3: The title compound **19** was prepared in analogy to **Example 10, Step 14** by using compound **19-2** and compound **1-31**. The product (5.6 mg, yield 36%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{149}H_{209}N_{19}O_{47}S_2$: 3082.52; found: 1542.0 $[M+2H]^{2+}$.

Example 20. Synthesis of (2S,2'S,3S,3'S,4S,4'S,5R,5'R,6S,6'S)-6,6'-((((2,2'-((2,2'-(((((2R,3R,4R,5R,6R)-2-((4-(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)-1H-1,2,3-triazol-1-yl)methyl)-6-((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-5-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(azanediyl))bis(acetyl))bis(azanediyl))bis(4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)-2,1-phenylene))bis(oxy))bis(3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid) (20)



Synthetic scheme 20

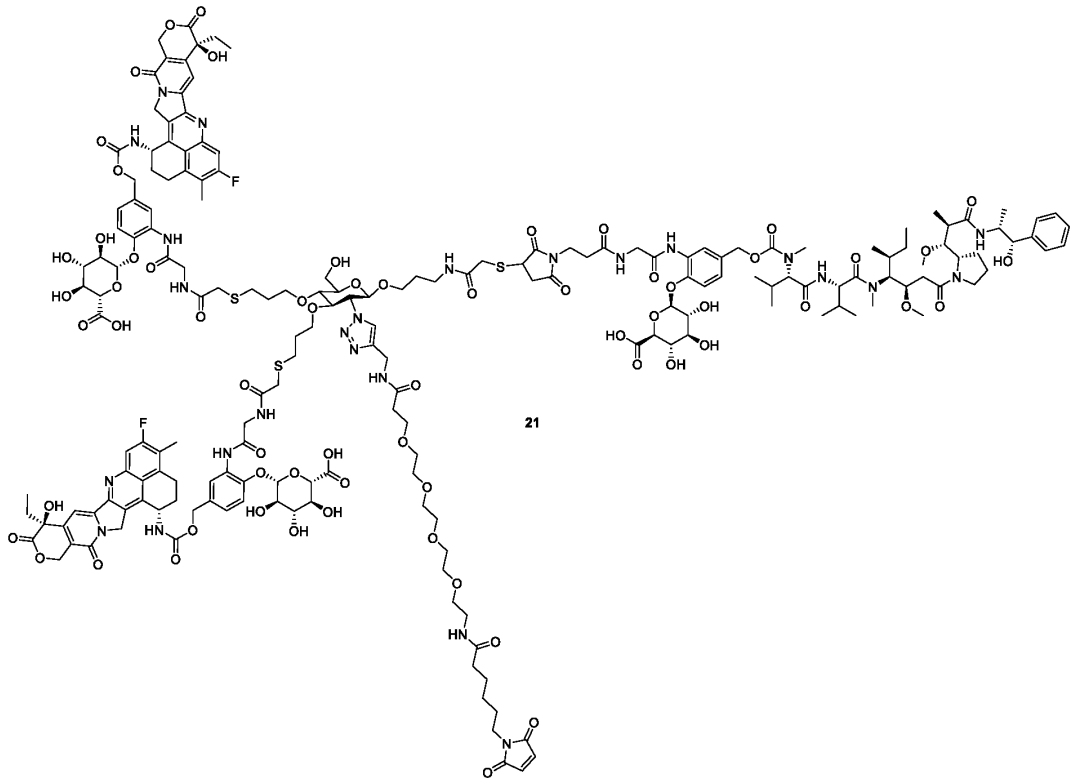


[00589] Step 1: The title compound **20-1** was prepared in analogy to **Example 18, Step 9** by using compound **17-15**. The crude product (111 mg, yield 96%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{61}H_{104}N_8O_{29}S_2$: 1477.65; found: 1478.76$[M+H]^+$.

[00590] Step 2: The title compound **20-2** was prepared in analogy to **Example 18, Step 10** by using compound **7-8** and compound **20-1**. The product (70 mg, yield 32%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{133}H_{174}F_2N_{16}O_{51}S_2$: 2915.03; found: 1458.42 $[M+2H]^{2+}$.

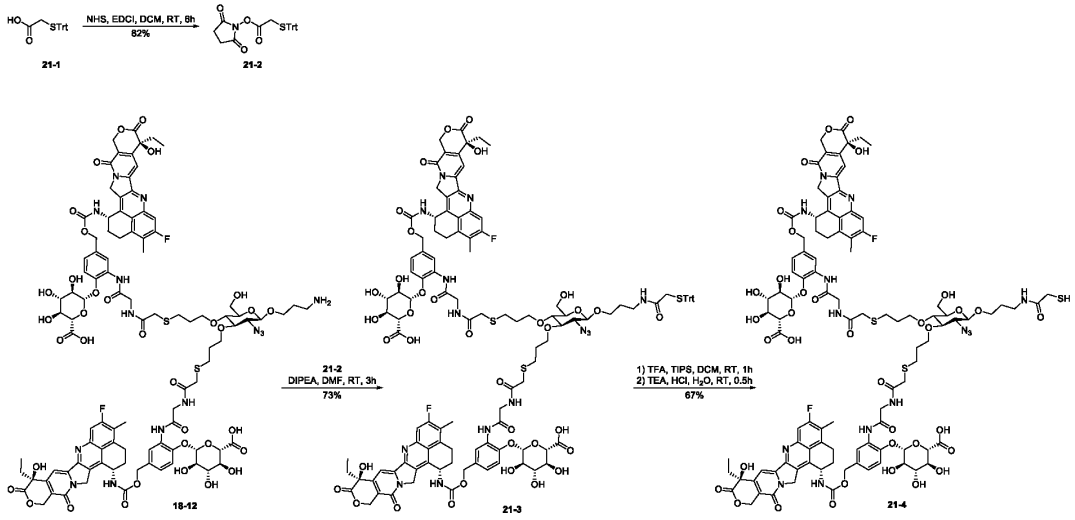
[00591] Step 3: The title compound **20** was prepared in analogy to **Example 10, Step 14** by using compound **20-2** and compound **1-31**. The product (24 mg, yield 51%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{157}H_{211}F_2N_{19}O_{59}S_2$: 3410.61; found: 1137.7 $[M+3H]^{3+}$.

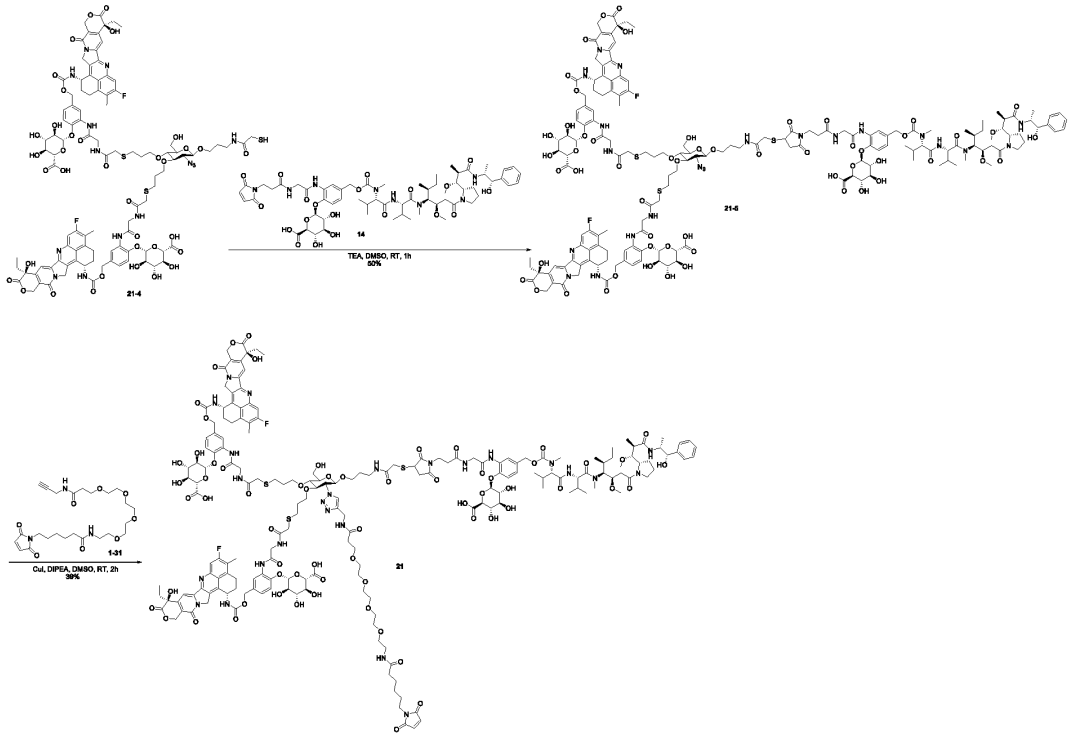
Example 21. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(4*R*,6*R*)-4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]propoxy]-5-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)tetrahydropyran-3-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (21)



21

Synthetic scheme 21





[00592] Step 1: The title compound **21-2** was prepared in analogy to **Example 11, Step 1** by using compound **21-1**. The product (3.2 g, yield 82%) was obtained as a white solid.

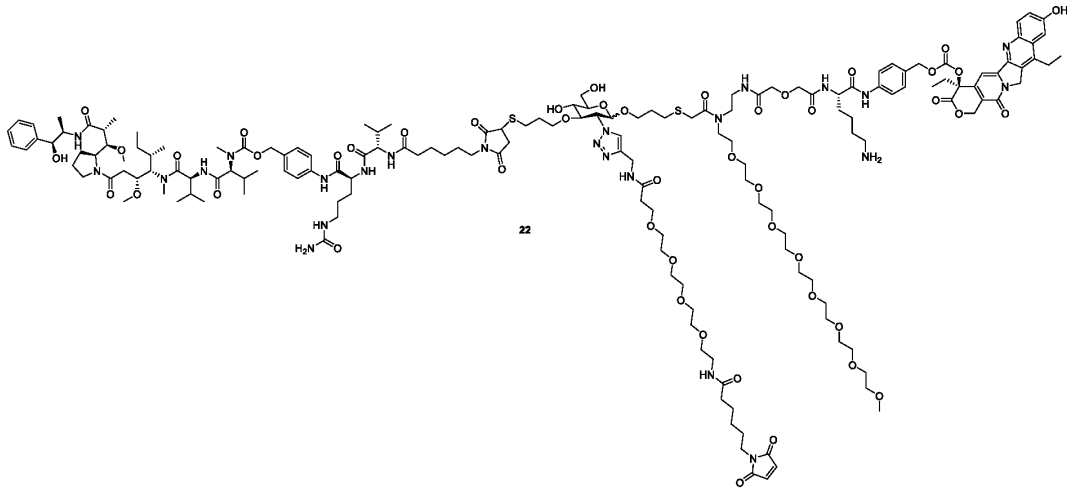
[00593] Step 2: The title compound **21-3** was prepared in analogy to **Example 11, Step 2** by using compound **18-12** and compound **21-1**. The product (38 mg, yield 73%) was obtained as a white solid. LCMS: m/z calculated for $C_{120}H_{126}F_2N_{14}O_{36}S_3$: 2474.57; found: 1237.8 $[M+2H]^{2+}$.

[00594] Step 3: To a suspension of compound **21-3** (38 mg, 0.015 mmol) in dichloromethane (1.6 mL) was added TFA (0.4 mL) and triisopropylsilane (15 uL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was adjusted pH to 7 with TEA and to 5 with 1 N HCl. The resulting mixture was concentrated. The residue was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **21-4** (23 mg, yield 67%) as a pale-yellow solid. LCMS: m/z calculated for $C_{101}H_{112}F_2N_{14}O_{36}S_3$: 2232.25; found: 1116.8 $[M+2H]^{2+}$.

[00595] Step 4: The title compound **21-5** was prepared in analogy to **Example 10, Step 10** by using compound **21-4** and compound **14**. The product (16.5 mg, yield 50%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{163}H_{202}F_2N_{22}O_{56}S_3$: 3499.68; found: 1719.8 $[M+2H]^{2+}$.

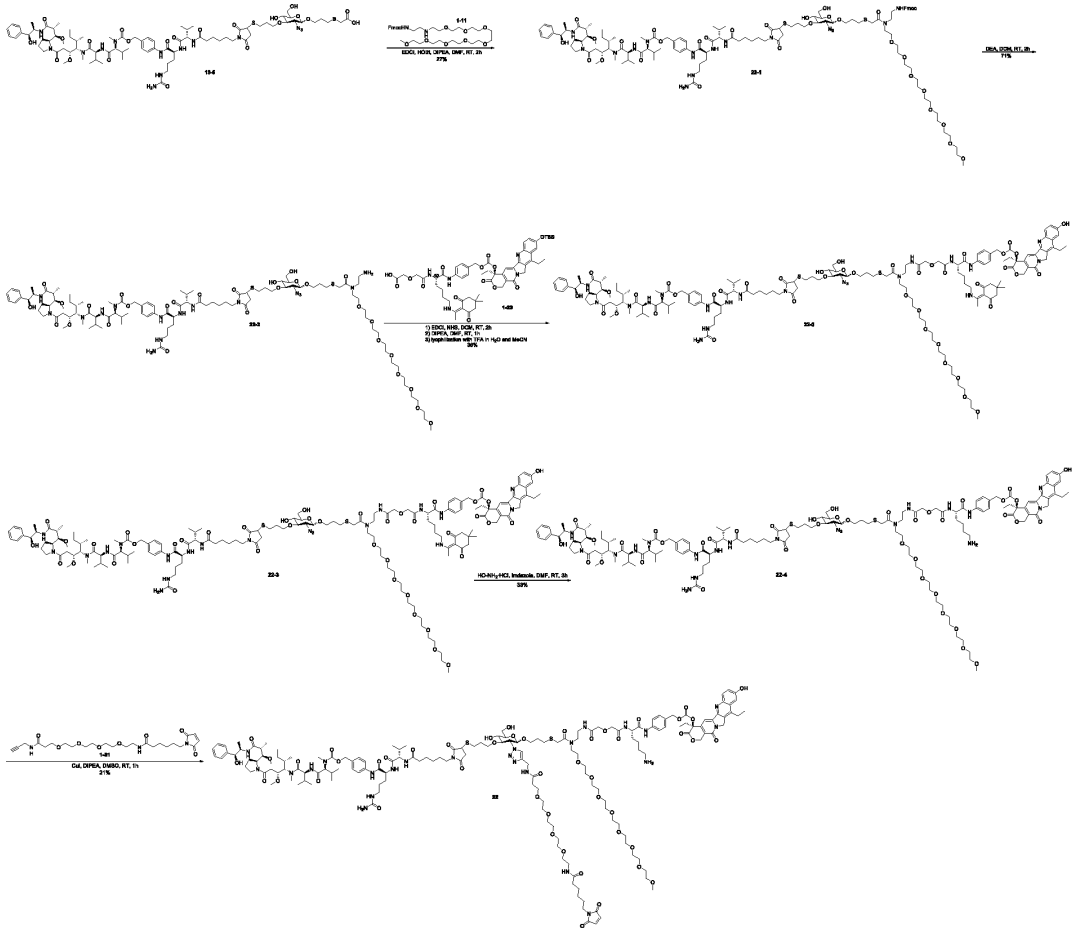
[00596] Step 5: The title compound **21** was prepared in analogy to **Example 10, Step 14** by using compound **21-5** and compound **1-31**. The product (6.7 mg, yield 39%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{187}H_{239}F_2N_{25}O_{64}S_3$: 3995.26; found: 1332.3 $[M+3H]^{3+}$.

Example 22. Synthesis of 4-((2S)-2-((2S)-2-(6-(3-((3-(((3R,4R,5S,6R)-2-((26-(2-(2-(2-(((S)-6-amino-1-((4-((((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-2-oxoethoxy)acetamido)ethyl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propyl)thio)-2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (22)



22

Synthetic scheme 22



[00597] Step 1: The title compound **22-1** was prepared in analogy to **Example 2, Step 5** by using compound **13-5** and compound **1-11**. The product (46 mg, yield 27%) was obtained as a white solid. LCMS: m/z calculated for $C_{116}H_{180}N_{16}O_{31}S_2$: 2358.92; found: 1181.22 $[M+2H]^{2+}$.

[00598] Step 2: To a solution of compound **22-1** (47 mg, 0.02 mmol) in DMF (2 mL) was added diethyl amine (DEA, 5 mg, 0.068 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound title compound **22-2** (30 mg, yield 71%) as a pale-yellow solid. LCMS: m/z calculated for $C_{101}H_{170}N_{16}O_{29}S_2$: 2136.67; found: 1070.15 $[M+2H]^{2+}$.

[00599] Step 3: The title compound **22-3** was prepared in analogy to **Example 18, Step 9** and **step 10** by using compound **22-2** and compound **1-23**. The product (25 mg, yield 36%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{151}H_{223}N_{21}O_{42}S_2$: 3068.67; found: 1535.53 $[M+2H]^{2+}$.

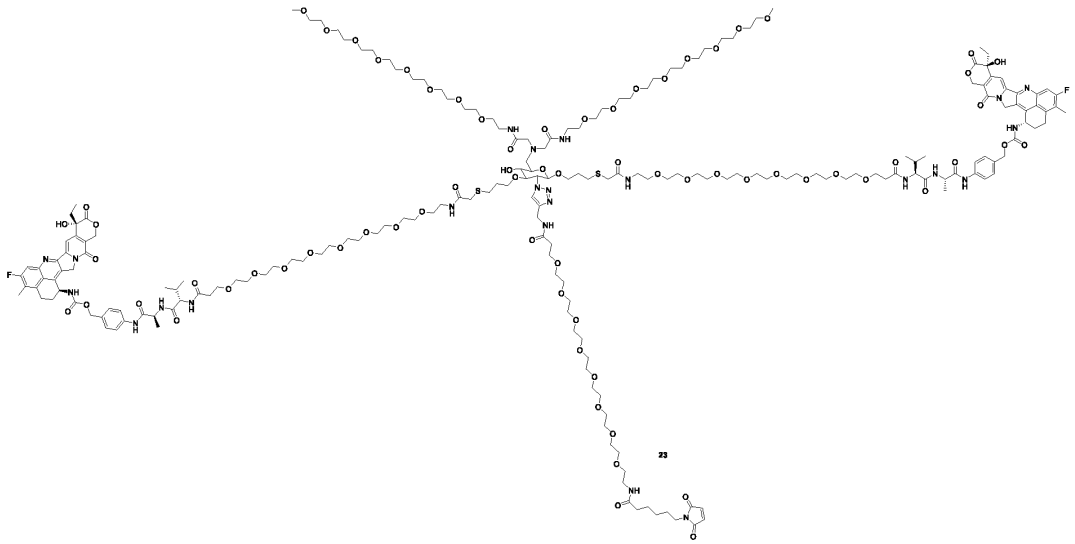
[00600] Step 4: The title compound **22-4** was prepared in analogy to **Example 1, Step 20** by using compound **22-3**. The product (7.8 mg, yield 33%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{141}H_{211}N_{21}O_{40}S_2$: 2904.47; found: 1454.27 $[M+2H]^{2+}$.

[00601] Step 5: The title compound **22** was prepared in analogy to **Example 7, Step 10** by using compound **22-4** and compound **1-31**. The product (2 mg, yield 21%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{165}H_{248}N_{24}O_{48}S_2$: 3400.04; found: 1135.05 $[M+3H]^{3+}$.

[00602] The below compounds were synthesized following similar procedures as shown above.

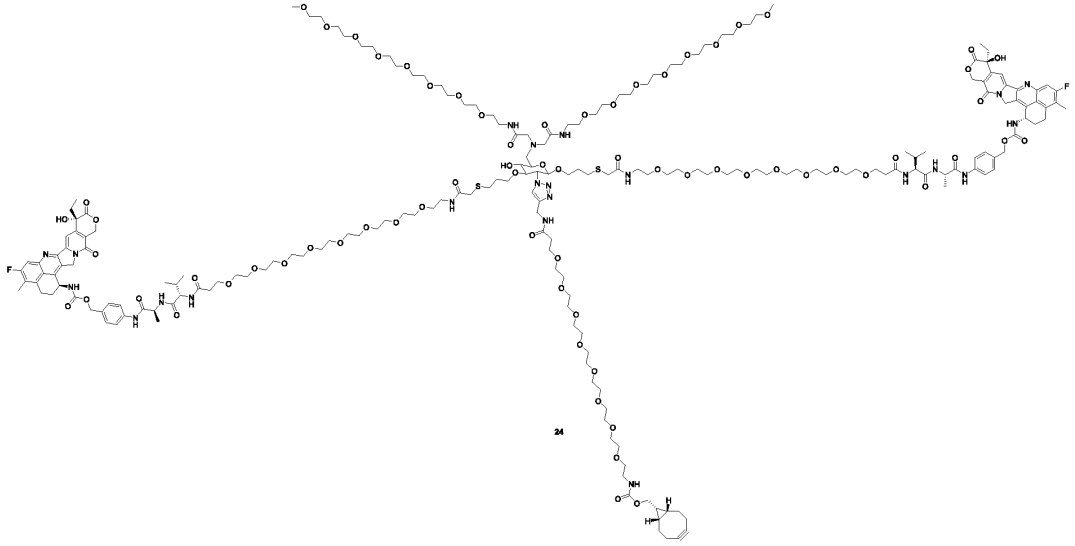
Example 23. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[2-[[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]methyl]-5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-

ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (23)



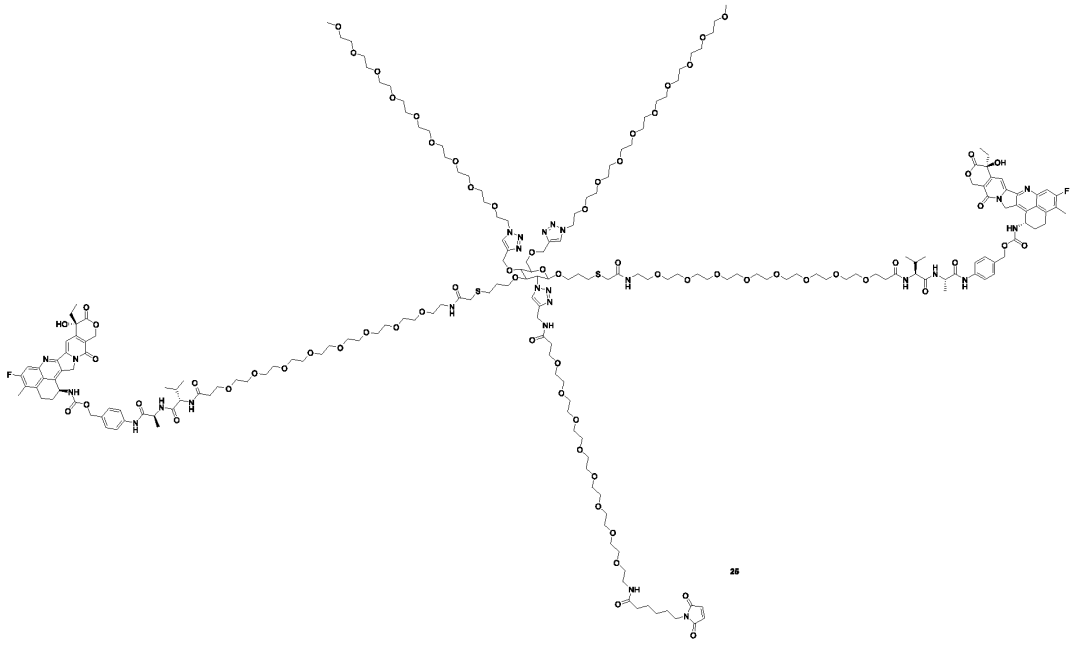
Example 24. Synthesis of [(1*S*,8*R*)-9-bicyclo[6.1.0]non-4-ynyl]methyl *N*-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[6-[[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]methyl]-2,4-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate (24)



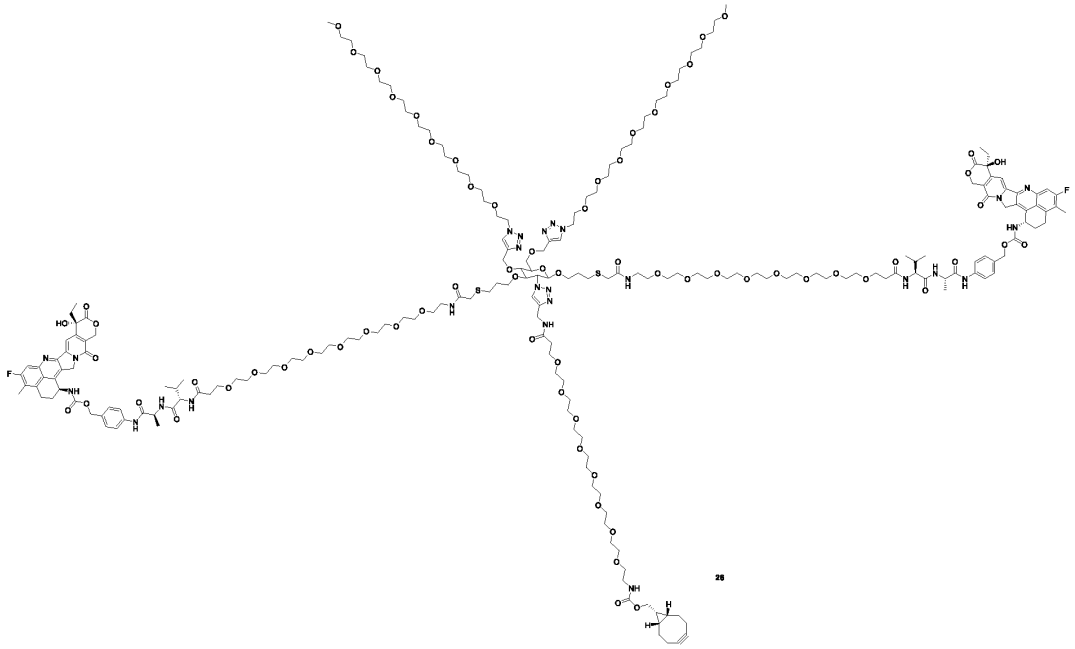
Example 25. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-

methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (25)



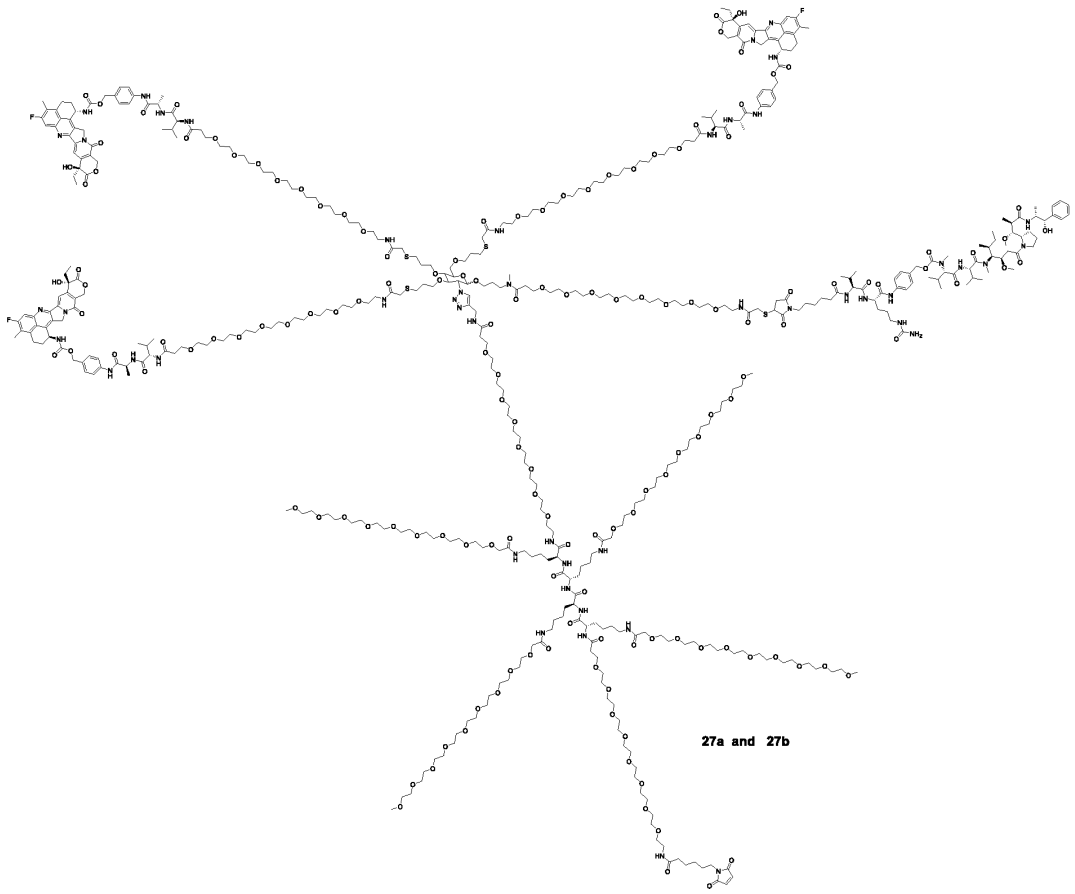
Example 26. Synthesis of [(1*S*,8*R*)-9-bicyclo[6.1.0]non-4-ynyl]methyl *N*-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[2,4-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-

propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate (26)

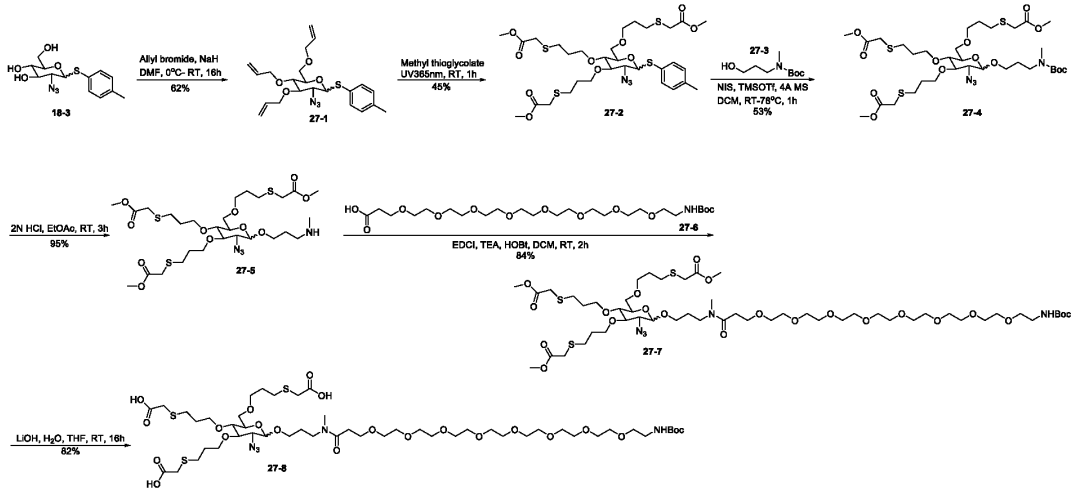


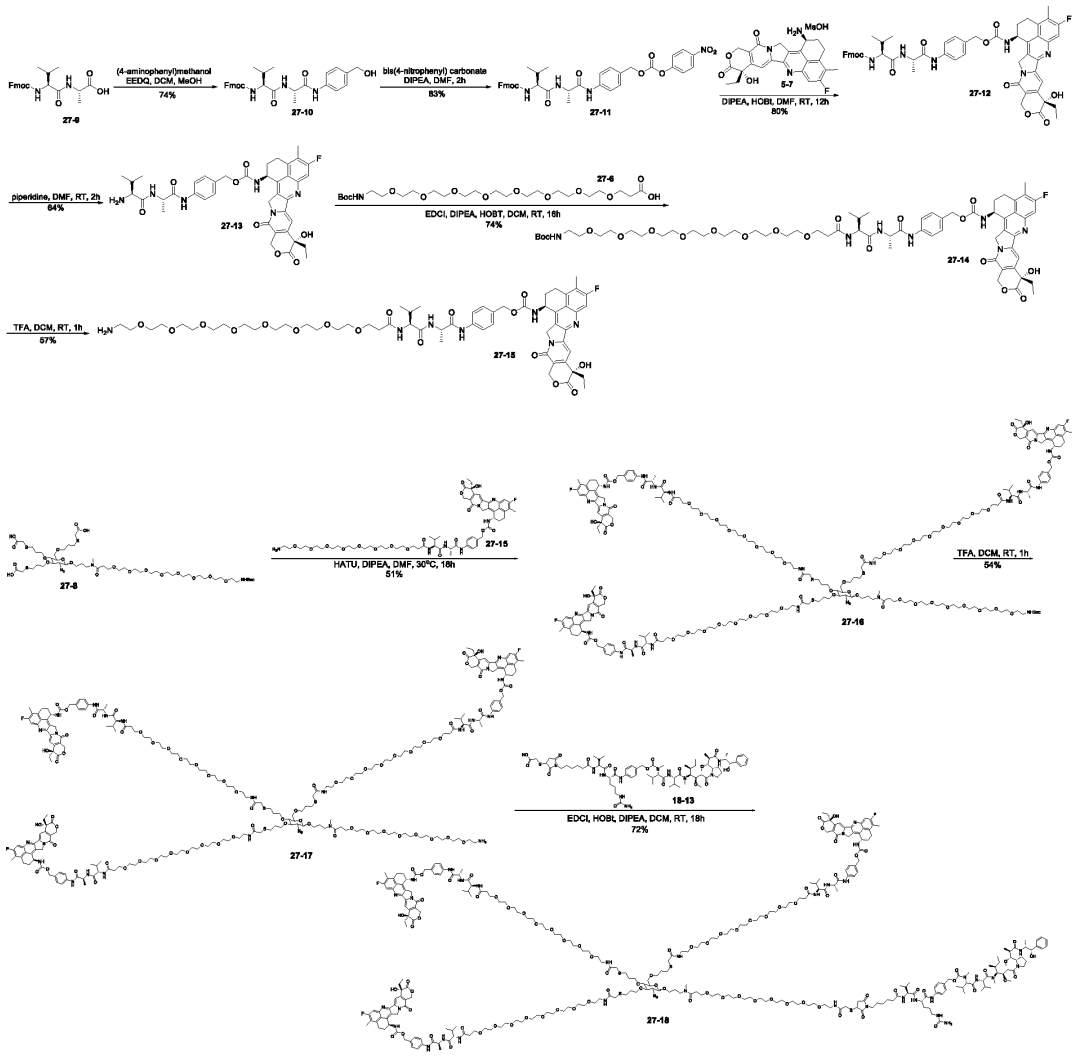
Example 27. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-

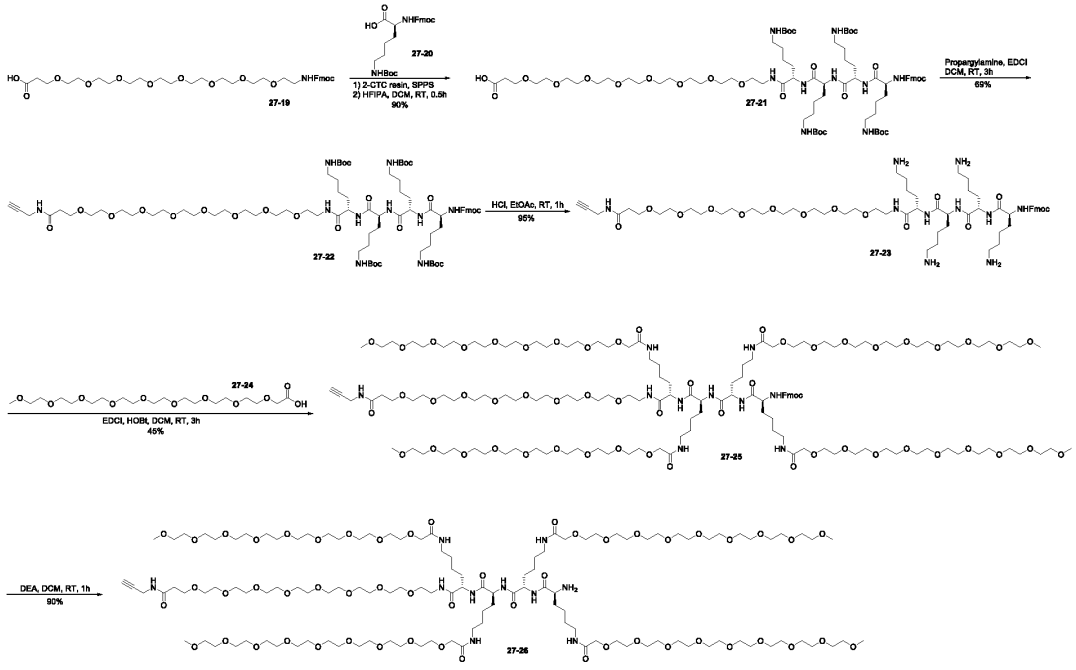
methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (two isomers: 27a and 27b)

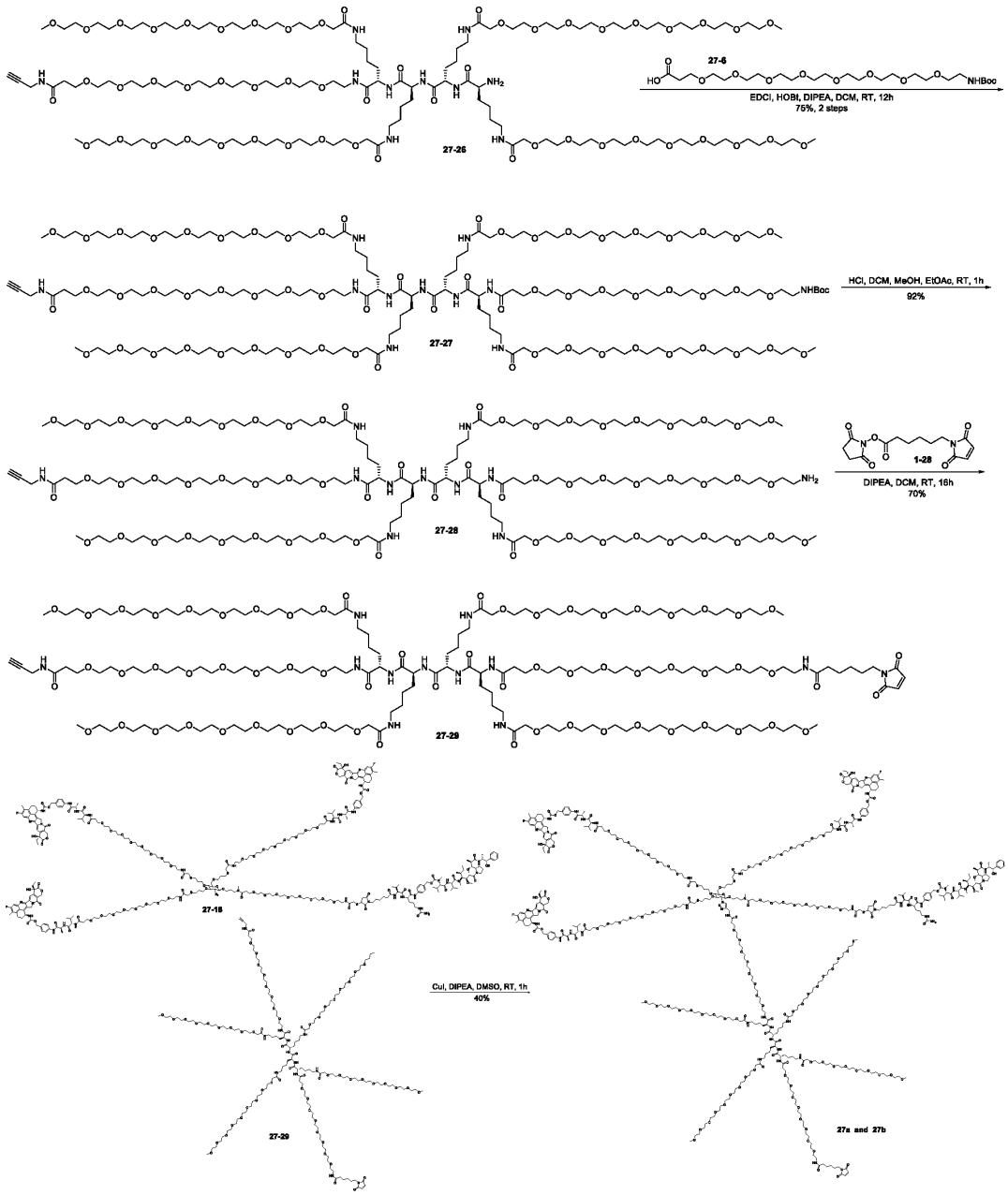


Synthetic scheme 27




Fmoc
27-9
(4-aminophenyl)methanol
EEDQ, DCM, MeOH
74%
27-10
bis(4-nitrophenyl) carbonate
DIPEA, DMF, 2h
83%
27-11
5-7
DIPEA, HOBt, DMF, RT, 12h
80%
27-12
piperidine, DMF, RT, 2h
64%
27-13
27-6
EDCI, DIPEA, HOBT, DCM, RT, 16h
74%
27-14
TFA, DCM, RT, 1h
57%
27-15
27-8
HATU, DIPEA, DMF, 30°C, 16h
51%
TFA, DCM, RT, 1h
54%
27-16
27-17
18-13
EDCI, HOBt, DIPEA, DCM, RT, 16h
72%
27-18


27-19
27-20
1) 2-CTC resin, SPPS
2) HFIPA, DCM, RT, 0.5h
90%
27-21
Propargylamine, EDCI
DCM, RT, 3h
69%
27-22
HCl, EtOAc, RT, 1h
95%
27-23
27-24
EDCI, HOBt, DCM, RT, 3h
45%
27-25
DEA, DCM, RT, 1h
90%
27-26



[00603] Step 1: The title compound **27-1** was prepared in analogy to **Example 18, Step 5** by using compound **18-3**. The product (7.32 g, yield 62%) was obtained as a colorless oil.

[00604] Step 2: The title compound **27-2** was prepared in analogy to **Example 18, Step 6** by using compound **27-1**. The product (5.73 g, yield 45%) was obtained as a colorless slurry.

[00605] Step 3: The title compound **27-4** was prepared in analogy to **Example 18, Step 7** by using compound **27-2** and tert-butyl (3-hydroxypropyl)(methyl)carbamate (**27-3**) instead of compound **18-7**. The product (3.1 g, yield 53%) was obtained as a colorless slurry. LCMS: m/z calculated for $C_{33}H_{58}N_4O_{13}S_3$: 815.02; found: 837.37 $[M+Na]^+$.

[00606] Step 4: The title compound **27-5** was prepared in analogy to **Example 27, Step 23** by using compound **27-4** in ethyl acetate. The product (2.57 g, yield 95%) was obtained as a colorless slurry. LCMS: m/z calculated for $C_{28}H_{50}N_4O_{11}S_3$: 714.91; found: 715.31 $[M+H]^+$.

[00607] Step 5: The title compound **27-7** was prepared in analogy to **Example 5, Step 5** by using compound **27-5** and compound **27-6** in dichloromethane instead of DMF. The product (3.98 g, yield 84%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{52}H_{95}N_5O_{22}S_3$: 1238.53; found: 1238.57 $[M+H]^+$.

[00608] Step 6: The title compound **27-8** was prepared in analogy to **Example 4, Step 5** by using compound **27-7**. The product (3.15 g, yield 82%) was obtained as a colorless slurry. LCMS: m/z calculated for $C_{49}H_{89}N_5O_{22}S_3$: 1196.44; found: 1196.60 $[M+H]^+$.

[00609] Step 7: The title compound **27-10** was prepared in analogy to **Example 1, Step 11** by using compound **27-9** in dichloromethane and methanol. The product (15.80 g, yield 73%) was obtained as a white solid. LCMS: m/z calculated for $C_{30}H_{33}N_3O_5$: 515.61; found: 516.31 $[M+H]^+$.

[00610] Step 8: The title compound **27-11** was prepared in analogy to **Example 7, Step 3** by using compound **27-10**. The product (17.35 g, yield 83%) was obtained as a white solid. LCMS: m/z calculated for $C_{37}H_{36}N_4O_9$: 680.71; found: 681.33 $[M+H]^+$.

[00611] Step 9: The title compound **27-12** was prepared in analogy to **Example 7, Step 4** by using compound **27-11** and compound **5-7**. The product (19.95 g, yield 80%) was obtained as a pale-brown solid. LCMS: m/z calculated for $C_{55}H_{53}FN_6O_{10}$: 977.06; found: 977.48 $[M+H]^+$.

[00612] Step 10: The title compound **27-13** was prepared in analogy to **Example 7, Step 6** by using compound **27-12**. The product (9.87 g, yield 80%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{40}H_{43}FN_6O_8$: 754.82; found: 755.38 $[M+H]^+$.

[00613] Step 11: The title compound **27-14** was prepared in analogy to **Example 5, Step 5** by using compound **27-13** in dichloromethane instead of DMF. The product (12.36 g, yield 74%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{64}H_{88}FN_7O_{19}$: 1278.44; found: 1278.58 $[M+H]^+$.

[00614] Step 12: To a solution of compound **27-14** (6.00 g, 4.69 mmol) in dichloromethane (45 mL) was added TFA (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane (200 mL) and concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **27-15** (3.15 g, yield 57%) as a yellow solid. LCMS: m/z calculated for $C_{59}H_{80}FN_7O_{17}$: 1178.32; found: 1178.54 $[M+H]^+$.

[00615] Step 13: The title compound **27-16** was prepared in analogy to **Example 8, Step 3** by using compound **27-8** and compound **27-15**. The product (345 mg, yield 51%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{226}H_{323}F_3N_{26}O_{70}S_3$: 4677.36; found: 1145.17 $[M+4H]^{4+}$.

[00616] Step 14: The title compound **27-17** was prepared in analogy to **Example 27, Step 13** by using compound **27-16**. The product (200 mg, yield 54%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{221}H_{315}F_3N_{26}O_{68}S_3$: 4577.24; found: 1526.58 $[M+3H]^{3+}$.

[00617] Step 15: The title compound **27-18** was prepared in analogy to **Example 5, Step 5** by using compound **18-13** and compound **27-17**. The product (180 mg, yield 72%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{291}H_{422}F_3N_{37}O_{84}S_4$: 5967.99; found: 1194.38 $[M+5H]^{5+}$.

[00618] Step 16: The title compound **27-21** was prepared in analogy to **Example 5, Step 4** by using compound **27-20** and **compound 27-19**. The product (8.3 g, yield 90%) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{78}H_{129}N_9O_{24}$: 1576.93; found: 1576.85 $[M+H]^+$.

[00619] Step 17: To a solution of compound **27-21** (3.5 g, 2.22 mmol) and propargylamine (245 mg, 4.44 mmol) in dichloromethane (30 mL) was added EDCI (640 mg, 3.33 mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was diluted with dichloromethane (50 mL) and washed with water (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 5% methanol in dichloromethane) to afford the title compound **27-22** (2.47 g, yield 69%) as a colorless oil. LCMS: m/z calculated for $C_{81}H_{132}N_{10}O_{23}$: 1613.99; found: 707.69 $[M+2H]^{2+}$.

[00620] Step 18: The title compound **27-23** was prepared in analogy to **Example 27, Step 23** by using compound **27-22** in ethyl acetate. The product (2.46 g, yield 95%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{61}H_{100}N_{10}O_{15}$: 1213.53; found: 607.74 $[M+2H]^{2+}$.

[00621] Step 19: The title compound **27-25** was prepared in analogy to **Example 2, Step 5** by using compound **27-24** and compound **27-23**. The product (1.55 g, yield 45%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{137}H_{244}N_{10}O_{55}$: 2911.47; found: 971.50 $[M+3H]^{3+}$.

[00622] Step 20: The title compound **27-26** was prepared in analogy to **Example 22, Step 2** by using compound **27-25** in dichloromethane instead of DMF. The concentrated crude product (1.8 g) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{122}H_{234}N_{10}O_{53}$: 2689.23; found: 897.37 $[M+3H]^{3+}$.

[00623] Step 21: The title compound **27-27** was prepared in analogy to **Example 2, Step 5** by using compound **27-26** and compound **27-6**. The product (1.29 g, yield 75% over 2 steps) was obtained as a colorless oil. LCMS: m/z calculated for $C_{146}H_{279}N_{11}O_{64}$: 3212.85; found:1071.93 $[M+3H]^{3+}$.

[00624] Step 22: To a solution of compound **27-28** (460 mg, 0.14 mmol) in dichloromethane (5 mL) and MeOH (0.5 mL) was added 4 N HCl in ethyl acetate (2.5 mL). The reaction mixture

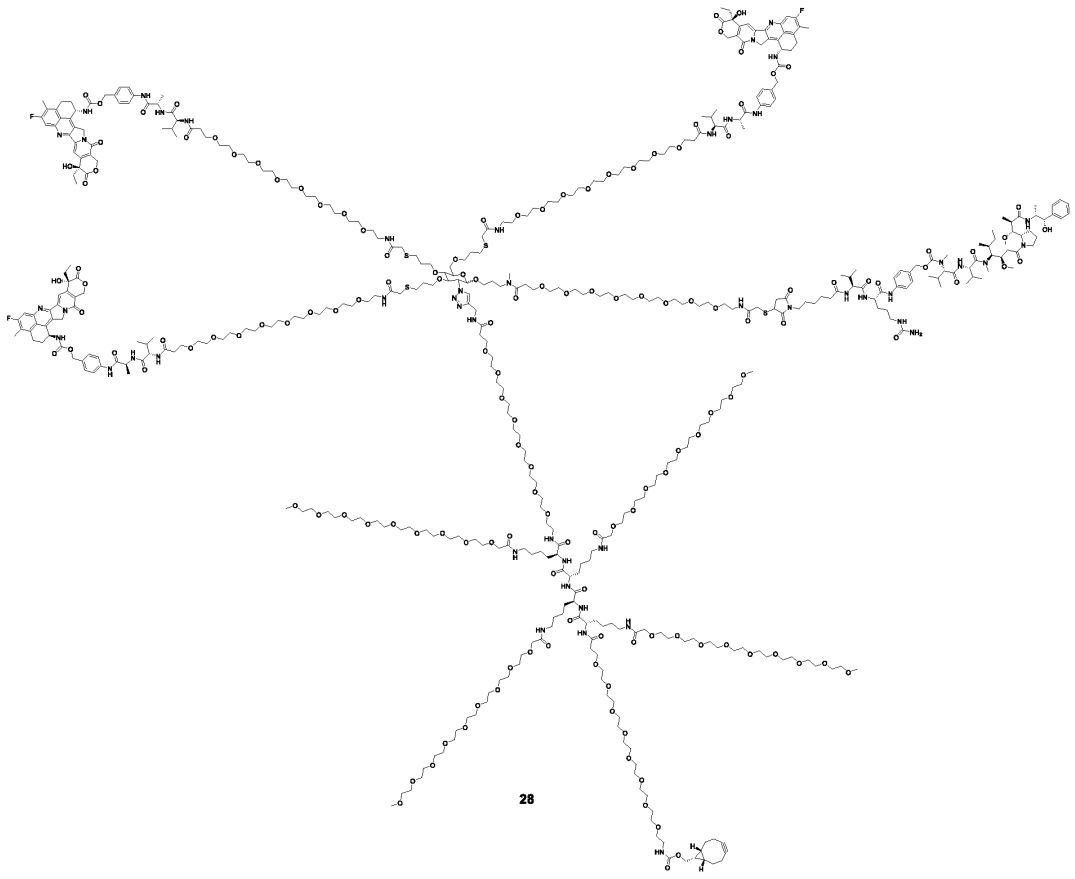
was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated to afford the title compound **27-28** (417 mg, yield 92%) as a colorless oil. The crude product was used into the next step without further purification. LCMS: m/z calculated for $C_{141}H_{271}N_{11}O_{62}$: 3211.89; found: 1039.48 $[M+3H]^{3+}$.

[00625] Step 23: The title compound **27-29** was prepared in analogy to **Example 1, Step 21** by using compound **27-28** and compound **1-28**. The product (687 mg, yield 70%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{151}H_{282}N_{12}O_{65}$: 3305.94; found: 1102.90 $[M+3H]^{3+}$.

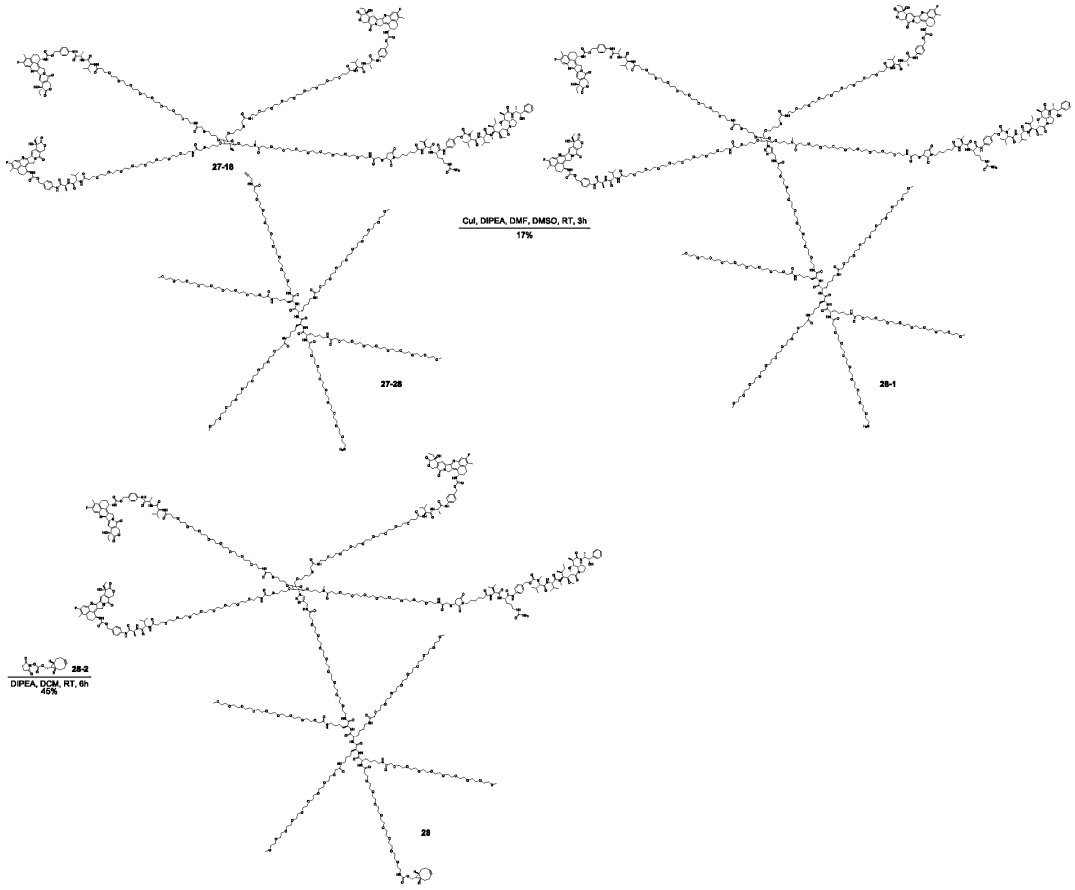
[00626] Step 24: Under nitrogen atmosphere, to a mixture of compound **27-18** (65 mg, 0.01 mmol) and compound **27-29** (38 mg, 0.01 mmol) in a flask was added dimethyl sulfoxide (2 mL), N,N-diisopropylethylamine (0.028 mL , 0.16 mmol) and cuprous iodide (8 mg , 0.04 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (1.5 mL). The resulting mixture was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give two isomers: compound **27a** (14 mg, yield 13%) as a pale-yellow oil. LCMS: m/z calculated for $C_{442}H_{704}F_3N_{49}O_{149}S_4$: 9273.92; found: 1855.55 $[M+5H]^{5+}$. compound **27b** (27 mg, yield 27%) as a pale-yellow oil. LCMS: m/z calculated for $C_{442}H_{704}F_3N_{49}O_{149}S_4$: 9273.92; found: 1855.56 $[M+5H]^{5+}$.

Example 28. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(1*S*,8*R*)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]

amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (28)



Synthetic scheme 28



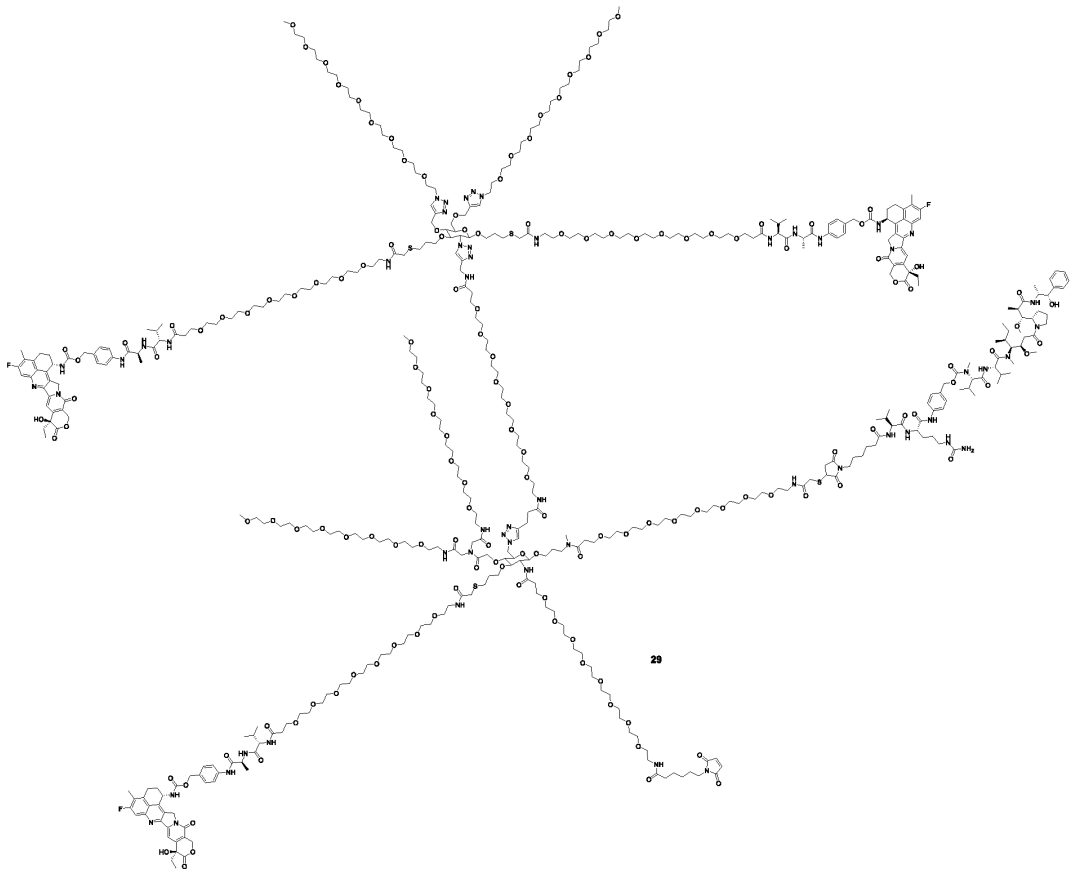
[00627] Step 1: The title compound **28-1** was prepared in analogy to **Example 10, Step 14** by using compound **27-18** and compound **27-28**. The product (26 mg, yield 17%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{432}H_{693}F_3N_{48}O_{146}S_4$: 9080.72; found: 1514.26 $[M+6H]^{6+}$.

[00628] Step 2: The title compound **28** was prepared in analogy to **Example 51, Step 1** by using compound **28-1** and compound ((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (**28-2**). The product (12 mg, yield 45%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{443}H_{705}F_3N_{48}O_{148}S_4$: 9256.94; found: 1543.74 $[M+6H]^{6+}$.

[00629] The below compounds were synthesized following similar procedures as shown above.

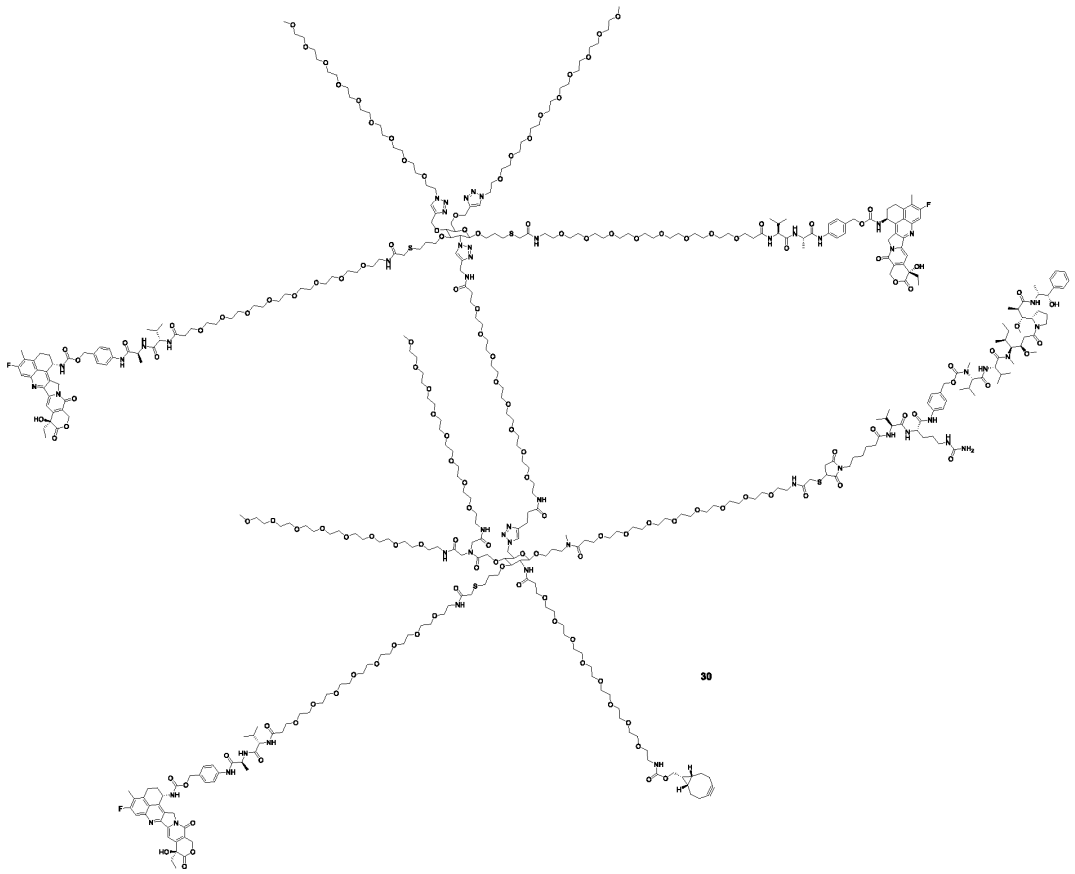
Example 29. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[6-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[2,4-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-3-oxo-propyl]triazol-1-yl]methyl]-5-[2-[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]-2-oxo-ethoxy]-3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-

[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (29)



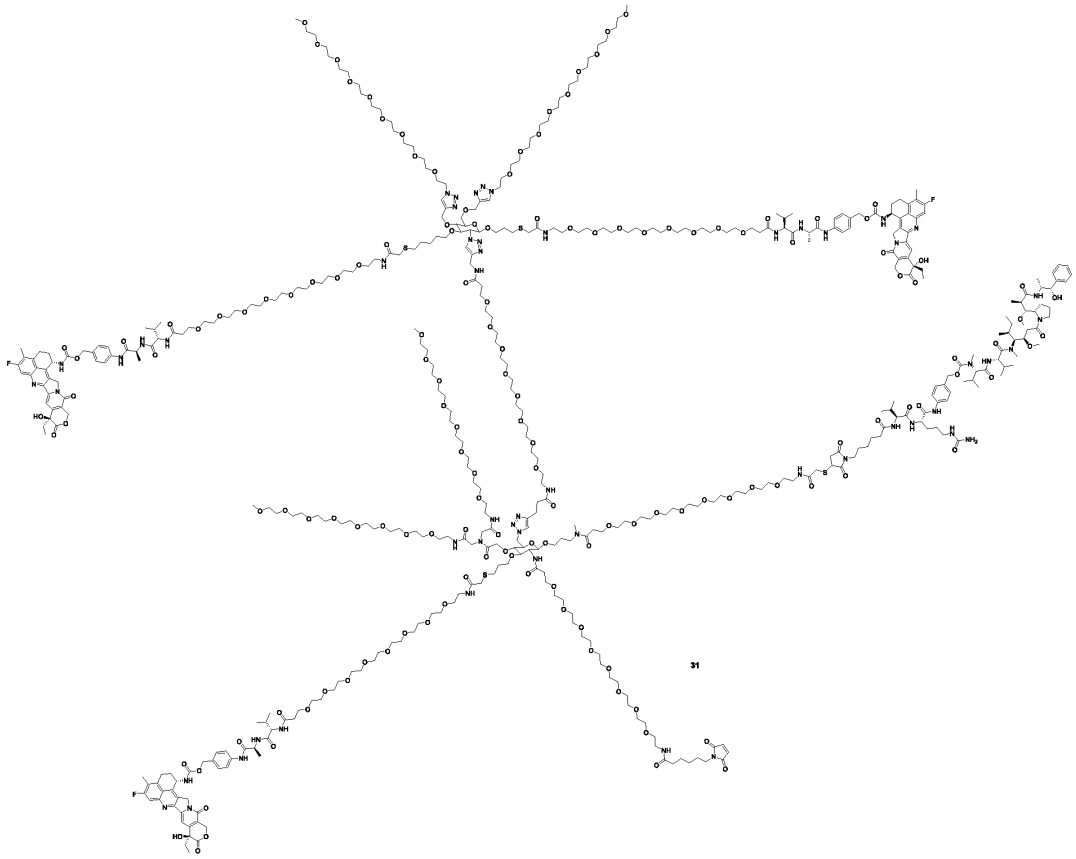
Example 30. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(1*S*,8*R*)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[2,4-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-

propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-3-oxo-propyl]triazol-1-yl]methyl]-5-[2-[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]-2-oxo-ethoxy]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (30)



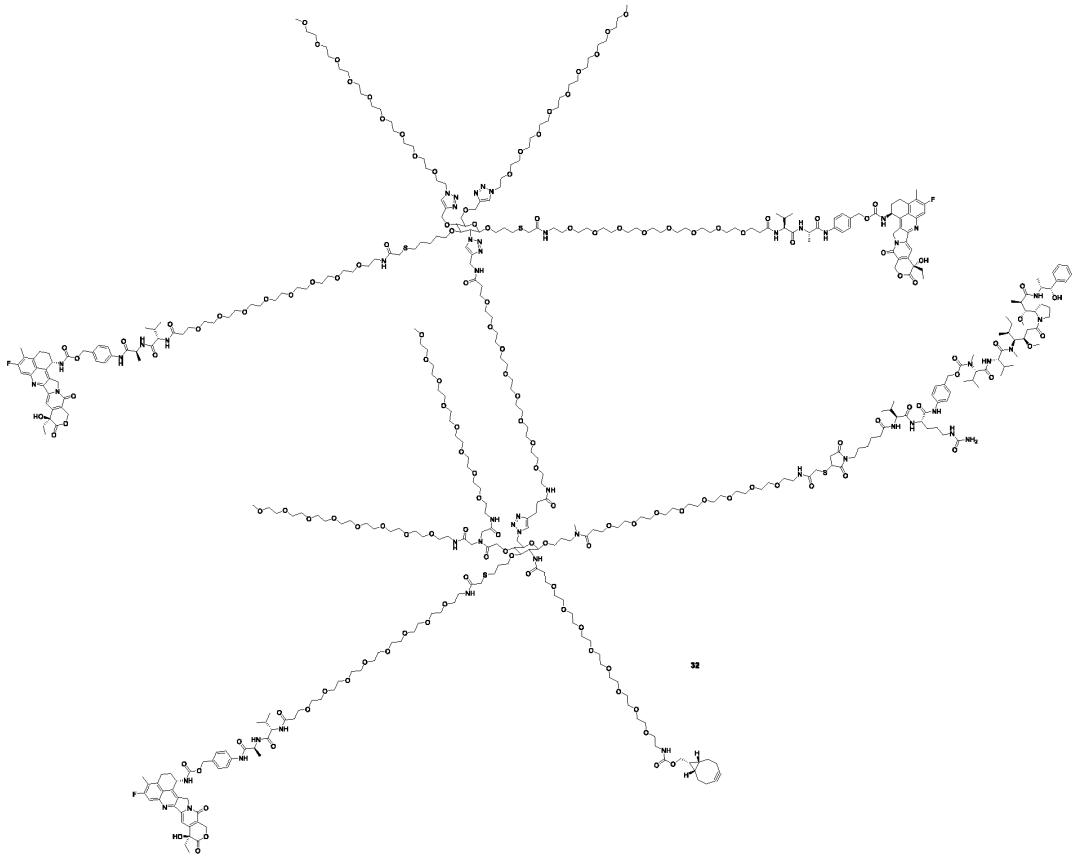
Example 31. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[5-[2-[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]-2-oxo-ethoxy]-3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[4-[5-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-

propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpentoxy]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-3-oxo-propyl]triazol-1-yl]methyl]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (31)



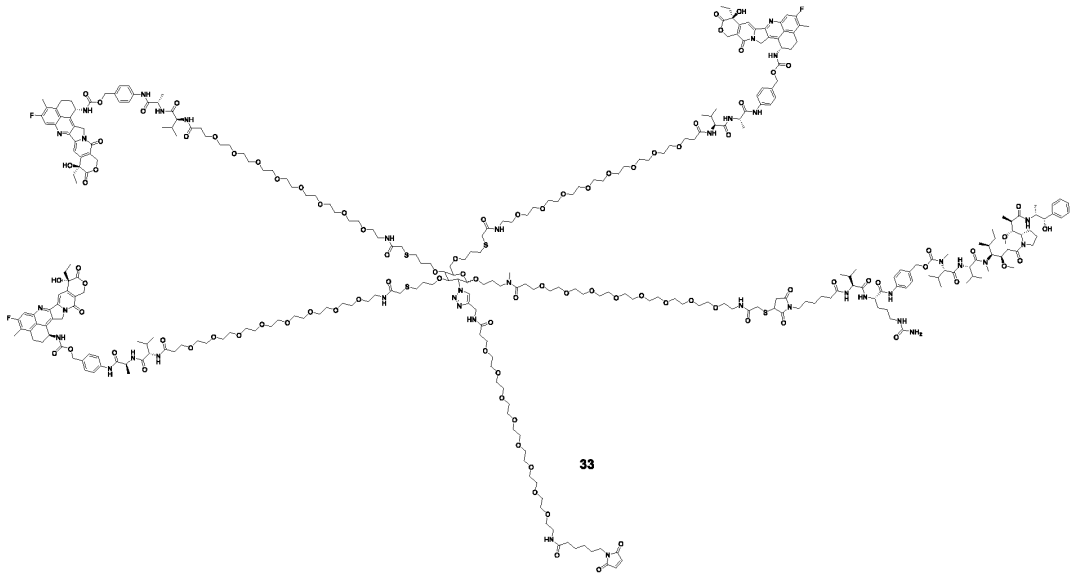
Example 32. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(1*S*,8*R*)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-[2-[bis[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]amino]-2-oxo-ethoxy]-6-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[4-[5-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-

ethyl]sulfanylpentoxy]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxy]-6-[[1-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]methoxymethyl]tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-3-oxo-propyl]triazol-1-yl]methyl]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (32)

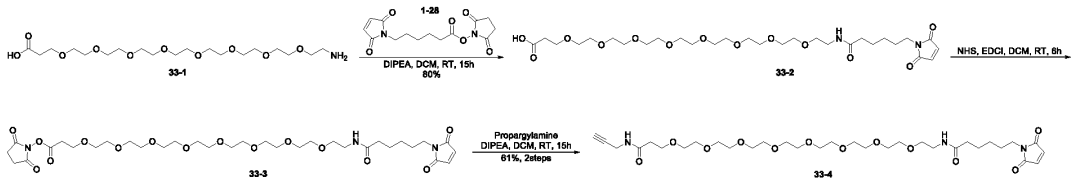


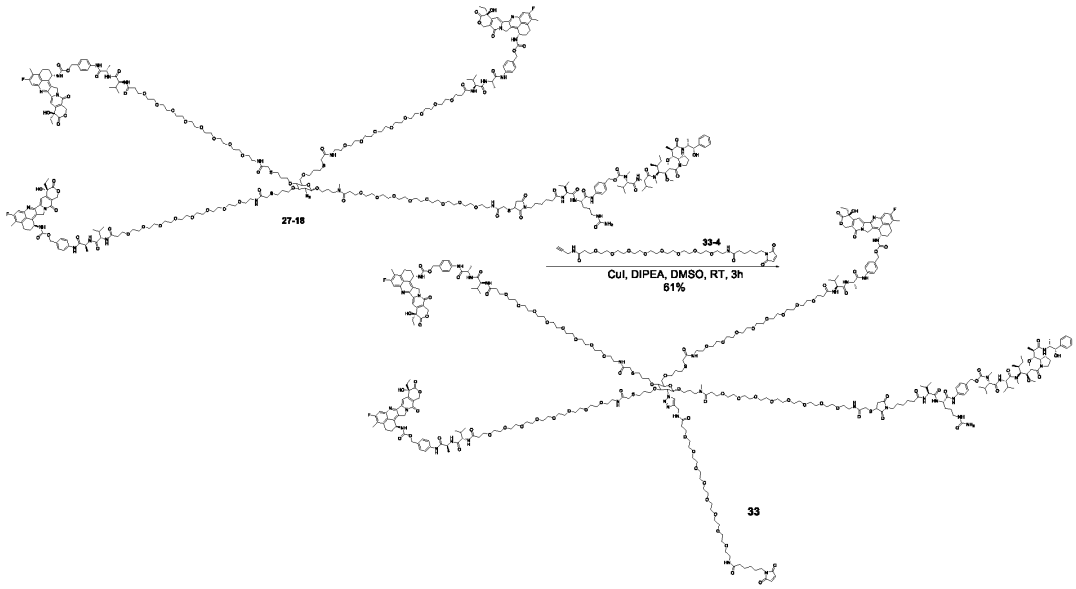
Example 33. Synthesis of 4-((2S)-2-((2S)-2-(6-(3-((34-(((3R,4R,5S,6R)-3-(4-(36-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,31-dioxo-6,9,12,15,18,21,24,27-octaoxa-2,30-diazahexatriacontyl)-1H-1,2,3-triazol-1-yl)-6-((38S,41S)-42-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-38-isopropyl-41-methyl-8,36,39,42-tetraoxo-2,12,15,18,21,24,27,30,33-nonaoxa-6-thia-9,37,40-triazadotetracontyl)-4,5-bis(((2S,5S)-1-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-5-isopropyl-2-methyl-1,4,7,35-tetraoxo-10,13,16,19,22,25,28,31-octaoxa-37-thia-3,6,34-triazatetracontan-40-yl)oxy)tetrahydro-2H-

pyran-2-yl)oxy)-31-methyl-2,30-dioxo-6,9,12,15,18,21,24,27-octaoxa-3,31-diazatetratriacontyl)thio)-2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (33)



Synthetic scheme 33





[00630] Step 1: The title compound **33-2** was prepared in analogy to **Example 1, Step 21** by using compound **33-1** and compound **1-28**. The product (574 mg, yield 80%) was obtained as an colorless oil. LCMS: m/z calculated for $C_{29}H_{50}N_2O_{13}$: 634.72; found: 636.01 $[M+H]^+$.

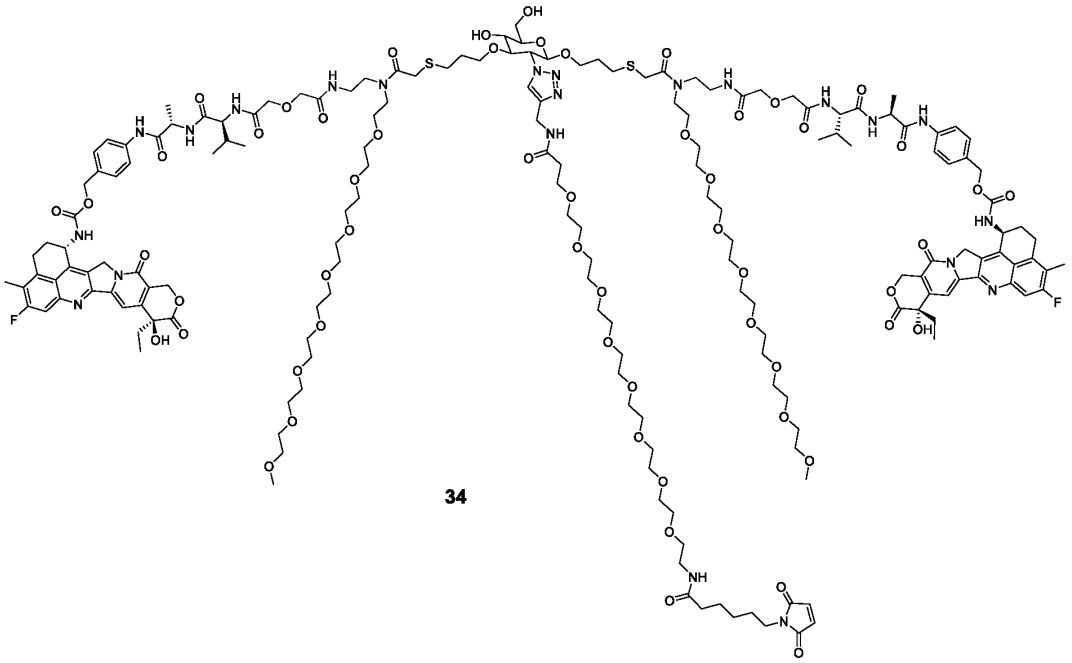
[00631] Step 2: The title compound **33-3** was prepared in analogy to **Example 1, Step 22** by using compound **33-3**. The product (690 mg, curde) was obtained as an colorless oil. LCMS: m/z calculated for $C_{33}H_{53}N_3O_{15}$: 731.79; found: 732.45 $[M+H]^+$.

[00632] Step 3: The title compound **33-4** was prepared in analogy to **Example 1, Step 23** by using compound **33-3**. The product (385 mg, yield 61% over 2 steps) was obtained as an off-white solid. LCMS: m/z calculated for $C_{32}H_{53}N_3O_{12}$: 671.79; found: 672.38 $[M+H]^+$.

[00633] Step 4: The title compound **33** was prepared in analogy to **Example 10, Step 14** by using compound **27-18** and compound **33-4**. The product (38 mg, yield 61%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{323}H_{475}F_3N_{40}O_{96}S_4$: 6639.77; found: 1328.91 $[M+5H]^{5+}$.

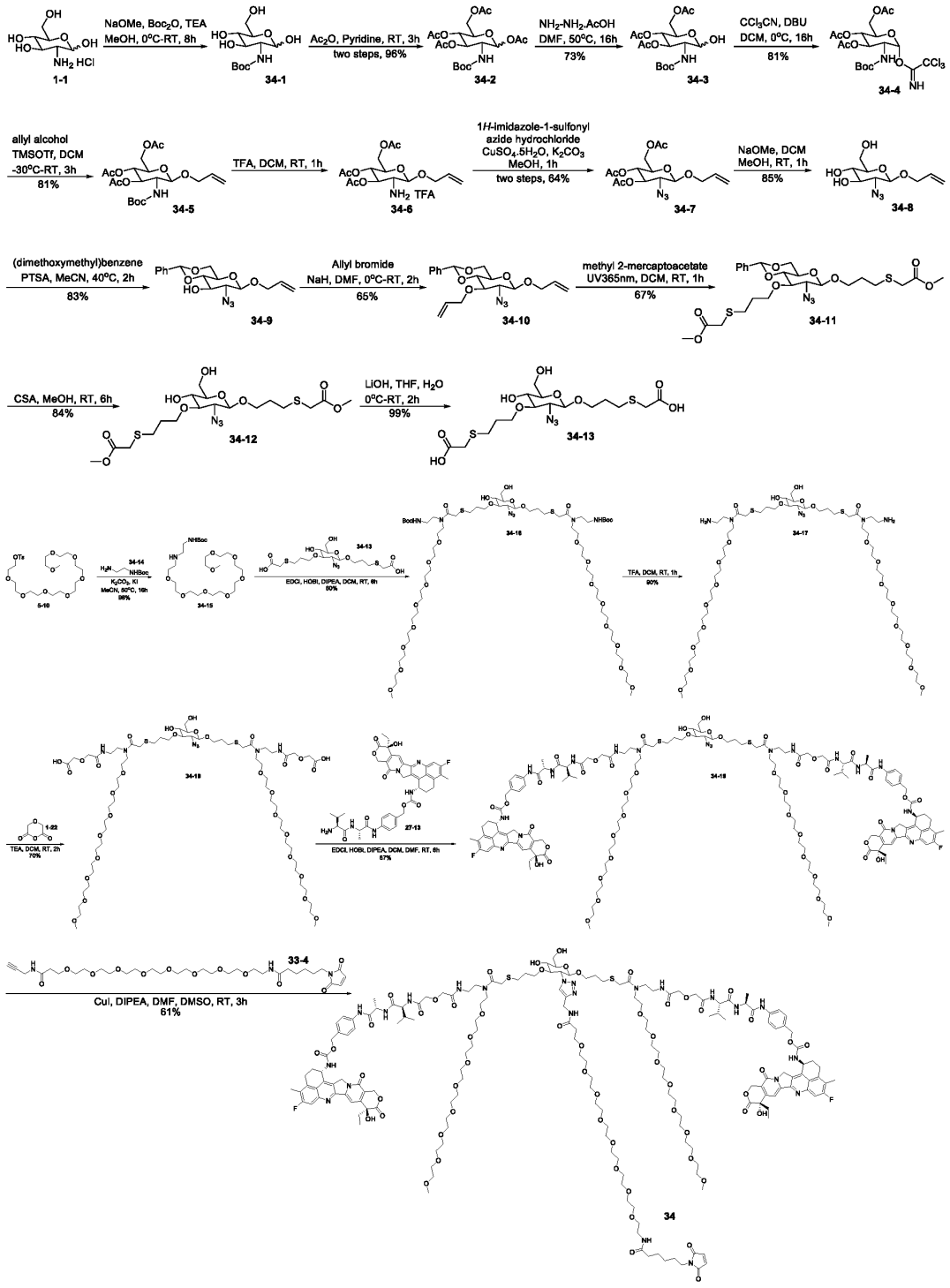
Example 34. Synthesis of ((((36S,36'S,39S,39'S)-26,26'-(2,2'-(((((2R,3R,4R,5S,6R)-3-(4-(36-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,31-dioxo-6,9,12,15,18,21,24,27-octaoxa-2,30-diazahexatriacontyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-

pyran-2,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(36-isopropyl-39-methyl-30,34,37-trioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35,38-tetraazatetracontan-40-oyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate) (34)



34

Synthetic scheme 34


NaOMe, Boc2O, TEA
MeOH, 0°C-RT, 8h
1-1
34-1
Ac2O, Pyridine, RT, 3h
two steps, 96%
34-2
NH2-NH2.AcOH
DMF, 50°C, 16h
73%
34-3
CCl3CN, DBU
DCM, 0°C, 16h
81%
34-4
allyl alcohol
TMSOTf, DCM
-30°C-RT, 3h
81%
34-5
TFA, DCM, RT, 1h
34-6
1H-imidazole-1-sulfonyl azide hydrochloride
CuSO4.5H2O, K2CO3
MeOH, 1h
two steps, 64%
34-7
NaOMe, DCM
MeOH, RT, 1h
85%
34-8
(dimethoxymethyl)benzene
PTSA, MeCN, 40°C, 2h
83%
34-9
Allyl bromide
NaH, DMF, 0°C-RT, 2h
65%
34-10
methyl 2-mercaptoacetate
UV365nm, DCM, RT, 1h
67%
34-11
CSA, MeOH, RT, 6h
84%
34-12
LiOH, THF, H2O
0°C-RT, 2h
99%
34-13
5-10
34-14
K2CO3, KI
MeCN, 50°C, 16h
86%
34-15
EDCI, HOBt, DIPEA, DCM, RT, 6h
80%
34-16
TFA, DCM, RT, 1h
90%
34-17
34-18
1-22
TEA, DCM, RT, 2h
70%
27-13
EDCI, HOBt, DIPEA, DCM, DMF, RT, 6h
67%
34-19
33-4
CuI, DIPEA, DMF, DMSO, RT, 3h
61%
34

[00634] Step 1: To a mixture of compound **1-1** (100 g, 0.46 mol) in methanol (1.3 L) was added sodium methoxide (27.32 g, 0.52 mol) and stirred at room temperature for 1 h. Then di-tert-butyl dicarbonate (120.47 g, 0.55 mol) and triethylamine (46.46 g, 0.46 mol) was added slowly at room temperature. The mixture was stirred for 16 h. After completion of the reaction, the mixture was filtered and washed cake with methanol and dichloromethane. The filtrate was concentrated under reduced pressure and purified by silica gel column (eluted with 14% methanol in dichloromethane) to afford the title compound **34-1** (155.00 g, crude) as a white solid. The crude product was directly used for the next step.

[00635] Step 2: To a mixture of compound **34-1** (100 g, 0.36 mol) in pyridine (800 mL) was added acetic anhydride (257.27 g, 2.52 mol) at 0°C and then stirred at room temperature for 12 h. After completion of the reaction, the mixture was concentrated under vacuum. The mixture was adjusted to pH=6 with dilute hydrochloric acid at 0°C and extracted with dichloromethane (800 mL x 3) and water (2.4 L). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 200% ethyl acetate in petroleum ether) to afford the title compound **34-2** (200 mg, yield 96% over 2 steps) as a colorless oil. The crude product was directly used for the next step. LCMS: m/z calculated for $C_{19}H_{29}NO_{11}$: 447.44; found: 470.21 $[M+H]^+$.

[00636] Step 3: To a mixture of compound **34-2** (100 g, 0.22 mol) in N,N-dimethylformamide (300 mL) was added hydrazinium acetate (20.26 g, 0.22 mol) at 0°C and then stirred at room temperature for 16 h. After completion of the reaction, the mixture was extracted with ethyl acetate (400 mL x 3) and water (1.5 L). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 100% ethyl acetate in petroleum ether) to afford the title compound **34-3** (66.14 g, 73%) as a white solid.

[00637] Step 4: To a mixture of **34-3** (5.0 g, 12.33 mmol) in dry dichloromethane (50 mL) was added 2,2,2-trichloroacetonitrile (8.90 g, 61.67 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-

ene (281.57 mg, 1.85 mmol) at 0°C under N_2 atmosphere. Then the mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was concentrated and purified by silica gel column (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **34-4** (5.5 g, yield 81%) as a white solid.

[00638] Step 5: To a mixture of **34-4** (5.50 g, 0.01 mol), allyl alcohol (2.00 g, 35.00 mmol) and 11.00 g 4Å in dry dichloromethane (150 mL) was added trimethylsilyl trifluoromethanesulfonate (1.10 g, 5.00 mmol) at -30 °C under N_2 atmosphere. Then the mixture was stirred at -30°C for 3 h. After completion of the reaction, the mixture was filtered. The filtrate was concentrated and purified by silica gel column (eluted with 33% ethyl acetate in petroleum ether) to afford the title compound **34-5** (3.60 g, yield 81%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ5.92 (dd, J = 17.5, 10.8 Hz, 1H), 5.34 (d, J = 17.2 Hz, 2H), 5.25 (d, J = 11.0 Hz, 1H), 5.08 (t, J = 9.7 Hz, 1H), 4.70 (d, J = 32.0 Hz, 2H), 4.40 (dd, J = 12.9, 5.0 Hz, 1H), 4.31 (dd, J = 11.4, 5.4 Hz, 1H), 4.15 (t, J = 13.3 Hz, 2H), 3.71 (d, J = 10.4 Hz, 1H), 3.56 (q, J = 10.3, 8.8 Hz, 1H), 2.13 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.47 (s, 9H).

[00639] Step 6: To a solution of **34-5** (3.40 g, 7.63 mmol) in dichloromethane (34 mL) was added trifluoroacetic acid (10.2 mL) at room temperature. Then the mixture was stirred for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure to afford the title compound **34-6** (4.80 g, crude) as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.93 (d, J = 6.5 Hz, 1H), 5.44–5.28 (m, 3H), 5.14 (t, J = 9.4 Hz, 1H), 4.91 (d, J = 8.2 Hz, 1H), 4.44 (dd, J = 12.4, 5.4 Hz, 1H), 4.35 (dd, J = 12.5, 4.4 Hz, 1H), 4.19 (dd, J = 12.8, 7.5 Hz, 2H), 3.83 (dd, J = 10.0, 4.0 Hz, 1H), 3.39 (t, J = 9.3 Hz, 1H), 2.16 (s, 3H), 2.09 (s, 6H).

[00640] Step 7: To a mixture of **34-6** (4.80 g, 7.63 mmol), potassium carbonate (4.21g, 30.52 mmol) and Copper sulfate pentahydrate (19.08 mg, 0.08 mmol) in methanol (34 mL) was added 1H-imidazole-1-sulfonyl azide hydrochloride (1.92 g, 9.16 mmol) at room temperature. Then the mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered, the filtrate was concentrated under reduced pressure and purified by silica gel

column (eluted with 33% ethyl acetate in petroleum ether) to afford the title compound **34-7** (1.84 g, two steps, yield 64%) as a white solid.

[00641] Step 8: To a solution of **34-7** (1.84 g, 49.55 mmol) and methanol (9 mL) in dichloromethane (9 mL) was added sodium methoxide (53.46 mg, 0.99 mmol) at 0 °C. Then the mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure and purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **34-8** (1.03 g, yield 85%) as a white solid. LCMS: m/z calculated for $C_9H_{15}N_3O_5$: 245.24; found: 308.70 $[M+H]^+$. ^1H NMR (400 MHz, Chloroform-*d*) δ 5.99 (ddt, J = 16.8, 11.0, 5.6 Hz, 1H), 5.39 (d, J = 17.3 Hz, 1H), 5.28 (d, J = 10.4 Hz, 1H), 4.45 (d, J = 7.2 Hz, 2H), 4.20 (dd, J = 12.7, 5.4 Hz, 1H), 3.92 (s, 2H), 3.67 (t, J = 9.1 Hz, 1H), 3.43 (dd, J = 14.2, 8.4 Hz, 2H), 3.35 (d, J = 9.2 Hz, 1H).

[00642] Step 9: To a solution of **34-8** (741 mg, 3.02 mmol) and p-toluenesulfonic acid (52.00 mg, 0.30 mmol) in acetonitrile (8 mL) was added (dimethoxymethyl)benzene (1.38 g, 9.06 mmol) at room temperature. Then the mixture was stirred at 40 °C for 2 h under N_2 atmosphere. After completion of the reaction, the mixture was quenched with triethylamine (30.30 mg, 0.30 mmol), concentrated under reduced pressure and purified by silica gel column (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **34-9** (830.00 mg, yield 83%) as a white solid. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.50 (dd, J = 6.6, 3.1 Hz, 2H), 7.40 (dd, J = 5.0, 1.9 Hz, 3H), 5.98 (dddd, J = 16.9, 10.5, 6.2, 5.3 Hz, 1H), 5.57 (s, 1H), 5.39 (dd, J = 17.2, 1.5 Hz, 1H), 5.29 (dd, J = 10.4, 1.3 Hz, 1H), 4.51 (d, J = 8.0 Hz, 1H), 4.44 (ddt, J = 12.7, 5.2, 1.5 Hz, 1H), 4.37 (dd, J = 10.5, 5.0 Hz, 1H), 4.20 (ddt, J = 12.7, 6.1, 1.4 Hz, 1H), 3.82 (t, J = 10.3 Hz, 1H), 3.70 (t, J = 9.4 Hz, 1H), 3.59 (t, J = 9.3 Hz, 1H), 3.49 – 3.41 (m, 2H).

[00643] Step 10: To a solution of **34-9** (830.00 mg, 2.49 mmol) in N,N-dimethylformamide (8 mL) was added sodium hydride (60% in mineral oil) (199.20 mg, 4.98 mmol) at 0°C under N_2 atmosphere and stirred for 1 h. Then **38-15** (602.48 mg, 4.98 mmol) was added to the above mixture and stirred at 0°C for 1 h under N_2 atmosphere. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (10 mL) at 0 °C. The mixture was

extracted with ethyl acetate (20 mL x 3) and water (70 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 10% ethyl acetate in petroleum ether) to afford the title compound **34-10** (605.00 mg, yield 65%) as a white solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.49 (dd, J = 7.5, 2.0 Hz, 2H), 7.43 – 7.37 (m, 3H), 6.02 – 5.93 (m, 2H), 5.58 (s, 1H), 5.38 (dd, J = 17.2, 1.5 Hz, 1H), 5.31 (d, J = 1.6 Hz, 1H), 5.28 (dd, J = 10.4, 1.3 Hz, 1H), 5.22 (dd, J = 10.4, 1.4 Hz, 1H), 4.41 (dd, J = 14.0, 7.3 Hz, 3H), 4.36 (dd, J = 10.5, 5.0 Hz, 1H), 4.27 (dd, J = 12.5, 6.0 Hz, 1H), 4.19 (dd, J = 12.8, 6.2 Hz, 1H), 3.81 (t, J = 10.3 Hz, 1H), 3.67 (t, J = 9.1 Hz, 1H), 3.53 – 3.44 (m, 2H), 3.40 (td, J = 9.8, 5.0 Hz, 1H).

[00644] Step 11: To a solution of **34-10** (3.00 g, 8.03 mmol) in dichloromethane (30 mL) was added methyl 2-mercaptoacetate (8.52 g, 80.30 mmol) and stirred at room temperature for 2 h under UV@365nm. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 10%~33% ethyl acetate in petroleum ether) to afford the title compound **34-11** (1.6 g, yield 67%) as a light-yellow oil.

[00645] Step 12: To a solution of **34-11** (2.20 g, 3.76 mmol) in methanol (50 mL) was added (1S)-bicyclo[2.2.1]heptane-1-methane sulfonic acid (1.75 g, 7.52 mmol) at room temperature and stirred for 1 h. After completion of the reaction, the mixture was adjusted to pH = 7 with saturated sodium bicarbonate at 0 °C and concentrated. The mixture was extracted with dichloromethane (80 mL x 3) and water (240 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **34-12** (1.58 g, yield 84%) as a yellow oil.

[00646] Step 13: To a solution of **34-12** (1.57 g, 3.16 mmol) in tetrahydrofuran (10 mL) and H_2O (5 mL) was added lithium hydroxide (378.63 mg, 15.78 mmol) and stirred at room temperature for 4 h. After completion of the reaction, the mixture was adjusted to pH=6 with 1 N hydrochloric acid at 0 °C and concentrated under reduced pressure. The residue was purified by

reverse flash chromatography (C18 silica gel column, 63.6% acetonitrile in water, HCl condition) to afford the title compound **34-13** (1.40 g, yield 99%) as a colorless oil. LCMS: m/z calculated for $C_{16}H_{27}N_3O_9S_2$: 469.52; found: 492.09 $[M+Na]^+$. ^1H NMR (400 MHz, DMSO-*d6*) δ 4.32 (d, J = 8.1 Hz, 1H), 3.82 (dt, J = 6.4, 3.2 Hz, 2H), 3.65 (d, J = 1.7 Hz, 2H), 3.62 (d, J = 2.0 Hz, 3H), 3.14 (s, 5H), 3.11 (s, 4H), 2.68 – 2.58 (m, 4H), 1.82–1.69 (m, 4H).

[00647] Step 14: To a solution of compound **34-14** (4.48 g, 27.96 mmol), potassium carbonate (3.85 g, 27.86 mmol) and potassium iodide (3.08 g, 18.56 mmol) in acetonitrile (100 mL) was added compound **5-10** (10.00 g, 18.56 mmol). The reaction mixture was stirred at 50 °C under nitrogen atmosphere for 16 h. After completion of the reaction, the mixture was filtered and concentrated. The residue was purified by silica gel column chromatography (eluted with 2% methanol in dichloromethane) to afford the title compound **34-15** (9.38 g, yield 96%) as a pale-yellow oil. LCMS: m/z calculated for $C_{24}H_{50}N_2O_{10}$: 526.67; found: 528.16 $[M+H]^+$.

[00648] Step 15: The title compound **34-16** was prepared in analogy to **Example 2, Step 5** by using compound **34-15** and compound **35-13**. The product (1.34 g, yield 60%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{64}H_{123}N_7O_{27}S_2$: 1486.83; found: 644.11 $[M-2Boc+2H]^{2+}$.

[00649] Step 16: The title compound **34-17** was prepared in analogy to **Example 27, Step 23** by using compound **34-16**. The product (2.5 g, yield 90%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{54}H_{107}N_7O_{23}S_2$: 1286.60; found: 644.15 $[M+2H]^{2+}$.

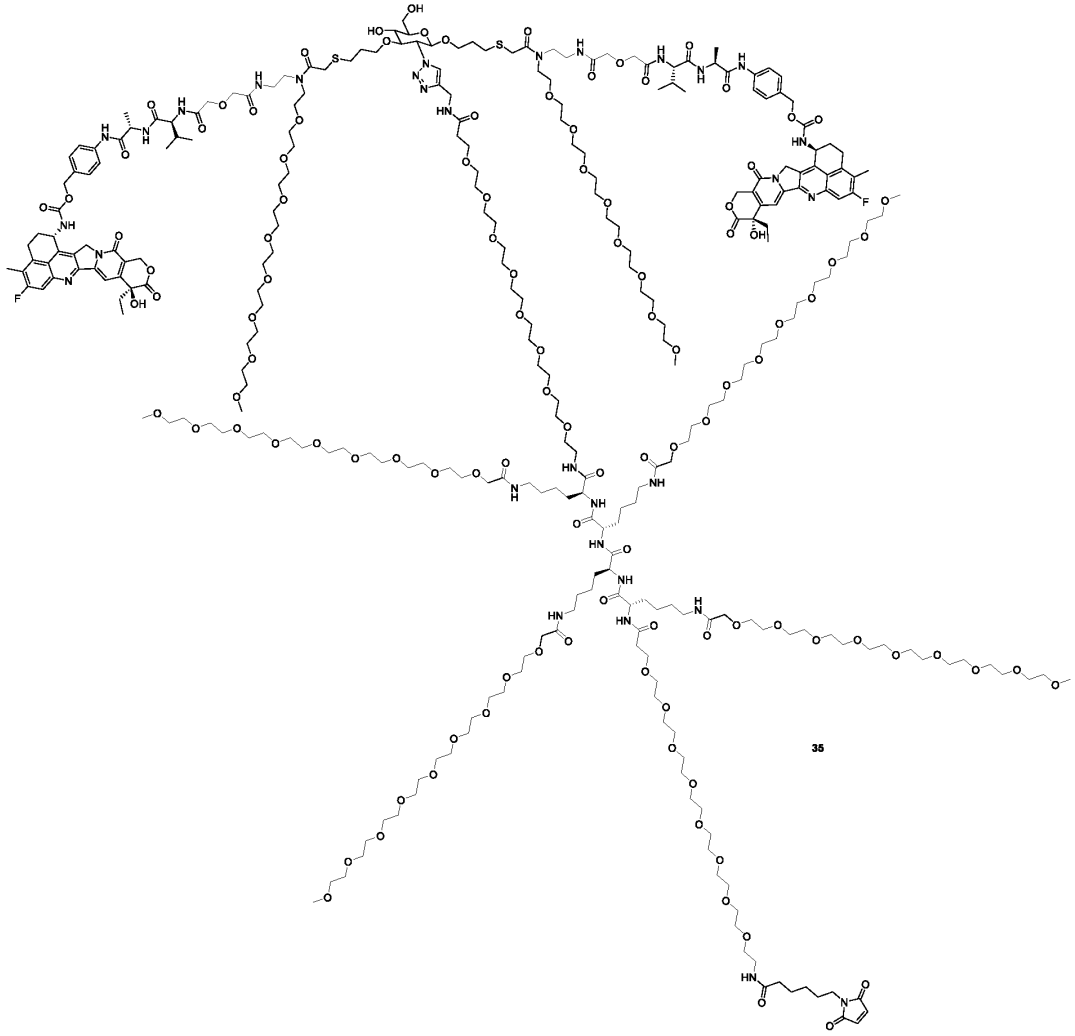
[00650] Step 17: The title compound **34-18** was prepared in analogy to **Example 2, Step 7** by using compound **34-17** and compound **1-22**. The product (390 mg, yield 70%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{62}H_{115}N_7O_{31}S_2$: 1518.74; found: 760.18 $[M+2H]^{2+}$.

[00651] Step 18: The title compound **34-19** was prepared in analogy to **Example 2, Step 5** by using compound **34-18** and compound **27-13**. The product (424 mg, yield 67%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{142}H_{197}F_2N_{19}O_{45}S_2$: 2992.34; found: 998.33 $[M+3H]^{3+}$.

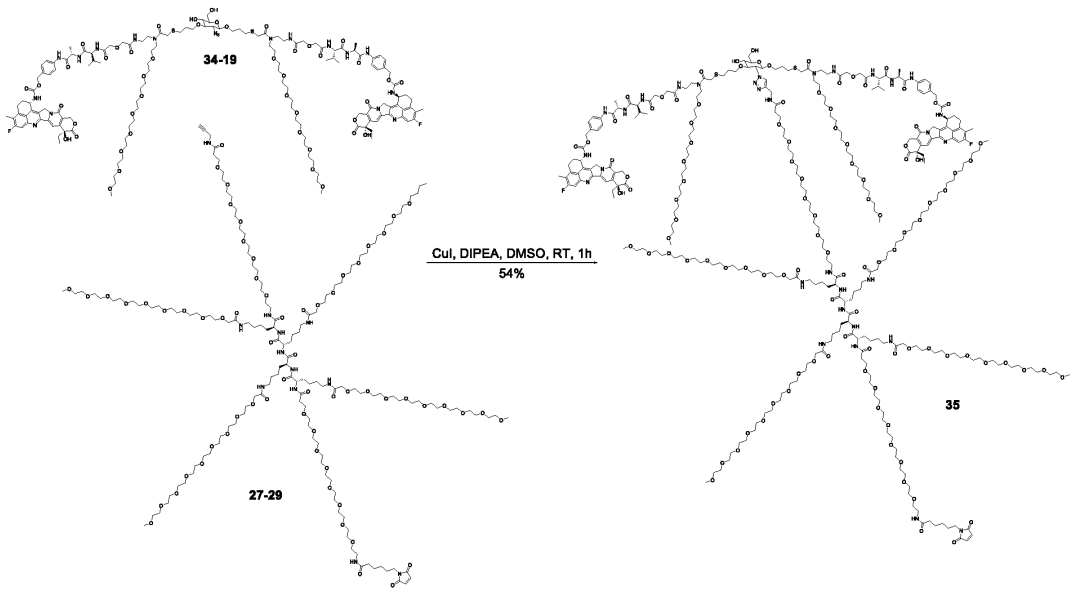
[00652] Step 19: The title compound **34** was prepared in analogy to **Example 10, Step 14** by using compound **34-19** and compound **33-4**. The product (38 mg, yield 61%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{174}H_{250}F_2N_{22}O_{57}S_2$: 3664.13; found: 1222.11 $[M+3H]^{3+}$.

Example 35. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[[2-[2-[2-[[2-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (35)

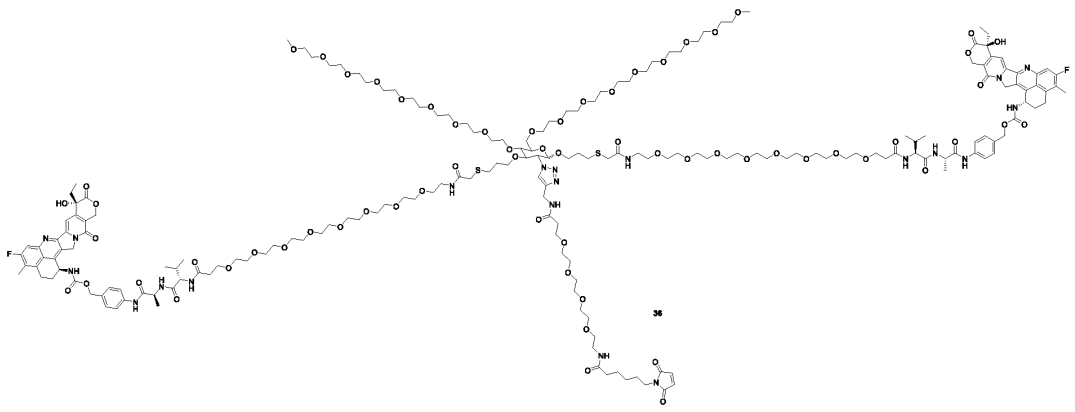


Synthetic scheme 35

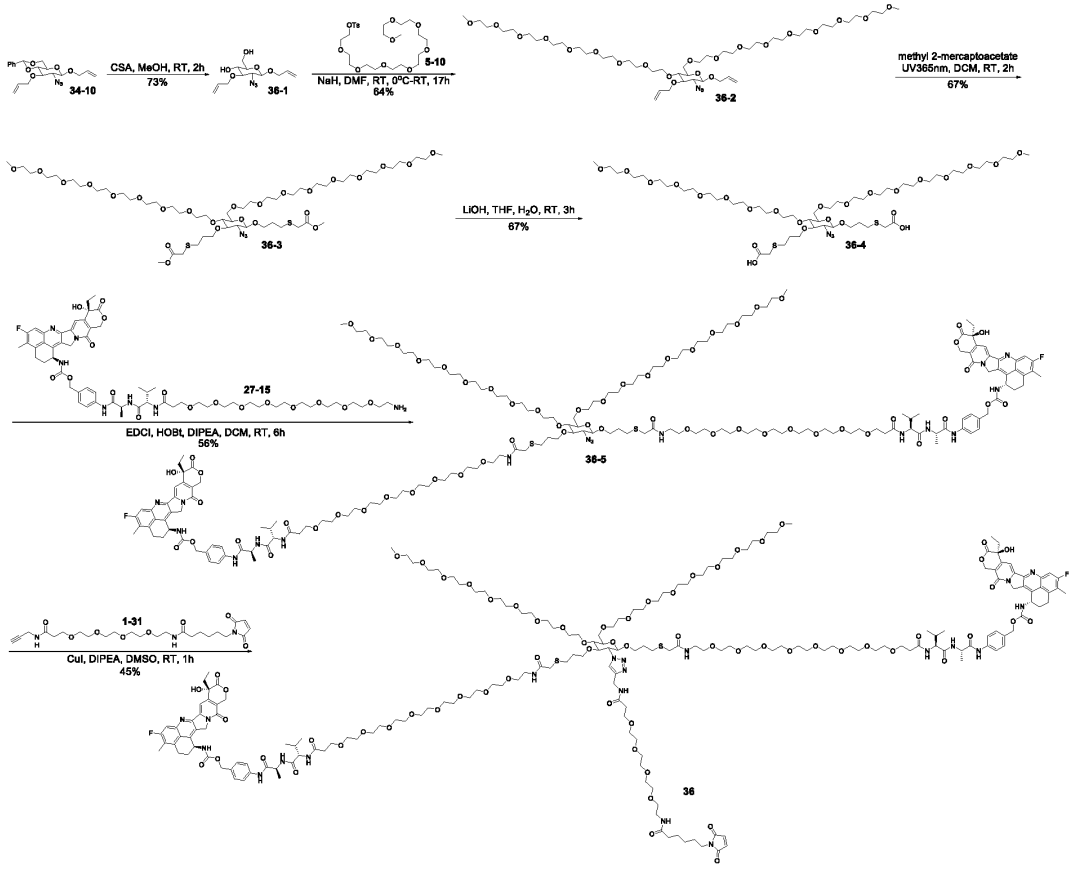


[00653] Step 1: The title compound **35** was prepared in analogy to **Example 10, Step 14** by using compound **34-19** and compound **27-29**. The product (57 mg, yield 54%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{293}H_{479}F_2N_{31}O_{110}S_2$: 6298.28; found: 1260.36 $[M+5H]^{5+}$.

Example 36. Synthesis of ((((2S,2'S,5S,5'S)-40,40'-(((2R,3R,4R,5S,6R)-5-((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-6-(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)tetrahydro-2H-pyran-2,4-diyl)bis(oxy))bis(5-isopropyl-2-methyl-4,7,35-trioxo-10',13',16',19',22',25',28',31'-octaoxa-37'-thia-3',6',34'-triazatetracontanoyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate) (36)



Synthetic scheme 36



[00654] Step 1: To a solution of **34-10** (7.80 g, 20.89 mmol) in methanol (80 mL) was added (1S)-bicyclo[2.2.1]heptane-1-methane sulfonic acid (4.85 g, 20.89 mmol) at room temperature

and stirred for 2 h. After completion of the reaction, the mixture was adjusted to pH = 7 with saturated sodium bicarbonate at 0°C and concentrated. The mixture was extracted with ethyl acetate (80 mL x 3) and water (240 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **36-1** (5.38 g, yield 73%) as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.00 (ddq, J = 16.6, 11.5, 6.0 Hz, 2H), 5.38 (dd, J = 17.2, 9.4 Hz, 2H), 5.28 (dd, J = 10.2, 4.4 Hz, 2H), 4.51–4.39 (m, 3H), 4.27 (dd, J = 12.6, 6.2 Hz, 1H), 4.21 (dd, J = 12.8, 6.0 Hz, 1H), 3.95 (dd, J = 11.8, 3.2 Hz, 1H), 3.85 (dd, J = 11.8, 4.7 Hz, 1H), 3.64 (t, J = 9.3 Hz, 1H), 3.43–3.34 (m, 2H), 3.21 (t, J = 9.4 Hz, 1H).

[00655] Step 2: To a solution of **36-1** (1.10 g, 3.86 mmol) in N,N-dimethylformamide (40 mL) was added sodium hydride (60% in mineral oil) (463.20 mg, 8.11 mmol) at 0°C under N_2 atmosphere and stirred for 1 h. Then **5-10** (4.37 g, 4.98 mmol) was added to the above mixture and stirred at 0°C for 1 h under N_2 atmosphere. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (50 mL) at 0°C. The mixture was extracted with ethyl acetate (40 mL x 3) and water (250 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **36-2**(2.50 g, yield 64%) as a yellow oil. LCMS: m/z calculated for $C_{46}H_{87}N_3O_{21}$: 1018.20; found: 1035.67 $[M+H_2O+H]^+$.

[00656] Step 3: To a solution of **36-2** (2.00 g, 1.96 mmol) in dichloromethane (12 mL) was added methyl 2-mercaptoacetate (1.04 g, 9.82 mmol) and stirred at room temperature for 2 h under UV@365nm. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **36-3** (1.6 g, yield 67%) as a light-yellow oil. LCMS: m/z calculated for $C_{52}H_{99}N_3O_{25}S_2$: 1230.48; found: 1247.69 $[M+H_2O+H]^+$.

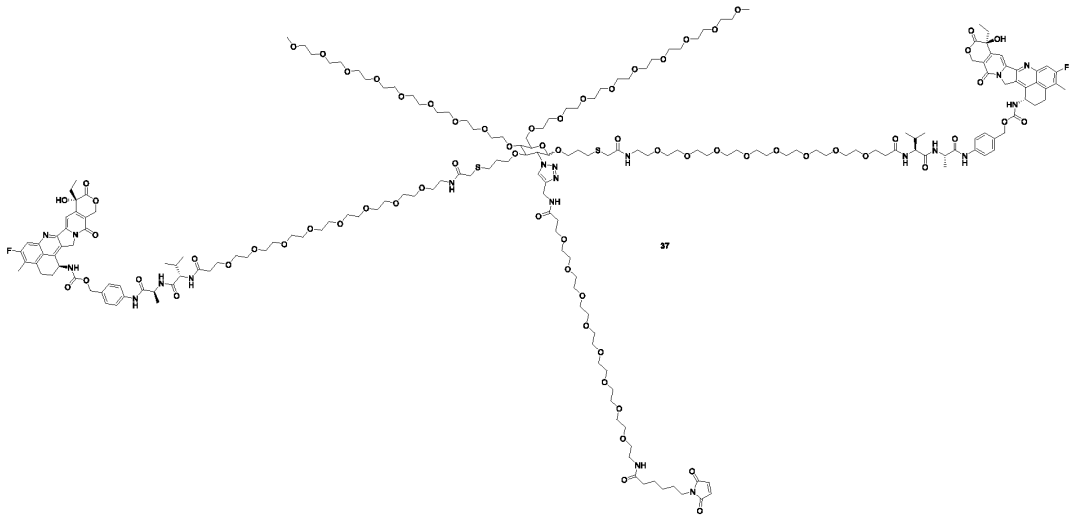
[00657] Step 4: To a solution of **36-3**(2.00 g, 1.30 mmol) in tetrahydrofuran (16 mL) and H_2O (16 mL) was added sodium hydroxide (156.00 mg, 3.90 mmol) and stirred at room temperature for 1 h. After completion of the reaction, the mixture was adjusted to pH=6 with dilute hydrochloric acid at 0°C and concentrated under reduced pressure. The residue was purified by reverse flash chromatography (C18 silica gel column, 64% acetonitrile in water, HCl condition) to afford the title compound **36-4** (1.05 g, yield 67%) as a light brown oil. LCMS: m/z calculated for $C_{52}H_{99}N_3O_{25}S_2$: 1230.48; found: 1247.69 $[M+H_2O+H]^+$. ^1H NMR (500 MHz, Chloroform-*d*) δ 4.23 (d, J = 7.9 Hz, 1H), 3.96 (ddd, J = 22.8, 10.1, 5.1 Hz, 2H), 3.91–3.85 (m, 2H), 3.77 (d, J = 10.9 Hz, 2H), 3.72 (dd, J = 16.1, 5.6 Hz, 4H), 3.69–3.64 (m, 56H), 3.61 (t, J = 4.8 Hz, 2H), 3.58 (dd, J = 5.7, 3.6 Hz, 4H), 3.40 (s, 6H), 3.35 (d, J = 4.9 Hz, 2H), 3.25 (d, J = 2.5 Hz, 4H), 2.81 (qd, J = 7.2, 2.7 Hz, 4H), 2.00–1.88 (m, 4H).

[00658] Step 5: To a solution of **36-4** (320.00 mg, 0.27 mmol), **27-15** (784.05 mg, 0.67 mmol) and N,N-diisopropylethylamine (417.96 mg, 3.24 mmol) in dichloromethane (14 mL) was added 1-hydroxybenzotriazole (145.80 mg, 1.08 mmol) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (310.55 mg, 1.62 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (C18 silica gel column, 54% acetonitrile in water, TFA condition) to afford the title compound **36-5** (736.00 mg, yield 79%) as a light brown solid. LCMS: m/z calculated for $C_{168}H_{251}F_2N_{17}O_{57}S_2$: 3523.03; found: 1175.44 $[M+3H]^+$.

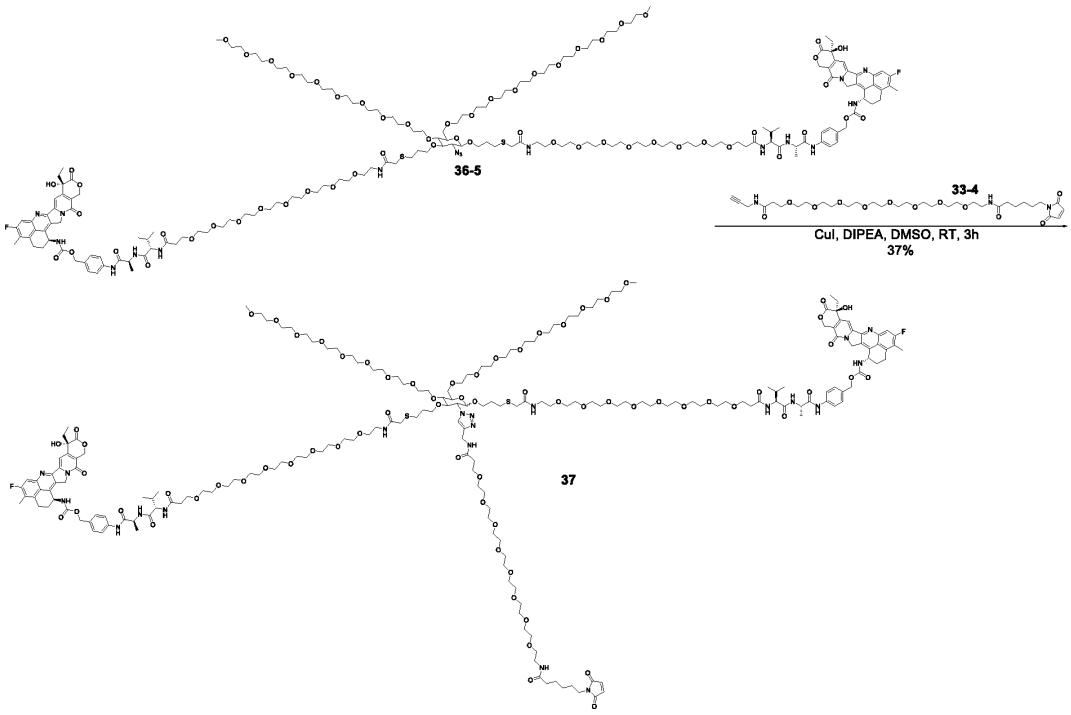
[00659] Step 6: The title compound **36** was prepared in analogy to **Example 10, Step 14** by using compound **36-5** and compound **1-31**. The product (16 mg, yield 45%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{192}H_{288}F_2N_{20}O_{65}S_2$: 4018.61; found: 1005.45 $[M+4H]^{4+}$.

Example 37. Synthesis of ((((2S,2'S,5S,5'S)-40,40'-(((3R,4R,5S,6R)-5-((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-3-(4-(36-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,31-dioxo-6,9,12,15,18,21,24,27-octaoxa-2,30-diazahexatriacontyl)-1H-1,2,3-triazol-1-yl)-6-

(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)tetrahydro-2H-pyran-2,4-diyl)bis(oxy))bis(5-isopropyl-2-methyl-4,7,35-trioxo-10',13',16',19',22',25',28',31'-octaoxa-37'-thia-3',6',34'-triazatetracontanoyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate) (37)



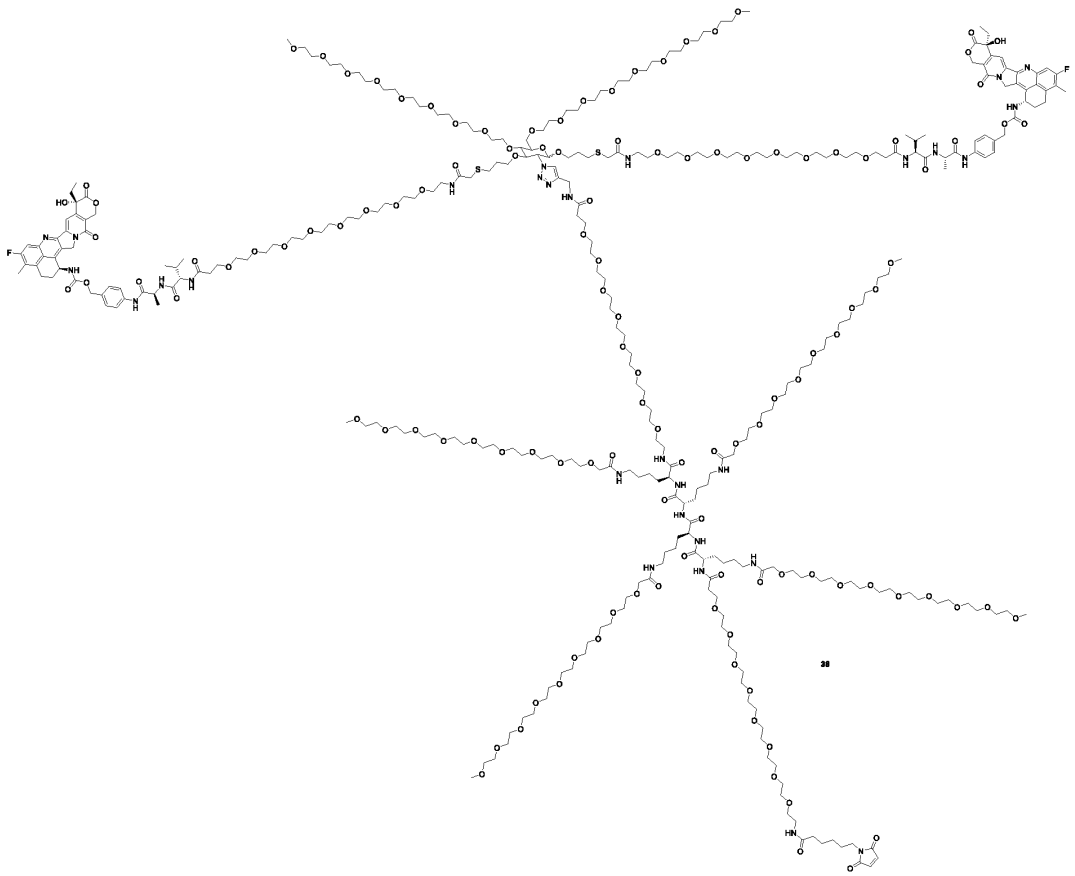
Synthetic scheme 37



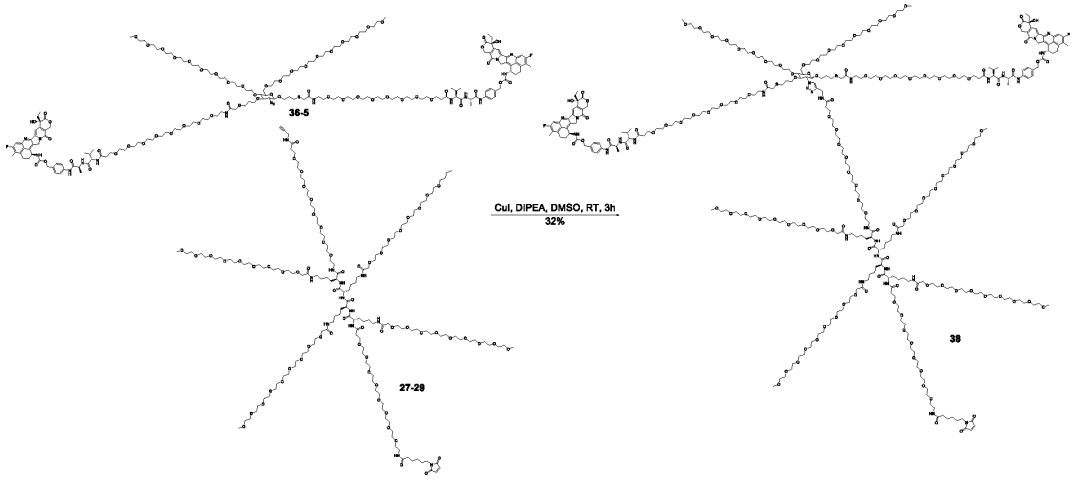
[00660] Step 1: The title compound **37** was prepared in analogy to **Example 10, Step 14** by using compound **36-5** and compound **33-4**. The product (18 mg, yield 37%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{200}H_{304}F_2N_{20}O_{69}S_2$: 4194.82; found: 1049.55 $[M+4H]^{4+}$.

Example 38. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]

amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-5-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]-6-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (38)

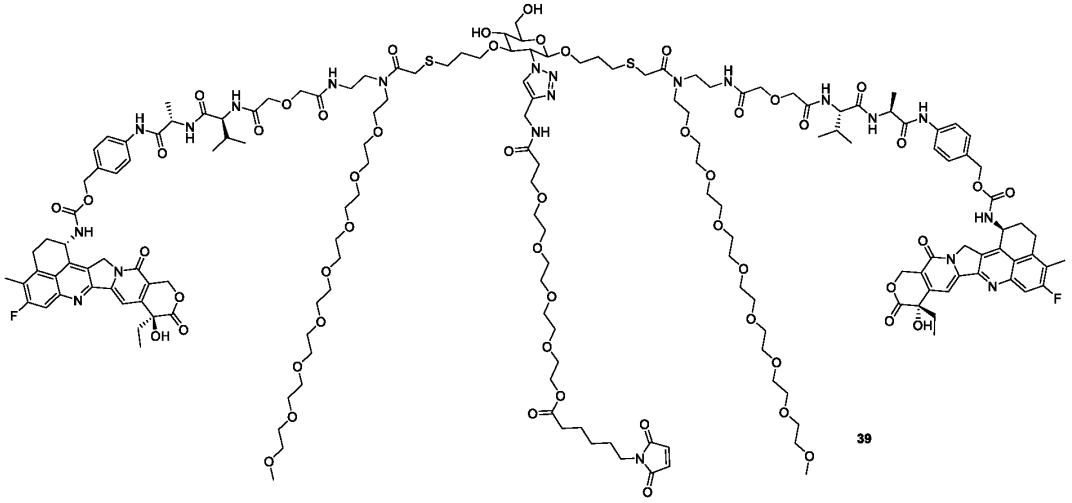


Synthetic scheme 38

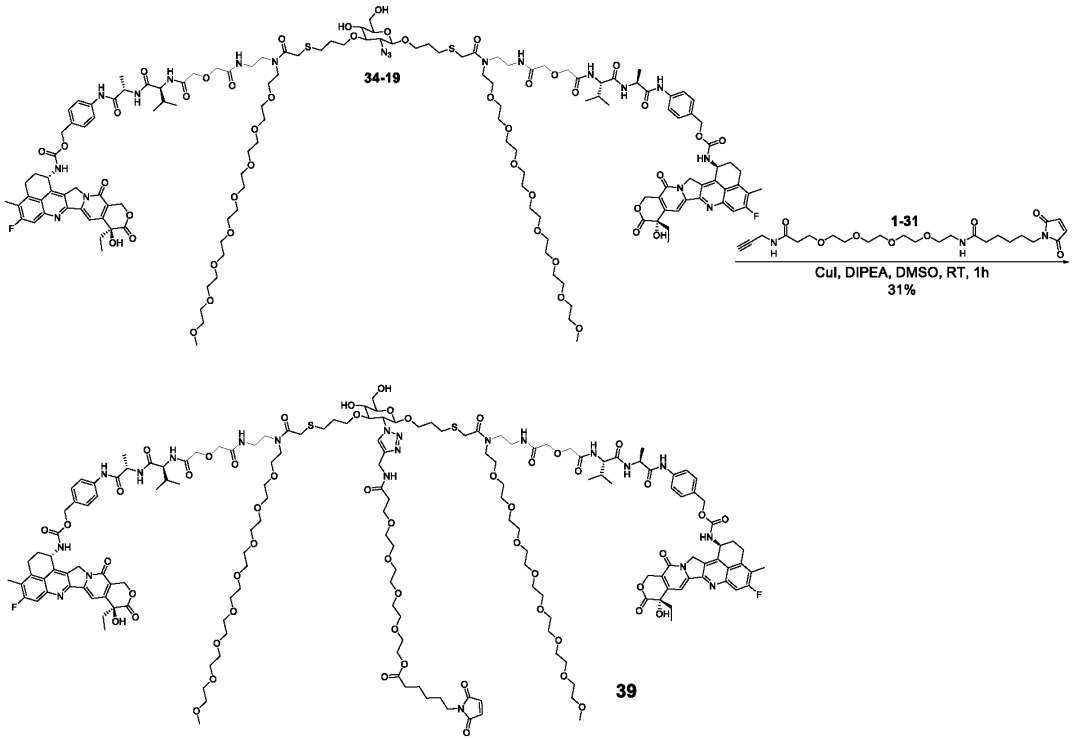


[00661] Step 1: The title compound **38** was prepared in analogy to **Example 10, Step 14** by using compound **36-5** and compound **27-29**. The product (16 mg, yield 32%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{319}H_{533}F_2N_{29}O_{122}S_2$: 6828.97; found: 1139.03 $[M+6H]^{6+}$.

Example 39. Synthesis of 1-(1-((2R,3R,4R,5S,6R)-2,4-bis((26-((2S,5S)-1-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-5-isopropyl-2-methyl-1,4,7,11-tetraoxo-9-oxa-3,6,12-triazatetradecan-14-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-1H-1,2,3-triazol-4-yl)-3-oxo-6,9,12,15-tetraoxa-2-azaheptadecan-17-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (39)



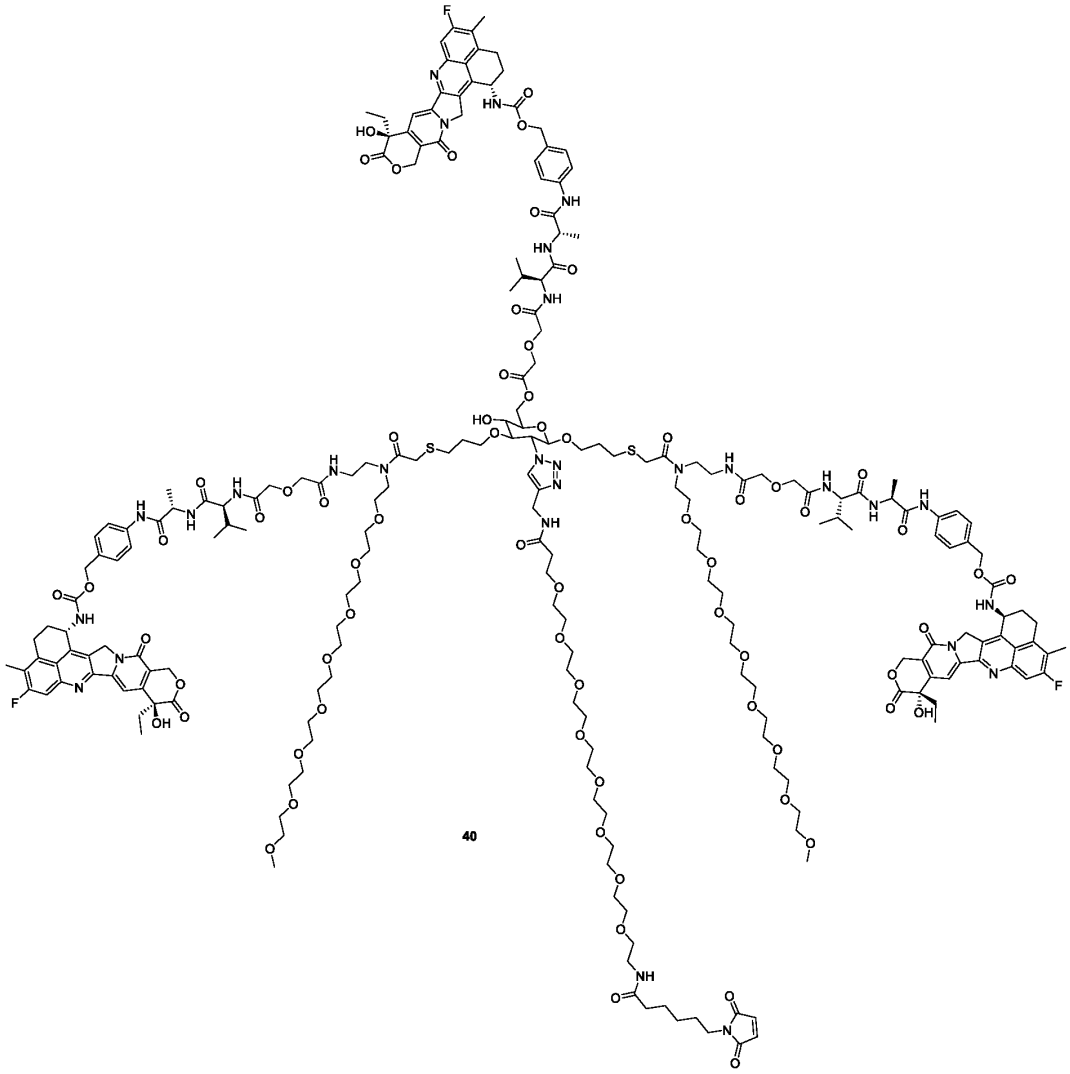
Synthetic scheme 39



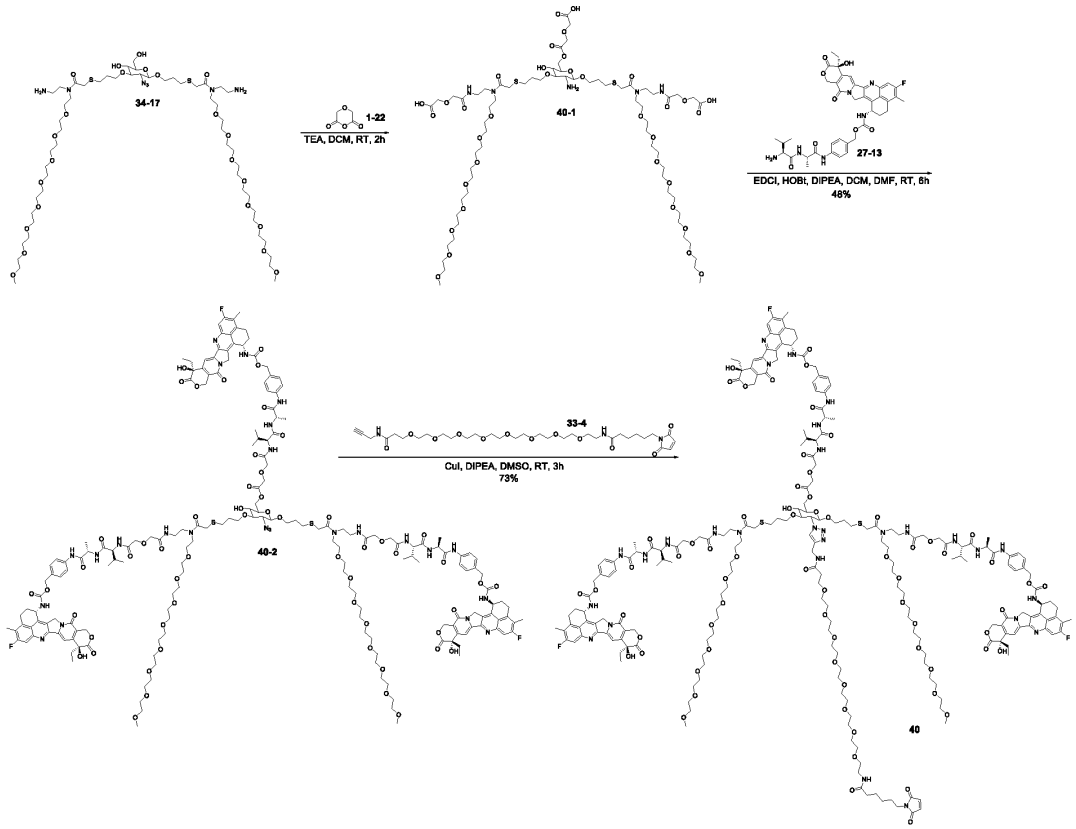
[00662] Step 1: The title compound **39** was prepared in analogy to **Example 10, Step 14** by using compound **34-19** and compound **1-31**. The product (12 mg, yield 31%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{166}H_{233}F_2N_{21}O_{54}S_2$: 3488.90; found: 1163.53 $[M+3H]^{3+}$.

Example 40. Synthesis of ((2R,3S,4R,5R,6R)-5-(4-(36-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,31-dioxo-6,9,12,15,18,21,24,27-octaoxa-2,30-diazahexatriacontyl)-1H-1,2,3-triazol-1-yl)-4,6-bis((26-((2S,5S)-1-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-5-isopropyl-2-methyl-1,4,7,11-tetraoxo-9-oxa-3,6,12-triazatetradecan-14-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-3-hydroxytetrahydro-2H-pyran-2-yl)methyl 2-(2-(((S)-1-(((S)-1-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-

yl)carbamoyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)acetate (40)



Synthetic scheme 40



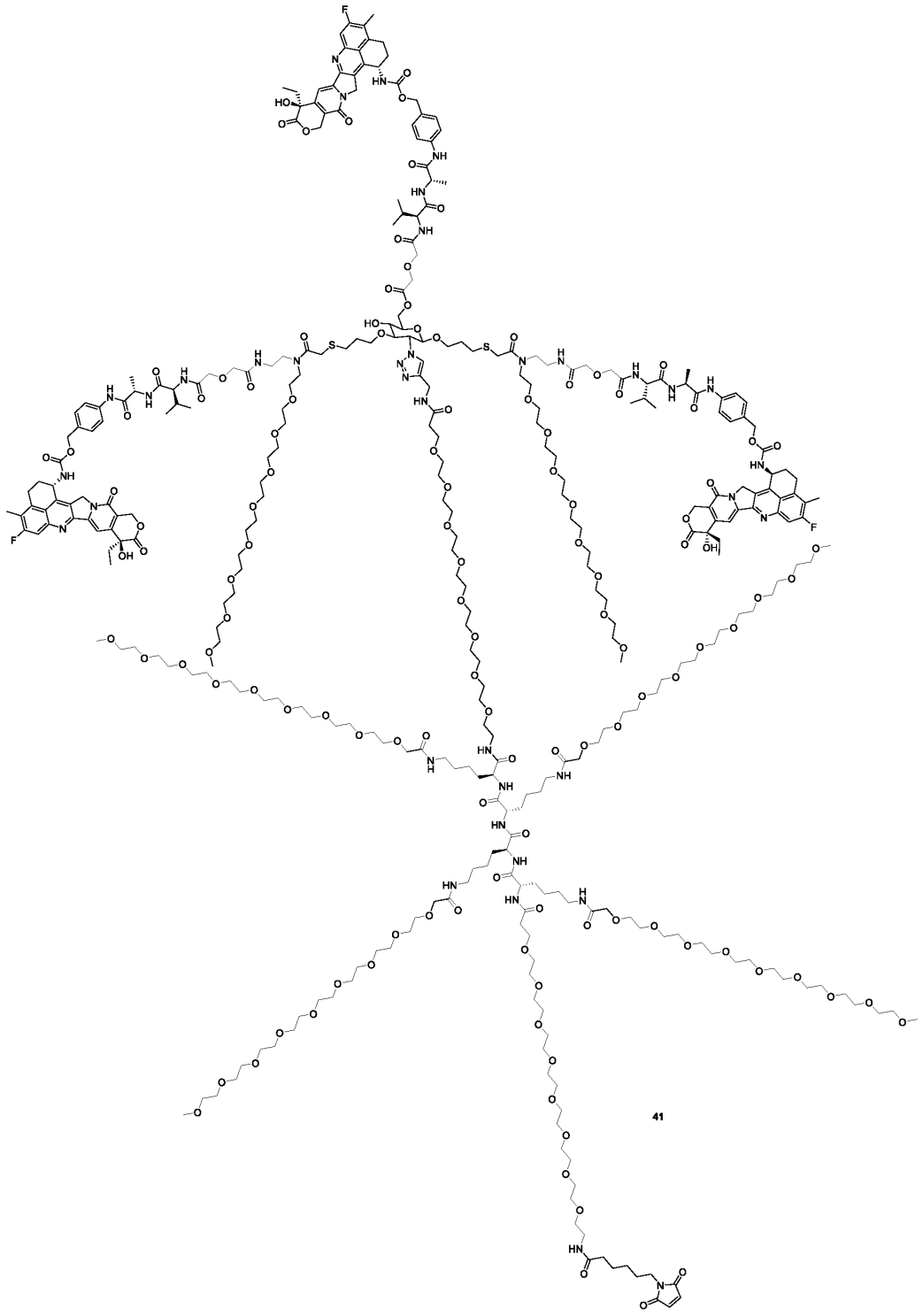
[00663] Step 1: The title compound **40-1** was prepared in analogy to **Example 2, Step 7** by using compound **34-17** and compound **1-22** (4 eq). The product (280 mg, yield 86%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{66}H_{121}N_5O_{35}S_2$: 1608.81; found: 817.47 $[M+2H]^{2+}$.

[00664] Step 2: The title compound **40-2** was prepared in analogy to **Example 2, Step 5** by using compound **40-1** and compound **27-13**. The product (320 mg, yield 48%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{186}H_{242}F_3N_{25}O_{56}S_2$: 3845.22; found: 1282.82 $[M+3H]^{3+}$.

[00665] Step 3: The title compound **40** was prepared in analogy to **Example 10, Step 14** by using compound **40-2** and compound **33-4**. The product (30 mg, yield 73%) was obtained as a

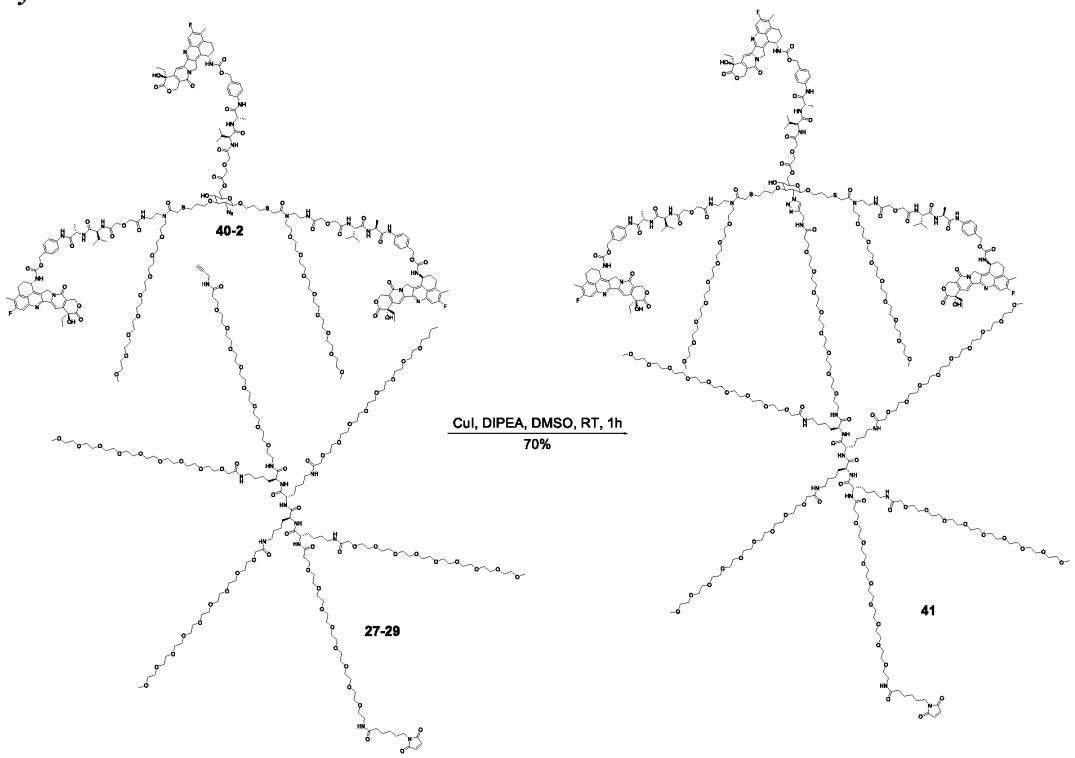
yellow solid. LCMS: m/z calculated for $C_{218}H_{295}F_3N_{28}O_{68}S_2$: 4517.00; found: 1506.41 $[M+3H]^{3+}$.

Example 41. Synthesis of [5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,6-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-tetrahydropyran-2-yl]methyl 2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetate (41)



41

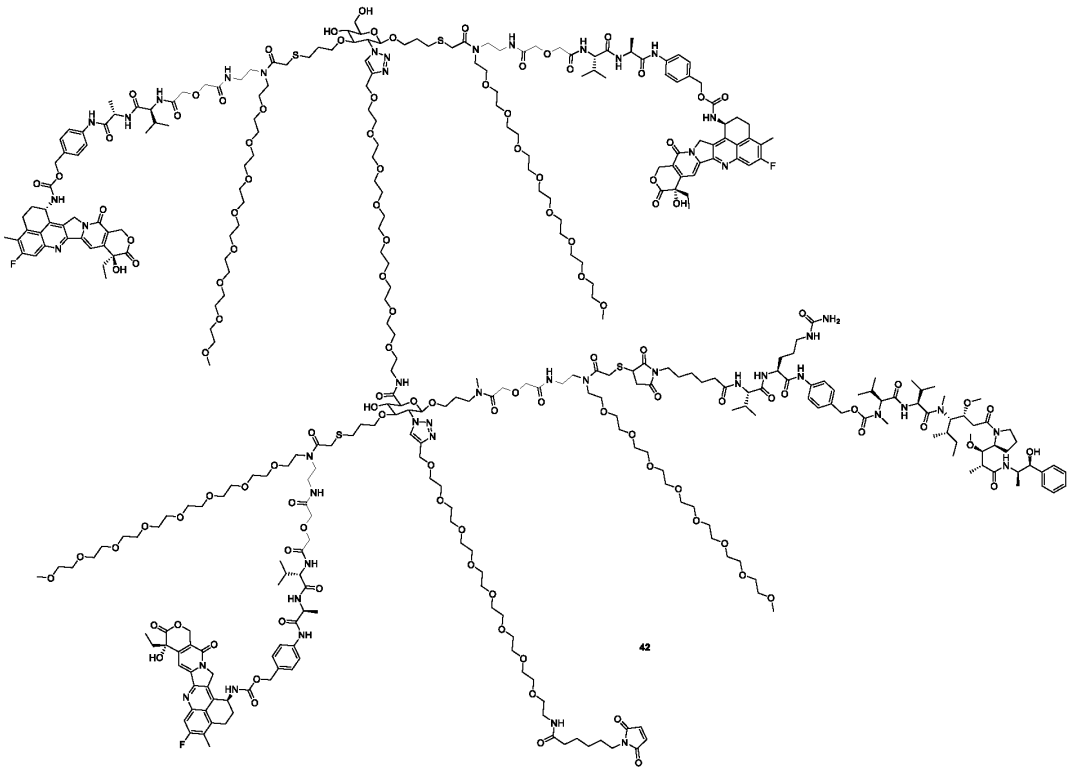
Synthetic scheme 41



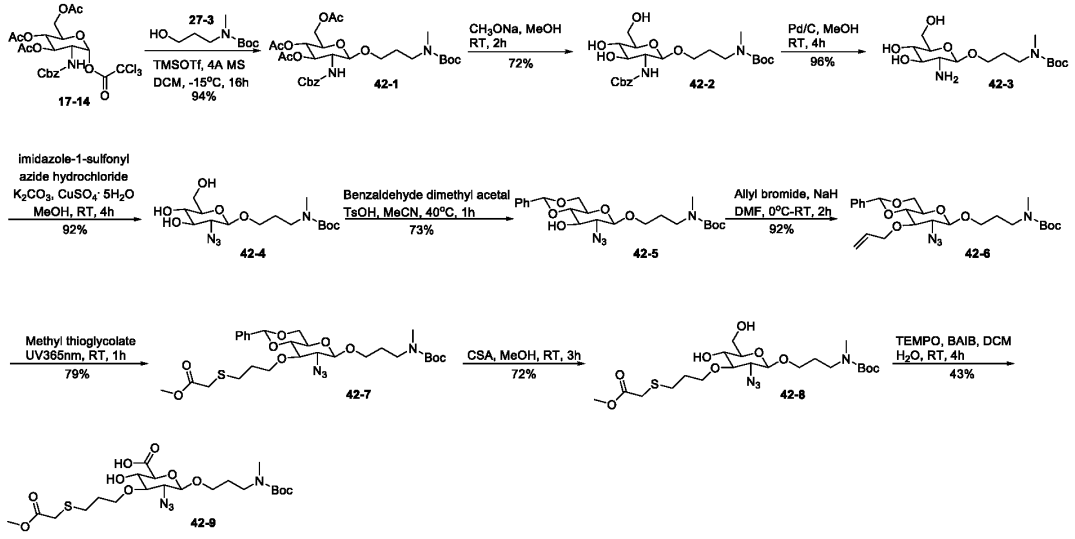
[00666] Step 1: The title compound **41** was prepared in analogy to **Example 10, Step 14** by using compound **40-2** and compound **27-29**. The product (29 mg, yield 70%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{337}H_{524}F_3N_{37}O_{121}S_2$: 7151.15; found: 1192.73 $[M+6H]^{6+}$.

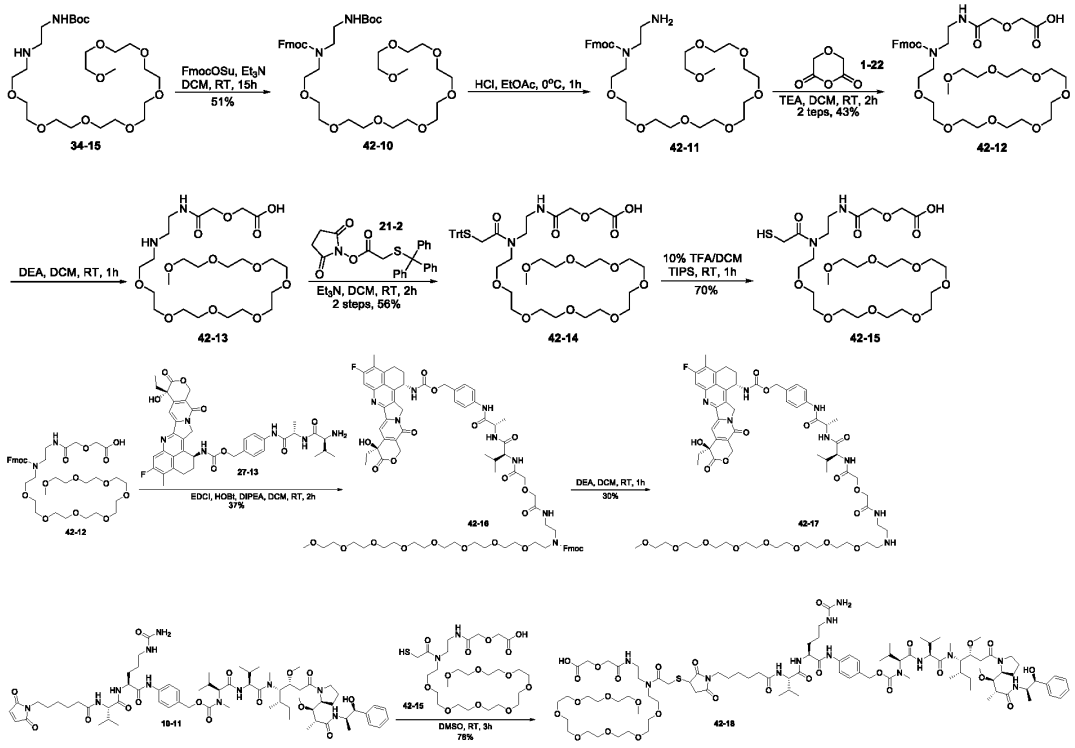
Example 42. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-

yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (42)

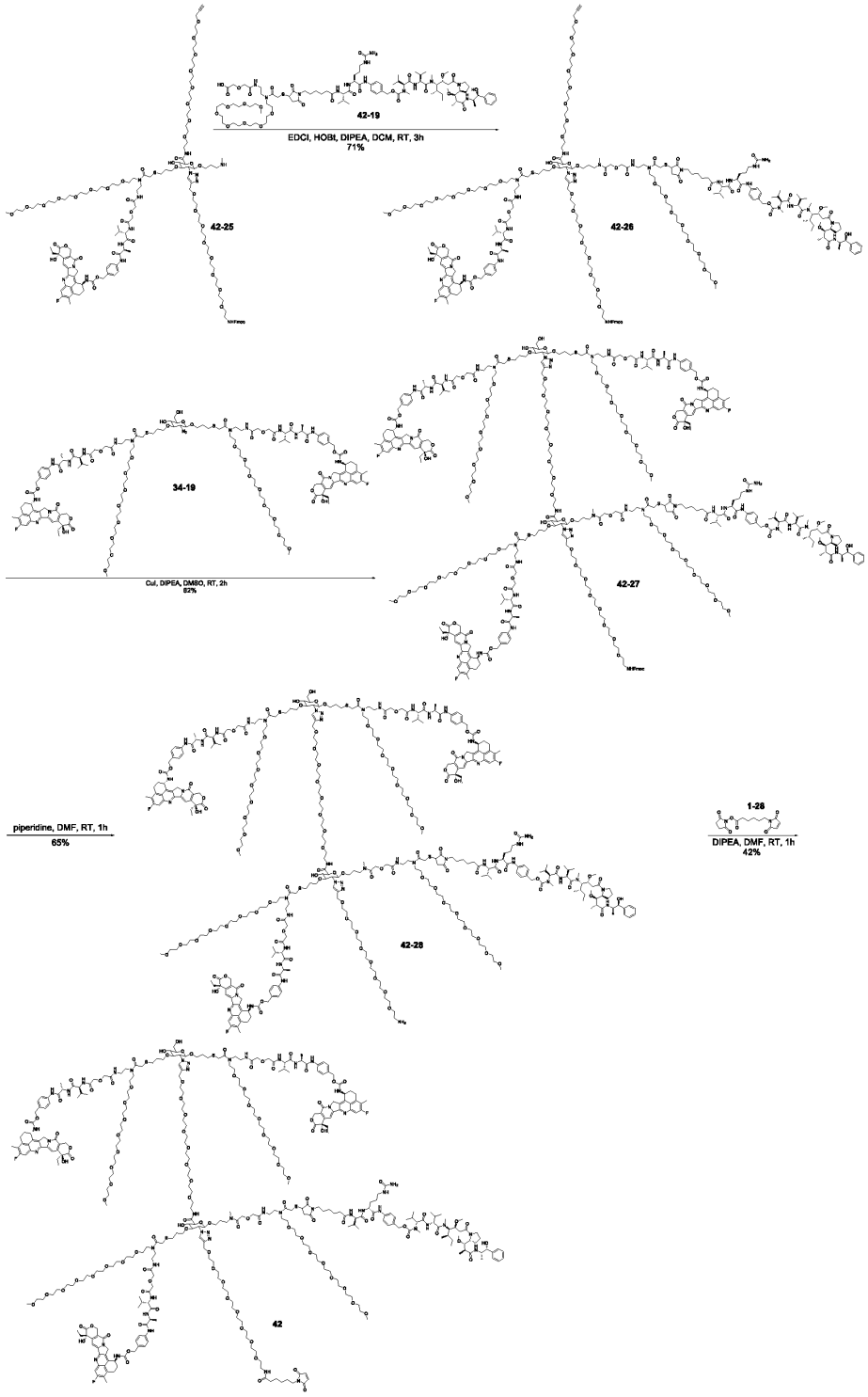


Synthetic scheme 42




34-15
FmocOSu, Et3N
DCM, RT, 15h
51%
42-10
HCl, EtOAc, 0°C, 1h
42-11
1-22
TEA, DCM, RT, 2h
2 teps, 43%
42-12
DEA, DCM, RT, 1h
42-13
21-2
Et3N, DCM, RT, 2h
2 steps, 56%
42-14
10% TFA/DCM
TIPS, RT, 1h
70%
42-15
27-13
EDCI, HOBt, DIPEA, DCM, RT, 2h
37%
42-16
DEA, DCM, RT, 1h
30%
42-17
18-11
42-15
DMSO, RT, 3h
78%
42-18


FmocOSu, TEA
DCM, RT, 16h
78%
42-9
CuI, DIPEA, THF, RT, 4h
83%
HATU, DIPEA, DMF, RT, 3h
60%
NaOH, CaCl2, IPA
H2O, 0°C, 5h
72%
42-19
42-20
42-21
42-22
42-23
42-17
EDCI, HOBt, DIPEA, DCM, RT, 4h
86%
TFA, DCM, RT, 1h
89%
42-24
42-25


42-19
EDCI, HOBt, DIPEA, DCM, RT, 3h
71%
42-25
42-26
34-19
CuI, DIPEA, DMBO, RT, 2h
82%
42-27
piperidine, DMF, RT, 1h
65%
1-26
DIPEA, DMF, RT, 1h
42%
42-28
42

[00667] Step 1: The 4A molecular sieve in flask was flamed for 30 minutes under reduced pressure. A solution of compound **17-14** (76.5 g, 131.04 mmol) and compound **27-3** (49.6 g, 262.08 mmol) in anhydrous dichloromethane (800 mL) was added to the flask under nitrogen atmosphere. The mixture was stirred at -15°C for 1.5h. Then TMSOTf (7.11 mL, 39.311 mmol) was added slowly to the reaction mixture. The mixture was stirred at -15°C for 16h. After completion of the reaction, the mixture was quenched with triethylamine (18.164 mL, 131.038 mmol) at 0°C, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **42-1** (75 g, yield 94%) as a colorless oil.

[00668] Step 2: To a mixture of compound **42-1** (65 g, 106.44 mmol) in methyl alcohol (80 mL) was added sodium methoxide solution 30% (0.395 mL, 2.13 mmol). The mixture was stirred at room temperature for 2h. After completion of the reaction, the mixture was concentrated. The crude product was purified silica gel column chromatography (eluted with ethyl acetate) to afford the title compound **42-2** (37.33 g, yield 72%) as a white solid.

[00669] Step 3: Under hydrogen, to a mixture of compound **42-2** (36.84 g, 76.03 mmol) in methyl alcohol (200 mL) was added Pd/C 10% (3.68 g). The mixture was stirred at room temperature for 4h. After completion of the reaction, the mixture was filtered with diatomite and concentrated to afford the title compound **42-3** (25.6 g, yield 96%) as a colorless oil. The crude product was directly used for the next step.

[00670] Step 4: To a mixture of compound **42-3** (24.9 g, 71.060 mmol) in methyl alcohol (300 mL) was added potassium carbonate (19.64 g, 142.119 mmol), copper sulfate pentahydrate (177 mg, 0.710 mmol) and imidazole-1-sulfonyl azide hydrochloride (17.87 g, 85.27 mmol). The mixture was stirred at room temperature for 4h. After completion of the reaction, the mixture was filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with ethyl acetate) to afford the title compound **42-4** (24.7 g, yield 92%) as a colorless oil.

[00671] Step 5: Under nitrogen atmosphere, to a mixture of compound **42-4** (11.1 g, 29.49 mmol) in anhydrous acetonitrile (100 mL) was added benzaldehyde dimethyl acetal (13.3 mL, 88.46 mmol) and 4-methylbenzenesulfonic acid (2.79 mL, 32.29 mmol). The mixture was stirred at 40°C for 2h. After completion of the reaction, the mixture was quenched with triethylamine (1.22 mL, 8.84 mmol) and concentrated. The mixture was poured into water (200 mL) and extracted with ethyl acetate (300 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **42-5** (10 g, yield 73%) as a colorless oil. LCMS: m/z calculated for $C_{22}H_{32}N_4O_7$: 464.52; found: 465.94 $[M+H]^+$.

[00672] Step 6: Under nitrogen atmosphere, to a mixture of compound **42-5** (10 g, 21.52 mmol) in anhydrous N,N-dimethylformamide (100 mL) was added sodium hydride (60%, 1.72 g, 43.05 mmol). The mixture was stirred at 0°C for 1h before allyl bromide (2.79 mL, 32.29 mmol) was added thereto. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched with cold water (700 mL) and extracted with ethyl acetate (800 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 16% ethyl acetate in petroleum ether) to afford the title compound **42-6** (10.05 g, yield 92%) as a colorless oil. LCMS: m/z calculated for $C_{25}H_{36}N_4O_7$: 504.58; found: 505.94 $[M+H]^+$.

[00673] Step 7: To a mixture of compound **42-6** (10 g, 19.818 mmol) in dichloromethane (80 mL) was added methyl thioglycolate (8.86 mL, 99.092 mmol). The mixture was stirred at room temperature for 1h under UV@365nm. After completion of the reaction, the mixture was concentrated. The crude product was purified silica gel column chromatography (eluted with 16% ethyl acetate in petroleum ether) to afford the title compound **42-7** (9.58 g, yield 79%) as a colorless oil. LCMS: m/z calculated for $C_{28}H_{42}N_4O_9S$: 610.72; found: 556.09 $[M-tBu+H]^+$.

[00674] Step 8: To a mixture of compound **42-7** (9.58 g, 15.68 mmol) in methyl alcohol (100 mL) was added camphorsulfonic acid (4.37 g, 18.82 mmol). The mixture was stirred at room temperature for 3h. After completion of the reaction, the mixture was adjusted pH to 7-8 with triethylamine and concentrated. The residue was poured into water (400 mL) and extracted with ethyl acetate (400 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 66% ethyl acetate in petroleum ether) to afford the title compound **42-8** (5.94 g, yield 72%) as a colorless oil. LCMS: m/z calculated for $C_{21}H_{38}N_4O_9S$: 522.62; found: 546.07 $[M+Na]^+$.

[00675] Step 9: To a mixture of compound **42-8** (5.94 g, 11.366 mmol) in dichloromethane (36 mL) and water (14.4 mL) was added 2,2,6,6-tetramethylpiperidinyl-1-oxide (TEMPO, 194 mg, 1.136 mmol) and [bis(acetoxy)iodo]benzene (BAIB, 7.32 g, 22.732 mmol) at 0°C. The mixture was stirred at 0°C for 20 min and then returned to room temperature for 40 min. After completion of the reaction, the mixture was poured into water (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified automatic column machine (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-9** (4.21 g, yield 69%) as a pale-yellow oil. LCMS: m/z calculated for $C_{21}H_{36}N_4O_{10}S$: 536.62; found: 537.91 $[M+H]^+$.

[00676] Step 10: Under nitrogen atmosphere, to a solution of compound **34-16** (11.54 g, 21.91 mmol) in dichloromethane (100 mL) was added triethylamine (8.86 g, 87.64 mmol) and FmocOSu (13.30 g, 39.44 mmol), the reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the mixture was poured into water (160 mL) and extracted with dichloromethane (160 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 2.5% dichloromethane in methanol) to afford the title

compound **42-10** (8.38 g, yield 51%) as a yellow oil. LCMS: m/z calculated for $C_{39}H_{60}N_2O_{12}$: 748.91; found: 649.41 $[(M\text{-Boc})+H]^+$.

[00677] Step 11: To a solution of compound **42-10** (8.38 g, 11.18 mmol) in ethyl acetate (8 mL) was added hydrochloride (4N in ethyl acetate, 8 mL). The reaction mixture was stirred at room temperature for 0.5 h. After completion of the reaction, the mixture was diluted with ethyl acetate and concentrated in vacuum. The residue was azeotroped with dichloromethane to afford the title compound **42-11** (10.52g, crude product) as a yellow oil. The crude product was used into the next step without further purification. LCMS: m/z calculated for $C_{34}H_{52}N_2O_{10}$: 648.79; found: 649.48 $[M+H]^+$.

[00678] Step 12: The title compound **42-12** was prepared in analogy to **Example 4, Step 8** by using compound **42-11** and compound **1-22**. The product (2.56g, yield 43% over 2 steps) was obtained as a yellow oil. LCMS: m/z calculated for $C_{38}H_{56}N_2O_{14}$: 764.87; found: 765.43 $[M+H]^+$.

[00679] Step 13: To a solution of compound **42-12** (2.54 g, 3.32 mmol) in dichloromethane (10 mL) was added diethylamine (5 mL). The reaction mixture was stirred at room temperature for 2 h, after completion of the reaction, the mixture was diluted with ethyl acetate and concentrated in vacuum. The residue was azeotroped with dichloromethane to afford the title compound **42-13** (2.87 g, crude product) as a yellow oil. The crude product was used into the next step without further purification. LCMS: m/z calculated for $C_{23}H_{46}N_2O_{12}$: 542.62; found: 543.40 $[M+H]^+$.

[00680] Step 14: To a solution of compound **42-13** (2.87g, crude) and compound **21-2** (2.02 g, 4.68 mmol) in dichloromethane (10 mL) was added triethylamine (0.95 g, 9.35 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated in vacuum. The crude product was purified by reverse phase flash (C18 column, eluted with 75% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **42-14** (1.59g,

yield 56% over 2 steps) as a yellow oil. LCMS: m/z calculated for $C_{44}H_{62}NO_{13}S$: 859.04; found: 617.31 $[(M-Trt)+H]^+$.

[00681] Step 15: To a solution of compound **42-14** (1.55 g, 1.80 mmol) in dichloromethane (20mL) was added TFA (5ml) and triisopropylsilane (1.84ml) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated in vacuum. The crude product was purified by reverse phase flash (C18 column, eluted with 50% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **42-15** (0.79 g, yield 70%) as a yellow oil. LCMS: m/z calculated for $C_{25}H_{48}N_2O_{13}S$: 616.72; found: 617.30 $[M+H]^+$.

[00682] Step 16: To a solution of compound **42-12** (3 g, 4.0 mmol) in dichloromethane (30 mL) was added **27-13** (3.6 g, 4.8 mmol), 1-hydroxybenzotriazole (0.54 g, 4.0 mmol), N,N-diisopropylethylamine (1.5 g, 11.9 mmol) and EDCI (1.5 g, 8.0 mmol) at temperature. The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was extracted with water (50 mL) and dichloromethane (50 mL x 3). The combined organic phases were concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 72% acetonitrile in water, 0.1% HCl condition). The desired components were lyophilized to afford the title compound **42-16** (2.64 g, yield 44%) as a yellow solid. LCMS: m/z calculated for $C_{78}H_{97}FN_8O_{21}$; found: 1501.66 $[M+H]^+$.

[00683] Step 17: To a solution of compound **42-16** (2.64 g, 1.8 mmol) in N,N-dimethylformamide (15 mL) was added piperidine (299 mg, 3.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse phase flash chromatography (C18 column, eluted with 50% acetonitrile in water, 0.1% HCl condition). The desired components were lyophilized to afford the title compound **42-17** (1.95 g, yield 87%) as a white solid. LCMS: m/z calculated for $C_{63}H_{87}FN_8O_{19}$; found: 1279.53 $[M+H]^+$.

[00684] Step 18: The title compound **42-18** was prepared in analogy to **Example 18, Step 12** by using compound **10-11** and compound **42-15**. The product (1.92 g, yield 78%) was obtained

as a yellow solid. LCMS: m/z calculated for C93H153N13O28S: 1933.37; found: 1934.08 $[M+H]^+$.

[00685] Step 19: To a mixture of compound **42-19** (4 g, 9.816 mmol) in dichloromethane (40 mL) was added triethylamine (4.07 mL, 29.45 mmol) and FmocOSu (6.62 g, 19.63 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was poured into water (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 2% methyl alcohol in dichloromethane). The desired components were lyophilized to give the title compound **42-20** (4.82 g, yield 78%) as a yellow oil. LCMS: m/z calculated for $C_{34}H_{47}NO_{10}$: 629.75; found: 631.28 $[M+H]^+$.

[00686] Step 20: Under nitrogen atmosphere, to a mixture of compound **42-13** (3.52 g, 6.56 mmol) and compound **42-20** (4.96 g, 7.872 mmol) in flask were added anhydrous tetrahydrofuran (40 mL), N,N-diisopropylethylamine (2.28 mL, 13.120 mmol) and cuprous iodide (624 mg, 3.280 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was poured into saturated ammonium chloride (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were washed with 0.5M hydrochloric acid water, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 9% methyl alcohol in dichloromethane). The desired components were lyophilized to give the title compound **42-21** (6.42 g, yield 83%) as a yellow oil. LCMS: m/z calculated for $C_{62}H_{83}N_5O_{20}S$: 1166.34; found: 1167.84 $[M+H]^+$.

[00687] Step 21: Under nitrogen atmosphere, to a mixture of compound **42-21** (6.42 g, 5.50 mmol) in anhydrous N,N-dimethylformamide (60 mL) was added compound N,N-diisopropylethylamine (1.91 mL , 11.009 mmol), HATU (1.02 g , 2.69 mmol). The mixture was stirred at room temperature for 10 min before compound **42-19** (2.47 g, 6.054 mmol) was added thereto. The mixture was stirred at room temperature for 3 h. After completion of the reaction,

the mixture was poured into water (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **42-22** (5.14 g, yield 60%) as a yellow oil. LCMS: m/z calculated for $C_{74}H_{118}N_6O_{27}S$: 1555.83; found: 1555.71 $[M+H]^+$.

[00688] Step 22: To a mixture of compound **42-22** (1.5 g, 0.96 mmol) in 0.8 M calcium chloride solution and isopropyl alcohol (v:v=3:7, 15.7 mL) was added sodium hydroxide (58 mg, 1.45 mmol). The mixture was stirred at room temperature for 5 h. After completion of the reaction, the reaction mixture was adjusted pH to 5-6 with 0.5N hydrochloric acid water. The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to give the title compound **42-23** (1.71 g, yield 72%) as a yellow oil. LCMS: m/z calculated for $C_{73}H_{116}N_6O_{27}S$: 1541.81; found: 1563.61 $[M+Na]^+$.

[00689] Step 23: Under nitrogen atmosphere, to a mixture of compound **42-23** (303 mg, 0.19 mmol) in anhydrous dichloromethane (5 mL) was added compound **42-17** (276 mg, 0.22 mmol), N,N-diisopropylethylamine (0.102 mL, 0.59 mmol), 1-hydroxybenzotriazole (26 mg, 0.19 mmol) and EDCI (75 mg, 0.39 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (150 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-24** (475 mg, yield 86%) as a yellow solid. LCMS: m/z calculated for $C_{136}H_{201}FN_{14}O_{45}S$: 2803.22; found: 1402.27 $[M+2H]^{2+}$.

[00690] Step 24: To a mixture of compound **42-24** (475 mg, 0.17 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.50 mL, 6.76 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with

dichloromethane (50 mL) and concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-25** (408 mg, yield 89%) as a yellow solid. LCMS: m/z calculated for $C_{131}H_{193}FN_{14}O_{43}S$: 2703.10; found: 1352.17 $[M+2H]^{2+}$.

[00691] Step 25: Under nitrogen atmosphere, to a mixture of compound **42-25** (228 mg, 0.084 mmol) in anhydrous dichloromethane (3 mL) was added compound **42-24** (179 mg, 0.093 mmol), N,N-diisopropylethylamine (0.044 mL, 0.25 mmol), 1-hydroxybenzotriazole (11 mg, 0.08 mmol) and EDCI (32 mg, 0.168 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (50 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-26** (276 mg, yield 71%) as a yellow oil. LCMS: m/z calculated for $C_{224}H_{344}FN_{27}O_{70}S_2$: 4618.45; found: 1540.41 $[M+3H]^{3+}$.

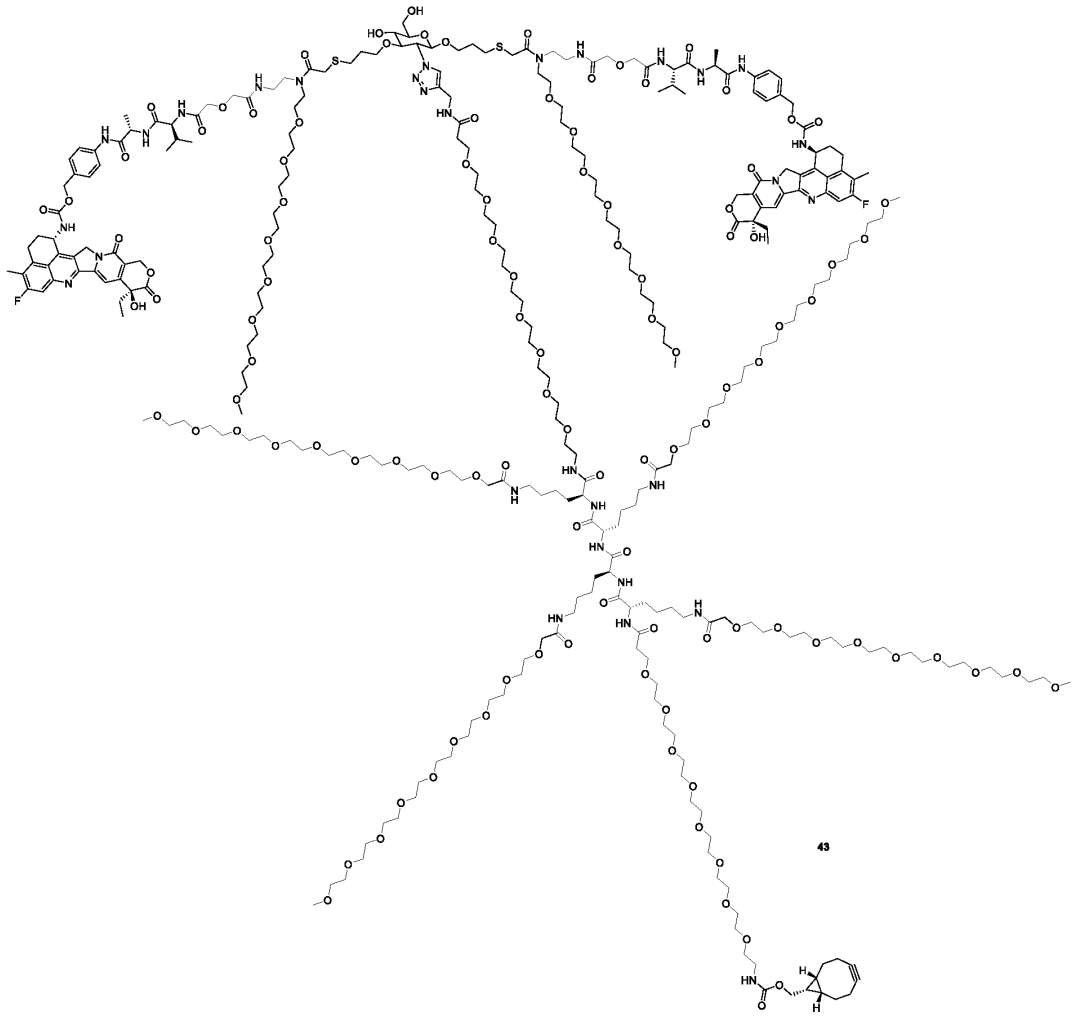
[00692] Step 26: Under nitrogen atmosphere, to a mixture of compound **42-26** (267 mg, 0.058 mmol) and compound **34-19** (144 mg, 0.048 mmol) in flask were added dimethyl sulfoxide (3 mL), N,N-diisopropylethylamine (0.067 mL, 0.384 mmol) and cuprous iodide (18 mg, 0.096 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride water (1 mL), filtered. The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-27** (240 mg, yield 62%) as a yellow solid. LCMS: m/z calculated for $C_{366}H_{541}F_3N_{46}O_{115}S_4$: 7620.80; found: 1903.54 $[M+4H]^{4+}$.

[00693] Step 27: Under nitrogen atmosphere, to a mixture of compound **42-27** (230 mg, 0.03 mmol) in anhydrous N,N-dimethylformamide (3 mL) was added piperidine (0.015 mL, 0.15 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was purified by reverse phase flash chromatography (C18 column, eluted with

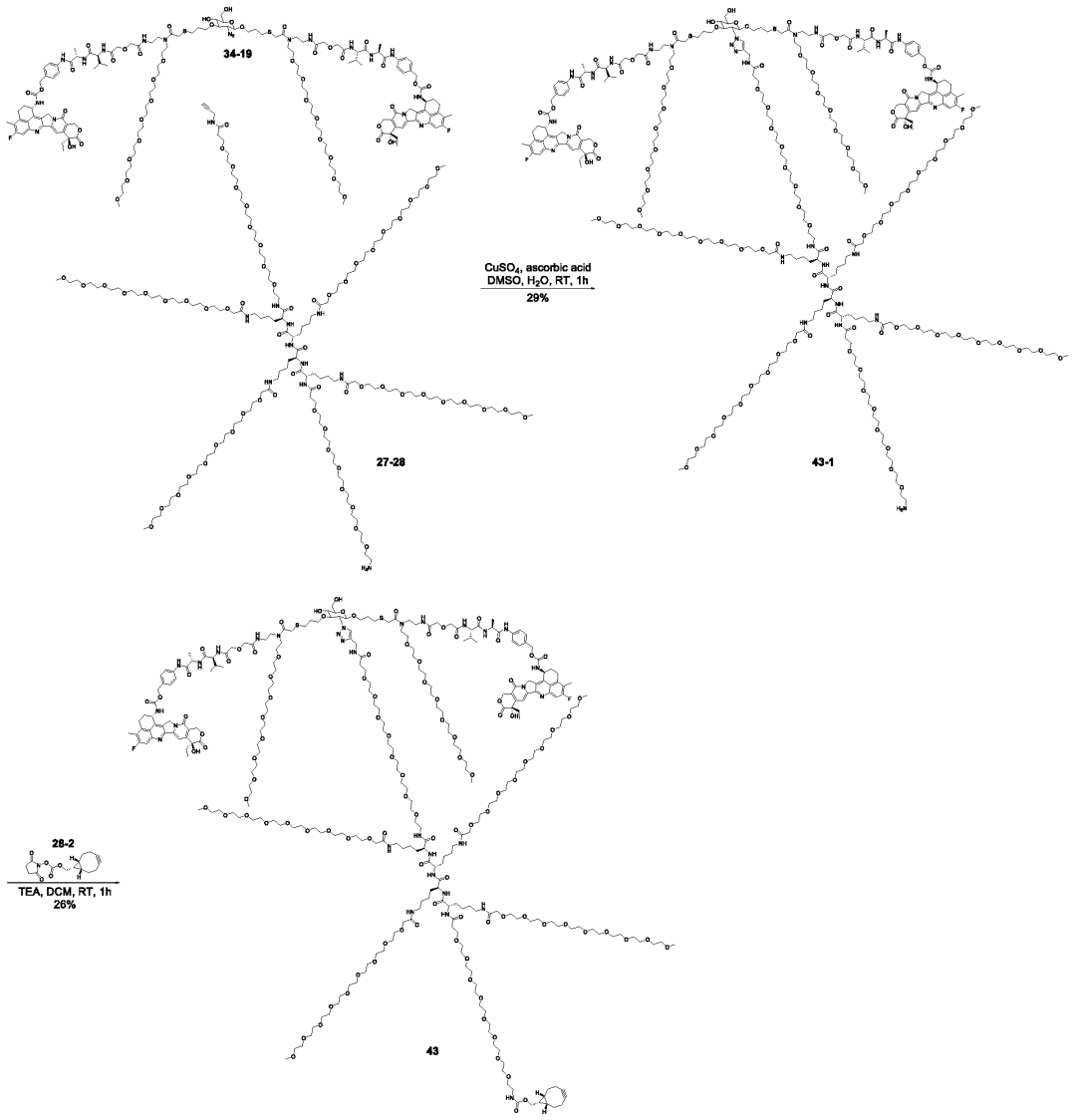
acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **42-28** (146 mg, yield 65%) as a yellow solid. LCMS: m/z calculated for $C_{351}H_{531}F_3N_{46}O_{113}S_4$: 7388.62; found: 1847.86 $[M+4H]^{4+}$.

[00694] Step 28: The title compound **42** was prepared in analogy to **Example 1, Step 21** by using compound **42-28** and compound **1-28** in DMF instead of dichloromethane. The product (40 mg, yield 42%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{361}H_{542}F_3N_{47}O_{116}S_4$: 7581.76; found: 1517.25 $[M+5H]^{5+}$.

Example 43. Synthesis of [(1*R*,8*S*)-9-bicyclo[6.1.0]non-4-ynyl]methyl *N*-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*)-1-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-5-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]pentyl]carbamoyl]-5-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]pentyl]carbamoyl]-5-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]pentyl]carbamoyl]-5-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]pentyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate (43)



Synthetic scheme 43

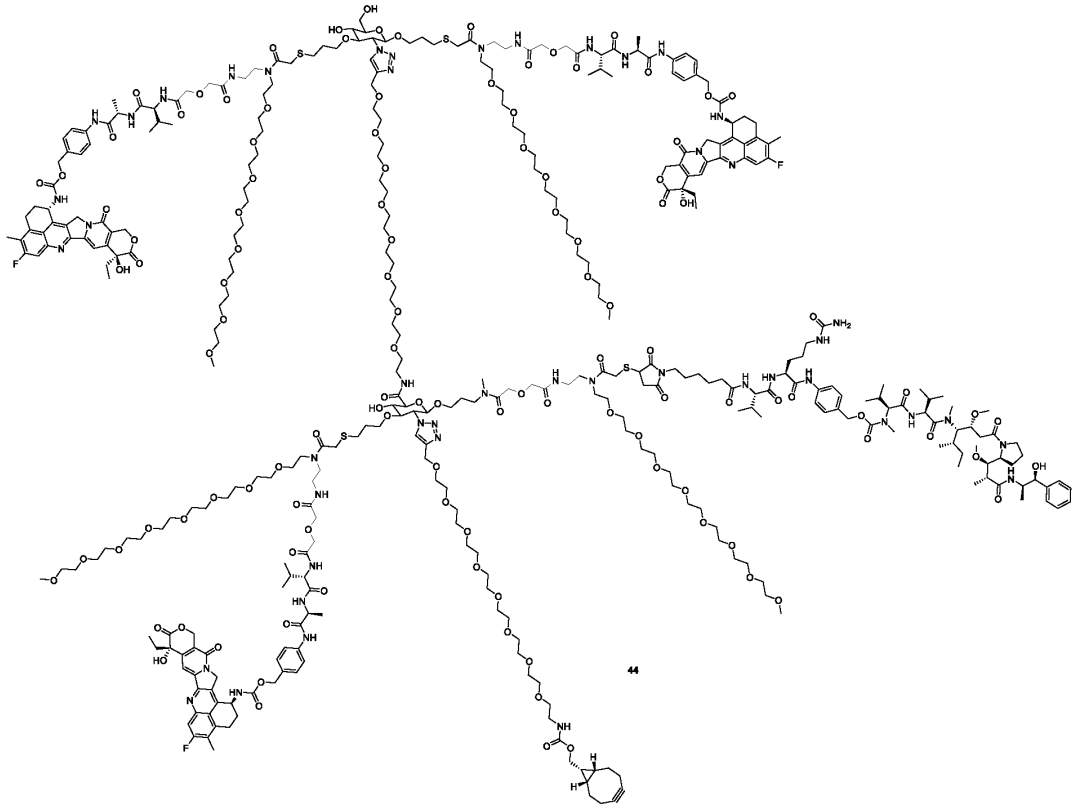


[00695] Step 1: The title compound **43-1** was prepared in analogy to **Example 1, Step 24** by using compound **34-19** and compound **27-28**. The product (9 mg, yield 29%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{283}H_{468}F_2N_{30}O_{107}S_2$: 6105.08; found: 1527.05 $[M+4H]^{4+}$.

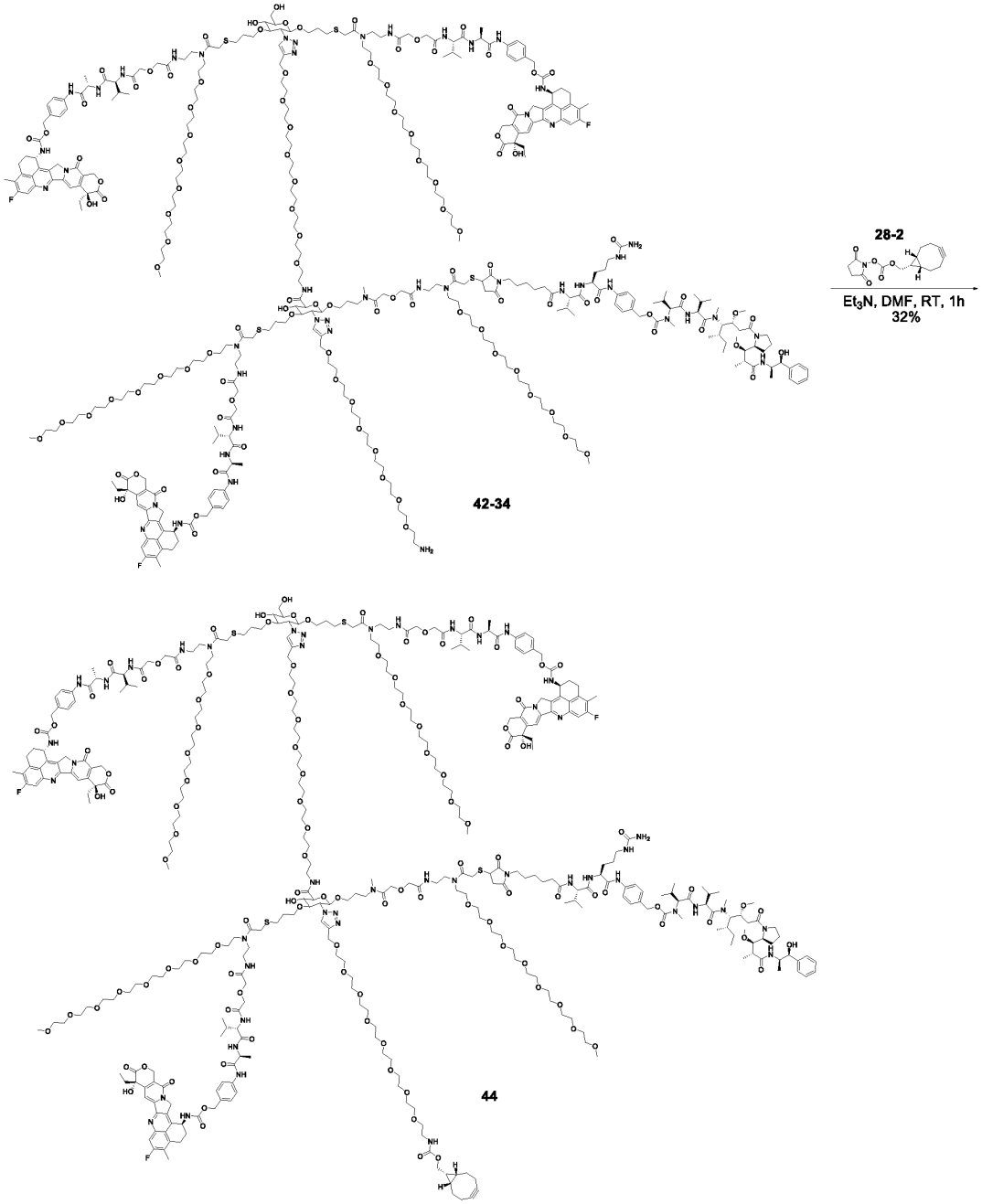
[00696] Step 2: The title compound **43** was prepared in analogy to **Example 51, Step 1** by using compound **43-1** and compound **28-2** with triethylamine instead of DIPEA. The product (9

mg, yield 26%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{294}H_{480}F_2N_{30}O_{109}S_2$: 6281.29; found: 1571.10 $[M+4H]^{4+}$.

Example 44. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[(1*R*,8*S*)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-6-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (44)



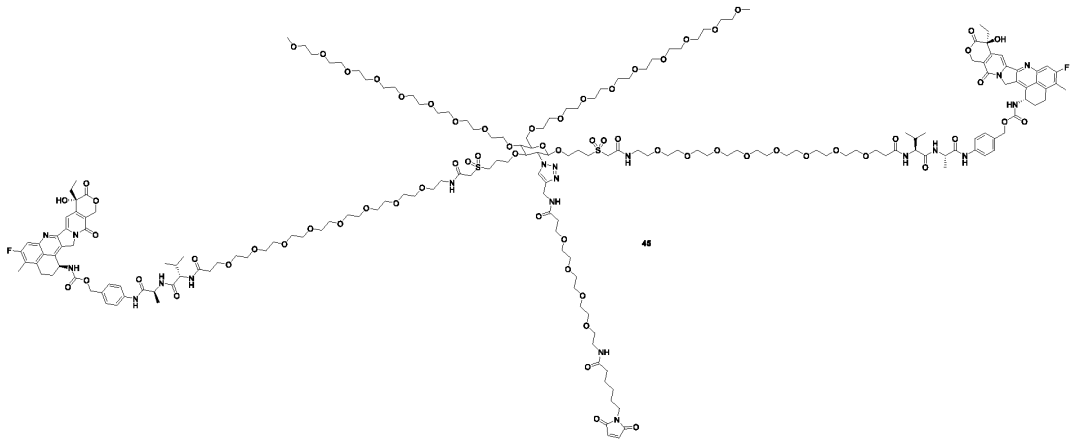
Synthetic scheme 44



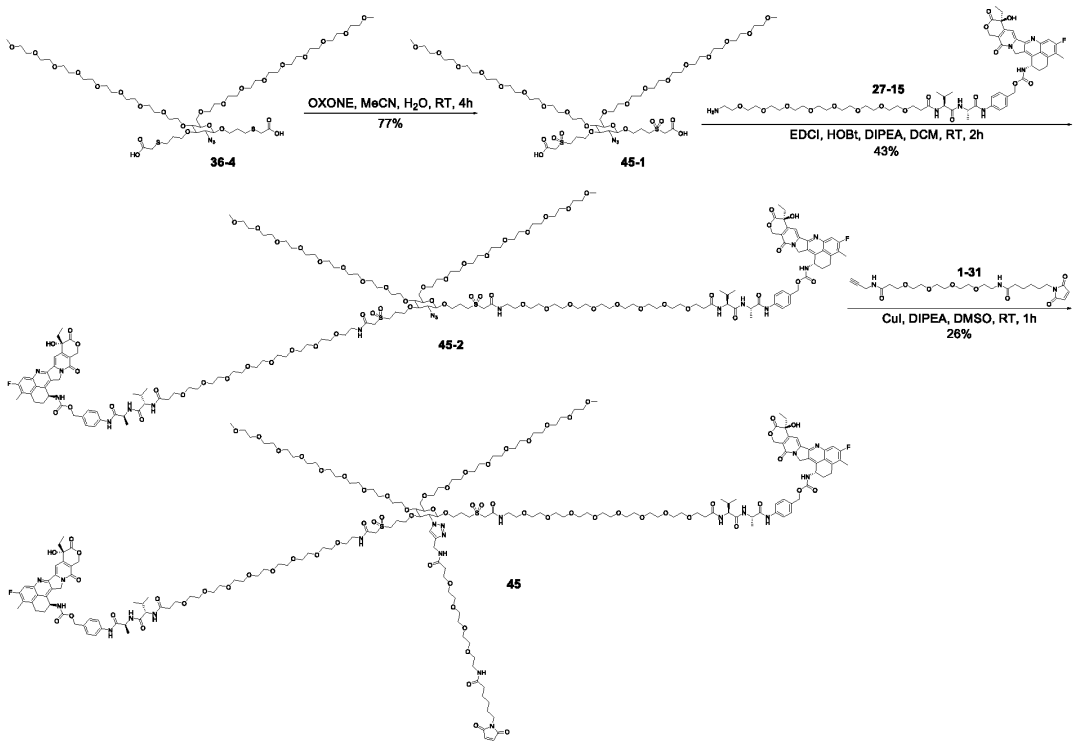
[00697] Step 1: The title compound **44** was prepared in analogy to **Example 51, Step 1** by using compound **42-34** and compound **28-2** in DMF with triethylamine instead of DIPEA. The

product (10.1 mg, yield 32%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{362}H_{543}F_3N_{46}O_{115}S_4$: 7564.77; found: 1513.80 $[M+5H]^{5+}$.

Example 45. Synthesis of 4-((32S,35S)-1-((3-(((2R,3R,4R,5S,6R)-5-((2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)oxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-4-(3-(((32S,35S)-36-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-32-isopropyl-35-methyl-2,30,33,36-tetraoxo-6,9,12,15,18,21,24,27-octaoxa-3,31,34-triazahexatriacontyl)sulfonyl)propoxy)-6-(2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)sulfonyl)-32-isopropyl-35-methyl-2,30,33-trioxo-6,9,12,15,18,21,24,27-octaoxa-3,31,34-triazahexatriacontan-36-amido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (45)



Synthetic scheme 45



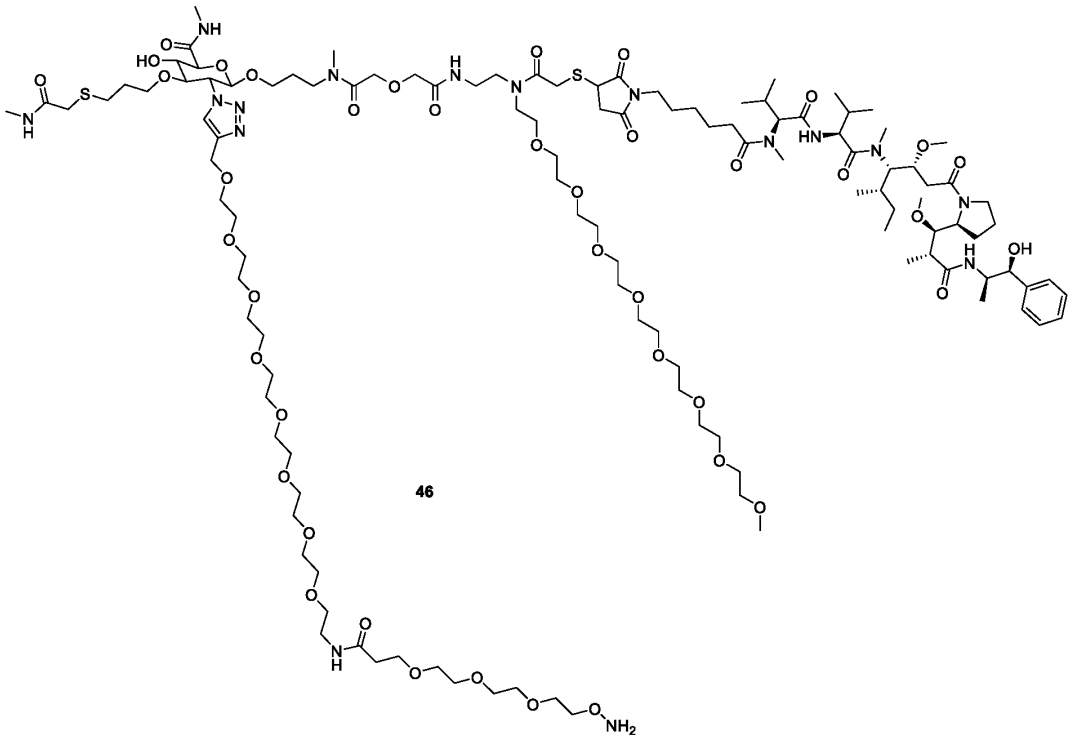
[00698] Step 1: To a solution of compound **36-4** (0.2 g, 0.166 mmol) in acetonitrile (2 mL) was added a solution of OXONE (0.409 g, 0.665 mmol) in H_2O (1 mL). The mixture was stirred at room temperature for 4 h. After completion, the mixture was filtered and concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **45-1** (167 mg, 77% yield) as a colorless oil.

[00699] Step 2: Compound **45-1** (153 mg, 0.121 mmol) was dissolved in dichloromethane (4 mL), followed by the sequential addition of HOBt (33 mg, 0.242 mmol), compound **27-15** (356 mg, 0.302 mmol), DIPEA (125 mg, 0.968 mmol) and EDCI (93 mg, 0.484 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 2 h. After completion, the mixture was filtered and concentrated under reduced pressure. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired

components were lyophilized to afford the title compound **45-2** (214 mg, 43% yield) as a white solid. LCMS: m/z calculated for $C_{168}H_{251}F_2N_{17}O_{61}S_2$: 3587.03; found: 1196.32 $[M+3H]^{3+}$.

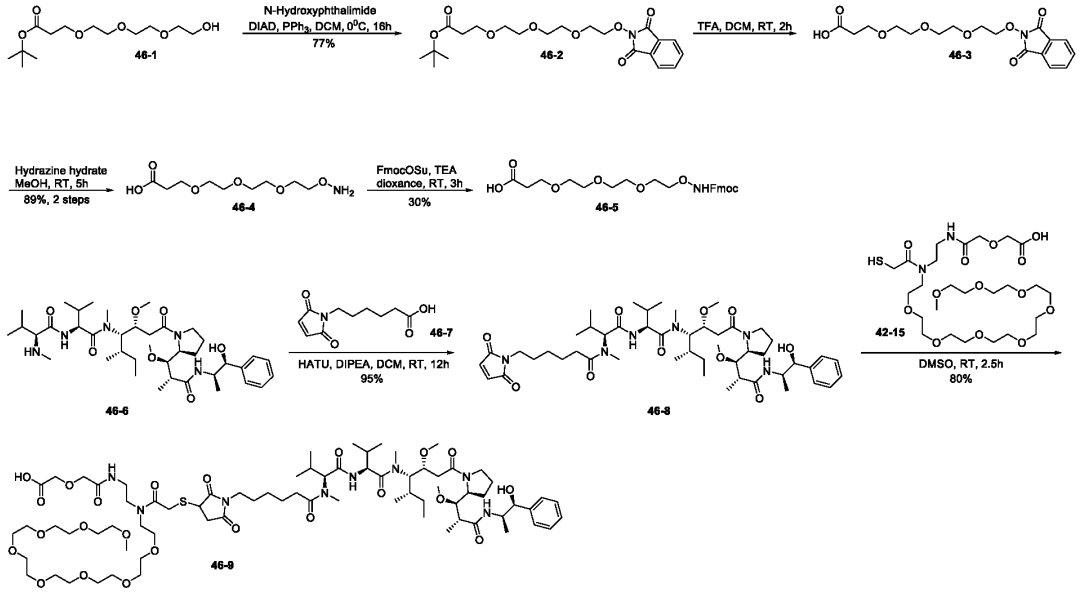
[00700] Step 3: To a solution of compound **45-2** (41 mg, 0.011 mmol) and compound **1-31** (8.5 mg, 0.017 mmol) in DMSO (1 mL) under a nitrogen (N_2) atmosphere was added CuI (4.3 mg, 0.023 mmol) and DIPEA (12 mg, 0.093 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered, and the filtrate was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **45** (12.3 mg, yield 46%) as a white solid. LCMS: m/z calculated for $C_{192}H_{288}F_2N_{20}O_{69}S_2$: 4082.60; found: 1361.93 $[M+3H]^{3+}$.

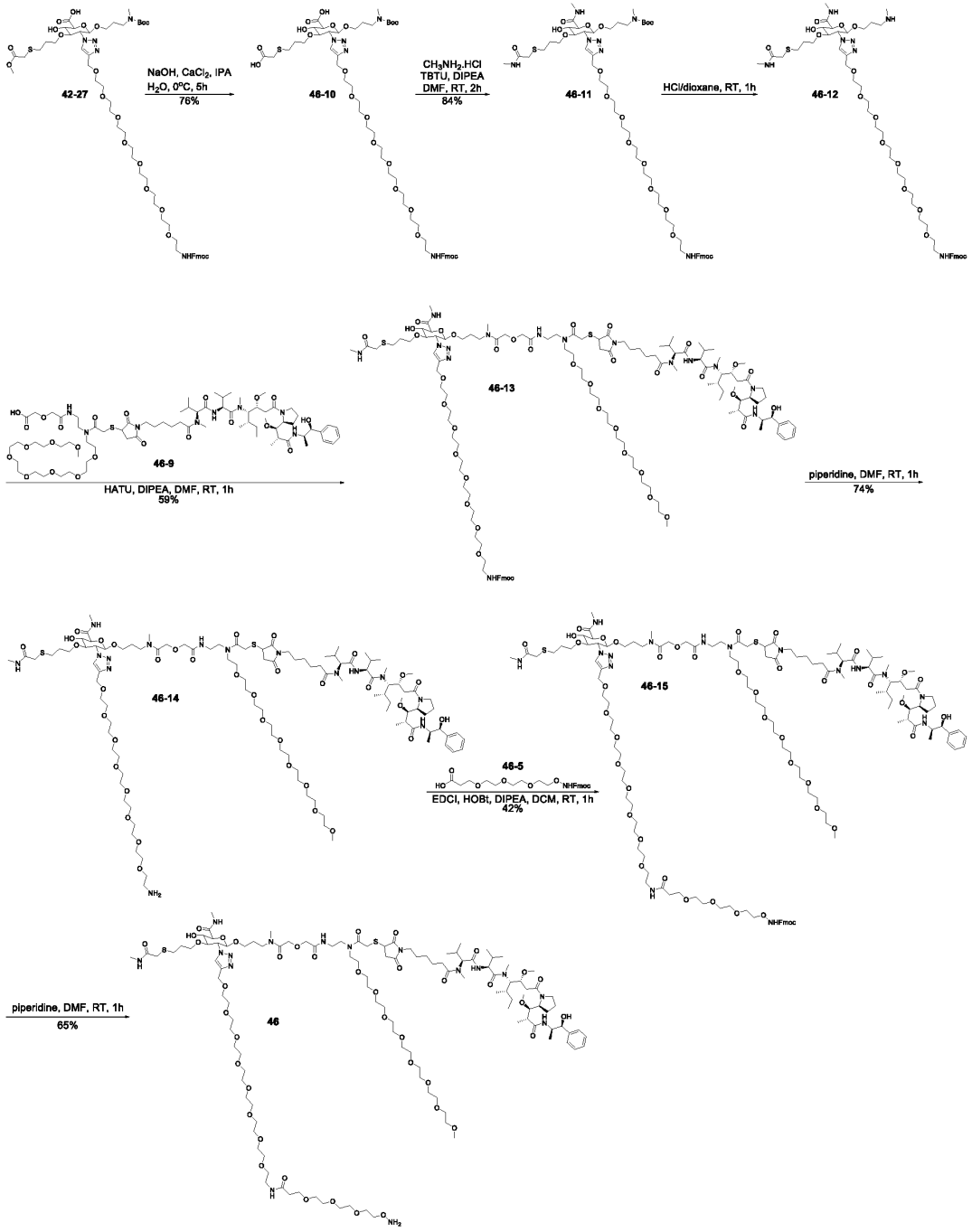
Example 46. Synthesis of (2S,3S,4R,5R,6R)-5-(4-(38-(aminooxy)-27-oxo-2,5,8,11,14,17,20,23,30,33,36-undecaoxa-26-azaoctatriacontyl)-1H-1,2,3-triazol-1-yl)-6-((26-(2-((1-((3R,4S,7S,10S)-4-((S)-sec-butyl)-3-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9,12-trioxo-2-oxa-5,8,11-triazaheptadecan-17-yl)-2,5-dioxopyrrolidin-3-yl)thio)acetyl)-35-methyl-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaoctatriacontan-38-yl)oxy)-3-hydroxy-N-methyl-4-(3-((2-(methylamino)-2-oxoethyl)thio)propoxy)tetrahydro-2H-pyran-2-carboxamide (46)



46

Synthetic scheme 46




42-27
NaOH, CaCl2, IPA
H2O, 0°C, 5h
76%
46-10
CH3NH2.HCl
TBTU, DIPEA
DMF, RT, 2h
84%
46-11
HCl/dioxane, RT, 1h
46-12
46-9
HATU, DIPEA, DMF, RT, 1h
59%
46-13
piperidine, DMF, RT, 1h
74%
46-14
46-15
46-5
EDCI, HOBt, DIPEA, DCM, RT, 1h
42%
piperidine, DMF, RT, 1h
65%
46

[00701] Step 1: Under nitrogen atmosphere, to a solution of compound **46-1** (4.01g, 14.40 mmol) in dichloromethane (20mL) was added N-hydroxyphthalimide (4.68 g, 28.68 mmol), PPh_3 (5.60 g, 21.34 mmol), followed by DIAD (4.36 g, 21.56 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated under vacuum. the crude product was purified by reverse phase flash chromatography (C18 column, eluted with 65% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **46-2** (4.71g, yield 77%) as a yellow solid. LCMS: m/z calculated for $C_{21}H_{29}NO_8$: 423.46; found: 368.07 $[M+H]^+$.

[00702] Step 2: To a solution of compound **46-2** (4.71 g, 11.09 mmol) in dichloromethane (9 mL) was added trifluoroacetic acid (21 mL) The reaction mixture was stirred at room temperature for 2 h, after completion of the reaction, the mixture was diluted with ethyl acetate and concentrated in vacuum. The residue was azeotroped with dichloromethane under reduced pressure to afford the title compound **46-3** (5.04 g, crude product) as a yellow oil. The crude product was used into the next step with further purification. LCMS: m/z calculated for $C_{17}H_{21}NO_8$: 367.35; found: 368.05 $[M+H]^+$.

[00703] Step 3: To a solution of compound **46-3** (5.04 g, crude) in methanol (10 mL) was added Hydrazine Hydrate (2.61 g, 52.13 mmol). The reaction mixture was stirred at room temperature for 16 h to form a white precipitate. After completion of the reaction, the mixture was filtered and concentrated in vacuum to afford the title compound **46-4** (2.85g, yield 89% over 2 steps) as a yellow oil. LCMS: m/z calculated for $C_9H_{19}NO_6$:237.25; found: 238.07 $[M+H]^+$.

[00704] Step 4: Under nitrogen atmosphere, to a solution of compound **46-4** (2.85 g, 12.01 mmol) in dichloromethane (25ml) was added triethylamine (3.64 g, 36.03 mmol) and FmocOSu (6.08 g, 18.02 mmol). The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (100 mL x 2). The combined organic layers were washed with brine, dried over

anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 55% acetonitrile in water, 0.1%TFAcondition). The desired components were lyophilized to afford the title compound **46-5** (1.62 g, yield 30%) as a yellow solid LCMS: m/z calculated for $C_{24}H_{29}NO_8$: 459.50; found: 460.13 $[M+H]^+$.

[00705] Step 5: Under nitrogen atmosphere, to a mixture of compound **46- 6** (400 mg, 0.55 mmol) and compound **46-7** (180 mg, 0.83 mmol) in dichloromethane (10 mL) was added DIPEA (0.13g, 0.99mmol) and HATU (0.32g, 0.83mmol), the reaction mixture was stirred at room temperature for 1 h, After completion of the reaction, the mixture was poured into water (30 mL) and extracted with dichloromethane (30 mL x 2), the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 5% dichloromethane in methanol) to afford the title compound **46-8** (0.49 g, yield 96%) as a white solid. LCMS: m/z calculated for $C_{49}H_{78}N_6O_{10}$: 911.20; found: 911.63 $[M+H]^+$.

[00706] Step 6: To a mixture of compound **46-8** (0.79g, 1.28mmol) and compound **42-21** (1.40g, 1.53mmol) in dimethyl sulfoxide (10 mL), then add triethylamine (0.13g, 1.27mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated under vacuum. The crude product was purified by reverse phase flash (C18 column, eluted with 58% acetonitrile in water). The desired components were lyophilized to afford the title compound **46-9** (1.57g, yield 80%) as a yellow oil LCMS: m/z calculated for $C_{74}H_{126}N_8O_{23}S$:1527.92; found: 764.71 $[M+2H]^{2+}$ 1527.88 $[M+H]^+$.

[00707] Step 7: To a solution of **42-27** (1.00 g, 0.86 mmol) and 0.8 N calcium chloride in isopropanol and H_2O (v:v=7:3, 14 mL) was added sodium hydroxide slowly (58.30 mg, 1.46 mmol) at 0°C. The reaction mixture was stirred for 5 h at 0 °C. After completion of the reaction, the mixture was adjusted to pH=6 with 1 N hydrochloric acid at 0 °C and concentrated under reduced pressure. The residue was purified by reverse flash chromatography (C18 column,

58.2% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **46-10** (749.00 mg, yield 76%) as a light-yellow oil. LCMS: m/z calculated for $C_{54}H_{81}N_5O_{20}S$: 1152.32; found: 1152.50 $[M+H]^+$.

[00708] Step 8: To a mixture of compound **46-10** (660.00 mg, 0.57 mmol), methylamine hydrochloride (115.46 mg, 1.71 mmol) and N,N-diisopropylethylamine (441.18 mg, 3.42 mmol) in N,N-dimethylformamide (8 mL) was added TBTU (549.05 mg, 1.71 mol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was purified by reverse flash chromatography (C18 silica gel column, 59% acetonitrile in water, HCl condition). The desired components were lyophilized to afford the title compound **46-11** (568.00 mg, yield 84%) as a light-yellow oil. LCMS: m/z calculated for $C_{56}H_{87}N_7O_{18}S$: 1178.40; found: 1178.58 $[M+H]^+$.

[00709] Step 9: A solution of compound **46-11** (568.00 mg, 0.48 mol) in 4 N HCl in dioxane (3 mL) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure to afford the title compound **46-12** (510.00 mg, 98%) as a yellow oil. LCMS: m/z calculated for $C_{51}H_{79}N_7O_{16}S$: 1078.29; found: 1078.55 $[M+H]^+$.

[00710] Step 10: To a solution of **46-9** (252.00 mg, 0.16 mmol) in N,N-dimethylformamide (5 mL) was added N,N-diisopropylethylamine (30.96 mg, 0.24 mmol) and HATU (91.20 mg, 0.24 mmol) and stirred at room temperature for 10 min. To the above mixture was added **46-12** (189.78 mg, 0.18 mmol). Then the mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse flash chromatography (C18 silica gel column, 55% acetonitrile in water, TFA condition) to afford the title compound **46-13** (251.00 mg, yield 59%) as a light-yellow oil. LCMS: m/z calculated for $C_{125}H_{203}N_{15}O_{38}S_2$: 2588.19; found: 1294.53 $[M+2H]^+$.

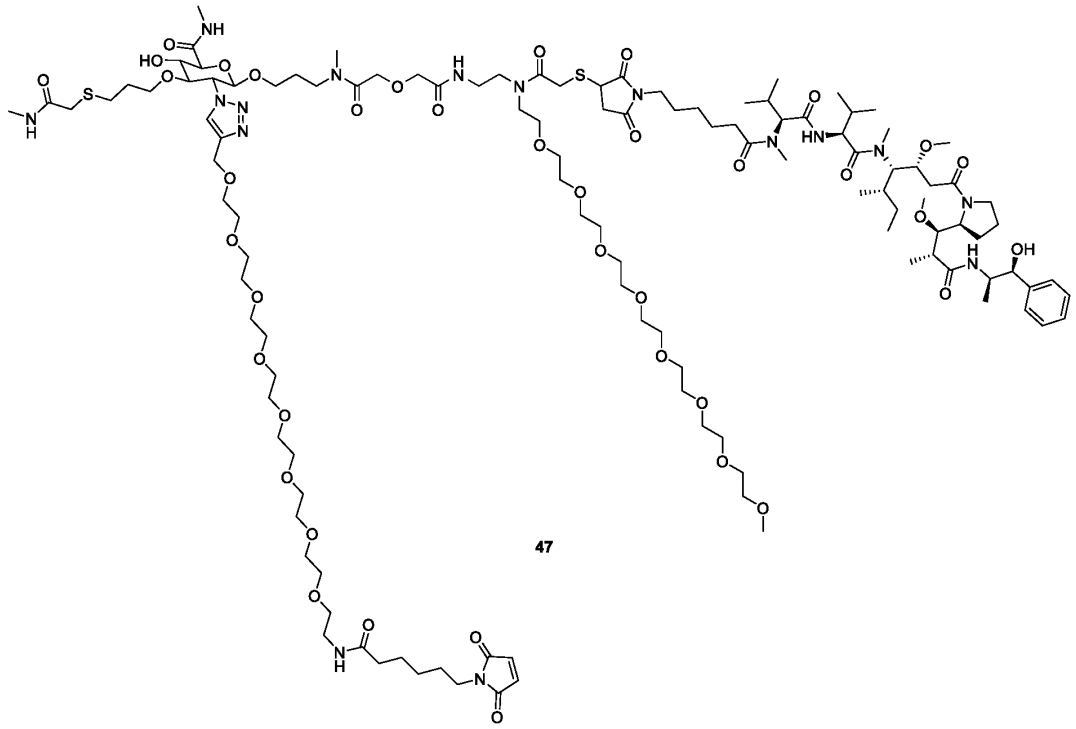
[00711] Step 11: A solution of **46-13** (251.00 mg, 0.01 mol) in N,N-dimethylformamide (2.5 mL) was added piperidine (41.29 mg, 0.48 mmol). Then the mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse flash chromatography (C18 silica gel column, 48% acetonitrile in water, TFA condition). The desired

components were lyophilized to afford the title compound **46-14** (169.00 mg, yield 74%) as a light-yellow solid. LCMS: m/z calculated for $C_{110}H_{193}N_{15}O_{36}S_2$: 2365.94; found: 1183.63 $[M+2H]^+$.

[00712] Step 12: To a solution of **46-14** (96.00 mg, 0.04 mmol), **46-5** (20.51 mg, 0.04 mmol) and N,N-diisopropylethylamine (15.48 mg, 0.12 mmol) in dichloromethane (3 mL) was added 1-hydroxybenzotriazole (5.94 mg, 0.04 mmol) and EDCI (8.43 mg, 0.04 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (C18 silica gel column, 62% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **46-15** (48.00 mg, yield 42%) as a light-yellow oil. LCMS: m/z calculated for $C_{134}H_{220}N_{16}O_{43}S_2$: 2807.42; found: 1404.35 $[M+2H]^+$.

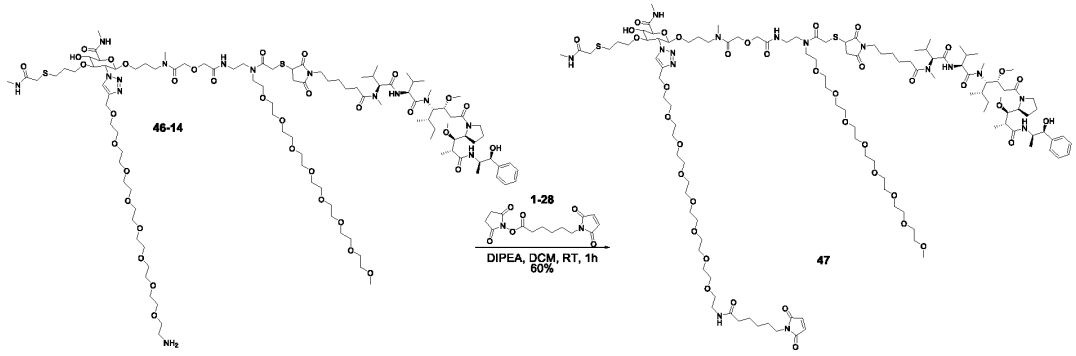
[00713] Step 13: A solution of **46-15** (48.00 mg, 0.02 mol) in N,N-dimethylformamide (1 mL) was added piperidine (8.52 mg, 0.10 mmol). Then the mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse flash chromatography (C18 silica gel column, 53.7% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **46** (28.90 mg, yield 65%) as a yellow oil. LCMS: m/z calculated for $C_{119}H_{210}N_{16}O_{41}S_2$: 2585.18; found: 1293.14 $[M+H]^+$.

Example 47. Synthesis of (2S,3S,4R,5R,6R)-6-((26-(2-((1-((3R,4S,7S,10S)-4-((S)-sec-butyl)-3-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9,12-trioxo-2-oxa-5,8,11-triazaheptadecan-17-yl)-2,5-dioxopyrrolidin-3-yl)thio)acetyl)-35-methyl-30,34-dioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35-triazaoctatriacontan-38-yl)oxy)-5-(4-(32-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-26-azadotriacontyl)-1H-1,2,3-triazol-1-yl)-3-hydroxy-N-methyl-4-(3-((2-(methylamino)-2-oxoethyl)thio)propoxy)tetrahydro-2H-pyran-2-carboxamide (47)



47

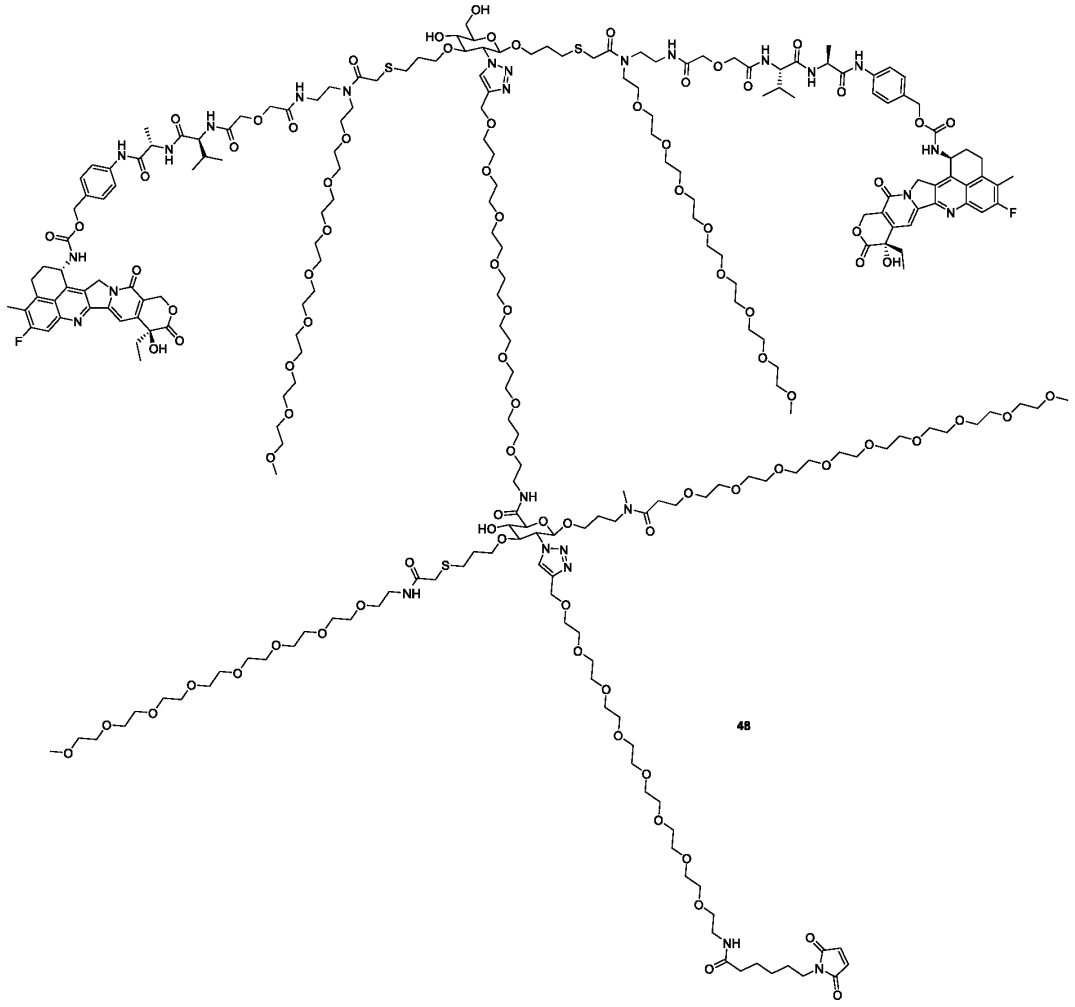
Synthetic scheme 47



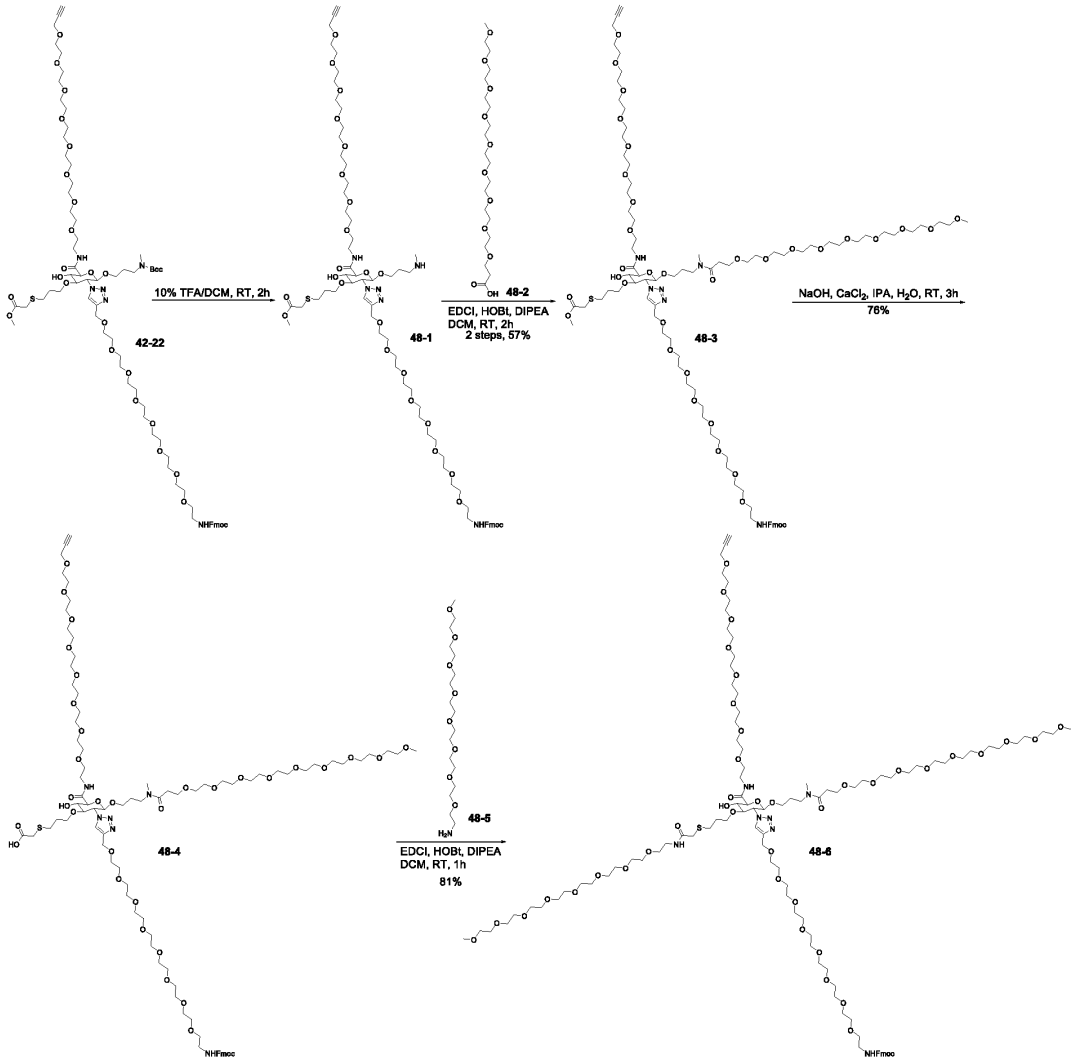
[00714] Step 1: To a solution of **46-14** (50.00 mg, 0.02 mmol) and N,N-diisopropylethylamine (6.82 mg, 0.05 mmol) in dichloromethane (2 mL) was added **47-1** (9.25 mg, 0.03 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (C18 silica gel column, 58% acetonitrile in water, TFA condition). The desired

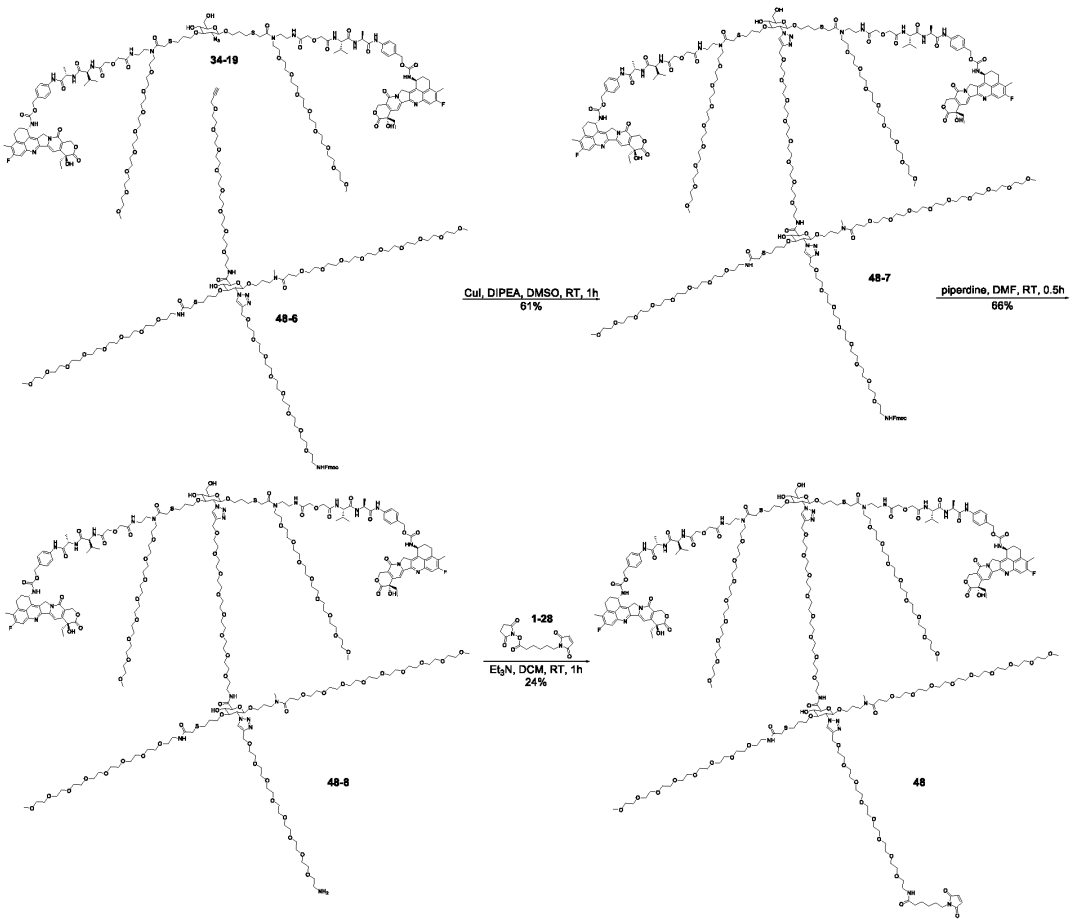
components were lyophilized to afford the title compound **47** (32.40 mg, yield 60%) as a colorless oil. LCMS: m/z calculated for $C_{120}H_{204}N_{16}O_{39}S_2$: 2559.15; found: 1280.29 $[M+2H]^+$.

Example 48. Synthesis of ((((36S,36'S,39S,39'S)-26,26'-(2,2'-(((((2R,3R,4R,5S,6R)-3-(4-(1-((2S,3S,4R,5R,6R)-5-(4-(32-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-26-azadotriacontyl)-1H-1,2,3-triazol-1-yl)-3-hydroxy-6-((30-methyl-29-oxo-2,5,8,11,14,17,20,23,26-nonaoxa-30-azatritriacontan-33-yl)oxy)-4-((27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)tetrahydro-2H-pyran-2-yl)-1-oxo-5,8,11,14,17,20,23,26-octaoxa-2-azaheptacosan-27-yl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(36-isopropyl-39-methyl-30,34,37-trioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35,38-tetraazatetracontan-40-oyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate) (48)



Synthetic scheme 48


10% TFA/DCM, RT, 2h
42-22
48-1
48-2
EDCI, HOBt, DIPEA
DCM, RT, 2h
2 steps, 57%
48-3
NaOH, CaCl2, IPA, H2O, RT, 3h
76%
48-4
48-5
EDCI, HOBt, DIPEA
DCM, RT, 1h
81%
48-6
NHFmoc



[00715] Step 1: To a solution of compound **42-22** (1.00 g, 0.64 mmol) in dichloromethane (9 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated to afford the title compound **48-1** (1.1 g) as a pale-yellow oil. The crude product was used into the next step without further purification. LCMS: m/z calculated for $C_{69}H_{110}N_6O_{25}S$: 1454.72; found: 1455.46 $[M+H]^+$.

[00716] Step 2: The title compound **48-3** was prepared in analogy to **Example 2, Step 5** by using compound **48-1** and compound **48-2**. The product (694 mg, yield 57% over 2 steps) was obtained as a colorless oil. LCMS: m/z calculated for $C_{89}H_{148}N_6O_{35}S$: 1894.23; found: 948.00 $[M+2H]^{2+}$.

[00717] Step 3: The title compound **48-4** was prepared in analogy to **Example 42, Step 22** by using compound **48-3**. The product (523 mg, yield 76%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{88}H_{146}N_6O_{35}S$: 1880.20; found: 940.78 $[M+2H]^{2+}$.

[00718] Step 4: The title compound **48-6** was prepared in analogy to **Example 2, Step 5** by using compound **48-4** and compound **48-5**. The product (508 mg, yield 81%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{105}H_{181}N_7O_{42}S$: 2245.67; found: 1124.07 $[M+2H]^{2+}$.

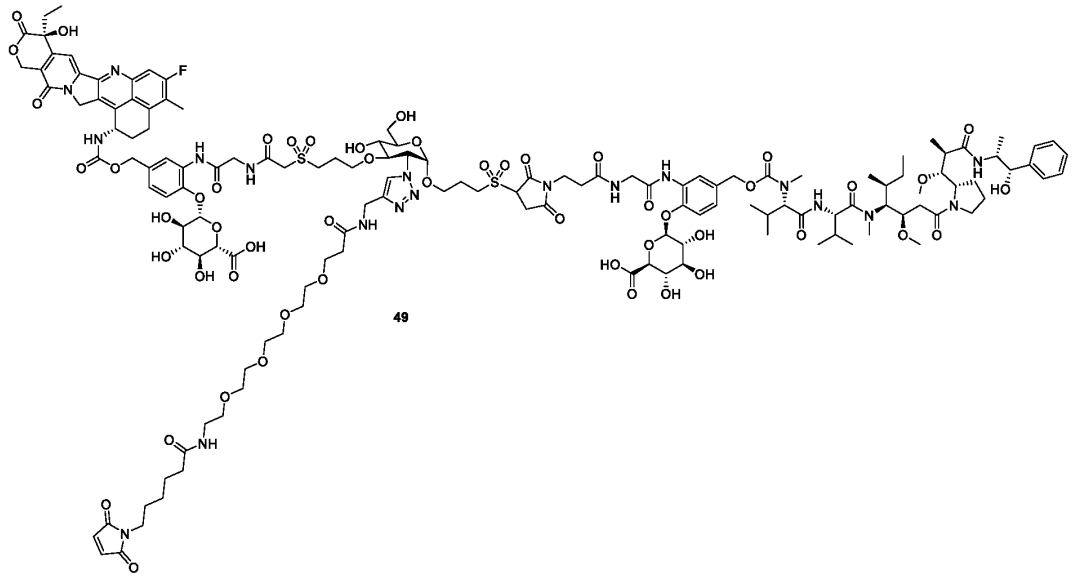
[00719] Step 5: The title compound **48-7** was prepared in analogy to **Example 10, Step 14** by using compound **34-19** and compound **48-6**. The product (212 mg, yield 61%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{247}H_{378}F_2N_{26}O_{87}S_3$: 5238.01; found: 1051.94 $[M+5H]^{5+}$.

[00720] Step 6: The title compound **48-8** was prepared in analogy to **Example 42, Step 27** by using compound **48-7**. The product (212 mg, yield 66%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{232}H_{368}F_2N_{26}O_{85}S_3$: 5015.77; found: 1004.14 $[M+5H]^{5+}$.

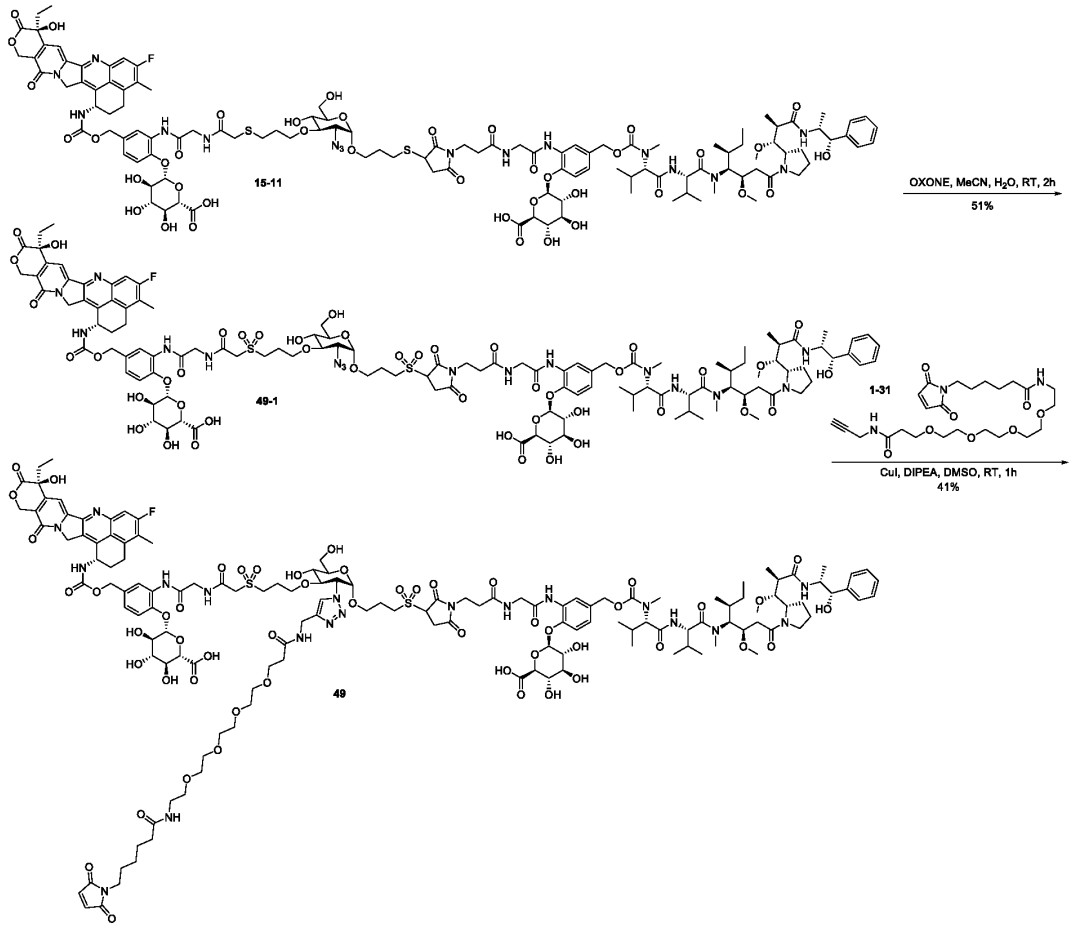
[00721] Step 7: The title compound **48** was prepared in analogy to **Example 47, Step 1** by using compound **1-28** and compound **48-8**. The product (8 mg, yield 24%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{242}H_{379}F_2N_{27}O_{88}S_3$: 5208.97; found: 1042.75 $[M+5H]^{5+}$.

Example 49. Synthesis of (2S,3S,4S,5R,6S)-6-(2-(2-(2-((3-(((2S,3R,4R,5S,6R)-2-(3-((1-(3-((2-((5-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-2-oxoethyl)amino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)sulfonyl)propoxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propyl)sulfonyl)acetamido)acetamido)-4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-

1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (49)



Synthetic scheme 49

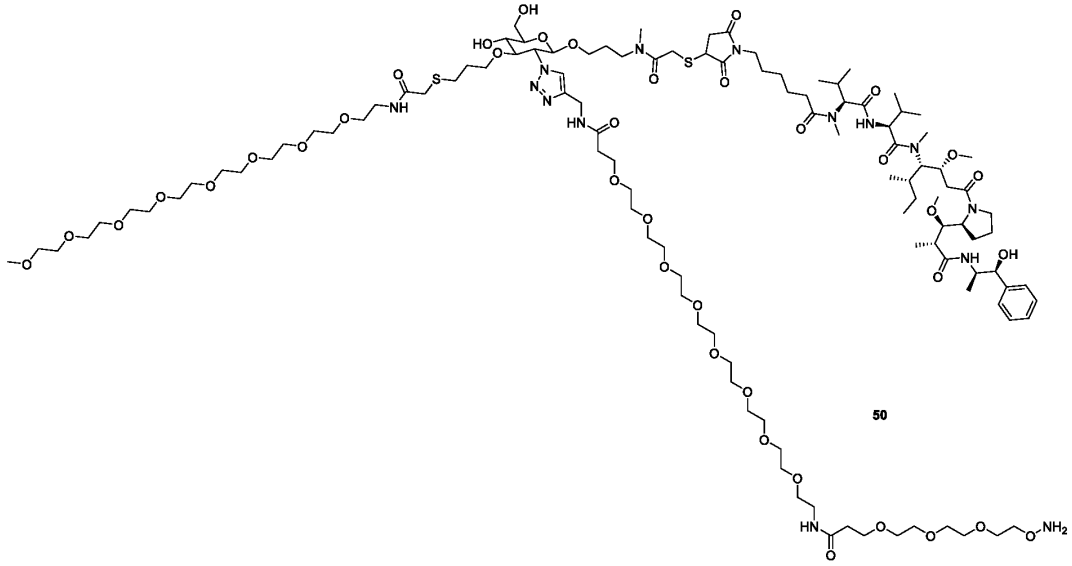


[00722] Step 1: To a solution of **15-11** (60 mg, 0.024 mmol) in acetonitrile (1 mL) was added a solution of OXONE (60 mg, 0.098 mmol) in H_2O (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C18 column, eluted with 50% acetonitrile in H_2O, 0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **49-1** (31 mg, yield 51%) as a light-yellow solid. LCMS: m/z calculated for $C_{116}H_{153}FN_{16}O_{44}S_2$: 2558.69; found: 1280.10 $[M+2H]^{2+}$.

[00723] Step 2: Under nitrogen atmosphere, to a solution of **49-1** (31 mg, 0.012 mmol) and **1-31** (8 mg, 0.016 mmol) in dimethyl sulfoxide (0.5 mL) was added N,N-diisopropylethylamine (6.25 mg, 0.048 mmol) and cuprous iodide (2.40 mg, 0.012 mmol) at room temperature. The

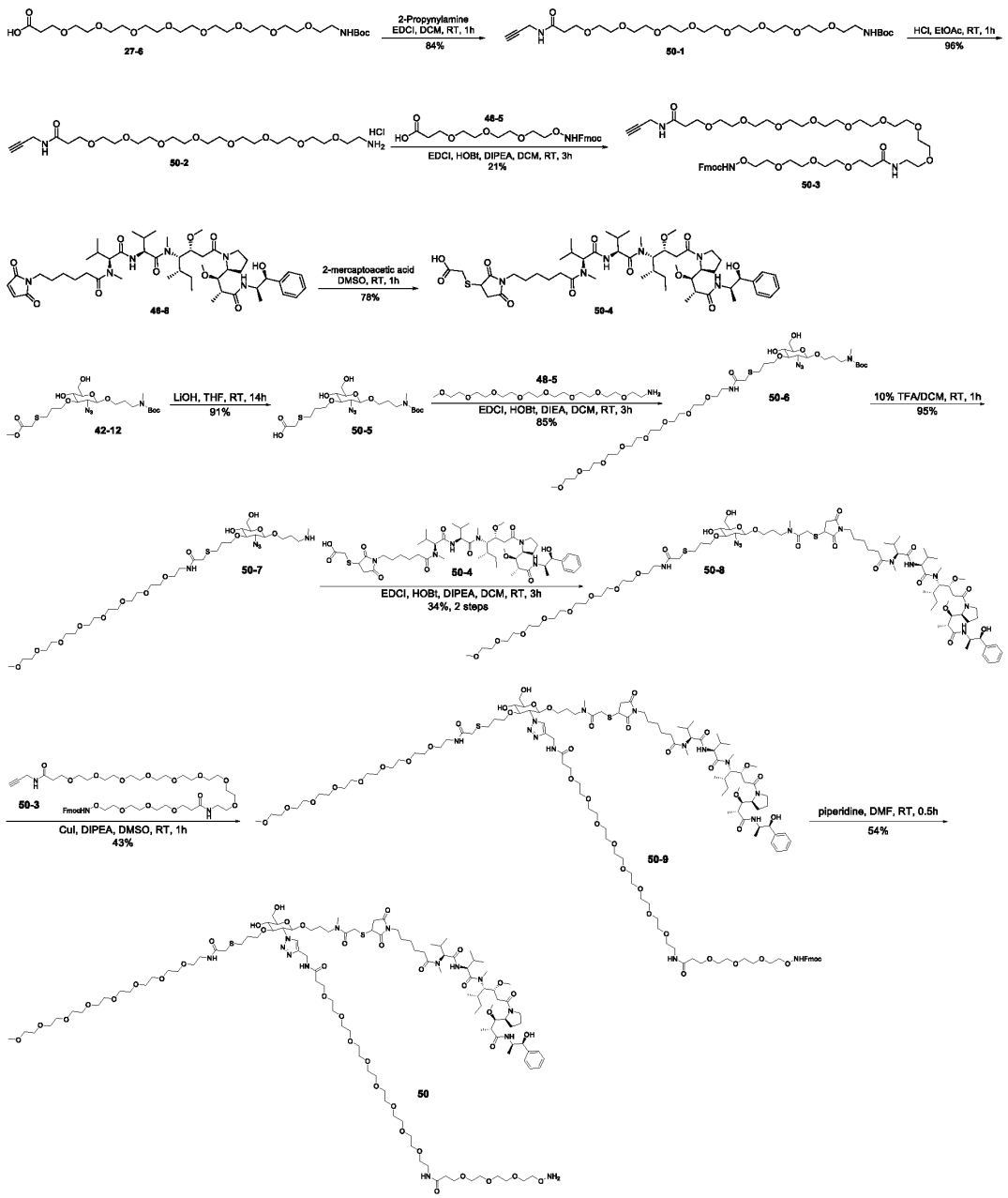
reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C18 column, eluted with 56% acetonitrile in H_2O, 0.1% trifluoroacetic acid condition). The desired components were lyophilized to afford the title compound **49** (15 mg, yield 41%) as a light-yellow solid. LCMS: m/z calculated for $C_{140}H_{190}FN_{19}O_{52}S_2$: 3054.26; found: 1527.41 $[M+2H]^{2+}$.

Example 50. Synthesis of 1-(3-(2-(2-(2-(aminooxy)ethoxy)ethoxy)ethoxy)propanamido)-N-((1-((2R,3R,4R,5S,6R)-2-(3-(2-((1-((3R,4S,7S,10S)-4-((S)-sec-butyl)-3-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9,12-trioxo-2-oxa-5,8,11-triazaheptadecan-17-yl)-2,5-dioxopyrrolidin-3-yl)thio)-N-methylacetamido)propoxy)-5-hydroxy-6-(hydroxymethyl)-4-((27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)tetrahydro-2H-pyran-3-yl)-1H-1,2,3-triazol-4-yl)methyl)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (50)



50

Synthetic scheme 50



[00724] Step 1: To a solution of **27-6** (1.50 g, 2.77 mmol) and 2-propynylamine (183.04 mg, 3.32 mmol) in dichloromethane (15 mL) was added EDCI (1.06 g, 5.54 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was

concentrated under reduced pressure and purified by silica gel column (eluted with 5% methanol in dichloromethane) to afford the title compound **50-1** (1.50 g, yield 94%) as a pale-yellow oil. LCMS: m/z calculated for $C_{27}H_{50}N_2O_{11}$: 578.70; found: 579.48 $[M+H]^+$.

[00725] Step 2: The title compound **50-2** was prepared in analogy to **Example 27**, **Step 23** by using compound **50-1** in 4 N hydrochloric acid in ethyl acetate. The concentrated crude product (1.29 g, yield 96%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{22}H_{42}N_2O_9$: 478.58; found: 479.15 $[M+3H]^+$.

[00726] Step 3: The title compound **50-3** was prepared in analogy to **Example 2**, **Step 5** by using compound **46-5** and compound **50-1**. The product (488 mg, yield 21%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{46}H_{69}N_3O_{16}$: 920.06; found: 920.51 $[M+H]^+$.

[00727] Step 4: The title compound **50-4** was prepared in analogy to **Example 18**, **Step 2** by using compound **46-8** and 2-mercaptoacetic acid. The product (1.29 g, yield 78%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{51}H_{82}N_6O_{12}S$: 1003.31; found: 1003.79 $[M+H]^+$.

[00728] Step 5: To a solution of compound **42-12** (387 mg, 0.74 mmol) in THF (5 mL) was added a solution of LiOH (62 mg, 1.48 mmol) in H_2O (1.5 mL). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 hour. After completion of the reaction, the mixture was adjusted pH to 3 with 0.4 N HCl. The resulting mixture was concentrated under reduced pressure, and the residue was extracted with ethyl acetate (10 mL × 3). The combined organic phases were concentrated to afford the title compound **50-5** (344 mg, yield 91%), which was used into the next step with further purification. LCMS: m/z calculated for $C_{20}H_{36}N_4O_9S$: 508.59; found: 409.52 $[M-Boc+H]^+$.

[00729] Step 6: To a solution of compound **50-5** (344mg, 0.67 mmol) and compound **48-5** (322 mg, 0.84 mmol) in dichloromethane (5 mL) was added HOBt (95 mg, 0.7 mmol), DIPEA (362 mg, 2.8 mmol) and EDCI (268 mg, 1.4 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 3 h. After completion of the reaction, the mixture was diluted with dichloromethane (50 mL) and washed with water (30 mL × 3). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The

residue was purified by silica gel column chromatography (eluted with 9% methanol in dichloromethane) to afford the title compound **50-6** (540 mg, yield 85%) as a colorless oil. LCMS: m/z calculated for $C_{37}H_{71}N_5O_{16}S$: 874.05; found: 874.57 $[M+H]^+$.

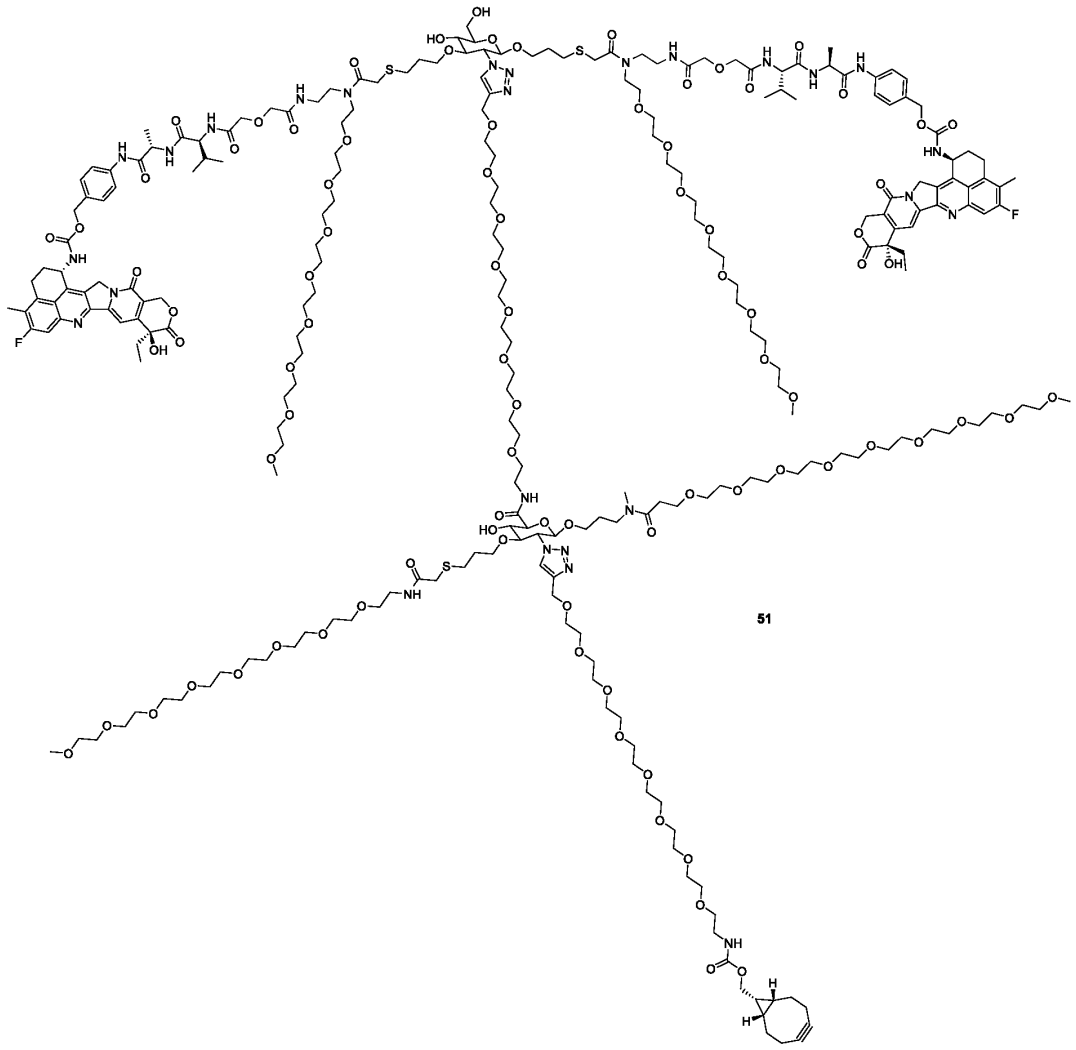
[00730] Step 7: To a solution of compound **50-6** (100 mg, 0.114 mmol) in dichloromethane (4 mL) was added TFA (0.4 mL). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was concentrated and azeotroped with dichloromethane (30 mL × 3) under reduced pressure to afford the title compound **50-7** (120 mg), which was used into the next step with further purification. LCMS: m/z calculated for $C_{32}H_{63}N_5O_{14}S$: 773.94; found: 774.50 $[M+H]^+$.

[00731] Step 8: To a solution of compound **50-7** (120 mg, 0.114 mmol) in dichloromethane (5 mL) was added compound **50-4** (125 mg, 0.124 mmol), HOBt (15 mg, 0.114 mmol), DIPEA (74 mg, 0.51 mmol) and EDCI (44 mg, 0.228 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 3 h. After completion of the reaction, the mixture was diluted with dichloromethane (50 mL) and washed with water (20 mL × 3). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **50-8** (69 mg, yield 34% over 2 steps) as a pale-yellow oil. LCMS: m/z calculated for $C_{83}H_{143}N_{11}O_{25}S_2$: 1759.23; found: 880.35 $[M+2H]^{2+}$.

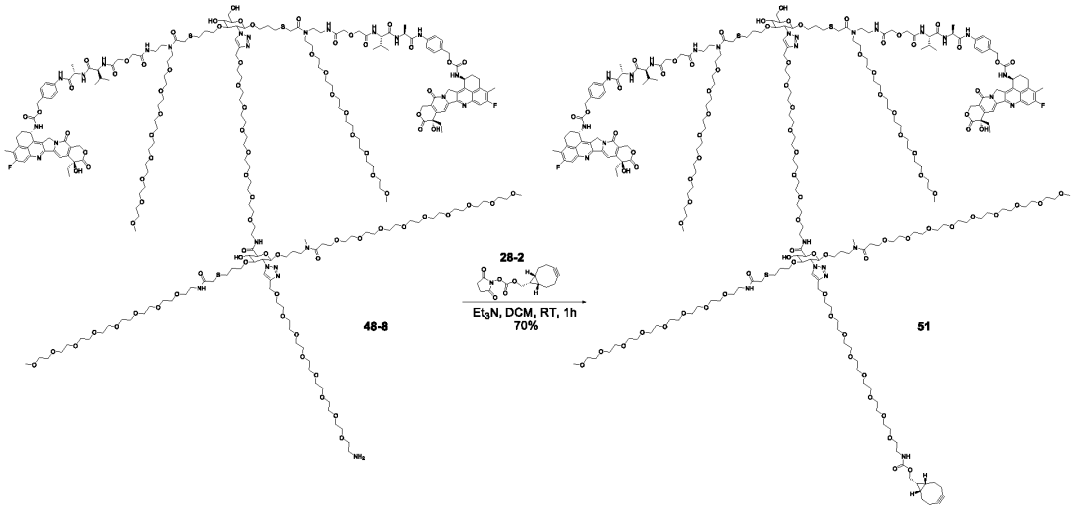
[00732] Step 9: under nitrogen atmosphere, to a solution of compound **50-8** (127 mg, 0.072 mmol) and compound **50-3** (81 mg, 0.088 mmol) in DMSO (1 mL) was added CuI (14 mg, 0.144 mmol) and DIEA (74 mg, 0.576 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **50-9** (40 mg, yield 43%) as a pale-yellow oil. LCMS: m/z calculated for $C_{129}H_{212}N_{14}O_{41}S_2$: 2679.29; found: 893.99 $[M+3H]^{3+}$.

[00733] Step 10: To a solution of compound **50-9** (40 mg, 0.0149 mmol) in DMF (1 mL) was added piperidine (5.6 mg, 0.0745 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 0.5 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C18 column, eluted with acetonitrile and water, 0.1% TFA condition). The desired components were lyophilized to afford the title compound **50** (20 mg, yield 54%) as a pale-yellow oil. LCMS: m/z calculated for $C_{114}H_{202}N_{14}O_{39}S_2$: 2457.05; found: 819.96 $[M+3H]^{3+}$.

Example 51. Synthesis of 4-((36S,39S)-26-(2-((3-(((2R,3R,4R,5S,6R)-3-(4-(1-((2S,3S,4R,5R,6R)-5-(4-(1-((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-azanonacosan-29-yl)-1H-1,2,3-triazol-1-yl)-3-hydroxy-6-((30-methyl-29-oxo-2,5,8,11,14,17,20,23,26-nonaoxa-30-azatritriacontan-33-yl)oxy)-4-((27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)tetrahydro-2H-pyran-2-yl)-1-oxo-5,8,11,14,17,20,23,26-octaoxa-2-azaheptacosan-27-yl)-1H-1,2,3-triazol-1-yl)-2-((26-((2S,5S)-1-((4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)phenyl)amino)-5-isopropyl-2-methyl-1,4,7,11-tetraoxo-9-oxa-3,6,12-triazatetradecan-14-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propyl)thio)acetyl)-36-isopropyl-39-methyl-30,34,37-trioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35,38-tetraazatetracontan-40-amido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (51)



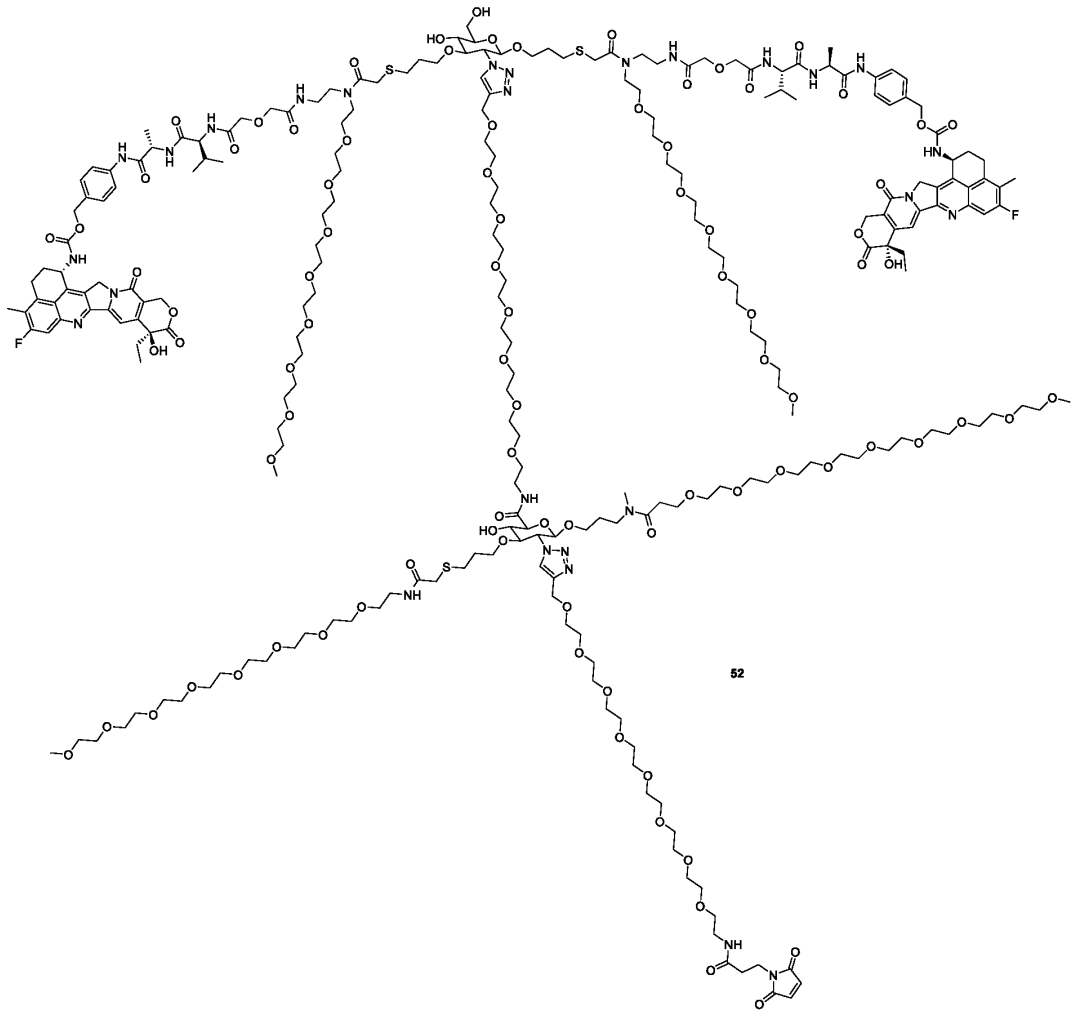
Synthetic scheme 51



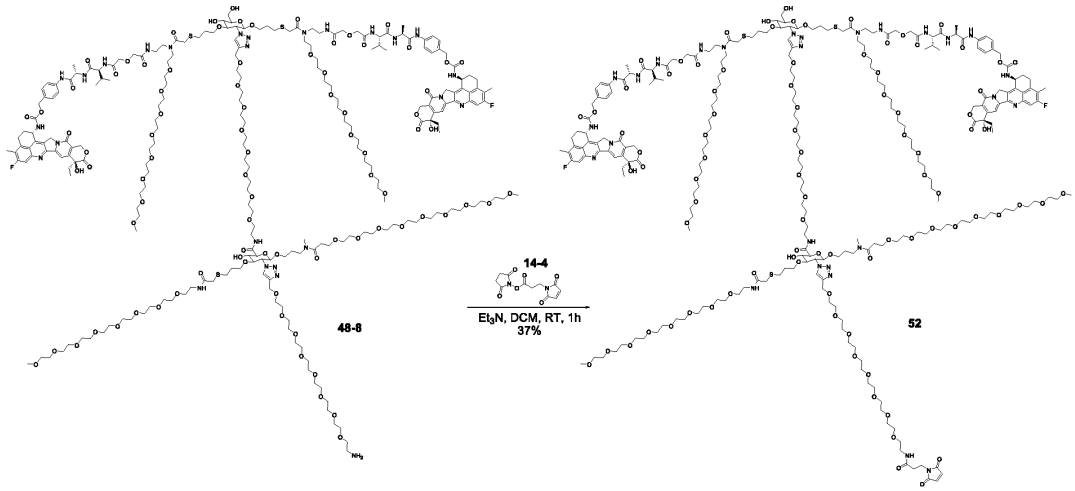
[00734] Step 1: To a solution of compound 48-8 (40.0 mg, 0.008 mmol) in dichloromethane (2 mL) was added triethylamine (1.6 mg, 0.016 mmol) and compound **28-2** (3.0 mg, 0.01 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was concentrated and purified by pre-HPLC (C18 column, eluted with acetonitrile and water, 0.1% AcOH condition). The desired components were lyophilized to afford the title compound **51** (29 mg, yield 70%) as a pale-yellow solid. LCMS: m/z calculated for $C_{243}H_{380}F_2N_{26}O_{87}S_3$: 5191.98; found: 1298.91 $[M+4H]^{4+}$.

Example 52. Synthesis of ((((36S,36'S,39S,39'S)-26,26'-(2,2'-(((((2R,3R,4R,5S,6R)-3-(4-(1-((2S,3S,4R,5R,6R)-5-(4-(29-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-27-oxo-2,5,8,11,14,17,20,23-octaoxa-26-azanonacosyl)-1H-1,2,3-triazol-1-yl)-3-hydroxy-6-((30-methyl-29-oxo-2,5,8,11,14,17,20,23,26-nonaoxa-30-azatritriacontan-33-yl)oxy)-4-((27-oxo-2,5,8,11,14,17,20,23-octaoxa-29-thia-26-azadotriacontan-32-yl)oxy)tetrahydro-2H-pyran-2-yl)-1-oxo-5,8,11,14,17,20,23,26-octaoxa-2-azaheptacosan-27-yl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(36-isopropyl-39-methyl-30,34,37-trioxo-2,5,8,11,14,17,20,23,32-nonaoxa-26,29,35,38-tetraazatetracontan-40-oyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene) bis(((1S,9S)-9-ethyl-5-fluoro-9-

hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate) (52)

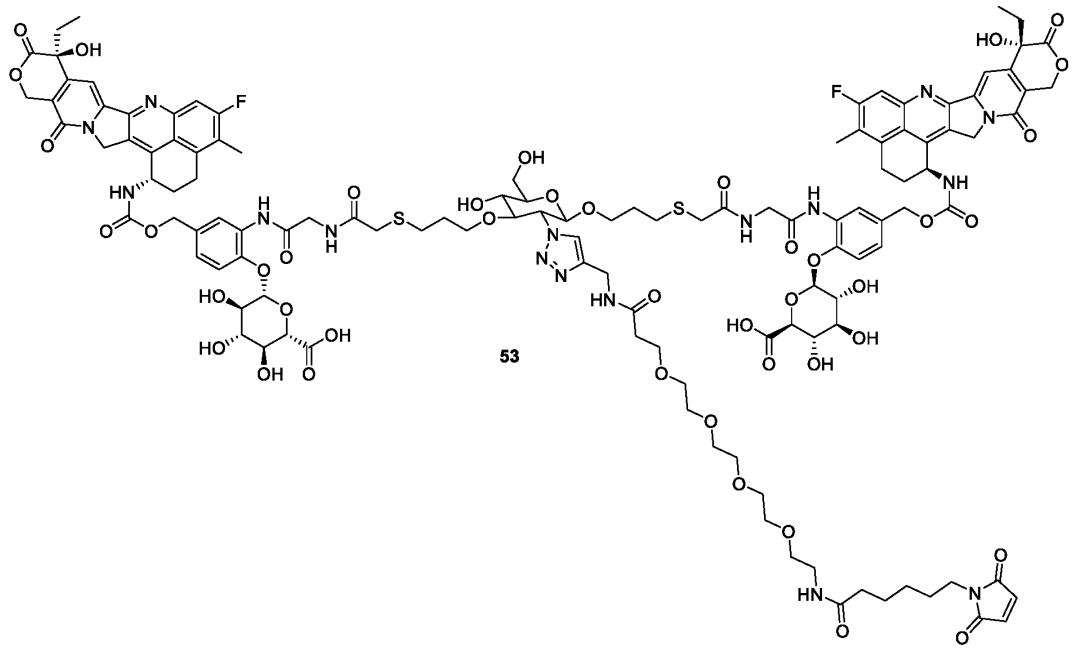


Synthetic scheme 52



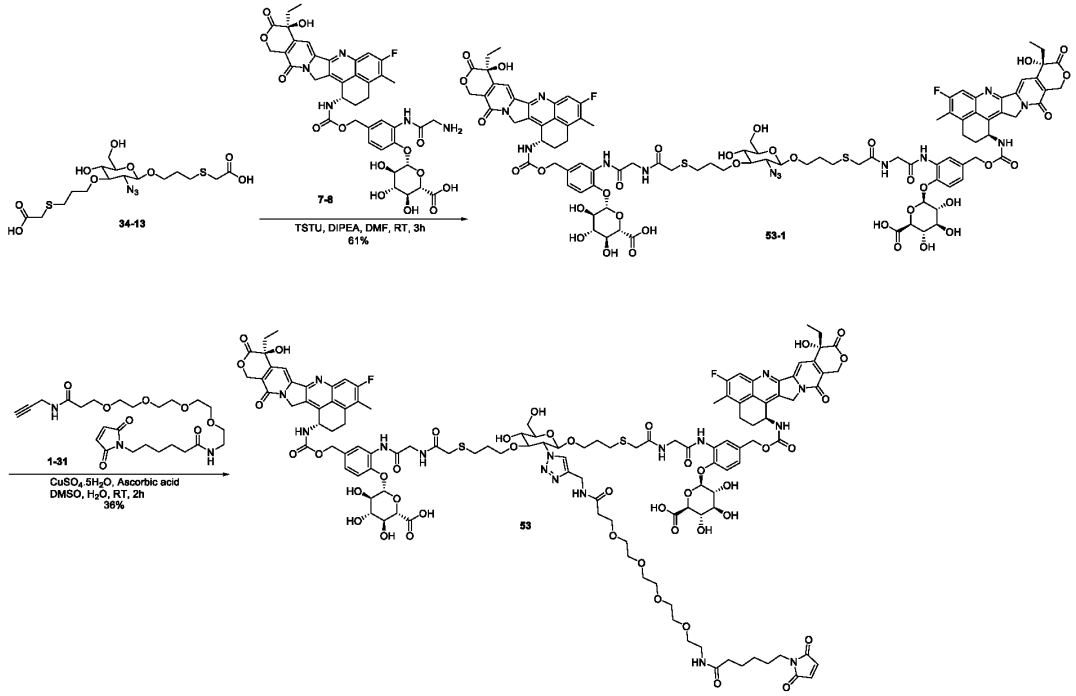
[00735] Step 1: The title compound **52** was prepared in analogy to **Example 1, Step 21** by using compound **14-4** and compound **48-8**. The product (12 mg, yield 37%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{239}H_{373}F_2N_{27}O_{88}S_3$: 5166.89; found: 1034.30 $[M+5H]^{5+}$.

Example 53. Synthesis of (2S,2'S,3S,3'S,4S,4'S,5R,5'R,6S,6'S)-6,6'-((((2,2'-((2,2'-(((((2R,3R,4R,5S,6R)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2,4-diyl)bis(oxy))bis(propane-3,1-diyl))bis(sulfanediyl))bis(acetyl))bis(azanediyl))bis(acetyl))bis(azanediyl))bis(4-(((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)-2,1-phenylene))bis(oxy))bis(3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid) (53)



53

Synthetic scheme 53

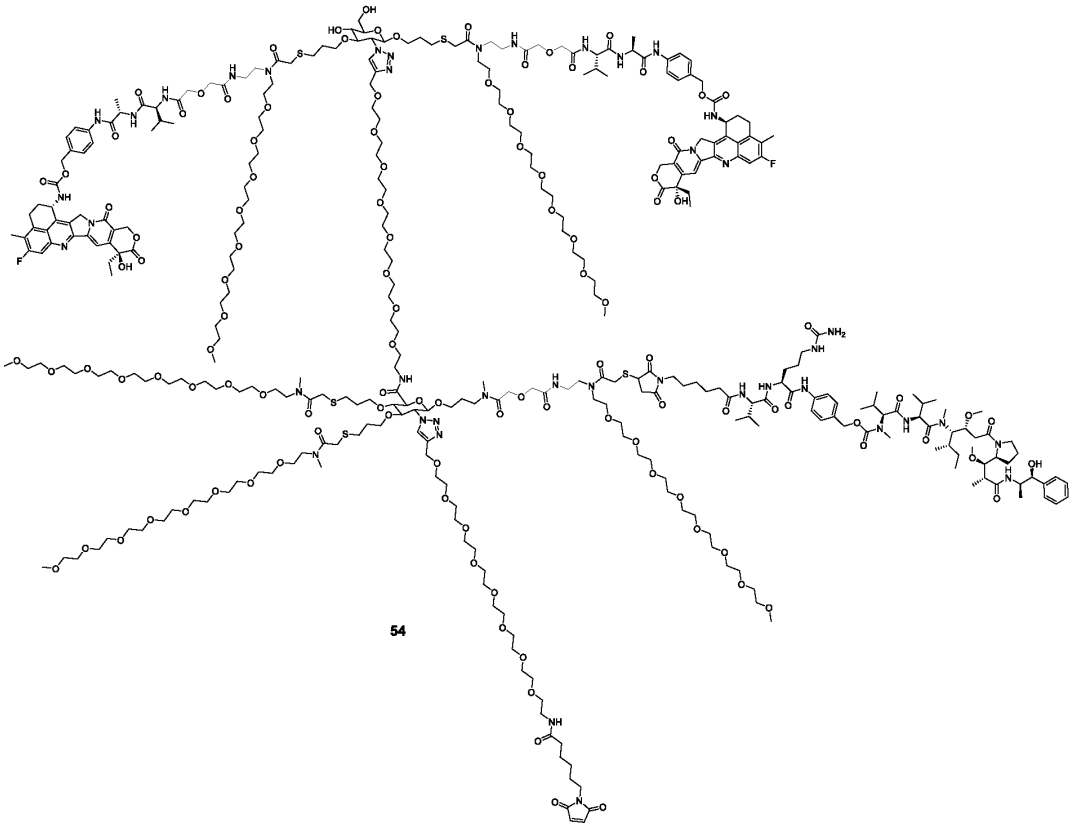


[00736] Step 1: To a solution of compound **34-13** (200.00 mg, 0.43 mmol) in N,N-dimethylformamide (3 mL) was added N,N-diisopropylethylamine (164.85 mg, 1.28 mmol) and TSTU (323.63 mg, 1.08 mmol). The reaction mixture was stirred at room temperature for 1 h. Then the above mixture was added dropwise to a solution of compound **7-8** (716.98 mg, 0.86 mmol) and N,N-diisopropylethylamine (55.47 mg, 0.43 mmol) in N,N-dimethylformamide (5 mL). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 47% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **53-1** (550.00 mg, yield 59%) as a yellow solid. LCMS: m/z calculated for $C_{96}H_{103}F_2N_{13}O_{35}S_2$: 2101.05; found: 1051.45 $[M+2H]^+$.

[00737] Step 2: To a solution of compound **53-1** (50.00 mg, 0.02 mmol) and compound **1-31** (11.79 mg, 0.02 mmol) in dimethyl sulfoxide (2 mL) and H_2O (0.3 mL) was added copper sulfate pentahydrate (19.98 mg, 0.08 mmol) and L(+)-ascorbic acid (31.70 mg, 0.18 mmol). The reaction mixture was stirred at room temperature under N_2 atmosphere for 2 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (0.3mL). The resulting mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile in water, TFA condition) to afford the title compound **53** (22.50 mg, yield 36%) as a yellow solid. LCMS: m/z calculated for $C_{120}H_{140}F_2N_{16}O_{43}S_2$: 2596.63; found: 1298.82 $[M+2H]^+$.

Example 54. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-

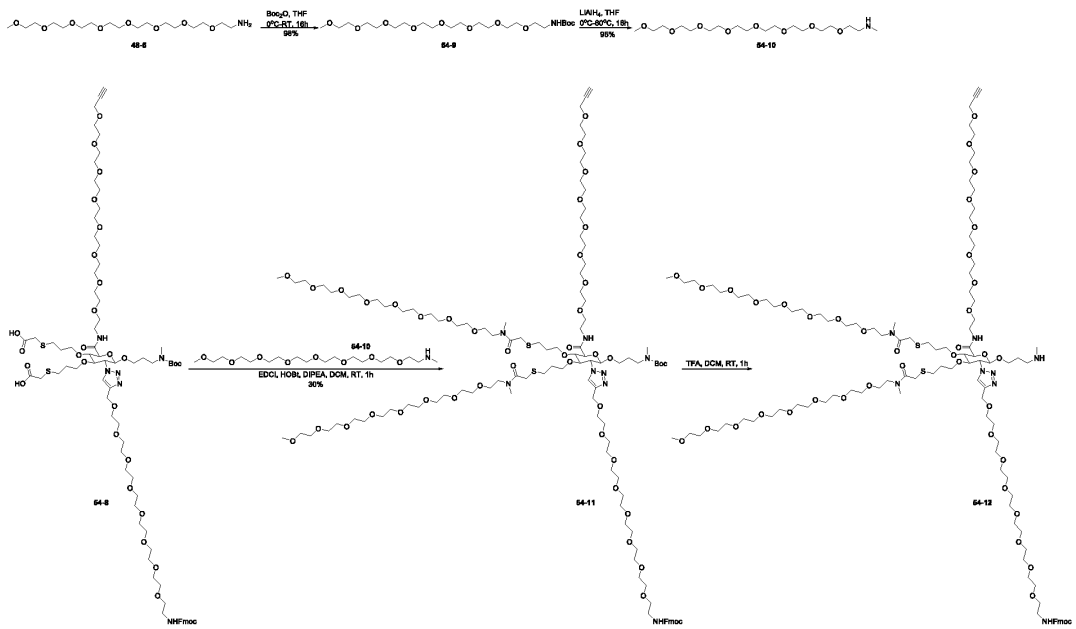
[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl-methyl-amino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (54)

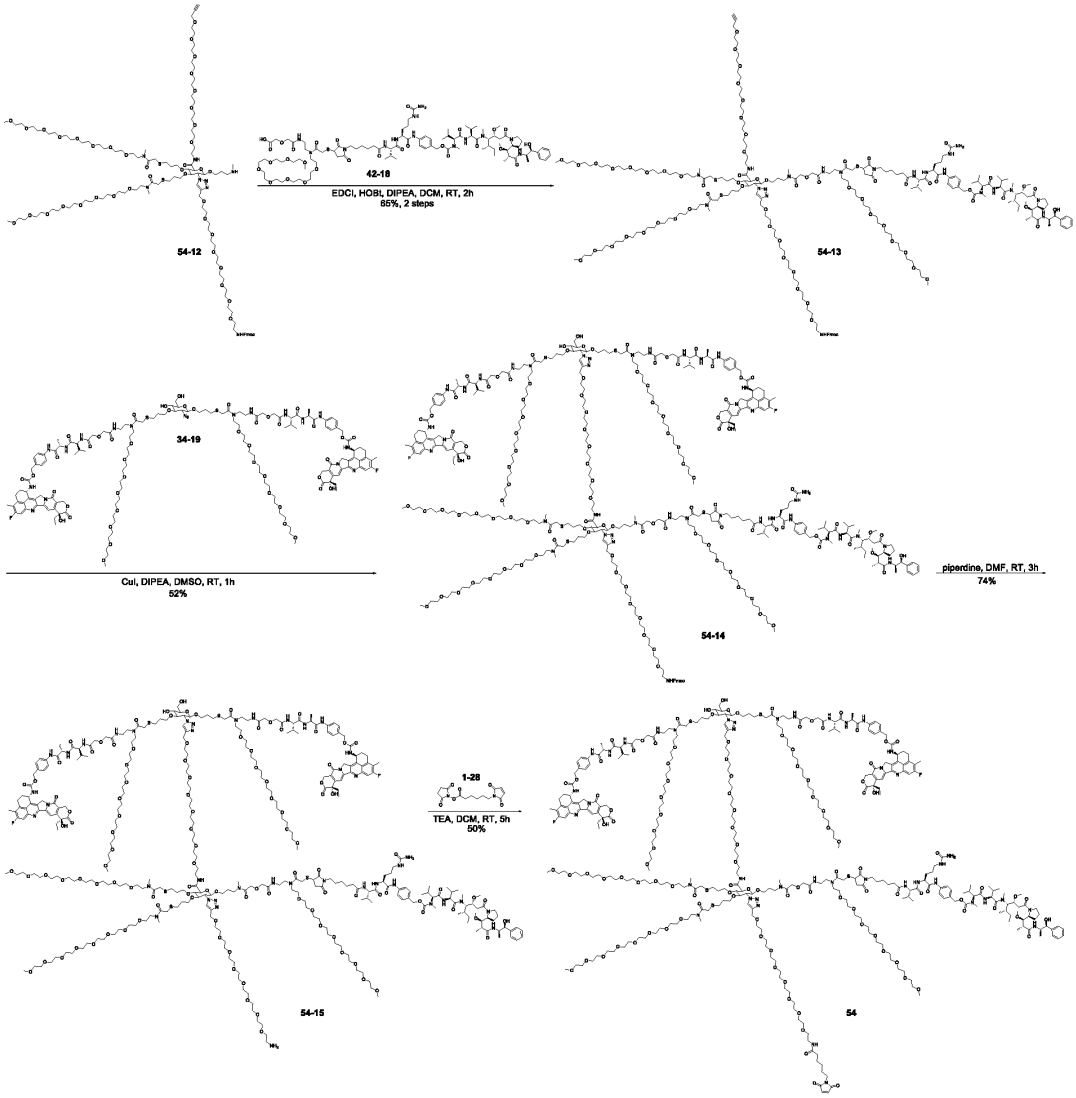


54

Synthetic scheme 54




Boc2O, THF
0°C-RT, 16h
98%
LiAlH4, THF
0°C-80°C, 16h
98%
48-5
54-9
54-10
64-10
EDCI, HOBt, DIPEA, DCM, RT, 1h
30%
TFA, DCM, RT, 1h
64-8
64-11
64-12



[00738] Step 1: The title compound **54-1** was prepared in analogy to **Example 9, Step 1** by using compound **42-4**, tert-butylchlorodimethylsilaneand triethylamine in dichloromethane**.** The product (19.3 g, yield 76%) was obtained as a colorless oil.

[00739] Step 2: The title compound **54-2** was prepared in analogy to **Example 1, Step 4** by using compound **54-1.** The product (19.2 g, yield 87%) was obtained as a pale-yellow oil.

[00740] Step 3: The title compound **54-3** was prepared in analogy to **Example 1, Step 5** by using compound **54-2.** The product (22.22 g, yield 86%) was obtained as a pale-yellow oil.

[00741] Step 4: To a mixture of compound **54-3** (10 g, 12.770 mmol) in anhydrous tetrahydrofuran (100 mL) was added 1M TBAF (THF solution, 19.155 mL, 19.155 mmol) in tetrahydrofuran at 0°C. The mixture was stirred at room temperature for 2h. After completion of the reaction, the mixture the mixture was poured into water (100 mL) and extracted with ethyl acetate (100 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to give the title compound **54-4** (7.21 g, yield 84%) as a pale-yellow oil. LCMS: m/z calculated for $C_{27}H_{48}N_4O_{11}S_2$: 668.82; found: 670.18 $[M+H]^+$.

[00742] Step 5: The title compound **54-5** was prepared in analogy to **Example 42, Step 9** by using compound **54-4.** The product (4.80 g, yield 66%) was obtained as a yellow oil. $C_{27}H_{46}N_4O_{12}S_2$: 682.80; found: 684.17 $[M+H]^+$.

[00743] Step 6: The title compound **54-6** was prepared in analogy to **Example 42, Step 20** by using compound **54-5** and compound **42-20**. The product (1.55 g, yield 76%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{61}H_{93}N_5O_{22}S_2$: 1312.55; found: 1313.90 $[M+H]^+$.

[00744] Step 7: Under nitrogen atmosphere, to a mixture of compound **54-6** (1.4 g, 1.066 mmol) in anhydrous dichloromethane (14 mL) was added compound **42-19** (521 mg, 1.279 mmol), N, N-diisopropylethylamine (0.926 mL, 5.330 mmol), 1-Hydroxybenzotriazole (144 mg, 1.066 mmol) and EDCI (613 mg , 3.198 mmol). The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (150 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 5% methyl alcohol in dichloromethane) to give the title compound **54-7** (1.15 g, yield 61%) as a yellow oil. LCMS: m/z calculated for $C_{80}H_{128}N_6O_{29}S_2$: 1702.04; found: 1703.65 $[M+H]^+$.

[00745] Step 8: The title compound **54-8** was prepared in analogy to Example **42**, Step 22 by using compound **54-7**. The product (793 mg, yield 80%) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{78}H_{124}N_6O_{29}S_2$ 1673.98; found: 1675.45 $[M+H]^+$.

[00746] Step 9: Under nitrogen atmosphere, to a mixture of compound **48-5** (3 g, 7.823 mmol) in tetrahydrofuran (30 mL) was added bis(tert-butoxycarbonyl)oxide (3.63 mL, 15.646 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was poured into water (200 mL) and extracted with dichloromethane (200 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 5% methyl alcohol in dichloromethane) to afford the title compound **54-9** (3.70 g, yield 98%) as a colorless oil. LCMS: m/z calculated for $C_{22}H_{45}NO_{10}$: 483.60; found: 485.08 $[M+H]^+$.

[00747] Step 10: Under nitrogen atmosphere, to a mixture of compound **54-9** (3.34 g, 6.906 mmol) in anhydrous tetrahydrofuran (35 mL) was added slowly lithium aluminum hydride (2.5M in THF, 8.29 mL, 20.72 mmol) at 0°C. The mixture was stirred at 60 °C for 16 h. After completion of the reaction, the mixture was diluted with anhydrous tetrahydrofuran (100 mL) and treated slowly with $Na_2SO_4.10H_2O$ at 0 °C until no bubble was formed. The mixture was treated with a small amount of water and stirred at room temperature for further 30 min. The resulting mixture was filtered and concentrated to afford the title compound **54-10** (2.60 g, yield 95%) as a yellow oil. LCMS: m/z calculated for $C_{18}H_{39}NO_8$: 397.51; found: 398.98 $[M+H]^+$.

[00748] Step 11: To a solution of **56-8** (668.00 mg, 0.40 mmol), **56-10** (333.11 mg, 0.84 mmol) and N,N-diisopropylethylamine (258.00 mg, 2.00 mmol) in dichloromethane (11 mL) was added 1-hydroxybenzotriazole (81.00 mg, 0.60 mmol) and EDCI (230.04 mg, 1.2 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 68% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **56-11** (291.00 mg, yield 30%)

as a light-yellow oil. LCMS: m/z calculated for $C_{114}H_{198}N_8O_{43}S_2$: 2432.97; found: 1217.19 $[M+2H]^+$.

[00749] Step 12: To a solution of **56-11** (291.00 mg, 0.12 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.6 mL) at room temperature. Then the mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure to afford the title compound **56-12** (514.00 mg, crude) as a yellow oil. LCMS: m/z calculated for $C_{109}H_{190}N_8O_{41}S_2$: 2332.85; found: 1167.24 $[M+2H]^+$.

[00750] Step 13: To a solution of **56-12** (514.00 mg, 0.12 mmol), **42-18** (231.22 mg, 0.12 mmol) and N,N-diisopropylethylamine (77.40 mg, 0.60 mmol) in dichloromethane (9 mL) was added 1-hydroxybenzotriazole (24.30 mg, 0.18 mmol) and EDCI (69.01 mg, 0.36 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **56-13** (330.00 mg, two steps, yield 65%) as a light-yellow oil. LCMS: m/z calculated for $C_{202}H_{341}N_{21}O_{68}S_3$: 4248.21; found: 1416.72 $[M+3H]^+$.

[00751] Step 14: Under nitrogen atmosphere, to a mixture of compound **34-19** (57 mg, 0.019 mmol) and compound **56-13** (97 mg, 0.023 mmol) in dimethyl sulfoxide (2 mL) was added N,N-diisopropylethylamine (0.026 mL, 0.152 mmol) and cuprous iodide (7 mg, 0.038 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **54-14** (73 mg, yield 52%) as a yellow oil. LCMS: m/z calculated for $C_{344}H_{538}F_2N_{40}O_{113}S_5$: 7240.55; found: 1810.84 $[M+4H]^{4+}$.

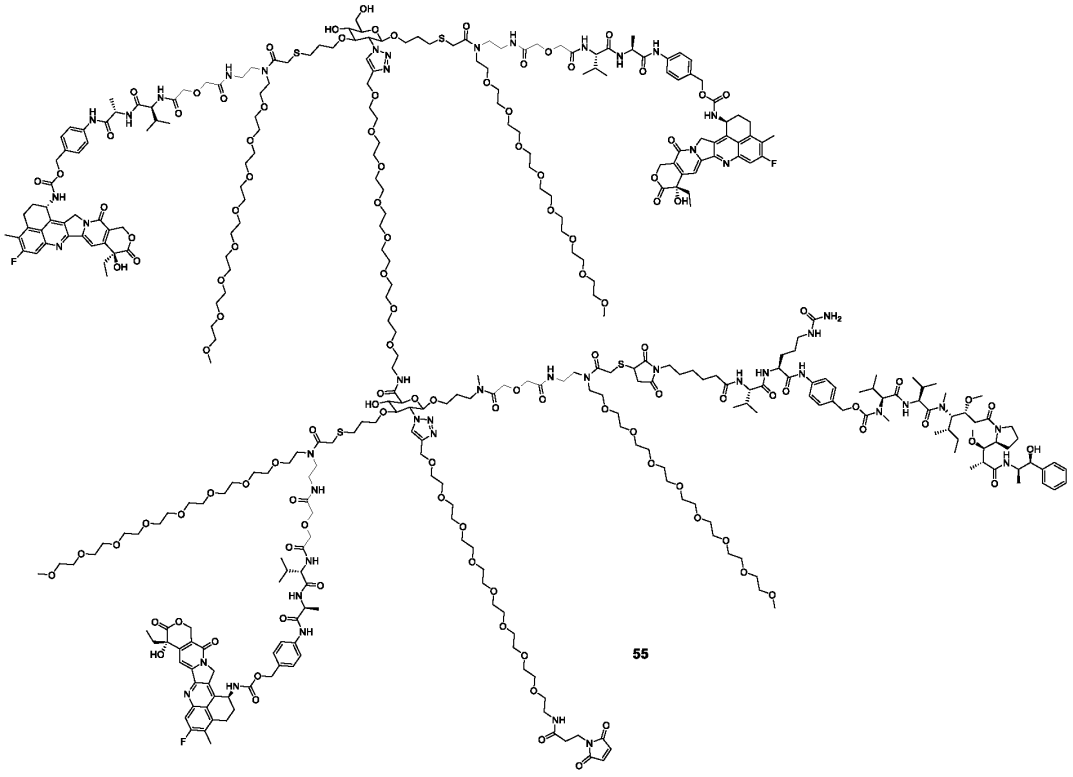
[00752] Step 15: Under nitrogen atmosphere, to a mixture of compound **54-14** (68 mg, 0.009 mmol) in anhydrous N, N-dimethylformamide (1 mL) was added piperidine (0.007 mL, 0.068mmol). The mixture was stirred at room temperature for 3 h. After completion of the

reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **54-15** (49.4 mg, yield 74%) as a yellow oil. LCMS: m/z calculated for $C_{329}H_{528}F_2N_{40}O_{111}S_5$: 7018.31; found: 1755.40 $[M+4H]^{4+}$.

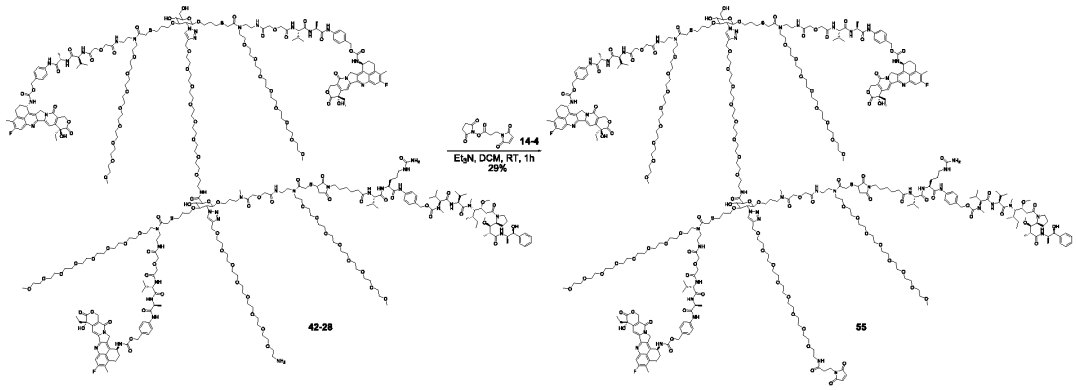
[00753] Step 16: Under nitrogen atmosphere, to a mixture of compound **54-15** (29 mg, 0.004 mmol) in anhydrous dichloromethane (2 mL) was added triethylamine (0.002 mL, 0.014mmol) and compound **1-28** (2.5 mg, 0.008 mmol). The mixture was stirred at room temperature for 5 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **54** (15.9 mg, yield 50%) as a colorless oil. LCMS: m/z calculated for $C_{339}H_{539}F_2N_{41}O_{114}S_5$: 7211.51; found: 1803.95 $[M+4H]^{4+}$.

Example 55. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-

ethyl]sulfanylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (55)



Synthetic scheme 55



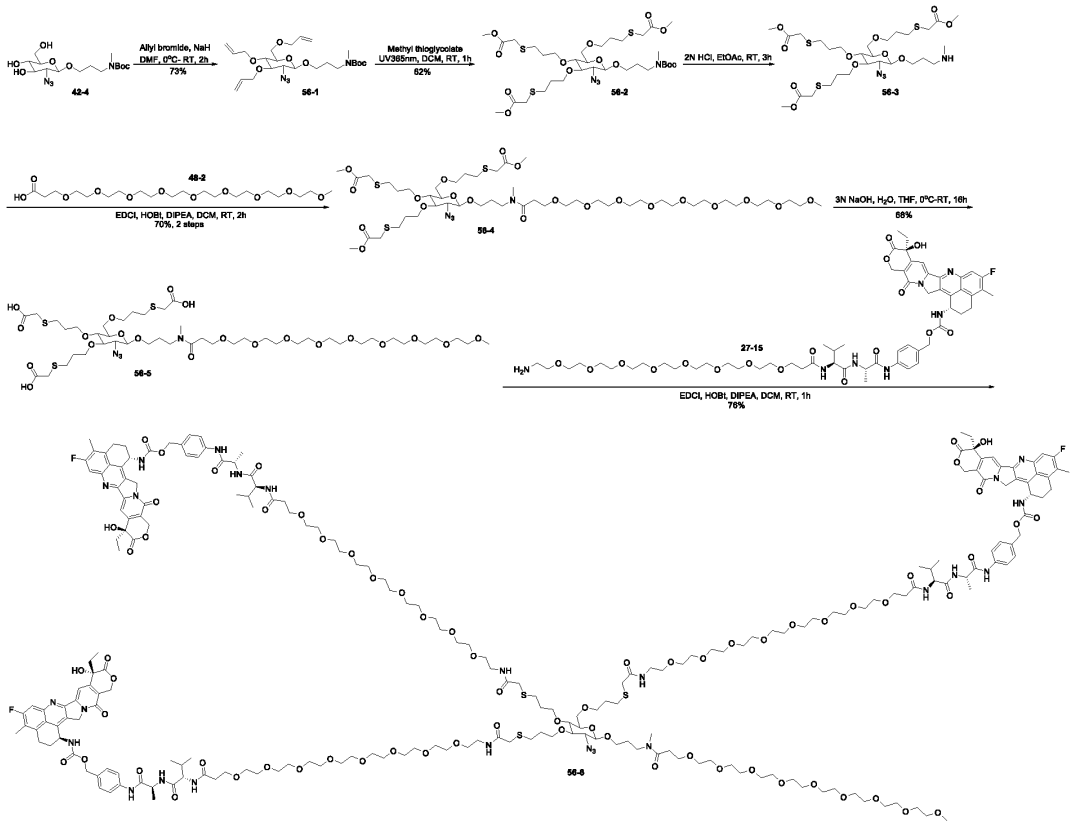
[00754] Step 1: The title compound **55** was prepared in analogy to **Example 1, Step 21** by using compound **42-29** and compound **14-4**. The product (9 mg, yield 29%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{358}H_{536}F_3N_{47}O_{116}S_4$: 7539.67; found: 1885.58 $[M+4H]^{4+}$.

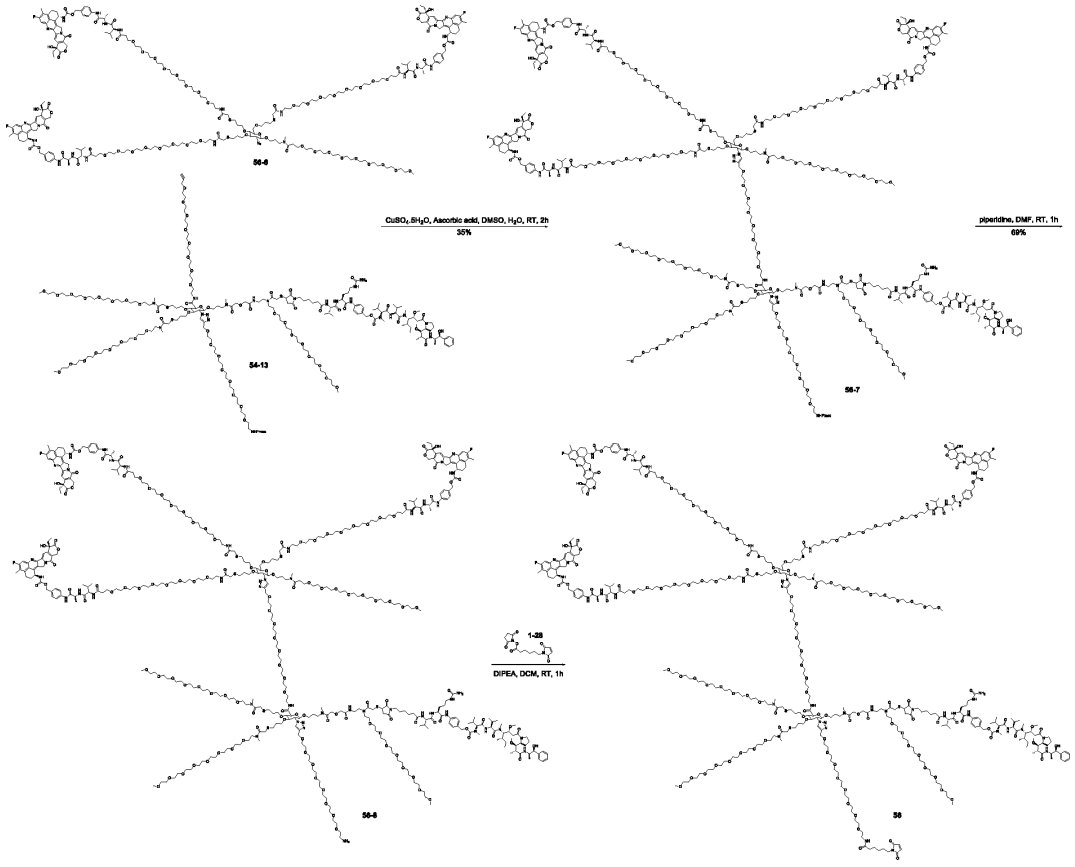
Example 56. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxymethyl]-2-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-3-yl]triazol-4-

yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl-methyl-amino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (56)



Synthetic scheme 56


Allyl bromide, NaH
DMF, 0℃- RT, 2h
73%
Methyl thioglycolate
UV365nm, DCM, RT, 1h
82%
2N HCl, EtOAc, RT, 3h
42-4
56-1
56-2
56-3
48-2
EDCI, HOBt, DIPEA, DCM, RT, 2h
70%, 2 steps
56-4
3N NaOH, H2O, THF, 0℃-RT, 16h
68%
56-5
27-15
EDCI, HOBt, DIPEA, DCM, RT, 1h
76%
56-6



[00755] Step 1: The title compound **56-1** was prepared in analogy to **Example 27, Step 1** by using compound **42-4**. The product (3.56 g, yield 73%) was obtained as a colorless oil.

[00756] Step 2: The title compound **56-2** was prepared in analogy to **Example 27, Step 2** by using compound **56-1**. The product (3.63 g, yield 62%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{33}H_{58}N_4O_{13}S_3$: 815.02; found: 837.37 $[M+Na]^+$.

[00757] Step 3: To a mixture of compound **56-2** (1 g, 1.227 mmol) in ethyl acetate (8 mL) was added 2M hydrochloric acid ethyl acetate solution (8 mL). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated to afford the title compound **56-3** (950 mg, crude) as a colorless oil, which was used into the next step without further purification. LCMS: m/z calculated for $C_{28}H_{50}N_4O_{11}S_3$: 714.90; found: 837.37 $[M+Na]^+$.

[00758] Step 4: Under nitrogen atmosphere, to a solution of compound **56-3** (885 mg, 1.179 mmol) in anhydrous dichloromethane (10 mL) was added compound **48-2** (591 mg, 1.297 mmol), N,N-diisopropylethylamine (0.819 mL, 4.716 mmol), 1-hydroxybenzotriazole (240 mg, 1.179 mmol) and EDCI (613 mg , 2.358 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (150 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 2% methyl alcohol in dichloromethane) to give the title compound **56-4** (1 g, yield 70% over 2steps) as a colorless oil. LCMS: m/z calculated for $C_{48}H_{88}N_4O_{21}S_3$: 1153.42; found: 1154.60 $[M+H]^+$.

[00759] Step 5: The title compound **56-5** was prepared in analogy to **Example 4, Step 5** by using compound **56-4**. The concentrated crude product (594 mg, yield 68%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{45}H_{82}N_4O_{21}S_3$: 1111.34; found: 1112.48 $[M+H]^+$.

[00760] Step 6: To a solution of **56-5** (102 mg, 0.09 mmol), **27-15** (424.20 mg, 0.36 mmol) and N,N-diisopropylethylamine (185.76 mg, 1.44 mmol) in dichloromethane (10 mL) was added 1-hydroxybenzotriazole (60.75 mg, 0.45 mmol) and EDCI (138.02 mg, 0.72 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 55% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **56-6** (319.00 mg, yield 76%) as a light-yellow solid. LCMS: m/z calculated for $C_{222}H_{316}F_3N_{25}O_{69}S_3$: 4592.25; found: 1531.35 $[M+3H]^+$.

[00761] Step 7: To a solution of **54-13** (143.39 mg, 0.03 mmol) and **56-6** (155.00 mg, 0.03 mmol) in dimethyl sulfoxide (2 mL) and H_2O (0.3 mL) was added copper sulfate pentahydrate (22.47 mg, 0.09 mmol) and L(+)-ascorbic acid (47.55 mg, 0.27 mmol). The mixture was stirred at RT under N_2 atmosphere for 2 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (0.3 mL) and filtered. The filtrate was purified by reverse

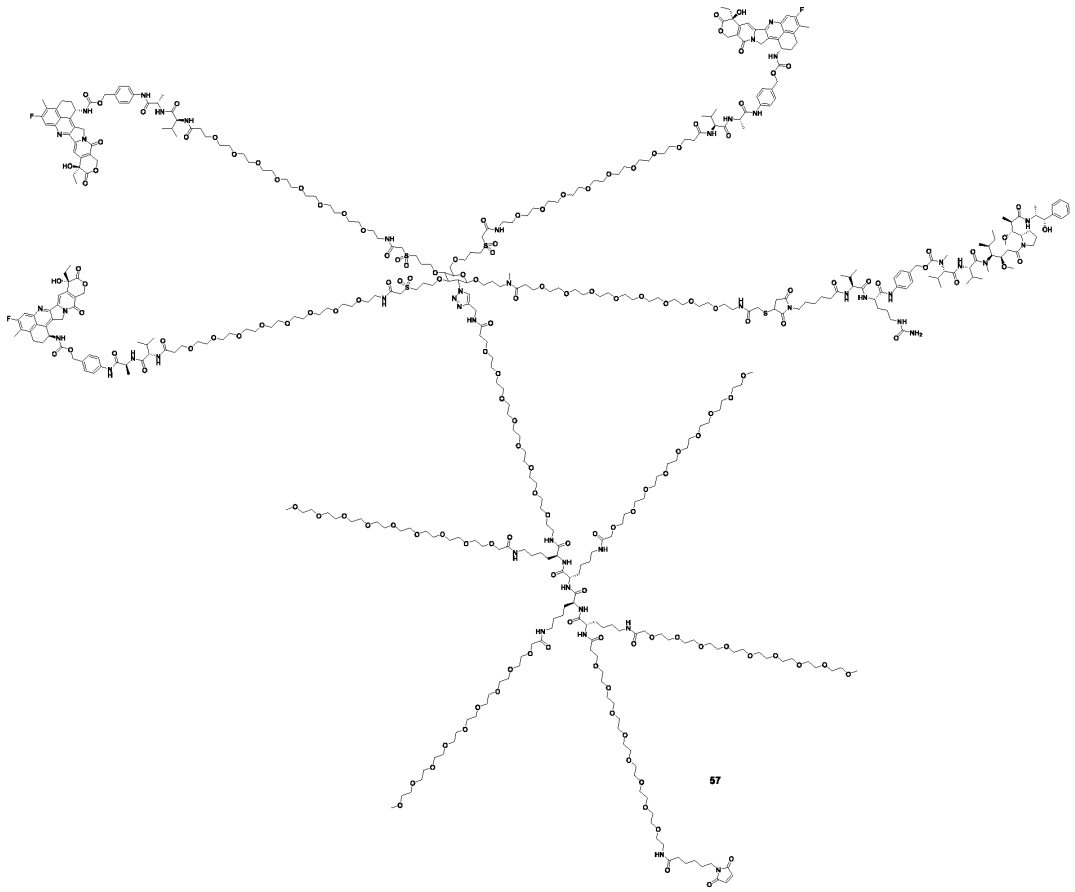
phase flash chromatography (C18 column, eluted with 60% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **56-7** (103.20 mg, yield 35%) as a yellow solid. LCMS: m/z calculated for $C_{424}H_{657}F_3N_{46}O_{137}S_6$: 8840.46; found: 1474.42 $[M+6H]^+$.

[00762] Step 8: To a solution of **56-7** (101.2 mg, 0.01 mol) in N,N-dimethylformamide (1 mL) was added piperidine (4.87 mg, 0.05 mmol). Then the mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 71% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **56-8** (68.3 mg, yield 69%) as a light-yellow solid. LCMS: m/z calculated for $C_{409}H_{647}F_3N_{46}O_{135}S_6$: 8618.22; found: 1437.28 $[M+6H]^+$.

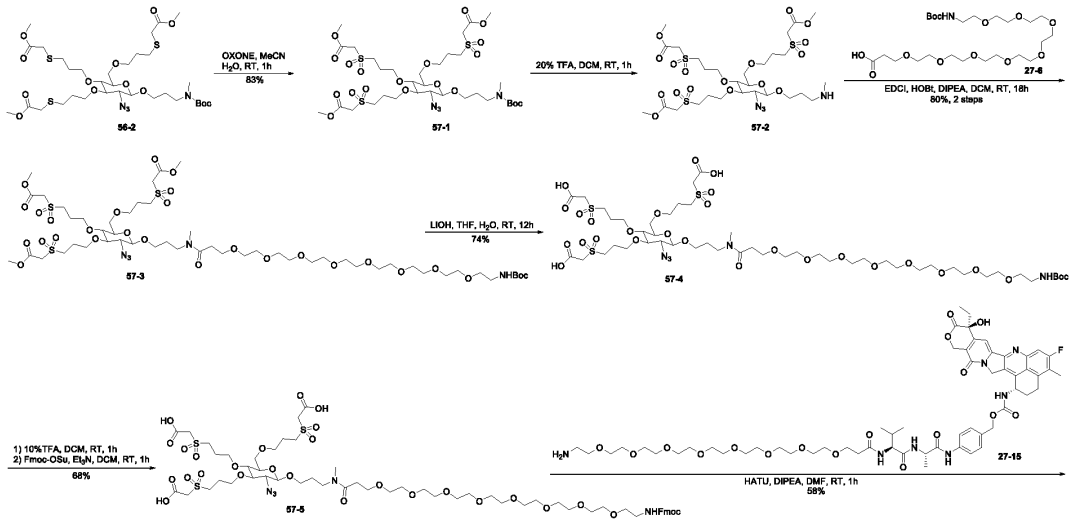
[00763] Step 9: To a solution of compound **56-8** (25.00 mg, 0.003 mmol) and N,N-diisopropylethylamine (0.94 mg, 0.07 mmol) in dichloromethane (1 mL) was added compound **1-28** (0.92 mg, 0.003 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by pre-HPLC (C18 silica gel column, eluted with 63% acetonitrile in water, AcOH condition) to afford the title compound **56** (16.00 mg, yield 63%) as a pale-yellow solid. LCMS: m/z calculated for $C_{419}H_{658}F_3N_{47}O_{138}S_6$: 8811.42; found: 1469.56 $[M+6H]^+$.

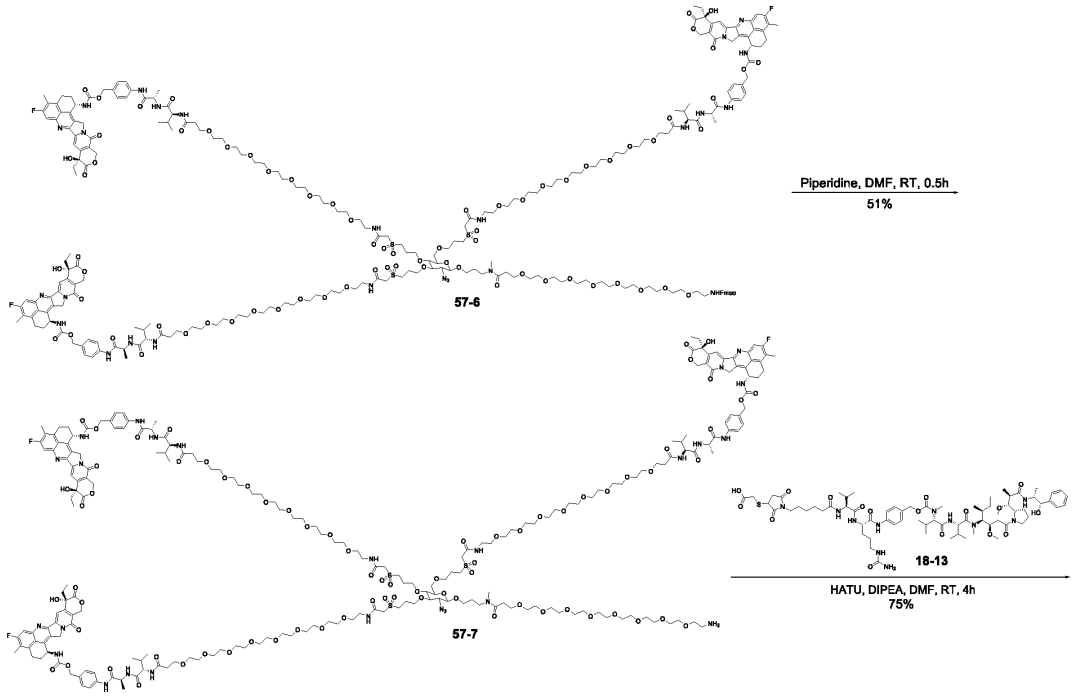
Example 57. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-

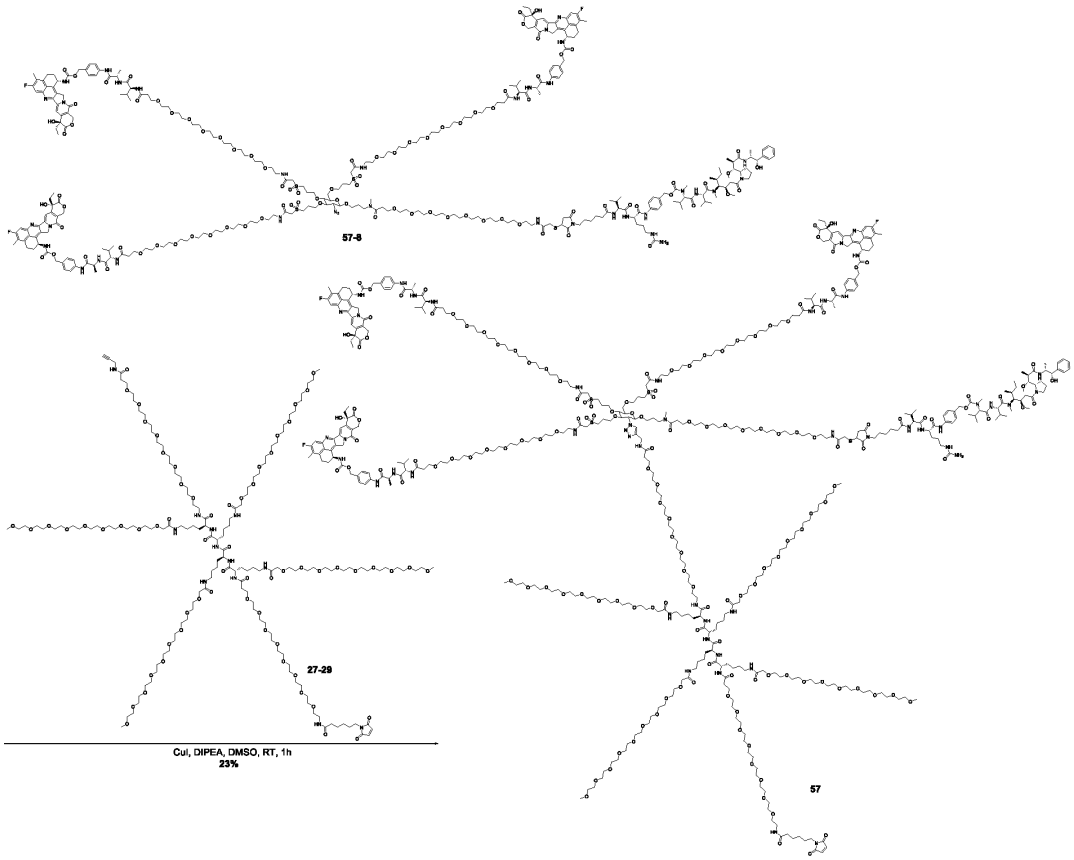
methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (57)



Synthetic scheme 57




Piperidine, DMF, RT, 0.5h
51%
57-6
57-7
18-13
HATU, DIPEA, DMF, RT, 4h
75%



[00764] Step 1: To a solution of compound **56-2** (2.00 g, 2.45 mmol) in acetonitrile (30 mL) was added a solution of OXONE (9.04 g, 14.70 mmol) in H_2O (50 mL). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and concentrated under reduced pressure. The left aqueous solution was extracted with ethyl acetate (50 mL x2). The organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 60% ethyl acetate in petroleum ether) to afford the title compound **57-1** (1.85 g, yield 83%) as a colorless oil. LCMS: m/z calculated for $C_{33}H_{58}N_4O_{19}S_3$: 911.02; found: 811.28 $[M\text{-}Boc+H]^+$.

[00765] Step 2: Compound **57-1** (1 g, 1.10 mmol) was dissolved in dichloromethane (4 mL), followed by the addition of TFA (1 mL). The reaction was stirred at room temperature for 1 h.

The reaction mixture was then diluted with dichloromethane (50 mL) and concentrated to afford the title compound **57-2** (1.34 g, crude), which was used into the next step without further purification. LCMS: m/z calculated for $C_{28}H_{50}N_4O_{17}S_3$: 810.90; found: 811.26 $[M+H]^+$.

[00766] Step 3: Compound **57-2** (1.34 g) was dissolved in dichloromethane (10 mL), followed by the sequential addition of compound **27-6** (1.07 g, 1.98 mmol), DIPEA (1.07 g, 8.25 mmol), HOBt (0.27 g, 1.98 mmol), and EDCI (0.63 g, 3.30 mmol). The reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column chromatography (eluted with 9% methanol in dichloromethane) to afford the title compound **57-3** (1.18 g, yield 80% over 2 steps) as a colorless oil. LCMS: m/z calculated for $C_{52}H_{95}N_5O_{28}S_3$: 1334.52; found: 617.95 $[M\text{-Boc}+2H]^{2+}$.

[00767] Step 4: Compound **57-3** (2.16 g, 1.62 mmol) was dissolved in THF (30 mL) and H_2O (30 mL), followed by the slow addition of LiOH (0.54 g, 12.95 mmol). The reaction was stirred at room temperature for 12 h. After completion of the reaction, the mixture was adjusted to pH to 5 with 1N HCl. The resulting mixture was filtered and concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **57-4** (1.09 g, yield 74%) as a colorless oil. LCMS: m/z calculated for $C_{49}H_{89}N_5O_{28}S_3$: 1292.44; found: 596.97 $[M\text{-Boc}+2H]^{2+}$.

[00768] Step 5: Compound **57-4** (1.6 g, 1.24 mmol) was dissolved in dichloromethane (16 mL), followed by the addition of TFA (4 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of reaction, the mixture was diluted with dichloromethane (100 mL) and concentrated to dryness under reduced pressure.

[00769] The crude intermediates were redissolved in dichloromethane (15 mL), followed by the sequential addition of DIPEA (481 mg, 3.72 mmol) and Fmoc-OSu (627 mg, 1.86 mmol). The reaction mixture was stirred at room temperature for 1 hour. After completion of reaction, the mixture was adjusted pH to 2 with 1N HCl. The mixture was extracted with dichloromethane (50 mL x2). The combined organic layers were washed with brine (50 mL), dried over

anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 30% methanol in dichloromethane) to afford the title compound **57-5** (1.2 g, yield 68%) as a yellow solid. LCMS: m/z calculated for $C_{59}H_{91}N_5O_{28}S_3$: 1414.56; found: 708.09 $[M+2H]^{2+}$.

[00770] Step 6: Compound **57-5** (70 mg, 0.05 mmol) was dissolved in DMF (1 mL), followed by the addition of HATU (95 mg, 0.25 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 15 minutes before was added to a mixture of compound **27-15** (234 mg, 0.2 mmol) and DIPEA (64 mg, 0.5 mmol) in DMF (1 mL). The resulting mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **57-6** (180 mg, yield 58%) as a yellow solid. LCMS: m/z calculated for $C_{236}H_{325}F_3N_{26}O_{76}S_3$: 4895.48; found: 1224.85 $[M+4H]^{4+}$.

[00771] Step 7: Compound **57-6** (170 mg, 0.035 mmol) was dissolved in DMF (0.5 mL), followed by the addition of piperidine (15 mg, 0.174 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 0.5 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **57-7** (59 mg, yield 51%) as a pale-yellow solid. LCMS: m/z calculated for $C_{221}H_{315}F_3N_{26}O_{74}S_3$: 4673.23; found: 1169.34 $[M+4H]^{4+}$.

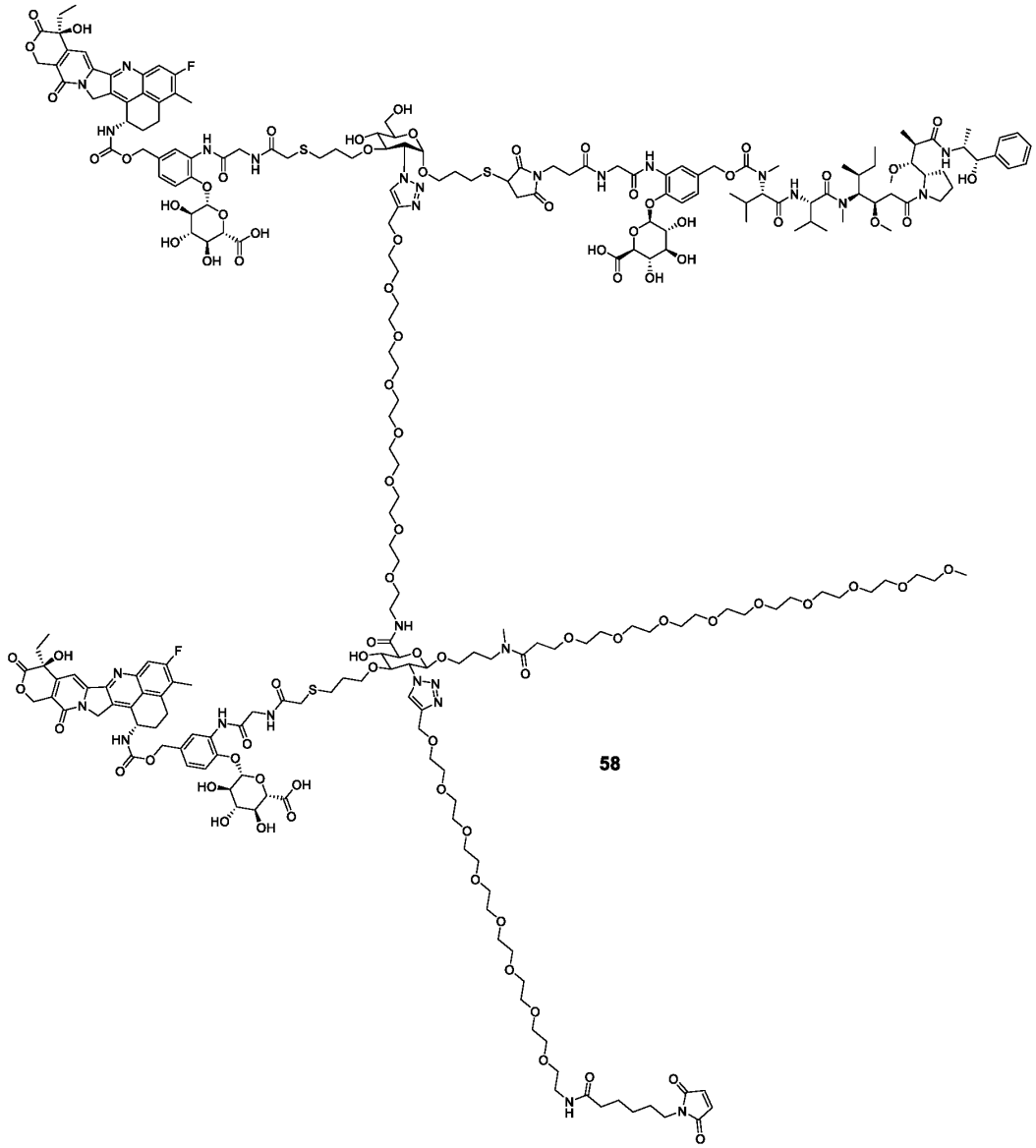
[00772] Step 8: Compound **57-7** (59 mg, 0.0148 mmol) was dissolved in dichloromethane (1 mL), followed by the sequential addition of DIPEA (74 mg, 0.51 mmol), compound **18-13** (21 mg, 0.0177 mmol) and HATU (9 mg, 0.0296 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 4 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the

title compound **57-8** (57 mg, yield 75%) as a pale-yellow solid. LCMS: m/z calculated for $C_{291}H_{422}F_3N_{37}O_{90}S_4$: 6063.98; found: 1213.69 $[M+5H]^{5+}$.

[00773] Step 9: Under nitrogen atmosphere, compound **57-8** (45 mg, 0.007 mmol) and compound **27-29** (32 mg, 0.010 mmol) were dissolved in DMSO (1 mL), followed by the sequential addition of CuI (2.0 mg, 0.011 mmol) and DIPEA (5.5 mg, 0.042 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **57** (16.1 mg, yield 23%) as a pale-yellow solid. LCMS: m/z calculated for $C_{442}H_{704}F_3N_{49}O_{155}S_4$: 9369.92; found: 1562.42 $[M+6H]^{6+}$.

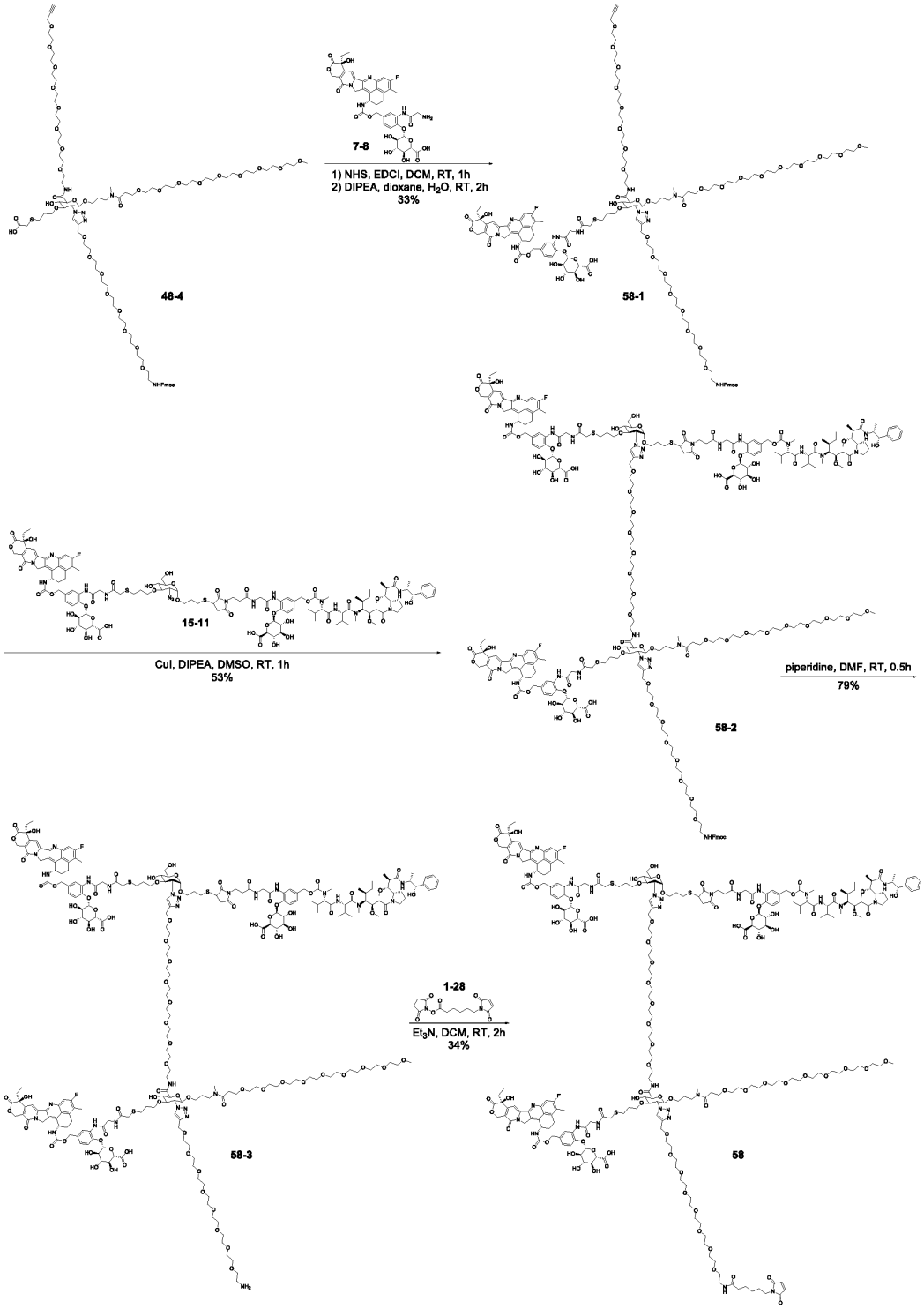
Example 58. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(2*S*,4*R*)-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-2-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-

pyrrolidin-3-yl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (58)



58

Synthetic scheme 58


48-4
7-8
1) NHS, EDCI, DCM, RT, 1h
2) DIPEA, dioxane, H2O, RT, 2h
33%
58-1
15-11
CuI, DIPEA, DMSO, RT, 1h
53%
58-2
piperidine, DMF, RT, 0.5h
79%
1-28
Et3N, DCM, RT, 2h
34%
58-3
58

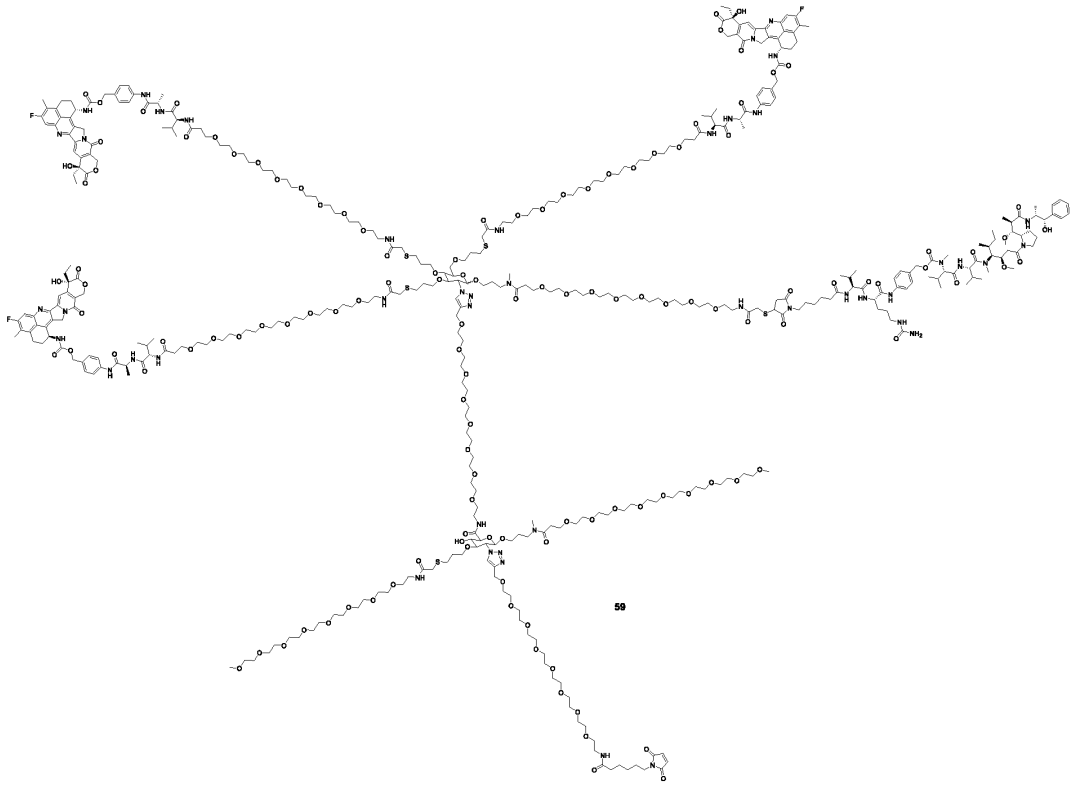
[00774] Step 1: The title compound **58-1** was prepared in analogy to **Example 18, Step 9** and **Step 10** by using compound **48-4** and compound **7-8**. The product (142 mg, yield 33%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{128}H_{184}FN_{11}O_{48}S$: 2695.97; found: 899.73 $[M+3H]^{3+}$. Step 2: The title compound **58-2** was prepared in analogy to **Example 10, Step 14** by using compound **15-11** and compound **58-1**. The product (106 mg, yield 53%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{244}H_{337}F_2N_{27}O_{88}S_3$: 5190.66; found: 1298.47 $[M+4H]^{4+}$.

[00775] Step 3: The title compound **58-3** was prepared in analogy to **Example 42, Step 27** by using compound **58-2**. The product (45 mg, yield 79%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{229}H_{327}F_2N_{27}O_{86}S_3$: 4968.41; found: 1242.93 $[M+4H]^{4+}$.

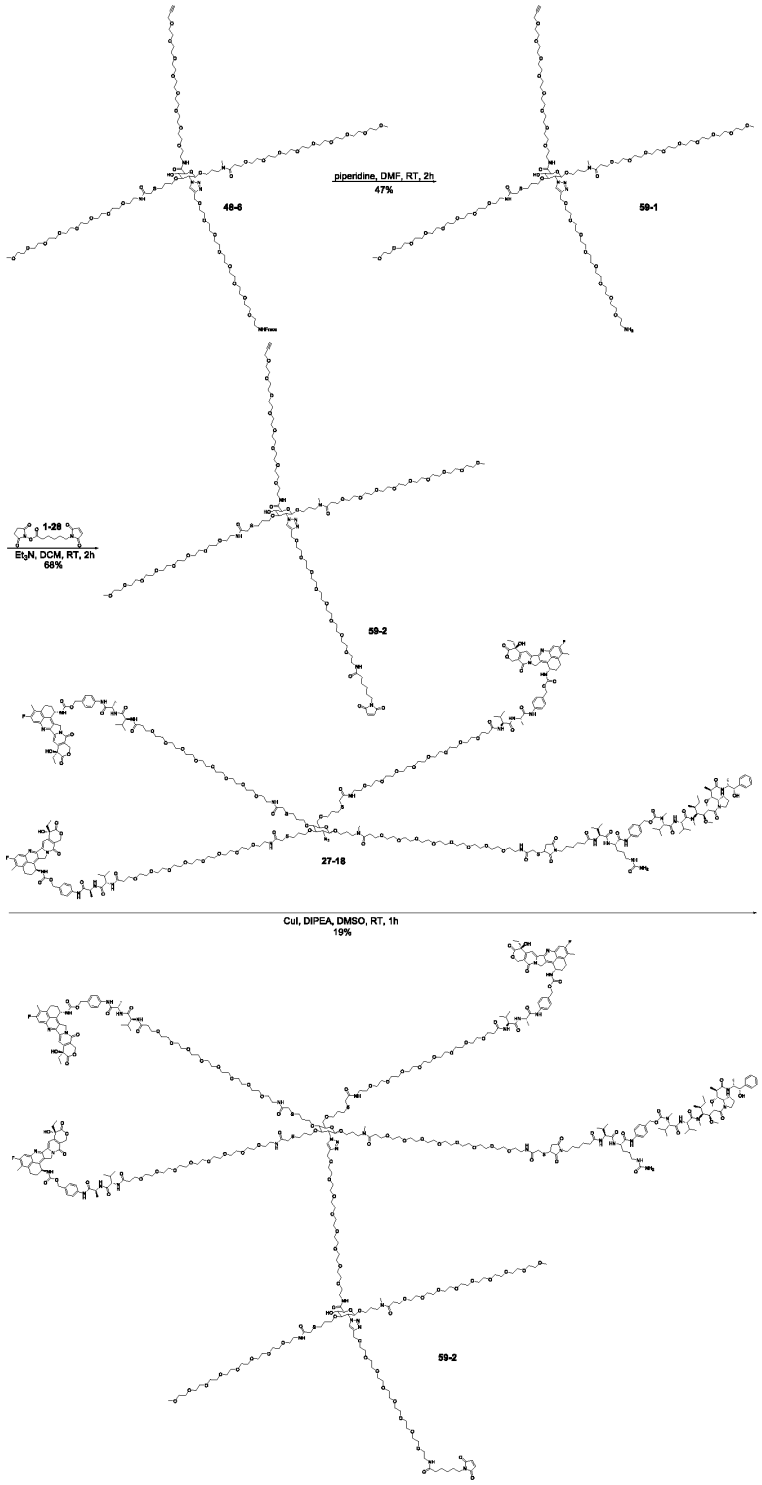
[00776] Step 4: The title compound **58** was prepared in analogy to **Example 1, Step 21** by using compound **1-28** and compound **58-3**. The product (24 mg, yield 34%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{239}H_{338}F_2N_{28}O_{89}S_3$: 5161.62; found: 1291.18 $[M+4H]^{4+}$.

Example 59. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-

propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (59)



Synthetic scheme 59


46-6
piperidine, DMF, RT, 2h
47%
59-1
1-28
Et3N, DCM, RT, 2h
68%
59-2
27-18
CuI, DIPEA, DMSO, RT, 1h
19%
59-2

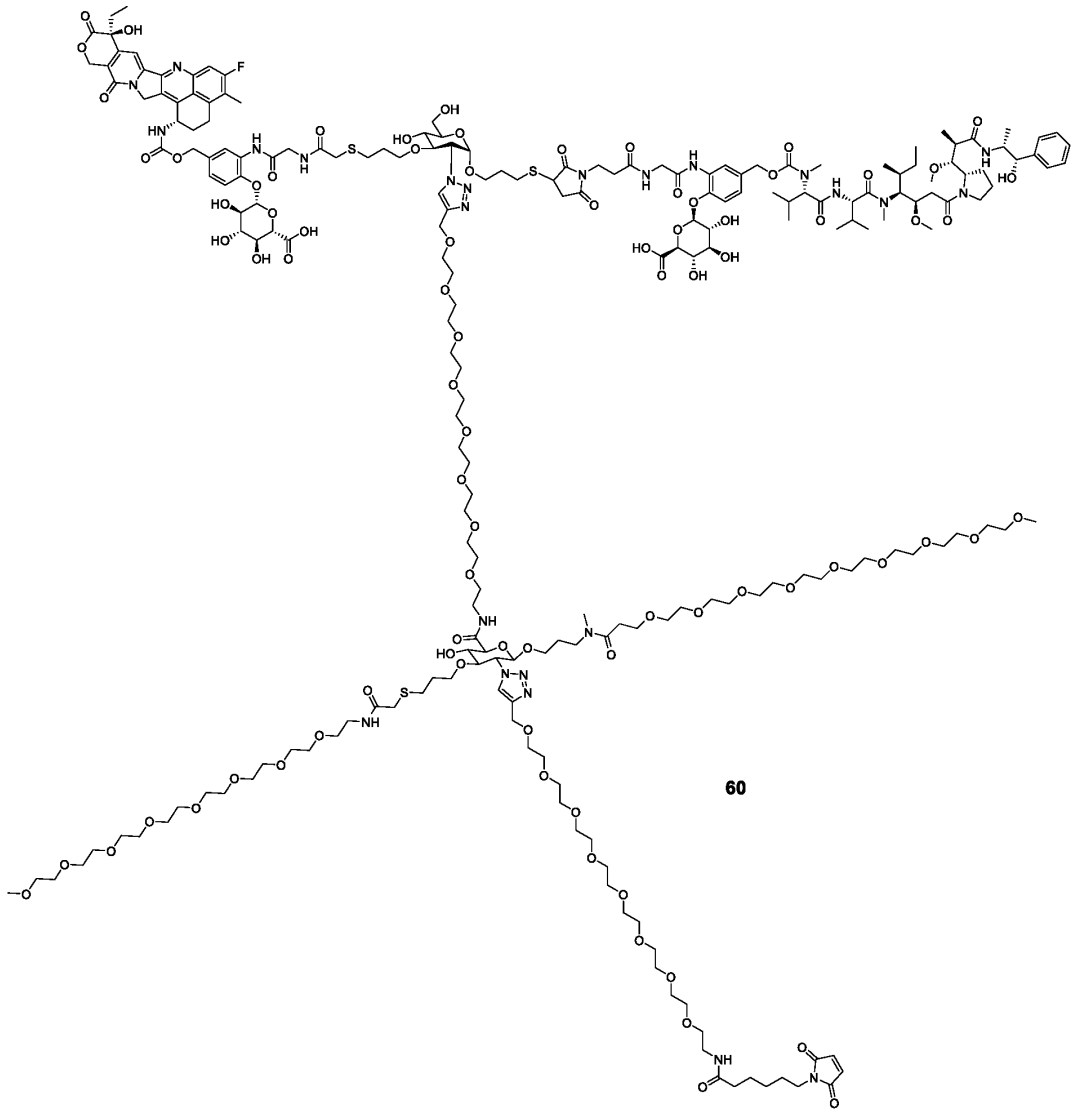
[00777] Step 1: Under nitrogen atmosphere, to a solution of compound **48-6** (472 mg, 0.210 mmol) in anhydrous N, N-dimethylformamide (4 mL) was added piperidine (0.030 mL, 0.630 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **59-1** (201 mg, yield 47%) as a yellow oil. LCMS: m/z calculated for $C_{90}H_{171}N_7O_{40}S$: 2023.43; found: 1012.67 $[M+2H]^{2+}$.

[00778] Step 2: Under nitrogen atmosphere, to a solution of compound **59-1** (142.5 mg, 0.070 mmol) in anhydrous dichloromethane (2 mL) was added triethylamine (0.024 mL, 0.176 mmol) and compound **1-28** (32 mg, 0.105 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **59-2** (106 mg, yield 68%) as a pale-yellow oil. LCMS: m/z calculated for $C_{100}H_{182}N_8O_{43}S$: 2216.63; found: 1109.14 $[M+2H]^{2+}$.

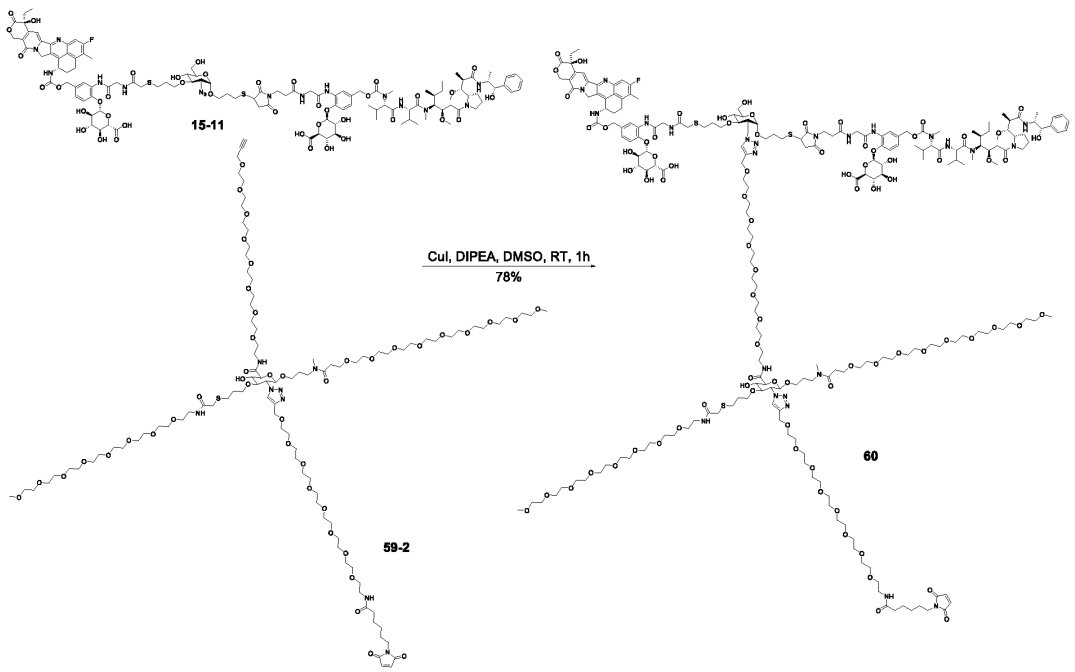
[00779] Step 3: Under nitrogen atmosphere, to a solution of compound **59-2** (14 mg, 0.006 mmol) and compound **27-18** (35 mg, 0.006 mmol) in dimethyl sulfoxide (2 mL) was added N, N-diisopropylethylamine (0.016 mL, 0.096 mmol) and cuprous iodide (4.5 mg, 0.024 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (1 mL), filtered and purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **59** (9 mg, yield 19%) as a pale-yellow oil. LCMS: m/z calculated for $C_{391}H_{604}F_3N_{45}O_{127}S_5$: 8184.62; found: 1637.69 $[M+5H]^{5+}$.

Example 60. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(2*S*,4*R*)-2-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-

carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (60)



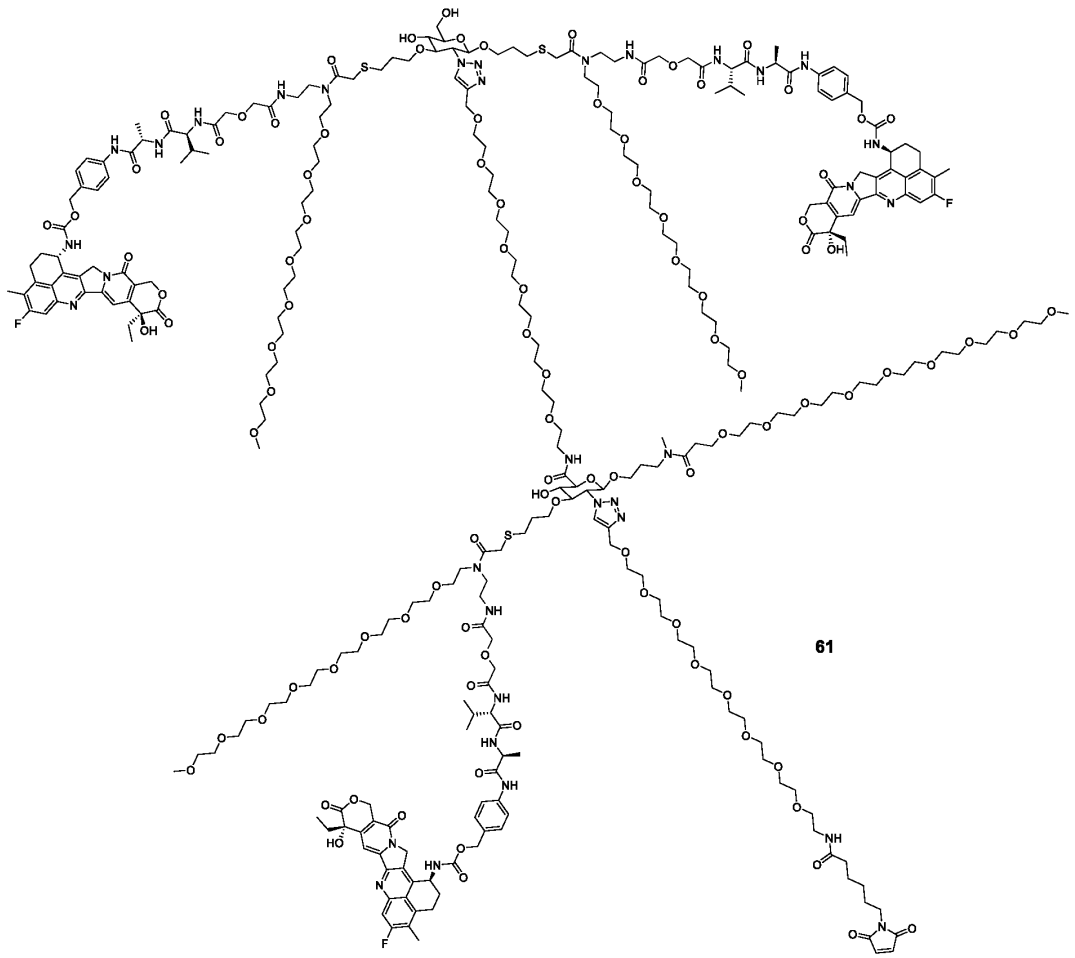
Synthetic scheme 60



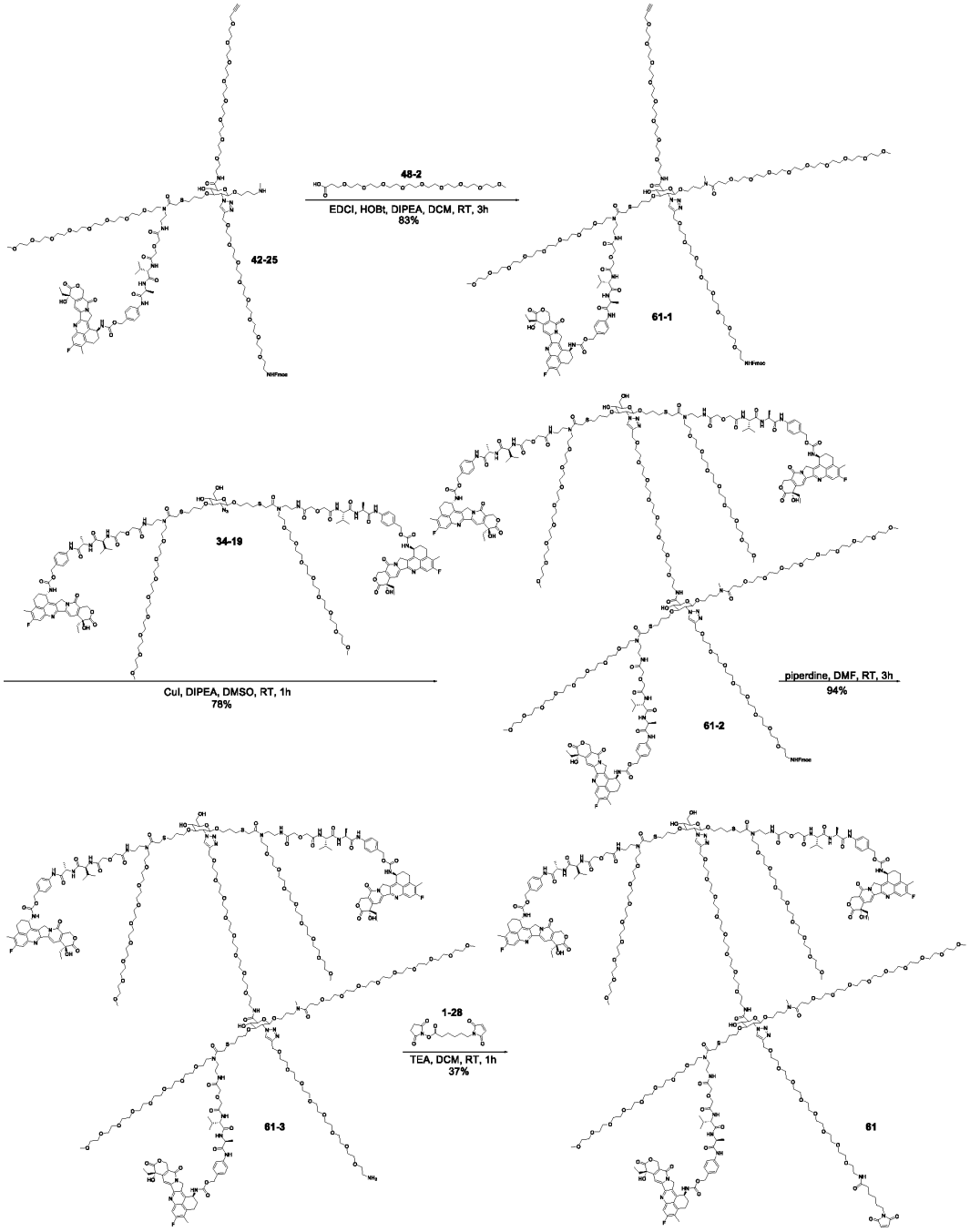
[00780] Step 1: Under nitrogen atmosphere, to a mixture of compound **59-2** (29 mg, 0.013 mmol) and compound **15-11** (30 mg, 0.012 mmol) in dimethyl sulfoxide (1 mL) was added N, N-diisopropylethylamine (0.033 mL, 0.192 mmol) and cuprous iodide (9 mg, 0.048 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (1 mL), filtered and purified by HPLC (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **60** (55.5 mg, yield 78%) as a pale-yellow oil. LCMS: m/z calculated for $C_{216}H_{335}FN_{24}O_{83}S_3$: 4711.32; found: 1570.92 $[M+3H]^{3+}$.

Example 61. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[[2-[2-[2-[[2-[3-[3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-

ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-2-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (61)



Synthetic scheme 61



[00781] Step 1: Under nitrogen atmosphere, to a solution of compound **42-25** (400 mg, 0.148 mmol) in anhydrous dichloromethane (5 mL) was added compound **48-2** (81 mg, 0.177 mmol),

N, N-diisopropylethylamine (0.077 mL, 0.444 mmol), 1-hydroxybenzotriazole (20 mg, 0.148 mmol) and EDCI (57 mg , 0.296 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (100 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **61-1** (387 mg, yield 83%) as a yellow solid. LCMS: m/z calculated for $C_{151}H_{231}FN_{14}O_{53}S$: 3141.61; found: 1571.31 $[M+2H]^{2+}$.

[00782] Step 2: Under nitrogen atmosphere, to a mixture of compound **61-1** (190 mg, 0.060 mmol) and compound **34-19** (164 mg, 0.055 mmol) in dimethyl sulfoxide (3 mL) was added N, N-diisopropylethylamine (0.076 mL, 0.440 mmol) and cuprous iodide (21 mg, 0.110 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (1.5 mL) and filtered. The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **61-2** (223 mg, yield 78%) as a yellow solid. LCMS: m/z calculated for $C_{293}H_{428}F_3N_{33}O_{98}S_3$: 6133.96; found: 1534.21 $[M+4H]^{4}+$.

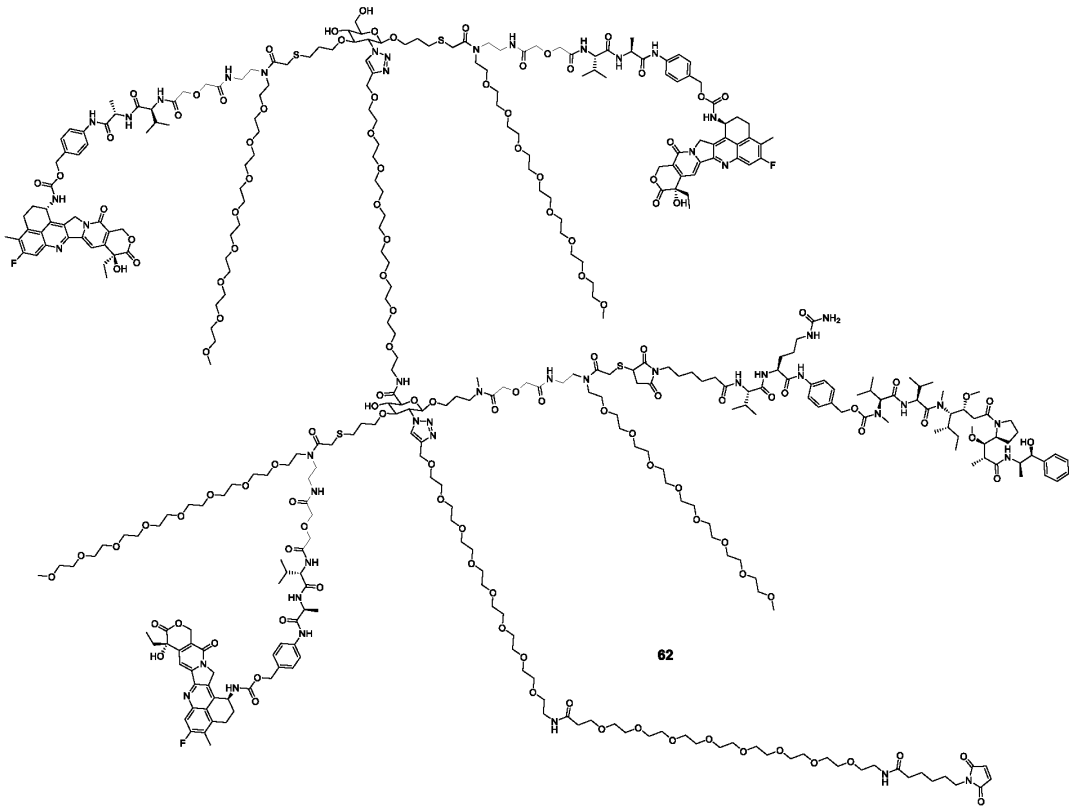
[00783] Step 3: Under nitrogen atmosphere, to a mixture of compound **61-2** (215 mg, 0.035 mmol) in anhydrous N, N-dimethylformamide (2 mL) was added piperidine (0.010 mL, 0.105 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **61-3** (196 mg, yield 94%) as a yellow oil. LCMS: m/z calculated for $C_{278}H_{418}F_3N_{33}O_{96}S_3$: 5911.71; found: 1971.02 $[M+3H]^{3+}$.

[00784] Step 4: Under nitrogen atmosphere, to a mixture of compound **61-3** (30 mg, 0.005 mmol) in anhydrous dichloromethane (1.5 mL) was added triethylamine (0.002 mL, 0.015 mmol) and compound **1-28** (3.2 mg, 0.01 mmol). The mixture was stirred at room temperature

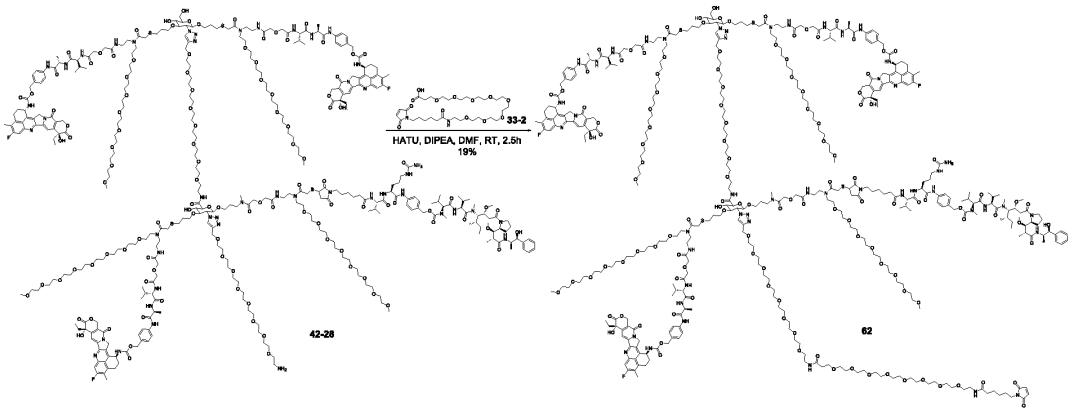
for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **61** (11.5 mg, yield 37%) as a pale-yellow oil. LCMS: m/z calculated for $C_{288}H_{429}F_3N_{34}O_{99}S_3$: 6104.91; found: 1526.54 $[M+4H]^{4+}$.

Example 62. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-

propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (62)



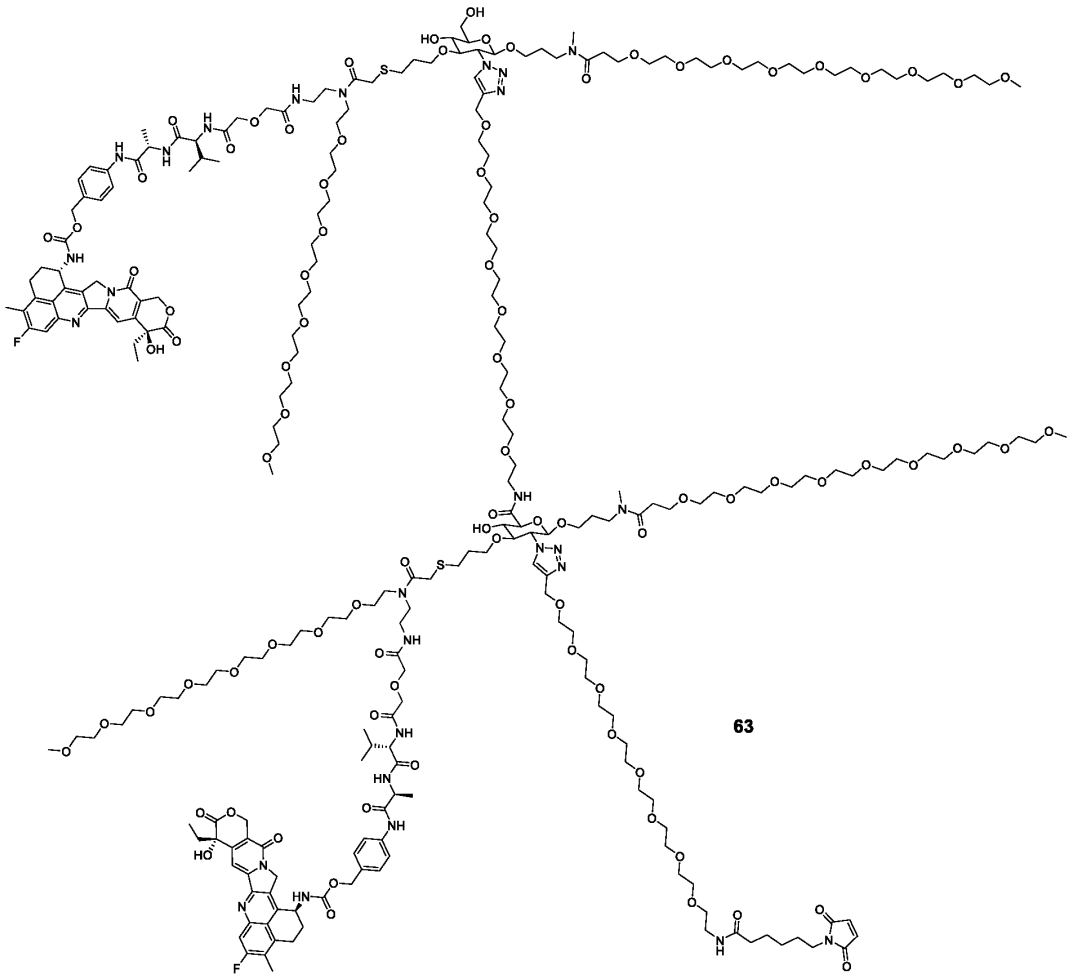
Synthetic scheme 62



[00785] Step 1: Under nitrogen atmosphere, to a mixture of compound **33-2** (2.3 mg, 0.004 mmol) in anhydrous N, N-dimethylformamide (1.8 mL) was added N, N-diisopropylethylamine (0.002 mL, 0.012 mmol), HATU (1.5 mg, 0.004 mmol). The mixture was stirred at room temperature for 10 min before compound **42-28** (20 mg, 0.003 mmol) was added thereto. The mixture was stirred at room temperature for 2.5 h. After completion of the reaction, the mixture was quenched with water and filtered. The resulting mixture was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **62** (4.1 mg, yield 19%) as a colorless oil. LCMS: m/z calculated for $C_{380}H_{579}F_3N_{48}O_{125}S_4$: 8005.26; found: 1601.90 $[M+5H]^{5+}$.

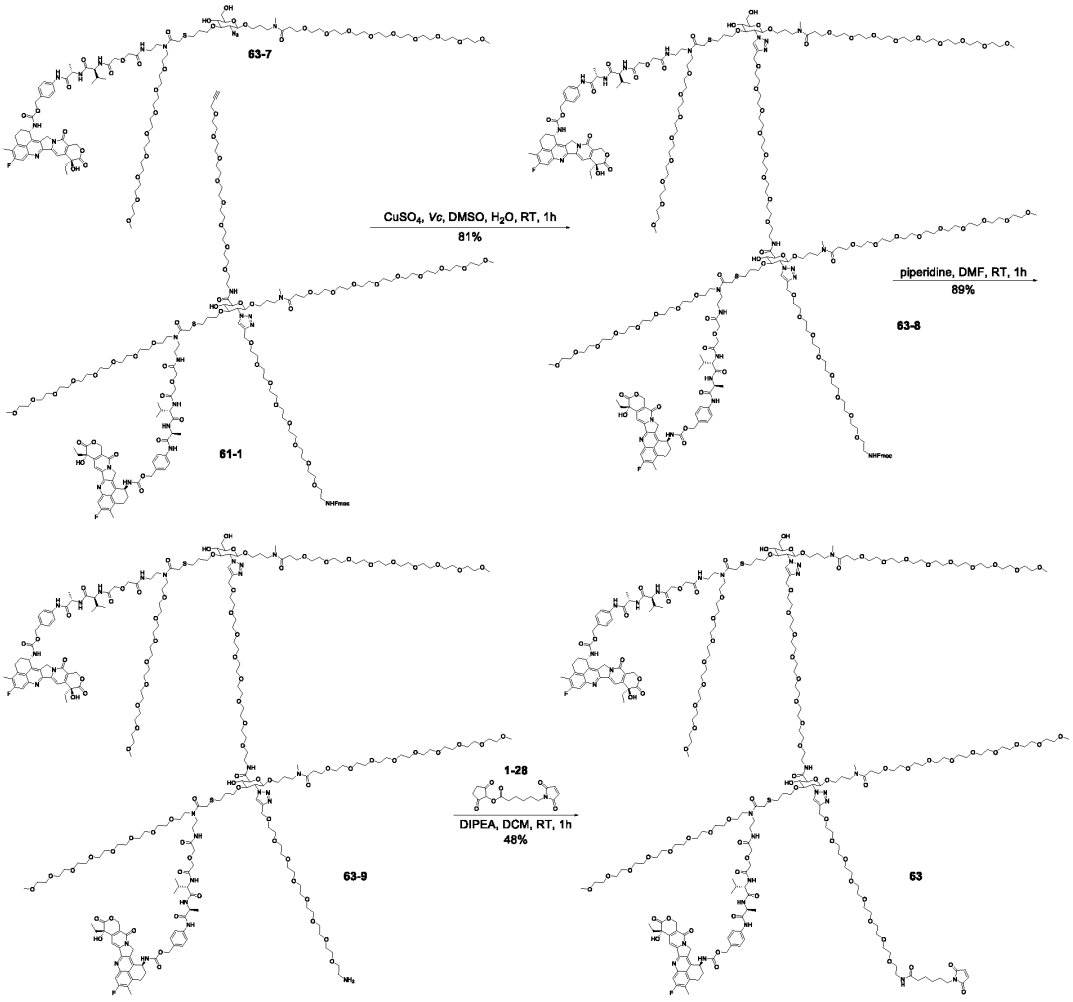
Example 63. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[[2-[2-[2-[[2-[3-[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-2-(hydroxymethyl)-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-

[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (63)



Synthetic scheme 63


27-13
1-22
DIPEA, DMF, RT, 1h
76%
63-1
42-8
TFA, DCM, RT, 1h
90%
63-2
48-2
EDCI, HOBt, DIPEA, DCM, RT, 2h
61%
63-3
LiOH, H2O, THF
RT, 0°C-RT, 16h
56%
63-4
34-15
HATU, DIPEA, DMF, RT, 3h
74%
63-5
HCl, MeOH, RT, 2h
90%
63-6
63-1
HATU, DIPEA, DMF, RT, 3h
55%
63-7



[00786] Step 1: The title compound **63-1** was prepared in analogy to **Example 2, Step 7** by using compound **27-13** and compound **1-22**. The product (820 mg, yield 76%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{44}H_{47}FN_6O_{12}$: 870.89; found: 872.08 $[M+H]^+$.

[00787] Step 2: The title compound **63-2** was prepared in analogy to **Example 57, Step 2** by using compound **42-8**. The product (720 mg, yield 90%) was obtained as a pale-yellow oil. LCMS: m/z calculated for C16H30N4O7S: 422.50; found: 423.54 $[M+H]^+$.

[00788] Step 3: Under nitrogen atmosphere, to a mixture of compound **63-2** (439 mg, 1.039 mmol) in anhydrous dichloromethane (5 mL) was added compound **48-2** (474 mg, 1.039 mmol), triethylamine (0.575 mL, 4.156 mmol), 1-Hydroxybenzotriazole (140 mg, 1.039 mmol) and

EDCI (398 mg, 2.078 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was poured into water (100 mL) and extracted with dichloromethane (50 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 6% methyl alcohol in dichloromethane) to afford the title compound **63-3** (554 mg, yield 61%) as a colorless oil. LCMS: m/z calculated for $C_{36}H_{68}N_4O_{17}S$: 861.01; found: 861.5 $[M+H]^+$.

[00789] Step 4: To a mixture of compound **63-3** (550 mg, 0.639 mmol) in tetrahydrofuran (6 mL) was added 1N LiOH (1.277 mL, 1.277 mmol). The mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was adjusted pH to 5-6 with 1N hydrochloric acid. The resulting mixture was concentrated. The crude product was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **63-4** (360 mg, yield 66%) as a colorless oil. LCMS: m/z calculated for $C_{35}H_{66}N_4O_{17}S$: 846.98; found: 847.44 $[M+H]^+$.

[00790] Step 5: Under nitrogen atmosphere, to a mixture of compound **63-4** (350 mg, 0.413 mmol) in anhydrous N,N-dimethylformamide (3 mL) was added compound **34-15** (326 mg, 0.620 mmol), N,N-diisopropylethylamine (0.287 mL , 1.652 mmol), HATU (314 mg , 0.826 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, 0.05%TFA condition). The desired components were lyophilized to afford the title compound **63-5** (414 mg, yield 74%) as a yellow oil. LCMS: m/z calculated for $C_{59}H_{114}N_6O_{26}S$: 1355.64; found: 1355.79 $[M+H]^+$.

[00791] Step 6: To a mixture of compound **63-5** (409 mg, 0.302 mmol) in methyl alcohol (2 mL) was added 3N HCl/MeOH (2 mL). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated to give the title compound

63-6 (350 mg, yield 90%) as a yellow solid. The crude product was directly used next step. LCMS: m/z calculated for $C_{54}H_{106}N_6O_{24}S$: 1255.52; found: 1255.66 $[M+H]^+$.

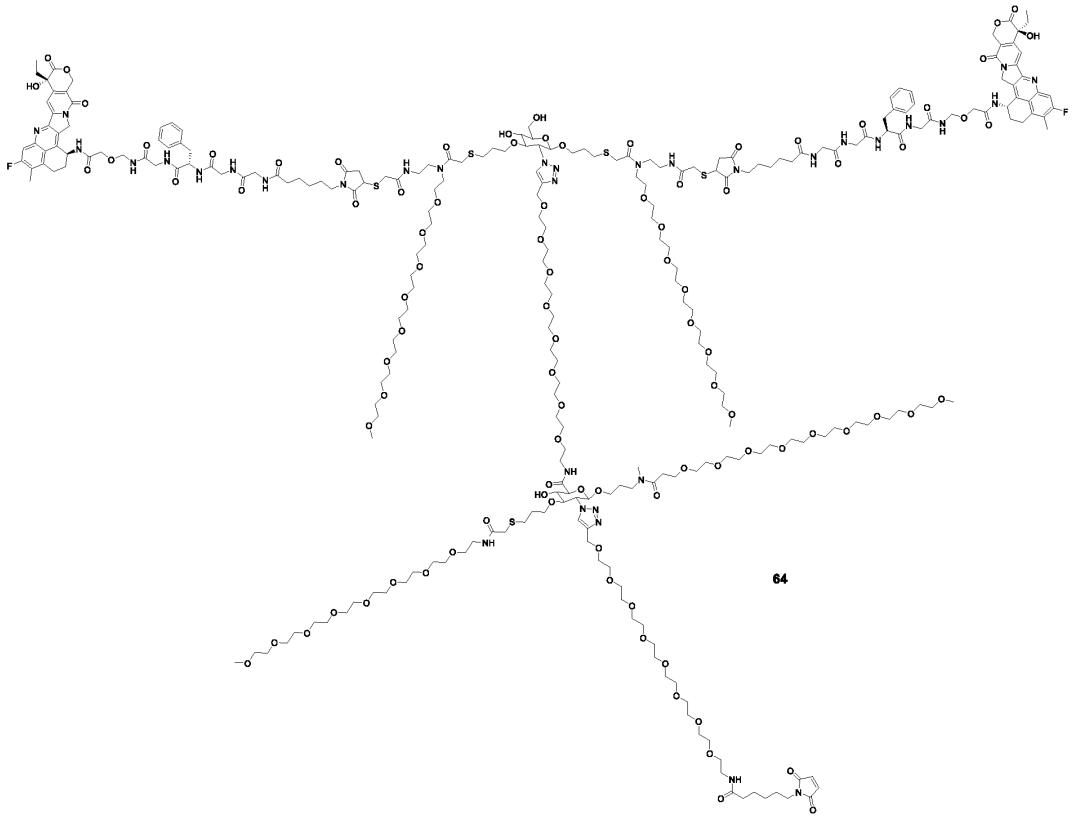
[00792] Step 7: Under nitrogen atmosphere, to a mixture of compound **63-1** (226 mg, 0.260 mmol) in anhydrous N,N-dimethylformamide (6 mL) was added N, N-diisopropylethylamine (0.181 mL , 1.040 mmol), HATU (148 mg , 0.390 mmol). The mixture was stirred at room temperaturefor 10 min before compound **63-6** (359 mg, 0.286 mmol) was added thereto. The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture filtered and purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **63-7** (350 mg, yield 55%) as a yellow oil. LCMS: m/z calculated for $C_{98}H_{151}FN_{12}O_{35}S$: 2108.39; found: 1054.97 $[M+2H]^{2+}$.

[00793] Step 8: Under nitrogen atmosphere, to a mixture of compound **63-7** (175 mg, 0.056 mmol) and compound **61-1** (141 mg, 0.067 mmol) in dimethyl sulfoxide (3 mL) was added 1M L-Ascorbic acid aqueous solution (0.672 mL, 0.672 mmol) and 1M copper sulfate aqueous solution (0.224 mL, 0.224 mmol). The mixture was stirred at room temperature under nitrogen atmosphere for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (1.5 mL), filtered. The resulting mixture was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **63-8** (236 mg, yield 81%) as a yellow oil. LCMS: m/z calculated for $C_{278}H_{418}F_3N_{33}O_{96}S_3$: 5911.71; found: 1478.73 $[M+4H]^{4+}$.

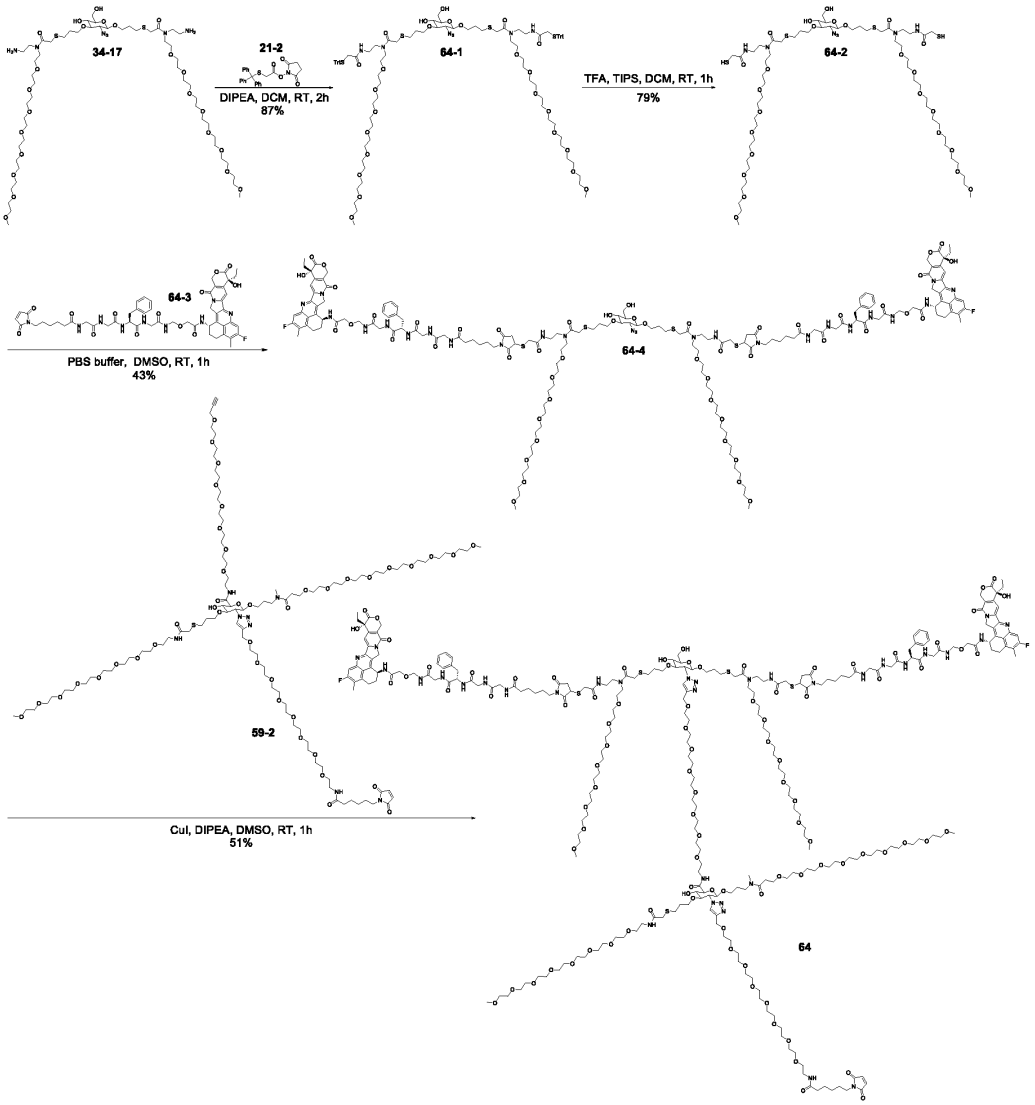
[00794] Step 9: Under nitrogen atmosphere, to a mixture of compound **63-8** (232 mg, 0.044 mmol) in anhydrous N, N-dimethylformamide (2 mL) was added piperidine (0.013 mL, 0.132 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **63-9** (199 mg, yield 89%) as a yellow oil. LCMS: m/z calculated for $C_{234}H_{372}F_2N_{26}O_{86}S_2$: 5027.76; found: 1257.86 $[M+4H]^{4+}$.

[00795] Step 10: Under nitrogen atmosphere, to a mixture of compound **63-9** (30 mg, 0.006 mmol) in anhydrous dichloromethane (1.5 mL) was added triethylamine (0.002 mL, 0.018 mmol) and compound **1-28** (2.8 mg, 0.009 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **63** (15.1 mg, yield 48%) as a colorless oil. LCMS: m/z calculated for $C_{244}H_{383}F_2N_{27}O_{89}S_2$: 5220.96; found: 1305.98 $[M+4H]^{4+}$.

Example 64. Synthesis of *N*-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-carboxamide (64)



Synthetic scheme 64



[00796] Step 1: Under nitrogen atmosphere, to a mixture of solution of **34-17** (0.98 g, 0.76 mmol) and DIPEA (0.59 g, 4.57 mmol) in dichloromethane (15 mL) was added dropwise a solution of compound **21-2** (0.72 g, 1.67 mmol) in dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 2 h. The mixture was poured into water (30 mL) and extracted with dichloromethane (30 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 5% dichloromethane in methanol) to

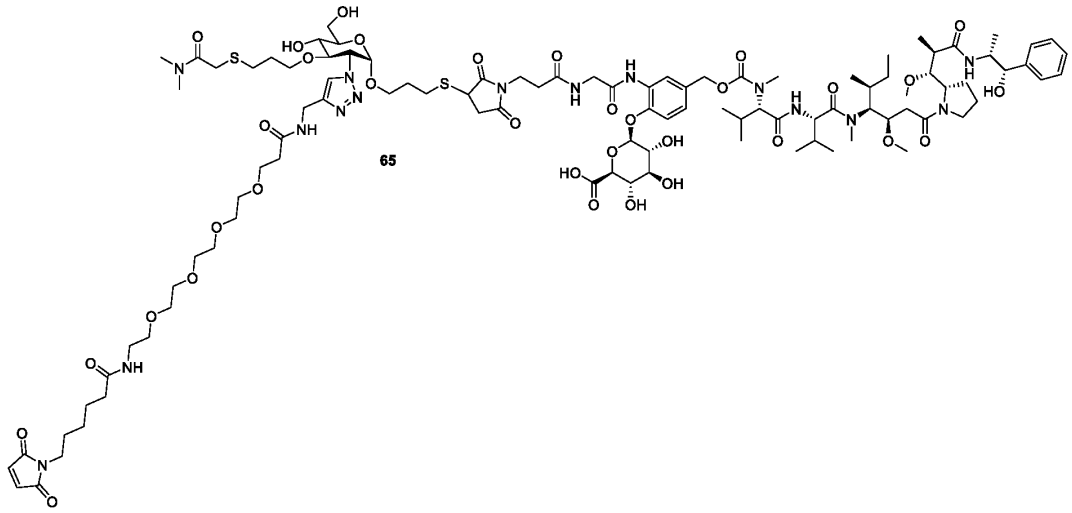
afford the title compound **64-1** (1.27 g, yield 87%) as a yellow oil. LCMS: m/z calculated for $C_{96}H_{139}N_7O_{25}S_4$: 1919.43; found: 839.11 $[(M\text{-}Trt)+2H]^{2+}$.

[00797] Step 2: To a solution of compound **64-1** (0.47 g, 0.25 mmol) in dichloromethane (9.5 mL) was added TFA (0.5 mL) and triisopropylsilane (0.5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated in vacuum. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 61% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **64-2** (0.28 g, yield 79%) as a yellow oil LCMS: m/z calculated for $C_{58}H_{111}N_7O_{25}S_4$: 1434.79; found: 718.87 $[M+2H]^{2+}$.

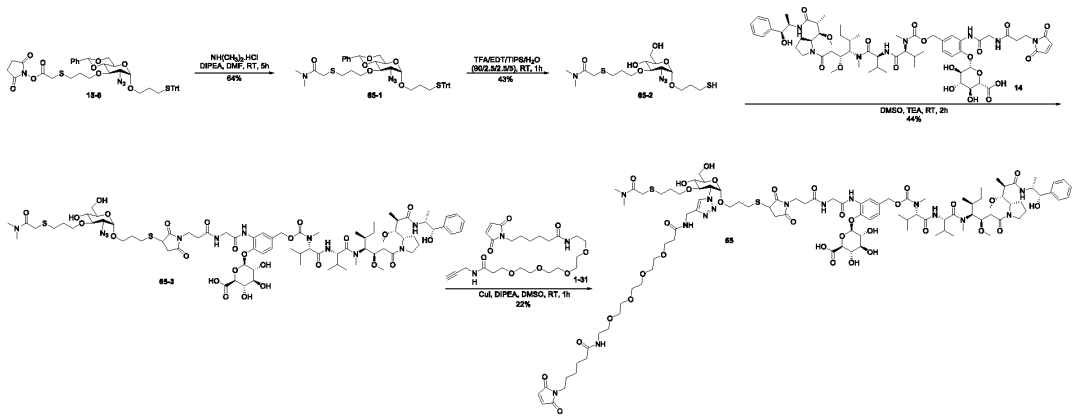
[00798] Step 3: Under nitrogen atmosphere, to a mixture of compound **64-2** (275 mg, 0.20 mmol) in PBS buffer (1 mL) and DMSO (1.5 mL) was added a solution of compound **64-3** (356mg，0.34mmol) in DMSO (3 mL) at 0 °C. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 2 h. After completion of the reaction, the reaction was filtered and purified by reverse phase flash (C18 column, eluted with 55% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **64-4** (290 mg, yield 43%) as a yellow solid LCMS: m/z calculated for $C_{162}H_{223}F_2N_{25}O_{51}S_4$: 3502.93; found: 1168.45 $[M+3H]^{3+}$.

[00799] Step 4: Under nitrogen atmosphere, to a solution of compound **64-4** (55 mg, 0.02 mmol) and **59-2** (25 mg, 0.01 mmol) in dimethyl sulfoxide (3 mL) was added ethyldiisopropylamine (24 mg, 0.20 mmol), CuI (9 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the reaction was filtered and purified by pre-HPLC (C18 column, eluted with 48% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **64** (46 mg, yield 51%) as a yellow oil LCMS: m/z calculated for $C_{262}H_{405}F_2N_{33}O_{94}S_5$: 5719.56; found: 1430.45 $[M+4H]^{4+}$.

Example 65. Synthesis of (2S,3S,4S,5R,6S)-6-(4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)-2-(2-(3-(3-((3-(((2S,3R,4R,5S,6R)-4-(3-((2-(dimethylamino)-2-oxoethyl)thio)propoxy)-3-(4-(24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,19-dioxo-6,9,12,15-tetraoxa-2,18-diazatetracosyl)-1H-1,2,3-triazol-1-yl)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)-2,5-dioxopyrrolidin-1-yl)propanamido)acetamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (65)



Synthetic scheme 65



[00800] Step 1: Under nitrogen atmosphere, to a solution of dimethylamine hydrochloride (54 mg, 0.66 mmol) in N,N-dimethylformamide (5 mL) was added N,N-diisopropylethylamine (307.51 mg, 2.38 mmol) and compound **15-8** (500 mg, 0.60 mmol) at room temperature. The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with 78% acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **65-1** (293 mg, yield 64%) as a white solid. LCMS: m/z calculated for $C_{42}H_{48}N_4O_6S_2$: 768.99; found: 791.29 $[M+Na]^+$

[00801] Step 2: To a mixture of trifluoroacetic acid, 1,2-ethanedithiol, triisopropylsilane and H_2O with a ratio of 90:2.5:2.5:5 (total 3 mL) was added compound **65-1** (283 mg, 0.37 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 10 minutes. After completion of the reaction, the mixture was diluted with cold tert-butyl methyl ether (30 mL) and concentrate under reduced pressure. The crude product was purified by pre-HPLC (C18 column, eluted with 70% acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **65-2** (71 mg, yield 43%) as a white solid. LCMS: m/z calculated for $C_{16}H_{30}N_4O_6S_2$: 438.56; found: 439.22 $[M+H]^+$.

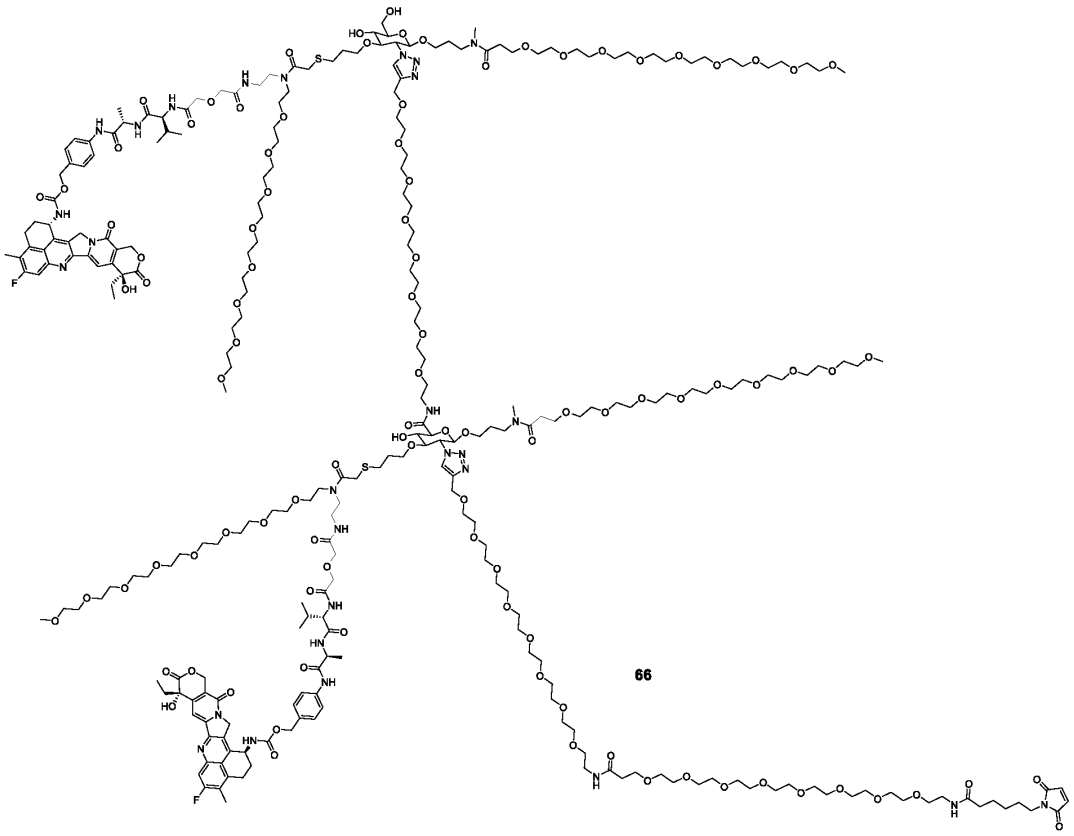
[00802] Step 3: Under nitrogen atmosphere, to a solution of compound **65-2** (71 mg, 0.16 mmol) in dimethyl sulfoxide (2 mL) was added compound **14** (216 mg, 0.17 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile and water, AcOH condition). The

desired components were lyophilized to afford the title compound **65-3** (120 mg, yield 44%) as a white solid. LCMS: m/z calculated for $C_{78}H_{120}N_{12}O_{26}S_2$: 1706.00; found: 1705.56 $[M+H]^+$.

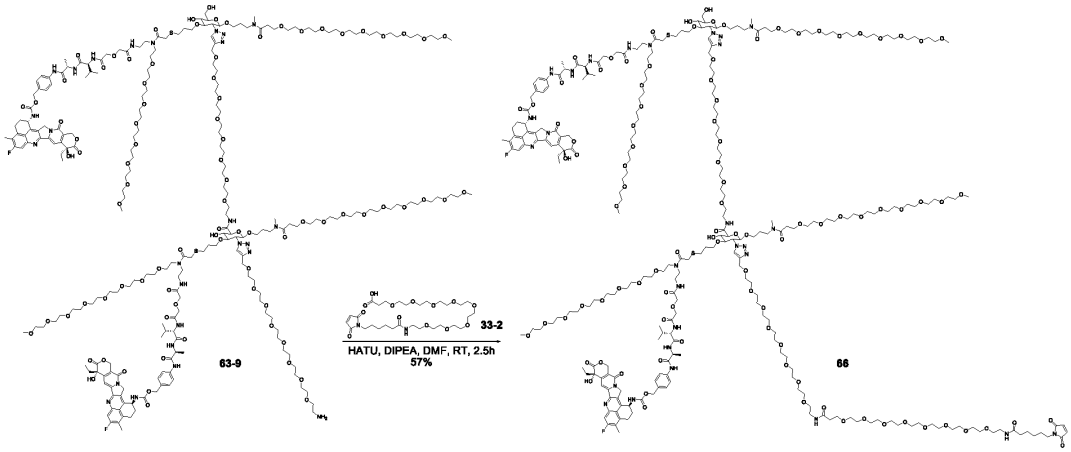
[00803] Step 4: Under nitrogen atmosphere, to a solution of compound **65-3** (50 mg, 0.03 mmol) and compound **1-31** (20 mg, 0.04 mmol) in DMSO (1 mL) was added N,N-diisopropylethylamine(15 mg, 0.12 mmol) and cuprous iodide (10 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by pre-HPLC (C18 column, eluted with 43% acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **65** (14 mg, yield 22%) as a white solid. LCMS: m/z calculated for $C_{102}H_{157}N_{15}O_{34}S_2$: 2201.57; found: 1101.51 $[M+2H]^{2+}$.

Example 66. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[[2-[2-[2-[[2-[3-[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-2-(hydroxymethyl)-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-

[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (66)



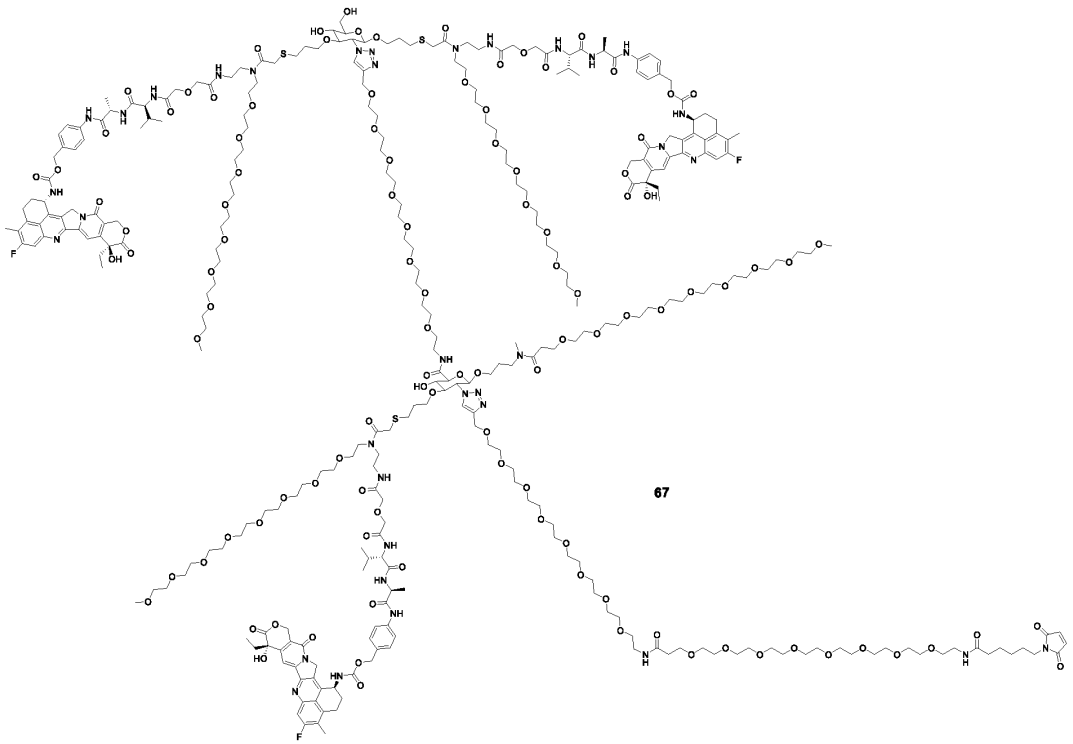
Synthetic scheme 66



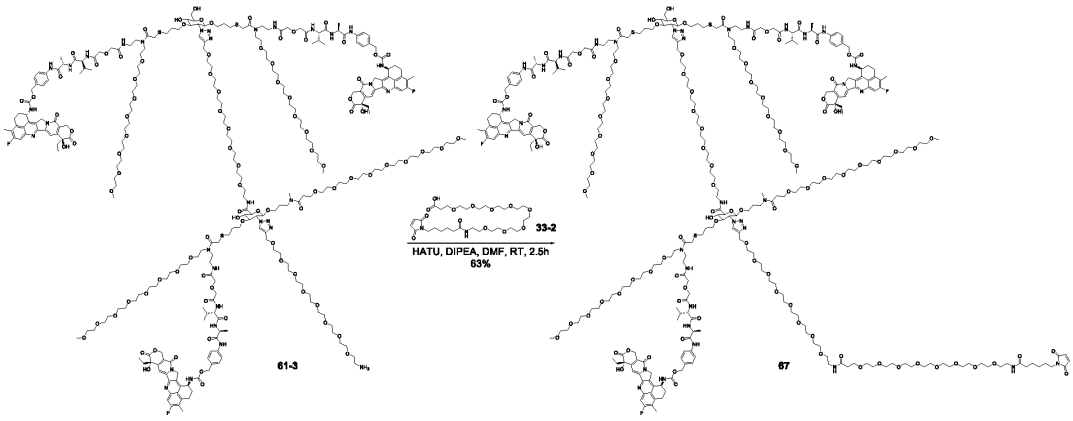
[00804] Step 1: Under nitrogen atmosphere, to a mixture of compound **33-2** (8.5 mg, 0.014 mmol) in anhydrous N,N-dimethylformamide (1.5 mL) was added compound N,N-diisopropylethylamine (0.006 mL , 0.036 mmol) and HATU (5.5 mg , 0.014 mmol). The mixture was stirred at room temperature for 5 min before compound **66-29** (45 mg, 0.009 mmol) was added thereto. The mixture was stirred at room temperature for 2.5 h. After completion of the reaction, the mixture was quenched with water (0.5 mL) and filtered. The mixture was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **66** (29 mg, yield 57%) as a pale-yellow oil. LCMS: m/z calculated for $C_{263}H_{420}F_2N_{28}O_{98}S_2$: 5644.47; found: 1411.99 $[M+4H]^{4+}$.

Example 67. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[[2-[2-[2-[2-[[2-[3-[3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-

methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-2-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamate (67)



Synthetic scheme 67

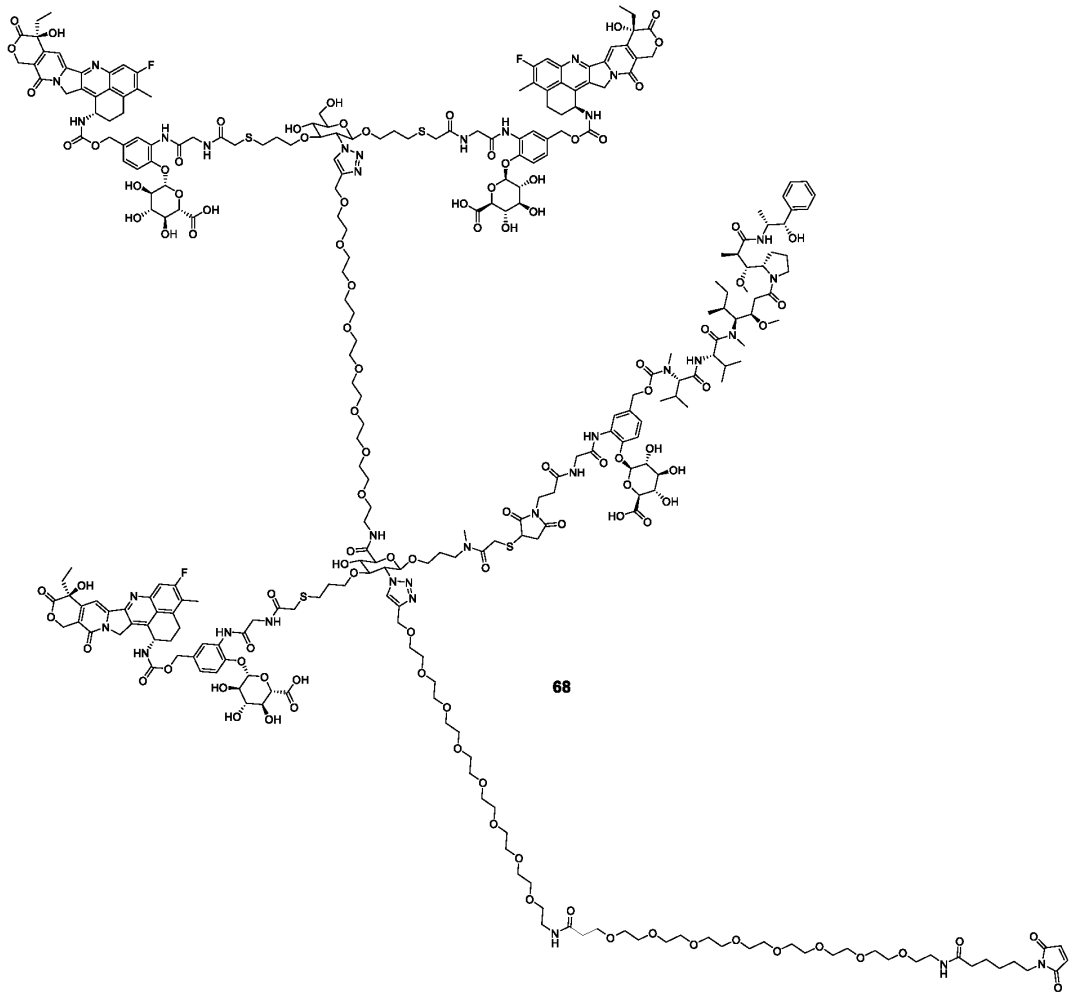


[00805] Step 1: Under nitrogen atmosphere, to a mixture of compound **33-2** (5 mg, 0.008 mmol) in anhydrous N,N-dimethylformamide (1.5 mL) was added compound N,N-diisopropylethylamine (0.004 mL , 0.021 mmol) and HATU (5 mg , 0.009 mmol). The mixture was stirred at room temperature for 5 min before compound **66-29** (40 mg, 0.007 mmol) was added thereto. After completion of the reaction, the mixture was quenched with water (0.5 mL) and filtered. The mixture was purified by pre-HPLC (C18 column, eluted with acetonitrile in

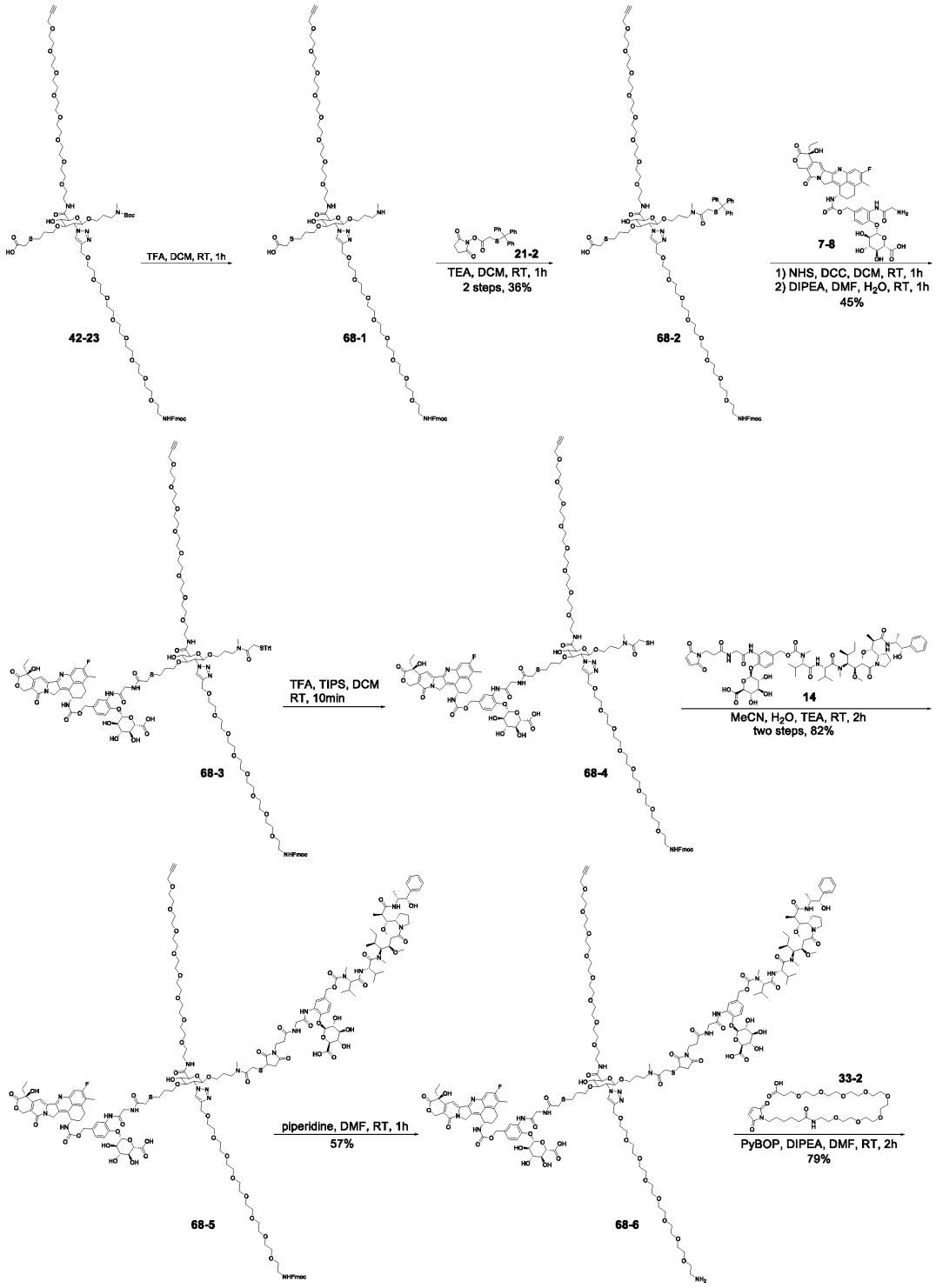
H_2O, AcOH condition). The desired components were lyophilized to give the title compound **67** (29 mg, yield 63%) as a pale-yellow oil. LCMS: m/z calculated for $C_{307}H_{466}F_3N_{35}O_{108}S_3$: 6528.42; found: 1306.79 $[M+5H]^{5+}$.

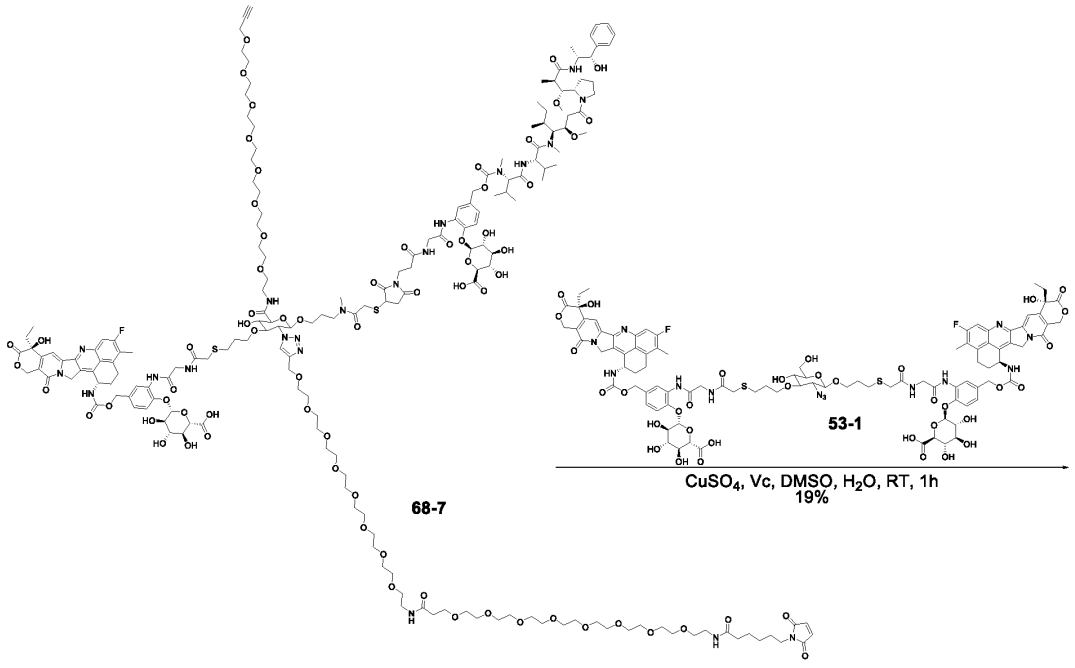
Example 68. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

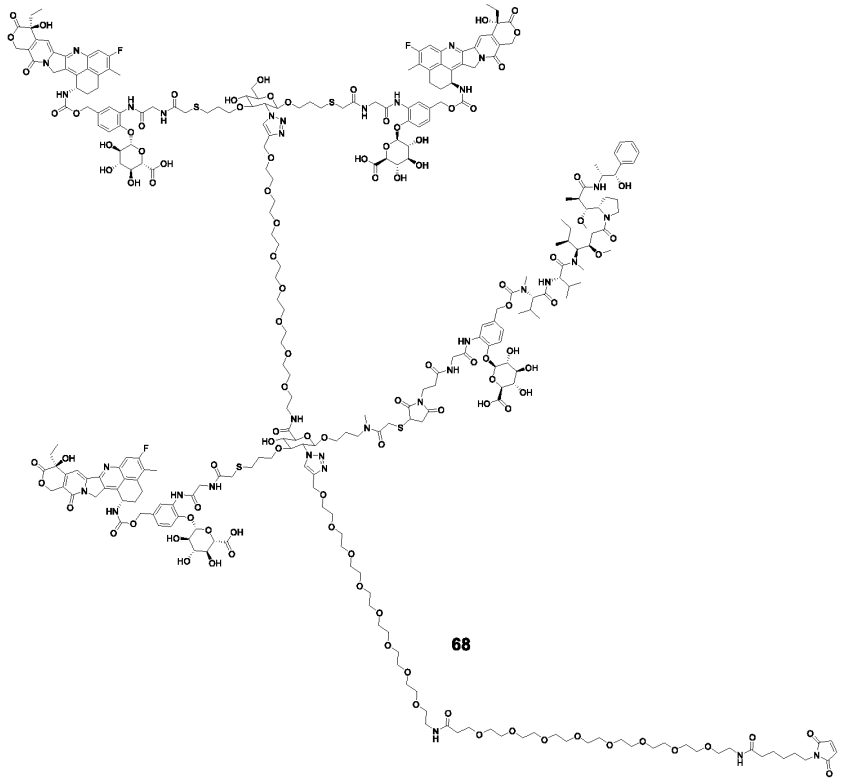
diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (68)



Synthetic scheme 68


TFA, DCM, RT, 1h
42-23
68-1
21-2
TEA, DCM, RT, 1h
2 steps, 36%
68-2
7-8
1) NHS, DCC, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 1h
45%
TFA, TIPS, DCM
RT, 10min
68-3
68-4
14
MeCN, H2O, TEA, RT, 2h
two steps, 82%
piperidine, DMF, RT, 1h
57%
68-5
68-6
33-2
PyBOP, DIPEA, DMF, RT, 2h
79%


68-7
53-1
CuSO4, Vc, DMSO, H2O, RT, 1h
19%


68

[00806] Step 1: The title compound **68-1** was prepared in analogy to **Example 57, Step 2** by using compound **42-23** and trifluoroacetic acid in dichloromethane. The crude product (1.30 g) was obtained as a yellow oil, which was used into the next step without further purification. LCMS: m/z calculated for $C_{68}H_{108}N_6O_{25}S$: 1441.69; found: 1441.62 $[M+H]^+$.

[00807] Step 2: The title compound **68-2** was prepared in analogy to **Example 11, Step 2** by using compound **68-1**, compound **21-7** and DIPEA in DMF. The product (400.00 mg, two steps, yield 36%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{89}H_{124}N_6O_{26}S_2$: 1758.11; found: 1758.71 $[M+H]^+$.

[00808] Step 3: The title compound **68-3** was prepared in analogy to **Example 18, Step 9 and step 10** by using N-hydroxy succinimide, EDCI, compound **68-2**, DIPEA and compound **7-8** in dichloromethane and DMF. The product (261.00 mg, yield 45%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{129}H_{162}FN_{11}O_{39}S_2$: 2573.87; found: 1287.31 $[M+2H]^+$.

[00809] Step 4: The title compound **68-4** was prepared in analogy to **Example 42, Step 15** by using compound **68-3**, triisopropylsilane and trifluoroacetic acid in dichloromethane. The product (318.00 mg, crude) was obtained as a yellow oil. LCMS: m/z calculated for $C_{110}H_{148}FN_{11}O_{39}S_2$: 2331.55; found: 1166.28 $[M+2H]^+$.

[00810] Step 5: The title compound **68-5** was prepared in analogy to **Example 18, Step 12** by using compound **68-4**, triethylamine and compound **14** in acetonitrile and H_2O. The product (300.00 mg, yield 82% over 2 steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{172}H_{238}FN_{19}O_{59}S_2$: 3598.99; found: 1799.57 $[M+2H]^+$.

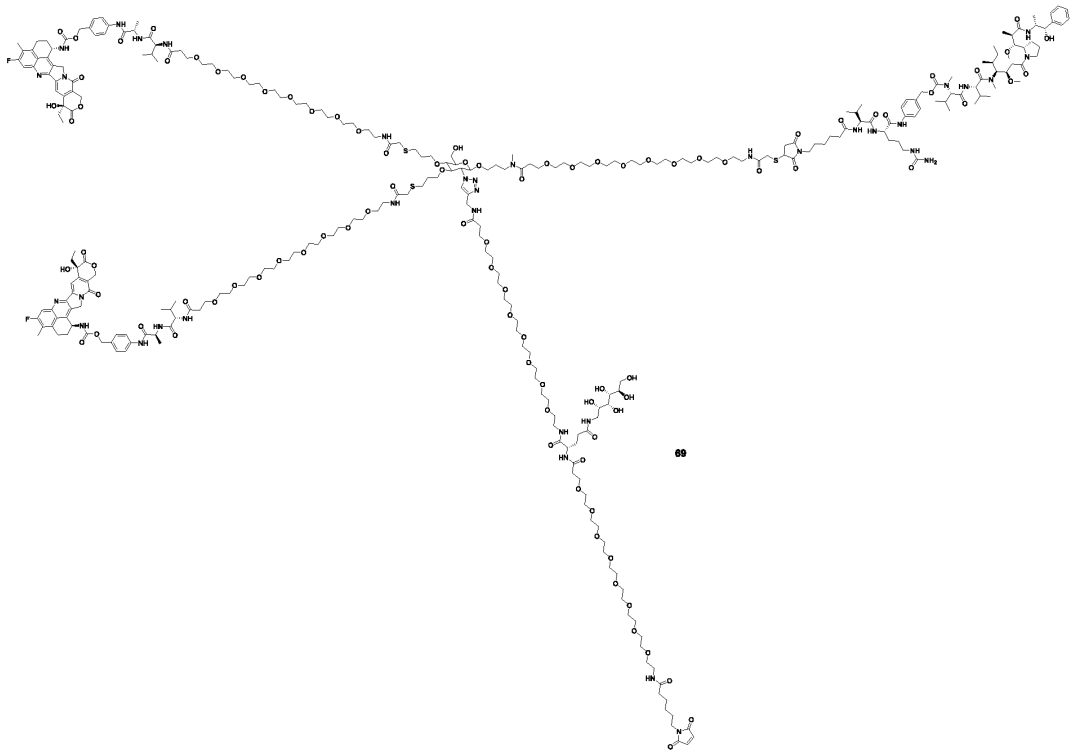
[00811] Step 6: The title compound **68-6** was prepared in analogy to **Example 42, Step 27** by using compound **68-5** and piperidine in DMF. The product (160.00 mg, yield 57%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{157}H_{228}FN_{19}O_{57}S_2$: 3376.75; found: 1688.65 $[M+H]^+$.

[00812] Step 7: The title compound **68-7** was prepared in analogy to **Example 62, Step 1** by using compound **33-2**, DIPEA, PyBOP and compound **68-6** in DMF. The product (75.00 mg, yield 79%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{186}H_{276}FN_{21}O_{69}S_2$: 3993.45; found: 1331.62 $[M+3H]^+$.

[00813] Step 8: The title compound **68** was prepared in analogy to **Example 1, Step 24** by using copper sulfate pentahydrate, L(+)-ascorbic acid, compound **53-1** and compound **68-7** in

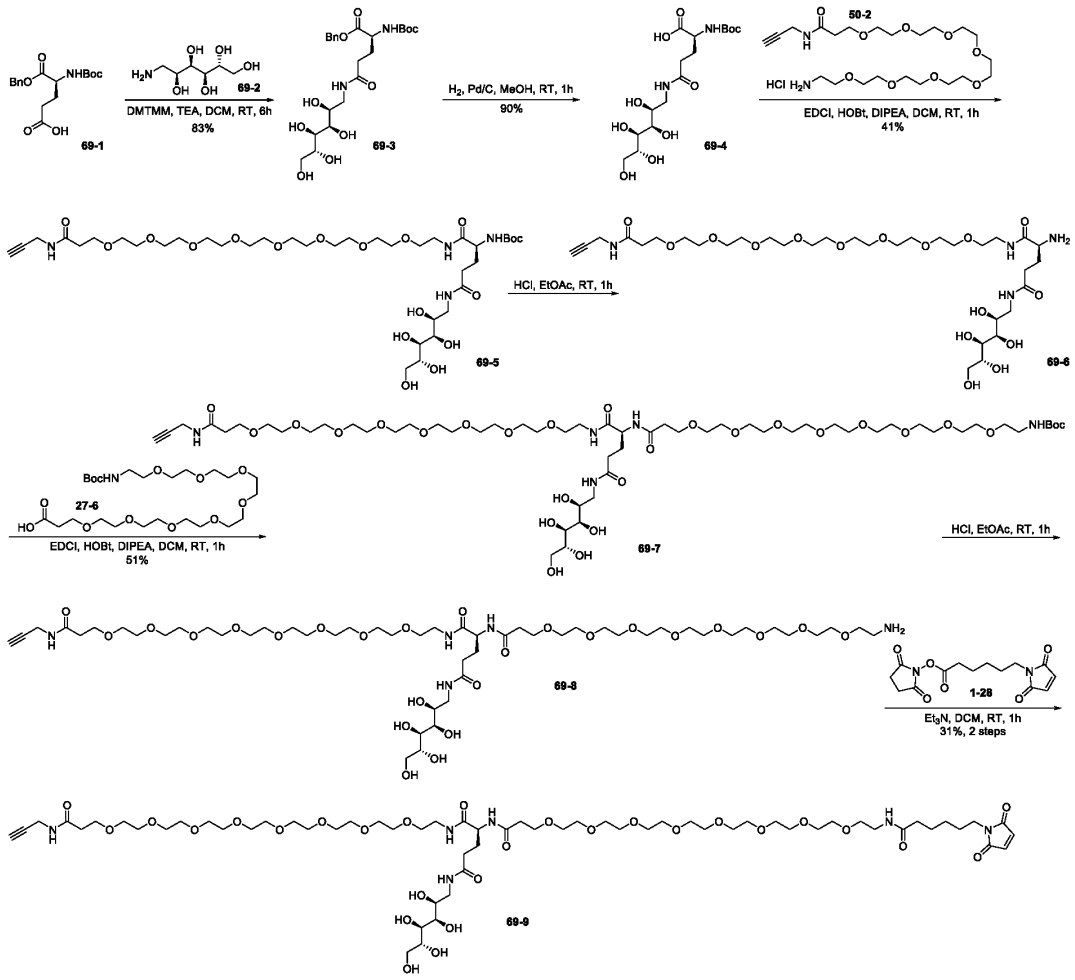
DMSO and H_2O. The product (11.30 mg, yield 19%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{282}H_{379}F_3N_{34}O_{104}S_4$: 6094.50; found: 1524.20 $[M+4H]^+$.

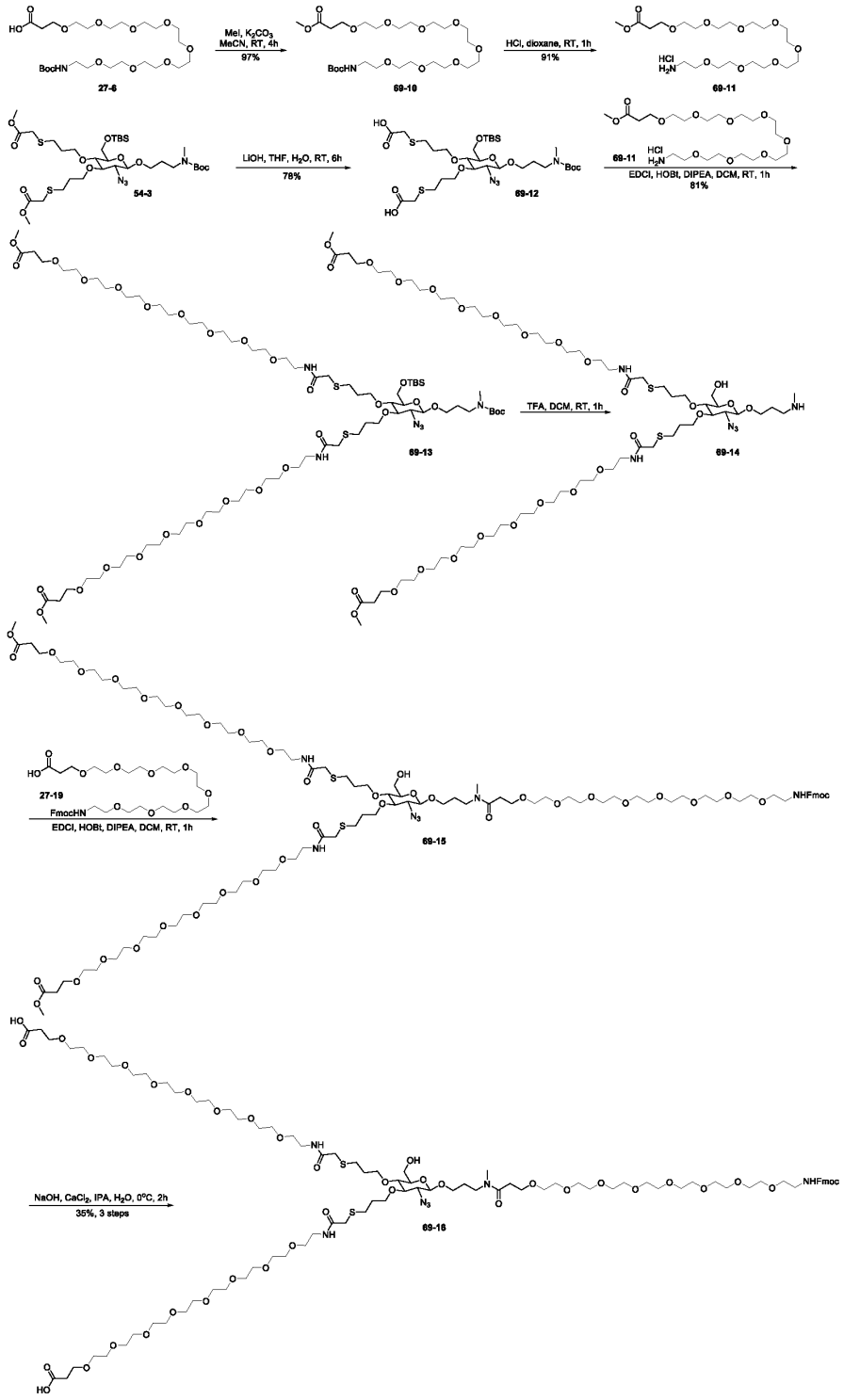
Example 69. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (69)

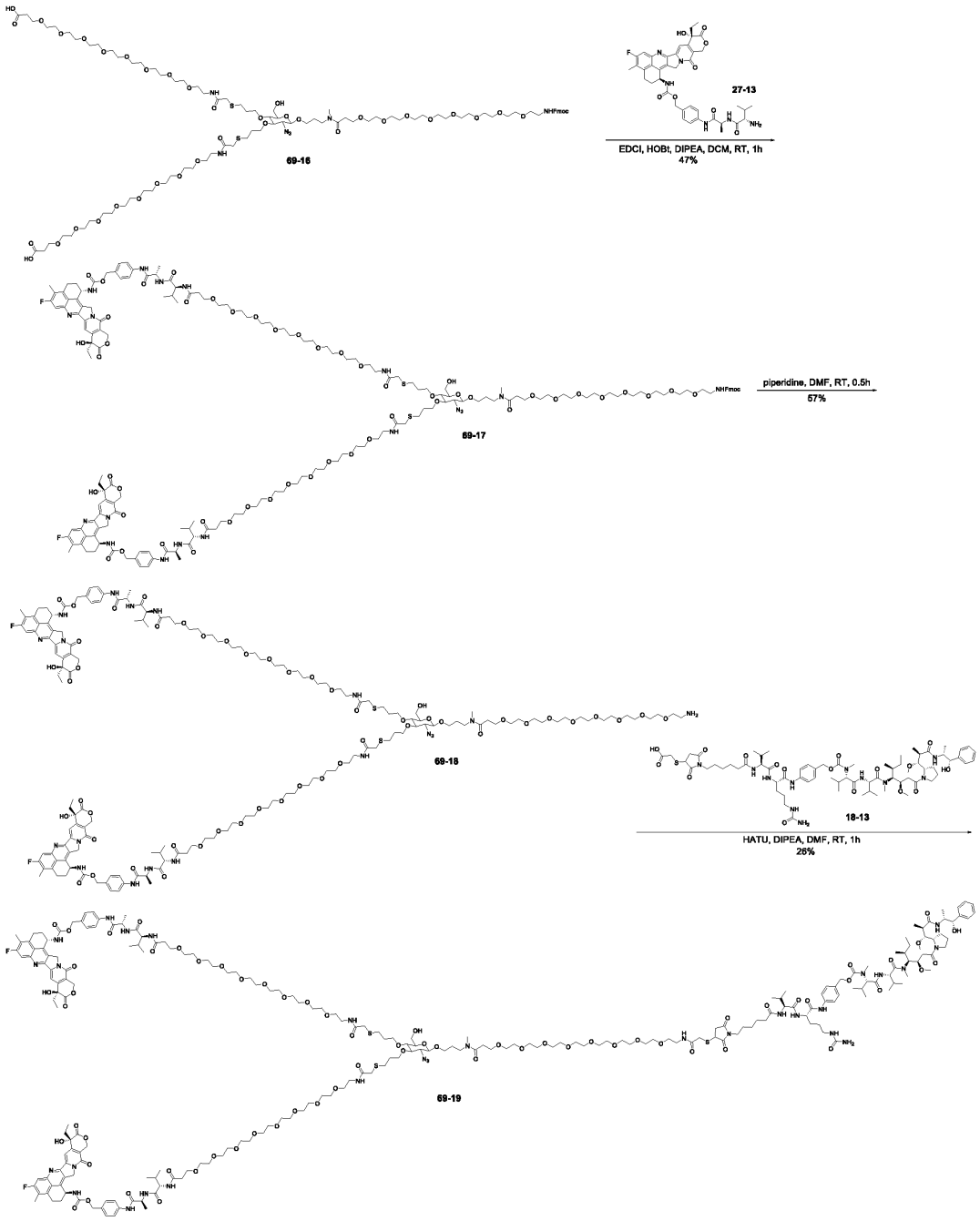


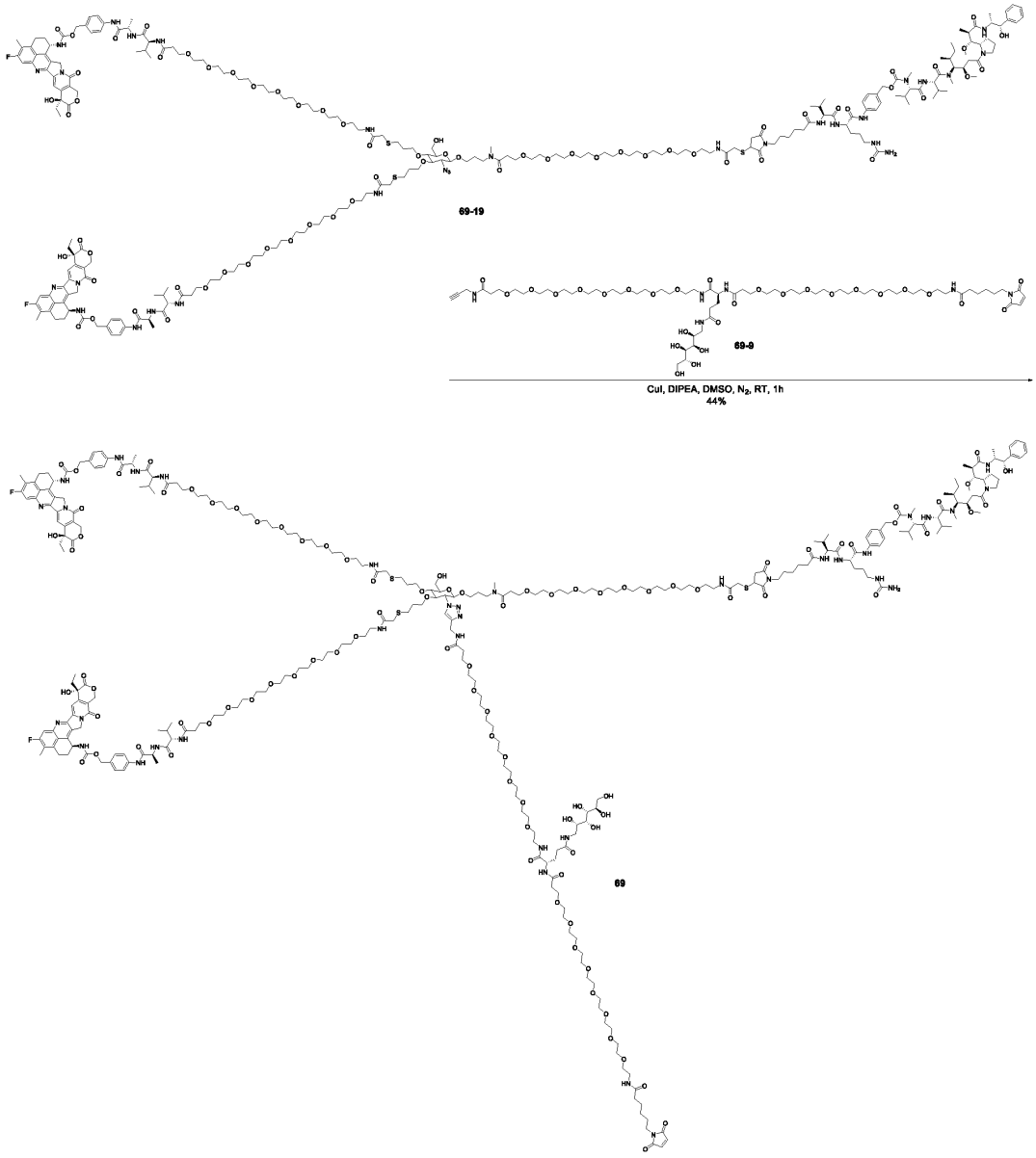
69

Synthetic scheme 69


69-1
69-2
DMTMM, TEA, DCM, RT, 6h
83%
69-3
H2, Pd/C, MeOH, RT, 1h
90%
69-4
50-2
EDCI, HOBt, DIPEA, DCM, RT, 1h
41%
HCl, EtOAc, RT, 1h
69-5
69-6
27-6
EDCI, HOBt, DIPEA, DCM, RT, 1h
51%
69-7
HCl, EtOAc, RT, 1h
69-8
1-28
Et3N, DCM, RT, 1h
31%, 2 steps
69-9


MeI, K2CO3
MeCN, RT, 4h
97%
HCl, dioxane, RT, 1h
91%
27-6
69-10
69-11
54-3
LiOH, THF, H2O, RT, 6h
78%
69-12
EDCI, HOBt, DIPEA, DCM, RT, 1h
81%
69-13
TFA, DCM, RT, 1h
69-14
27-19
FmocHN
EDCI, HOBt, DIPEA, DCM, RT, 1h
69-15
NaOH, CaCl2, IPA, H2O, 0°C, 2h
35%, 3 steps
69-16


69-16
27-13
EDCI, HOBt, DIPEA, DCM, RT, 1h
47%
69-17
piperidine, DMF, RT, 0.5h
57%
69-18
18-13
HATU, DIPEA, DMF, RT, 1h
26%
69-19



[00814] Step 1: Step 5: To a solution of (S)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid **(69-1)** (5.00 g, 14.82 mmol) and and (2R,3R,4R,5S)-6-aminohexane-1,2,3,4,5-pentaol **(69-2)** (2.95 g, 16.30 mmol) in dichloromethane (120 mL) was added triethylamine (2.99 g, 29.64 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMTMM, 4.51 g, 16.30 mmol). The reaction was stirred at room temperature for 6 h. After completion of the reaction, the mixture was concentrated under

reduced pressure. The residue was purified by silica gel column chromatography (eluted with 20~25% methanol in dichloromethane) to afford the title compound **69-3** (5.00 g, yield 68%) as a yellow oil. LCMS: m/z calculated for $C_{23}H_{36}N_2O_{10}$: 500.55; found: 501.32 $[M+H]^+$.

[00815] Step 2: To a solution of compound **69-3** (4.50 g, 8.99 mmol) in methanol (70 mL) was added 10% Pd/C (957.78 mg, 0.90 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and the cake was washed with methanol. The filtrate was concentrated under reduced pressure to afford the title compound **69-4** (3.30 g, yield 90%) as a yellow oil. LCMS: m/z calculated for $C_{16}H_{30}N_2O_{10}$: 410.42; found: 411.25 $[M+H]^+$.

[00816] Step 3: The title compound **69-5** was prepared in analogy to **Example 2, Step 5** by using compound **50-2** and compound **69-4**. The product (687 mg, yield 41%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{38}H_{70}N_4O_{18}$: 870.99; found: 871.51 $[M+H]^+$.

Step 4: The title compound **69-6** was prepared in analogy to **Example 27**, **Step 23** by using compound **69-5** in 4 N hydrochloric acid in ethyl acetate. The concentrated crude product (1.85 g) was obtained as a yellow oil. LCMS: m/z calculated for $C_{33}H_{62}N_4O_{16}$: 770.87; found: 771.48 $[M+H]^+$.

[00817] Step 5: The title compound **69-7** was prepared in analogy to **Example 2, Step 5** by using compound **69-6** and compound **27-6**. The product (1.50 g, yield 51% over 2 steps) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{57}H_{107}N_5O_{27}$: 1294.49; found: 1294.59 $[M+H]^+$.

[00818] Step 6: The title compound **69-8** was prepared in analogy to **Example 27**, **Step 23** by using compound **69-7** in 4 N hydrochloric acid in ethyl acetate. The concentrated crude product (768.00 mg) was obtained as a yellow oil. LCMS: m/z calculated for $C_{52}H_{99}N_5O_{25}$: 1194.37; found: 1194.70 $[M+H]^+$.

[00819] Step 7: The title compound **69-9** was prepared in analogy to **Example 1**, **Step 21** by using compound **69-8** and compound **1-28** with triethylamine instead of N,N-diisopropylethylamine. The product (243 mg, yield 31% over 2 steps) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{62}H_{110}N_6O_{28}$: 1387.58; found: 1387.65 $[M+H]^+$.

[00820] Step 8: To a solution of **27-6** (10.00 g, 18.46 mmol) and potassium carbonate (5.10 g, 36.92 mmol) in acetonitrile (100 mL) was added methyl iodide (5.24 g, 36.92 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the

mixture was filtered and the cake was washed with acetonitrile. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluted with 5% methanol in dichloromethane) to afford the title compound **69-10** (10.00 g, yield 97%) as a yellow oil. LCMS: m/z calculated for $C_{25}H_{49}NO_{12}$: 555.66; found: 456.37 $[M\text{-Boc}+H]^+$.

[00821] Step 9: A solution of **69-10** (10.00 g, 18.00 mmol) and 4 N hydrochloric acid in dioxane (10 mL) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure to afford the title compound **69-11** (8.1 g, yield 91%) as a yellow oil. LCMS: m/z calculated for $C_{20}H_{41}NO_{10}$: 455.55; found: 456.25 $[M+H]^+$.

[00822] Step 10: The title compound **69-12** was prepared in analogy to **Example 4**, **Step 5** by using compound **54-3**. The product (1.50 g, yield 78%) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{31}H_{58}N_4O_{11}S_2Si$: 755.03; found: 655.38 $[M\text{-Boc}+H]^+$.

[00823] Step 11: The title compound **69-13** was prepared in analogy to **Example 2**, **Step 5** by using compound **69-11** and compound **69-12**. The product (2.60 g, yield 81%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{71}H_{136}N_6O_{29}S_2Si$: 1630.09; found: 1629.76 $[M+H]^+$.

[00824] Step 12: The title compound **69-14** was prepared in analogy to **Example 57 Step 2** by using compound **69-13**. The concentrated crude product (3.30 g) was obtained as a yellow oil. LCMS: m/z calculated for $C_{60}H_{114}N_6O_{27}S_2$: 1415.71; found: 1415.66 $[M+H]^+$.

[00825] Step 13: The title compound **69-15** was prepared in analogy to **Example 2**, **Step 5** by using compound **69-14** and compound **27-19**. The crude product (1.50 g) was obtained as a yellow oil. LCMS: m/z calculated for $C_{94}H_{161}N_7O_{38}S_2$: 2061.45; found: 1031.16 $[M+2H]^+$.

[00826] Step 14: The title compound **69-16** was prepared in analogy to **Example 42**, **Step 22** by using compound **69-15**. The product (1.16 g, three steps, yield 35%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{92}H_{157}N_7O_{38}S_2$: 2033.40; found: 1017.15 $[M+2H]^+$.

[00827] Step 15: The title compound **69-17** was prepared in analogy to **Example 2**, **Step 5** by using compound **69-16** and compound **27-13**. The product (487.00 mg, yield 47%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{172}H_{239}F_2N_{19}O_{52}S_2$: 3507.00; found: 1169.94 $[M+3H]^+$.

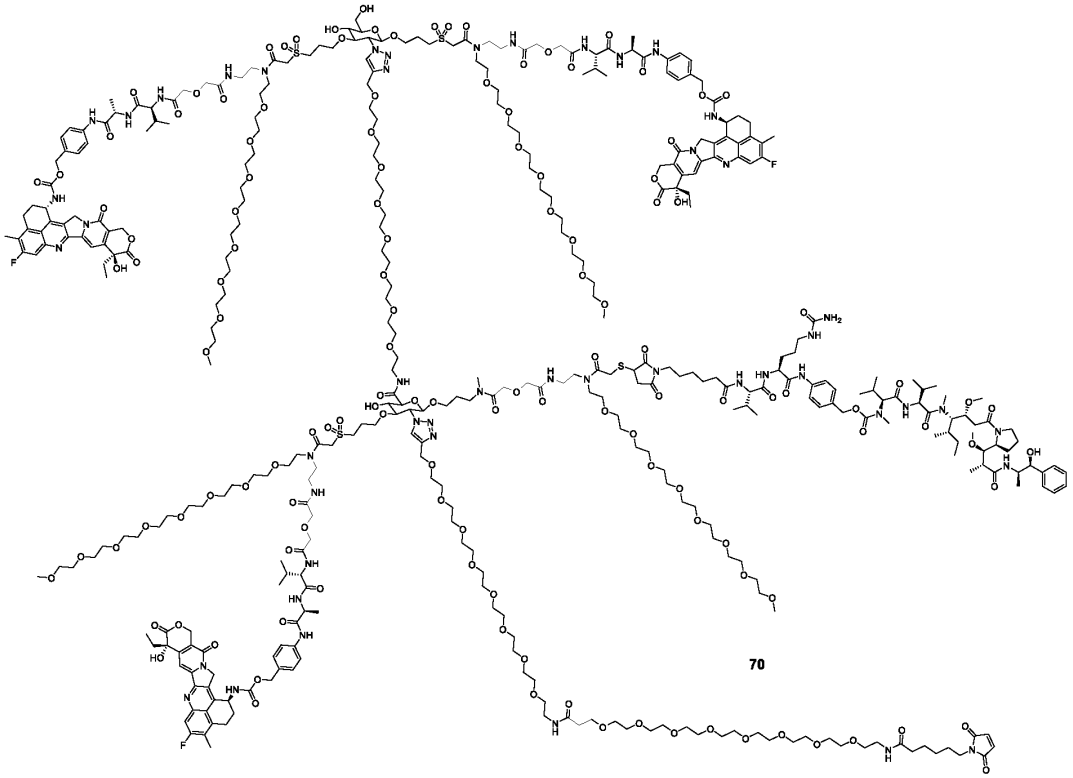
[00828] Step 16: The title compound **69-18** was prepared in analogy to **Example 42**, **Step 27** by using compound **69-17**. The product (261.00 mg, yield 57%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{157}H_{229}F_2N_{19}O_{50}S_2$: 3284.76; found: 1642.76 $[M+2H]^+$.

[00829] Step 17: The title compound **69-19** was prepared in analogy to **Example 13**, **Step 6** by using compound **69-18** and compound **18-13**. The product (95.00 mg, yield 26%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{227}H_{336}F_2N_{30}O_{66}S_3$: 4675.51; found: 1170.86 $[M+4H]^+$.

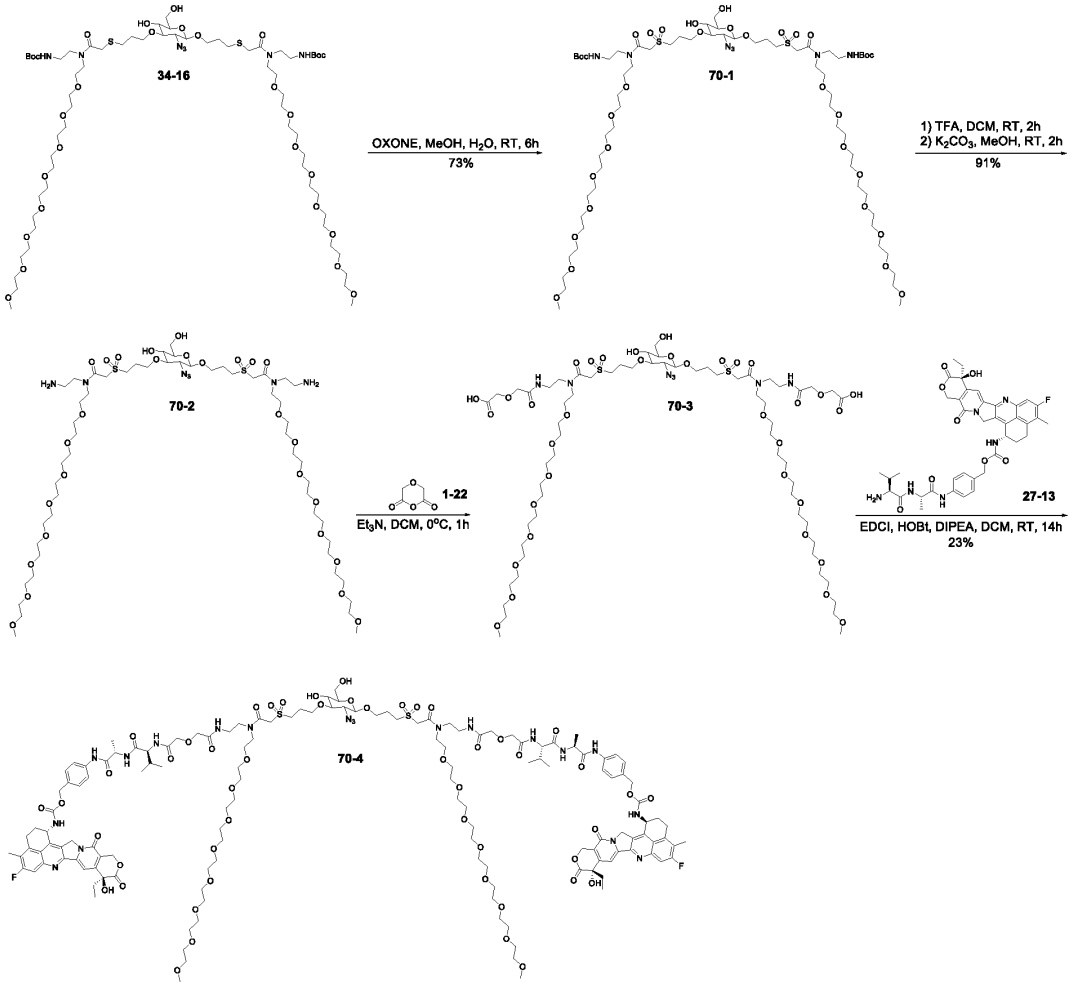
[00830] Step 18: To a solution of compound **69-19** (30.00 mg, 0.006 mmol) and compound **69-9** (8.90 mg, 0.006 mmol) in dimethyl sulfoxide (1 mL) was added copper(I) iodide (1.14 mg, 0.006 mmol) and N,N-diisopropylethylamine (3.10 mg, 0.03 mmol). The mixture was stirred at room temperature under N_2 atmosphere for 1 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (0.2 mL) and filtered. The resulting mixture was purified by pre-HPLC (C18 column, eluted with 69% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **69** (17.20 mg, yield 44%) as a pale-yellow solid. LCMS: m/z calculated for $C_{289}H_{446}F_2N_{36}O_{94}S_3$: 6063.08; found: 1213.38 $[M+5H]^+$.

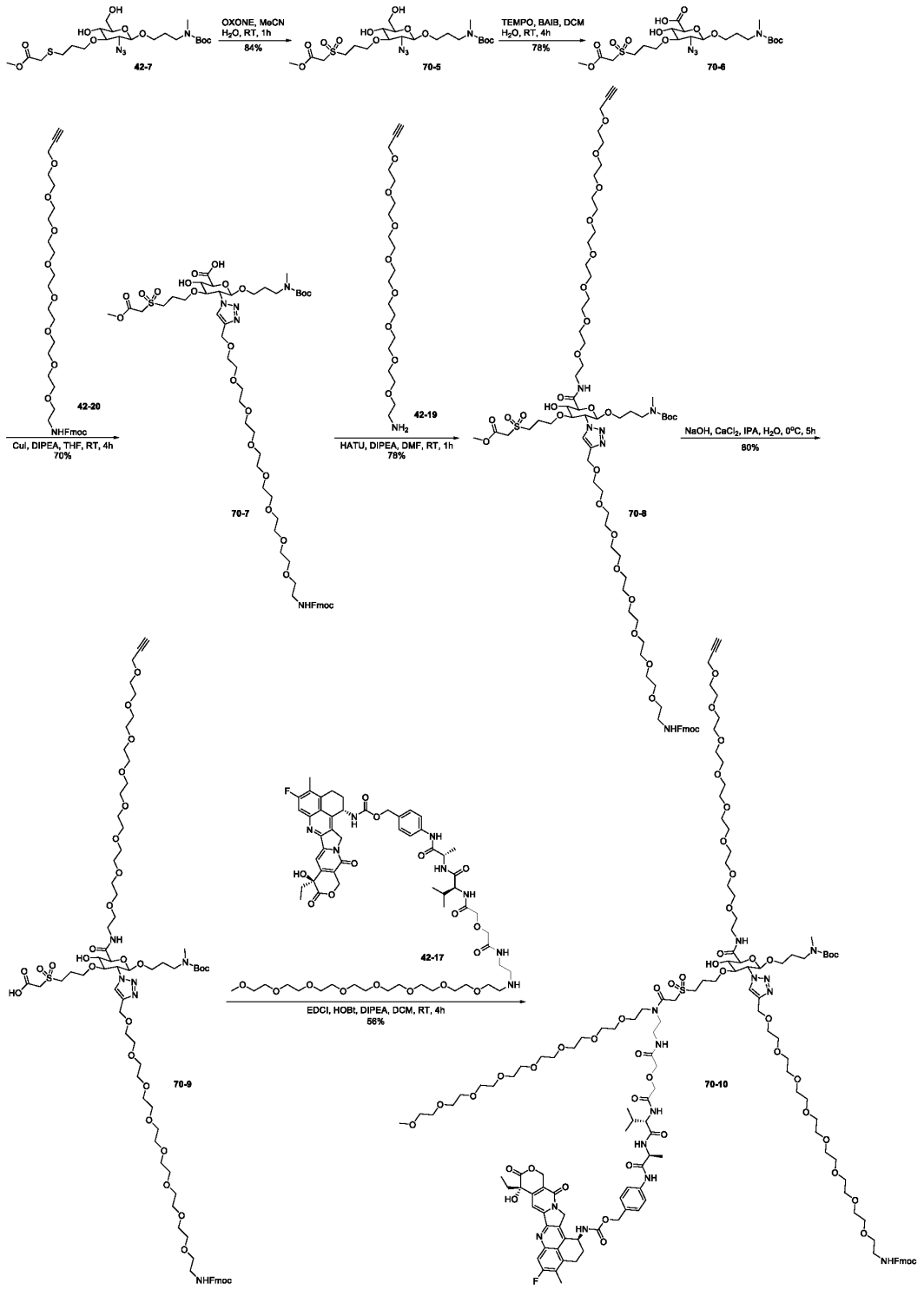
Example 70. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-

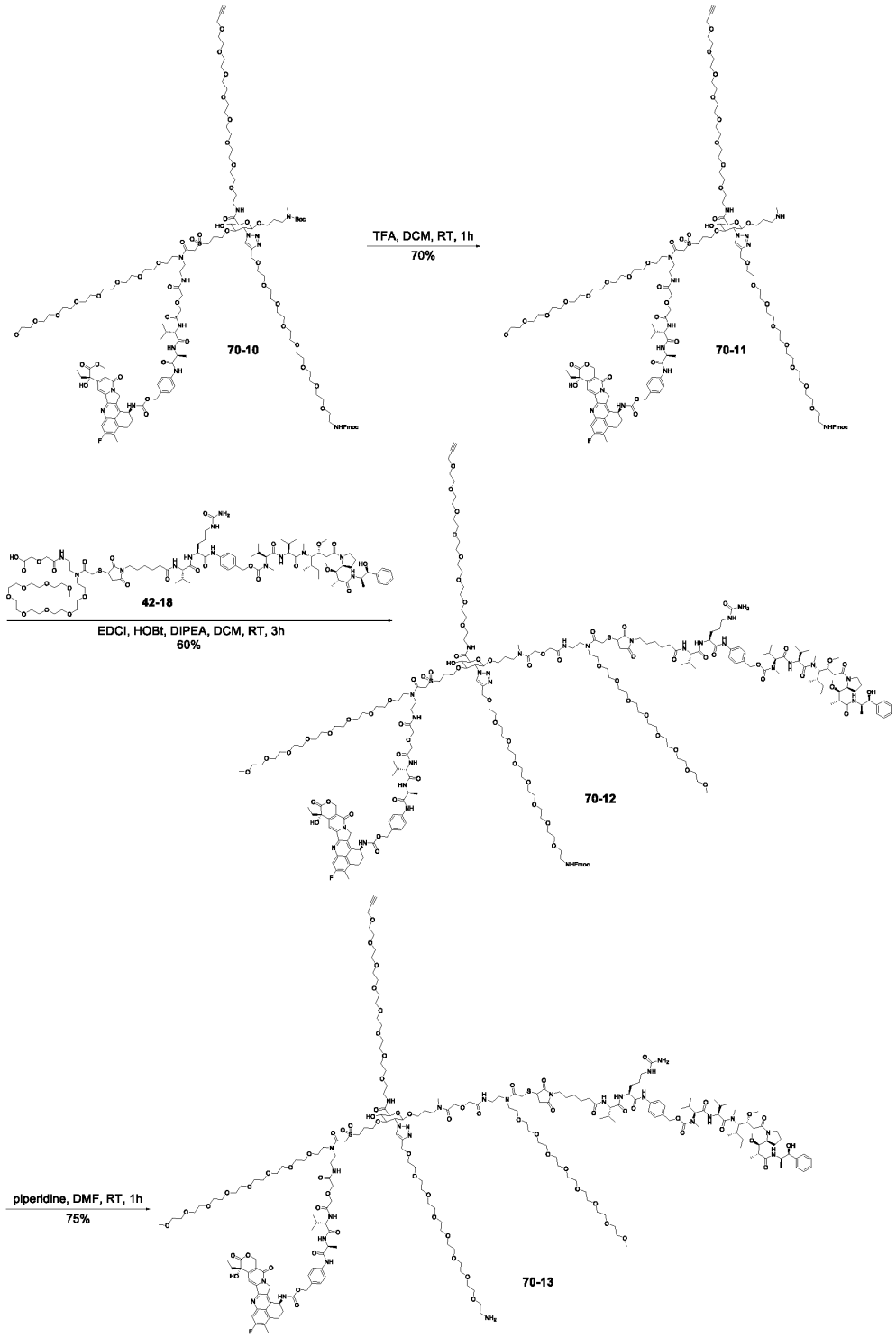
[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (70)

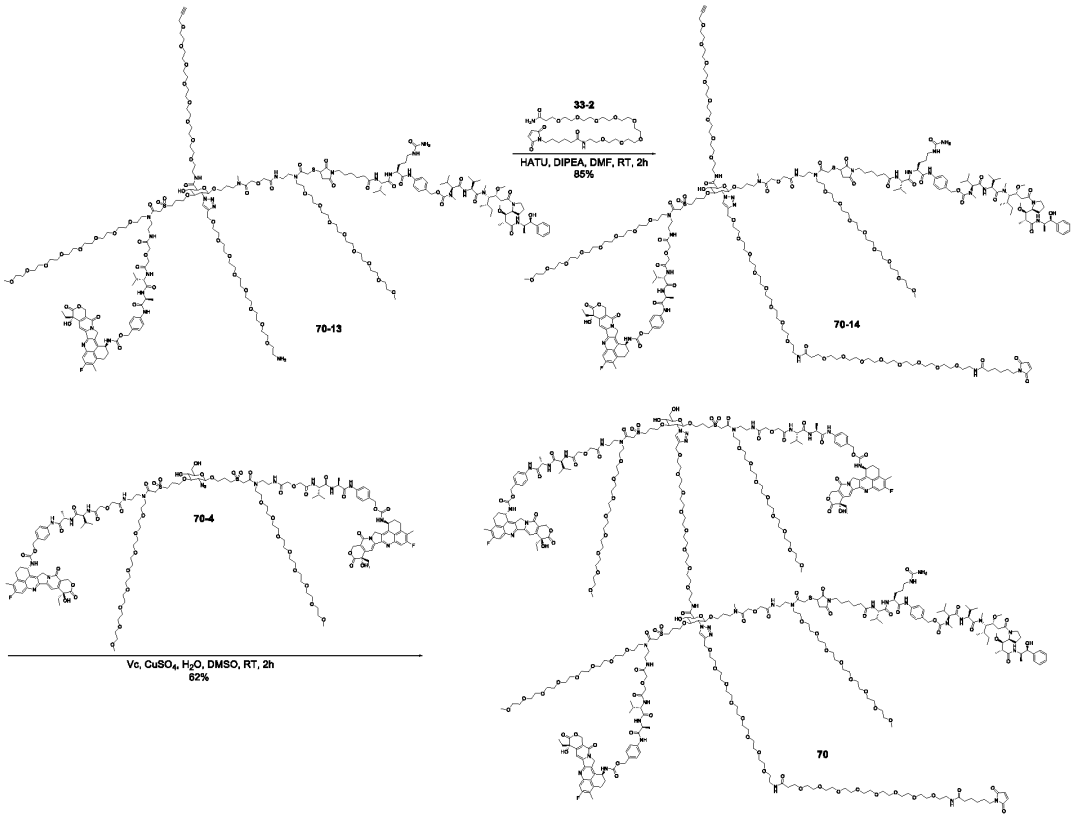


Synthetic scheme 70


34-16
OXONE, MeOH, H2O, RT, 6h
73%
70-1
1) TFA, DCM, RT, 2h
2) K2CO3, MeOH, RT, 2h
91%
70-2
1-22
Et3N, DCM, 0°C, 1h
70-3
27-13
EDCI, HOBt, DIPEA, DCM, RT, 14h
23%
70-4


OXONE, MeCN
H2O, RT, 1h
84%
TEMPO, BAIB, DCM
H2O, RT, 4h
78%
42-7
70-5
70-6
42-20
CuI, DIPEA, THF, RT, 4h
70%
70-7
42-19
HATU, DIPEA, DMF, RT, 1h
78%
70-8
NaOH, CaCl2, IPA, H2O, 0°C, 5h
80%
42-17
EDCI, HOBt, DIPEA, DCM, RT, 4h
56%
70-9
70-10


70-10
TFA, DCM, RT, 1h
70%
70-11
42-18
EDCI, HOBt, DIPEA, DCM, RT, 3h
60%
70-12
piperidine, DMF, RT, 1h
75%
70-13



[00831] Step 1: The title compound **70-1** was prepared in analogy to **Example 45, Step 1** by using compound **34-16** in methanol. The product (780 mg, yield 73%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{64}H_{123}N_7O_{31}S_2$: 1550.83; found: 1550.49 $[M+H]^+$.

[00832] Step 2: The title compound **70-2** was prepared in analogy to **Example 18, Step 11** by using compound **70-1**. The product (618 mg, yield 91%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{54}H_{107}N_7O_{27}S_2$: 1350.59; found: 1350.44 $[M+H]^+$.

[00833] Step 3: The title compound **70-3** was prepared in analogy to **Example 2, Step 7** by using compound **70-2** and compound **1-22**. The product (600 mg, yield 83%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{62}H_{115}N_7O_{35}S_2$: 1582.74; found: 1582.38 $[M+H]^+$.

[00834] Step 4: The title compound **70-4** was prepared in analogy to **Example 2, Step 7** by using compound **70-2** and compound **1-22**. The product (267 mg, yield 23%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{142}H_{197}F_2N_{19}O_{49}S_2$: 3056.34; found: 1528.34 $[M+2H]^{2+}$.

[00835] Step 5: To a mixture of compound **42-7** (3.05 g, 5.836 mmol) in acetonitrile (30 mL) and water (30 mL) was added OXONE (7.17 g, 11.672 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and concentrated. The aqueous mixture was poured into water (100 mL) and extracted with dichloromethane (100 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified silica gel column chromatography (eluted with 75% ethyl acetate in petroleum ether) to afford the title compound **70-5** (3.29 g, yield 84%) as a yellow oil. LCMS: m/z calculated for $C_{21}H_{38}N_4O_{11}S$: 554.61; found: 555.95 $[M+H]^+$.

[00836] Step 6: To a mixture of compound **70-5** (2.64 g, 4.760 mmol) in dichloromethane (20 mL) and water (8 mL) was added TEMPO (81 mg, 0.476 mmol) and BAIB (3.07 g, 9.520 mmol) at 0 °C. The mixture was stirred at 0 °C for 20 min and then warmed to room temperature for 40 min. After completion of the reaction, the mixture was poured into water (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-6** (2.01 g, yield 74%) as a yellow oil. LCMS: m/z calculated for $C_{21}H_{36}N_4O_{12}S$: 568.60; found: 569.95 $[M+H]^+$.

[00837] Step 7: Under nitrogen atmosphere, to a mixture of compound **70-6** (1.21 g, 2.128 mmol) and compound **42-20** (1.608 g, 2.553 mmol) in anhydrous tetrahydrofuran (23 mL) was added N, N-diisopropylethylamine (0.740 mL, 4.256 mmol) and cuprous iodide (101 mg, 1.064 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was poured into saturated ammonium chloride (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were washed with 0.5 M hydrochloric acid, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 9% methyl alcohol in dichloromethane) to give the title compound **70-7** (2.15 g, yield 70%) as a yellow oil. LCMS: m/z calculated for $C_{55}H_{83}N_5O_{22}S$: 1198.34; found: 1198.46 $[M+H]^+$.

[00838] Step 8: Under nitrogen atmosphere, to a mixture of compound **70-7** (2.15 g, 1.794 mmol) in anhydrous N, N-dimethylformamide (25 mL) was added N, N-diisopropylethylamine

(1.091 mL, 6.279 mmol), HATU (1.02 g, 2.691 mmol). The mixture was stirred at room temperature for 10 min before compound **42-19** (804 mg, 1.973 mmol) was added. The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was poured into water (300 mL) and extracted with dichloromethane (400 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-8** (2.22 g, yield 78%) as a yellow oil. LCMS: m/z calculated for $C_{74}H_{118}N_6O_{29}S$: 1587.83; found: 1587.66 $[M+H]^+$.

[00839] Step 9: To a mixture of compound **70-8** (2.15 g, 1.354 mmol) in 0.8M calcium chloride solution and isopropyl alcohol (v/v=3:7, 22 mL) was added sodium hydroxide (81 mg, 2.031 mmol). The mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was adjusted pH to 5-6 with 0.5M hydrochloric acid. The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-9** (1.71 g, yield 80%) as a yellow oil. LCMS: m/z calculated for $C_{73}H_{116}N_6O_{29}S$: 1573.80; found: 1573.59 $[M+H]^+$.

[00840] Step 10: Under nitrogen atmosphere, to a mixture of compound **70-9** (565 mg, 0.359 mmol) in anhydrous dichloromethane (10 mL) was added compound **42-17** (505 mg, 0.395 mmol), N, N-diisopropylethylamine (0.187 mL, 1.516 mmol), 1-Hydroxybenzotriazole (48 mg, 0.359 mmol) and EDCI (137 mg, 0.718 mmol). The mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was poured into water (50 mL) and extracted with dichloromethane (50 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, HCl condition). The desired components were lyophilized to afford the title compound **70-10** (605 mg, yield 59%) as a yellow solid. LCMS: m/z calculated for $C_{136}H_{201}FN_{14}O_{47}S$: 2835.21; found: 1418.15 $[M+2H]^{2+}$.

[00841] Step 11: To a mixture of compound **70-10** (589 mg, 0.208 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.560 mL, 7.539 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column,

eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-11** (477 mg, yield 70%) as a yellow oil. LCMS: m/z calculated for $C_{131}H_{193}FN_{14}O_{45}S$: 2735.10; found: 1369.06 $[M+2H]^{2+}$.

[00842] Step 12: Under nitrogen atmosphere, to a mixture of compound **70-11** (472 mg, 0.172 mmol) in anhydrous dichloromethane (8 mL) was added compound **42-18** (367 mg, 0.190 mmol), N, N-diisopropylethylamine (0.090 mL, 0.516 mmol), 1-Hydroxybenzotriazole (24 mg, 0.172 mmol) and EDCI (67 mg, 0.345 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-12** (483 mg, yield 60%) as a yellow solid. LCMS: m/z calculated for $C_{224}H_{344}FN_{27}O_{72}S_2$: 4650.45; found: 1163.59 $[M+4H]^{4+}$.

[00843] Step 13: Under nitrogen atmosphere, to a mixture of compound **70-12** (470 mg, 0.101 mmol) in anhydrous N,N-dimethylformamide (3 mL) was added piperidine (0.030 mL, 0.303 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-13** (339 mg, yield 75%) as a yellow oil. LCMS: m/z calculated for $C_{209}H_{334}FN_{27}O_{70}S_2$: 4428.21; found: 1476.74 $[M+3H]^{3+}$.

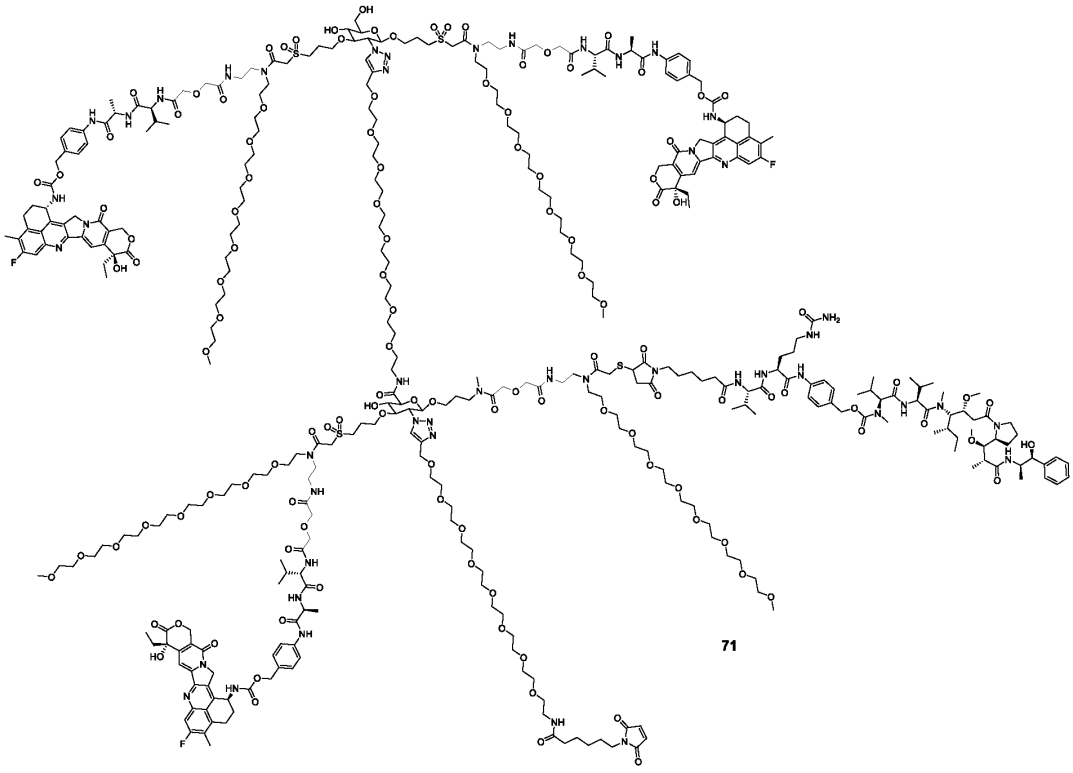
[00844] Step 14: Under nitrogen atmosphere, to a mixture of compound **33-2** (8 mg, 0.013 mmol) in anhydrous N, N-dimethylformamide (2 mL) was added compound N,N-diisopropylethylamine (0.005 mL , 0.030 mmol) and HATU (5 mg , 0.013 mmol). The mixture was stirred at room temperature for 10 min before compound **70-13** (47 mg, 0.010 mmol) was added. The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **70-14** (45.7 mg, yield 85%) as a yellow solid. LCMS: m/z calculated for $C_{238}H_{382}FN_{29}O_{82}S_2$: 5044.91; found: 1261.99 $[M+4H]^{4+}$.

[00845] Step 15: Under nitrogen atmosphere, to a mixture of compound **70-14** (17 mg, 0.003 mmol) and compound **70-4** (9.6 mg, 0.003 mmol) in dimethyl sulfoxide (1 mL) was added 0.5M L-Ascorbic acid aqueous solution (0.108 mL , 0.054 mmol) and 0.5M copper sulfate

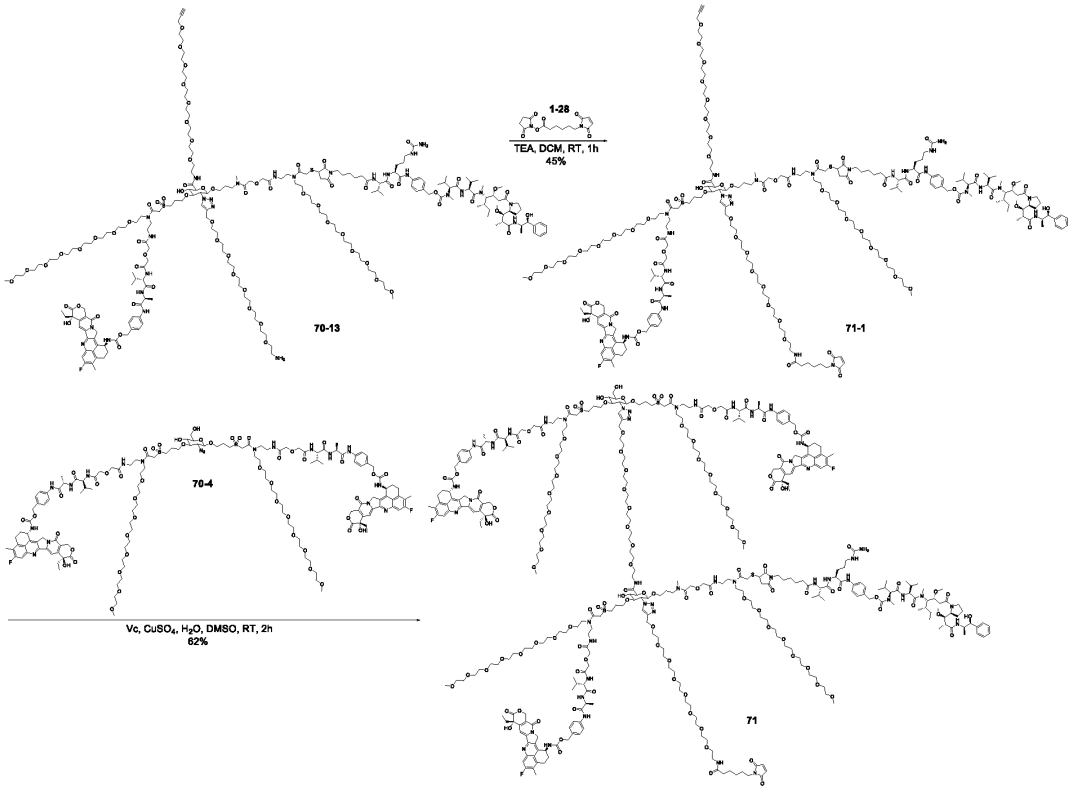
aqueous solution (0.036 mL , 0.018 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **70** (15.9 mg, yield 62%) as a yellow solid. LCMS: m/z calculated for $C_{380}H_{579}F_3N_{48}O_{131}S_4$: 8101.25; found: 1351.01 $[M+6H]^{6+}$.

Example 71. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-

propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (71)



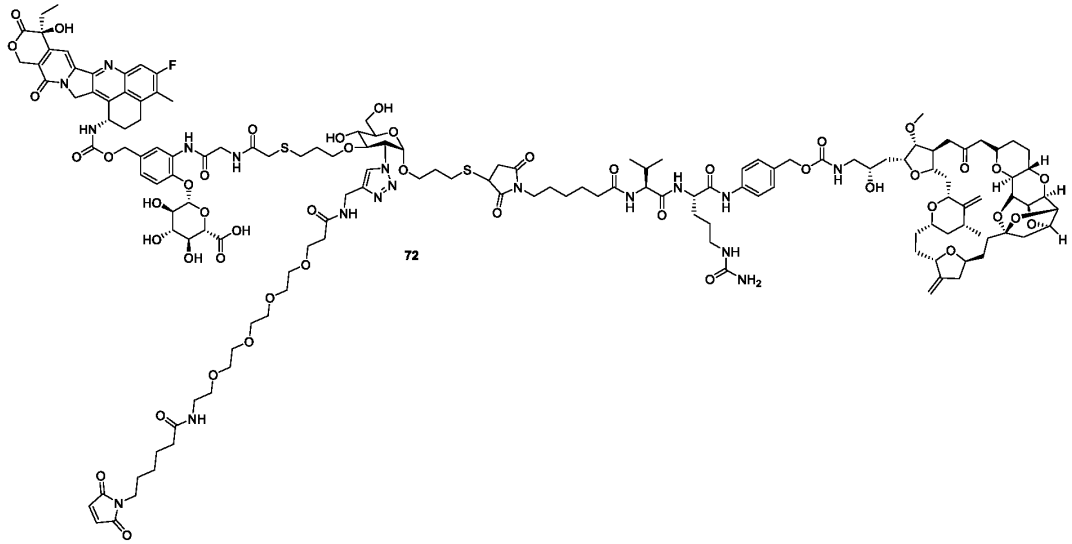
Synthetic scheme 71



[00846] Step 1: Under nitrogen atmosphere, to a mixture of compound **70-13** (47 mg, 0.011 mmol) in anhydrous dichloromethane (2 mL) was added triethylamine (0.006 mL, 0.042mmol) and compound **1-28** (5 mg, 0.016 mmol). The mixture was stirred at room temperature or 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound 71-1 (17 mg, yield 34%) as a white solid. LCMS: m/z calculated for $C_{219}H_{345}FN_{28}O_{73}S_2$: 4621.41; found: 1156.26 $[M+4H]^{4+}$.

[00847] Step 2: Under nitrogen atmosphere, to a mixture of compound **71-1** (17 mg, 0.003 mmol) and compound **70-4** (10.4 mg, 0.003 mmol) in dimethyl sulfoxide (1 mL) was added 0.5M L-Ascorbic acid aqueous solution (0.122 mL, 0.061 mmol) and 0.5M copper sulfate aqueous solution (0.041 mL , 0.020 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the reaction mixture was filtered and purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **71** (15.9 mg, yield 62%) as a yellow solid. LCMS: m/z calculated for $C_{361}H_{542}F_3N_{47}O_{122}S_4$: 7677.75; found: 1536.51 $[M+5H]^{5+}$.

Example 72. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(2*S*,4*R*)-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-2-[3-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[4-[[(2*S*)-2-hydroxy-3-[(3*S*,6*S*,9*S*,12*S*,14*R*,16*R*,20*R*,21*R*,22*S*,29*S*,31*R*,32*S*,33*R*,35*R*,36*S*)-21-methoxy-14-methyl-8,15-dimethylene-24-oxo-2,19,30,34,37,39,40,41-octaoxanonacyclo[24.9.2.$1^{3,32}$.$1^{3,33}$.$1^{6,9}$.$1^{12,16}$.$0^{18,22}$.$0^{29,36}$.$0^{31,35}$]hentetracontan-20-yl]propyl]carbamoyloxymethyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (72)



72

Synthetic scheme 72



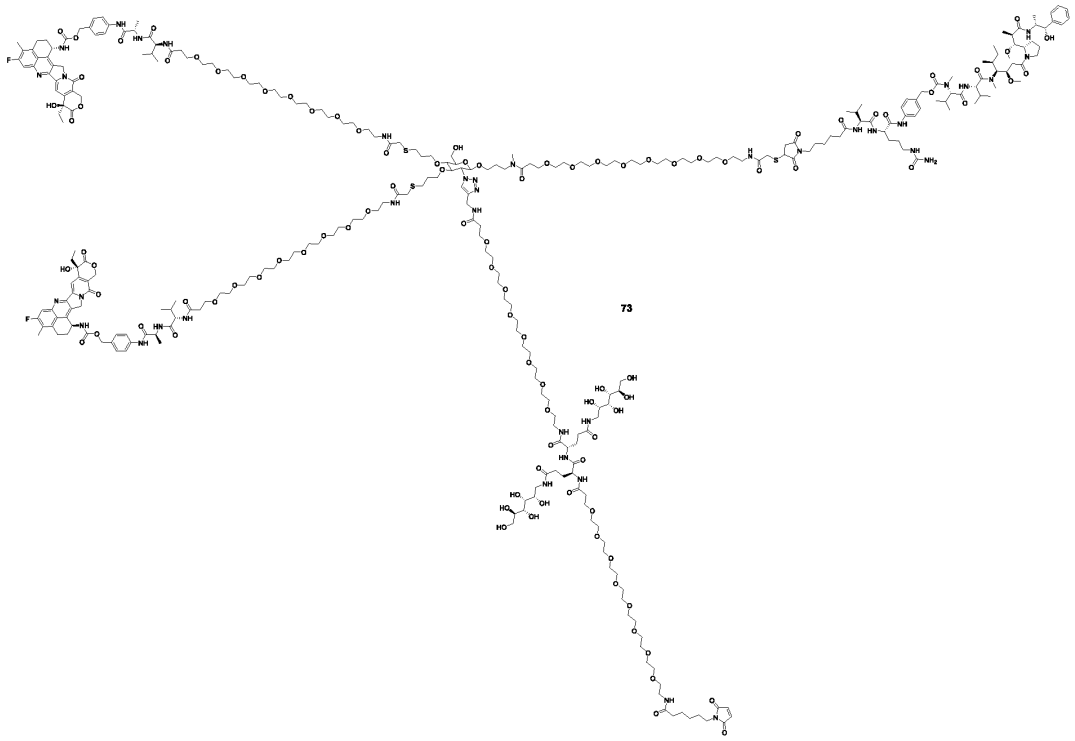
[00848] Step 1: The title compound **72-3** was prepared in analogy to **Example 7, Step 4** by using compound eribulin.MsOH (**72-1**) and compound MC-VC-PAB-PNP (**72-2**). The product (128 mg, yield 74%) was obtained as a white solid. LCMS: m/z calculated for $C_{69}H_{97}N_7O_{19}$: 1328.57; found: 1328.67 $[M+2]^+$.

[00849] Step 2: Under nitrogen atmosphere, to a solution of compound **15-10** (108 mg, 0.09 mmol) in dimethyl sulfoxide (2 mL) was added compound **72-3** (117 mg, 0.09 mmol) and triethylamine (27 mg, 0.26 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **72-4** (140 mg, yield 62%) as a light-yellow solid. LCMS: m/z calculated for $C_{123}H_{160}FN_{15}O_{39}S_2$: 2555.82; found: 1281.31 $[M+2H]^{2+}$.

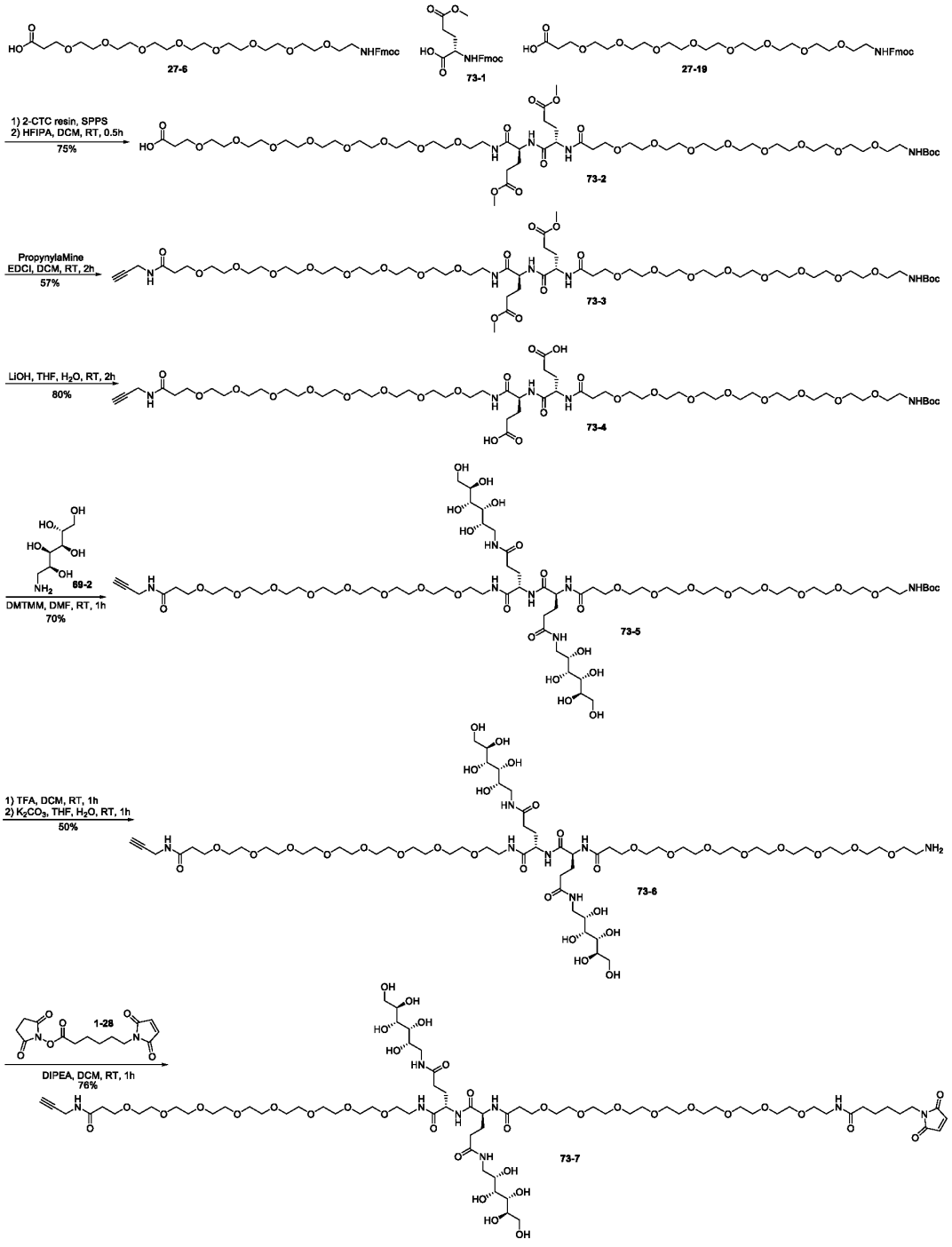
[00850] Step 3: Under nitrogen atmosphere, to a solution of compound **72-4** (20 mg, 0.008 mmol) and compound **1-31** (5 mg, 0.010 mmol) in dimethyl sulfoxide (0.5 mL) was added N,N-diisopropylethylamine (4 mg, 0.031 mmol) and cuprous iodide(1.5 mg, 0.008 mmol) at room

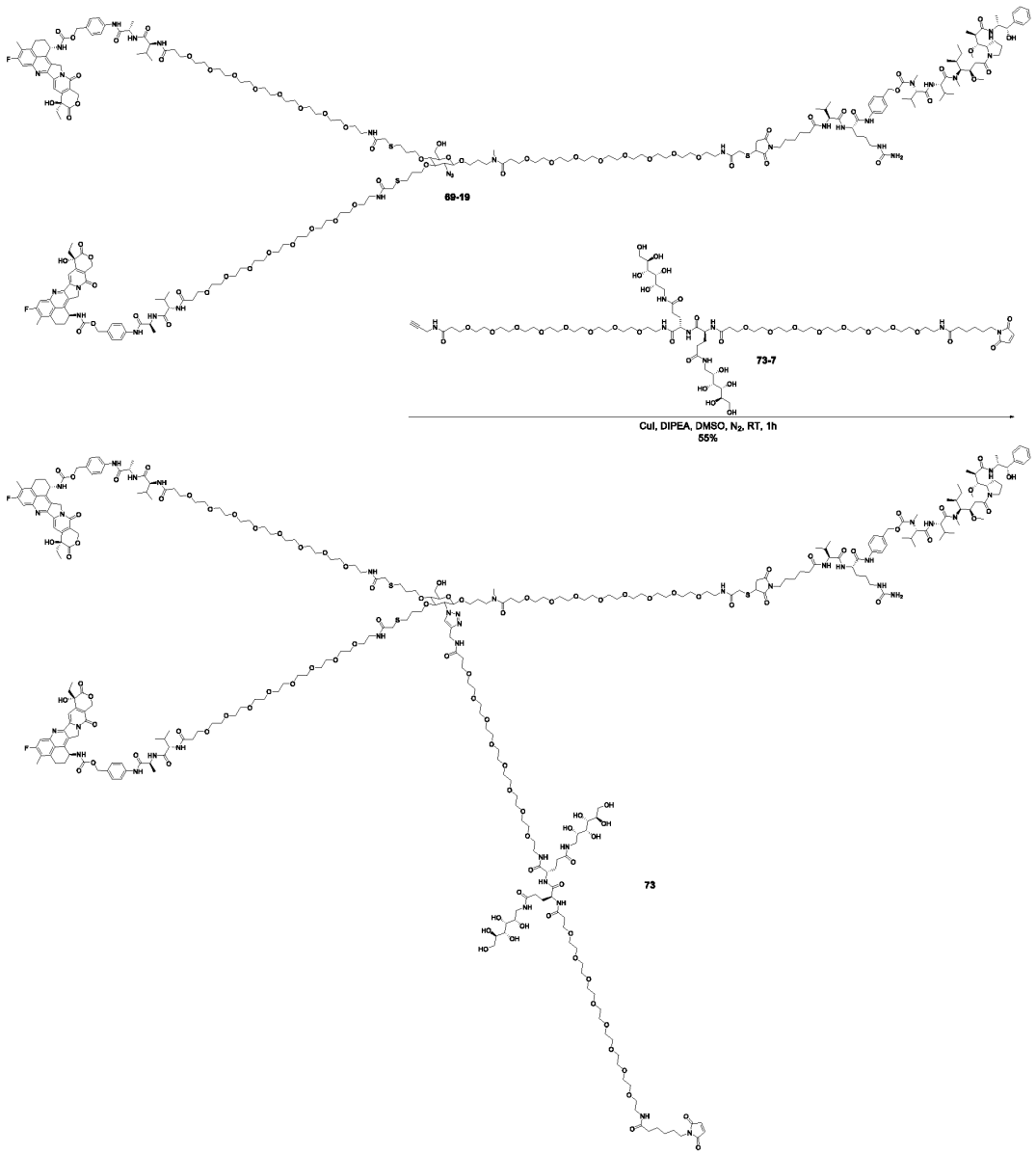
temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by pre-HPLC (C18 column, eluted with acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **72** (8 mg, yield 33%) as a white solid. LCMS: m/z calculated for $C_{147}H_{197}FN_{18}O_{47}S_2$: 3051.39; found: 1018.33 $[M+3H]^{3+}$.

Example 73. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (73)



Synthetic scheme 73


27-6
73-1
27-19
1) 2-CTC resin, SPPS
2) HFIPA, DCM, RT, 0.5h
75%
73-2
PropynylaMine
EDCI, DCM, RT, 2h
57%
73-3
LiOH, THF, H2O, RT, 2h
80%
73-4
69-2
DMTMM, DMF, RT, 1h
70%
73-5
1) TFA, DCM, RT, 1h
2) K2CO3, THF, H2O, RT, 1h
50%
73-6
1-28
DIPEA, DCM, RT, 1h
76%
73-7



[00851] Step 1: The title compound **73-2** was prepared in analogy to **Example 5, Step 4** by using compound **27-6**, compound **73-1** and compound **27-19**. The product (1.8 g, yield 75%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{55}H_{102}N_4O_{27}$: 1251.42; found: 1251.70 $[M+H]^+$.

[00852] Step 2: The title compound **73-3** was prepared in analogy to **Example 27, Step 18** by using compound **73-2** and 2-propynylamine. The product (1.08 g, yield 57%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{58}H_{105}N_5O_{26}$: 1288.49; found: 1289.96 $[M+H]^+$.

[00853] Step 3: The title compound **73-4** was prepared in analogy to **Example 4**, **Step 5** by using compound **73-3**. The product (780.00 mg, yield 80%) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{56}H_{101}N_5O_{26}$: 1260.43; found: 1260.69 $[M+H]^+$.

[00854] Step 4: To a solution of **73-4** (740.00 mg, 0.59 mmol) and **69-2** (425.51 mg, 2.35 mmol) in N,N-dimethylformamide (12 mL) was added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMTMM, 375.51 mg, 1.36 mmol). The reaction was stirred at room temperature for 1 h. After completion of the reaction, the mixture was purified by reverse phase flash chromatography (C18 column, eluted with 30% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **73-5** (652.00 mg, yield 70%) as a colorless oil. LCMS: m/z calculated for $C_{68}H_{127}N_7O_{34}$: 1586.78; found: 744.13 $[M\text{-Boc}+H]^+$.

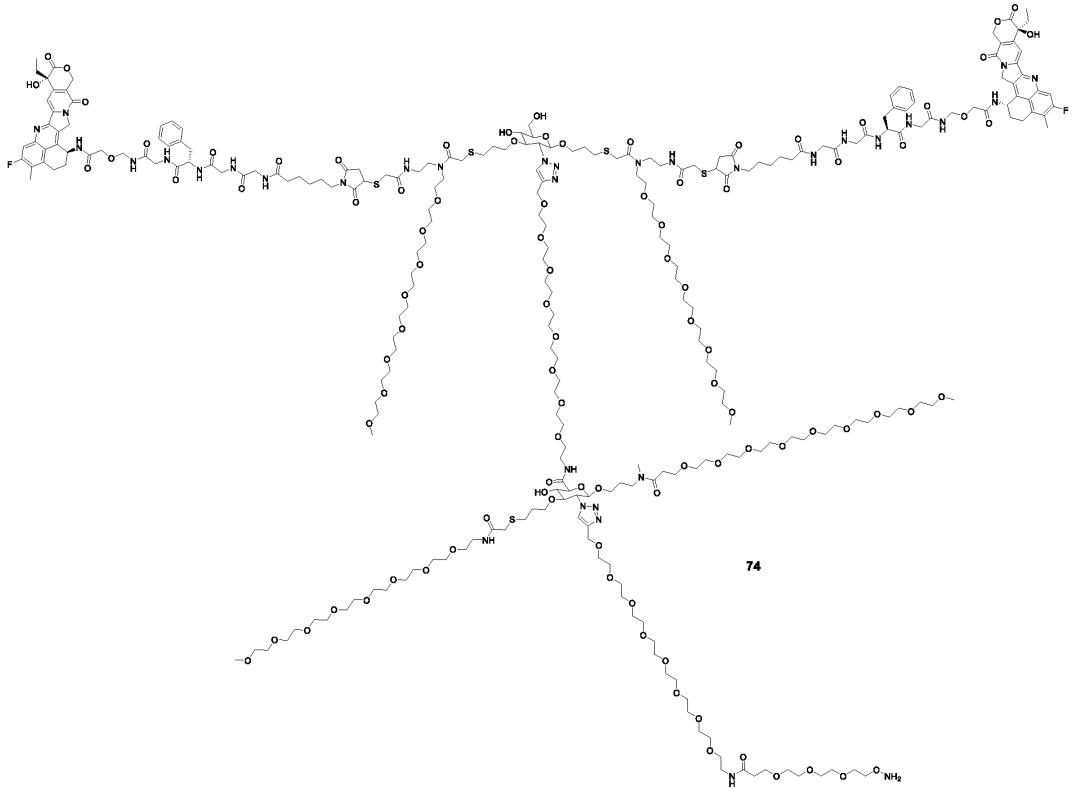
[00855] Step 5: The title compound **73-6** was prepared in analogy to **Example 18**, **Step 11** by using compound **73-5**. The product (233.00 mg, yield 50%) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{63}H_{119}N_7O_{32}$: 1486.66; found: 744.20 $[M+2H]^+$.

[00856] Step 6: The title compound **73-7** was prepared in analogy to **Example 1**, **Step 21** by using compound **1-28** and compound **73-6**. The product (168.00 mg, yield 76%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{73}H_{130}N_8O_{35}$: 1679.86; found: 840.60 $[M+2H]^+$.

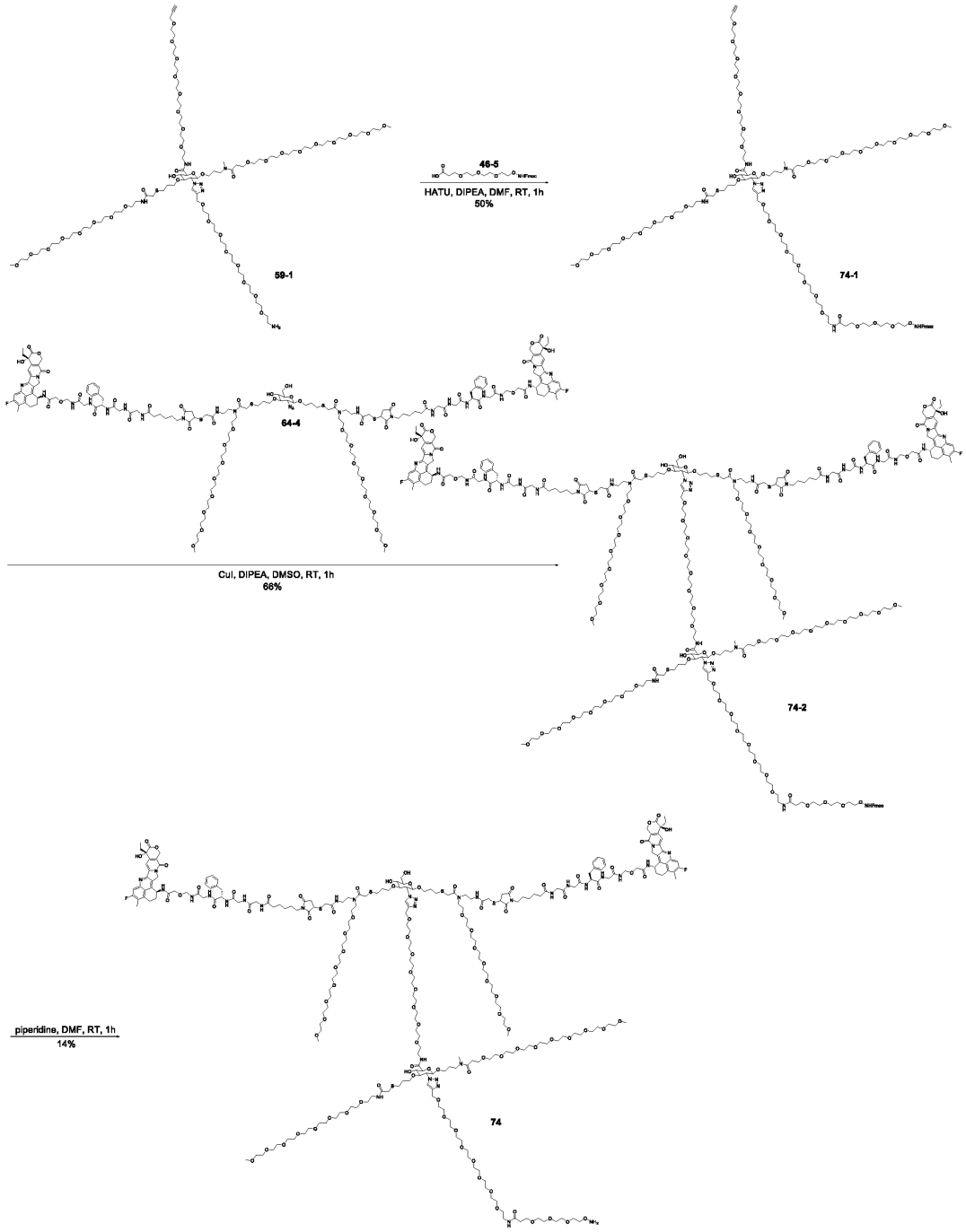
[00857] Step 7: The title compound **73** was prepared in analogy to **Example 69**, **Step 18** by using compound **69-19** and compound **73-7**. The product (24.60 mg, yield 55%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{300}H_{466}F_2N_{38}O_{101}S_3$: 6355.37; found: 1271.89 $[M+5H]^+$.

Example 74. Synthesis of 5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-(2-aminooxyethoxy)ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-*N*-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-

ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-4-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]tetrahydropyran-2-carboxamide (74)



Synthetic scheme 74



[00858] Step 1: A mixture of compound **46-5** (19 mg, 0.04 mmol), ethyldiisopropylamine (18 mg, 0.14 mmol) and HATU (16 mg, 0.04 mmol) in N,N-dimethylformamide (2 mL) was

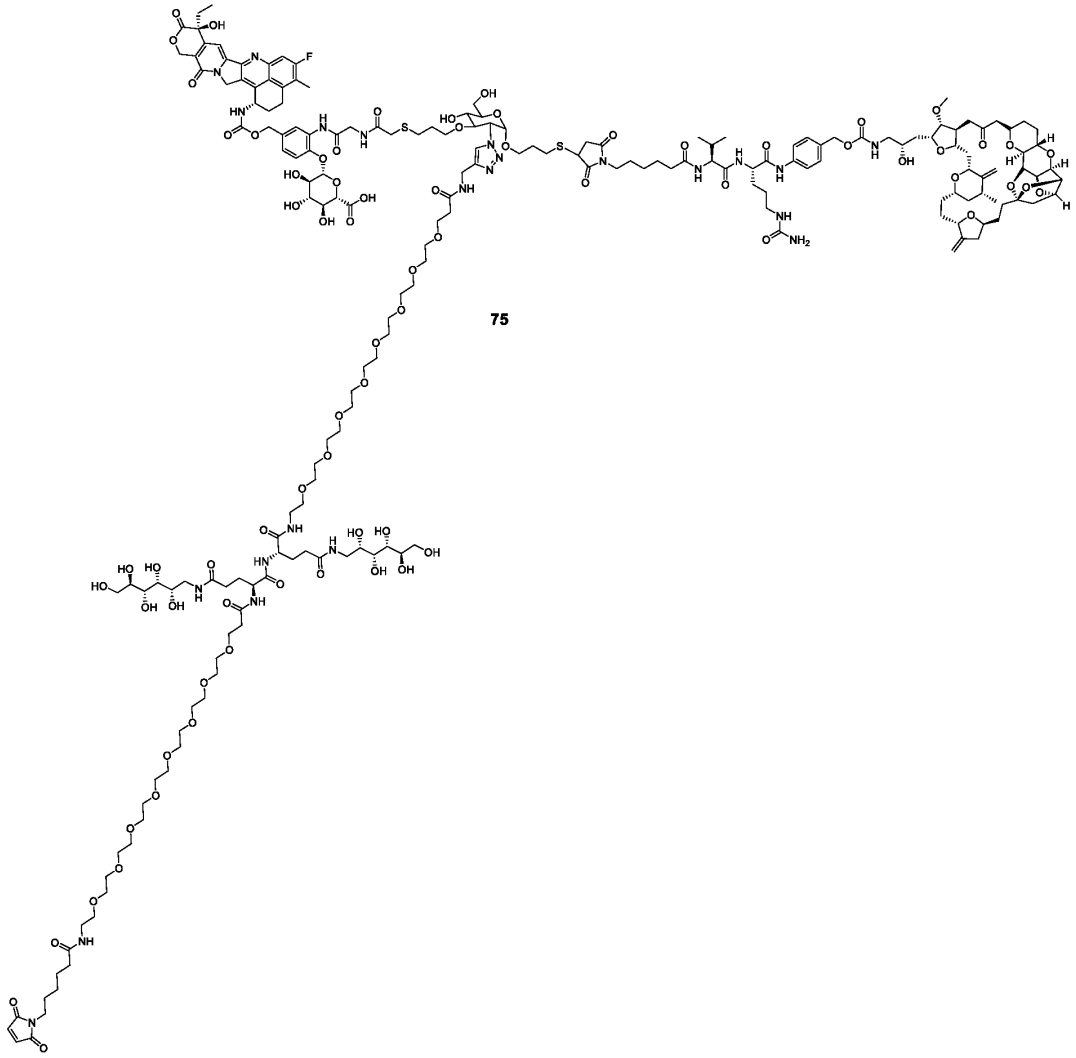
stirred at room temperature under nitrogen atmosphere for 10 min. Then compound **59-1** (70 mg, 0.03 mmol) was added thereto. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 70% acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **74-1** (44 mg, yield 51%) as a yellow oil LCMS: m/z calculated for $C_{114}H_{198}N_8O_{47}S$: 2464.91; found: 1233.01 $[M+2H]^{2+}$.

[00859] Step 2: To a solution of compound **64-4** (62 mg, 0.02 mmol) and **74-1** (44 mg, 0.02 mmol) in dimethyl sulfoxide(3ml) was added ethyldiisopropylamine (31 mg, 0.24 mmol) and CuI (11 mg, 0.06 mmol) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with 55% acetonitrile and water, AcOH condition). The desired components were lyophilized to afford the title compound **74-2** (70 mg, yield 66%) as a yellow oil LCMS: m/z calculated for $C_{276}H_{421}F_2N_{33}O_{98}S_5$: 5719.56; found: 1194.34 $[M+5H]^{5+}$.

[00860] Step 3: To a solution of compound **74-2** (68 mg, 0.01 mmol) in N,N-dimethylformamide (3 mL) was added piperidine (2 mg, 0.02 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C18 column, eluted with 44% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **74** (21mg, yield 32%) as a yellow oil LCMS: m/z calculated for $C_{261}H_{411}F_2N_{33}O_{96}S_5$: 5745.59; found: 1149.98 $[M+5H]^{5+}$.

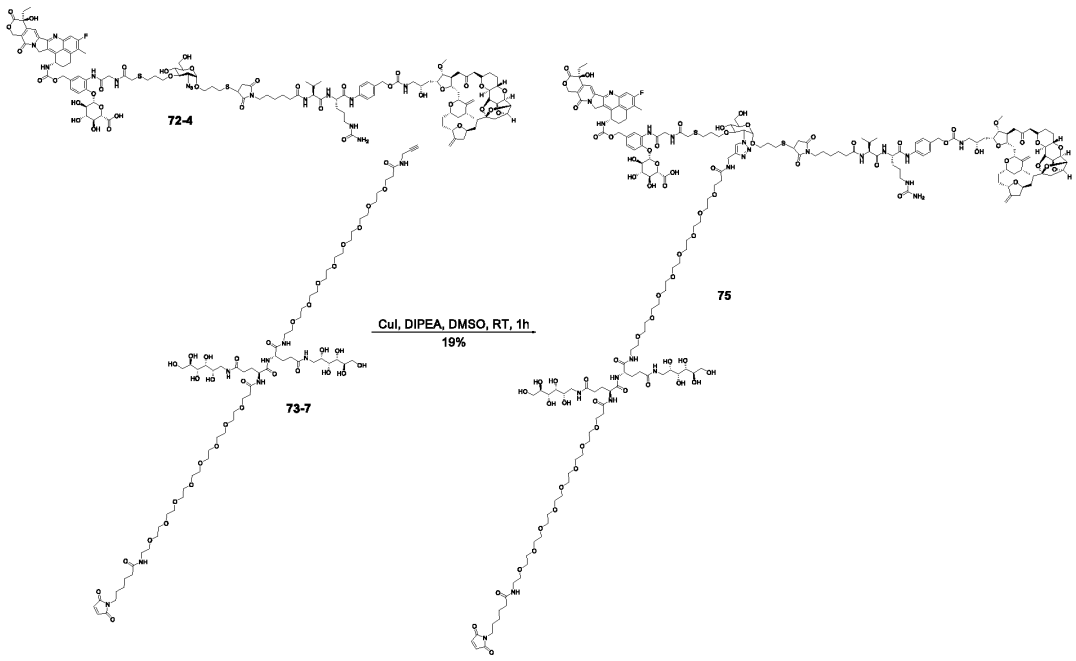
Example 75. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-2-[3-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[4-[[(2*S*)-2-hydroxy-3-[(3*S*,6*S*,9*S*,12*S*,14*R*,16*R*,20*R*,21*R*,22*S*,29*S*,31*R*,32*S*,33*R*,35*R*,36*S*)-21-

methoxy-14-methyl-8,15-dimethylene-24-oxo-2,19,30,34,37,39,40,41-octaoxanonacyclo[24.9.2.1^{3,32}.1^{3,33}.1^{6,9}.1^{12,16}.0^{18,22}.0^{29,36}.0^{31,35}]hentetracontan-20-yl]propyl]carbamoyloxymethyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (75)



75

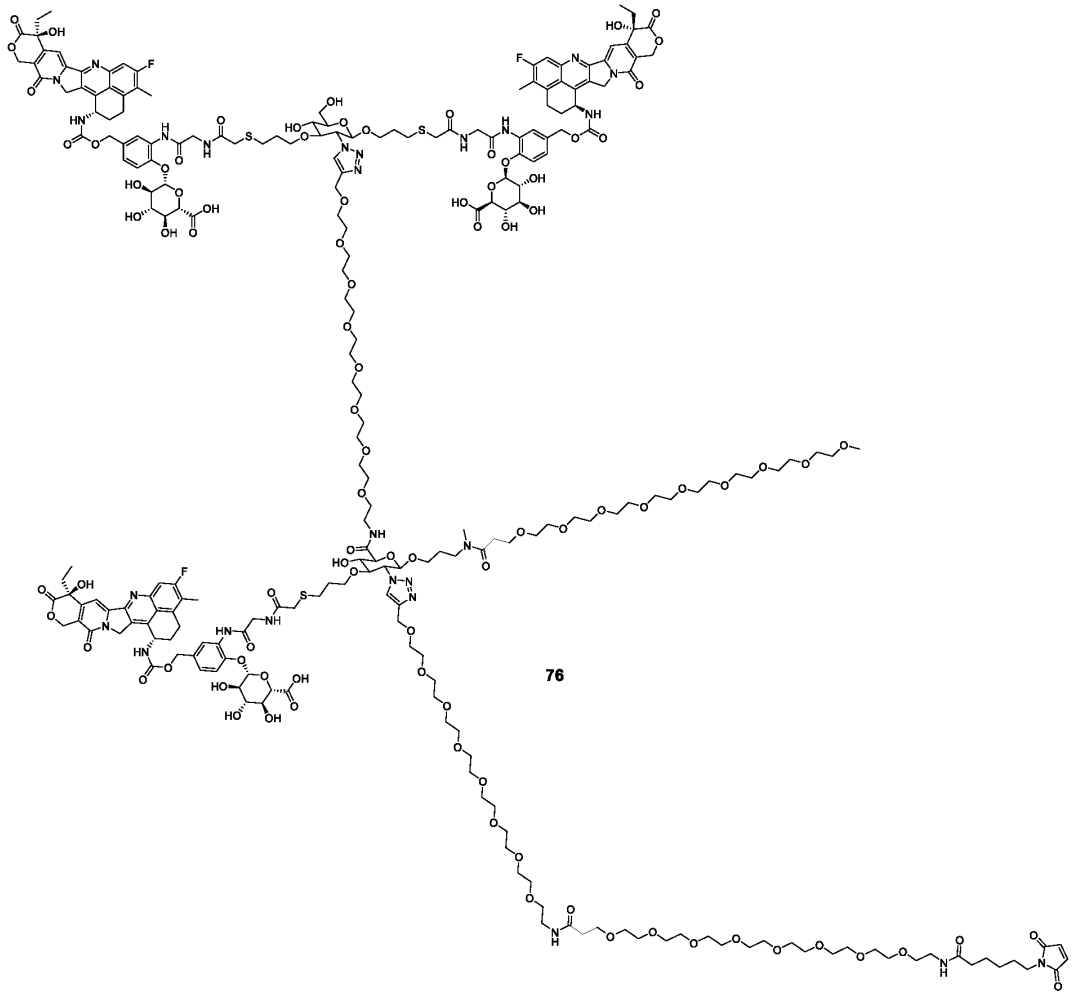
Synthetic scheme 75



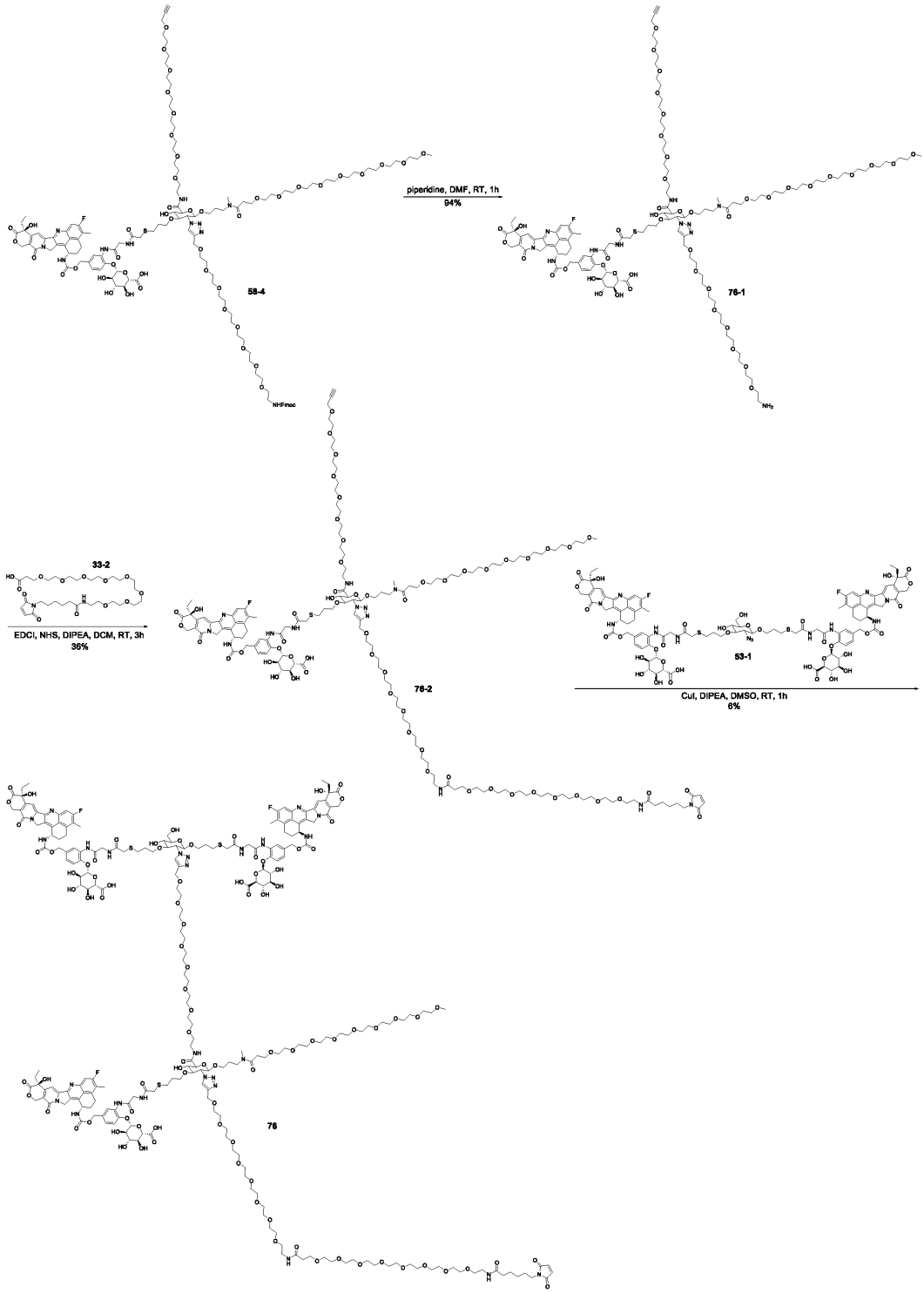
[00861] Step 1: The title compound **75** was prepared in analogy to **Example 7, Step 10** by using compound **72-4** and compound **73-7**. The product (8 mg, yield 19%) was obtained as a white solid. LCMS: m/z calculated for $C_{196}H_{290}FN_{23}O_{74}S_2$: 4235.68; found: 1413.86 $[M+3H]^{3+}$.

Example 76. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylam

ino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (76)



Synthetic scheme 76


piperidine, DMF, RT, 1h
94%
58-4
76-1
33-2
EDCI, NHS, DIPEA, DCM, RT, 3h
36%
76-2
53-1
CuI, DIPEA, DMSO, RT, 1h
6%
76

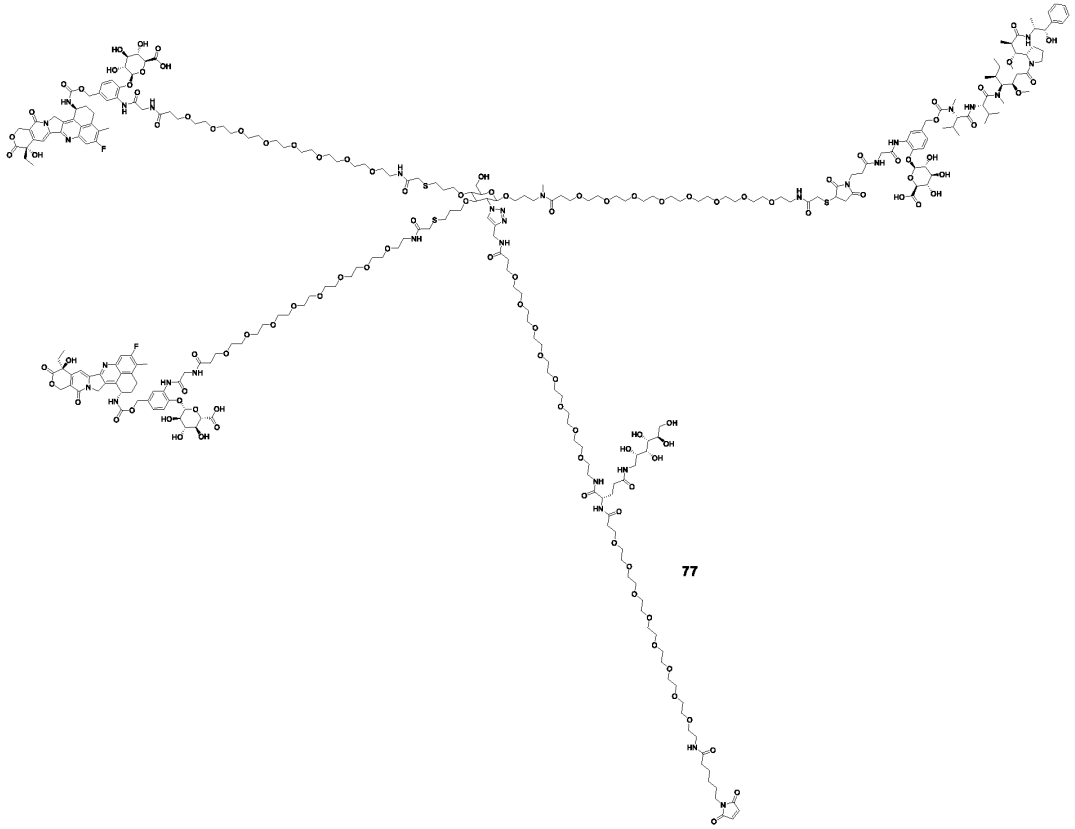
[00862] Step 1: The title compound **76-1** was prepared in analogy to **Example 42**, **Step 27** by using compound **58-4**. The product (95.00 mg, yield 94%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{113}H_{174}FN_{11}O_{46}S$: 2473.72; found: 1238.50 $[M+2H]^+$.

[00863] Step 2: The title compound **76-2** was prepared in analogy to **Example 18**, **Step 9** and **Example 18**, **Step 10** by using compound **76-1** and compound **33-2**. The product (40.00 mg, yield 36%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{142}H_{222}FN_{13}O_{58}S$: 3090.43; found: 1031.06 $[M+3H]^+$.

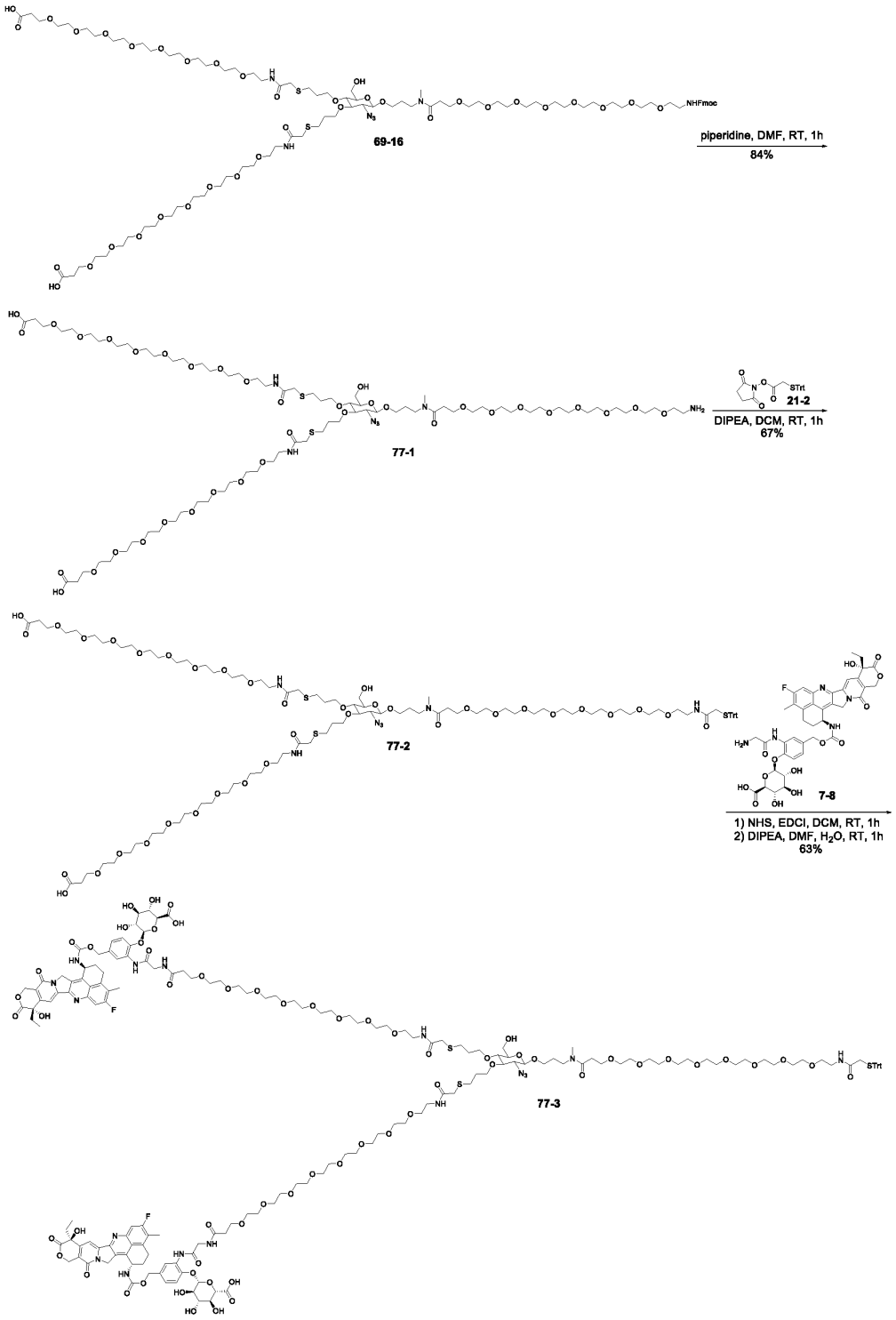
[00864] Step 3: The title compound **76** was prepared in analogy to **Example 10**, **Step 14** by using compound **76-2** and compound **53-1**. The product (3.80 mg, yield 6%) was obtained as a white solid. LCMS: m/z calculated for $C_{238}H_{325}F_3N_{26}O_{93}S_3$: 5191.48; found: 1298.76 $[M+4H]^+$.

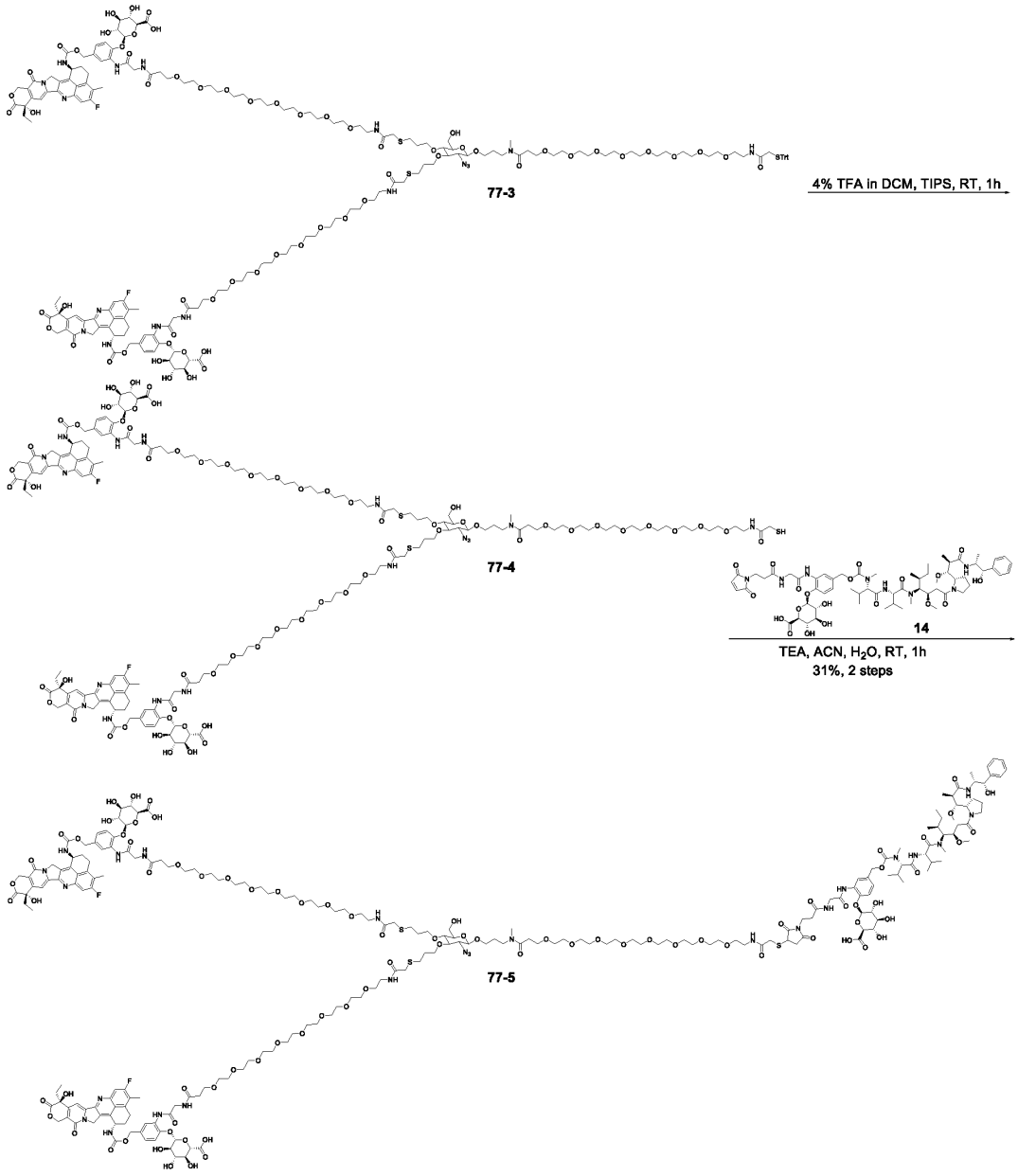
Example 77. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]et

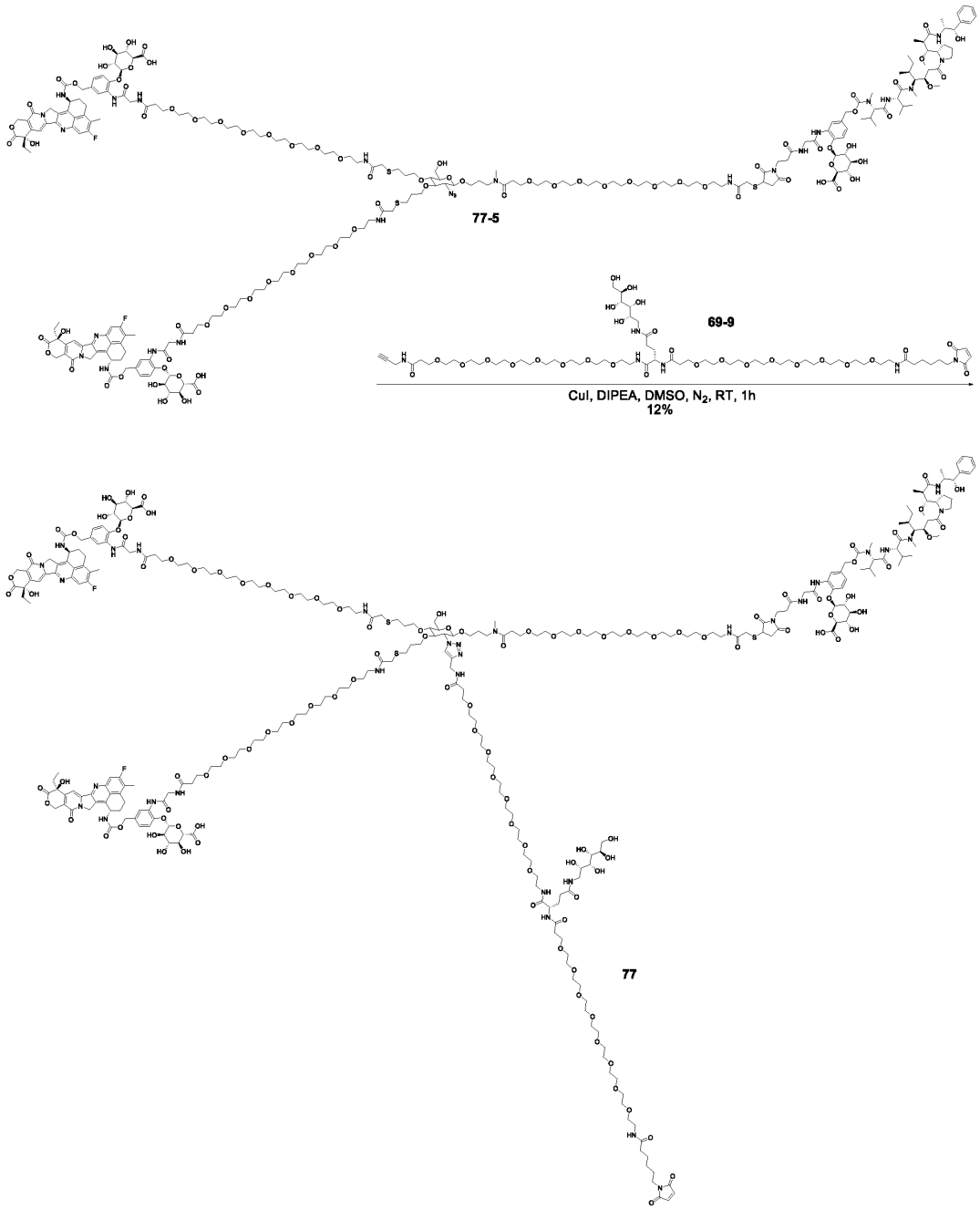
hoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)tetrahydropyran-3-yl]oxypropylsulfanyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (77)



Synthetic scheme 77


69-16
piperidine, DMF, RT, 1h
84%
77-1
21-2
DIPEA, DCM, RT, 1h
67%
77-2
7-8
1) NHS, EDCI, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 1h
63%
77-3


77-3
4% TFA in DCM, TIPS, RT, 1h
77-4
14
TEA, ACN, H2O, RT, 1h
31%, 2 steps
77-5



[00865] Step 1: The title compound **77-1** was prepared in analogy to **Example 42**, **Step 27** by using compound **69-16**. The product (621.00 mg, yield 84%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{77}H_{147}N_7O_{36}S_2$: 1811.16; found: 906.19 $[M+2H]^+$.

[00866] Step 2: To a solution of **77-1** (621.00 mg, 0.34 mmol) and N,N-diisopropylethylamine (88.46 mg, 0.69 mmol) in dichloromethane (8 mL) was added **21-2** (176.06 mg, 0.41 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 silica gel column, 55% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **77-2** (488.00 mg, two steps, yield 67%) as a colorless oil. LCMS: m/z calculated for $C_{98}H_{163}N_7O_{37}S_3$: 2127.57; found: 1064.26 $[M+2H]^+$.

[00867] Step 3: The title compound **77-3** was prepared in analogy to **Example 18**, **Step 9** and **Example 18**, **Step 10** by using compound **77-2** and compound **7-8**. The product (533.50 mg, yield 63%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{178}H_{239}F_2N_{17}O_{63}S_3$: 3759.10; found: 1880.42 $[M+2H]^+$.

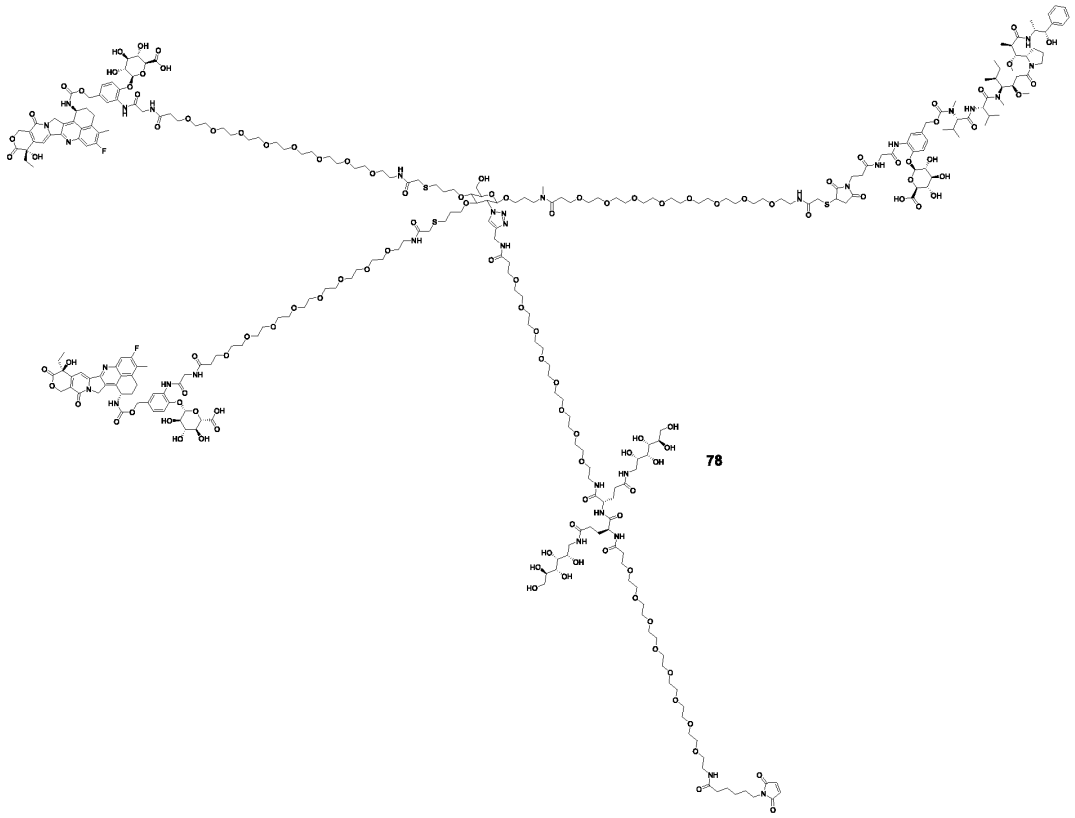
[00868] Step 4: The title compound **77-4** was prepared in analogy to **Example 57**, **Step 2** by using compound **77-3**. The crude product (100 mg) was obtained as a yellow solid. LCMS: m/z calculated for $C_{159}H_{225}F_2N_{17}O_{63}S_3$: 3516.78; found: 1758.18 $[M+2H]^+$.

[00869] Step 5: The title compound **77-5** was prepared in analogy to **Example 10**, **Step 10** by using compound **77-4** and compound **14** in acetonitrile and H_2O. The product (42.00 mg, yield 31% over 2 steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{221}H_{315}F_2N_{25}O_{83}S_3$: 4784.22; found: 1595.50 $[M+3H]^+$.

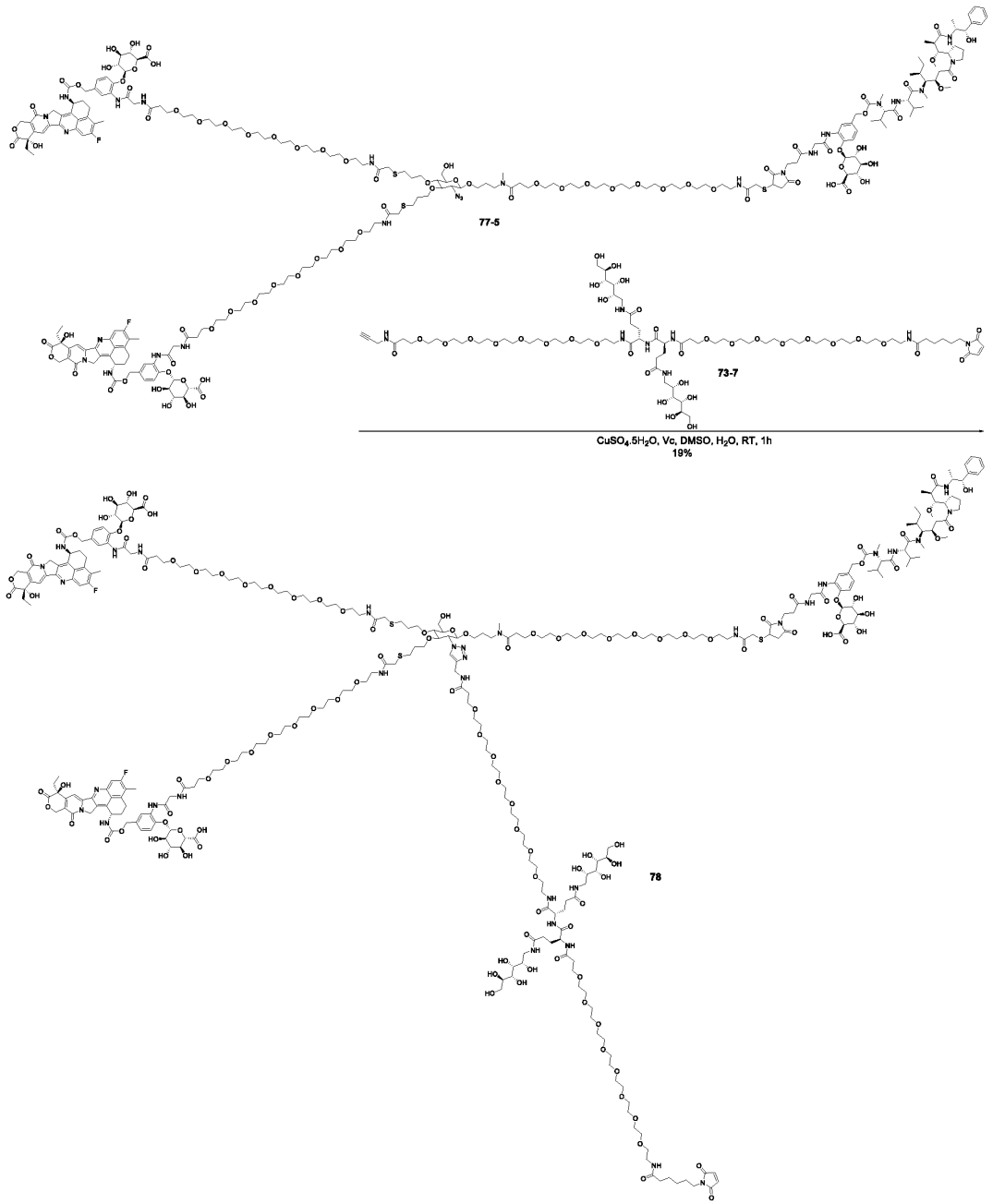
[00870] Step 6: The title compound **77** was prepared in analogy to **Example 7**, **Step 10** by using compound **77-5** and compound **69-9**. The product (5.20 mg, yield 12%) was obtained as a white solid. LCMS: m/z calculated for $C_{283}H_{425}F_2N_{31}O_{111}S_3$: 6171.80; found: 1543.83 $[M+4H]^+$.

Example 78. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-

carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)tetrahydropyran-3-yl]oxypropylsulfanyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (78)



Synthetic scheme 78



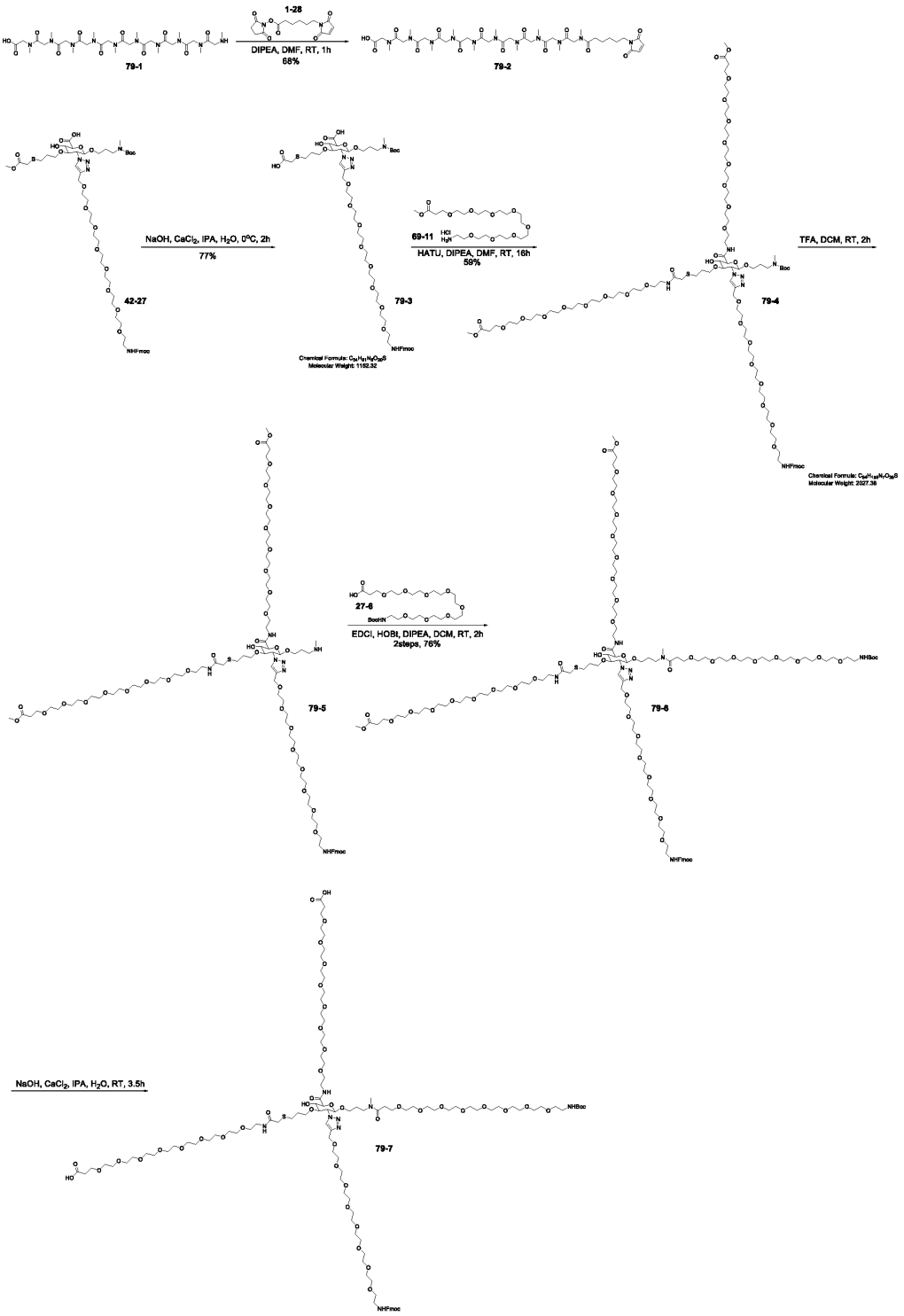
[00871] Step 1: The title compound **78** was prepared in analogy to **Example 1**, **Step 24** by using compound **77-5** and compound **73-7**. The product (6.70 mg, yield 19%) was obtained as a

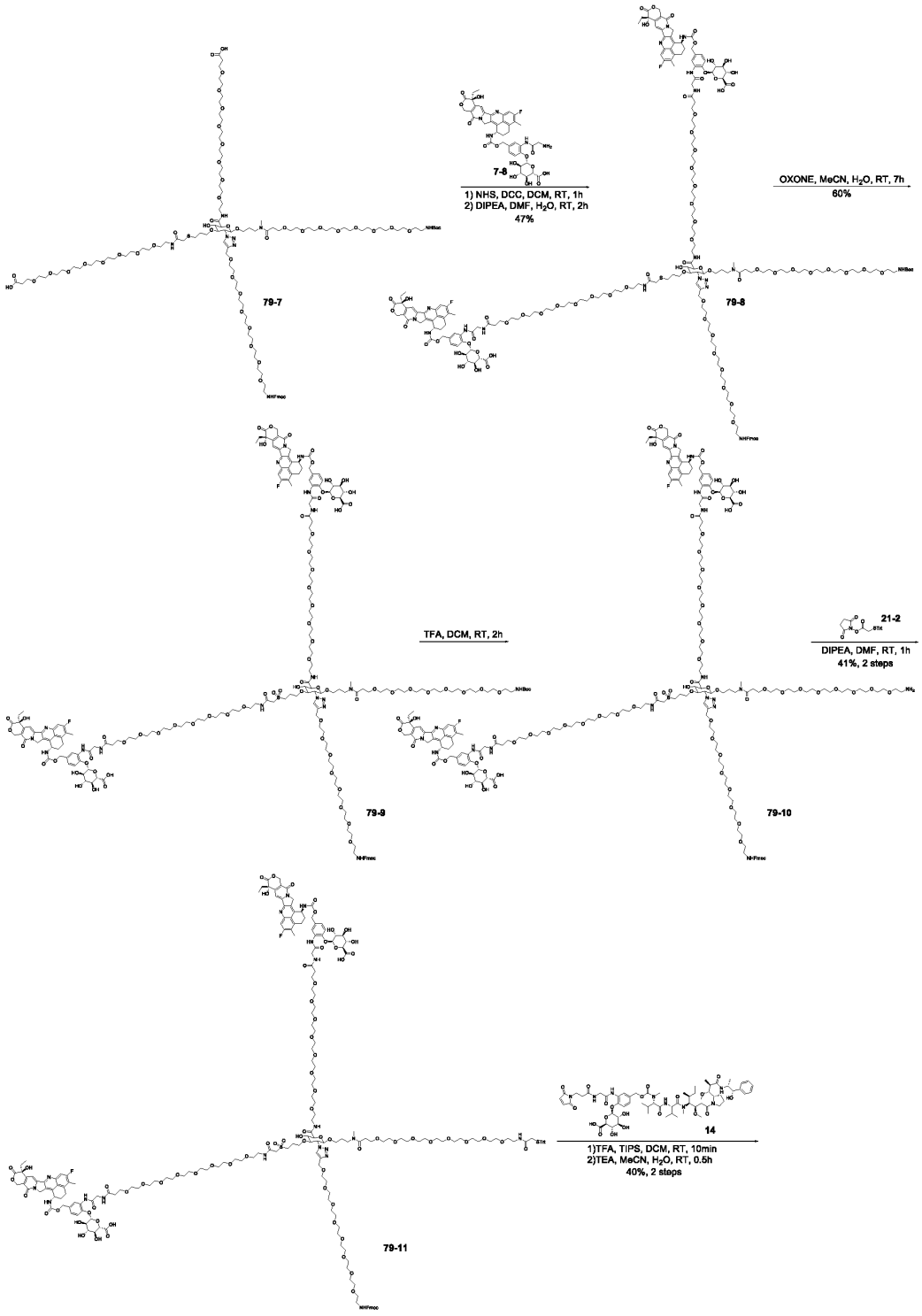
pale-yellow solid. LCMS: m/z calculated for $C_{294}H_{445}F_2N_{33}O_{118}S_3$: 6464.08; found: 1616.70 $[M+4H]^+$.

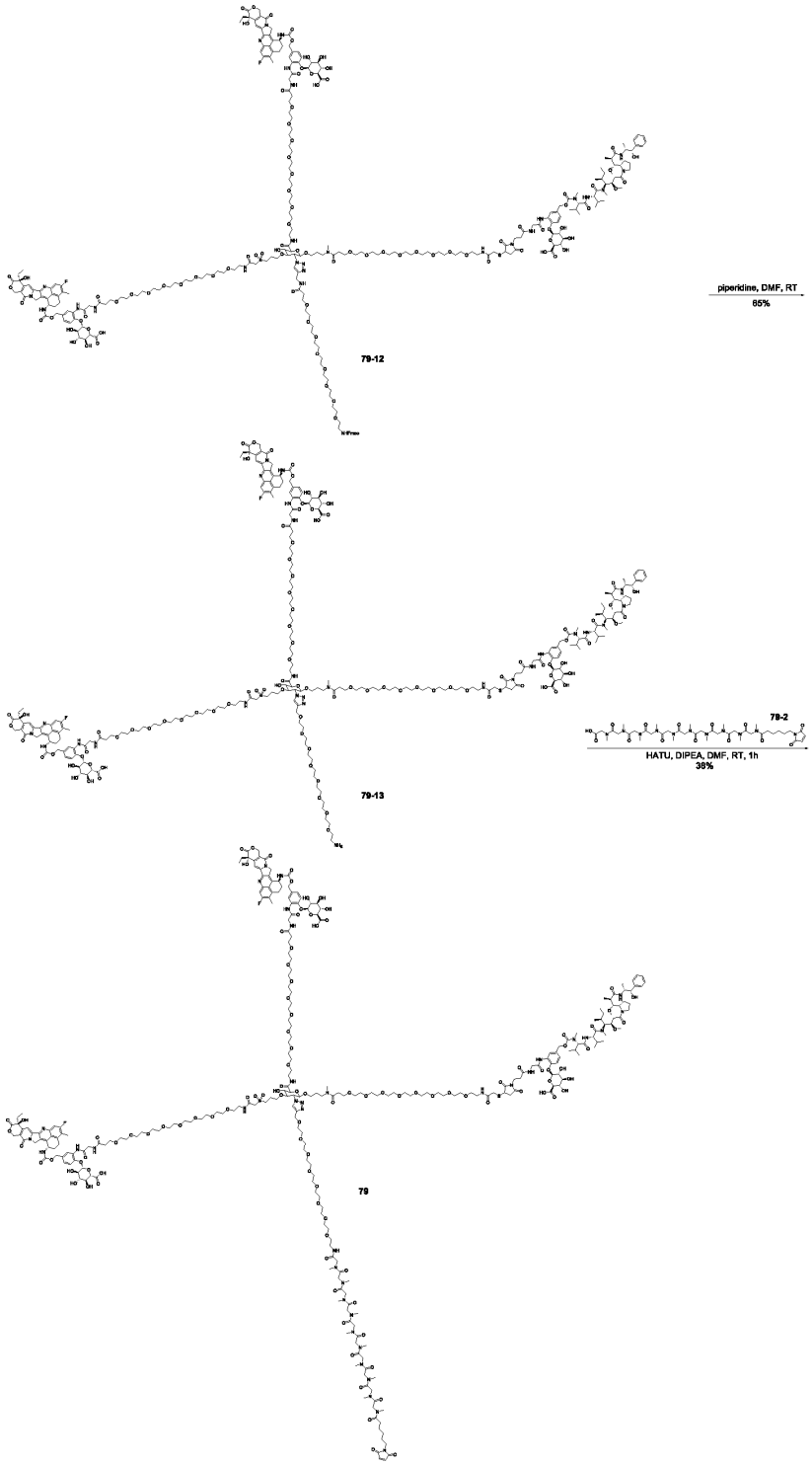
Example 79. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[6-(2,5-dioxopyrrol-1-yl)hexanoyl-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (79)



Synthetic scheme 79


1-28
DIPEA, DMF, RT, 1h
68%
79-1
79-2
NaOH, CaCl2, IPA, H2O, 0°C, 2h
77%
42-27
79-3
69-11
HATU, DIPEA, DMF, RT, 16h
59%
TFA, DCM, RT, 2h
79-4
27-6
EDCI, HOBt, DIPEA, DCM, RT, 2h
2steps, 76%
79-5
79-6
NaOH, CaCl2, IPA, H2O, RT, 3.5h
79-7


7-8
1) NHS, DCC, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 2h
47%
OXONE, MeCN, H2O, RT, 7h
60%
79-7
79-8
TFA, DCM, RT, 2h
21-2
DIPEA, DMF, RT, 1h
41%, 2 steps
79-9
79-10
14
1)TFA, TIPS, DCM, RT, 10min
2)TEA, MeCN, H2O, RT, 0.5h
40%, 2 steps
79-11


piperidine, DMF, RT
85%
79-12
79-2
HATU, DIPEA, DMF, RT, 1h
38%
79-13
79

[00872] Step 1: The title compound **79-2** was prepared in analogy to **Example 1, Step 21** by using 5,8,11,14,17,20,23,26,29-nonamethyl-4,7,10,13,16,19,22,25,28-nonaoxo-2,5,8,11,14,17,20,23,26,29-decaazahentriacontan-31-oic acid (H-Sar10-OH, **79-1**) and compound **1-28** in DMF. The product (142 mg, yield 68%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{40}H_{63}N_{11}O_{14}$: 922.01; found: 922.45 $[M+H]^+$.

[00873] Step 2: The title compound **79-3** was prepared in analogy to **Example 42, Step 22** by using compound **42-27**. The product (1.50 g, yield 77%) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{54}H_{81}N_5O_{20}S$: 1152.32; found: 1153.78 $[M+H]^+$.

[00874] Step 3: The title compound **79-4** was prepared in analogy to **Example 8, Step 3** by using compound **69-11** and compound **79-3**. The product (1.57 g, yield 59%) as a pale-yellow oil. LCMS: m/z calculated for $C_{94}H_{159}N_7O_{38}S$: 2027.38; found: 965.23 $[M-Boc+2H]^{2+}$.

[00875] Step 4: The title compound **79-5** was prepared in analogy to **Example 57, Step 2** by using compound **79-4**. The crude product was concentrated and directly used in the next step. LCMS: m/z calculated for $C_{89}H_{151}N_7O_{36}S$: 1925.99; found: 1926.78 $[M+H]^+$.

[00876] Step 5: The title compound **79-6** was prepared in analogy to **Example 2, Step 5** by using compound **79-5** and compound **27-6**. The product (1.4 g, yield 76% over 2 steps) as a pale-brown oil. LCMS: m/z calculated for $C_{113}H_{196}N_8O_{47}S$: 2450.88; found: 1126.05 $[M+2H]^{2+}$.

[00877] Step 6: The title compound **79-7** was prepared in analogy to **Example 42, Step 22** by using compound **79-6**. The product (1.5 g, yield 75%) as a pale-brown oil. LCMS: m/z calculated for $C_{111}H_{192}N_8O_{47}S$: 2422.83; found: 1211.98 $[M+2H]^{2+}$.

[00878] Step 7: Under nitrogen atmosphere, to a mixture of compound **79-7** (720 mg, 0.297 mmol) in anhydrous dichloromethane (8 mL) was added dicyclohexylcarbodiimide (245 mg, 1.188 mmol) and N-Hydroxy succinimide (75 mg, 0.653 mmol). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and concentrated to give the NHS ester as a yellow solid.

[00879] To a mixture of compound **7-8** (544 mg, 0.653 mmol) in anhydrous N, N-dimethylformamide (7 mL) and H_2O (0.7 mL) was added N, N-diisopropylethylamine (0.31 mL, 1.782 mmol) was added a solution of the above NHS ester in N, N-dimethylformamide (2 mL). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to

afford the title compound **79-8** (49 mg, yield 47%) as a yellow solid. LCMS: m/z calculated for $C_{191}H_{268}F_2N_{18}O_{73}S$: 4054.35; found: 1352.44 $[M+3H]^{3+}$.

[00880] Step 8: The title compound **79-9** was prepared in analogy to **Example 45, Step 1** by using compound **79-8**. The product (120 mg, yield 60%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{191}H_{268}F_2N_{18}O_{75}S$: 4086.35; found: 1363.03 $[M+3H]^{3+}$.

[00881] Step 9: The title compound **79-10** was prepared in analogy to **Example 57, Step 2** by using compound **79-9**. The crude product was concentrated and directly used in the next step. LCMS: m/z calculated for $C_{186}H_{260}F_2N_{18}O_{73}S$: 3986.24; found: 1993.70 $[M+2H]^{2+}$.

[00882] Step 10: The title compound **79-11** was prepared in analogy to **Example 77, Step 2** by using N, N-diisopropylethylamine, compound **79-10** and compound **21-2** in DMF. The product (48 mg, yield 41% over 2steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{207}H_{276}F_2N_{18}O_{74}S_2$: 4302.65; found: 1435.06 $[M+3H]^{3+}$.

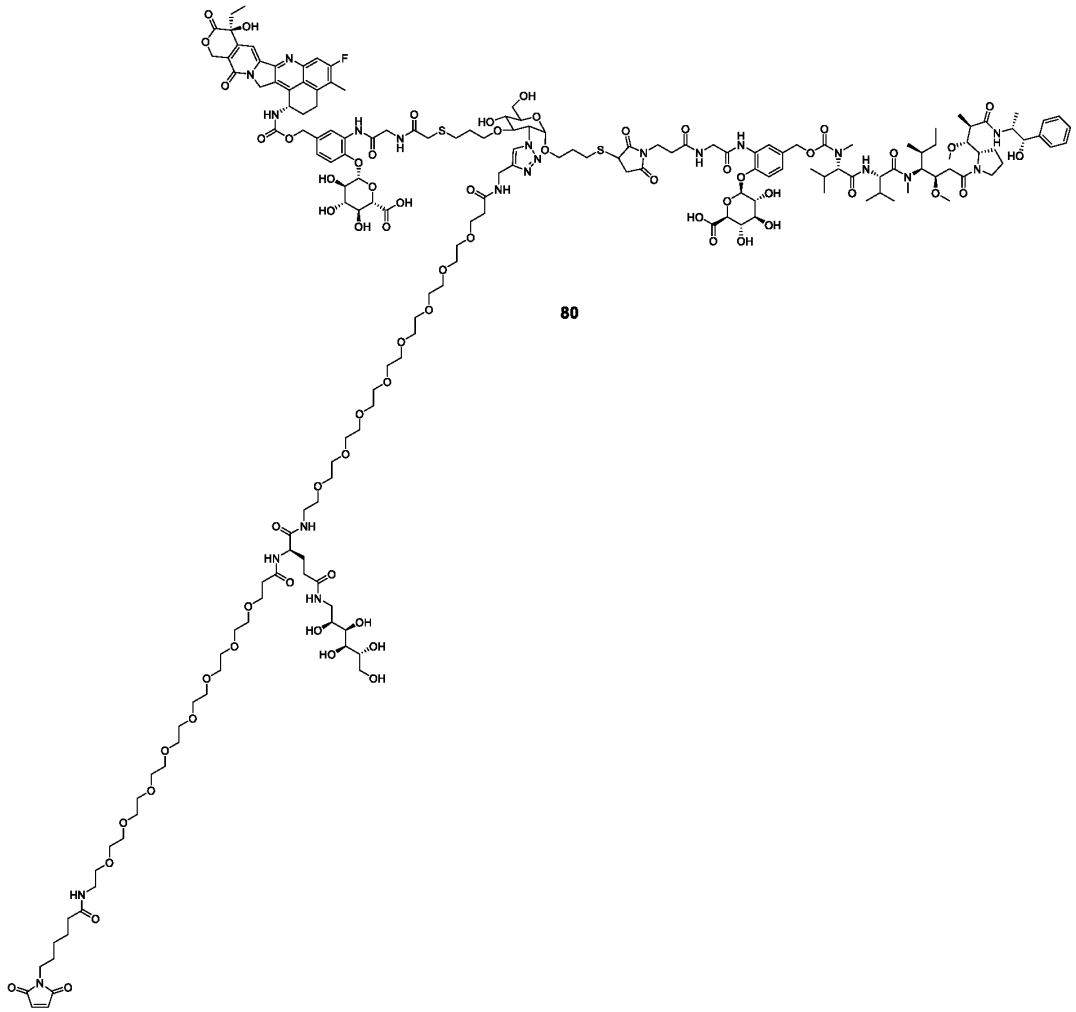
[00883] Step 11: The title compound **79-12** was prepared in analogy to **Example 42, Step 15 and Step 18** by using triisopropylsilane and trifluoroacetic acid to take off the Boc-group of compound **79-11**, and followed by reacting with DIPEA and compound **14** in DMF. The product (24 mg, yield 40%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{253}H_{357}F_2N_{27}O_{95}S_2$: 5398.85; found: 1176.38 $[M+3H]^{3+}$.

[00884] Step 12: The title compound **79-13** was prepared in analogy to **Example 42, Step 27** by using compound **79-12**. The product (15 mg, yield 65%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{235}H_{342}F_2N_{26}O_{92}S_2$: 5105.53; found: 1277.33 $[M+4H]^{4+}$.

[00885] Step 13: The title compound **79** was prepared in analogy to **Example 70, Step 8** by using compound **79-2** and compound **79-13**. The product (6.7 mg, yield 38%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{275}H_{403}F_2N_{37}O_{105}S_2$: 6009.52; found: 1915.26 $[M-265+3H]^{3+}$.

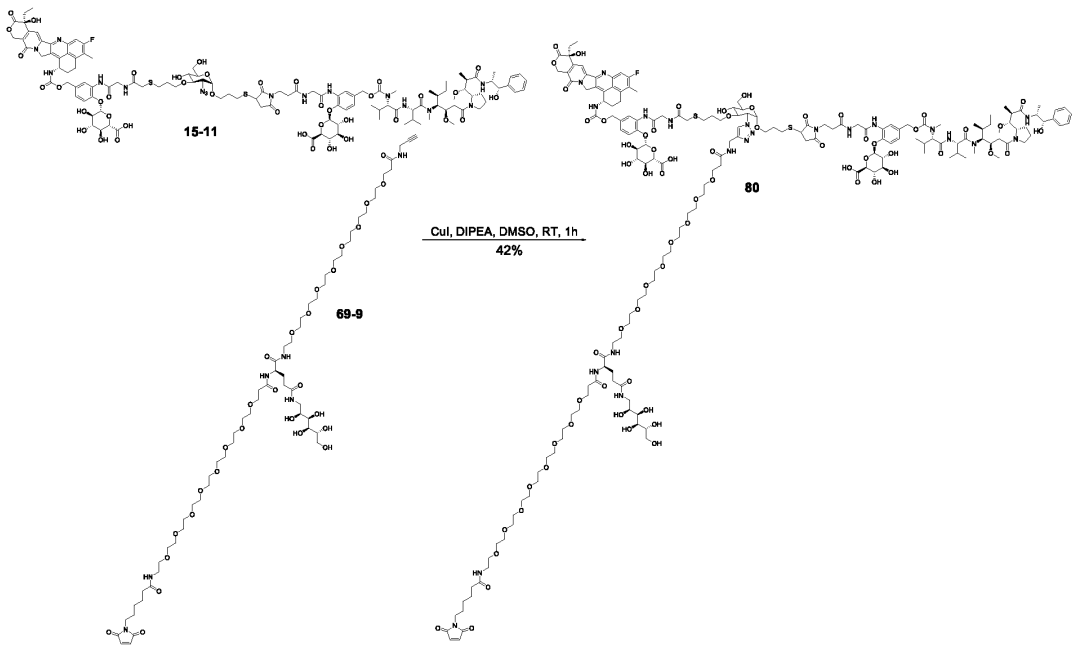
Example 80. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-

yl]sulfanylpropoxy]-3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[[(2*R*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (80)



80

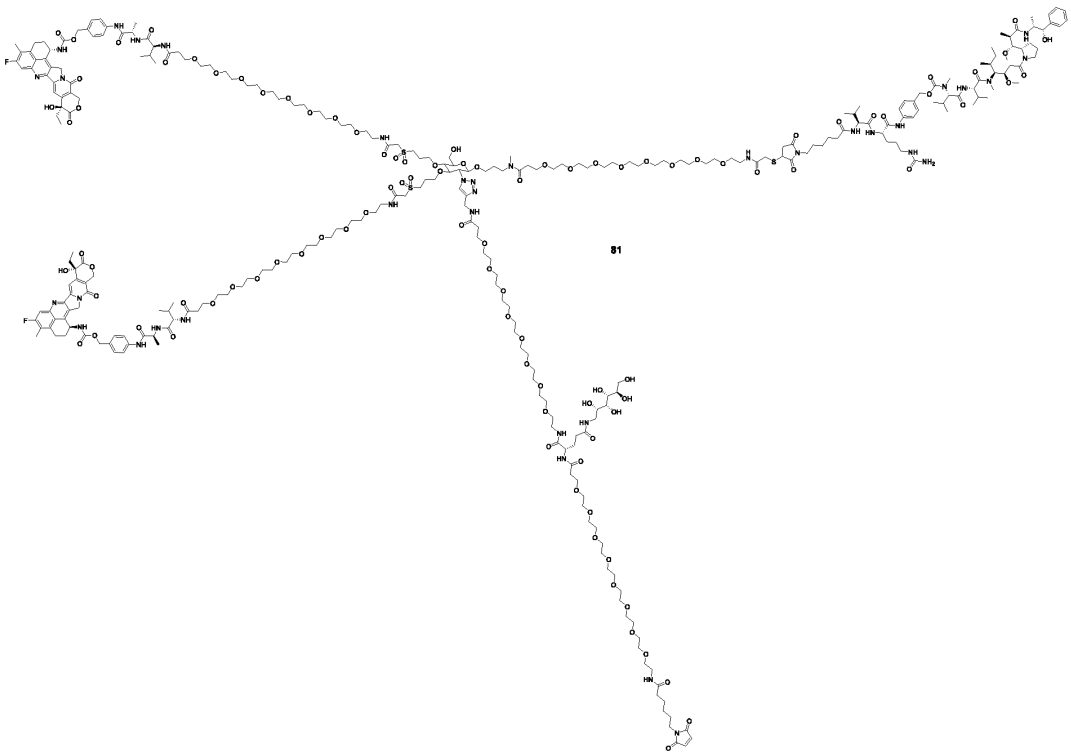
Synthetic scheme 80



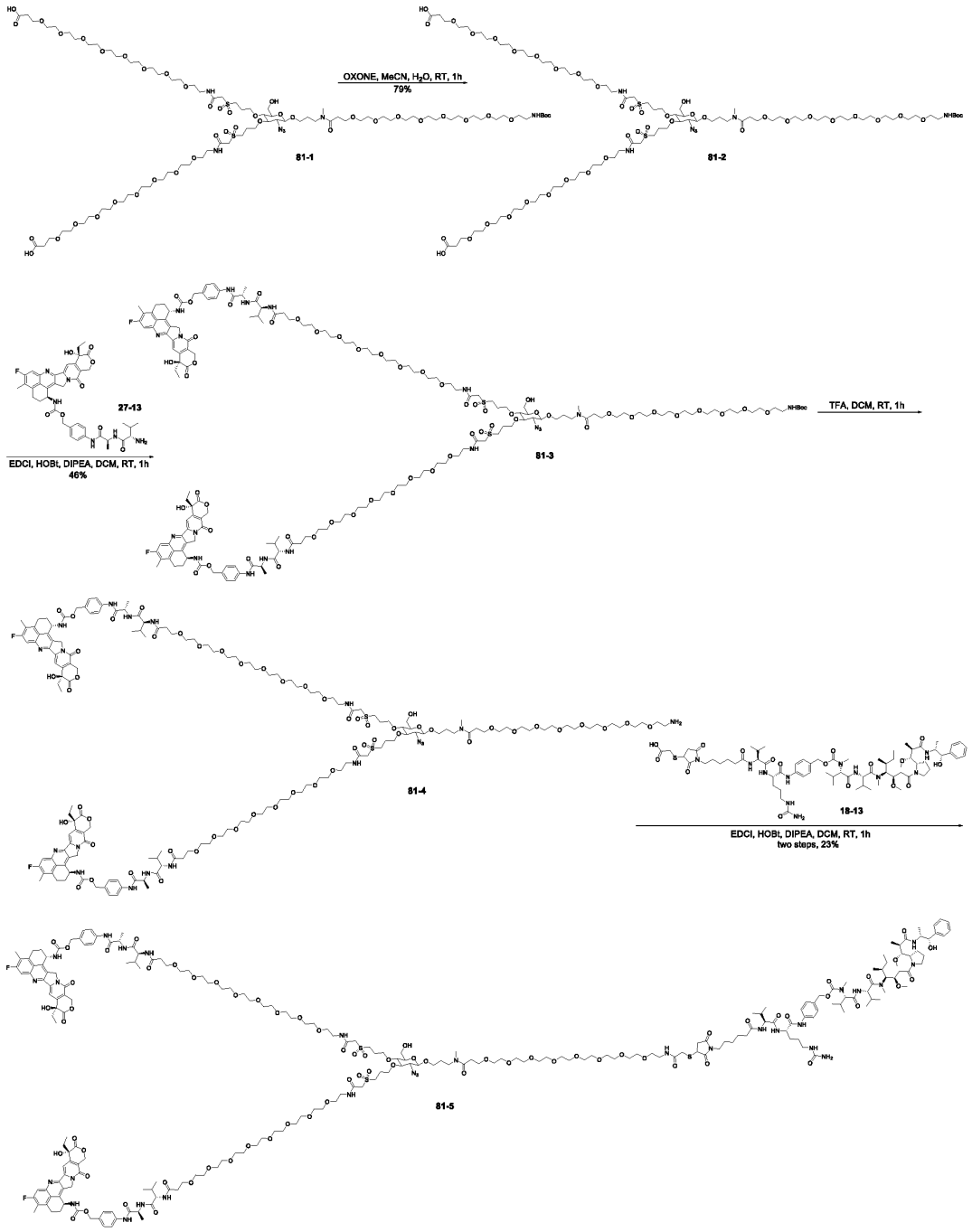
[00886] Step 1: The title compound **80** was prepared in analogy to **Example 10, Step 14** by using compound **15-11** and compound **69-9**. The product (27 mg, yield 42%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{178}H_{263}FN_{22}O_{68}S_2$: 3882.27; found: 1294.95 $[M+3H]^{3+}$.

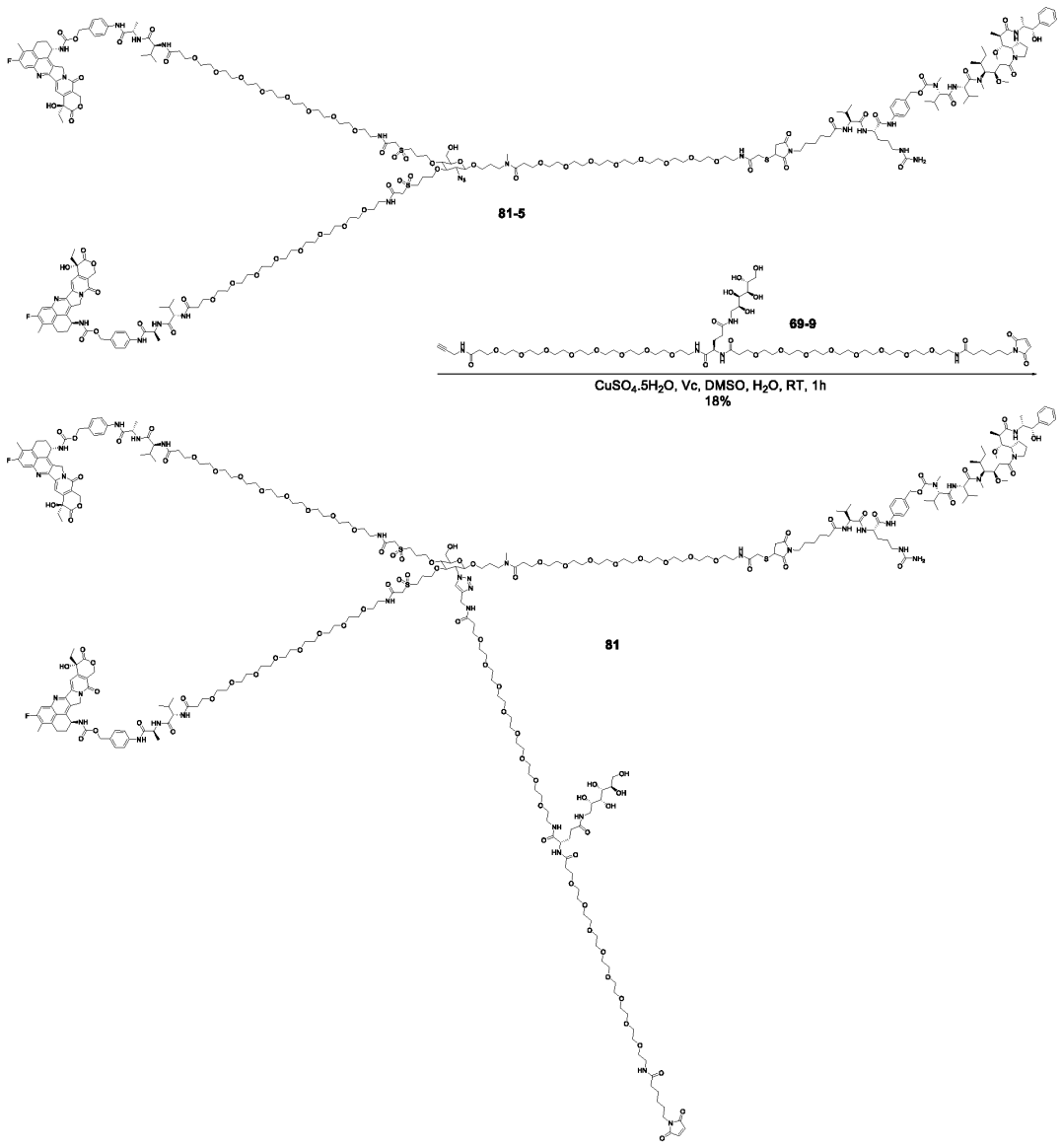
Example 81. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropyl-methyl-amino]-

3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (81)



Synthetic scheme 81


OXONE, MeCN, H2O, RT, 1h
79%
81-1
81-2
27-13
EDCI, HOBt, DIPEA, DCM, RT, 1h
46%
81-3
TFA, DCM, RT, 1h
81-4
18-13
EDCI, HOBt, DIPEA, DCM, RT, 1h
two steps, 23%
81-5



[00887] Step 1: The title compound **81-1** was prepared in analogy to compound **69-16** by using compound **69-14** and compound **27-6** instead of compound **27-19**. Compound **81-1** (1.48 g) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{82}H_{155}N_7O_{42}S_2$: 1975.27; found: 938.18 $[M+2H]^{2+}$.

[00888] To a mixture of compound **81-1** (995.00 mg, 0.52 mol) in acetonitrile (5 mL) and H_2O (5 mL) was added OXONE (1.28 g, 2.08 mol) and then stirred at room temperature for 1 h.

After completion of the reaction, the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by reverse flash phase chromatography (C18 column, 60% acetonitrile in water, TFA condition). The desired components were lyophilized to afford the title compound **81-2** (810.00 mg, 79%) as a yellow oil. LCMS: m/z calculated for $C_{82}H_{155}N_7O_{42}S_2$: 1975.27; found: 938.08 $[M\text{-}Boc+2H]^+$.

[00889] Step 2: The title compound **81-3** was prepared in analogy to **Example 2**, **Step 5** by using compound **81-2** and compound **27-13**. The product (240.00 mg, yield 46%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{162}H_{237}F_2N_{19}O_{56}S_2$: 3448.87; found: 1724.73 $[M+3H]^+$.

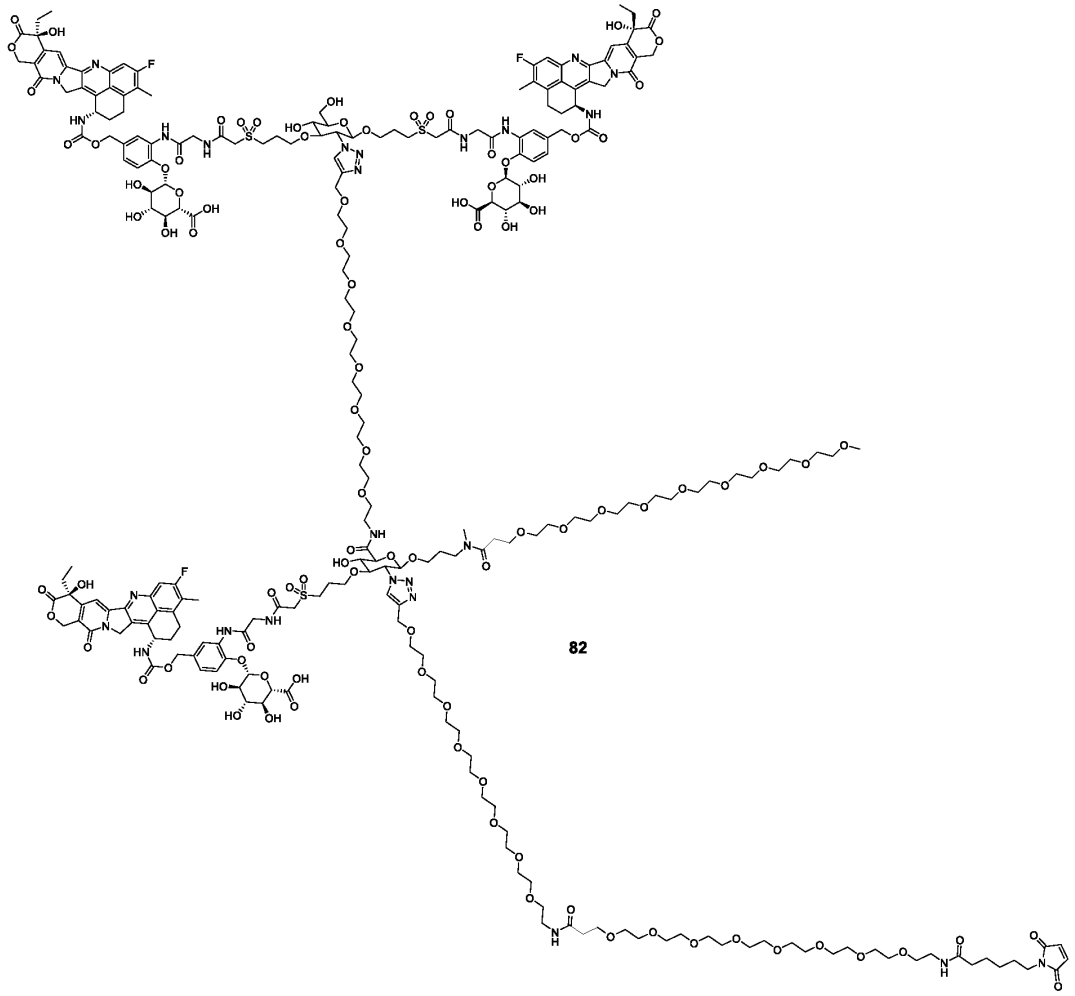
[00890] Step 3: The title compound **81-4** was prepared in analogy to **Example 57**, **Step 2** by using compound **81-3**. The crude product (255.00 mg) was obtained as a yellow oil. LCMS: m/z calculated for $C_{157}H_{229}F_2N_{19}O_{54}S_2$: 3348.75; found: 1117.08 $[M+3H]^+$.

[00891] Step 4: The title compound **81-5** was prepared in analogy to **Example 2**, **Step 5** by using compound **81-4** and compound **18-13**. The product (75.00 mg, yield 23% over 2 steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{227}H_{336}F_2N_{30}O_{70}S_3$: 4739.50; found: 1185.63 $[M+4H]^+$.

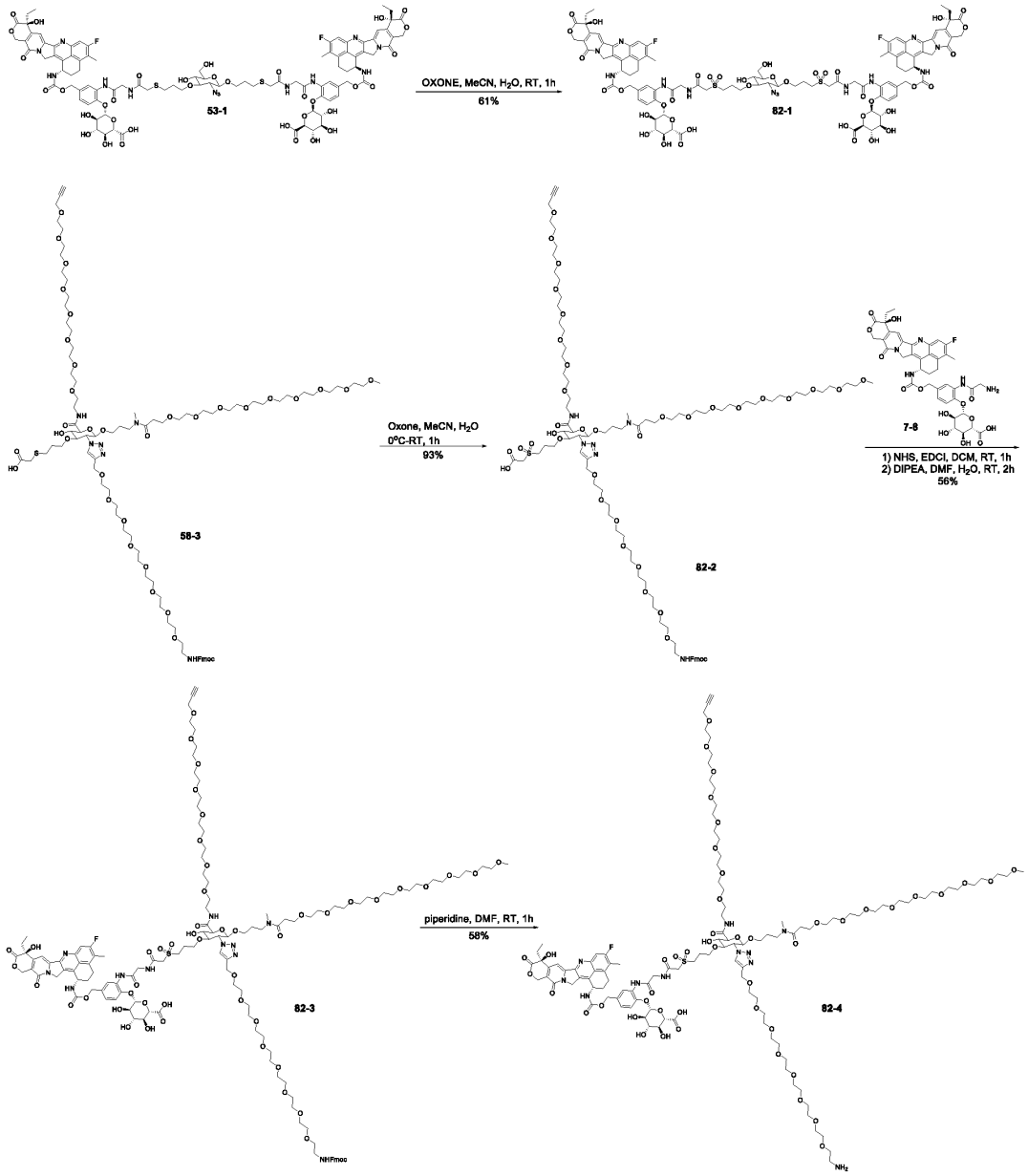
[00892] Step 5: The title compound **81** was prepared in analogy to **Example 1**, **Step 24** by using compound **81-5** and compound **69-9**. The product (3.50 mg, yield 18%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{289}H_{446}F_2N_{36}O_{98}S_3$: 6127.08; found: 1226.36 $[M+5H]^+$.

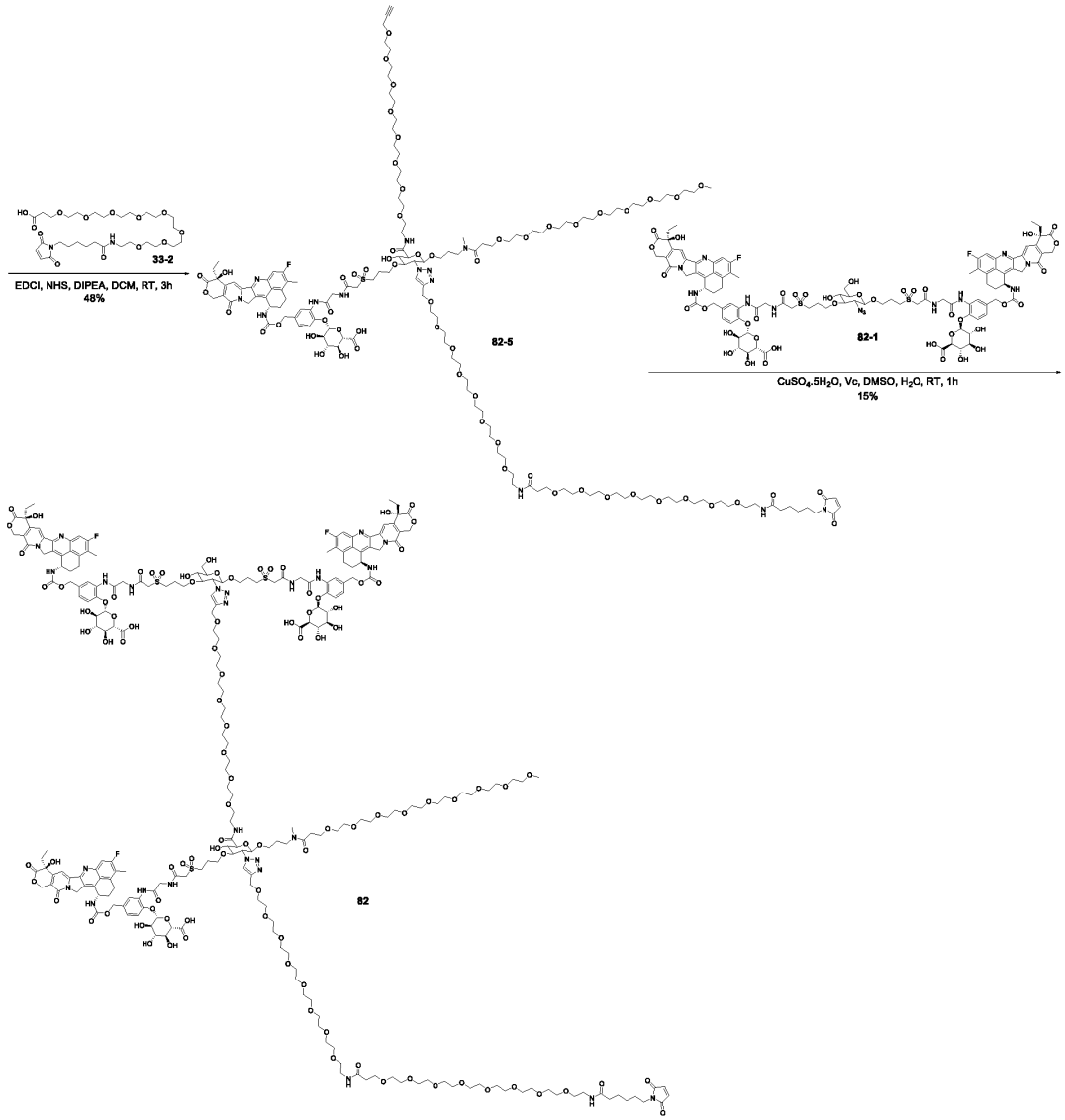
Example 82. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-

2-oxo-ethyl]sulfonylpropoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (82)



Synthetic scheme 82


53-1
OXONE, MeCN, H2O, RT, 1h
61%
82-1
58-3
Oxone, MeCN, H2O
0°C-RT, 1h
93%
82-2
7-8
1) NHS, EDCI, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 2h
56%
82-3
piperidine, DMF, RT, 1h
58%
82-4



[00893] Step 1: The title compound **82-1** was prepared in analogy to **Example 45**, **Step 1** by using compound **53-1**. The product (63.00 mg, yield 61%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{96}H_{103}F_2N_{13}O_{39}S_2$: 2165.05; found: 1083.26 $[M+2H]^+$.

[00894] Step 2: The title compound **82-2** was prepared in analogy to **Example 45**, **Step 1** by using compound **58-3**. The product (202.00 mg, yield 93%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{88}H_{146}N_6O_{37}S$: 1912.20; found: 957.84 $[M+2H]^+$.

[00895] Step 3: The title compound **82-3** was prepared in analogy to **Example 18**, **Step 9** and **Example 18**, **Step 9** by using compound **7-8** and compound **82-2** with dicyclohexylmethanediimine instead of EDCI. The product (137.00 mg, yield 56%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{128}H_{184}FN_{11}O_{50}S$: 2727.97; found: 910.16 $[M+3H]^+$.

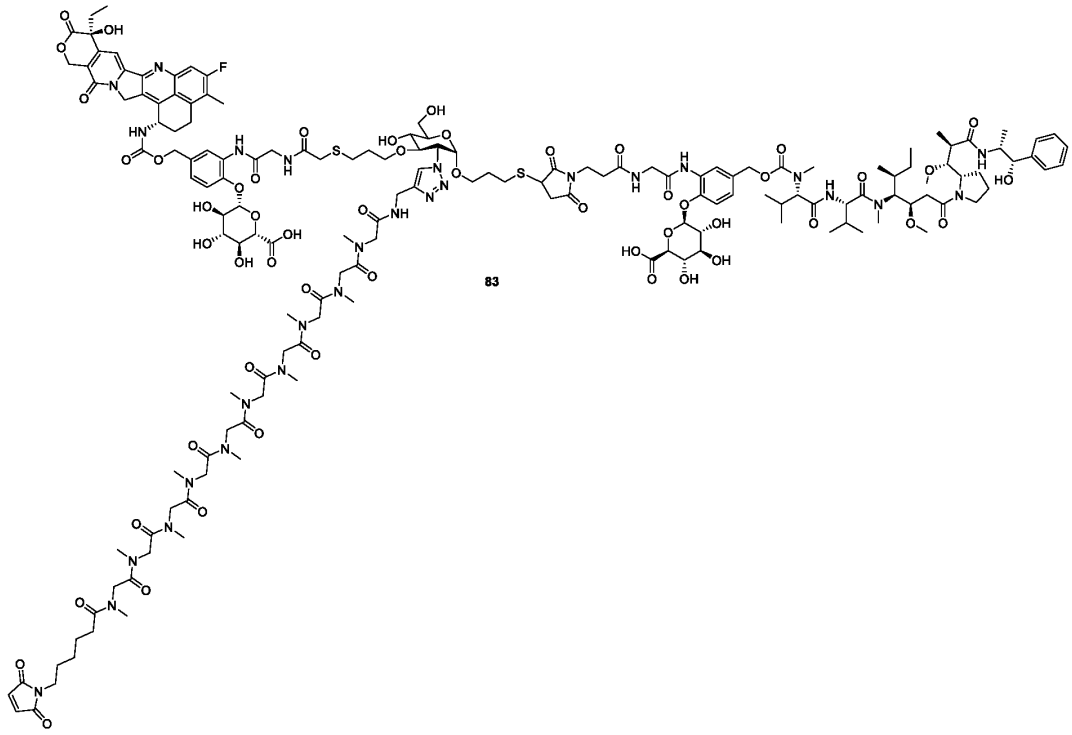
[00896] Step 4: The title compound **82-4** was prepared in analogy to **Example 42**, **Step 27** by using compound **82-3**. The product (73.00 mg, yield 58%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{113}H_{174}FN_{11}O_{48}S$: 2505.72; found: 1254.63 $[M+2H]^+$.

[00897] Step 5: The title compound **82-5** was prepared in analogy to **Example 70**, **Step 8** by using compound **82-4** and compound **33-2**. The product (44.00 mg, yield 48%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{142}H_{222}FN_{13}O_{60}S$: 3122.43; found: 1041.71 $[M+3H]^+$.

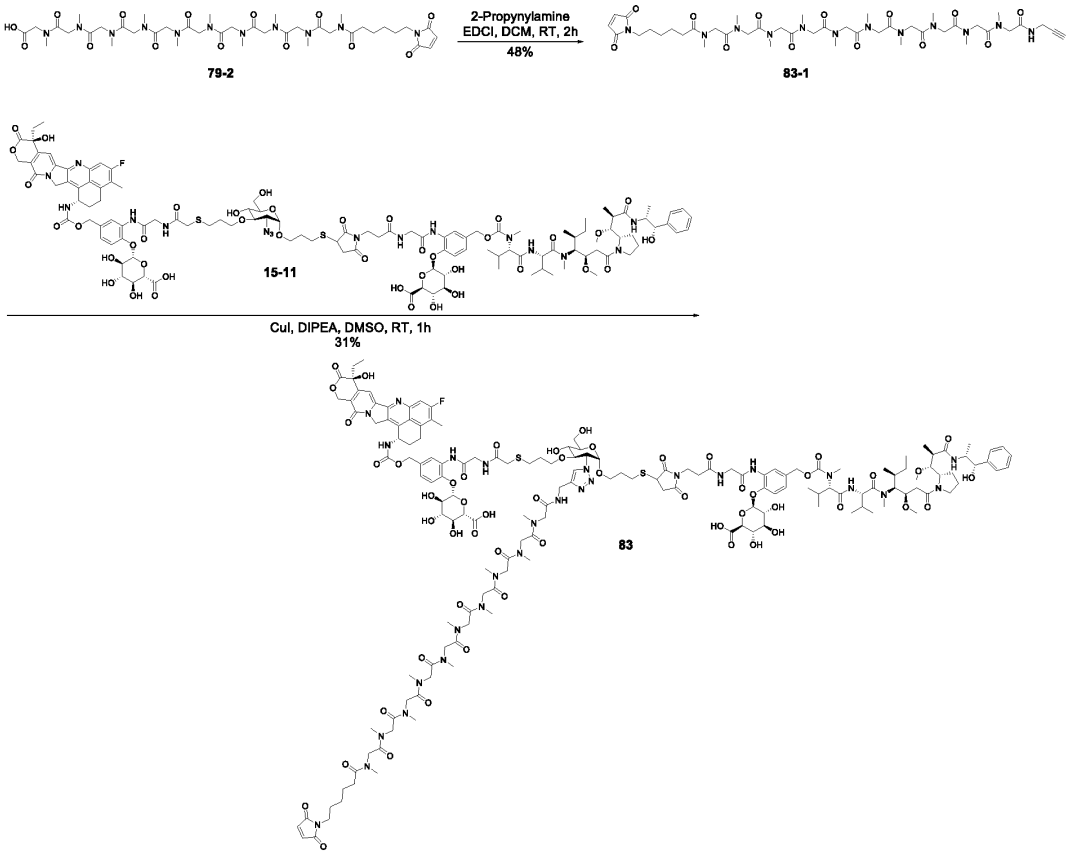
[00898] Step 6: The title compound **82** was prepared in analogy to **Example 1**, **Step 24** by using compound **82-1** and compound **82-5**. The product (13.80 mg, yield 15%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{238}H_{325}F_3N_{26}O_{99}S_3$: 5287.48; found: 1322.78 $[M+4H]^+$.

Example 83. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(2*S*,4*R*)-2-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-3-[4-[[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[6-(2,5-dioxopyrrol-1-yl)hexanoyl-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]amino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfanyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (83)



Synthetic scheme 83

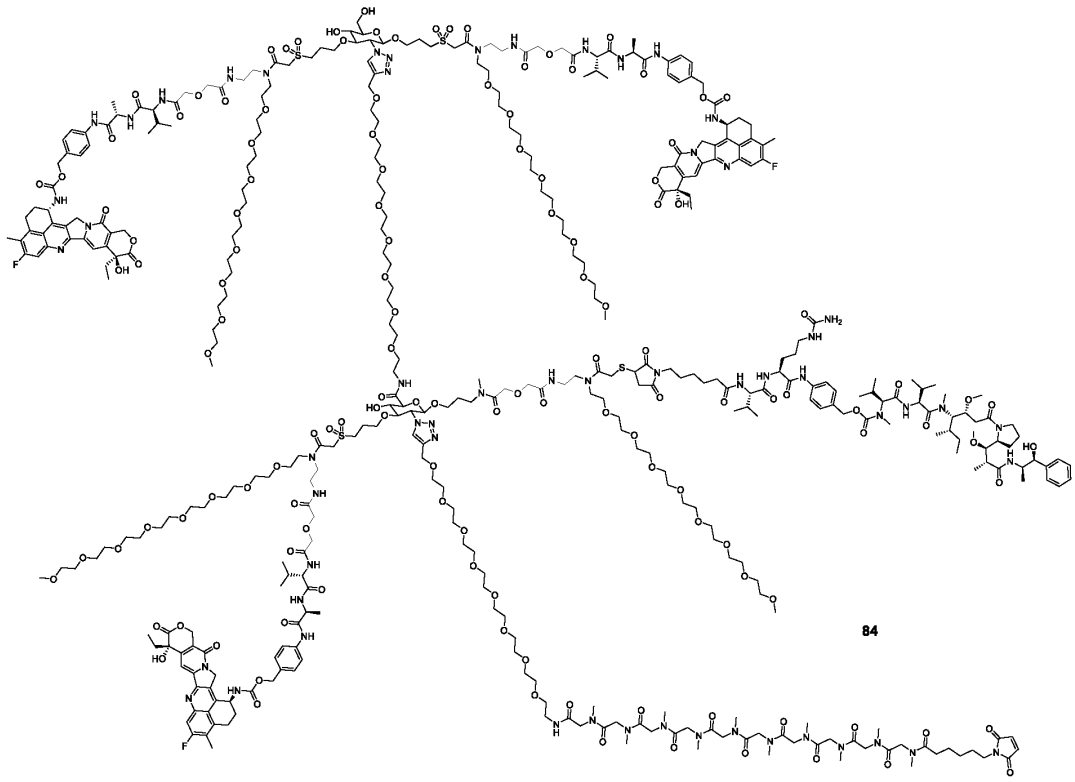


[00899] Step 1: The title compound **83-1** was prepared in analogy to **Example 27, Step 18** by using compound **79-2** and 2-propynylamine. The product (9 mg, yield 29%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{43}H_{66}N_{12}O_{13}$: 959.07; found: 959.73 $[M+H]^+$.

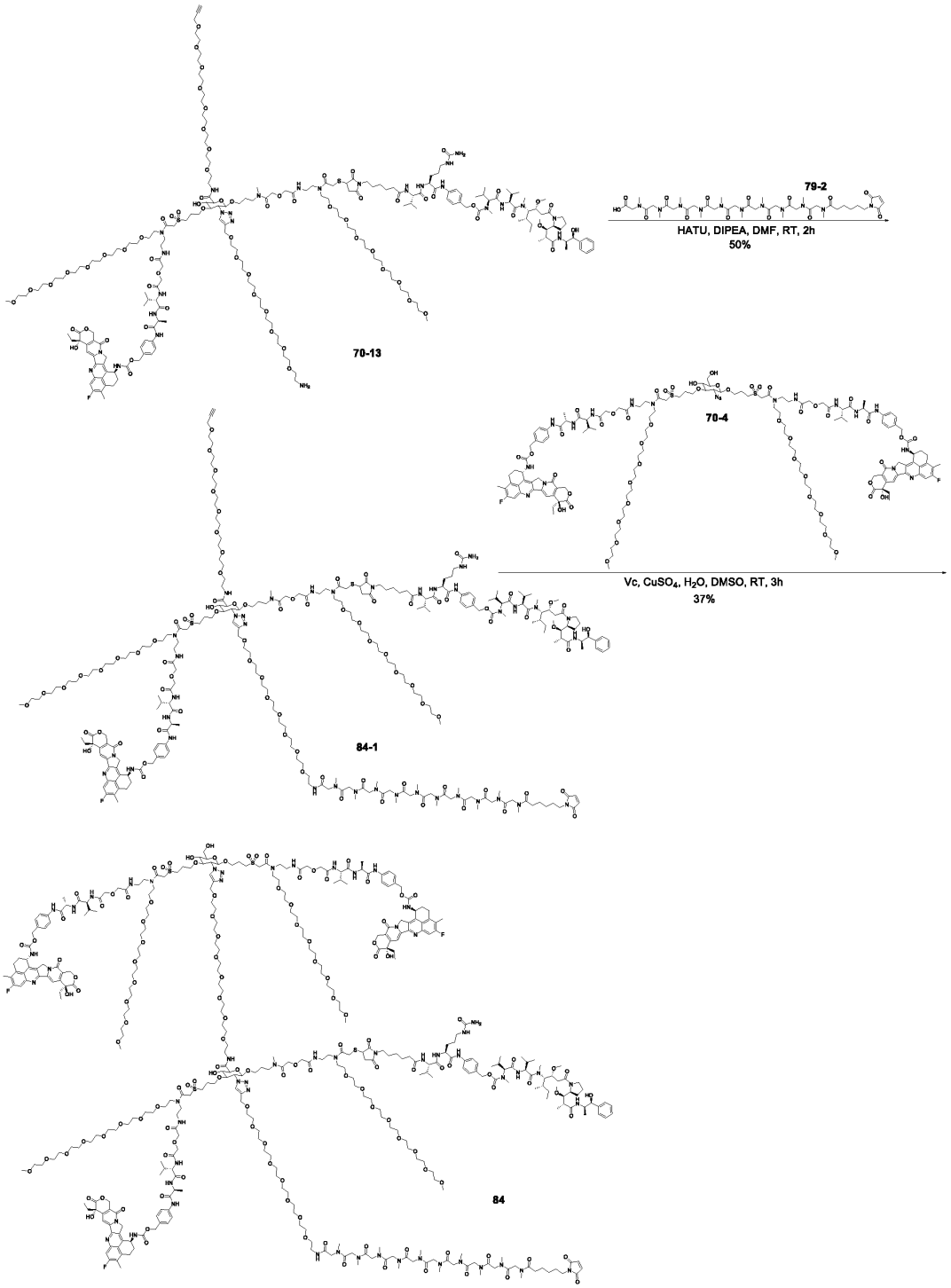
[00900] Step 2: The title compound **83** was prepared in analogy to **Example 10, Step 14** by using N, N-diisopropylethylamine, cuprous iodide, compound **15-11** and compound **83-1** in dimethyl sulfoxide. The product (20 mg, yield 31%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{159}H_{219}FN_{28}O_{53}S_2$: 3453.76; found: 1152.18 $[M+3H]^{3+}$.

Example 84. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[[2-[2-[3-[6-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-

[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[6-(2,5-dioxopyrrol-1-yl)hexanoyl-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[2-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-tetrahydropyran-2-yl]oxypropyl-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (84)



Synthetic scheme 84


79-2
HATU, DIPEA, DMF, RT, 2h
50%
70-13
70-4
Vc, CuSO4, H2O, DMSO, RT, 3h
37%
84-1
84

[00901] Step 1: Under nitrogen atmosphere, to a mixture of compound **79-2** (31mg, 0.034 mmol) in anhydrous N, N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.015 mL, 0.090 mmol) and HATU (19 mg , 0.050 mmol). The mixture was stirred at room temperature for 10 min before compound **70-13** (100 mg, 0.022 mmol) was added. The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **84-1** (61 mg, yield 56%) as a yellow solid. LCMS: m/z calculated for $C_{249}H_{395}FN_{38}O_{83}S_2$: 5332.20; found: 1778.10 $[M+3H]^{3+}$.

[00902] Step 2: Under nitrogen atmosphere, to a mixture of compound **84-30** (33 mg, 0.006 mmol) and compound **84-31** (16 mg, 0.005 mmol) in dimethyl sulfoxide (1.5 mL) was added 0.5M L-Ascorbic acid aqueous solution (0.377 mL, 0.188 mmol) and 0.5M copper sulfate aqueous solution (0.125 mL, 0.063 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the reaction mixture was quenched with saturated ammonium chloride (0.747 mL). The mixture was precipitated in methyl tert-butyl ether (30 mL) before centrifugation. The left brown solid was purified by pre-HPLC (C18 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **84** (16.3 mg, yield 37%) as a white solid. LCMS: m/z calculated for $C_{391}H_{592}F_3N_{57}O_{132}S_4$: 8388.54; found: 1399.11 $[M+6H]^{6+}$.

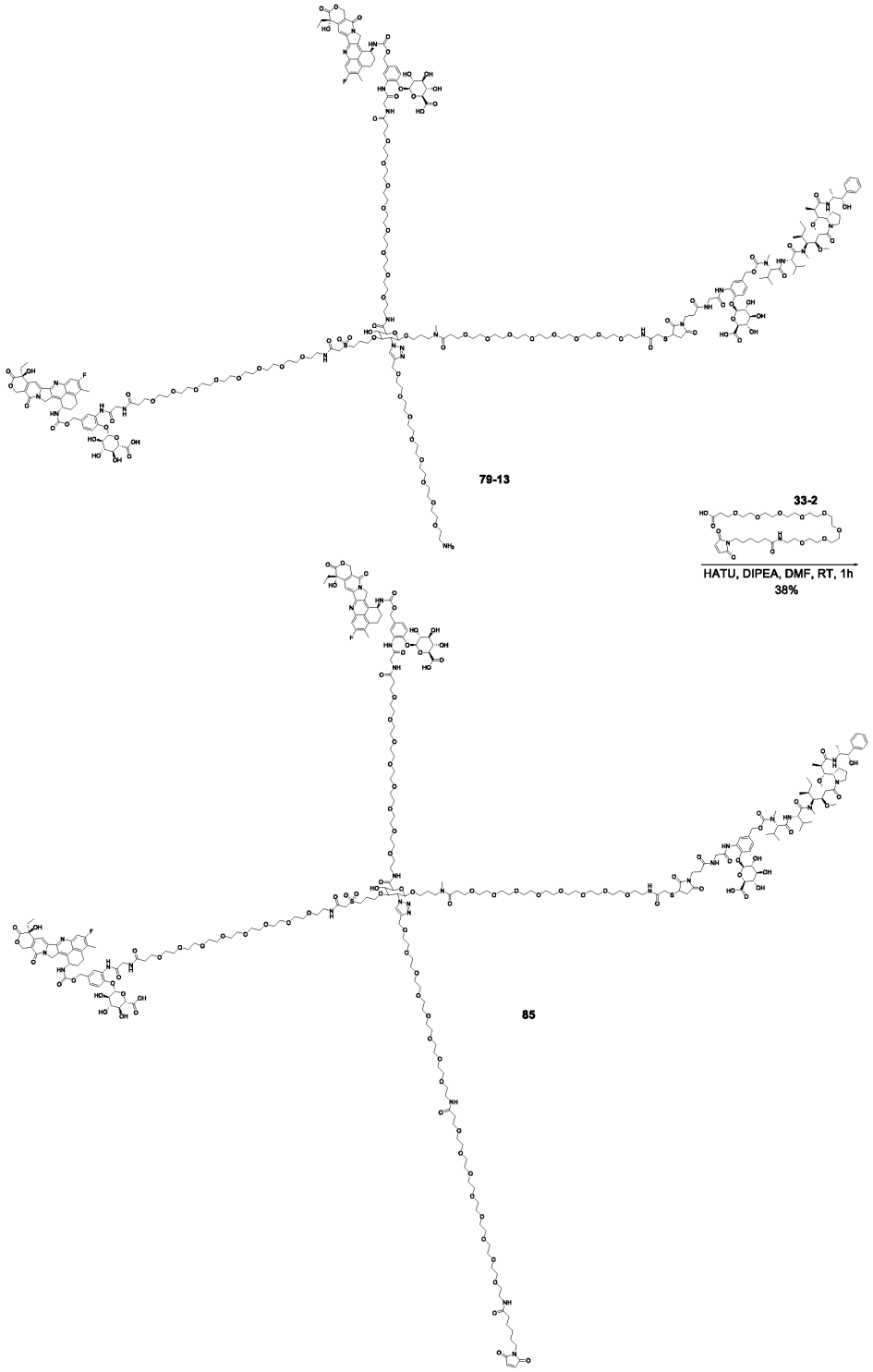
Example 85. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylcarbamoyl]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-

propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (85)



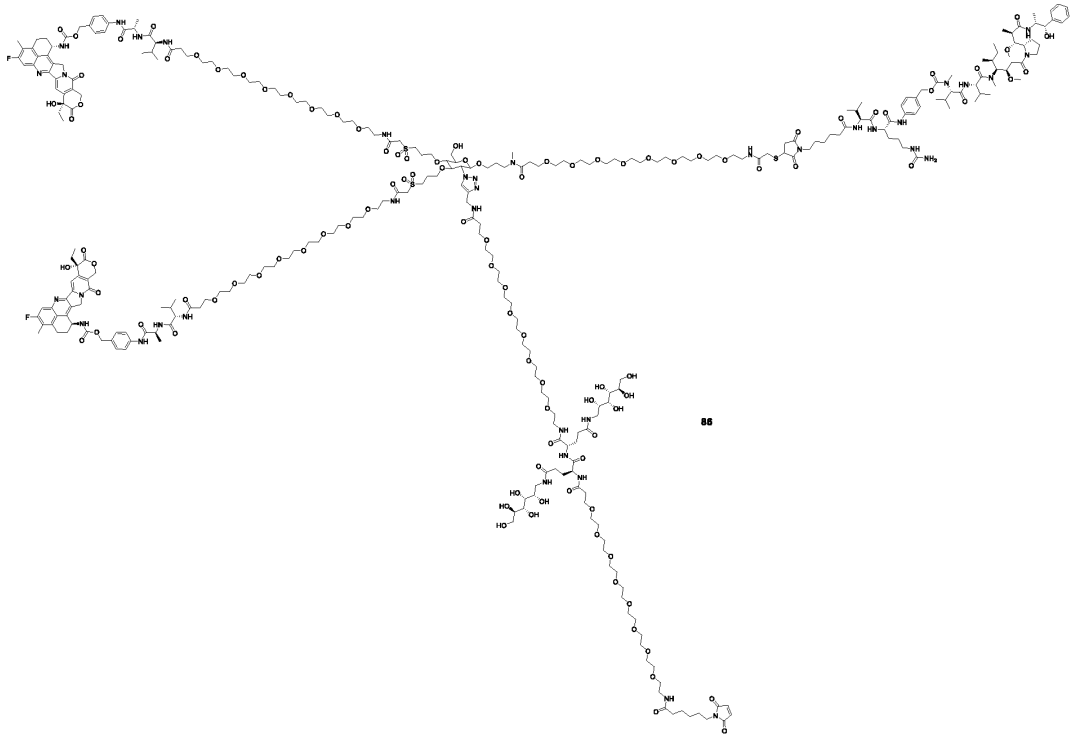
85

Synthetic scheme 85

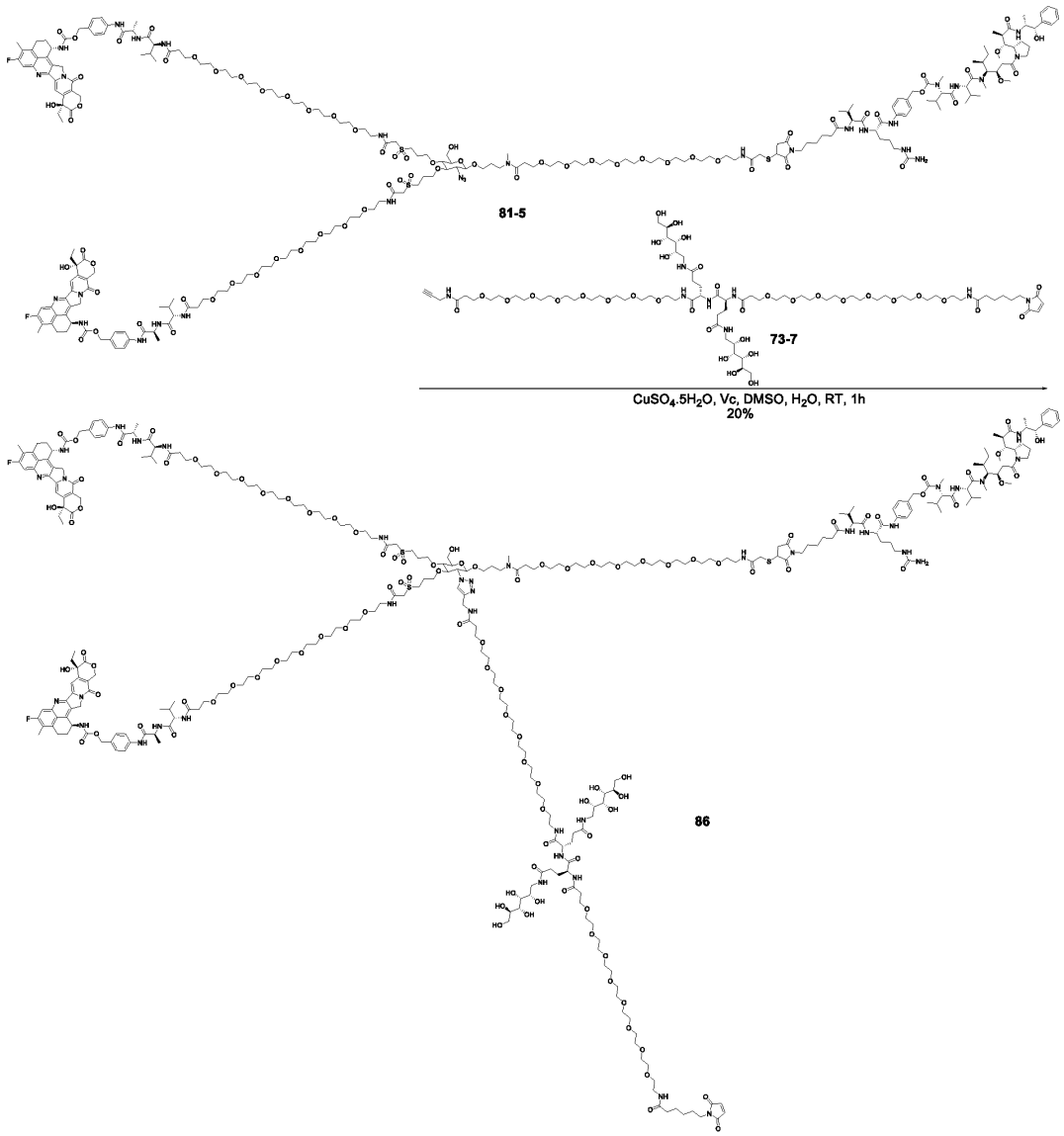

79-13
33-2
HATU, DIPEA, DMF, RT, 1h
38%
85

[00903] Step 1: The title compound **85** was prepared in analogy to **Example 70, Step 8** by using compound **33-2** and compound **79-13**. The product (6.7 mg, yield 38%) as a pale-yellow solid. LCMS: m/z calculated for $C_{264}H_{390}F_2N_{28}O_{104}S_2$: 5722.23; found: 1503.35 $[M+4H]^{4+}$.

Example 86. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (86)

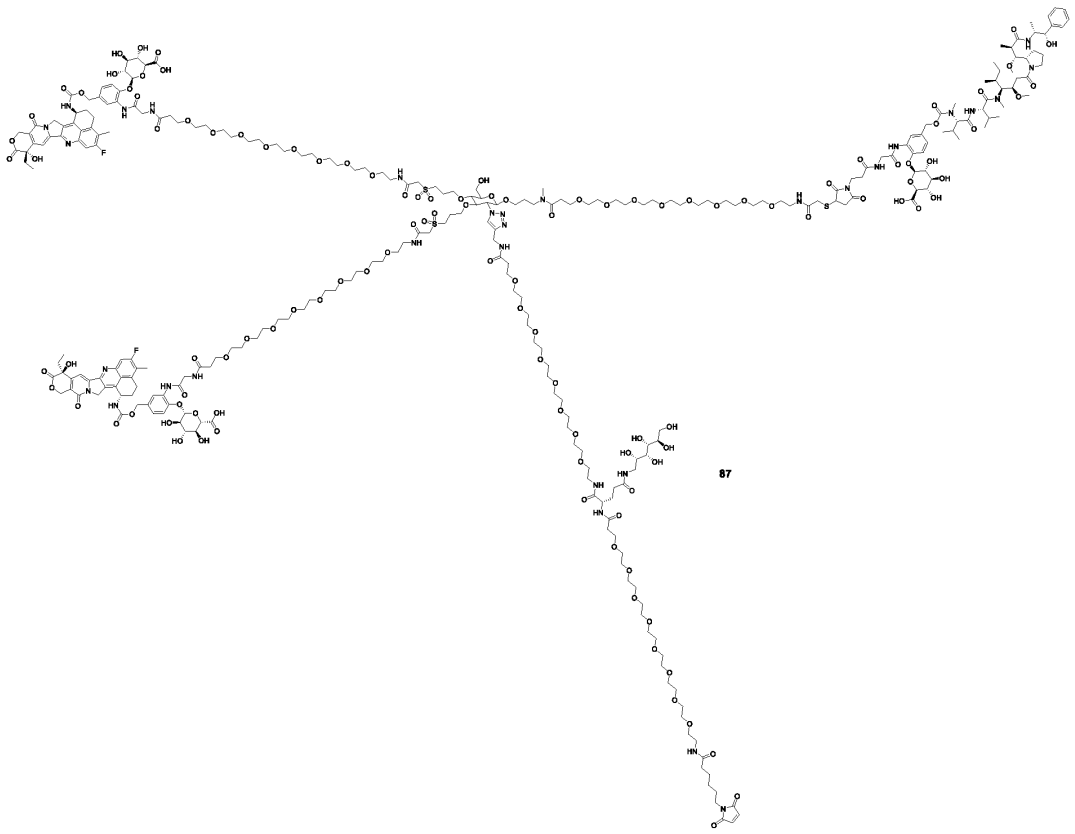


Synthetic scheme 86

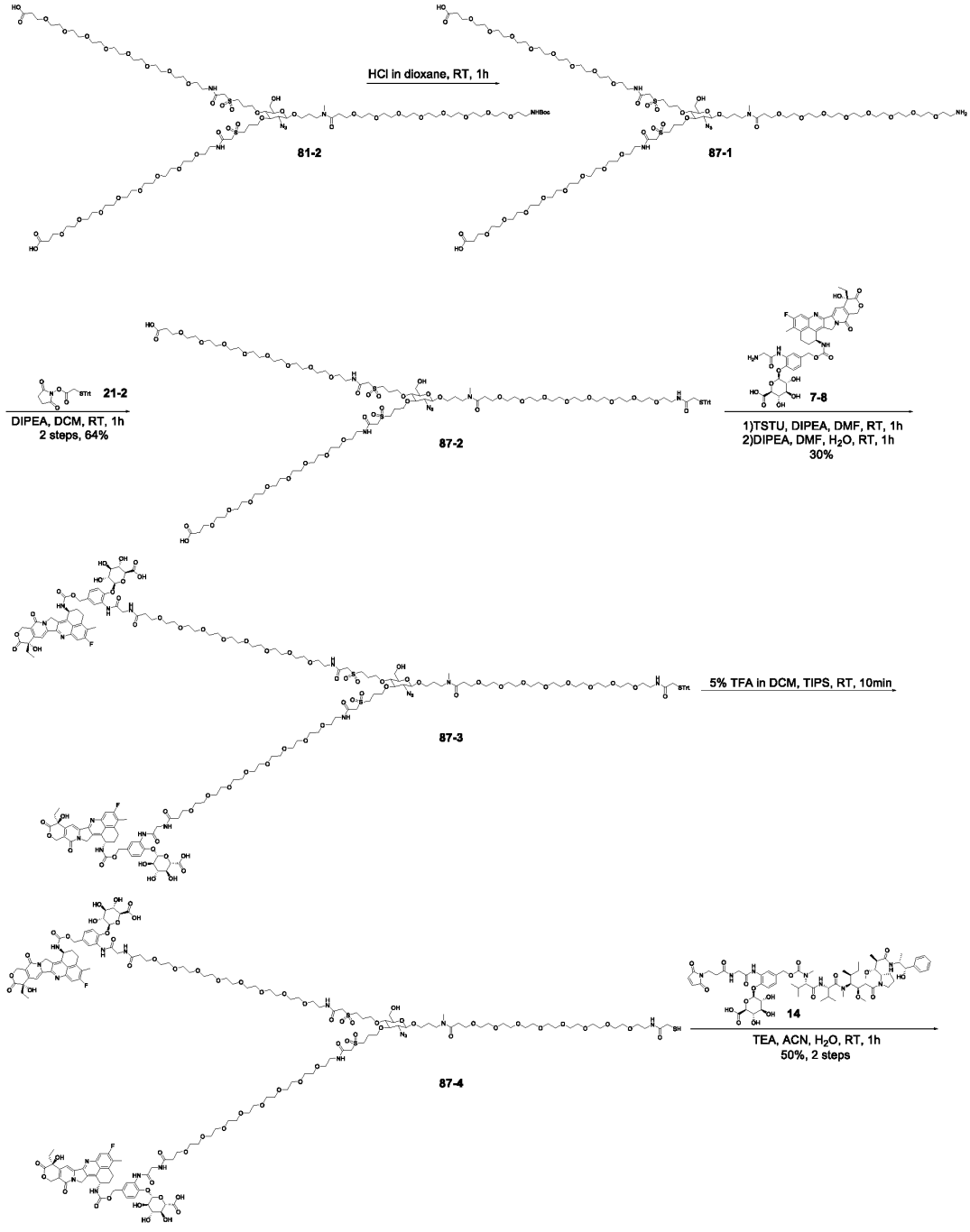


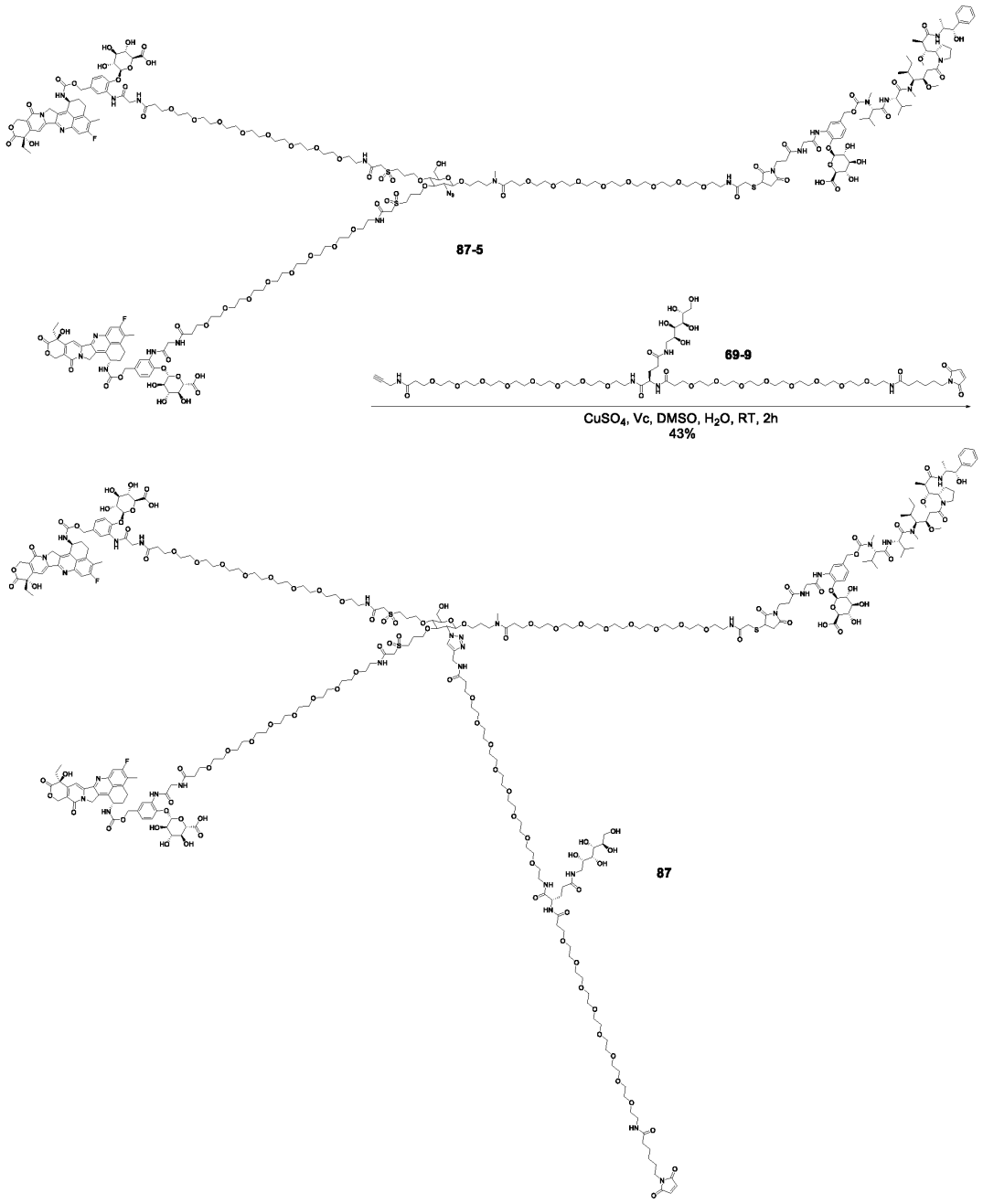
[00904] Step 1: The title compound **86** was prepared in analogy to **Example 1**, **Step 24** by using compound **81-5** and compound **73-7**. The product (4 mg, yield 20%) was obtained as a pale-yellow solid. LCMS: m/z calculated for $C_{300}H_{466}F_2N_{38}O_{105}S_3$: 6419.37; found: 1284.91 $[M+5H]^+$.

Example 87. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)tetrahydropyran-3-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (87)



Synthetic scheme 87


HCl in dioxane, RT, 1h
81-2
87-1
21-2
DIPEA, DCM, RT, 1h
2 steps, 64%
87-2
7-8
1)TSTU, DIPEA, DMF, RT, 1h
2)DIPEA, DMF, H2O, RT, 1h
30%
5% TFA in DCM, TIPS, RT, 10min
87-3
14
TEA, ACN, H2O, RT, 1h
50%, 2 steps
87-4



[00905] Step 1: The title compound **87-1** was prepared in analogy to **Example 69**, **Step 4** by using compound **81-2** in dioxane. The concentrated crude product (691.00 mg) was obtained as a yellow oil. LCMS: m/z calculated for $C_{77}H_{147}N_7O_{40}S_2$: 1875.15; found: 938.50 $[M+2H]^+$.

[00906] Step 2: The title compound **87-2** was prepared in analogy to **Example 77**, **Step 2** by using compound **87-1** and compound **21-2**. The product (498.00 mg, yield 64% over 2 steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{98}H_{163}N_7O_{41}S_3$: 2191.57; found: 1096.49 $[M+2H]^+$.

[00907] Step 3: The title compound **87-3** was prepared in analogy to **Example 53**, **Step 1** by using compound **87-2** and compound **7-8**. The product (130.78 mg, yield 30%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{178}H_{239}F_2N_{17}O_{67}S_3$: 3823.10; found: 1911.73 $[M+2H]^+$.

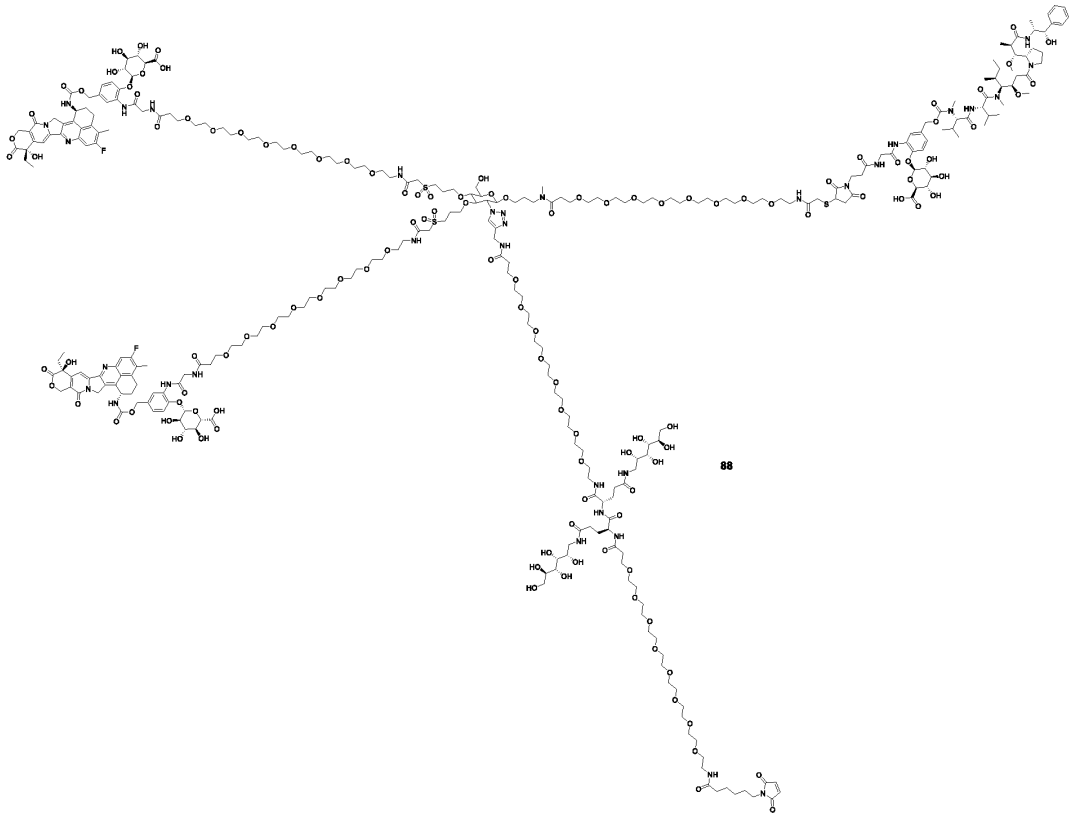
[00908] Step 4: The title compound **87-4** was prepared in analogy to **Example 42**, **Step 15** by using compound **87-3**. The crude product (80.00 mg) was obtained as a yellow solid. LCMS: m/z calculated for $C_{159}H_{225}F_2N_{17}O_{67}S_3$: 3580.78; found: 1194.46 $[M+H]^+$.

[00909] Step 5: The title compound **87-5** was prepared in analogy to **Example 18**, **Step 12** by using compound **87-4** and compound **14** in acetonitrile and H_2O. The product (44.00 mg, yield 50% over 2 steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{221}H_{315}F_2N_{25}O_{87}S_3$: 4848.22; found:1212.86 $[M+H]^+$.

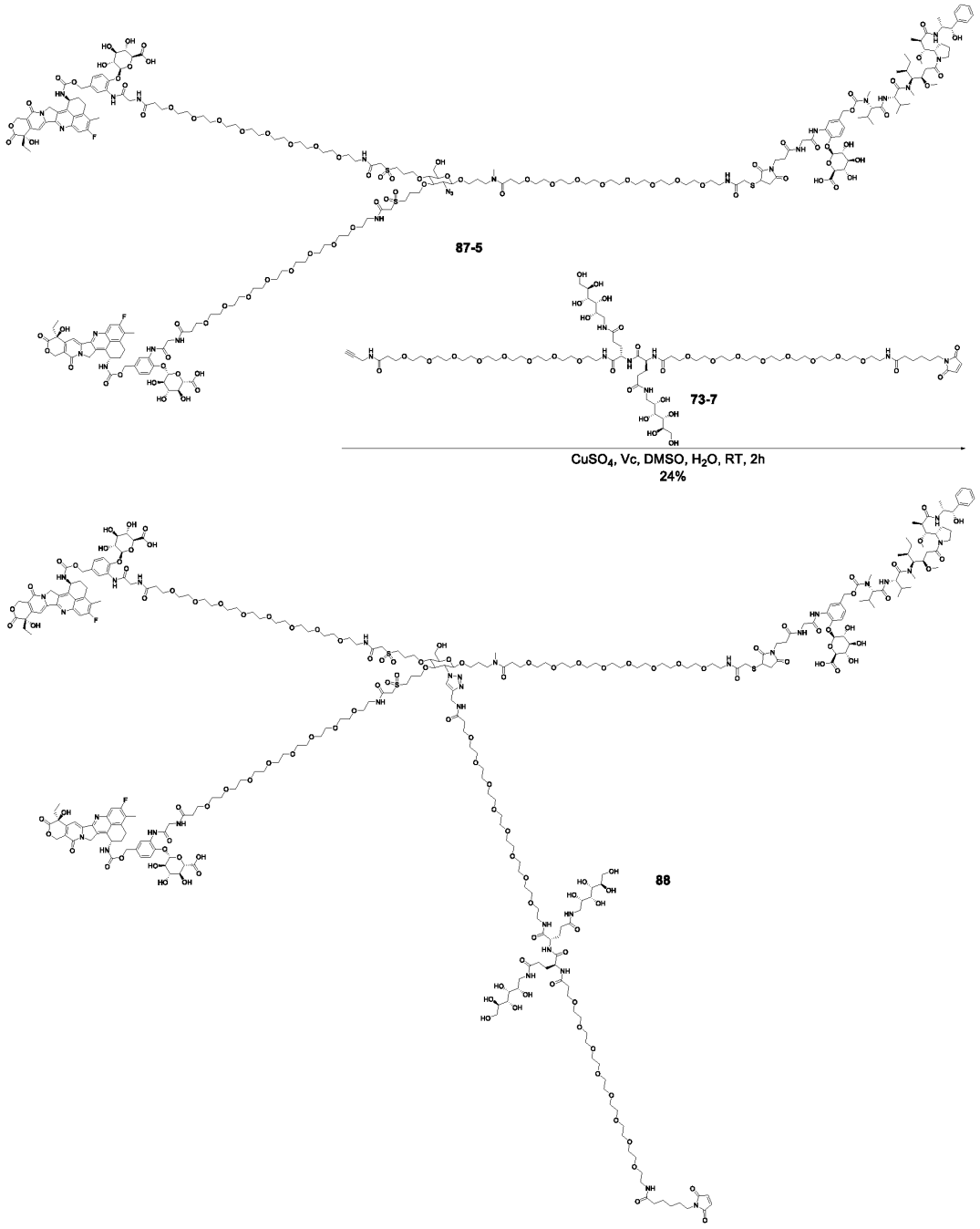
[00910] Step 6: The title compound **87** was prepared in analogy to **Example 1**, **Step 24** by using compound **87-5** and compound **69-9**. The product (13.20 mg, yield 43%) was obtained as a pale-brown solid. LCMS: m/z calculated for $C_{283}H_{425}F_2N_{31}O_{115}S_3$: 6235.79; found: 1559.66 $[M+4H]^+$.

Example 88. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-

carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]-5-oxo-5-[[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]amino]pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-2-(hydroxymethyl)tetrahydropyran-3-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (88)



Synthetic scheme 88

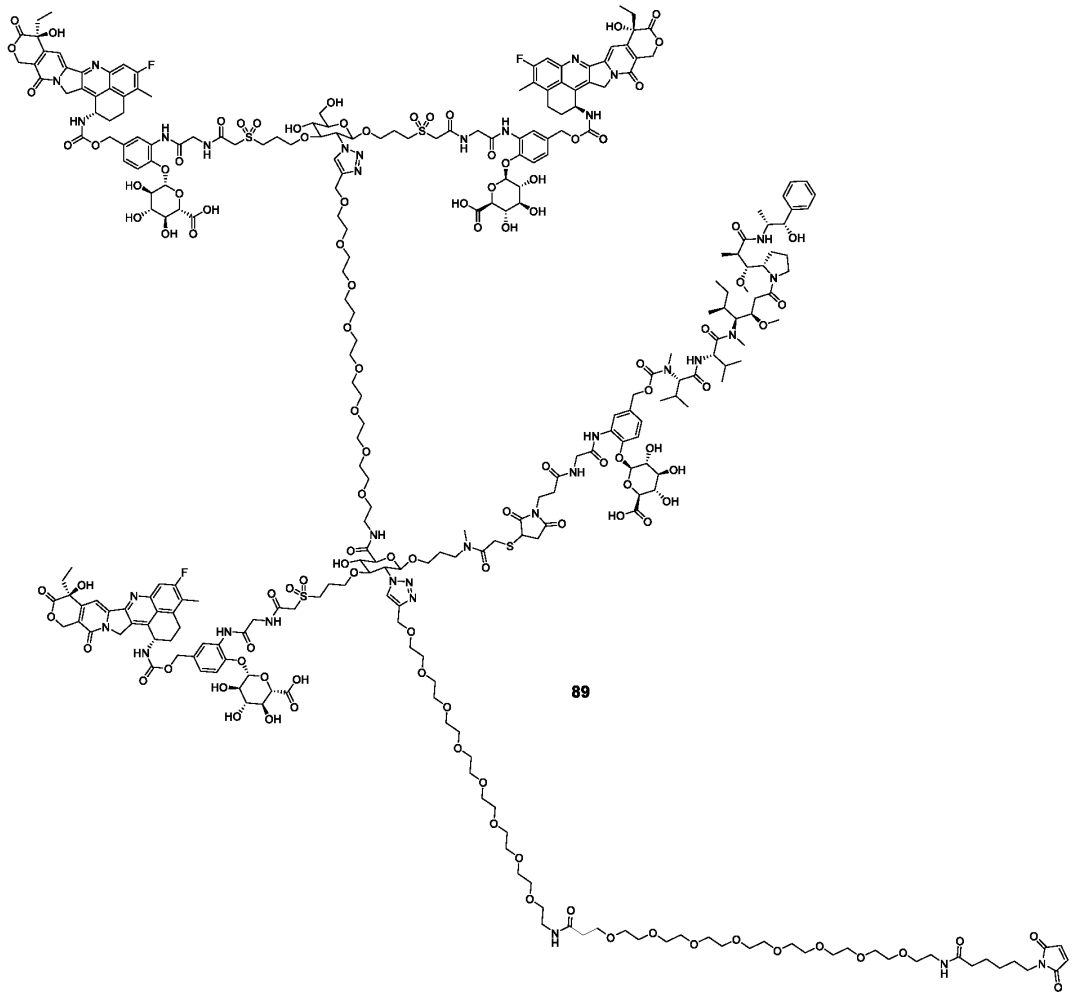


[00911] Step 1: The title compound **88** was prepared in analogy to **Example 1**, **Step 24** by using compound **87-5** and compound **73-7**. The product (9.90 mg, yield 24%) was obtained as a

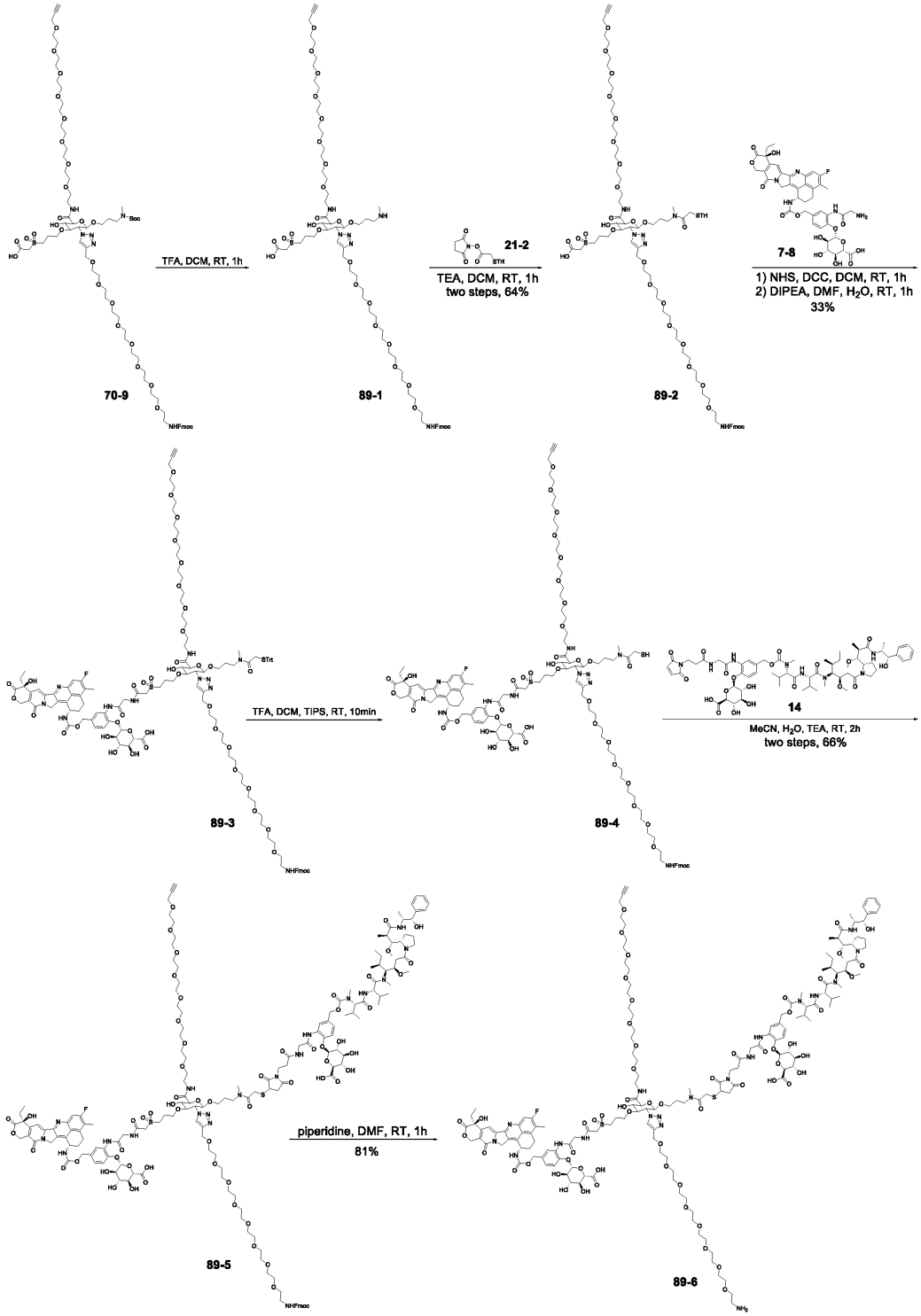
pale-brown solid. LCMS: m/z calculated for $C_{294}H_{445}F_2N_{33}O_{122}S_3$: 6528.08; found: 1632.62 $[M+4H]^+$.

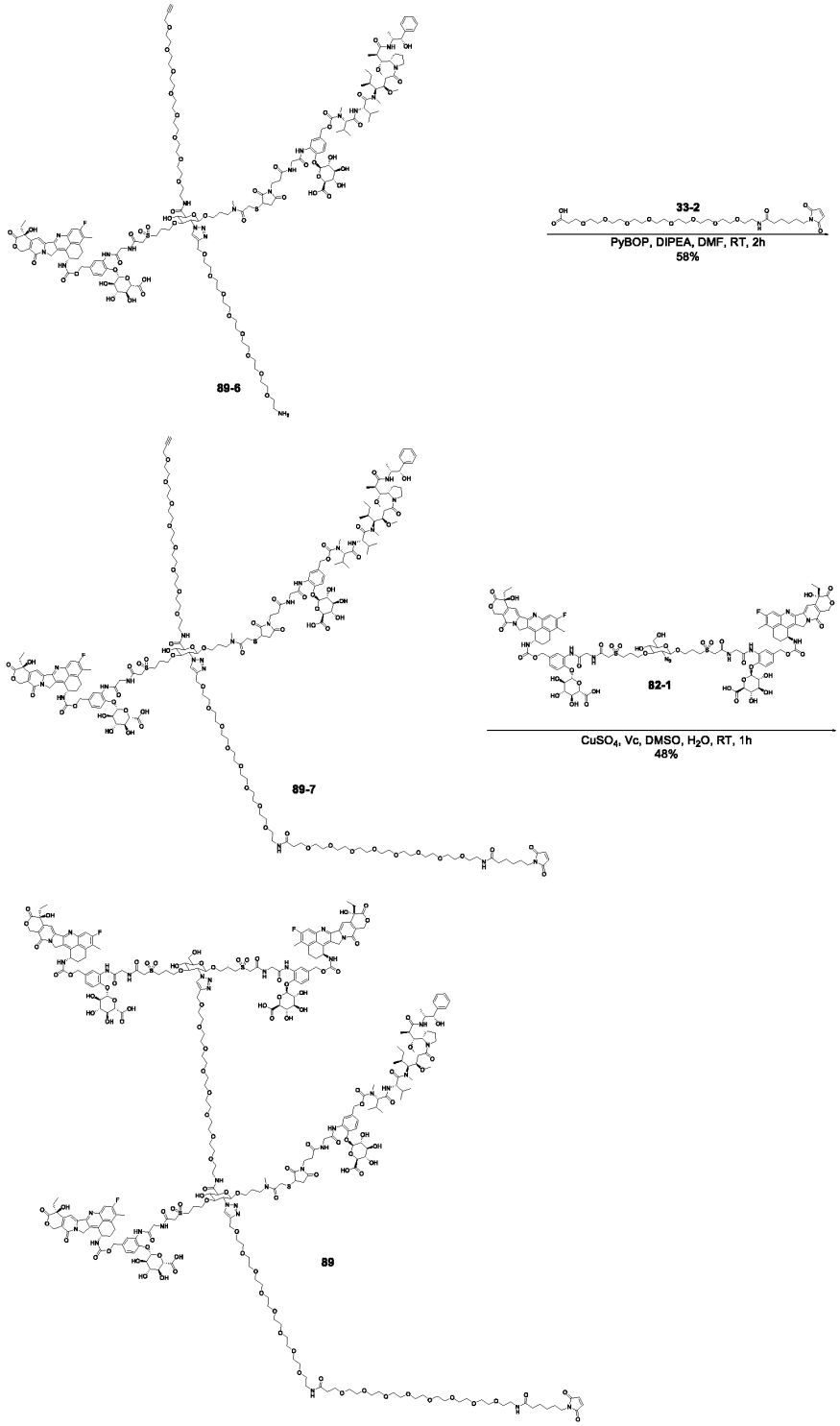
Example 89. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (89)



Synthetic scheme 89


TFA, DCM, RT, 1h
21-2
TEA, DCM, RT, 1h
two steps, 64%
7-8
1) NHS, DCC, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 1h
33%
70-9
89-1
89-2
TFA, DCM, TIPS, RT, 10min
14
MeCN, H2O, TEA, RT, 2h
two steps, 66%
89-3
89-4
piperidine, DMF, RT, 1h
81%
89-5
89-6


89-6
33-2
PyBOP, DIPEA, DMF, RT, 2h
58%
89-7
82-1
CuSO4, Vc, DMSO, H2O, RT, 1h
48%
89

[00912] Step 1: The title compound **89-1** was prepared in analogy to **Example 57, Step 2** by using compound **70-9**. The concentrated crude product (1.90 g) was obtained as a yellow oil. LCMS: m/z calculated for $C_{68}H_{108}N_6O_{27}S$: 1473.69; found: 1474.54 $[M+H]^+$.

[00913] Step 2: The title compound **89-2** was prepared in analogy to **Example 11, Step 2** by using compound **21-2** and compound **89-1**. The product (483.00 mg, yield 64% over two steps) was obtained as a yellow oil. LCMS: m/z calculated for $C_{89}H_{124}N_6O_{28}S_2$: 1790.11; found: 1790.59 $[M+H]^+$.

[00914] Step 3: The title compound **89-3** was prepared in analogy to **Example 18, Step 9 and Step 10** by using compound **7-8** and compound **89-2** with dicyclohexylcarbodiimide instead of EDCI. The product (245.00 mg, yield 33%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{129}H_{162}FN_{11}O_{41}S_2$: 2605.87; found: 1303.38 $[M+2H]^+$.

[00915] Step 4: The title compound **89-4** was prepared in analogy to **Example 42**, **Step 15** by using compound **89-3**. The crude product (311.00 mg) was obtained as a yellow solid. LCMS: m/z calculated for $C_{110}H_{148}FN_{11}O_{41}S_2$: 2363.55; found: 1182.35 $[M+2H]^+$.

[00916] Step 5: The title compound **89-5** was prepared in analogy to **Example 18**, **Step 12** by using compound **89-4** and compound **14**. The product (223.50 mg, two steps, yield 66%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{172}H_{238}FN_{19}O_{61}S_2$: 3630.99; found: 1211.27 $[M+3H]^+$.

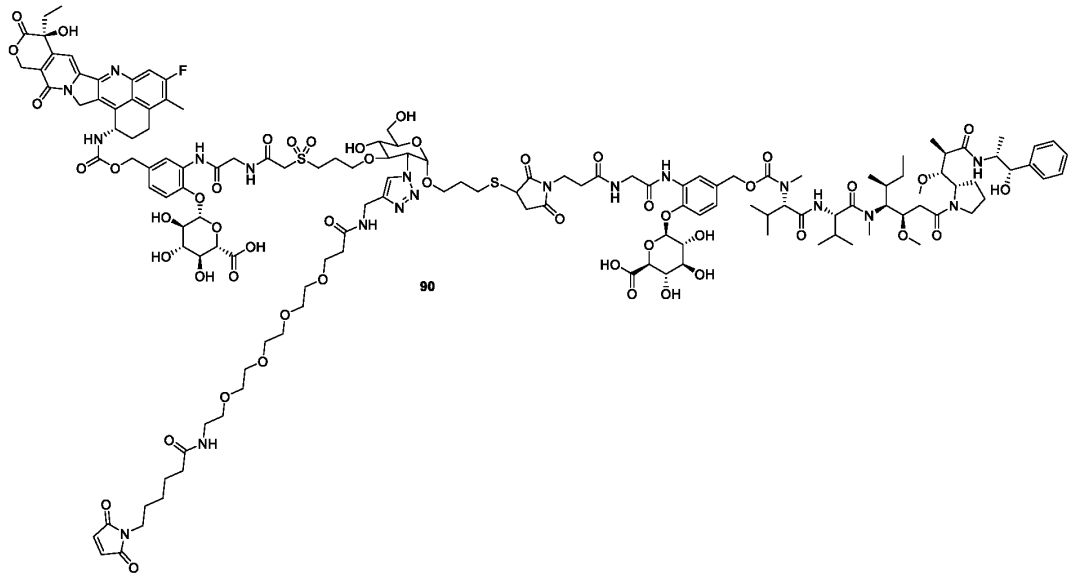
[00917] Step 6: The title compound **89-6** was prepared in analogy to **Example 42**, **Step 27** by using compound **89-5**. The product (170.00 mg, yield 81%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{157}H_{228}FN_{19}O_{59}S_2$: 3408.74; found: 1136.94 $[M+3H]^+$.

[00918] Step 7: The title compound **89-7** was prepared in analogy to **Example 70**, **Step 8** by using compound **33-2** and compound **89-6** The product (52.00 mg, yield 58%) was obtained as a pale-brown solid. LCMS: m/z calculated for $C_{186}H_{276}FN_{21}O_{71}S_2$: 4025.45; found: 1342.43 $[M+3H]^+$.

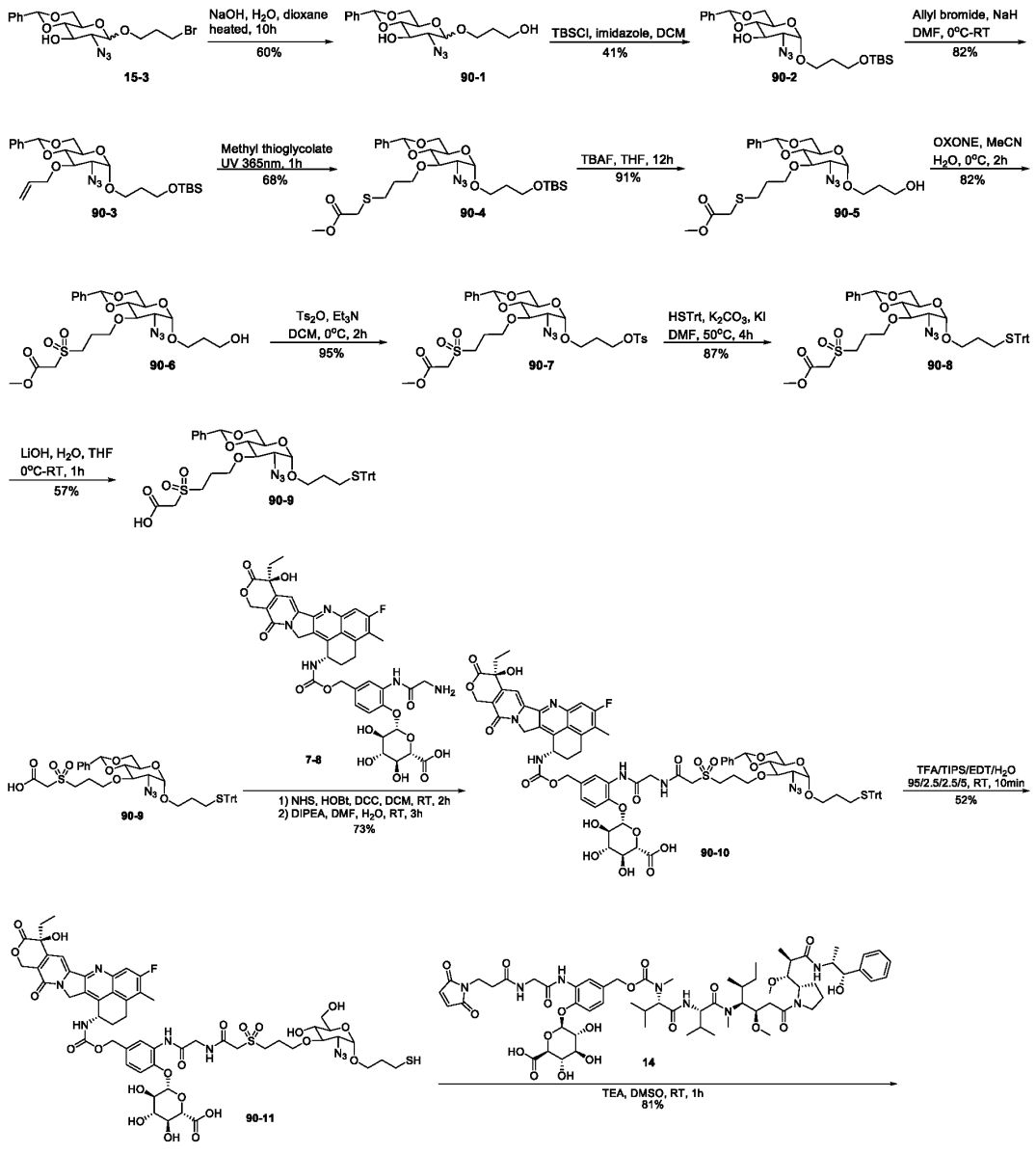
[00919] Step 8: The title compound **89** was prepared in analogy to **Example 1**, **Step 24** by using compound **89-7** and compound **82-1** The product (23.50 mg, yield 48%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{282}H_{379}F_3N_{34}O_{110}S_4$: 6190.50; found:1548.33 $[M+4H]^+$.

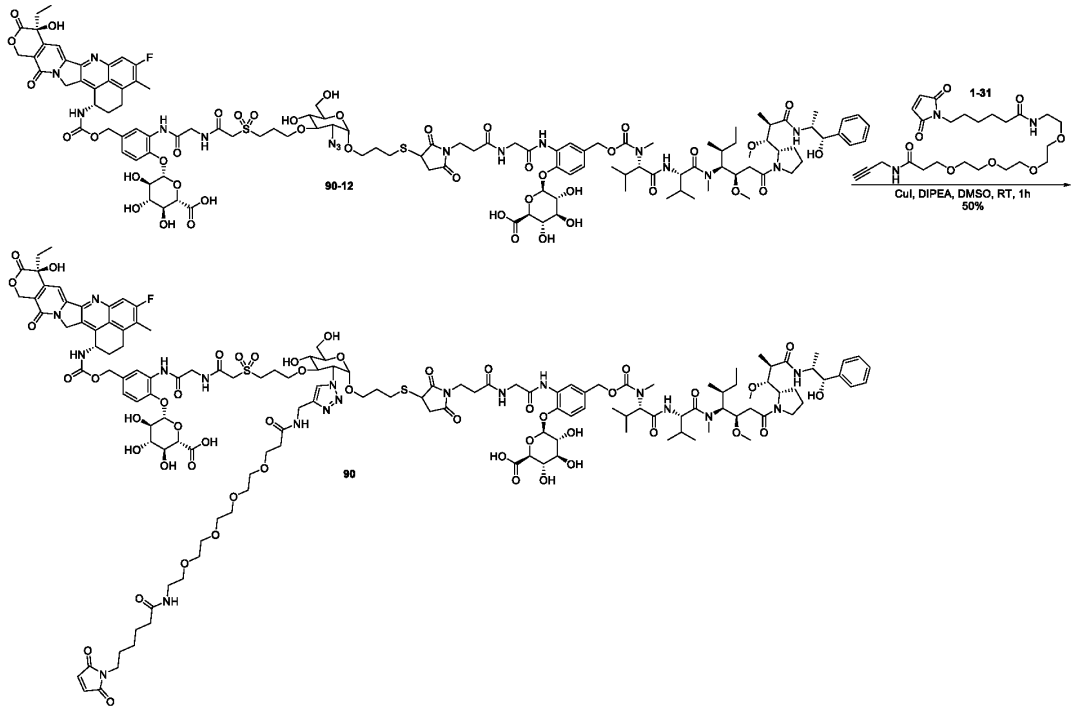
Example 90. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-

1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (90)



Synthetic scheme 90


NaOH, H2O, dioxane
heated, 10h
60%
TBSCl, imidazole, DCM
41%
Allyl bromide, NaH
DMF, 0°C-RT
82%
Methyl thioglycolate
UV 365nm, 1h
68%
TBAF, THF, 12h
91%
OXONE, MeCN
H2O, 0°C, 2h
82%
Ts2O, Et3N
DCM, 0°C, 2h
95%
HSTrt, K2CO3, KI
DMF, 50°C, 4h
87%
LiOH, H2O, THF
0°C-RT, 1h
57%
1) NHS, HOBt, DCC, DCM, RT, 2h
2) DIPEA, DMF, H2O, RT, 3h
73%
TFA/TIPS/EDT/H2O
95/2.5/2.5/5, RT, 10min
52%
TEA, DMSO, RT, 1h
81%
15-3
90-1
90-2
90-3
90-4
90-5
90-6
90-7
90-8
90-9
7-8
90-10
90-11
14



[00920] Step 1: To a solution of sodium hydroxide (1.48 g, 37.00 mmol) in H_2O (5 mL) was added a solution of compound **15-3** (2.20 g, 5.31 mmol) in dioxane (5 mL). The reaction mixture was heated and stirred for 10 h. After complete reaction, the mixture was cooled to room temperature, and extracted with water (10 mL) and ethyl acetate (20 mL x 2). The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **90-1** (1.12 g, yield 60%) as a light-yellow oil.

[00921] Step 2: To a solution of compound **90-1** (1.12 g, 3.19 mmol) in dichloromethane (10 mL) was added imidazole (330 mg, 4.85 mmol) and tert-butyldimethylsilyl chloride (580 mg, 3.85 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was extracted with water (10 mL) and dichloromethane (10 mL x 2). The combined the organic phases was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluted with 20% ethyl acetate in petroleum ether) to afford the title compound **90-2** (570 mg, yield 41%) as a light-yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (d, *J*

= 4.6 Hz, 2H), 7.39 (d, *J* = 4.6 Hz, 3H), 5.55 (s, 1H), 4.92 (d, *J* = 3.1 Hz, 1H), 4.31 – 4.19 (m, 2H), 3.93 – 3.83 (m, 2H), 3.74 (t, *J* = 10.7 Hz, 3H), 3.60 – 3.49 (m, 2H), 3.24 (d, *J* = 12.7 Hz, 1H), 1.90 – 1.81 (m, 2H), 0.90 (s, 9H), 0.07 (s, 6H). LCMS: m/z calculated for $C_{22}H_{35}N_3O_6Si$: 465.62 found: 466.20 $[M+H]^+$.

[00922] Step 3: The title compound **90-3** was prepared in analogy to **Example 1**, **Step 4** by using sodium hydrogen, allyl bromide and compound **90-2** in N,N-dimethylformamide. The product (650 mg, yield 82%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.51 – 7.46 (m, 2H), 7.42 – 7.34 (m, 3H), 6.04 – 5.90 (m, 1H), 5.56 (s, 1H), 5.31 (d, *J* = 18.6 Hz, 1H), 5.20 (d, *J* = 10.4 Hz, 1H), 4.87 (d, *J* = 3.5 Hz, 1H), 4.44 (dd, *J* = 12.4, 5.7 Hz, 1H), 4.26 (dd, *J* = 13.7, 3.9 Hz, 2H), 3.97 (t, *J* = 9.5 Hz, 1H), 3.85 (dd, *J* = 14.6, 9.6 Hz, 2H), 3.78 – 3.70 (m, 3H), 3.64 (t, *J* = 9.3 Hz, 1H), 3.55 (d, *J* = 9.7 Hz, 1H), 3.31 (d, *J* = 13.5 Hz, 1H), 1.88 – 1.81 (m, 2H), 0.90 (s, 9H), 0.07 (s, 6H).

[00923] Step 4: The title compound **90-4** was prepared in analogy to **Example 1**, **Step 5** by using methyl thioglycolate and compound **90-3** in dichloromethane. The product (500 mg, yield 68%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (d, *J* = 6.3 Hz, 2H), 7.38 (d, *J* = 6.9 Hz, 3H), 5.56 (s, 1H), 4.87 (d, *J* = 3.5 Hz, 1H), 4.27 (dd, *J* = 10.1, 4.7 Hz, 1H), 4.02 (d, *J* = 9.6 Hz, 1H), 3.93 – 3.86 (m, 2H), 3.85 – 3.80 (m, 2H), 3.78 (s, 3H), 3.75 (s, 2H), 3.71 (s, 3H), 3.63 – 3.57 (m, 2H), 3.55 (dd, *J* = 9.7, 6.5 Hz, 1H), 2.74 (t, *J* = 7.3 Hz, 2H), 1.93 – 1.82 (m, 4H), 0.91 (s, 9H), 0.07 (s, 6H).

[00924] Step 5: The title compound **90-5** was prepared in analogy to **Example 10**, **Step 5** by using tetrabutylammonium fluoride and compound **90-4** in tetrahydrofuran. The product (370 mg, yield 92%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (dd, *J* = 39.5, 5.4 Hz, 5H), 5.56 (s, 1H), 4.91 – 4.86 (m, 1H), 4.27 (dd, *J* = 10.0, 4.6 Hz, 1H), 4.05 – 3.97 (m, 1H), 3.95 – 3.86 (m, 2H), 3.85 – 3.76 (m, 5H), 3.74 (s, 2H), 3.70 (s, 3H), 3.60 (t, *J* = 9.3 Hz, 2H), 3.39 (d, *J* = 13.2 Hz, 1H), 2.72 (t, *J* = 7.3 Hz, 2H), 1.87 (dd, *J* = 13.5, 7.3 Hz, 4H).

[00925] Step 6: The title compound **90-6** was prepared in analogy to **Example 57**, **Step 1** by using OXONE and compound **90-5** in acetonitrile and H_2O. The product (320 mg, yield 82%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.50 – 7.43 (m, 2H), 7.42 – 7.35 (m, 3H), 5.56 (s, 1H), 4.90 (d, *J* = 3.3 Hz, 1H), 4.27 (dd, *J* = 9.8, 4.5 Hz, 1H), 4.02 (d, *J* = 9.6 Hz, 1H), 3.93 (d, *J* = 4.7 Hz, 2H), 3.89 – 3.81 (m, 6H), 3.79 (s, 3H), 3.73 (d, *J* = 9.9 Hz, 1H),

3.64 (d, J = 5.6 Hz, 1H), 3.60 (d, J = 8.2 Hz, 2H), 3.42 – 3.37 (m, 2H), 2.18 – 2.09 (m, 2H), 1.94 – 1.87 (m, 2H).

[00926] Step 7: To a solution of compound **90-6** (300 mg, 0.57 mmol) in dichloromethane (3 mL) was added triethylamine (110 mg, 1.13 mmol) and 4-methylbenzenesulfonic anhydride (270 mg, 0.83 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was extracted with water (5 mL) and dichloromethane (4 mL x 3). The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the title compound **90-7** (287 mg, yield 95%) as a yellow oil.

[00927] Step 8: The title compound **90-8** was prepared in analogy to **Example 10**, **Step 7** by using triphenylmercaptan, potassium carbonate, potassium iodide and compound **90-7** in N,N-dimethylformamide. The product (290 mg, yield 87%) was obtained as a light-yellow oil.

[00928] Step 9: The title compound **90-9** was prepared in analogy to **Example 1**, **Step 6** by using lithium hydroxide and compound **90-8** in THF and H_2O. The product (224 mg, yield 57%) was obtained as a white solid. LCMS: m/z calculated for $C_{40}H_{43}N_3O_9S_2$: 773.92; found:796.28 $[M+Na]^+$.

[00929] Step 10: The title compound **90-10** was prepared in analogy to **Example 11**, **Step 1 and Step 2** by using N-hydroxysuccinimide, 1-hydroxybenzotriazole and dicyclohexylcarbodiimide to activate compound **90-9** in dichloromethane, followed by coupling with compound **7-8** and N,N-diisopropylethylamine in N,N-dimethylformamide (3 mL). The product (75 mg, yield 73%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{80}H_{81}FN_8O_{22}S_2$: 1589.68; found: 1589.73 $[M+H]^+$.

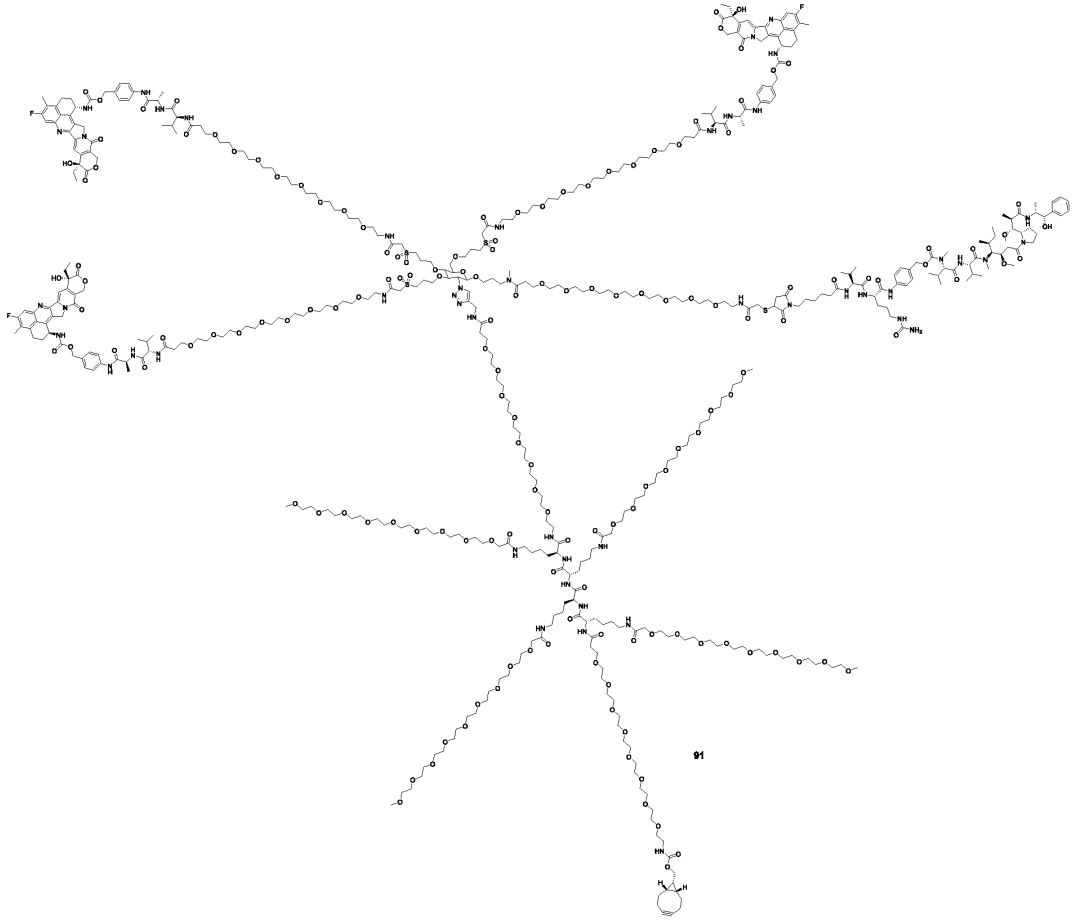
[00930] Step 11: The title compound **90-11** was prepared in analogy to **Example 7**, **Step 9** by using compound **90-10** in TFA, H_2O, EDT and TIPS. The product (23 mg, yield 57%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{54}H_{63}FN_8O_{22}S_2$: 1529.25; found: 1529.58 $[M+H]^+$.

[00931] Step 12: The title compound **90-12** was prepared in analogy to **Example 10**, **Step 10** by using triethylamine, compound **14** and compound **90-11** in dimethyl sulfoxide. The product (16.5 mg, yield 81%) was obtained as a white solid. LCMS: m/z calculated for $C_{116}H_{153}FN_{16}O_{42}S$: 2526.69; found: 1263.79 $[M+2H]^{2+}$.

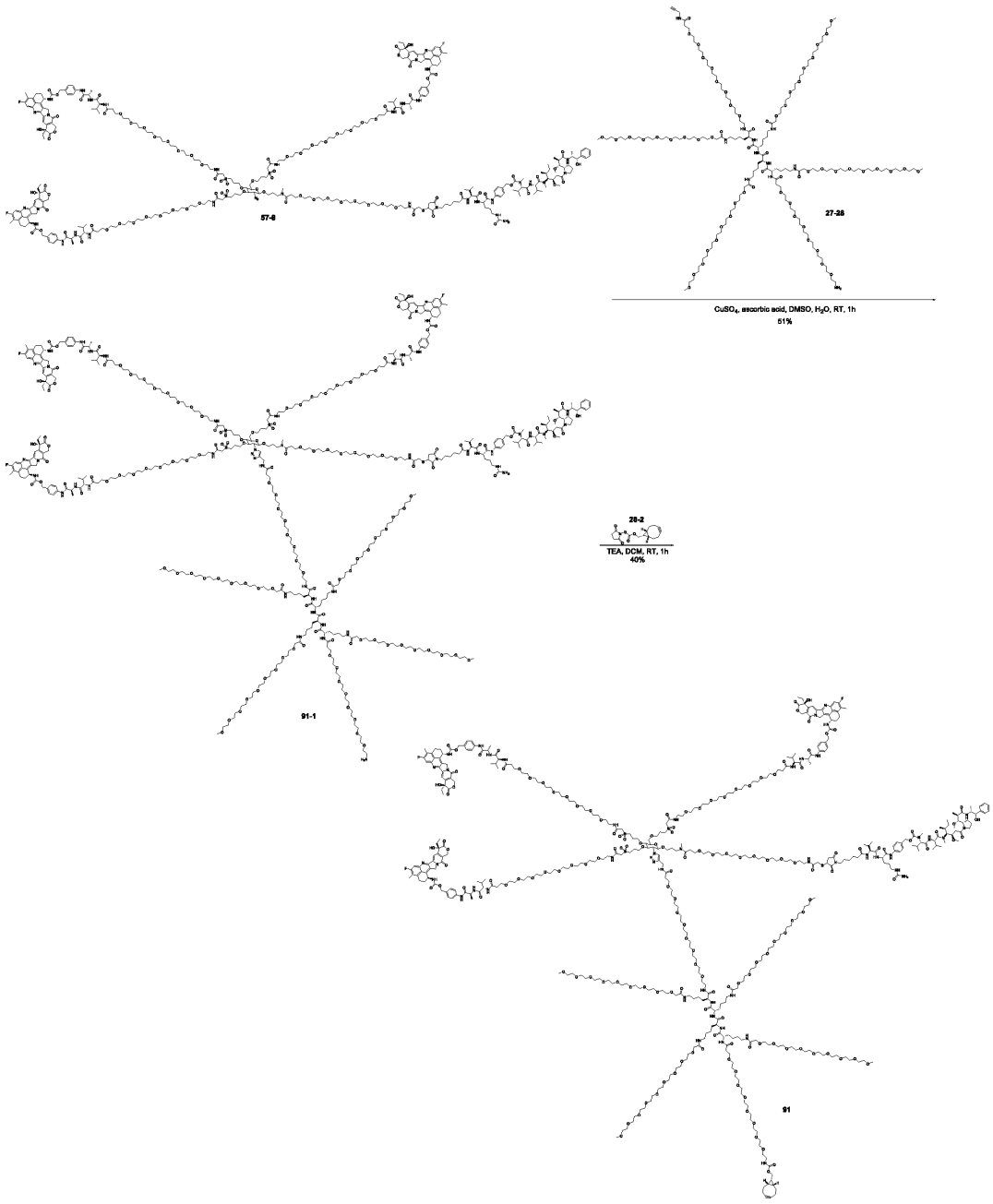
[00932] Step 13: The title compound **90** was prepared in analogy to **Example 10**, **Step 14** by using N,N-diisopropylethylamine, cuprous iodide, compound **1-31** and compound **90-12** in dimethyl sulfoxide. The product (5 mg, yield 50%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{140}H_{190}FN_{19}O_{50}S_2$: 3022.26; found: 1008.08 $[M+3H]^{3+}$.

Example 91. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(1*R*,8*S*)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-

propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (91)



Synthetic scheme 91



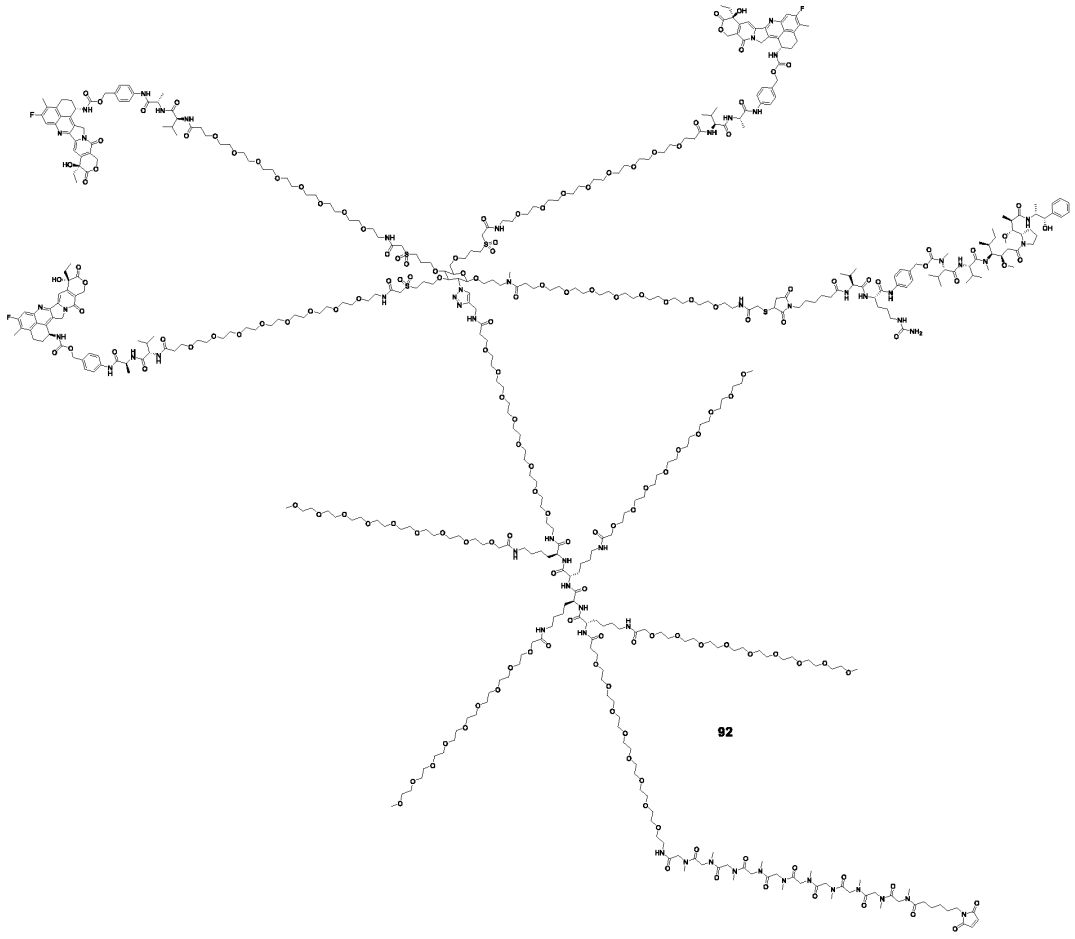
[00933] Step 1: Under nitrogen atmosphere, to a mixture of compound **57-8** (150 mg, 0.025 mmol) and compound **27-28** (100 mg, 0.033 mmol) in dimethyl sulfoxide (2 mL) was added 0.5M L-Ascorbic acid aqueous solution (0.750 mL, 0.750 mmol) and 0.5M copper sulfate

aqueous solution (0.250 mL, 0.250 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was quenched with saturated ammonium chloride (0.5 mL), filtered. The resulting mixture was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **91-1** (118 mg, yield 51%) as a yellow solid. LCMS: m/z calculated for $C_{432}H_{693}F_3N_{48}O_{152}S_4$: 9176.72; found: 1529.98 $[M+6H]^{6+}$.

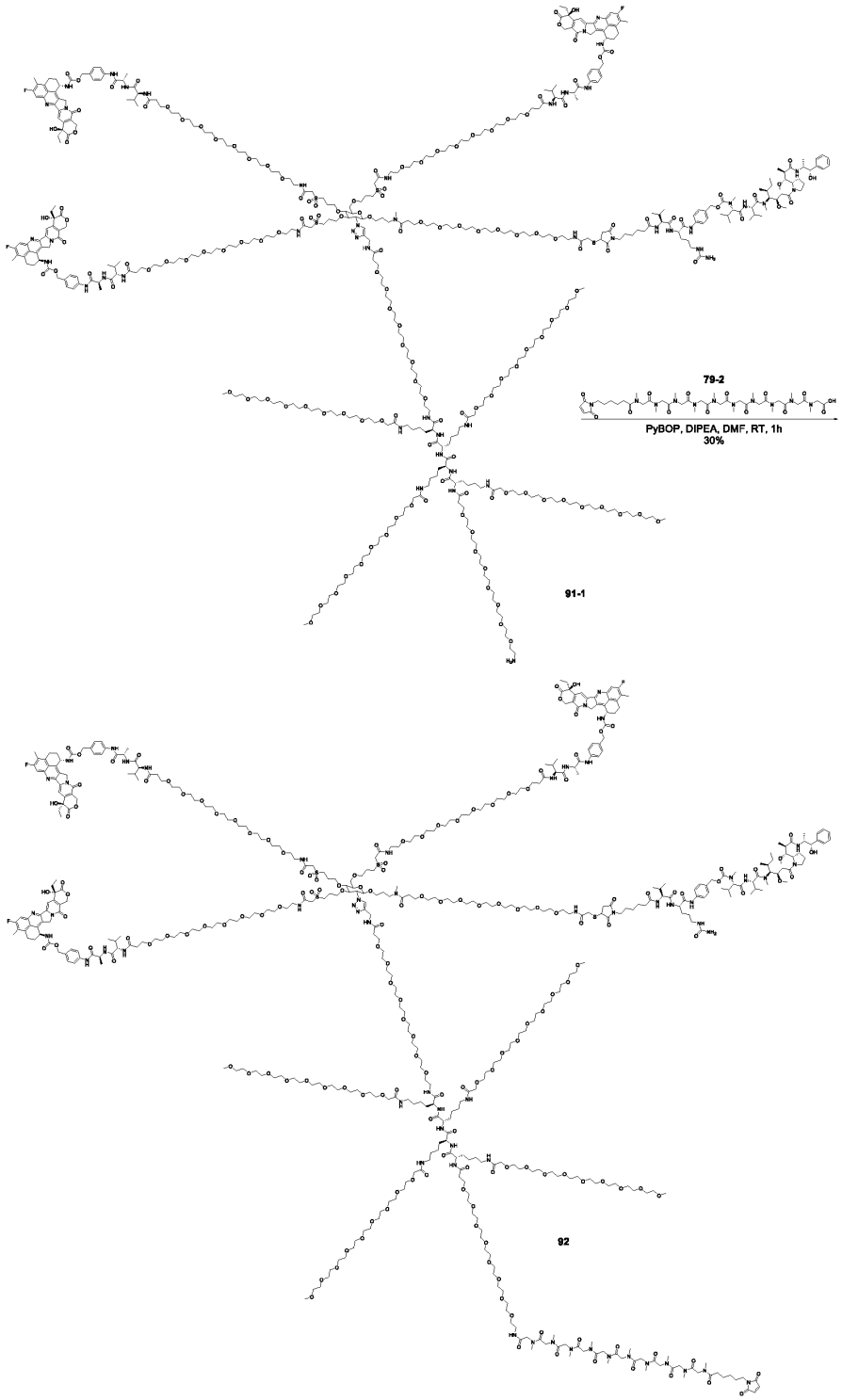
[00934] Step 2: Under nitrogen atmosphere, to a mixture of compound **91-1** (30 mg, 0.003 mmol) in anhydrous dichloromethane (1 mL) was added triethylamine (0.001 mL, 0.009mmol) and compound **28-2** (1.3 mg, 0.004 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by pre-HPLC (C4 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to give the title compound **91** (12.4 mg, yield 40%) as a pale-yellow solid. LCMS: m/z calculated for $C_{443}H_{705}F_3N_{48}O_{154}S_4$: 9352.93; found: 1559.43 $[M+6H]^{6+}$.

Example 92. Synthesis of [4-[[(2*S*)-2-[[(2*S*)-2-[6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[3-[3-[4-[[3-[2-[2-[2-[2-[2-[2-[2-[2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[6-(2,5-dioxopyrrol-1-yl)hexanoyl-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl] amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl] amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl] amino]-6-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]acetyl]amino]hexanoyl] amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]

triazol-1-yl]-4,5-bis[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(1*S*)-1-[[(1*S*)-2-[4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxymethyl]tetrahydropyran-2-yl]oxypropyl-methyl-amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfanyl-2,5-dioxo-pyrrolidin-1-yl]hexanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl *N*-[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (92)



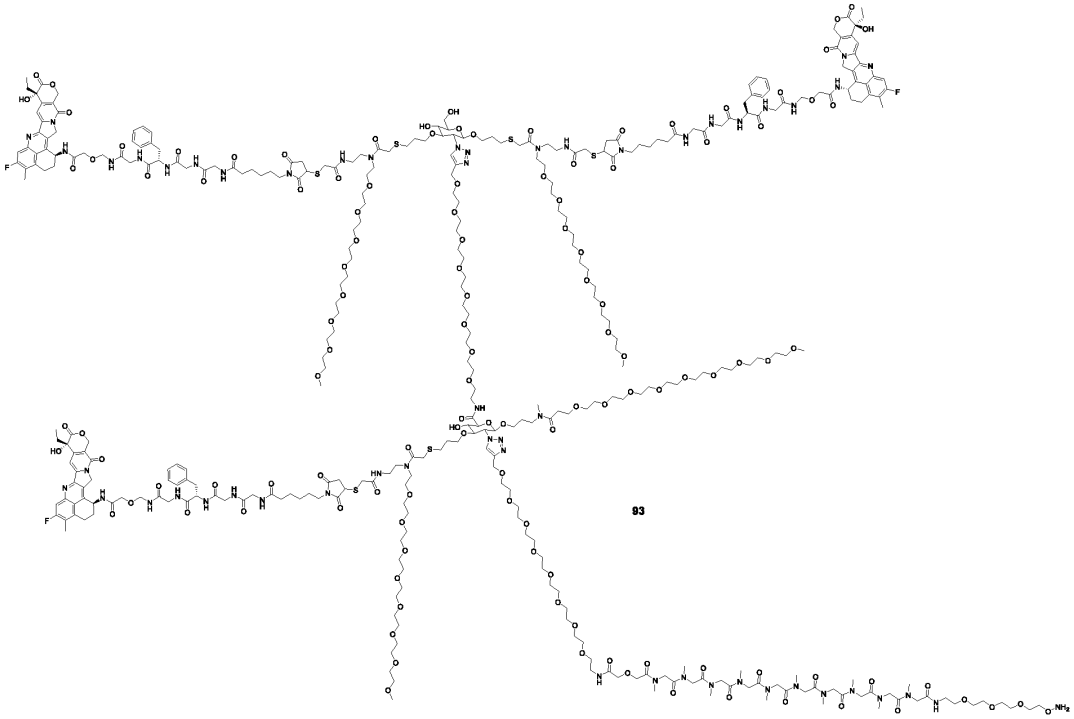
Synthetic scheme 92


79-2
PyBOP, DIPEA, DMF, RT, 1h
30%
91-1
92

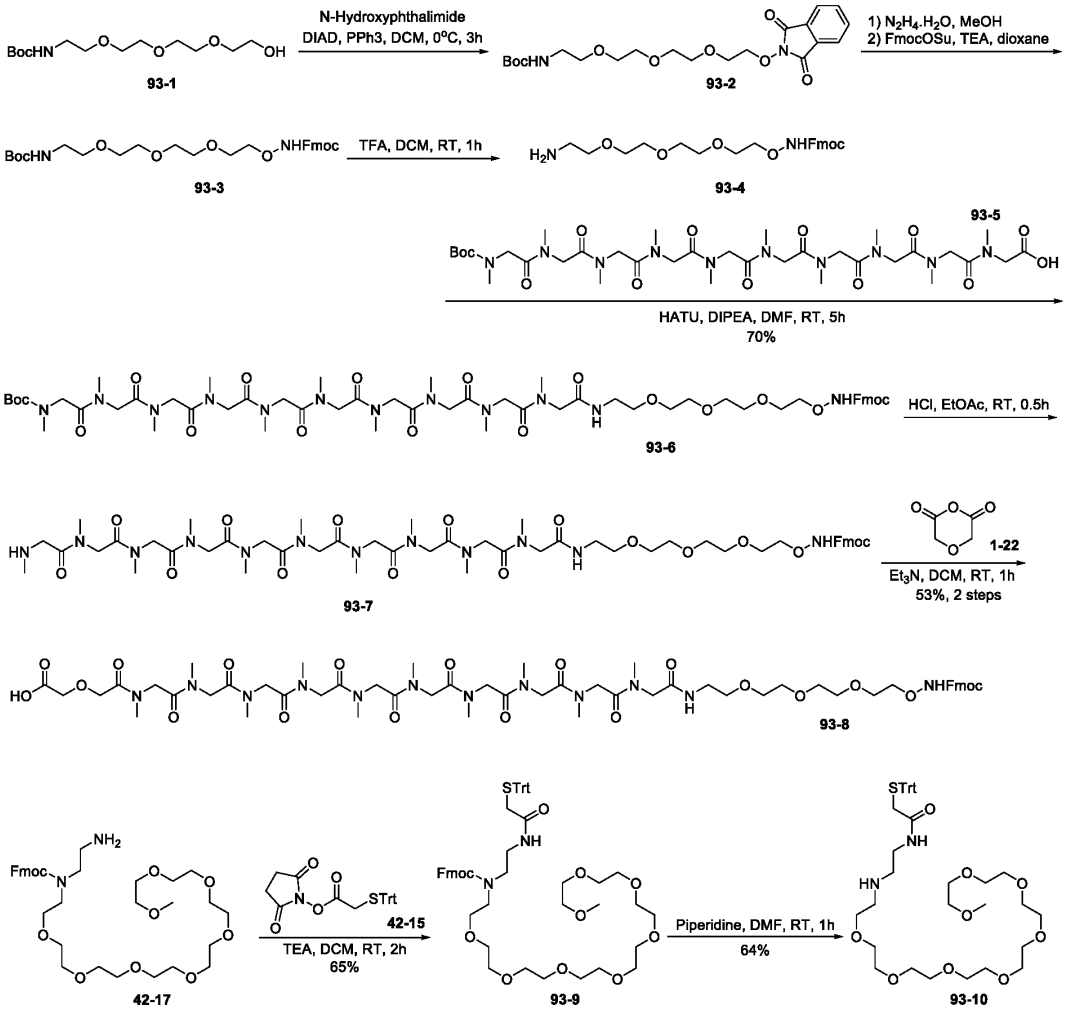
[00935] Step 1: Under nitrogen atmosphere, to a solution of compound **91-1** (30 mg, 0.003 mmol) in anhydrous N, N-dimethylformamide (1 mL) was added N, N-diisopropylethylamine (0.002 mL, 0.006 mmol), PyBOP (65 mg, 0.007 mmol) and compound **79-2** (3.6 mg, 0.004 mmol). The mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was precipitated in cold Methyl tert-Butyl Ether (5 mL) before centrifugation. The left solid was purified by pre-HPLC (C4 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **92** (9.9 mg, yield 30%) as a pale-yellow solid. LCMS: m/z calculated for $C_{472}H_{754}F_3N_{59}O_{165}S_4$: 10080.71; found: 1440.99 $[M+7H]^{7+}$.

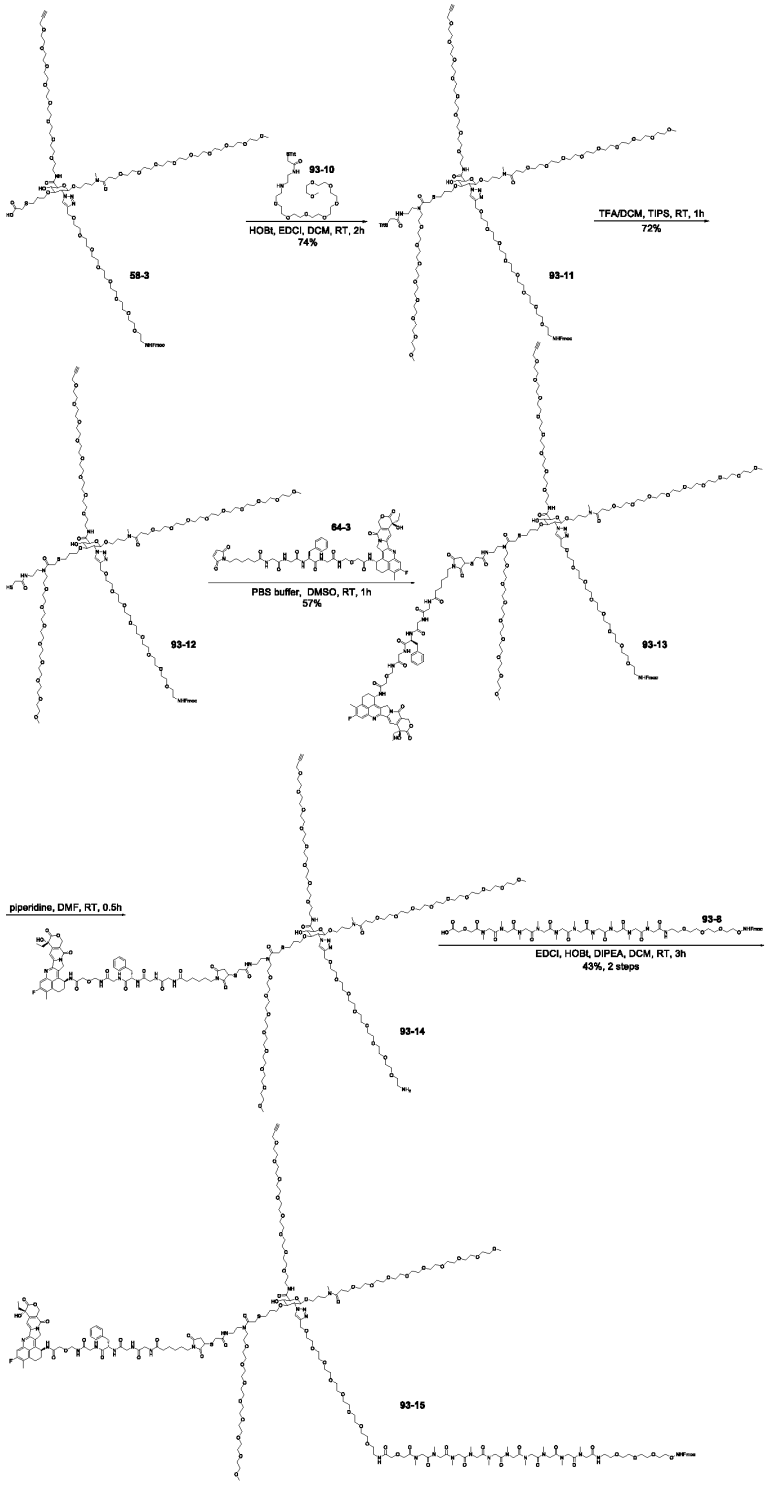
Example 93. Synthesis of 5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[2-[2-[2-[2-(2-aminooxyethoxy)ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-*N*-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-

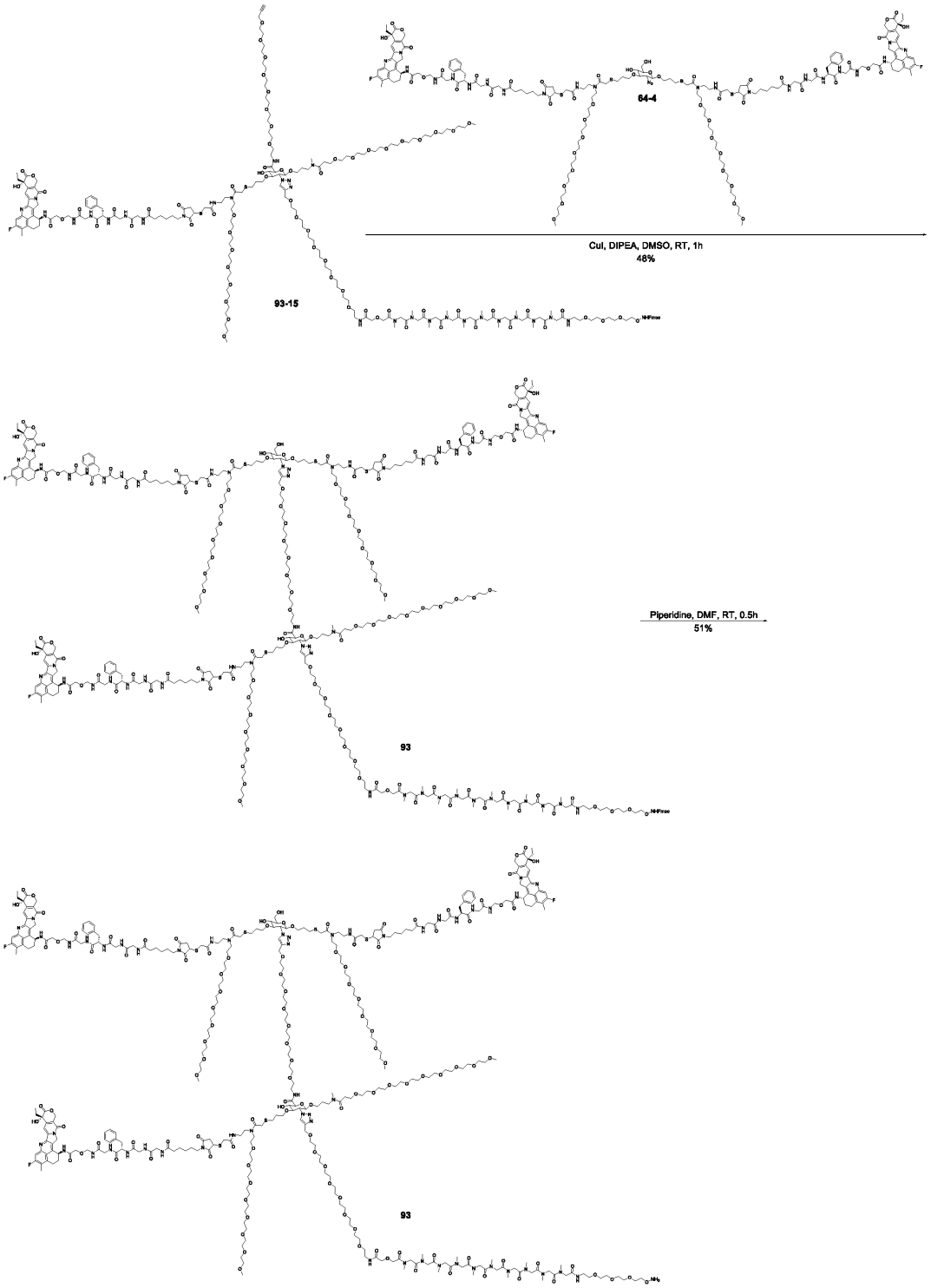
methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carboxamide (93)



Synthetic scheme 93


N-Hydroxyphthalimide
DIAD, PPh3, DCM, 0°C, 3h
1) N2H4.H2O, MeOH
2) FmocOSu, TEA, dioxane
TFA, DCM, RT, 1h
HATU, DIPEA, DMF, RT, 5h
70%
HCl, EtOAc, RT, 0.5h
Et3N, DCM, RT, 1h
53%, 2 steps
TEA, DCM, RT, 2h
65%
Piperidine, DMF, RT, 1h
64%


93-10
HOBt, EDCI, DCM, RT, 2h
74%
58-3
TFA/DCM, TIPS, RT, 1h
72%
93-11
64-3
PBS buffer, DMSO, RT, 1h
57%
93-12
93-13
piperidine, DMF, RT, 0.5h
93-14
93-8
EDCI, HOBt, DIPEA, DCM, RT, 3h
43%, 2 steps
93-15


64-4
CuI, DIPEA, DMSO, RT, 1h
48%
93-15
Piperidine, DMF, RT, 0.5h
51%
93
93

[00936] Step 1: The title compound **93-2** was prepared in analogy to **Example 46**, **Step 1** by using compound **93-1**. The product (1.24 g, yield 45%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{21}H_{30}N_2O_8$: 438.48; found: 339.21 $[(M\text{-}Boc)+H]^+$.

[00937] Step 2: The title compound **93-3** was prepared in analogy to **Example 46**, **Step 3** and **Step 4** by using compound **93-2**. The product (902 mg, yield 60%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{28}H_{38}N_2O_8$: 530.62; found: 431.52 $[(M\text{-}Boc)+H]^+$.

[00938] Step 3: The title compound **93-4** was prepared in analogy to **Example 46**, **Step 2** by using compound **93-3**. The product (850 mg, yield 90%) was obtained as a colorless oil. LCMS: m/z calculated for $C_{23}H_{30}N_2O_8$: 430.50; found: 431.25 $[M+H]^+$.

[00939] Step 4: Under nitrogen atmosphere, to a solution of compound **93-5** (800 mg, 0.96 mmol) in N,N-dimethylformamide (5 mL) was added DIPEA (374mg, 2.89mmol) and HATU (439 mg, 1.15 mmol) at room temperature. The mixture was stirred for 10 min before compound **93-4** (498 mg, 1.15 mmol) was added thereto. the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 62% acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **93-6** (834 mg, yield 70%) as a white solid. LCMS: m/z calculated for $C_{58}H_{88}N_{12}O_{18}$: 1241.41; found: 1241.50 $[M+H]^+$.

[00940] Step 5: To a mixture of compound **93-6** (834 mg, 0.67 mmol) in ethyl acetate (5 mL) was added 4N hydrochloride in ethyl acetate (5 mL), the reaction mixture was stirred at room temperature for 0.5 h. After completion of the reaction, the mixture was diluted with ethyl acetate and concentrated in vacuum. The residue was azeotroped with dichloromethane to afford the title compound **93-7** (849 mg, crude) as a colorless oil. The crude product was directly used into the next step. LCMS: m/z calculated for $C_{58}H_{88}FN_{12}O_{18}$: 1141.29; found: 1141.30 $[M+H]^+$.

[00941] Step 6: Under nitrogen atmosphere, to a mixture of compound **93-7** (849 mg, crude) and triethylamine (260mg, 2.01mmol) in dichloromethane (3 mL) was added compound **1-22** (86 mg, 0.74 mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated in vacuum. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 40% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound

93-8 (451mg, yield 53% over two steps) as a white solid. LCMS: m/z calculated for $C_{57}H_{84}N_{12}O_{20}$: 1257.50; found: 629.42 $[M+2H]^{2+}$, 1257.39 $[M+H]^{+}$.

[00942] Step 7: The title compound **93-9** was prepared in analogy to **Example 21**, **Step 2** by using compound **42-15** and compound **42-17** in dichloromethane. The product (4.5 g, yield 65%) as a yellow oil. LCMS: m/z calculated for $C_{55}H_{68}N_2O_{11}S$: 965.21; found: 723.39 $[(M-Trt)+H]^{+}$.

[00943] Step 8: Under nitrogen atmosphere, to a solution of compound **93-9** (0.82 g, 0.85 mmol) in N,N-dimethylformamide (0.22 g, 2.54 mmol) was added piperidine (0.22g, 2.54mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the crude product was purified by reverse phase flash chromatography (C18 column, eluted with 48% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **93-10** (0.41 g, yield 64%) as a yellow oil. LCMS: m/z calculated for $C_{40}H_{58}N_2O_9S$: 742.97; found: 501.33 $[(M-Trt)+H]^{+}$.

[00944] Step 9: Under nitrogen atmosphere, to a solution of compound **58-3** (0.72 g, 0.38 mmol) and **93-10** (0.34 g, 0.46 mmol) in dichloromethane (10 mL) was sequentially added DIPEA (0.12g, 0.095mmol), HOBt (0.05g, 0.38mmol) and EDCI (0.13g, 0.68mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated in vacuum. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 46% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **93-11** (0.72 g, yield 74%) as a yellow oil. LCMS: m/z calculated for $C_{128}H_{202}N_8O_{43}S_2$: 2605.16; found: 1303.16 $[M+2H]^{2+}$.

[00945] Step 10: To a mixture of compound **93-11** (0.72 g, 0.27 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.25 mL) and triisopropylsilane (0.28 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated in vacuum. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 46% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **93-12** (0.72 g, yield 72%) as a yellow oil. LCMS: m/z calculated for $C_{109}H_{188}N_8O_{43}S_2$: 2362.84; found: 1182.12 $[M+2H]^{2+}$.

[00946] Step 11: Under nitrogen atmosphere, to a mixture of compound **93-12** (0.49 g, 0.21 mmol) in PBS buffer (1 mL) and DMSO (2 mL) was added a solution of compound **64-3** in DMSO solution (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction. The reaction was filtered and purified by reverse phase flash

chromatography (C18 column, eluted with 58% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **93-13** (0.40 g, yield 57%) as a yellow solid. LCMS: m/z calculated for $C_{163}H_{246}F_2NO_{56}S_2$: 3394.93; found: 1133.16 $[M+3H]^{3+}$.

[00947] Step 12: To a solution of compound **93-13** (0.40 g, 0.12 mmol) in N,N-dimethylformamide (5 mL) was added piperidine (20 mg, 0.23 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was precipitated in methyl tert-butyl ether before centrifugation to afford the title compound **93-14** (0.35 g, crude) as a yellow solid. The crude product is directly used into the next step. LCMS: m/z calculated for $C_{146}H_{234}FN_{17}O_{54}S_2$: 3174.66; found: 1058.96 $[M+3H]^{3+}$.

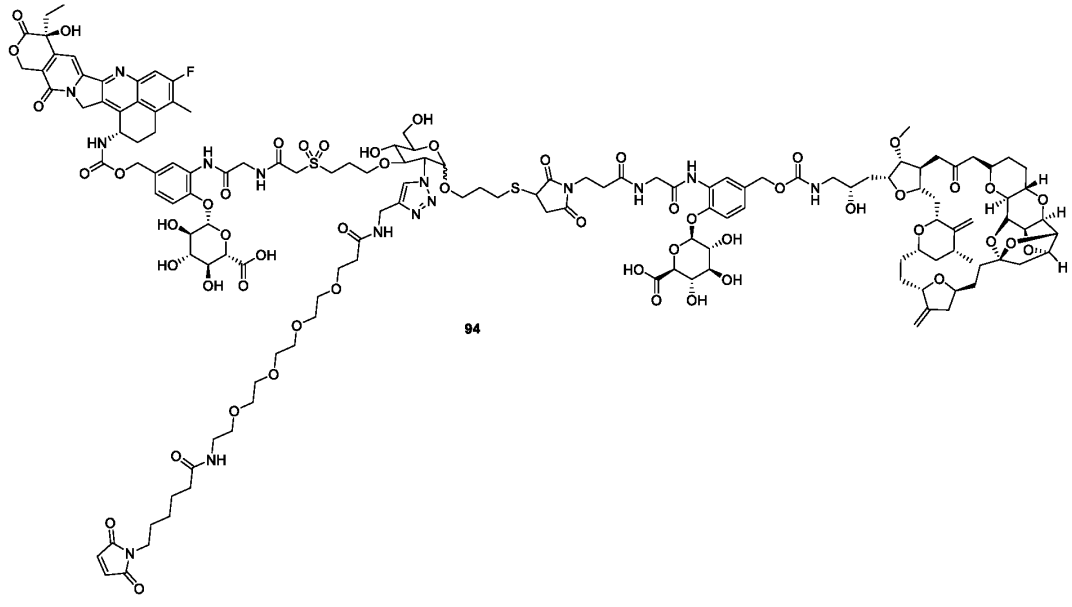
[00948] Step 13: Under nitrogen atmosphere, to a mixture of compound **93-14** (350 mg, crude) and **93-8** (148 mg, 0.12 mmol) in dichloromethane (5 mL) was sequentially added DIPEA (40 mg, 0.29 mmol), HOBt (17 mg, 0.13 mmol) and EDCI (41mg, 0.21mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated. The crude product was purified by pre-HPLC (C4 column, eluted with 48% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **93-15** (225mg, yield 43% over two steps) as a yellow oil LCMS: m/z calculated for $C_{203}H_{316}FN_{29}O_{73}S_2$: 4414.04; found: 1104.37 $[M+4H]^{4+}$.

[00949] Step 14: Under nitrogen atmosphere, a solution of compound **93-15** (206 mg, 0.05 mmol) and compound **64-4** (115 mg, 0.03 mmol) in dimethyl sulfoxide (3 mL) was added ethyldiisopropylamine (45mg, 0.35mmol) and CuI (27 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 48% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **93-16** (125 mg, yield 50%) as a white solid. LCMS: m/z calculated for $C_{365}H_{539}F_3N_{54}O_{124}S_6$: 7916.94; found: 1320.36 $[M+6H]^{6+}$.

[00950] Step 15: To a mixture of compound **94-16** (125 mg, 0.016 mmol) in N,N-dimethylformamide (2 mL) was added piperidine (3 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 0.5 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 46% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **93** (60 mg,

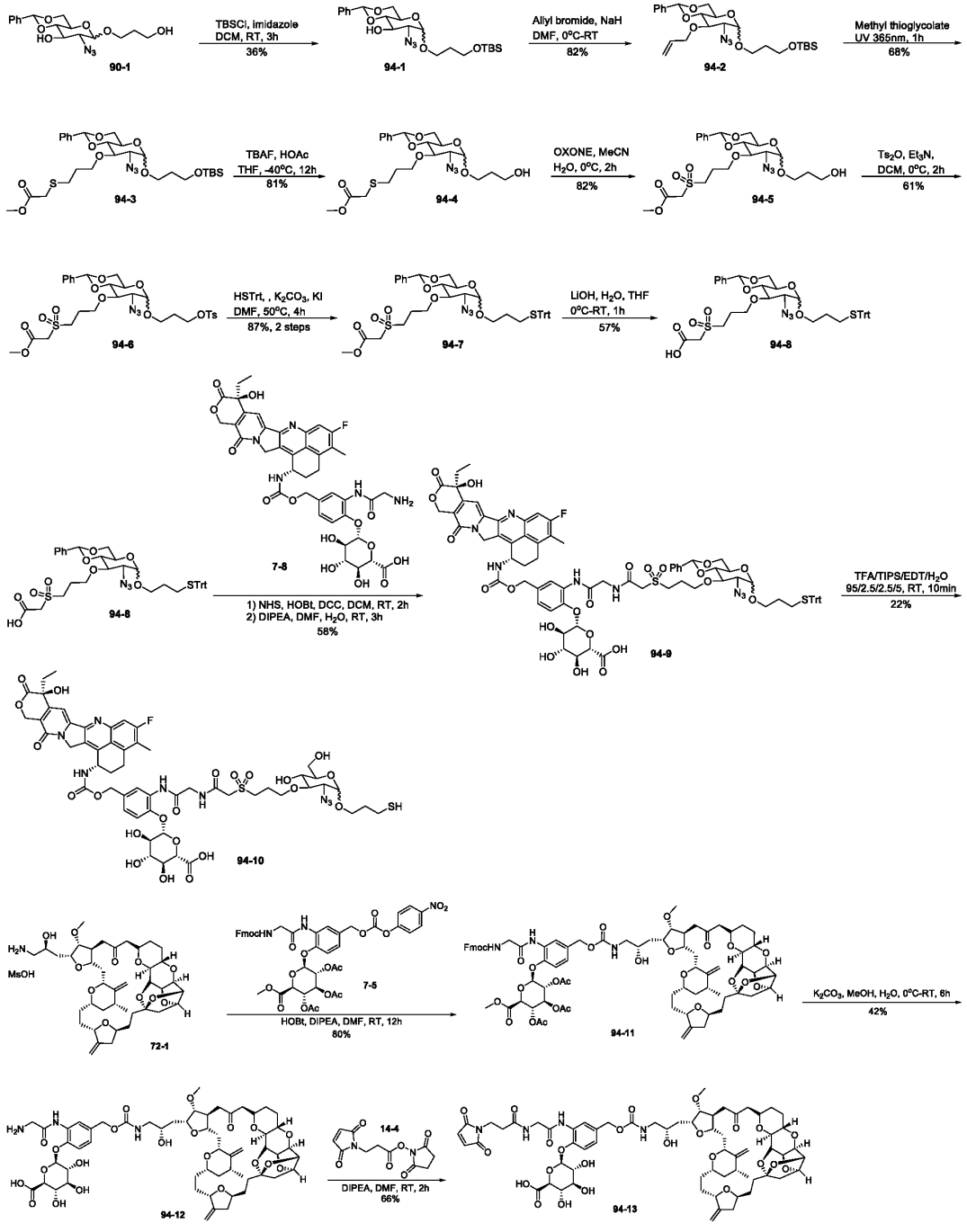
yield 50%) as a yellow oil LCMS: m/z calculated for $C_{350}H_{529}F_3N_{54}O_{124}S_6$: 7694.19; found: 1283.23 $[M+6H]^{6+}$.

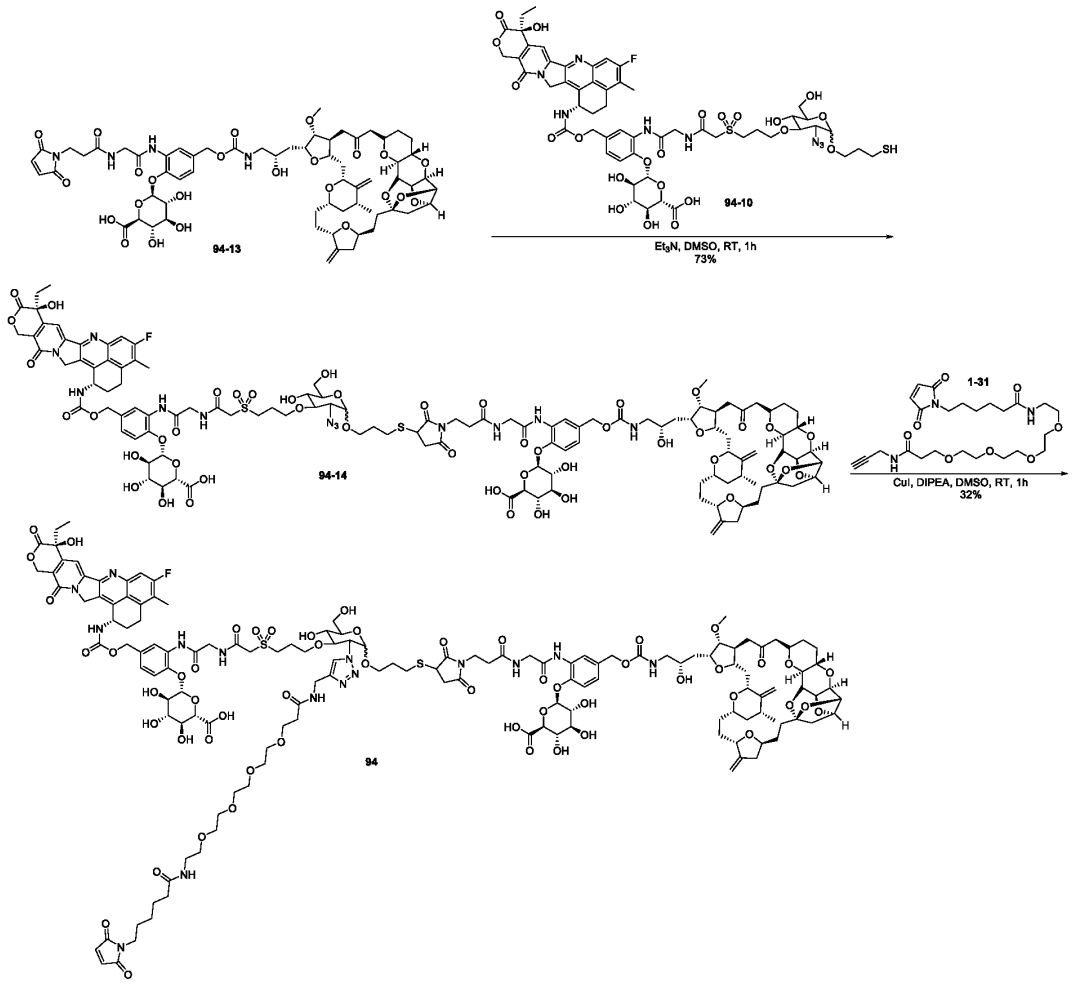
Example 94. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[3-[3-[4-[3-[2-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropylsulfanyl]-2,5-dioxo-pyrrolidin-1-yl]propanoylamino]acetyl]amino]-4-[[(2*S*)-2-hydroxy-3-[(3*S*,6*S*,9*S*,12*S*,14*R*,16*R*,20*R*,21*R*,22*S*,29*S*,31*R*,32*S*,33*R*,35*R*,36*S*)-21-methoxy-14-methyl-8,15-dimethylene-24-oxo-2,19,30,34,37,39,40,41-octaoxanonacyclo[24.9.2.$1^{3,32}$.$1^{3,33}$.$1^{6,9}$.$1^{12,16}$.$0^{18,22}$.$0^{29,36}$.$0^{31,35}$]hentetracontan-20-yl]propyl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (94)



94

Synthetic scheme 94





[00951] Step 1: The title compound **94-1** (racemic) was prepared in analogy to **Example 90**, **Step 2** by using compound **90-1**. The chirality of compound **94-1** was not separated, and the racemic mixture was used directly in the later steps.

[00952] Step 2: The title compound **94-2** (racemic) was prepared in analogy to **Example 1**, **Step 4** by using compound **94-1**. The chirality of compound **94-2** was not separated, and the racemic mixture was used directly in the later steps.

[00953] Step 3: The title compound **94-3** (racemic) was prepared in analogy to **Example 1**, **Step 5** by using compound **94-2**. The chirality of compound **94-3** was not separated, and the racemic mixture was used directly in the later steps.

[00954] Step 4: The title compound **94-4** (racemic) was prepared in analogy to **Example 10**, **Step 5** by using compound **94-3**. The chirality of compound **94-4** was not separated, and the racemic mixture was used directly in the later steps.

[00955] Step 5: The title compound **94-5** (racemic) was prepared in analogy to **Example 57**, **Step 1** by using compound **94-4**. The chirality of compound **94-5** was not separated, and the racemic mixture was used directly in the later steps.

[00956] Step 6: The title compound **94-6** (racemic) was prepared in analogy to **Example 90**, **Step 7** by using compound **94-5**. The chirality of compound **94-6** was not separated, and the racemic mixture was used directly in the later steps.

[00957] Step 7: The title compound **94-7** (racemic) was prepared in analogy to **Example 10**, **Step 7** by using compound **94-6**. The chirality of compound **94-7** was not separated, and the racemic mixture was used directly in the later steps.

[00958] Step 8: The title compound **94-8** (racemic) was prepared in analogy to **Example 1**, **Step 6** by using compound **94-7**. The chirality of compound **94-8** was not separated, and the racemic mixture was used directly in the later steps.

[00959] Step 9: The title compound **94-9** (racemic) was prepared in analogy to **Example 11**, **Step 1 and Step 2** by using compound **7-8** and compound **94-8**. The chirality of compound **94-9** was not separated, and the racemic mixture was used directly in the later steps.

[00960] Step 10: The title compound **94-10** (racemic) was prepared in analogy to **Example 7**, **Step 9** by using compound **94-9**. The chirality of compound **94-10** was not separated, and the racemic mixture was used directly in the later steps.

[00961] Step 11: To a solution of compound **72-1** (100 mg, 0.121 mmol) and compound **7-5** (130 mg, 0.144 mmol) in anhydrous DMF (3 mL) was added DIPEA (47 mg, 0.364 mmol) and HOBt (16 mg, 0.118 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was precipitated in MTBE before centrifugation. The left white crude products were dried in vacuum to afford the title compound **94-11** (145 mg, yield 80%) as a white solid. LCMS: m/z calculated for $C_{78}H_{95}N_3O_{26}$: 1490.61; found: 1490.42 $[M+H]^+$.

[00962] Step 12: The title compound **94-12** was prepared in analogy to **Example 14**, **Step 2** by using potassium carbonate and compound **94-11** in methanol and H_2O. The product (52 mg,

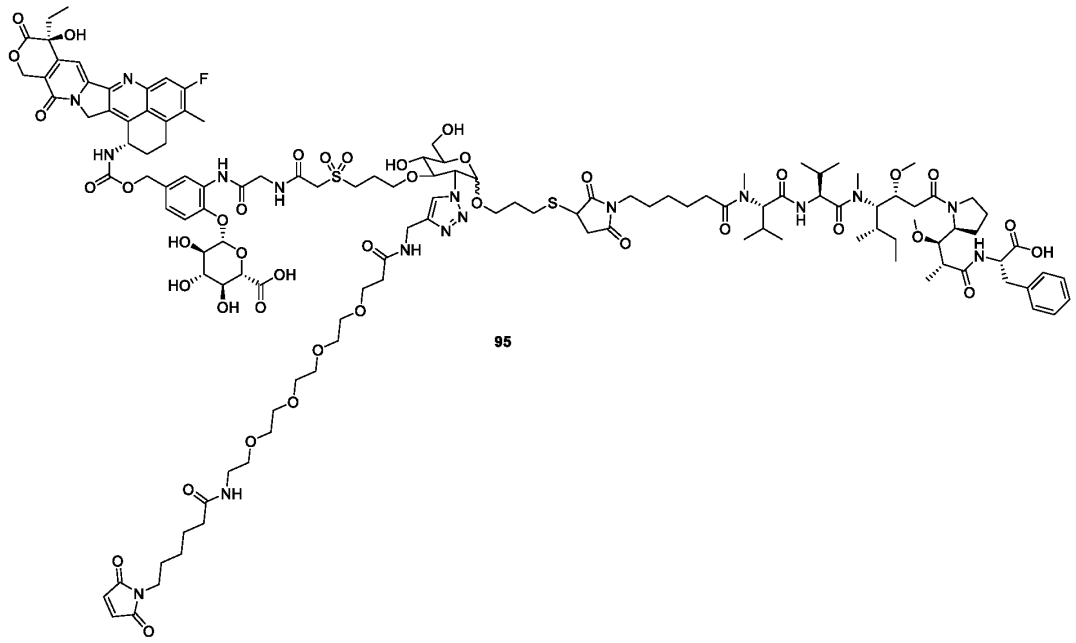
yield 63%) was obtained as a white solid. LCMS: m/z calculated for $C_{56}H_{77}N_3O_{21}$: 1128.23; found: 1129.42 $[M+H]^+$.

[00963] Step 13: The title compound **94-13** was prepared in analogy to **Example 14**, **Step 3** by using compound **14-4** and compound **94-12**. The product (37 mg, yield 66%) was obtained as a white solid. LCMS: m/z calculated for $C_{63}H_{82}N_4O_{24}$: 1279.35; found: 1279.27 $[M+H]^+$.

[00964] Step 14: The title compound **94-14** was prepared in analogy to **Example 10**, **Step 10** by using triethylamine, compound **94-10** and compound **94-13** in dimethyl sulfoxide. The product (30 mg, yield 73%) was obtained as a white solid. LCMS: m/z calculated for $C_{117}H_{145}FN_{12}O_{46}S_2$: 2538.60; found: 1270.33 $[M+2H]^{2+}$.

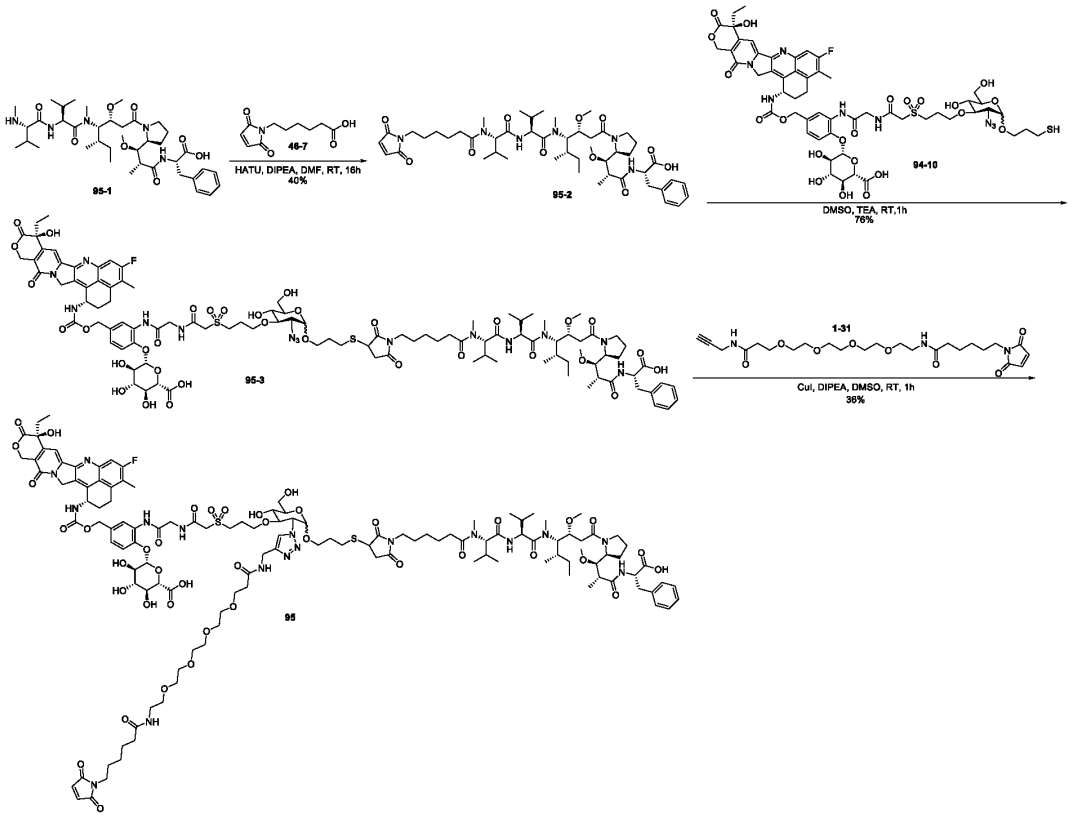
[00965] Step 15: The title compound **94** was prepared in analogy to **Example 10**, **Step 14** by using N,N-diisopropylethylamine, cuprous iodide, compound **1-31** and compound **94-14** in dimethyl sulfoxide. The product (12 mg, yield 32%) was obtained as a white solid. LCMS: m/z calculated for $C_{141}H_{182}FN_{15}O_{54}S_2$: 3034.18; found: 1517.59 $[M+2H]^{2+}$.

Example 95. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*S*)-1-carboxy-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (95)



95

Synthetic scheme 95



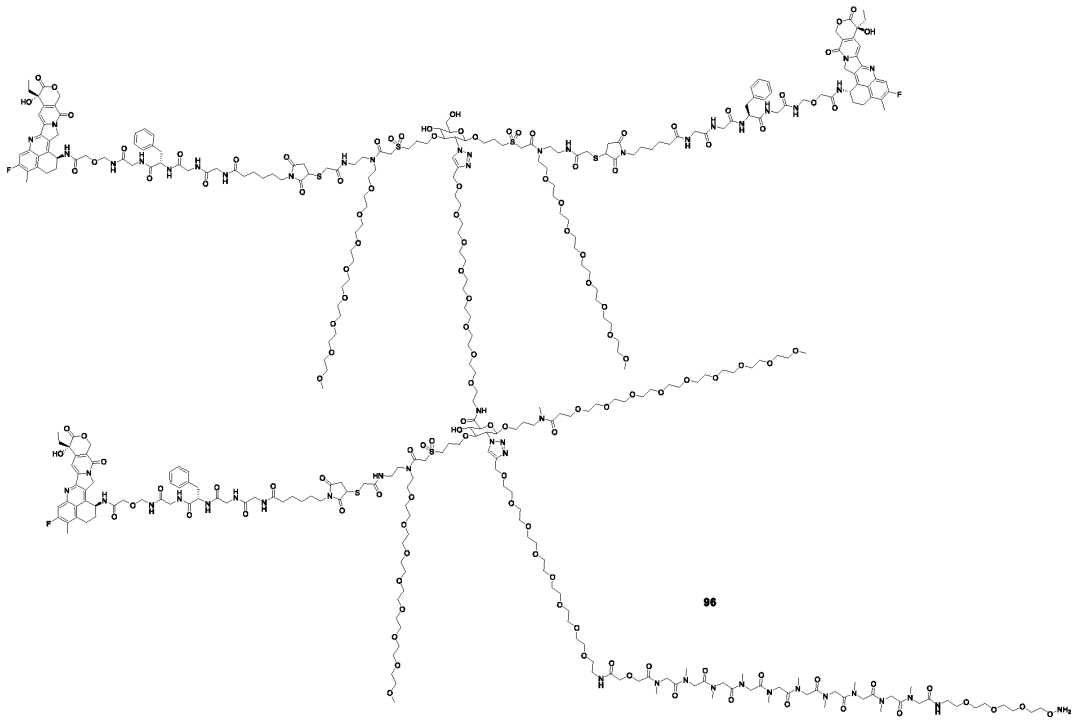
[00966] Step 1: The title compound **95-2** was prepared in analogy to **Example 70**, **Step 8** by using compound **95-1** and compound **46-7**. The product (2.54 g, yield 40%) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{49}H_{76}N_6O_{11}$: 925.18; found: 926.49 $[M+H]^+$.

[00967] Step 2: The title compound **95-3** was prepared in analogy to **Example 10**, **Step 10** by using triethylamine, compound **94-10** and compound **95-2** in dimethyl sulfoxide. The product (29 mg, yield 76%) was obtained as a white solid. LCMS: m/z calculated for $C_{103}H_{139}FN_{14}O_{33}S_2$: 2184.43; found: 1092.85 $[M+2H]^{2+}$.

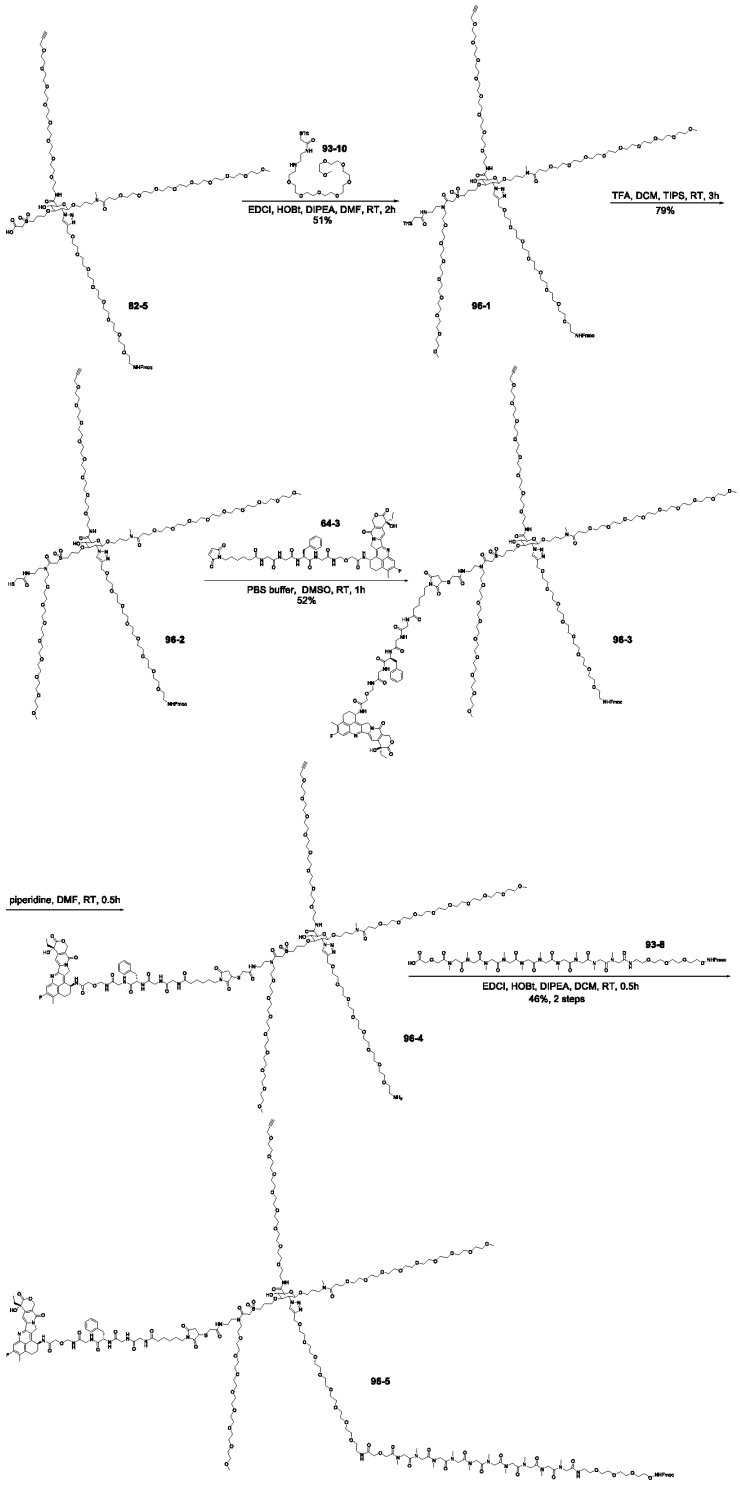
[00968] Step 3: The title compound **95** was prepared in analogy to **Example 7**, **Step 10** by using N,N-diisopropylethylamine, cuprous iodide, compound **1-31** and compound **95-3** in dimethyl sulfoxide. The product (13 mg, yield 36%) was obtained as a white solid. LCMS: m/z calculated for $C_{127}H_{176}FN_{17}O_{41}S_2$: 2680.00; found: 1340.26 $[M+2H]^{2+}$.

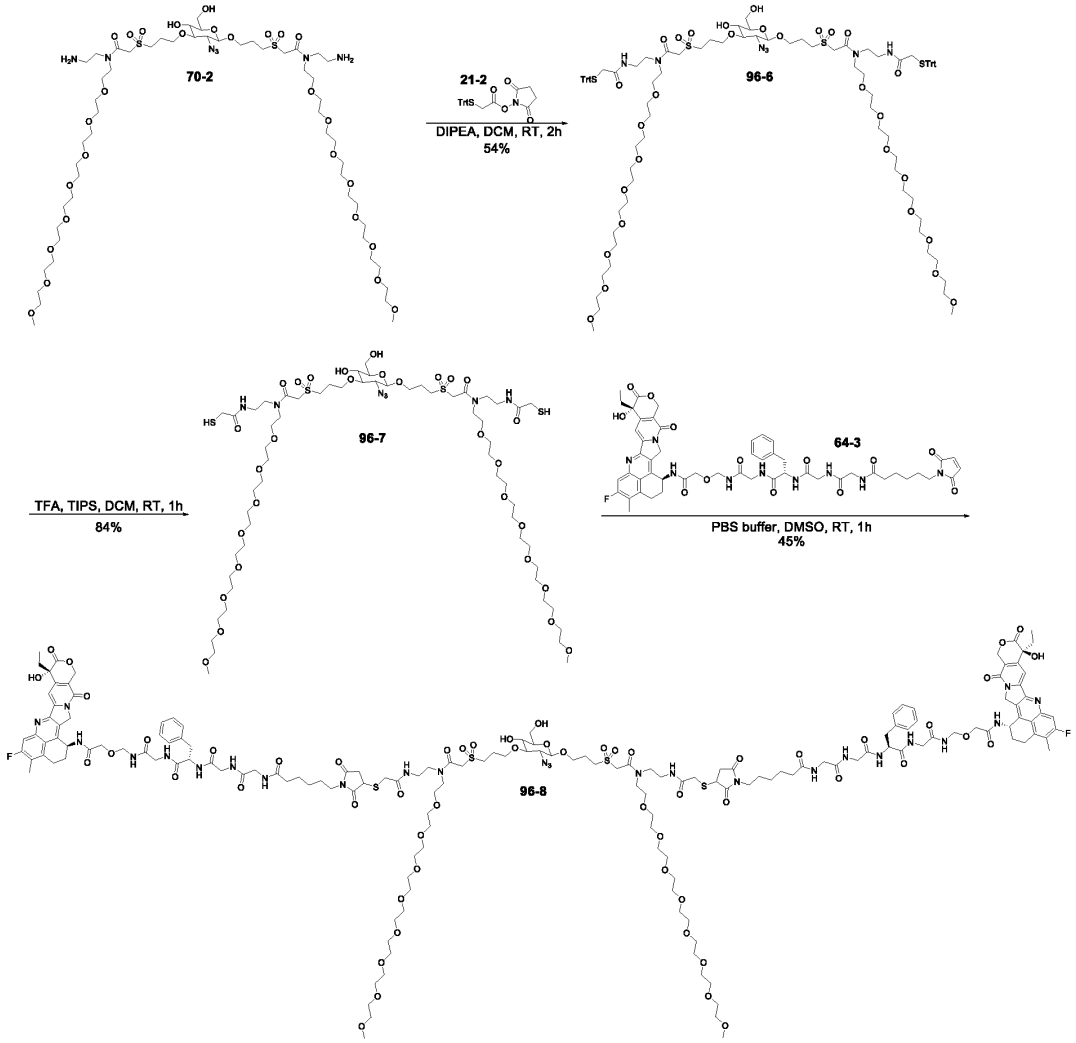
Example 96. Synthesis of 5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[2-[2-[2-(2-aminooxyethoxy)ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]-methyl-amino]-2-

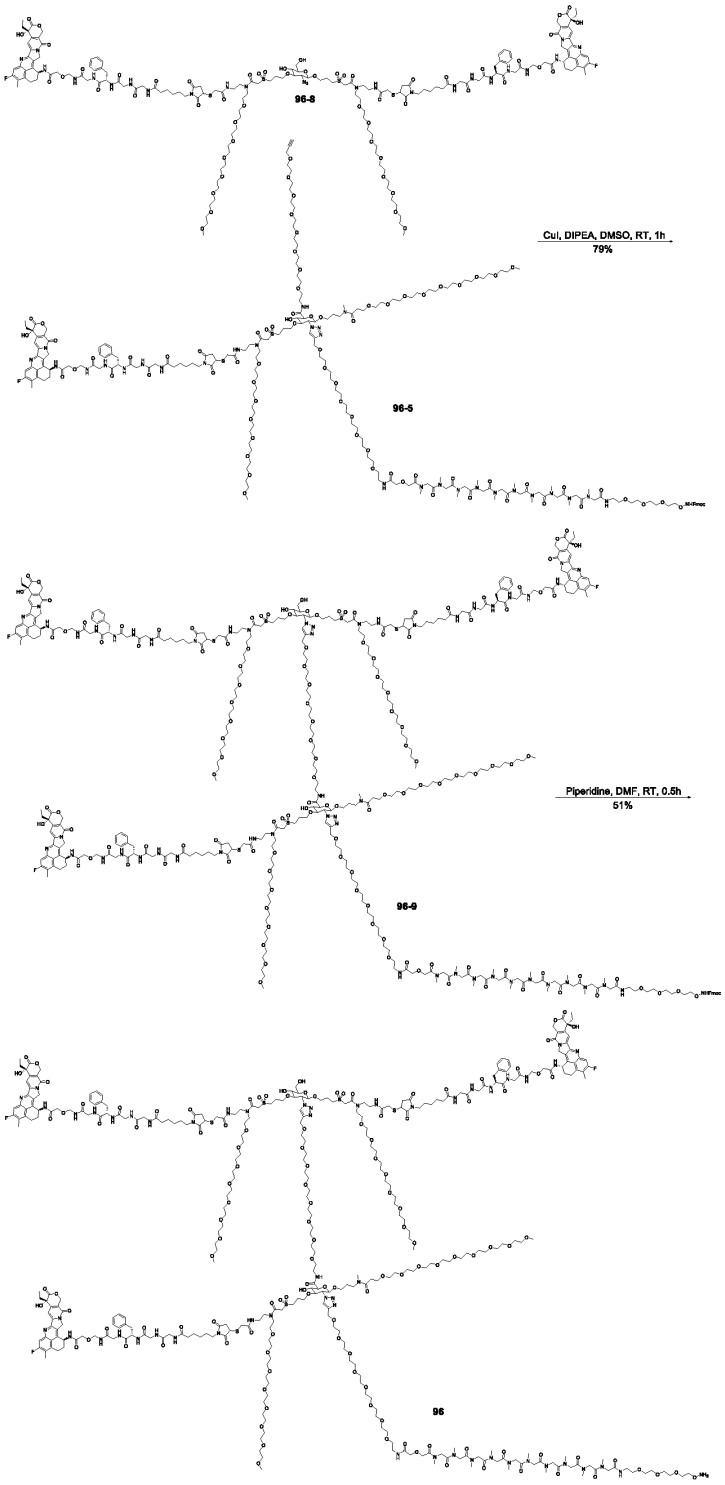
oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-*N*-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-hydroxy-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]tetrahydropyran-2-carboxamide (96)



Synthetic scheme 96


93-10
EDCI, HOBt, DIPEA, DMF, RT, 2h
51%
82-5
96-1
TFA, DCM, TIPS, RT, 3h
79%
64-3
PBS buffer, DMSO, RT, 1h
52%
96-2
96-3
piperidine, DMF, RT, 0.5h
93-8
EDCI, HOBt, DIPEA, DCM, RT, 0.5h
46%, 2 steps
96-4
96-5


70-2
21-2
DIPEA, DCM, RT, 2h
54%
96-6
96-7
TFA, TIPS, DCM, RT, 1h
84%
64-3
PBS buffer, DMSO, RT, 1h
45%
96-8


96-8
CuI, DIPEA, DMSO, RT, 1h
79%
96-5
Piperidine, DMF, RT, 0.5h
51%
96-9
96

[00969] Step 1: Under nitrogen atmosphere, compounds **82-5** (0.66 mg, 0.34 mmol) and **93-10** (0.31g, 0.41 mmol) were dissolved in dichloromethane (10 mL), followed by sequential addition of DIPEA (0.11g, 0.86mmol), HOBt (0.05g, 0.34mmol) and EDCI (0.18g, 0.61mmol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated in vacuum. The crude product was purified by reverse phase flash chromatography (C18 column, eluted with 68% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **96-1** (0.47 g, yield 51%) as a yellow oil. LCMS: m/z calculated for $C_{128}H_{202}N_8O_{45}S_2$: 2637.15; found: 1198.03 [(M-Trt)+2H]$^{2+}$.

[00970] Step 2: To a mixture of compound **96-1** (465mg, 0.17 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.25 mL), triisopropylsilane (0.36 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated in vacuum. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **96-2** (334 mg, yield 79%) as a yellow oil LCMS: m/z calculated for $C_{109}H_{188}N_8O_{45}S_2$: 2394.83; found: 1197.78 [M+2H]$^{2+}$.

[00971] Step 3: Under nitrogen atmosphere, to a mixture of compound **96-2** (334 mg, 0.14 mmol) in PBS buffer (1 mL) and DMSO (2 mL) was added a solution of compound **64-3** (144 mg, 0.14 mmol) in DMSO (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, after completion of the reaction. The reaction was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96-3** (248 mg, yield 52%) as a yellow solid LCMS: m/z calculated for $C_{161}H_{244}FN17O_{58}S_2$: 3428.90; found: 1143.79 [M+3H]$^{3+}$.

[00972] Step 4: To a mixture of compound **96-3** (0.24g, 0.07 mmol) in N,N-dimethylformamide (1 mL) was added piperidine (12 mg, 0.14 mol). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was precipitated in methyl tert-butyl ether before centrifugation. The residue was dried in vacuum to afford the title compound **96-4** (0.42 g, crude) as a yellow solid. The crude product is directly used into the next step. LCMS: m/z calculated for $C_{146}H_{234}FN_{17}O_{56}S_2$: 3206.66; found: 1069.59 [M+3H]$^{3+}$.

[00973] Step 5: Under nitrogen atmosphere, compounds **93-4** (420 mg, crude) and **93-8** (91 mg, 0.07 mmol) were dissolved in dichloromethane (5 mL), followed by sequentially addition of DIPEA (20 mg, 0.15 mmol), HOBt (10mg, 0.07mmol) and EDCI (25 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was concentrated in vacuum. The residue was purified by reverse phase flash chromatography (C4 column, eluted with 42% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96-5** (148 mg, yield 46% over two steps) as a yellow oil. LCMS: m/z calculated for $C_{203}H_{316}FN_{29}O_{73}S_2$: 4446.01; found: 1112.24 $[M+4H]^{4+}$.

[00974] Step 6: Under nitrogen atmosphere, to a mixture of compound **70-2** (2.23 g, 1.65 mmol) and compound **21-2** (1.56 g, 3.61 mmol) in dichloromethane (15 mL) was added DIPEA (1.07 g, 8.27 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was poured into water (30 mL) and extracted with dichloromethane (30 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 10% dichloromethane in methanol) to afford the title compound **96-6** (1.76 g, yield 54%) as a yellow oil. LCMS: m/z calculated for $C_{96}H_{139}N_7O_{29}S_4$: 1983.43; found: 1498.52 $[(M\text{-}Dxd*2)+H]^+$.

[00975] Step 7: To a solution of compound **96-6** (1.76 g, 0.89 mmol) in dichloromethane (9 mL) was added TFA (1 mL) and triisopropylsilane (1.78 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane and concentrated in vacuum. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 58% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96-7** (1.12g, yield 84%) as a yellow oil LCMS: m/z calculated for $C_{58}H_{111}N_7O_{25}S_4$: 1498.79; found: 1498.45 $[M+H]^+$.

[00976] Step 8: Under nitrogen atmosphere, to a mixture of compound **96-7** (0.15 g, 0.10 mmol) in PBS buffer (1 mL) and DMSO (2 mL) was added a solution of compound **64-3** (0.19 g, 0.18 mmol) in DMSO solution (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 43% acetonitrile in water,

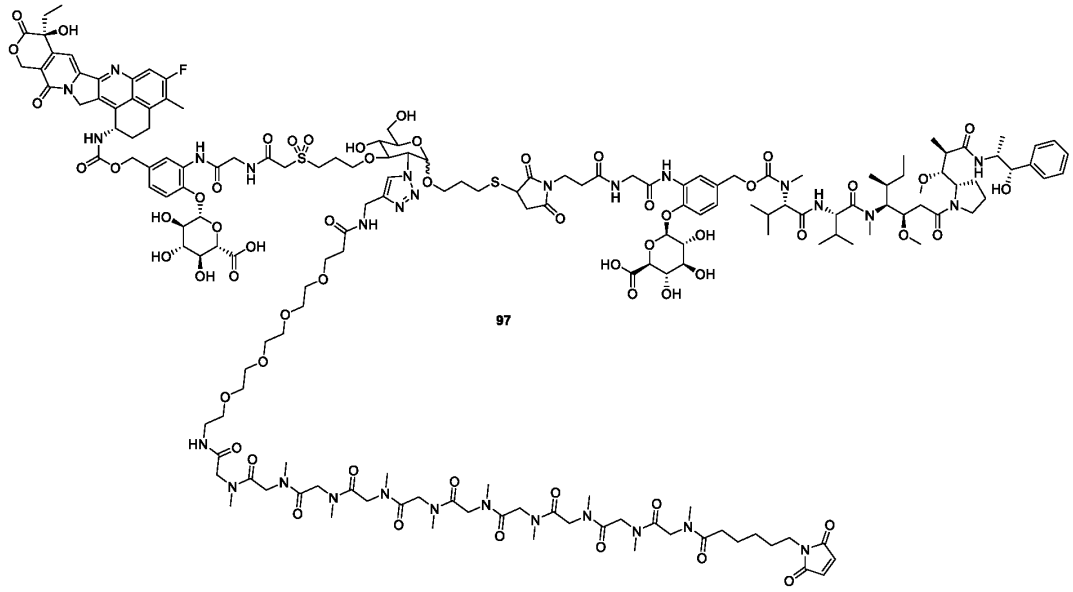
0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96-8** (0.16 g, yield 45%) as a white solid LCMS: m/z calculated for $C_{162}H_{223}F_2N_{25}O_{55}S_4$: 3566.92; found: 1189.53 $[M+3H]^{3+}$.

[00977] Step 9: Under nitrogen atmosphere, to a mixture of compounds **96-5** (40 mg, 0.01 mmol) and **96-8** (28 mg, 0.01 mmol) in dimethyl sulfoxide (3 mL) was added 1M ascorbic acid aqueous solution (0.07 mL, 0.09 mmol) and 1M $CuSO_4$ aqueous solution (0.03 mL, 0.03 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 47% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96-9** (50 mg, yield 79%) as a white solid. LCMS: m/z calculated for $C_{365}H_{539}F_3N_{54}O_{130}S_6$: 8012.93; found: 1504.63 $[M+6H]^{6+}$.

[00978] Step 10: To a mixture of compound **96-9** (50 mg, 0.006 mmol) in N,N-dimethylformamide (2 mL) was added piperidine (1 mg, 0.012 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 45% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **96** (24 mg, yield 50%) as a yellow oil LCMS: m/z calculated for $C_{350}H_{529}F_3N_{54}O_{128}S_6$: 7790.69; found: 1299.37 $[M+6H]^{6+}$.

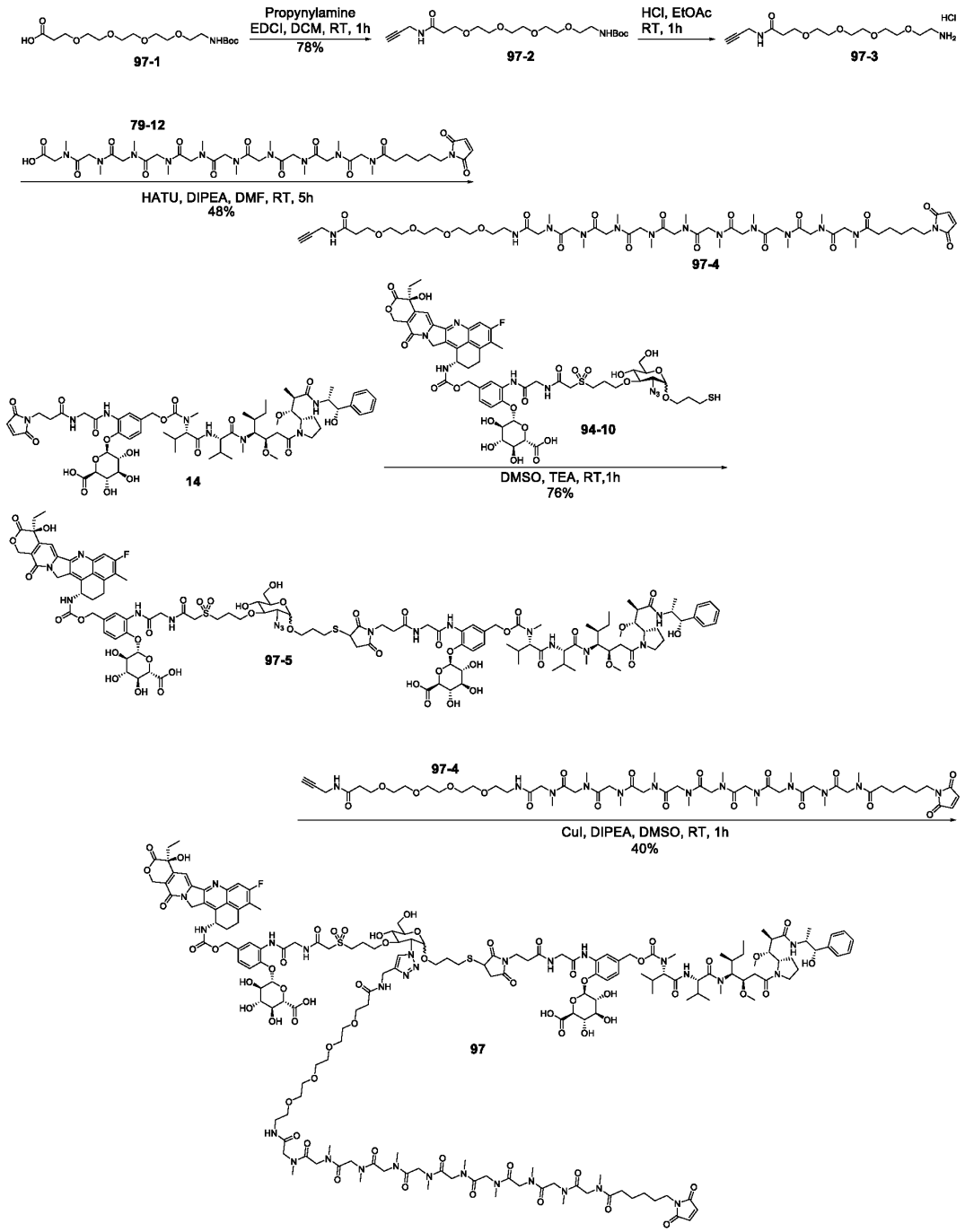
Example 97. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]-3-[4-[[3-[2-[2-[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[6-(2,5-dioxopyrrol-1-yl)hexanoyl-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]-methyl-amino]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-

yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (97)



97

Synthetic scheme 97



[00979] Step 1: To a solution of compound **97-1** (2.00 g, 5.48 mmol) in anhydrous dichloromethane (20 mL) was added 2-propargylamine (450 mg, 8.17 mmol) and EDCI (1.58 g,

8.24 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was diluted with dichloromethane (100 mL) and washed with water (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column (eluted with 4% methanol in dichloromethane) to afford the title compound **97-2** (1.72 g, yield 78%) as a colorless oil.

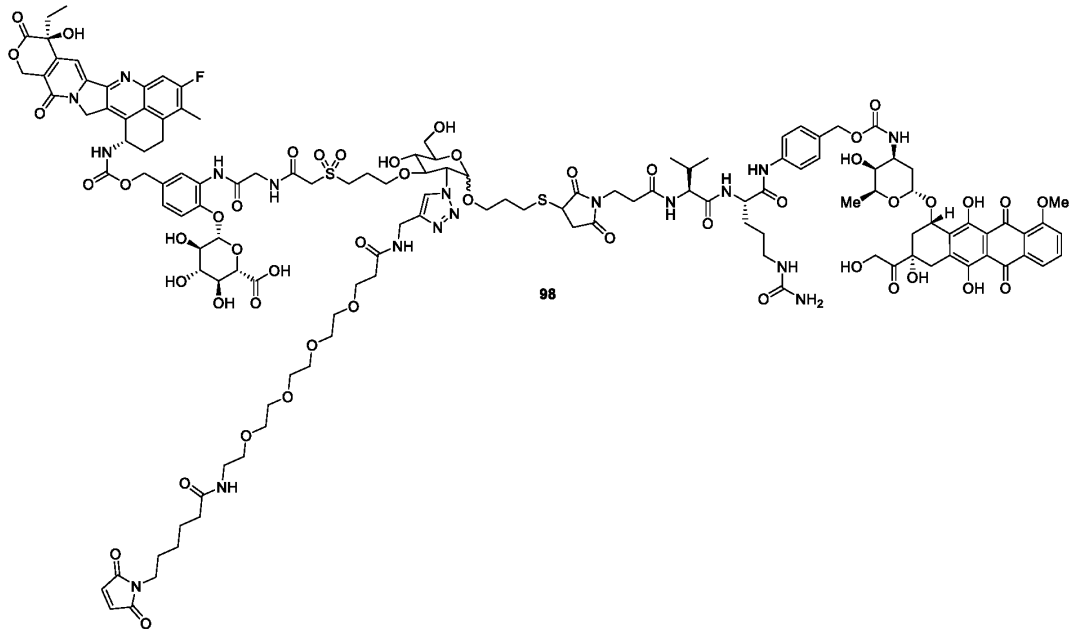
[00980] Step 2: To a solution of compound **97-2** (1.10 g, 2.73 mmol) in ethyl acetate (12 mL) was added 2 N HCl/ethyl acetate (6 mL). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure to give the title compound **97-3** (1.30 g) as a pale-yellow oil. The crude product was used into the next step without further purification.

[00981] Step 3: Under nitrogen atmosphere, to a solution of compound **79-12** (142 mg, 0.154 mmol) in N, N-dimethylformamide (2.5 mL) was added N, N-diisopropylethylamine (0.080 mL, 0.462 mmol) and HATU (88 mg, 0.231 mmol). The mixture was stirred at room temperature for 5 min before a solution of compound **97-3** (80 mg, 0.231 mmol) in anhydrous N, N-dimethylformamide added thereto. The reaction mixture was stirred at room temperature for 1 hour. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to afford the title compound **97-4** (90 mg, yield 48%) as a colorless oil. LCMS: m/z calculated for $C_{54}H_{87}N_{13}O_{18}$: 1206.36; found: 1206.42 $[M+H]^+$.

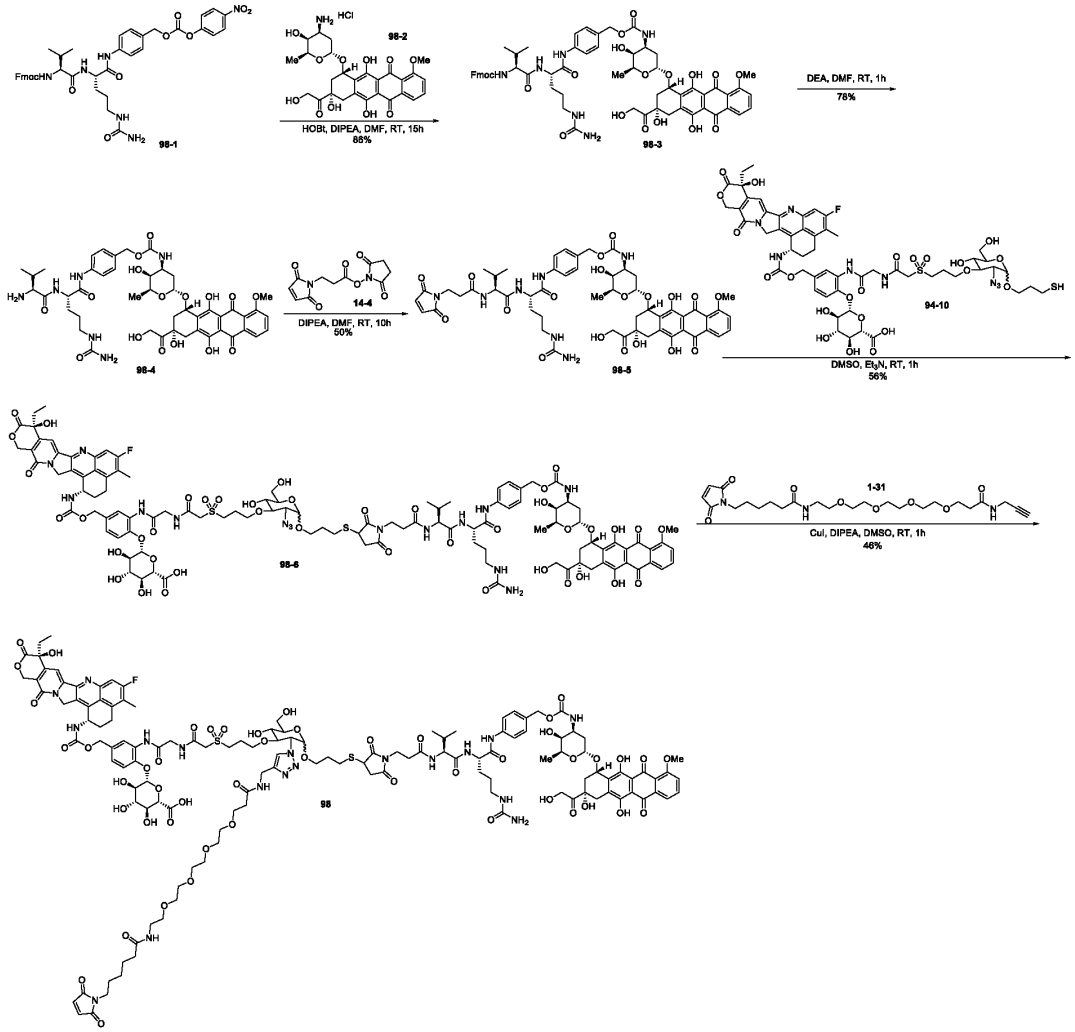
[00982] Step 4: The title compound **97-5** (racemic) was prepared in analogy to **Example 10**, **Step 10** by using compound **14** and compound **97-4**. The chirality of compound **94-5** was not separated, and the racemic mixture was used directly in the later steps.

[00983] Step 5: Under nitrogen atmosphere, to a solution of **97-5** (20 mg, 0.008 mmol) and **97-4** (11 mg, 0.009 mmol) in dimethyl sulfoxide (0.5 mL) was added N,N-diisopropylethylamine (4.1 mg, 0.032 mmol) and cuprous iodide (1.6 mg, 0.008 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 44% acetonitrile in water, AcOH condition). The desired components were lyophilized to afford the title compound **97** (13 mg, yield 40%) as a white solid. LCMS: m/z calculated for $C_{170}H_{240}FN_{29}O_{60}S_2$: 3733.05; found: 1244.86 $[M+3H]^{3+}$.

Example 98. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[5-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-3-hydroxy-2-(hydroxymethyl)-6-[3-[1-[3-[[(1*S*)-1-[[(1*S*)-1-[[4-[[(2*S*,3*S*,4*S*,6*R*)-3-hydroxy-2-methyl-6-[[(1*S*,3*S*)-3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-2,4-dihydro-1*H*-tetracen-1-yl]oxy]tetrahydropyran-4-yl]carbamoyloxymethyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylpropoxy]tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (98)



Synthetic scheme 98



[00984] Step 1: The title compound **98-3** was prepared in analogy to **Example 7**, **Step 4** by using compound **98-1** and compound **98-2**. The reaction mixture was precipitated in MTBE before centrifugation. The left residue was triturated in dichloromethane and ethyl acetate to afford the title compound **98-3** (15.36 g, yield 86%) as a red solid. LCMS: m/z calculated for $C_{61}H_{66}N_6O_{18}$: 1171.22; found: 1172.30 $[M+H]^+$.

[00985] Step 2: The title compound **98-4** was prepared in analogy to **Example 7**, **Step 6** by using compound **98-3** with diethylamine instead of piperidine. The reaction mixture was precipitated in MTBE before centrifugation. The left residue was triturated in ethyl acetate to

afford the title compound **98-4** (3.16 g, yield 78%) as a red solid. LCMS: m/z calculated for $C_{46}H_{56}N_6O_{16}$: 948.98; found: 949.95 $[M+H]^+$.

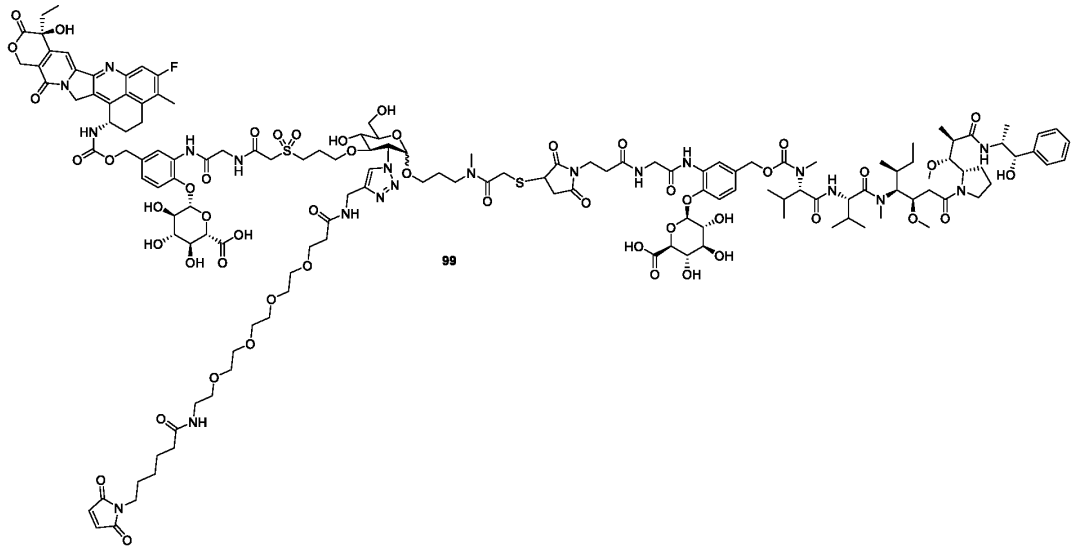
[00986] Step 3: To a solution of compound **98-4** (500 mg, 0.53 mmol) in DMF (5 mL) was added compound **14-4** (210 mg, 0.79 mmol) and DIPEA (136 mg, 1.05 mmol). The reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with acetonitrile and water, TFA condition). The desired components were lyophilized to afford the title compound **98-5** (220 mg, yield 38%) as a red solid. LCMS: m/z calculated for $C_{53}H_{61}N_7O_{19}$: 1100.10; found: 1100.31 $[M+H]^+$.

[00987] Step 4: Under nitrogen atmosphere, to a solution of compound **94-10** (20 mg, 0.016 mmol) in dimethyl sulfoxide (0.5 mL) was added compound **98-5** (18 mg, 0.016 mmol) and triethylamine (6.3 mg, 0.049 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 47% acetonitrile in water, 0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **98-6** (21 mg, yield 56%) as a red solid. LCMS: m/z calculated for $C_{107}H_{124}FN_{15}O_{41}S_2$: 2359.35; found: 1180.46 $[M+2H]^{2+}$.

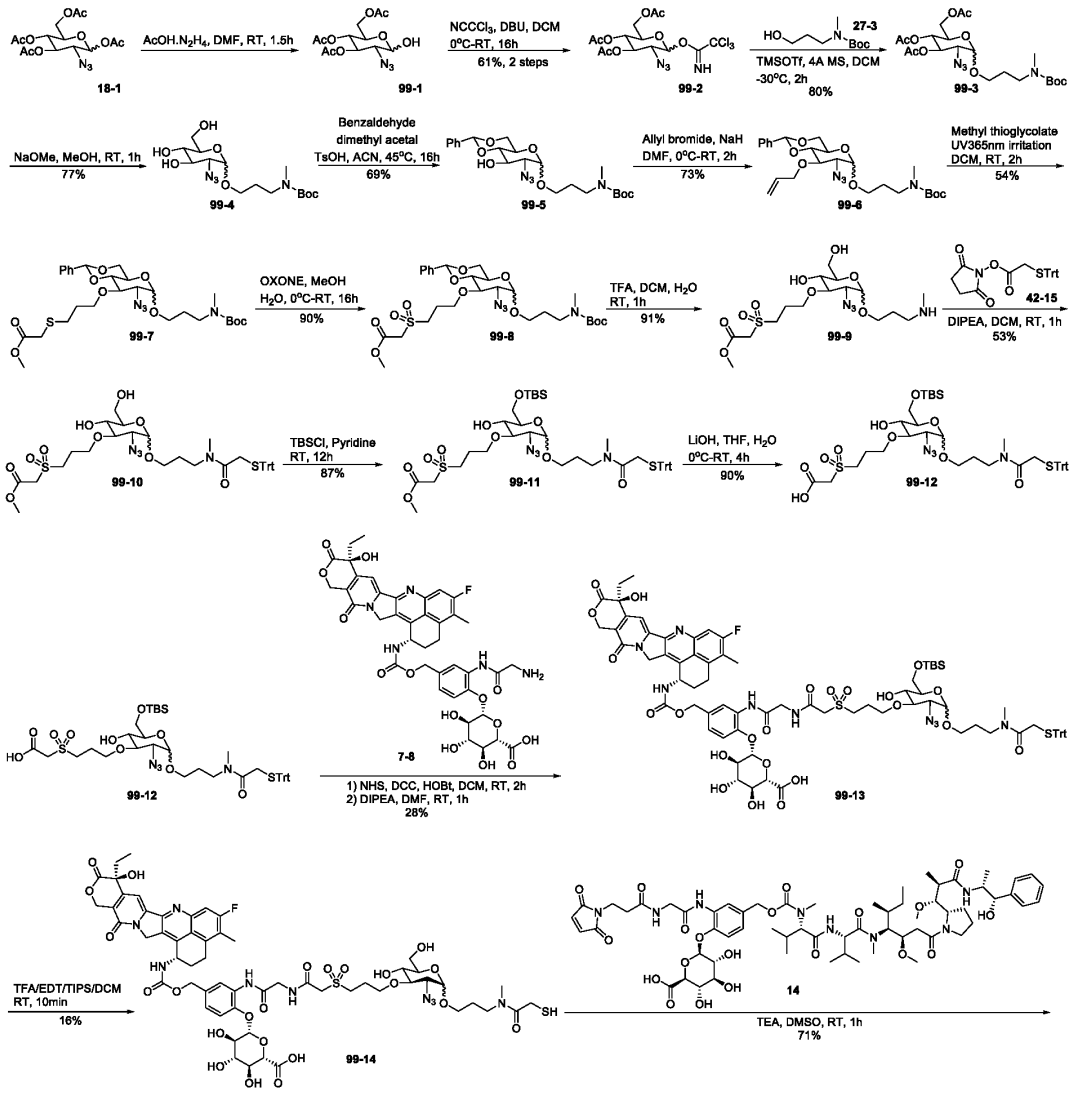
[00988] Step 5: Under nitrogen atmosphere, to a solution of compound **98-6** (26 mg, 0.011 mmol) and compound **1-31** (7 mg, 0.014 mmol) in dimethyl sulfoxide (0.5 mL) was added N,N-diisopropylethylamine (5.7 mg, 0.044 mmol) and cuprous iodide (2.2 mg, 0.011 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 34% acetonitrile in water, 0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **98** (3 mg, yield 10%) as a red solid. LCMS: m/z calculated for $C_{131}H_{161}FN_{18}O_{49}S_2$: 2854.92; found: 1428.46 $[M+2H]^{2+}$.

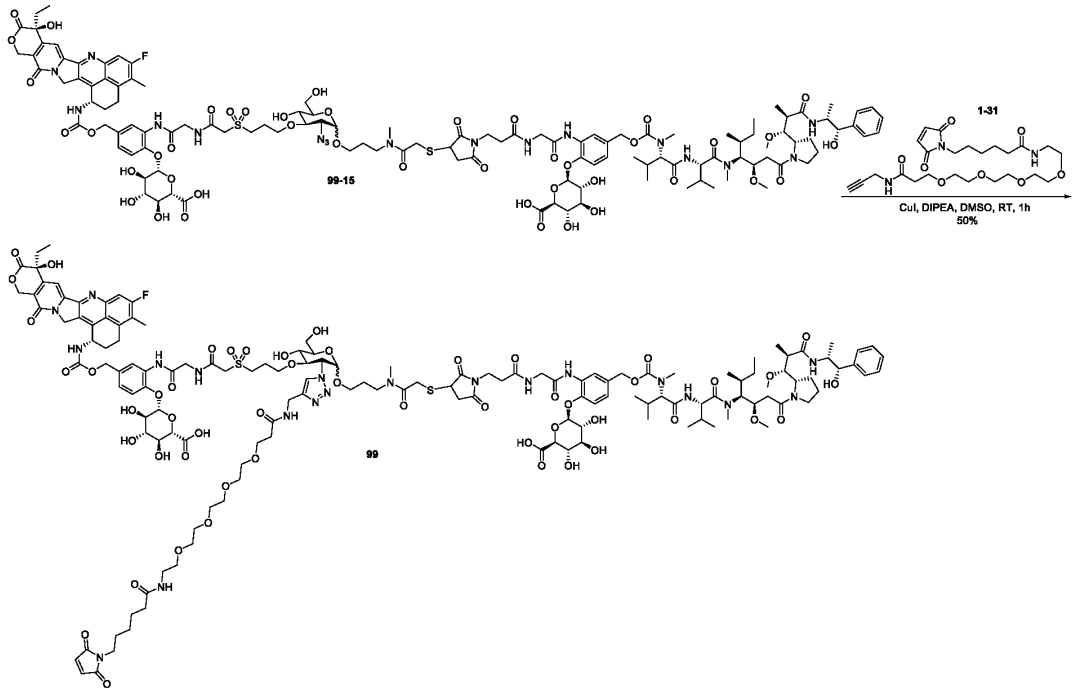
Example 99. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[(2*S*,4*R*)-2-[3-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-

carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]-methyl-amino]propoxy]-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (99)



Synthetic scheme 99


AcOH.N2H4, DMF, RT, 1.5h
NCCCl3, DBU, DCM
0°C-RT, 16h
61%, 2 steps
27-3
TMSOTf, 4A MS, DCM
-30°C, 2h
80%
NaOMe, MeOH, RT, 1h
77%
Benzaldehyde
dimethyl acetal
TsOH, ACN, 45°C, 16h
69%
Allyl bromide, NaH
DMF, 0°C-RT, 2h
73%
Methyl thioglycolate
UV365nm irritation
DCM, RT, 2h
54%
OXONE, MeOH
H2O, 0°C-RT, 16h
90%
TFA, DCM, H2O
RT, 1h
91%
42-15
DIPEA, DCM, RT, 1h
53%
TBSCl, Pyridine
RT, 12h
87%
LiOH, THF, H2O
0°C-RT, 4h
90%
7-8
1) NHS, DCC, HOBt, DCM, RT, 2h
2) DIPEA, DMF, RT, 1h
28%
TFA/EDT/TIPS/DCM
RT, 10min
16%
14
TEA, DMSO, RT, 1h
71%
18-1
99-1
99-2
99-3
99-4
99-5
99-6
99-7
99-8
99-9
99-10
99-11
99-12
99-13
99-14



[00989] Step 1： To a solution of compound **18-1** (20.00 g, 53.57 mmol) in anhydrous DMF (160 mL) was added hydrazine acetate (7.40 g, 80.35 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1.5 h. After completion of the reaction, the mixture was diluted with ethyl acetate (400 mL) and washed with water (500 mL). The organic layer was washed again with saturated $NaHCO_3$ solution and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the title compound **99-1** (17.9 g, crude) as a yellow oil.

[00990] Step 2: Under nitrogen atmosphere, to a solution of compound **99-1** (17.9 crude) and trichloroacetonitrile (23.0 g, 159.29 mmol) in anhydrous dichloromethane (130 mL) was added DBU (390 mg, 2.56 mmol) at 0 °C. The reaction mixture was warmed slowly to room temperature and stirred for 16 h. After completion of the reaction, the mixture was diluted with dichloromethane (400 mL) and washed with water (500 mL) and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was crystallized in n-hexane and ethyl acetate (v:v=1/3) to afford the title compound **99-2** (15.63 g, yield 61 over 2 steps) as a white solid.

[00991] Step 3: Under nitrogen atmosphere, to a flamed flask weighted activated 4A MS (23 g) was added a solution of compound **99-2** (15.00 g, 31.53 mmol) and compound **27-3** (10.0 g, 52.84 mmol) in anhydrous dichloromethane (150 mL). The mixture was stirred at room temperature for 1 h before trimethylsilyl trifluoromethane-sulfonate (3.50 g, 15.75 mmol) was added dropwise at -30 °C. The reaction mixture was warmed slowly to room temperature and stirred for 2 h. After completion of the reaction, the mixture was quenched by adding triethylamine (3.18 g, 31.48 mmol) with further stirring for 0.5 h. The resulting mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **99-3** (12.68 g, yield 80%) as a colorless oil.

[00992] Step 4: To a solution of compound **99-3** (12.7 g, 25.2 mmol) in anhydrous methanol (100 mL) was added a 5.4 M methanol solution of sodium methoxide (0.093 mL, 0.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The crude product was purified by silica gel column (eluted with 10% methanol in dichloromethane) to afford the title compound **99-4** (7.38 g, yield 77%) as a colorless oil.

[00993] Step 5: Under nitrogen atmosphere, to a solution of compound **99-4** (7.38 g, 19.6 mmol) in acetonitrile (60 mL) was added p-toluenesulfonic acid (1.0 g, 5.9 mmol) and benzaldehyde dimethyl acetal (8.95 g, 58.8 mmol) at room temperature. The reaction mixture was stirred at 45 °C for 16 h. After completion of the reaction, the mixture was adjusted pH to 5~7 with triethylamine before concentration under reduced pressure. The residue was purified by silica gel column (eluted with 10% methanol in dichloromethane) to afford the title compound **99-5** (6.3 g, yield 69%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (d, *J* = 3.5 Hz, 2H), 7.38 (d, *J* = 3.3 Hz, 3H), 5.55 (s, 1H), 4.90 (d, *J* = 3.4 Hz, 1H), 4.31 – 4.19 (m, 2H), 3.86 (d, *J* = 4.5 Hz, 1H), 3.76 (t, *J* = 9.6 Hz, 2H), 3.56 – 3.45 (m, 2H), 3.32 (t, *J* = 6.8 Hz, 2H), 3.25 (dd, *J* = 10.1, 3.3 Hz, 1H), 2.87 (s, 3H), 2.73 (s, 1H), 1.93 – 1.82 (m, 2H), 1.46 (s, 9H). LCMS: m/z calculated for $C_{22}H_{32}N_4O_7$: 464.52; found: 365.22 $[M-Boc+H]^+$.

[00994] Step 6: Under nitrogen atmosphere, to a solution of **99-5** (6.3 g, 13.6 mmol) in anhydrous N,N-dimethylformamide (30 mL) was added slowly sodium hydrogen (500 mg *2, 27.1 mmol) at 0 °C. After stirring for 1 h, allyl bromide (2.5 g, 20.3 mmol) was added thereto. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction,

the mixture was extracted with water (300 mL) and ethyl acetate (300 mL x 3) The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 25% ethyl acetate in petroleum ether) to afford the title compound **99-6** (6.55 g, yield 96%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 – 7.35 (m, 5H), 5.97 (dd, *J* = 16.9, 9.8 Hz, 1H), 5.57 (s, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.20 (d, *J* = 10.4 Hz, 1H), 4.90 – 4.83 (m, 1H), 4.45 (dd, *J* = 12.1, 4.8 Hz, 1H), 4.32 – 4.20 (m, 2H), 3.97 (t, *J* = 9.5 Hz, 1H), 3.90 – 3.81 (m, 1H), 3.74 (t, *J* = 9.8 Hz, 2H), 3.64 (t, *J* = 8.9 Hz, 1H), 3.48 (d, *J* = 6.1 Hz, 1H), 3.31 (s, 3H), 2.87 (s, 3H), 1.87 (s, 2H), 1.46 (s, 9H). LCMS: m/z calculated for $C_{25}H_{36}N_4O_7$: 504.58; found: 527.30 $[M+Na]^+$.

[00995] Step 7: Under nitrogen atmosphere, to a solution of compound **99-6** (6.55 g, 13.0 mmol) in dichloromethane (30 mL) was added methyl thioglycolate (5.51 g, 51.9 mmol) at room temperature. The reaction mixture was stirred under UV@365nm for 2 h. After completion of the reaction, the mixture was concentrated. The residue was purified by silica gel column chromatography (eluted with 33% ethyl acetate in petroleum ether) to afford the title compound **99-7** (4.6 g, yield 58%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (d, *J* = 41.5 Hz, 5H), 5.56 (s, 1H), 4.85 (s, 1H), 4.26 (d, *J* = 12.2 Hz, 1H), 4.02 (d, *J* = 7.9 Hz, 1H), 3.91 – 3.74 (m, 5H), 3.72 – 3.68 (m, 3H), 3.60 (d, *J* = 18.1 Hz, 1H), 3.47 (d, *J* = 9.3 Hz, 1H), 3.30 (s, 3H), 3.17 (s, 2H), 2.87 (s, 3H), 2.73 (t, *J* = 6.1 Hz, 2H), 1.92 – 1.81 (m, 5H), 1.45 (s, 9H).

[00996] Step 8: To a solution of OXONE (10.1 g, 16.4 mmol) in H_2O (45 mL) was added dropwise a solution of compound **99-7** (5.0 g, 8.2 mmol) in methanol (45 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. After completion of the reaction, the mixture was filtered and the filtrate was extracted with water (100 mL) and dichloromethane (90 mL x 3). The combined organic layers were washed with brine (200 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (eluted with 50% ethyl acetate in petroleum ether) to afford the title compound **99-8** (4.0 g, yield 76%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.33 (m, 5H), 4.37 – 4.31 (m, 1H), 3.80 – 3.77 (m, 3H), 3.41 – 3.26 (m, 5H), 2.87 (s, 3H), 2.13 (s, 1H), 1.87 (s, 1H), 1.46 (t, *J* = 2.6 Hz, 9H). LCMS: m/z calculated for $C_{28}H_{42}N_4O_{11}S$: 642.72; found: 665.22 $[M+Na]^+$.

[00997] Step 9: To a solution of compound **99-8** (3 g, 4.7 mmol) in dichloromethane (24 mL) was added a mixture of trifluoroacetic acid and H_2O (v:v=8/2, 12 mL) at 0 °C. The reaction

mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was precipitated in methyl tert-butyl ether before centrifugation. The left was dried under reduced pressure to afford the title compound **99-9** (1.93 g, yield 91%) as a colorless oil. LCMS: m/z calculated for $C_{16}H_{30}N_4O_9S$: 454.50; found: 455.15 $[M+H]^+$.

[00998] Step 10: To a solution of compound **99-9** (1.9 g, 4.2 mmol) and N,N-diisopropylethylamine (1.1 g, 8.4 mmol) in anhydrous dichloromethane (20 mL) was added slowly a solution of compound **99-15** (2.0 g, 4.6 mmol) in dichloromethane (20 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was extracted with water (40 mL) and dichloromethane (40 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluted with 5% methanol in dichloromethane) to afford the title compound **99-10** (1.7 g, yield 53%) as a white foam. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.83 (d, *J* = 3.3 Hz, 1H), 4.14 – 4.03 (m, 2H), 4.02 (s, 2H), 3.83 (d, *J* = 1.7 Hz, 3H), 3.71 (d, *J* = 10.1 Hz, 2H), 3.57 (td, *J* = 11.6, 9.1, 5.2 Hz, 4H), 3.42 (h, *J* = 8.2 Hz, 4H), 3.09 (t, *J* = 8.9 Hz, 2H), 2.69 (d, *J* = 1.6 Hz, 3H), 2.20 (s, 2H), 1.79 (s, 2H). LCMS: m/z calculated for $C_{37}H_{46}N_4O_{10}S_2$: 770.91; found: 793.22 $[M+Na]^+$.

[00999] Step 11: To a solution of compound **99-10** (1.7 g, 2.2 mmol) in pyridine (8 mL) was added tert-butyldimethylsilyl chloride (0.5 g, 3.3 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was extracted with ammonium chloride (100 mL) and dichloromethane (100 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluted with 3% methanol in dichloromethane) to afford the title compound **99-11** (1.7 g, yield 87%) as a colorless oil. LCMS: m/z calculated for $C_{43}H_{60}N_4O_{10}S_2Si$; found: 907.32 $[M+Na]^+$.

[001000] Step 12: To a solution of compound **99-11** (1.7 g, 1.9 mmol) in tetrahydrofuran (15 mL) was added dropwise 1N lithium hydroxide (2.8 mL, 2.8 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was adjusted pH to 5-6 with 0.5 N HCl at 0 °C. The resulting mixture was extracted with water (50 mL) and ethyl acetate (50 mL x 2). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude title

compound **99-12** (1.5 g, yield 89%) was obtained as a white foam. LCMS: m/z calculated for $C_{42}H_{58}N_4O_{10}S_2Si$: 871.15; found: 893.34 $[M+Na]^+$.

[001001] Step 13: To a solution of compound **99-12** (500 mg, 0.57 mmol), N-hydroxysuccinimide (132 mg, 1.15 mmol) and 1-hydroxybenzotriazole (78 mg, 0.57 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (237 mg, 1.15 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and the filtrate was extracted with cold water (10 mL) and dichloromethane (10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the NHS ester.

[001002] To a mixture of compound **7-8** (478 mg, 0.57 mmol) and N,N-diisopropylethylamine (222 mg, 1.72 mmol) in N,N-dimethylformamide (3 mL) and H_2O (0.5 mL) was added the above NHS ester in dichloromethane (2 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified directly by reverse phase flash chromatography (C18 column, eluted with 89% acetonitrile in water, 0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **99-13** (269 mg, yield 28%) as a yellow solid. LCMS: m/z calculated for $C_{82}H_{96}FN_9O_{23}S_2Si$: 1686.91; found: 1687.19 $[M+H]^+$.

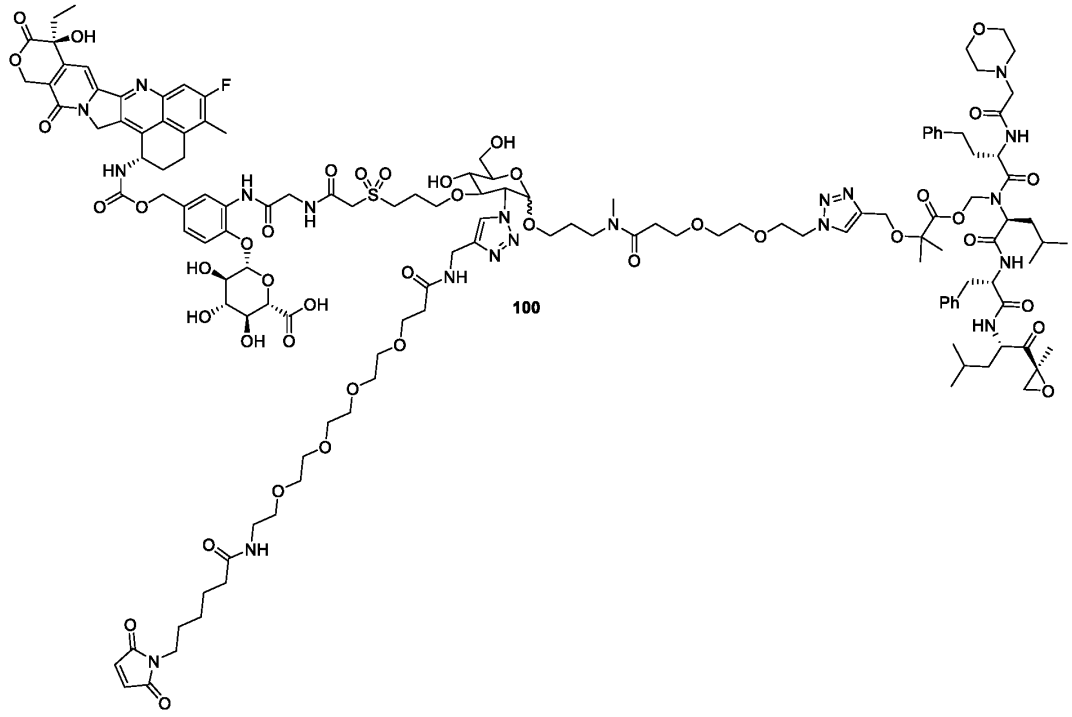
[001003] Step 14: To a mixture of trifluoroacetic acid, 1,2-ethanedithiol, triisopropylsilane and H_2O with a ratio of 90/ 2.5/ 2.5/ 5 (total 1 mL) was added compound **99-13** (100 mg, 0.059 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 10 min. After completion of the reaction, the mixture was precipitated in cold tert-butyl methyl ether before centrifugation. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 50% acetonitrile in water, 0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **9-14** (12.6 mg, yield 16%) as a light-yellow solid. LCMS: m/z calculated for $C_{76}H_{82}FN_9O_{23}S_2$: 1330.33; found: 1330.15 $[M+H]^+$.

[001004] Step 15: Under nitrogen atmosphere, to a solution of compound **99-14** (12 mg, 0.009 mmol) in dimethyl sulfoxide (0.5 mL) was added compound **14** (11.4 mg, 0.009 mmol) and triethylamine (2.7 mg, 0.027 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 59% acetonitrile in water,

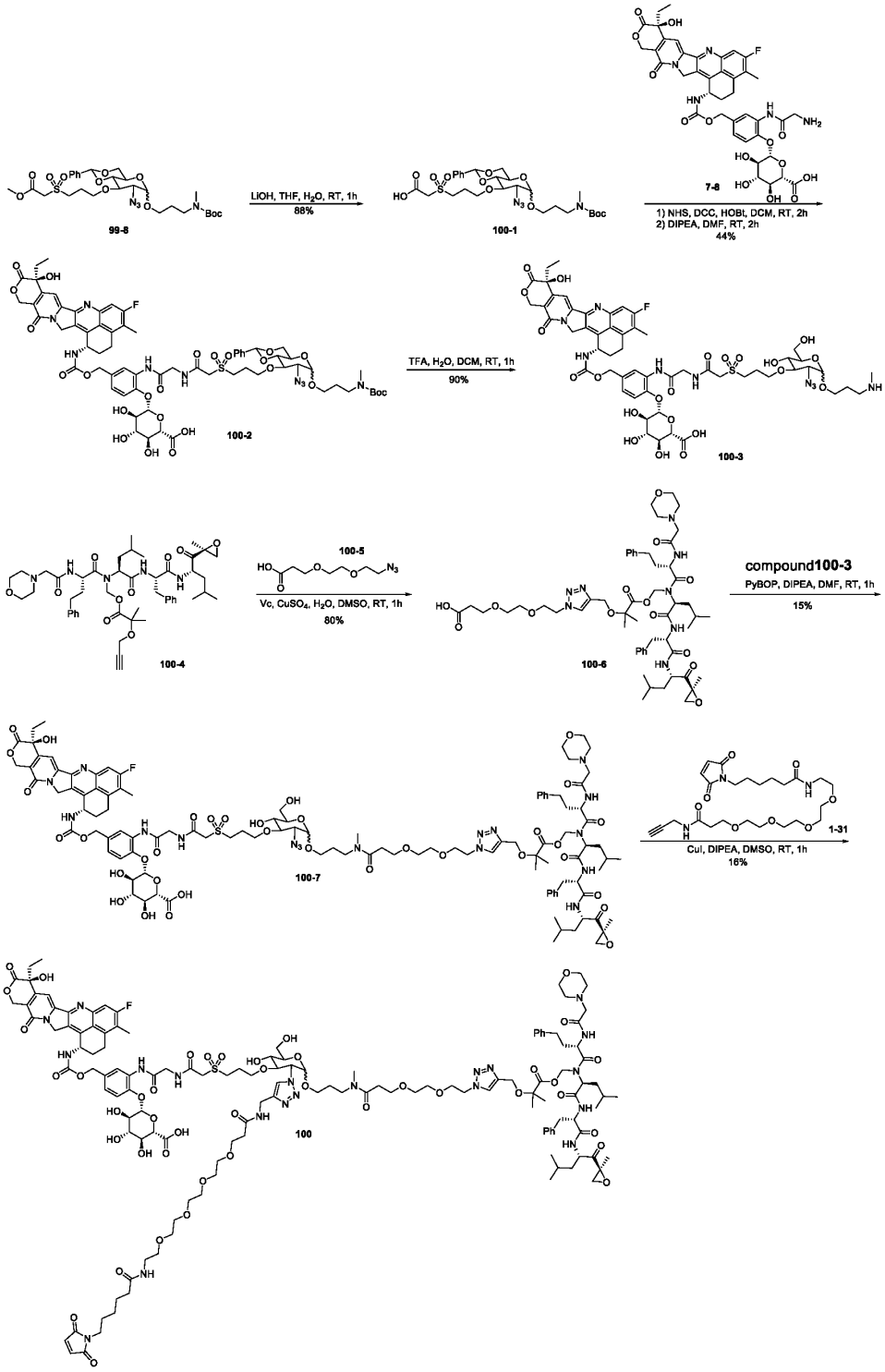
0.1% acetic acid condition). The desired components were lyophilized to afford the title compound **99-15** (17 mg, yield 71%) as a white solid. LCMS: m/z calculated for $C_{119}H_{158}FN_{17}O_{43}S_2$: 2597.77; found: 1299.28 $[M+2H]^{2+}$.

[001005] Step 16: Under nitrogen atmosphere, to a solution of compound **99-15** (17 mg, 0.007 mmol) and compound **1-31** (4.2 mg, 0.009 mmol) in dimethyl sulfoxide (0.5 mL) was added N,N-diisopropylethylamine (3.4 mg, 0.026 mmol) and cuprous iodide(1.3 mg, 0.007 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with 47% acetonitrile in water, 0.1% trifluoroacetic acid condition). The desired components were lyophilized to afford the title compound **99** (10 mg, yield 50%) as a white solid. LCMS: m/z calculated for $C_{143}H_{195}FN_{20}O_{51}S_2$: 3093.34; found: 1546.83 $[M+2H]^{2+}$.

Example 100. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[[2-[3-[2-[3-[3-[2-[2-[4-[[2-[[[(1*S*)-1-[[(1*S*)-1-benzyl-2-[[(1*S*)-3-methyl-1-[(2*S*)-2-methyloxirane-2-carbonyl]butyl]amino]-2-oxo-ethyl]carbamoyl]-3-methyl-butyl]-[(2*S*)-2-[(2-morpholinoacetyl)amino]-4-phenyl-butanoyl]amino]methoxy]-1,1-dimethyl-2-oxo-ethoxy]methyl]triazol-1-yl]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-3-[4-[[3-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (100)



Synthetic scheme 100


99-8
LiOH, THF, H2O, RT, 1h
88%
100-1
7-8
1) NHS, DCC, HOBt, DCM, RT, 2h
2) DIPEA, DMF, RT, 2h
44%
100-2
TFA, H2O, DCM, RT, 1h
90%
100-3
100-4
100-5
Vc, CuSO4, H2O, DMSO, RT, 1h
80%
100-6
compound100-3
PyBOP, DIPEA, DMF, RT, 1h
15%
100-7
1-31
CuI, DIPEA, DMSO, RT, 1h
16%
100

[001006] Step 1: The title compound **100-1** was prepared in analogy to **Example 99**, **Step 12** by using compound **99-8**. The product (530 mg, yield 88%) was obtained as a white solid. LCMS: m/z calculated for $C_{27}H_{40}N_4O_{11}S$: 628.69; found: 529.18 $[M\text{-}Boc+H]^+$.

[001007] Step 2: The title compound **100-2** was prepared in analogy to **Example 99**, **Step 13** by using compound **7-8** and compound **100-1**. The product (202 mg, yield 44%) was obtained as a white solid. LCMS: m/z calculated for $C_{67}H_{78}FN_9O_{24}S$: 1444.46; found: 1444.38 $[M+H]^+$.

[001008] Step 3: To a mixture of compound **100-2** (86 mg, 0.059 mmol) in dichloromethane (0.9 mL) was added 80% trifluoroacetic acid in water (0.3 mL). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was precipitated in cold tert-butyl methyl ether before centrifugation. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **100-3** (75 mg, yield 90%) as a yellow solid. LCMS: m/z calculated for $C_{55}H_{66}FN_9O_{22}S$: 1256.23; found: 1256.25 $[M+H]^+$.

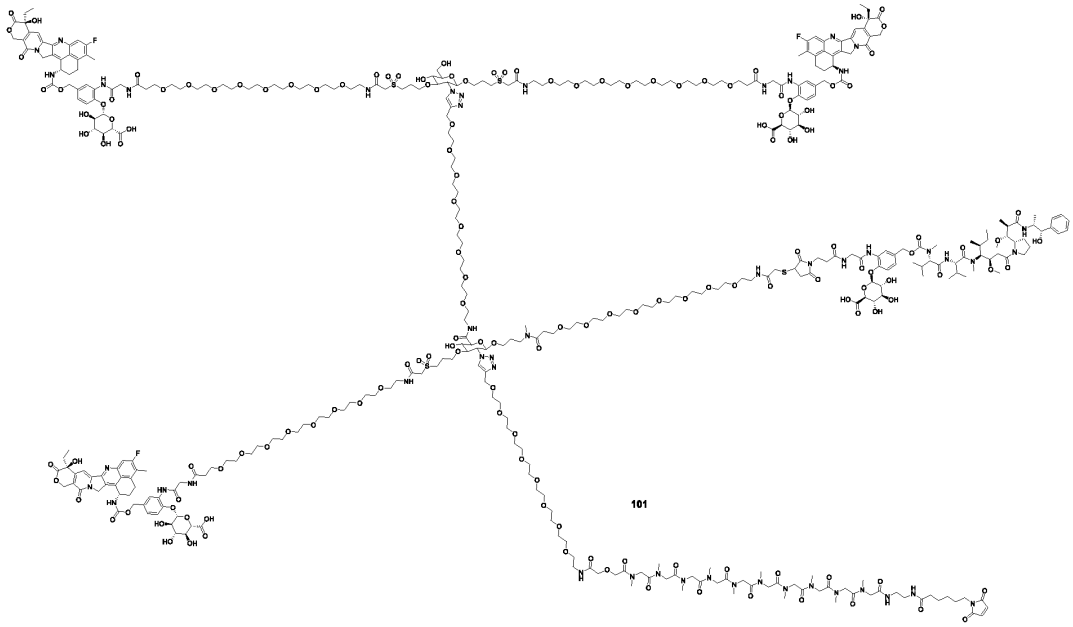
[001009] Step 4: Under nitrogen atmosphere, to a mixture of compound **100-4** (100 mg, 0.114 mmol) and compound **100-5** (26 mg, 0.005 mmol) in dimethyl sulfoxide (2 mL) was added 1M L-Ascorbic acid aqueous solution (0.686 mL, 0.686 mmol) and 1M copper sulfate aqueous solution (0.229 mL, 0.229 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to give the title compound **100-6** (98.5 mg, yield 80%) as a pale-yellow solid. LCMS: m/z calculated for $C_{55}H_{80}N_8O_{14}$: 1077.29; found: 1077.52 $[M+H]^+$.

[001010] Step 5: Under nitrogen atmosphere, to a solution of compound **100-6** (68.5 mg, 0.054 mmol) in anhydrous N,N-dimethylformamide (2 mL) was added N, N-Diisopropylethylamine (0.030 mL, 0.163 mmol) and PyBOP (43 mg, 0.082 mmol). The reaction mixture was stirred at room temperature for 25 min before a solution of compound **100-3** (65 mg, 0.060 mmol) in anhydrous N,N-dimethylformamide (0.5 mL) was added. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to afford the title compound **100-7** (19 mg, yield 15%) as a white solid. LCMS: m/z calculated for $C_{110}H_{144}FN_{17}O_{35}S$: 2315.50; found: 1158.85 $[M+2H]^{2+}$.

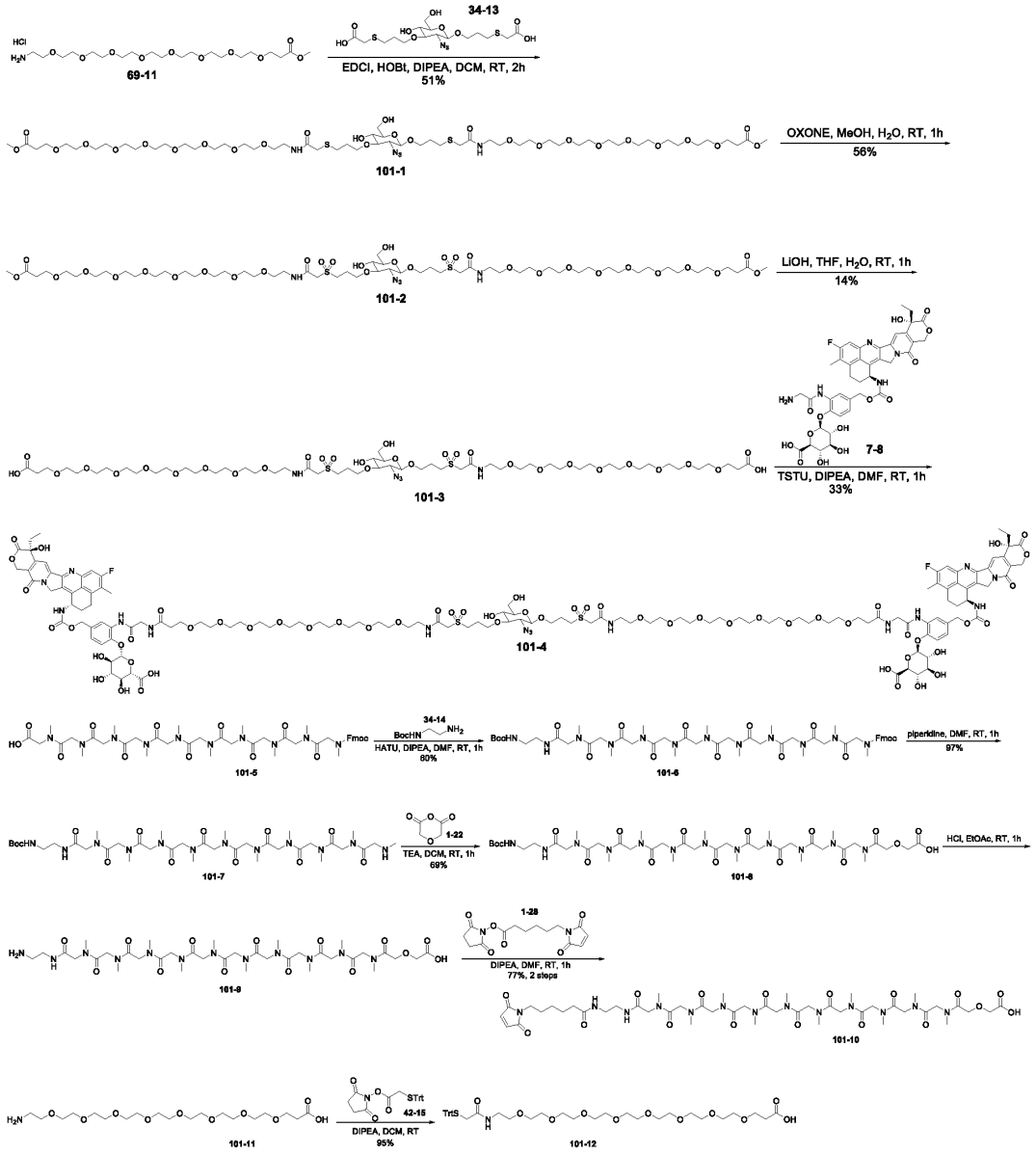
[001011] Step 6: Under nitrogen atmosphere, to a mixture of compound **100-6** (19 mg, 0.008 mmol) and compound **1-31** (5 mg, 0.009 mmol) in dimethyl sulfoxide (0.5 mL) was added N, N-diisopropylethylamine (0.003 mL, 0.016 mmol) and cuprous iodide (0.8 mg, 0.004 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by pre-HPLC (C4 column, eluted with acetonitrile in H_2O, AcOH condition). The desired components were lyophilized to afford the title compound **100** (3.6 mg, yield 16%) as a white solid. LCMS: m/z calculated for $C_{134}H_{181}FN_{20}O_{43}S$: 2811.08 found: 1406.33 $[M+2H]^{2+}$.

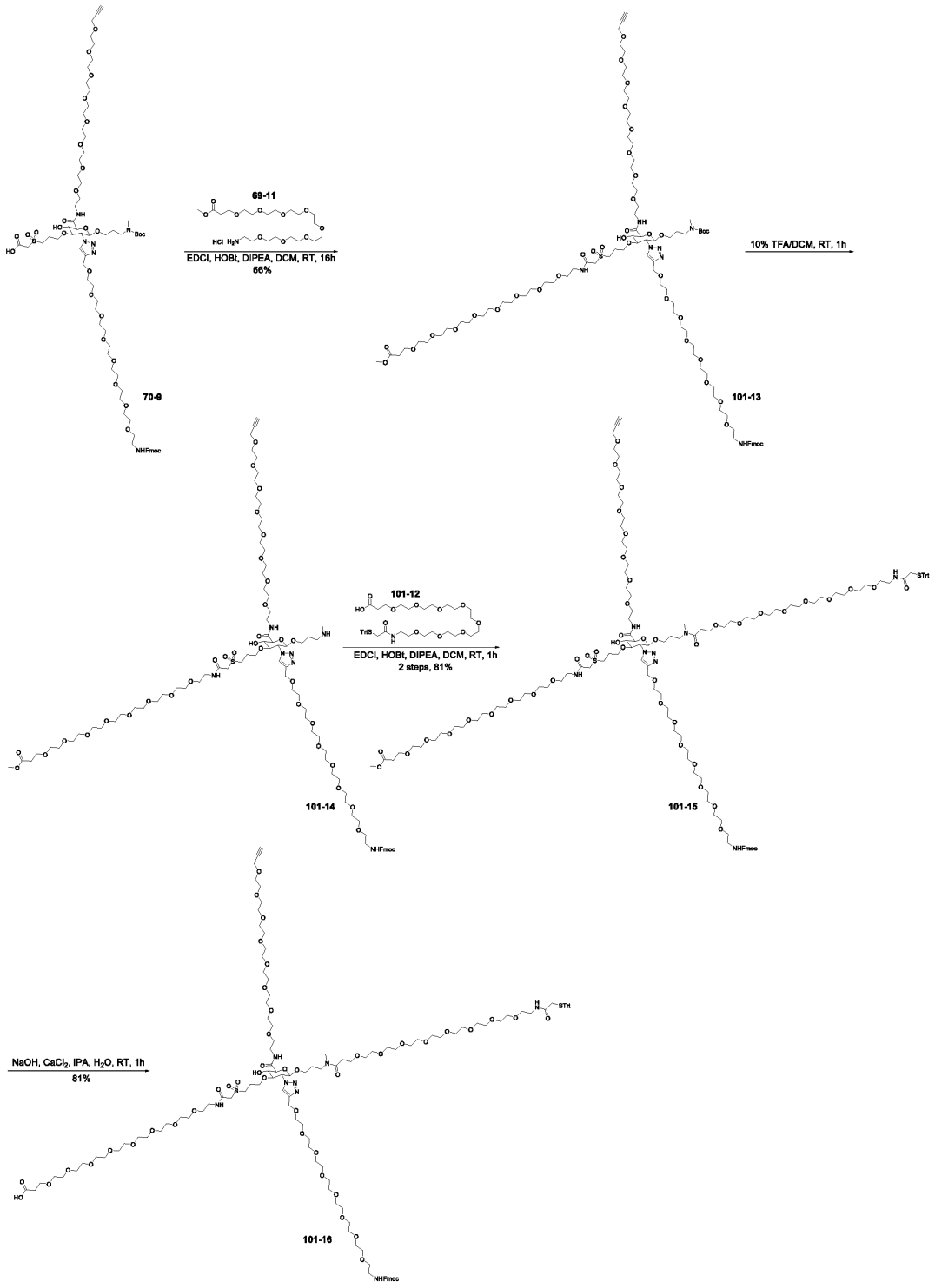
Example 101. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-

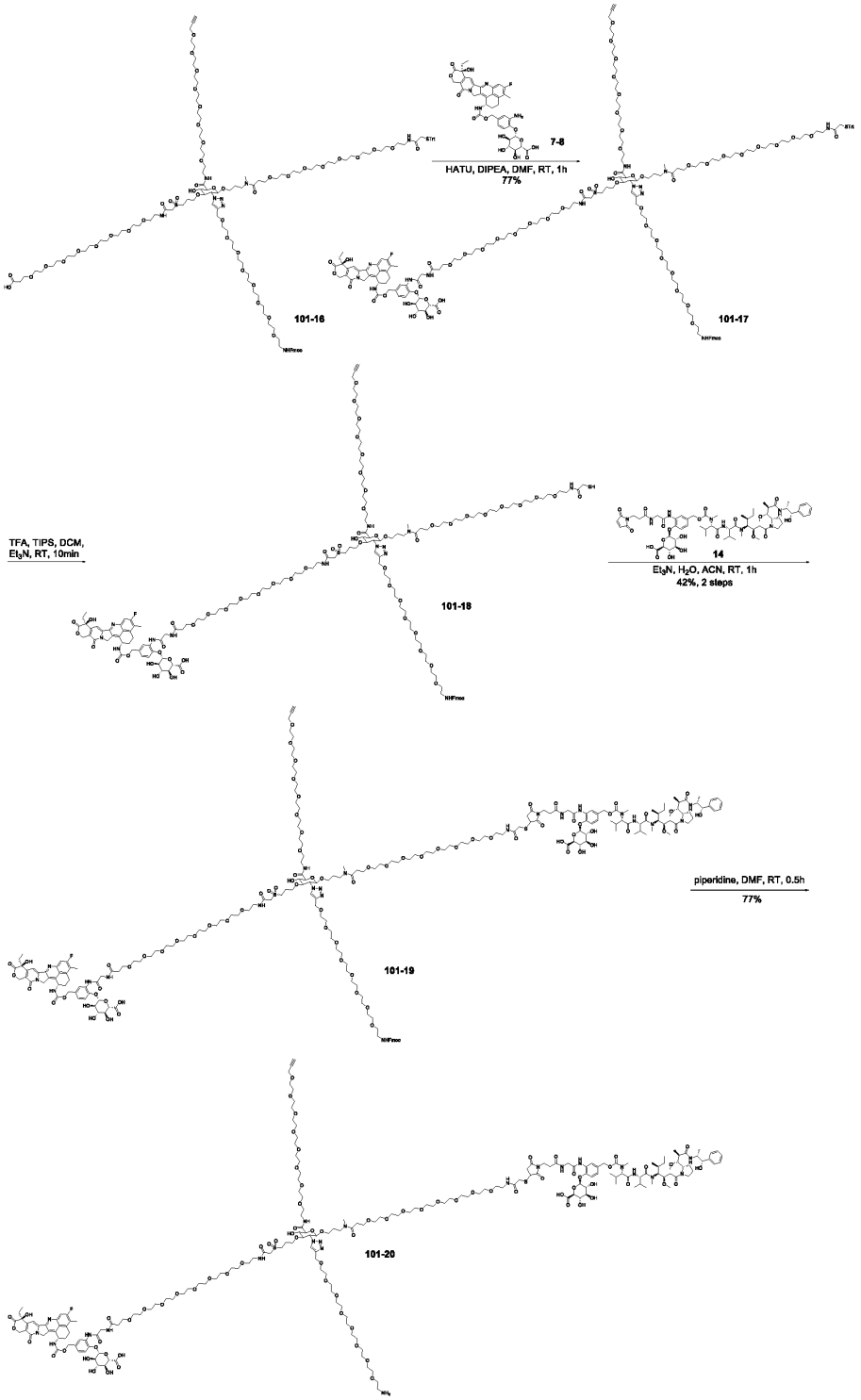
[[2-[[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethylamino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0^{2,14}.0^{4,13}.0^{6,11}.0^{20,24}]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (101)

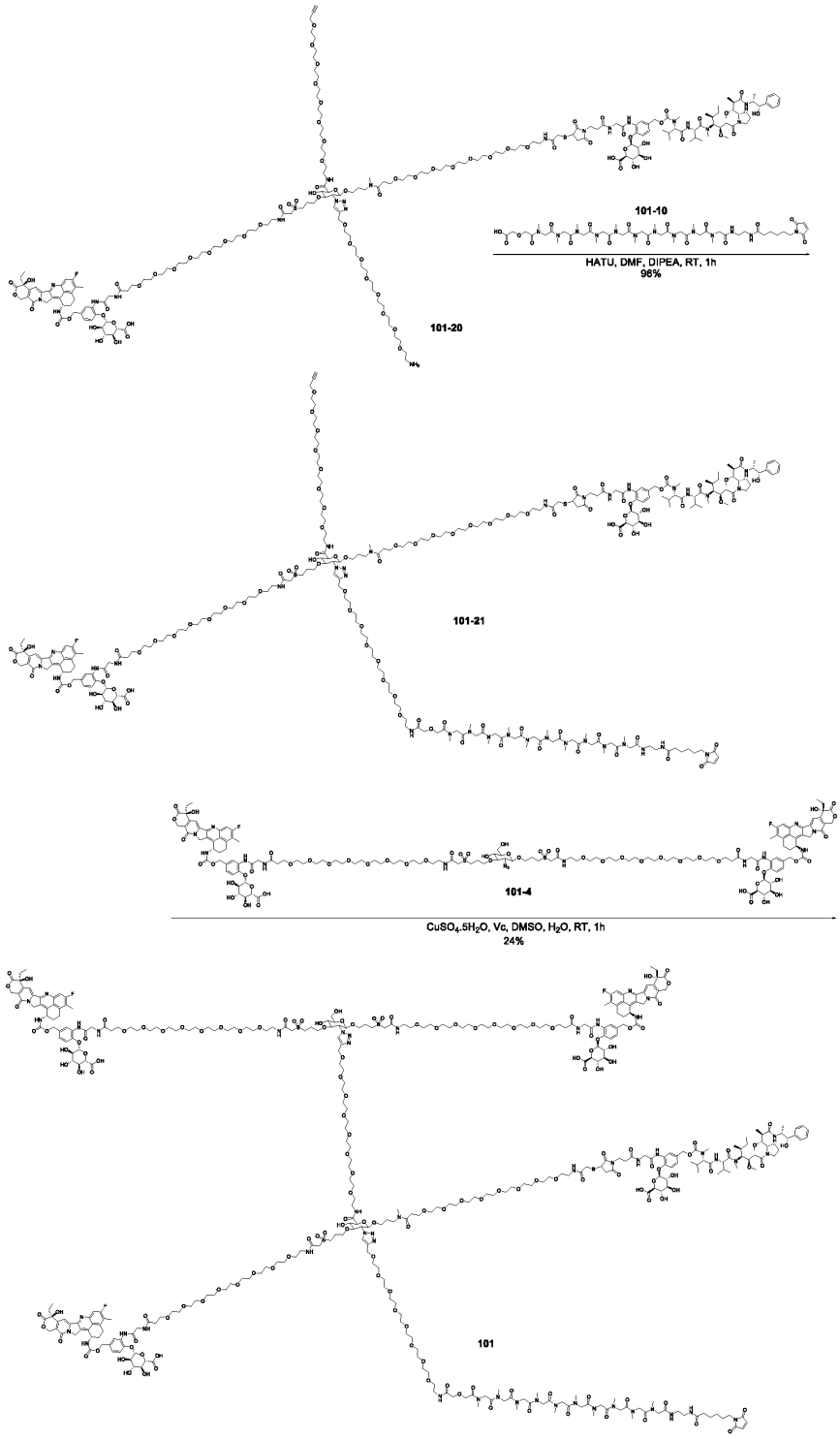


Synthetic scheme 101


34-13
69-11
EDCI, HOBt, DIPEA, DCM, RT, 2h
51%
101-1
OXONE, MeOH, H2O, RT, 1h
56%
101-2
LiOH, THF, H2O, RT, 1h
14%
7-8
101-3
TSTU, DIPEA, DMF, RT, 1h
33%
101-4
34-14
HATU, DIPEA, DMF, RT, 1h
80%
101-5
101-6
piperidine, DMF, RT, 1h
97%
1-22
TEA, DCM, RT, 1h
69%
101-7
101-8
HCl, EtOAc, RT, 1h
101-9
1-28
DIPEA, DMF, RT, 1h
77%, 2 steps
101-10
42-15
DIPEA, DCM, RT
95%
101-11
101-12


69-11
HCl H2N
EDCI, HOBt, DIPEA, DCM, RT, 16h
66%
70-9
10% TFA/DCM, RT, 1h
101-13
101-12
EDCI, HOBt, DIPEA, DCM, RT, 1h
2 steps, 81%
101-14
101-15
NaOH, CaCl2, IPA, H2O, RT, 1h
81%
101-16


7-8
HATU, DIPEA, DMF, RT, 1h
77%
101-16
101-17
TFA, TIPS, DCM,
Et3N, RT, 10min
101-18
14
Et3N, H2O, ACN, RT, 1h
42%, 2 steps
piperidine, DMF, RT, 0.5h
77%
101-19
101-20


101-10
HATU, DMF, DIPEA, RT, 1h
98%
101-20
101-21
101-4
CuSO4.5H2O, Vc, DMSO, H2O, RT, 1h
24%
101

[001012] Step 1: The title compound **101-1** was prepared in analogy to **Example 2**, **Step 5** by using compound **34-13** and compound **69-11**. The product (2.94 g, yield 51%) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{56}H_{105}N_5O_{27}S_2$: 1344.58; found: 1344.52 $[M+H]^+$.

[001013] Step 2: The title compound **101-2** was prepared in analogy to **Example 45**, **Step 1** by using compound **101-1**. The product (1.7 g, yield 56%) was obtained as a pale-yellow oil. LCMS: m/z calculated for $C_{56}H_{105}N_5O_{31}S_2$: 1408.58; found: 1409.52 $[M+H]^+$.

[001014] Step 3: The title compound **101-3** was prepared in analogy to **Example 4**, **Step 5** by using compound **101-2**. The product (342.00 mg, yield 14%) was obtained as a light-yellow oil. LCMS: m/z calculated for $C_{54}H_{101}N_5O_{31}S_2$: 1380.53; found: 1380.52 $[M+H]^+$.

[001015] Step 4: The title compound **101-4** was prepared in analogy to **Example 53**, **Step 1** by using compound **101-3 and** compound **7-8**. The product (81.00 mg, two steps, yield 33%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{134}H_{177}F_2N_{15}O_{57}S_2$: 3012.05; found: 1506.24 $[M+2H]^+$.

[001016] Step 5: Under nitrogen atmosphere, to a solution of 1-(9H-fluoren-9-yl)-4,7,10,13,16,19,22,25,28,31-decamethyl-3,6,9,12,15,18,21,24,27,30-decaoxo-2-oxa-4,7,10,13,16,19,22,25,28,31-decaazatritriacontan-33-oic acid (Fmoc-Sar10-OH, **101-5**, 1.81 g, 1.903 mmol) in N,N-dimethylformamide (20 mL) was added N,N-diisopropylethylamine (737 mg, 5.709 mmol), HATU (870 mg, 2.284 mmol). The mixture was stirred at room temperature for 15 min before compound **34-14** (460 mg, 2.854 mmol) was added thereto. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to afford the title compound **101-6** (1.3 g, yield 60%) as a white solid. LCMS: m/z calculated for $C_{52}H_{76}N_{12}O_{14}$: 1092.56; found: 1093.44 $[M+H]^+$.

[001017] Step 6: To a solution of compound **101-6** (1.3 g, 1.189 mmol) in N,N-dimethylformamide (4 mL) was added piperidine (0.353 mL, 3.567 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was precipitated in cold tert-butyl methyl ether before centrifugation. The residue was dried in vacuum to afford the title compound **101-7** (1.01 g, yield 97%) as a white solid. LCMS: m/z calculated for $C_{37}H_{66}N_{12}O_{12}$: 870.49; found: 871.46 $[M+H]^+$.

[001018] Step 7: To a solution of compound **101-7** (1.01 g, 1.159 mmol) in dichloromethane (10 mL) was added triethylamine (0.326 mL, 2.319 mmol) and compound **1-22** (148 mg, 1.275 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to afford the title compound **101-8** (811 mg, yield 69%) as a white solid. LCMS: m/z calculated for $C_{41}H_{70}N_{12}O_{16}$: 987.08; found: 987.52 $[M+H]^+$.

[001019] Step 8: To a solution of compound **101-8** (805 mg, 0.820 mmol) in dichloromethane (8 mL) was added 4N hydrochloric acid ethyl acetate solution (6 mL) at 0 °C. The reaction mixture was stirred at room temperature for 15 min. After completion of the reaction, the mixture was precipitated in cold ethyl acetate (20 mL) before centrifugation. The residue was dried under reduced pressure to afford the title compound **101-9** (962 mg, crude) as a yellow solid. LCMS: m/z calculated for $C_{36}H_{62}N_{12}O_{14}$: 886.96; found: 887.44 $[M+H]^+$.

[001020] Step 9: To a solution of compound **101-9** (715 mg, 0.820 mmol) in N,N-dimethylformamide (8 mL) was added N,N-diisopropylethylamine (208 mg, 1.610 mmol) and compound **1-28** (277 mg, 0.900 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 hour. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with acetonitrile in H_2O, TFA condition). The desired components were lyophilized to afford the title compound **101-10** (684 mg, yield 77% over 2 steps) as a white solid. LCMS: m/z calculated for $C_{46}H_{73}N_{13}O_{17}$: 1080.16; found: 1081.33 $[M+H]^+$.

[001021] Step 10: The title compound **101-12** was prepared in analogy to **Example 21**, **Step 2** by using compound **101-11** and compound **21-2**. The product (2.34 g, yield 95%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{40}H_{55}NO_{11}S$: 757.94; found: 758.37 $[M+1H]^{1+}$.

[001022] Step 11: The title compound **101-13** was prepared in analogy to **Example 2**, **Step 5** by using compound **70-9** and compound **69-11**. The product (1.70 g, yield 66%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{93}H_{155}N_7O_{38}S$: 2011.33; found: 1006.11 $[M+2H]^{2+}$.

[001023] Step 12: The title compound **101-14** was prepared in analogy to **Example 57**, **Step 2** by using compound **101-13**. The product was obtained as a yellow oil and directly used into the next step. LCMS: m/z calculated for $C_{88}H_{147}N_7O_{36}S$: 1911.22; found: 1911.60 $[M+H]^+$.

[001024] Step 13: The title compound **101-15** was prepared in analogy to **Example 87**, **Step 19** by using compound **101-12** and compound **101-14**. The product (1.77 g, yield 81% over 2 steps) was obtained as a brown oil. LCMS: m/z calculated for $C_{128}H_{200}N_8O_{46}S_2$: 2651.14; found: 1325.93 $[M+2H]^{2+}$.

[001025] Step 14: The title compound **101-16** was prepared in analogy to **Example 42**, **Step 22** by using compound **101-15**. The product (1.52 g, yield 81%) was obtained as a brown oil. LCMS: m/z calculated for $C_{127}H_{198}N_8O_{46}S_2$: 2637.11; found: 1319.38 $[M+2H]^{2+}$.

[001026] Step 15: The title compound **101-17** was prepared in analogy to **Example 70**, **Step 8** by using compound **101-16** and compound **7-8**. The product (244.00 mg, yield 91%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{167}H_{236}FN_{13}O_{59}S_2$: 3452.88; found: 1606.07 $[M\text{-}Trt+2H]^{+}$.

[001027] Step 16: The title compound **101-18** was prepared in analogy to **Example 42**, **Step 15** by using compound **101-17**. The crude product (256.20 mg) was obtained as a yellow solid.

[001028] Step 17: The title compound **101-19** was prepared in analogy to **Example 10**, **Step 10** by using compound **14** and compound **101-18**. The product (132.00 mg, yield 42% over two steps) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{210}H_{312}FN_{21}O_{79}S_2$: 4477.99; found: 1493.27 $[M+3H]^{+}$.

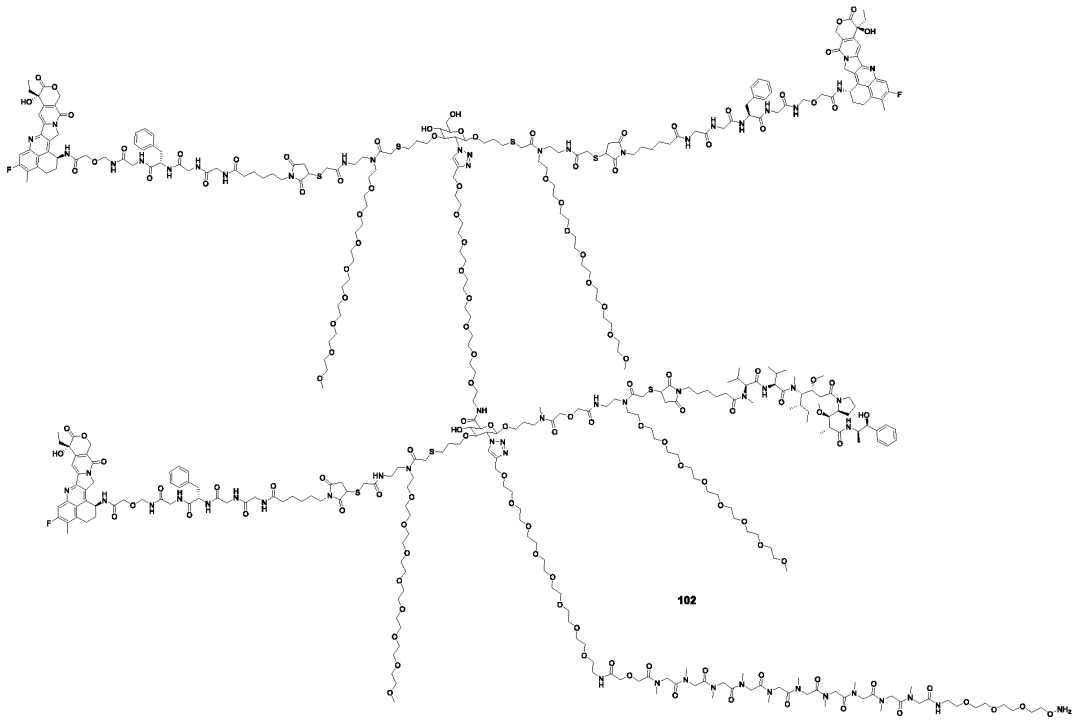
[001029] Step 18: The title compound **101-20** was prepared in analogy to **Example 42**, **Step 27** by using compound **101-19**. The product (97.30 mg, yield 77%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{195}H_{302}FN_{21}O_{77}S_2$: 4255.75; found:1064.78 $[M+3H]^{+}$.

[001030] Step 19: The title compound **101-21** was prepared in analogy to **Example 70**, **Step 8** by using compound **101-10** and compound **101-20**. The product (60.30 mg, yield 96%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{241}H_{373}FN_{34}O_{93}S_2$: 5317.90; found: 1773.38 $[M+3H]^{+}$.

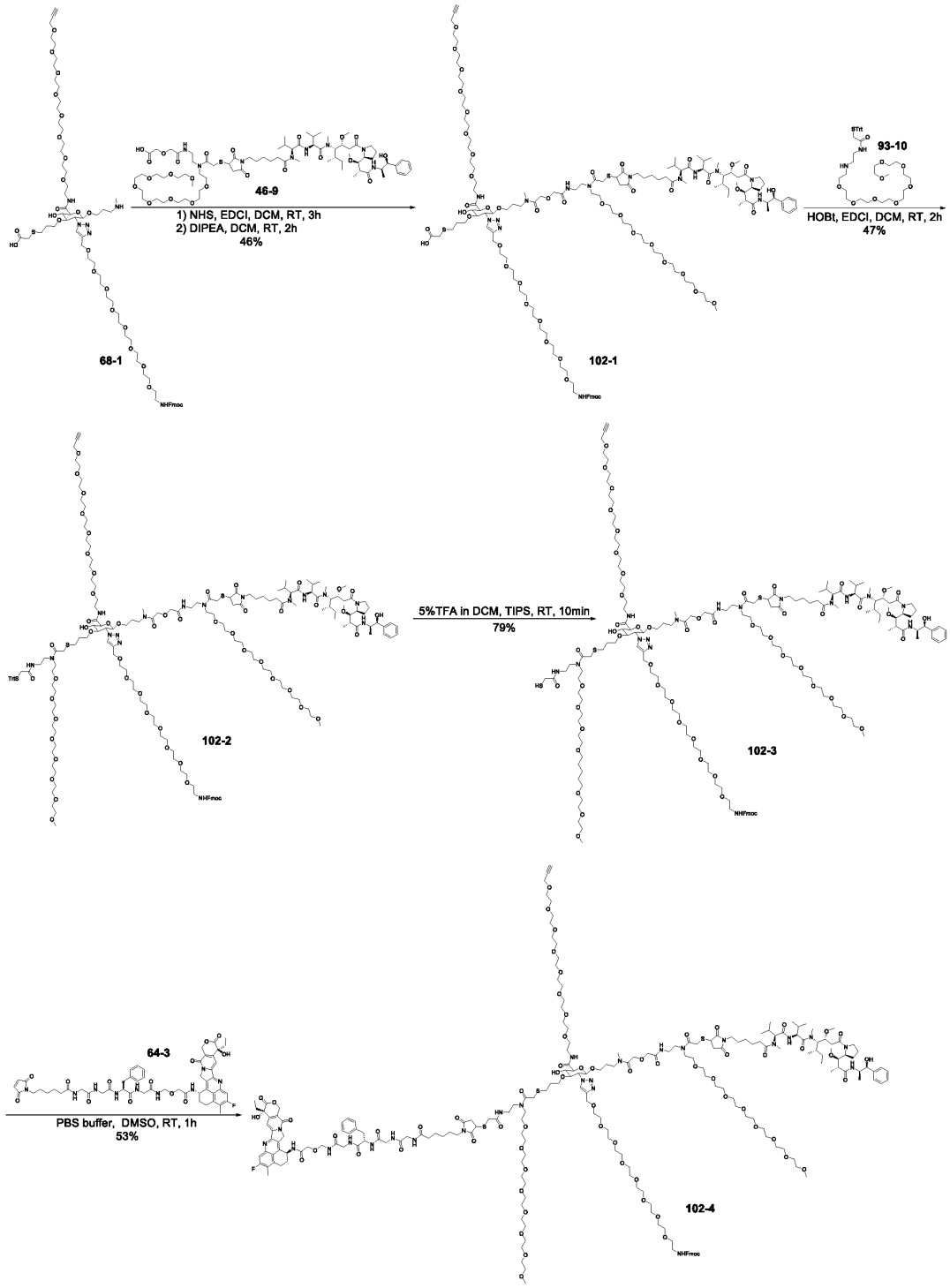
[001031] Step 20: The title compound **101** was prepared in analogy to **Example 1**, **Step 24** by using compound **101-4** and compound **101-21.** The product (22.30 mg, yield 24%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{375}H_{550}F_3N_{49}O_{150}S_4$: 8329.95; found: 1666.51 $[M+5H]^{+}$.

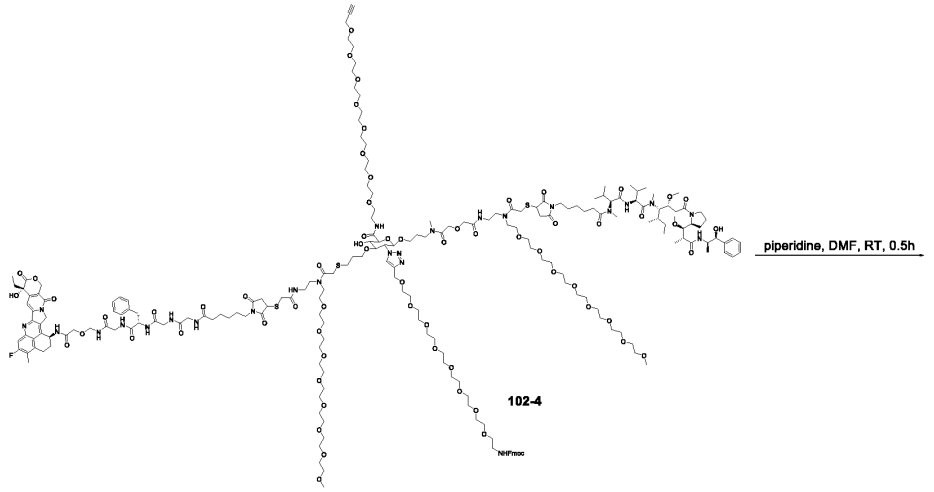
Example 102. Synthesis of 5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[2-[2-[2-[2-(2-aminooxyethoxy)ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]-methyl-amino]-2-

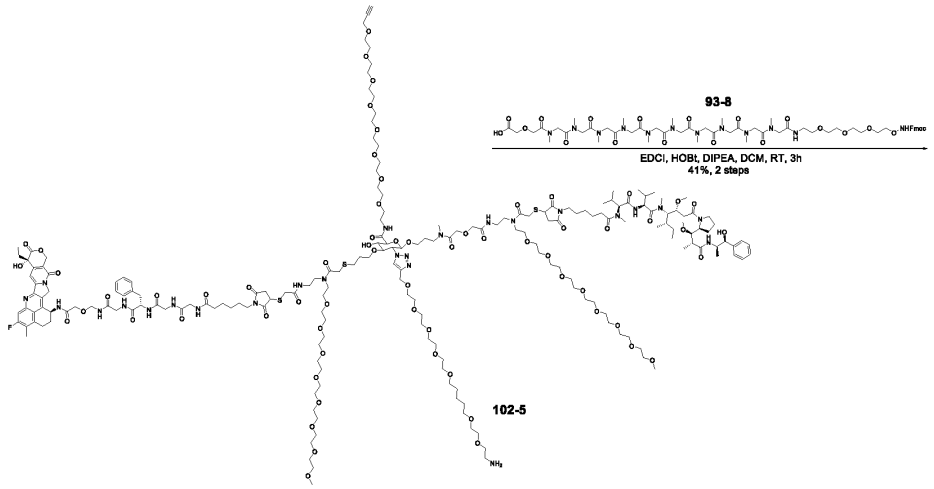
oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-*N*-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfanylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-hydroxy-6-[3-[[2-[2-[2-[[2-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]-methyl-amino]propoxy]tetrahydropyran-2-carboxamide (102)

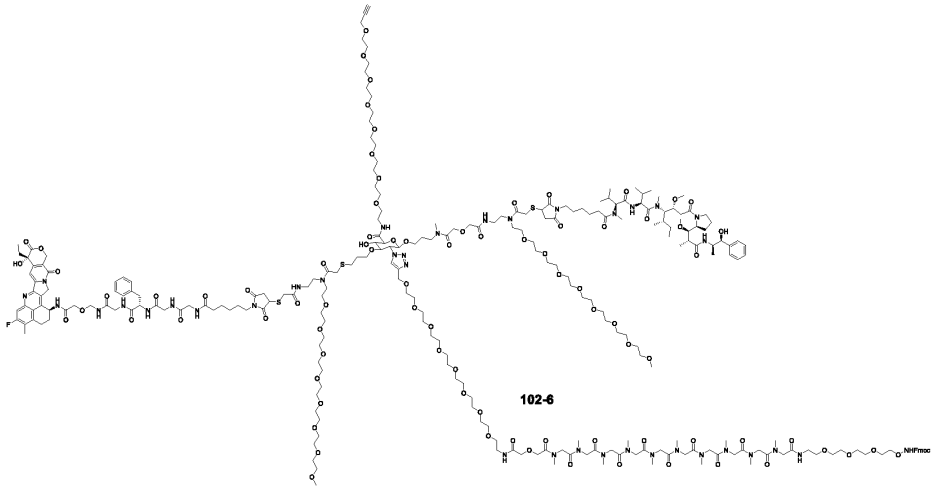


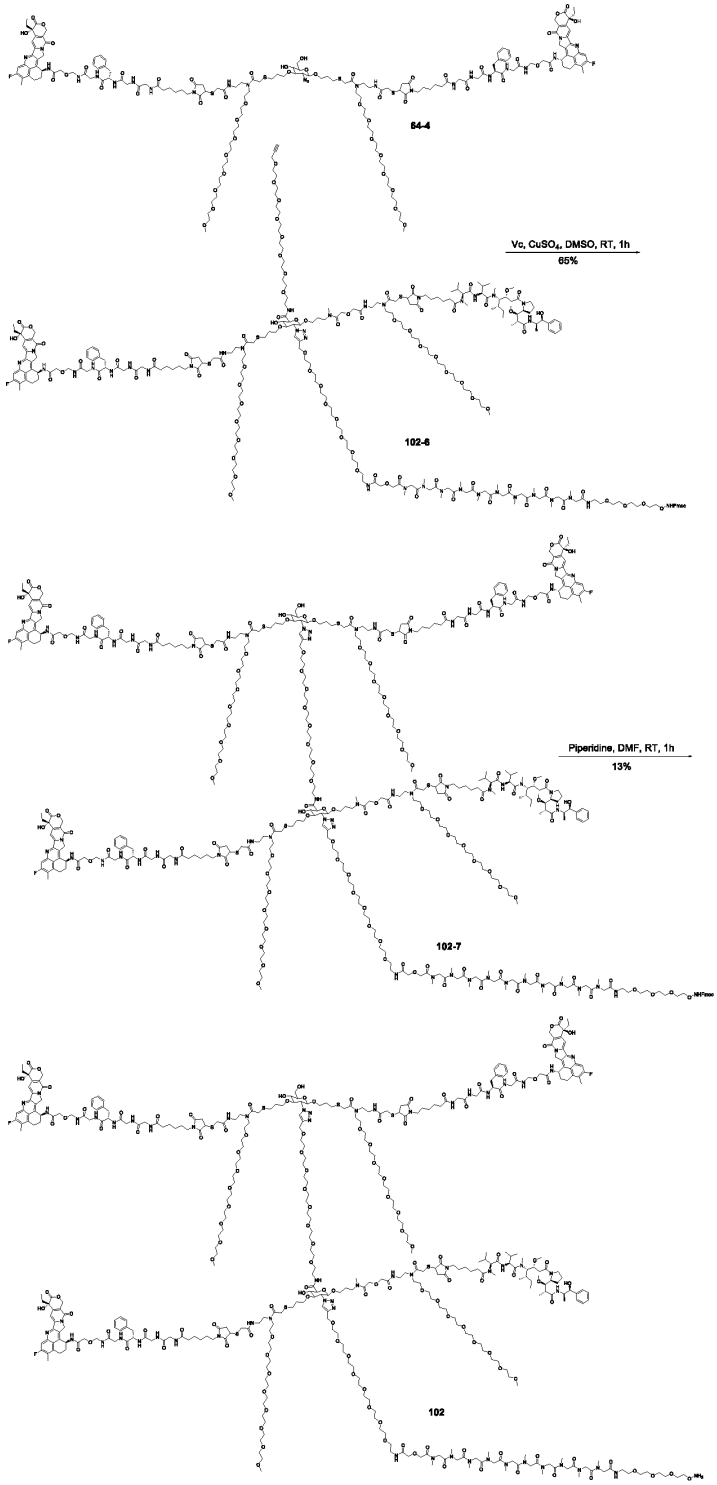
Synthetic scheme 102


68-1
46-9
1) NHS, EDCI, DCM, RT, 3h
2) DIPEA, DCM, RT, 2h
46%
102-1
93-10
HOBt, EDCI, DCM, RT, 2h
47%
102-2
5%TFA in DCM, TIPS, RT, 10min
79%
102-3
64-3
PBS buffer, DMSO, RT, 1h
53%
102-4


piperidine, DMF, RT, 0.5h
102-4
NHFmoc


93-8
EDCI, HOBt, DIPEA, DCM, RT, 3h
41%, 2 steps
102-5


102-6
NHFmoc


84-4
Vc, CuSO4, DMSO, RT, 1h
65%
102-6
Piperidine, DMF, RT, 1h
13%
102-7
102

[001032] Step 1: Under nitrogen atmosphere, a mixture of compound **46-9** (0.50 g, 0.32 mmol), DIPEA (0.13 g, 0.98 mmol) and HATU (0.14 g, 0.36 mmol) in N,N-dimethylformamide (10 mL) was stirred at room temperature for 20 min, followed by the addition of compound **68-1** (0.67 g, 0.46 mmol) in N,N-dimethylformamide (2 mL). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 68% acetonitrile in water). The desired components were lyophilized to afford the title compound **102-1** (0.45 g, yield 46%) as a yellow oil LCMS: m/z calculated for $C_{142}H_{232}N_{14}O_{47}S_2$:2951.59; found: 985.69 $[M+3H]^{3+}$.

[001033] Step 2: Under nitrogen atmosphere, a mixture of compound **102-1** (0.45 g, 0.15 mmol), **93-10** (0.14 g, 0.18 mmol), DIPEA (0.05 g, 0.38 mmol), HOBt (0.02 g, 0.15 mmol) and EDCI (0.05 g, 0.27 mmol) in dichloromethane (10 mL) was stirred at room temperature for 5 h. After completion of the reaction, the mixture was concentrated in vacuum. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 80% acetonitrile in water, 0.1%TFA condition). The desired components were lyophilized to afford the title compound **102-2** (0.27 g, yield 47%) as a yellow oil LCMS: m/z calculated for $C_{182}H_{288}N_{16}O_{55}S_3$: 3676.54; found: 1227.66 $[M+3H]^{3+}$.

[001034] Step 3: To a mixture of compound **102-2** (468 mg, 0.13 mmol) in dichloromethane (66.5 mL) was added trifluoroacetic acid (3.5 mL) and triisopropylsilane (0.15 mL). The reaction mixture was stirred at room temperature for 10 min. After completion of the reaction, the mixture was concentrated in vacuum. The residue was purified by reverse phase flash chromatography (C18 column, eluted with 100% acetonitrile in water, 0.1%AcOH condition). The desired components were lyophilized to afford the title compound **102-3** (345mg, yield 79%) as a yellow oil LCMS: m/z calculated for $C_{163}H_{274}N_{16}O_{55}S_3$: 3432.25; found: 859.38$[M+3H]^{3+}$.

[001035] Step 4: The title compound **102-4** was prepared in analogy to **Example 96, Step 3** by using compound **64-3** and compound **102-3**. The product (90 mg, yield 53%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{215}H_{330}FN_{25}O_{68}S_3$: 4468.29; found: 1490.08 $[M+3H]^{3+}$.

[001036] Step 5: The title compound **102-5** was prepared in analogy to **Example 96, Step 4** by using compound **102-4**. The product (150 mg, crude) was obtained as a yellow solid. LCMS: m/z calculated for $C_{201}H_{322}FN_{25}O_{65}S_3$: 4244.08; found: 1415.93 $[M+3H]^{3+}$.

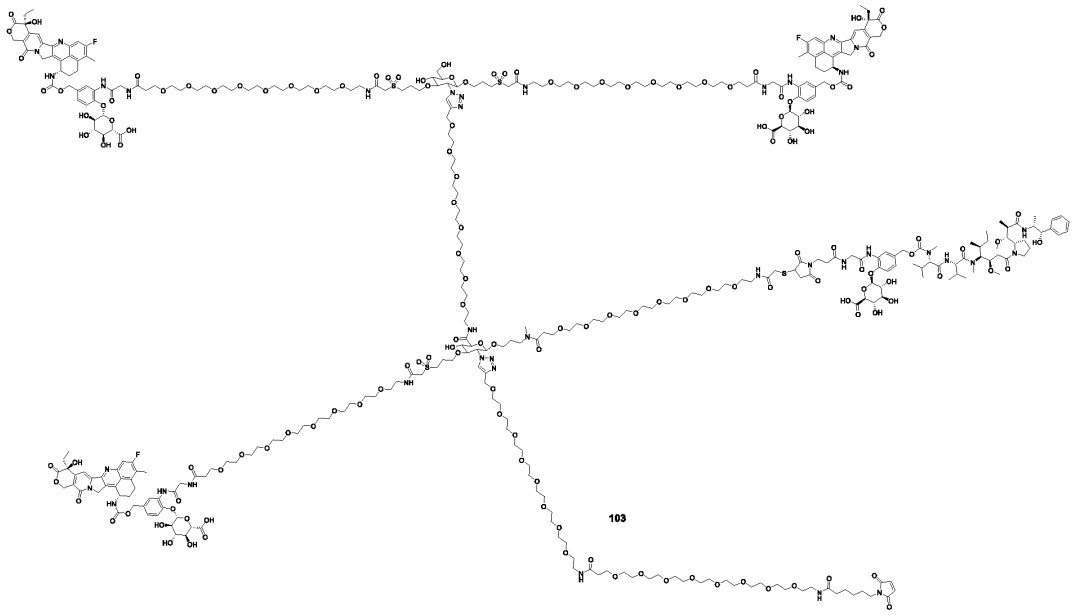
[001037] Step 6: The title compound **102-6** was prepared in analogy to **Example 96, Step 5** by using compound **93-8** and compound **102-5**. The product (45mg, yield 41% over two steps) was obtained as a yellow solid. LCMS: m/z calculated for $C_{257}H_{402}FN_{37}O_{85}S_3$: 5485.40; found: 1371.66 $[M+4H]^{4+}$.

[001038] Step 7: The title compound **102-7** was prepared in analogy to **Example 96, Step 9** by using compound **64-4** and compound **102-6**. The product (43mg, yield 65%) was obtained as a white solid. LCMS: m/z calculated for $C_{419}H_{625}F_3N_{62}O_{136}S_7$: 8988.32; found: 1498.65 $[M+6H]^{6+}$.

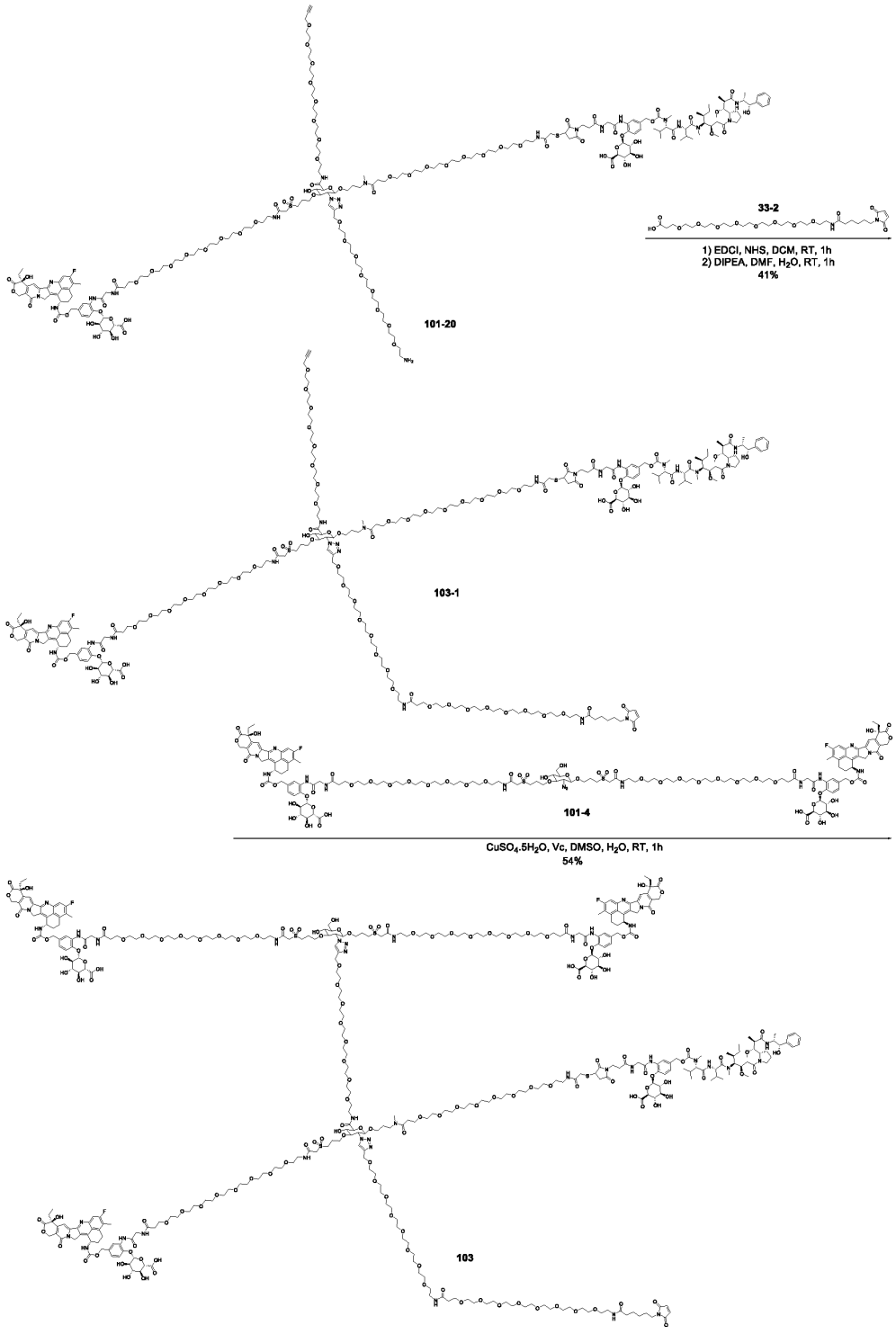
[001039] Step 8: The title compound **102** was prepared in analogy to **Example 96, Step 10** by using compound **102-7**. The product (5mg, yield 13%) was obtained as a white solid. LCMS: m/z calculated for $C_{404}H_{615}F_3N_{62}O_{134}S_7$: 8766.08; found: 1462.66 $[M+6H]^{6+}$.

Example 103. Synthesis of (2*S*,3*S*,4*S*,5*R*,6*S*)-6-[2-[[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[3-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-3-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]sulfonylpropoxy]-6-[3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[1-[3-[[2-[2-[(2*S*,3*R*,4*S*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-5-[[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-

carbamoyl]oxymethyl]anilino]-2-oxo-ethyl]amino]-3-oxo-propyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl-methyl-amino]propoxy]-5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-3-hydroxy-tetrahydropyran-2-carbonyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-4-yl]oxypropylsulfonyl]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]acetyl]amino]-4-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]carbamoyloxymethyl]phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid (103)



Synthetic scheme 103

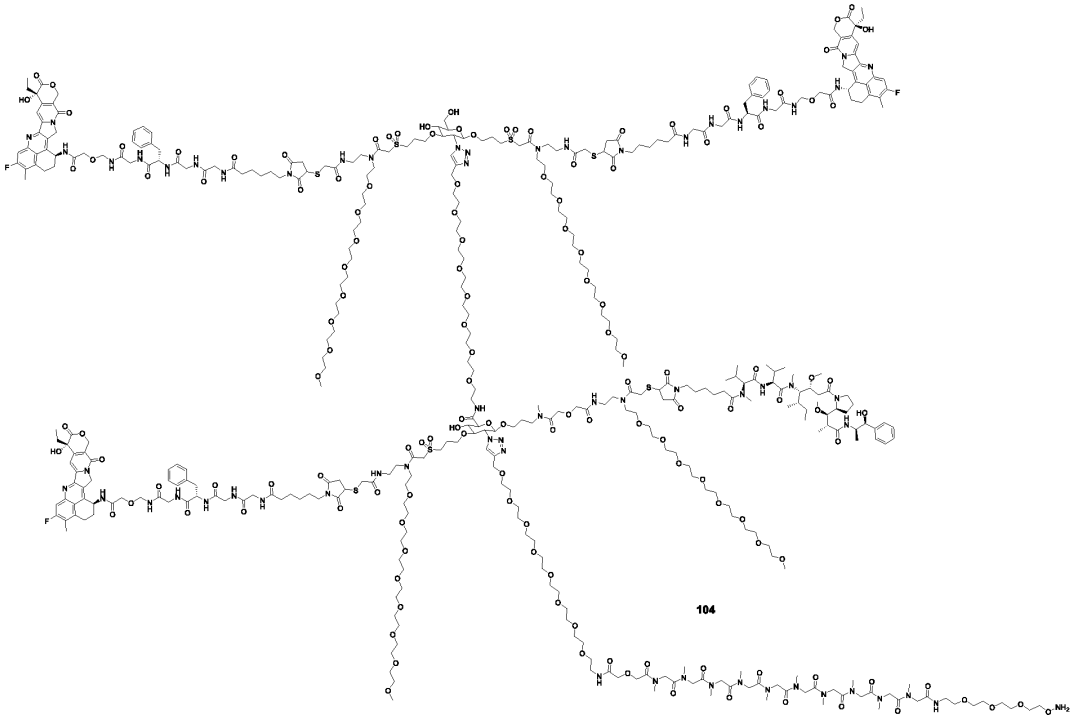

33-2
1) EDCI, NHS, DCM, RT, 1h
2) DIPEA, DMF, H2O, RT, 1h
41%
101-20
103-1
101-4
CuSO4.5H2O, Vc, DMSO, H2O, RT, 1h
54%
103

[001040] Step 1: The title compound **103-1** was prepared in analogy to **Example 18**, **Step 9** and **Example 18**, **Step 10** by using compound **33-2** and compound **101-20**. The product (20.00 mg, yield 41%) was obtained as a light-yellow solid. LCMS: m/z calculated for $C_{224}H_{350}FN_{23}O_{89}S_2$: 4872.45; found: 1218.80 $[M+4H]^+$.

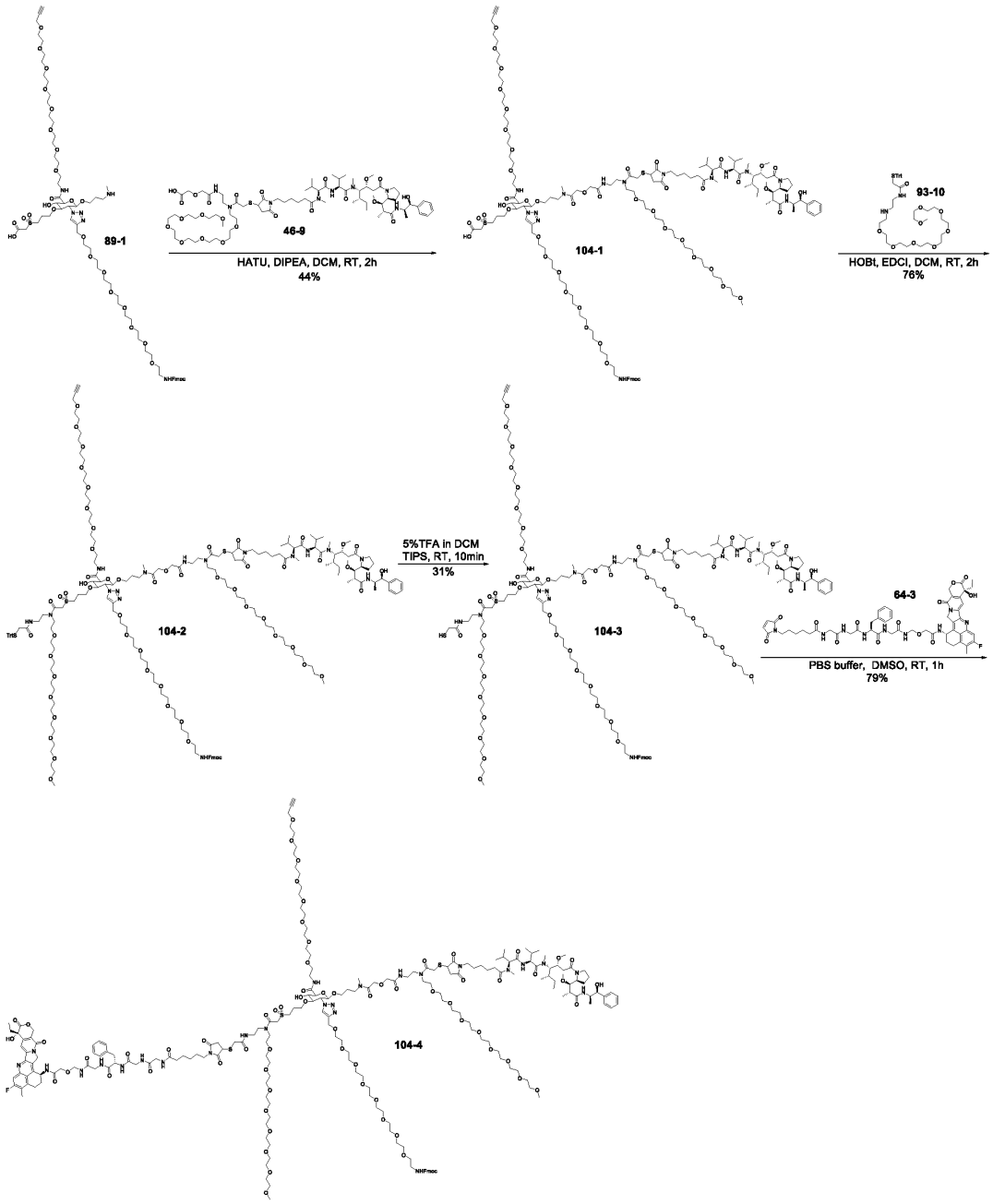
[001041] Step 2: The title compound **103** was prepared in analogy to **Example 1**, **Step 24** by using compound **101-4** and compound **103-1** The product (17.60 mg, yield 54%) was obtained as a yellow solid. LCMS: m/z calculated for $C_{358}H_{527}F_3N_{38}O_{146}S_4$: 7884.51; found: 1577.77 $[M+5H]^+$.

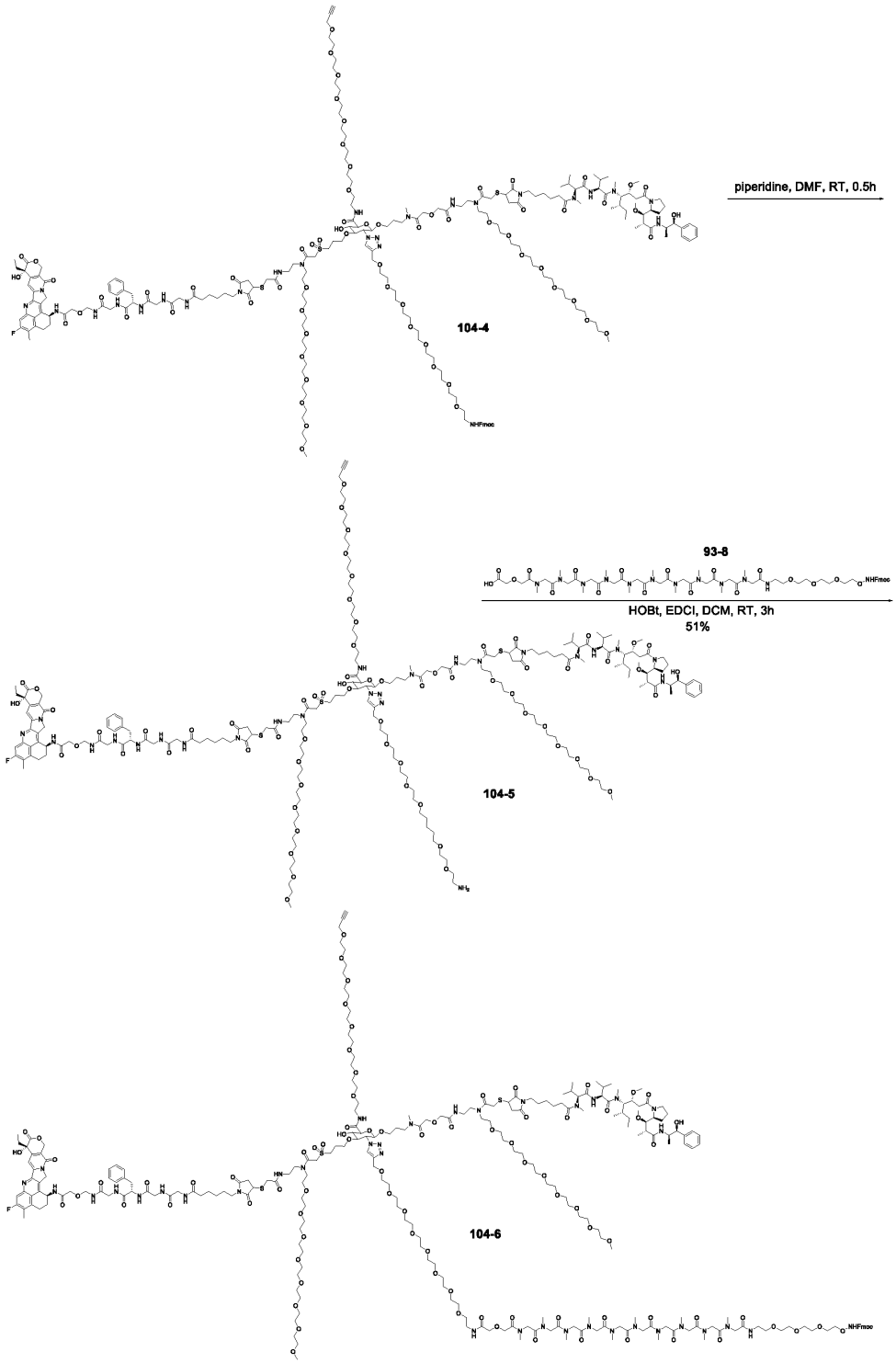
Example 104. Synthesis of 5-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[[2-[2-[2-[2-(2-aminooxyethoxy)ethoxy]ethoxy]ethylamino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethyl]-methyl-amino]-2-oxo-ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]-4-[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-ethyl]sulfonylpropoxy]-*N*-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[2,4-bis[3-[2-[2-[[2-[1-[6-[[2-[[2-[[(1*S*)-1-benzyl-2-[[2-[[2-[[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.$0^{2,14}$.$0^{4,13}$.$0^{6,11}$.$0^{20,24}$]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxo-ethoxy]methylamino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]amino]ethyl-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]-2-oxo-

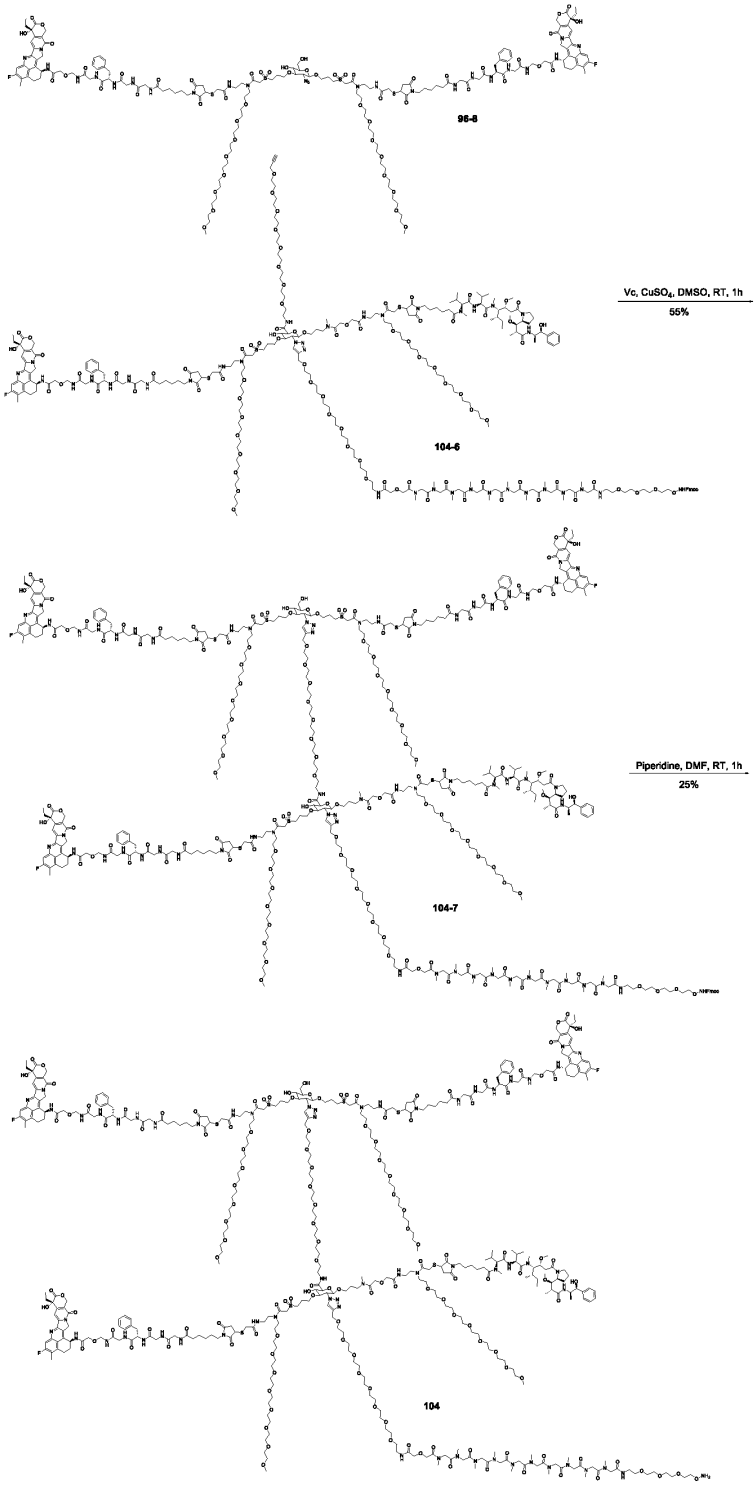
ethyl]sulfonylpropoxy]-5-hydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-hydroxy-6-[3-[[2-[2-[2-[[2-[1-[6-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]carbamoyl]-2-methyl-propyl]-methyl-amino]-6-oxo-hexyl]-2,5-dioxo-pyrrolidin-3-yl]sulfanylacetyl]-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]amino]ethylamino]-2-oxo-ethoxy]acetyl]-methyl-amino]propoxy]tetrahydropyran-2-carboxamide (104)



Synthetic scheme 104


89-1
46-9
HATU, DIPEA, DCM, RT, 2h
44%
104-1
93-10
HOBt, EDCI, DCM, RT, 2h
76%
104-2
5%TFA in DCM
TIPS, RT, 10min
31%
104-3
64-3
PBS buffer, DMSO, RT, 1h
79%
104-4


piperidine, DMF, RT, 0.5h
104-4
93-8
HOBt, EDCI, DCM, RT, 3h
51%
104-5
104-6


98-8
Vc, CuSO4, DMSO, RT, 1h
55%
104-6
Piperidine, DMF, RT, 1h
25%
104-7
104

[001042] Step 1: Under nitrogen atmosphere, a mixture of compound **46-9** (0.49 g, 0.32 mmol), DIPEA (0.12 g, 0.95 mmol) and HATU (0.13 g, 0.35 mmol) in N,N-dimethylformamide (10 mL) was stirred at room temperature for 20 min, followed by the addition of compound **89-1** (0.67g, crude) in N,N-dimethylformamide (3 mL). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was filtered and purified by reverse phase flash chromatography (C18 column, eluted with 68% acetonitrile in water，0.1%AcOH condition). The desired components were lyophilized to afford the title compound **104-1** (0.41 g, yield 44%) as a yellow oil LCMS: m/z calculated for $C_{142}H_{232}N_{14}O_{49}S_2$:2983.59; found: 1492.17 $[M+2H]^{2+}$.

[001043] Step 2: The title compound **104-2** was prepared in analogy to **Example 96, Step 1** by using compound **93-10** and compound **104-1**. The product (116 mg, 76%) was obtained as a yellow oil. LCMS: m/z calculated for $C_{182}H_{288}N_{16}O_{57}S_3$: 3708.54; found: 1236.88 $[M+3H]^{3+}$.

[001044] Step 3: The title compound **104-3** was prepared in analogy to **Example 96, Step 2** by using compound **104-2**. The product (34 mg, 31%) was obtained as a white solid. LCMS: m/z calculated for $C_{163}H_{274}N_{16}O_{57}S_3$:3466.22; found: 1156.66 $[M+3H]^{3+}$.

[001045] Step 4: The title compound **104-4** was prepared in analogy to **Example 96, Step 3** by using compound **64-3** and compound **104-3**. The product (35 mg, yield 79%) was obtained as a white solid. LCMS: m/z calculated for $C_{215}H_{330}FN_{25}O_{70}S_3$: 4500.29; found: 1500.79 $[M+3H]^{3+}$.

[001046] Step 5: The title compound **104-5** was prepared in analogy to **Example 96, Step 4** by using compound **104-4**. The product (52 mg, crude) was obtained as a yellow solid. LCMS: m/z calculated for $C_{201}H_{322}FN_{25}O_{67}S_3$: 4276.07; found: 1426.89 $[M+3H]^{3+}$.

[001047] Step 6: The title compound **104-6** was prepared in analogy to **Example 96, Step 5** by using compound **93-8** and compound **104-5**. The product (22 mg, yield 51% over two steps) was obtained as a white solid. LCMS: m/z calculated for $C_{257}H_{402}FN_{37}O_{87}S_3$:5517.39; found: 1380.15 $[M+4H]^{4+}$.

[001048] Step 7: The title compound **104-7** was prepared in analogy to **Example 96, Step 9** by using compound **96-8** and compound **104-6**. The product (20 mg, yield 55%) was obtained as a white solid. LCMS: m/z calculated for $C_{419}H_{625}F_3N_{62}O_{142}S_7$: 9084.32; found: 1515.00 $[M+6H]^{6+}$.

[001049] Step 8: The title compound **104** was prepared in analogy to **Example 96, Step 10** by using compound **104-7**. The product (5 mg, yield 25%) was obtained as a white solid. LCMS: m/z calculated for $C_{404}H_{615}F_3N_{62}O_{140}S_7$: 8862.07; found: 1477.86 $[M+6H]^{6+}$.

Example 105. General Conjugation procedure of SL to monoclonal Antibody

[001050] The general conjugates of SL's and monoclonal antibodies were formed using reported conjugation condition (Doronina, *et al.* **2003** *Nature Biotechnology* 21(7):778-84, Method A). The mAbs were reduced with TCEP and then alkylated with the maleimido-containing SL's forming the corresponding ADC conjugates (**Table 1.1**). Using trastuzumab in the above conjugation method, ADCs were produced as shown in **Table 1.2**. The hydrophobic interaction chromatography (HIC) chromatographs and size exclusion high-performance liquid chromatography (SEC-HPLC) chromatographs were shown in the figures.

Table 1.1

ADC#	mAb	SL	DAR
ADC-4	Trastuzumab	2	15
ADC-7	Trastuzumab	3	20
ADC-9	Trastuzumab	4	9
ADC-10	Trastuzumab	7	5
ADC-11	Trastuzumab	6	5
ADC-3	Trastuzumab	CL2A-SN38	8
ADC-12	Trastuzumab	10	exatecan x 6 + MMAE x 6
ADC-12	Trastuzumab	10	exatecan x 4 + MMAE x 4
ADC-13	Trastuzumab	11	16
ADC-14	Trastuzumab	mc-vc-PAB-MMAE	5
ADC-15	Trastuzumab	12	5.5
ADC-16	Trastuzumab	13	4
ADC-17	Trastuzumab	15	exatecan x 3.5 + MMAE x 3.5
ADC-17	Trastuzumab	15	exatecan x 4 + MMAE x 4
ADC-18	Trastuzumab	16	5
ADC-18	Trastuzumab	16	7

ADC-19	Trastuzumab	17	8
ADC-19	Trastuzumab	17	16
ADC-20	Trastuzumab	mc-GGFG-Dxd	8
ADC-21	Trastuzumab	18	exatecan x 6.8 + MMAE x 3.4
ADC-22	Human IgG1 isotype	15	exatecan x 5 + MMAE x 5
ADC-23	Trastuzumab	21	exatecan x 10 + MMAE x 5
ADC-24	Human IgG1 isotype	21	exatecan x 8 + MMAE x 4
ADC-25	Trastuzumab	20	8

Example 106. General Conjugation method to produce homogenous drug-to-antibody ratio (DAR) 2 ADC (method B).

[001051] Relative Reconstitute Herceptin (Roche) antibody lyophilized powder according to the instructions. Dilute the reconstituted antibody to 10 mg/ml using 1x PBS (Sigma Aldrich, Phosphate buffered saline) pH 7.2-7.4. Add 1.2 molar equivalents of TCEP (commercially available from Thermo Fisher) and EDTA (ethylenediaminetetraacetic acid, commercially available from Thermo Fisher, 0.5M stock solution) to a final concentration of 2 mM, relative to the antibody. Mix the above mixture thoroughly and react at 37 °C for 1 hour to reduce interchain disulfide bonds of the antibody.

[001052] Dissolve LP using DMSO (dimethyl sulfoxide, commercially available from Energy Chemical, 99.8%). Add the LP solution to the reduced antibody reaction mixture at 3 molar equivalents relative to the antibody and react at room temperature for 30 min.

[001053] Analyze the drug-to-antibody ratio (DAR) and product distribution using HIC-HPLC. Separate and purify components with different drug-to-antibody ratios using hydrophobic interaction chromatography (HIC) on a HiScreen Butyl FF HIC column (Cytiva) at a flow rate of 1.2 mL/min at ambient temperature. Solvent A is 0.7 M potassium phosphate pH 7. Solvent B is 0.01 M potassium phosphate pH 7. Equilibrate the column with 5 column volumes of solvent A beforehand.

[001054] Mix 1 volume of ADC with 0.5 volume of 0.7 M potassium phosphate and 0.5 volume of 1.4 M potassium phosphate (pH 7.0), centrifuge to remove insoluble matter, and apply the supernatant to the column. Elute species with different DAR ratios using a linear gradient. Collect the fraction with a drug-to-antibody ratio (DAR) of 2, concentrate using a 50 kDa ultrafiltration tube (Sartorius), and exchange the buffer to a histidine-sucrose buffer (20 mM Histidine, 8% sucrose, pH 6.0, powder purchased from Pfanstiehl). Flash freeze in liquid nitrogen and store at -80 degrees Celsius. ADC conjugates are shown in **Table 1.2.** The hydrophobic interaction chromatography (HIC) chromatographs and size exclusion high-performance liquid chromatography (SEC-HPLC) chromatographs were shown in the figures.

Table 1.2

SL#	ADC#	DAR	Exatecan : MMAE
27a	ADC-25	4.8	3:1
27b	ADC-26	5.0	3:1
34	ADC-27	3.9	2:0
37	ADC-30	4.0	2:0
35	ADC-28	3.9	2:0
39	ADC-31	4.4	2:0
36	ADC-29	6.0	2:0
42	ADC-32	2.0	3:1
45	ADC-33	5.0	2:0
48	ADC-34	4.0	2:0
49	ADC-35	4.3	1:1
54	ADC-36	4.3	2:1
56	ADC-37	7.6	3:1
57	ADC-38	7.2	3:1
58	ADC-39	3.4	2:1
62	ADC-42	6.9	3:1
15	ADC-40	2.0	1:1
58	ADC-41	2.0	2:1
77	ADC-43	1.9	2:1
78	ADC-44	2.0	2:1

68	ADC-45	1.9	3:1
80	ADC-46	1.8	1:1
79	ADC-47	2.1	2:1
82	ADC-48	2.2	3:0
83	ADC-49	2.0	1:1
65	ADC-50	2.0	0:1
75	ADC-51	2.2	eribulin 1 : exatecan 1
89	ADC-52	2.0	3:1
90	ADC-53	2.0	1:1
87	ADC-54	2.2	2:1
95	ADC-55	1.9	MMAF 1 : exatecan 1
94	ADC-56	1.9	eribulin 1 : exatecan 1
97	ADC-57	2.0	1:1
99	ADC-58	2.0	1:1
98	ADC-59	6.9	doxorubicin 1 : exatecan 1
101	ADC-60	2.2	3:1
103	ADC-61	2.0	3:1

Example 107. Hydrophilicity measure by high-performance liquid chromatography (HPLC)

[001055] Relative hydrophilicity of SL1 comparing to its monomer SL2 was measured by HPLC (Valkó *et al.* **1997** *Analytical Chemistry* 69 (11), 2022–2029). As shown in **FIG. 9** and **FIG. 10**, the retention times of both SL1 and its monomer SL2 are about the same (8.05 min vs. 8.26 min), indicating that although there are three more lipophilic SN-38 drugs in the sugar core of SL1, the SL1 has the similar hydrophilicity properties as its monomer SL2. This property is important for reducing ADC aggregation and maintaining its native Ab's pharmacokinetic properties (Lyon *et al.* **2015** *Nature Biotechnology* 33, 733–735).

Example 107. *In vitro* serum stability assays

[001056] Materials: CD-1 Mouse Serum; Human Serum

[001057] Compound Information: Test Compound: 2.3 mg/mL; Propantheline bromide as Control Compound: 10.0 mM.

[001058] Assay Procedures:

1. On the day of experiment, thaw the serum under running cold tap water and centrifuge at 3000×g, 20°C for 5 minutes to remove any clots, filtered aseptic membrane at biosafety cabinet.

2. For test compound, spike aliquots of stock solution into 1x PBS to achieve the concentration (200 μg/mL) of working solution and mix thoroughly.

3. For control compound Propantheline bromide, aliquots of stock solution into MeOH to achieve the concentration (50 μM) of working solution and mix thoroughly.

4. Add 950 μL of blank serum (experimental group, n=2) and 980 μL of blank serum (positive control group) into the corresponding well of sample plates.

5. Pre-incubate the sample plates at 37°C for approximately 10 min.

6. For T0 sample plates, firstly, add 150 μL stop solution to the corresponding wells. Secondly, add 50.0 μL (experimental group) or 20 μL (positive control group) of the compound working solution to achieve final concentration Propantheline bromide aliquots 1.00 μM (positive control group) and the testing ADC 10 μg/mL (experimental group) and shake well. Finally take aliquots of 50 μL serum samples to T0 sample plate.

7. After 5, 15, 30, 60 and 120 minutes of reaction (37°C, 600 rpm) for positive control group, 0.5h, 1h, 4h, 8h, 24h, 2d, 4d and 7d of reaction (37°C, 600 rpm) for experimental group, take aliquots of 50 μL serum samples to the corresponding wells of the sample plate (add 150 μL stop solution) to terminate the reaction.

8. Shake all sample plates, and centrifuge at 3000×g for 10 minutes.

9. Add the supernatant to dilution solution and then shake for LC-MS/MS analysis.

[001059] ADCs' serum stability data is shown in **Table 2**.

Table 2

Compound Name	Matrix (Serum)	expulsion rate (T=168h,%)
ADC-23	CD-1 Mouse	4.32 (MMAE), 2.14 (Exatecan)
	Human	2.07 (MMAE), 0.810 (Exatecan)

ADC-17	CD-1 Mouse	4.11 (MMAE), 0.00 (Exatecan）
	Human	0.00 (MMAE), 0.00 (Exatecan)
ADC-16	CD-1 Mouse	19.9 (MMAE), 0.00 (DXD)
	Human	0.232 (MMAE), 0.00 (DXD)
ADC-13	CD-1 Mouse	0.00 (Exatecan)
ADC-12	CD-1 Mouse	22.4 (MMAE), 0.00 (Exatecan)
	Human	0.00 (MMAE), 0.00 (Exatecan)

Example 108. *In-vivo* mouse PK Procedure

Formulation Preparation

[001060] The test article lyophilized powder was redissolved with sterile water to obtain the stock solution. The stock solution was diluted into 0.2 mg/mL using the solvent PBS (pH 7.2-7.4) or Trehalose Polysorbate 80 pH 6.5 for dosing.

Animal Acquisition

[001061] Total of 9 male ICR mouse (CD-1), 4 to 6 weeks old, approximately 18-25 g of bodyweight, were purchased from SPF(Beijing) BIOTECHNOLOGY Co., Ltd. The animals were free access to food and water.

Dosing and Dose Level

[001062] The dose level of the test article in this study was set at 2 mg/kg.

[001063] Nine mice were selected according to body weight and randomly divided into 3 groups (G1-G3) with 3 mice per group. All animals were administered by a single caudal intravenous injection. Animals do not fast and drink freely before administration. The dosage volume was 10 mL/kg.

Sample Collection, Processing and Storage

[001064] Blood collection time points of PK in G1 group were: 5 min ± 30s after administration, D1 ± 20 min a day, D4 ± 30 min a day, a total of 3 points. If it is not collected within the specified time, it should be recorded.

[001065] Blood collection time points of PK in G2 group were as follows: 2 hr ± 5 min after administration, D2 ± 30 min and D7 ± 1 hr. If it is not collected within the specified time, it should be recorded.

[001066] Blood collection time points of PK in G3 group were: 7 hr ± 10 min after administration, D3 ± 30 min and D14 ± 1 hr. If it is not collected within the specified time, it should be recorded.

[001067] PK blood collection time point before administration: Another 3 spare mice were selected for blood collection as PK samples before administration.

[001068] Blood was collected from the mice through orbital venous plexus into a coagulation stimulating tube, and about 0.12 mL whole blood was collected at each time point. After blood collection, the coagulation stimulating tube was left for at least 10 min to completely coagulate the samples, and the samples were centrifuged at 4°C within 0.5 hr after blood collection, rotating speed was 3000 g, and centrifuged for 5 min. Serum was taken immediately after centrifugation and each blood sample was placed into a new labeled tube. The freezing tube is placed on ice, and the serum sample is frozen to the -60 ~ -90°C refrigerator as soon as possible within 1 hr (if the serum sample can not be stored in the -60 ~ -90°C refrigerator in time, it can be temporarily stored in the -10 ~ -30°C refrigerator within 1 hr, and the temporary storage time is not more than 24 h).

[001069] Blood was collected from the mice through the orbital venous plexus into the EDTA-K2 anticoagulant tube, about 0.06 mL whole blood was collected at each time point, and 20 μL whole blood was immediately added into the tube containing 60 μL acetonitrile (duplicated), vortex mixed, and within 1 hr, freeze the whole blood sample to the -60 ~ -90°C refrigerator as soon as possible (if the whole blood sample can not be stored in the -60 ~ -90°C refrigerator in time, it can be temporarily stored in the -10 ~ -30°C refrigerator within 1 h, and the temporary storage time is not more than 24 h). Samples were collected, processed and stored away from light.

[001070] Labels for whole blood and serum samples contain information such as topic number, animal number, group, substrate type, and time point.

Sample Analysis

[001071] In this study, ADC and total antibody were detected by ELISA, in which ADC was detected by anti-MMAE and/or anti-DXD and/or anti-SN-38 antibodies respectively. During

sample analysis, a standard curve containing at least 6 concentration levels of the substance to be tested is prepared for each batch and accompanied by one or two sets of 3 low, medium and high concentration quality control (QC) samples. Calibration standard samples and quality control samples were measured by double and double holes, and their average values were taken, and other samples were measured by single holes.

[001072] The concentrations of free MMAE、SN-38、DXD and/or exatecan (MsOH) in whole blood were detected by LC-MS/MS method.

Example 109. Anti-proliferation assay in NCI-N87 cell line (method 1)

Experimental Design

[001073] The plating of cells and test article treatments are shown below in the plate map.

Table 3. Plate map for cell viability assay (μM)

"	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
B	Blank	Staurosporine, top concentration: 1 μM, 3-fold serial dilution, 9 points, duplicate									Vehicle control	PBS
C												PBS
D	Blank	Test article, top concentration: 1 μM, 3-fold serial dilution, 9 points, duplicate									Vehicle control	PBS
E												PBS
F	Blank	Test article, top concentration: 1 μM, 3-fold serial dilution, 9 points, duplicate									Vehicle control	PBS
G												PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

Blank, culture medium only. Vehicle control, cells without test articles.

Test articles with different solvents used their own vehicle control for analyses.

Cell Lines and Propagation

Table 4.

Cell line	Cancer type	Vendor	Growth Properties	Medium & Serum
NCI-N87	Stomach (Gastric carcinoma)	ATCC-CRL5822	Adherent	RPMI 1640 + 10% FBS

Culture Medium

Table 5.

Culture medium or reagents	Manufacturer	Catalog#
RPMI 1640	Gibco	22400-089
RPMI 1640, Powder	Gibco	31800-022
Dulbecco's PBS	Corning	21-031-CVC
FBS	EXCELL BIO	FND500
Antibiotic-antimycotic	Gibco	15240-062
DMSO	Sigma	D2650
0.25% Trypsin-EDTA	Gibco	25200-072

[001074] The NCI-N87 cancer cells were maintained in culture conditions at 37ºC in an atmosphere of 5% CO_2 in air. The tumor cells were routinely sub-cultured. The cells growing in an exponential growth phase were harvested and counted for plating.

[001075] Cell Plating and Compound Treatment

(1). Count cells by hemocytometer with Trypan blue staining.

(2). Adjust the cell concentration to proper cell density.

(3). Plate 150 μL of cell suspension into the assay plates according to the plate map. Add 150 μL of assay medium into the Blank wells.

(4). Incubate the plates at 37°C, 5% CO_2, 95% air and 100% relative humidity overnight.

Test article stock plate preparation and Test article Treatment

[001076] ADCs:

(1). Prepare 2x working stock of ADCs

(2). Prepare 2x working medium by dissolving medium powder.

(3). Remove the cell supernatant in the assay plates.

(4). Add 75 μL of the 2x working medium and 75 μL of the 2x ADC working stock of each well into the cells in 96-well assay plate according to the plate map.

Compounds and Staurosporine:

(5). Dissolve compounds into 10mM stock with DMSO.

(6). Dilute compound stock into 0.1mM working stock and dilute other compounds' stocks into 1mM working stocks with DMSO.

(7). Add working stocks into plate with Tecan D300e Digital Dispenser. The final DMSO concentration was 0.25%.

(8). Return the assay plates into incubator and incubate for 7 days.

CellTiter-Glo Luminescent Cell Viability Assay

The procedures were performed according to the Promega CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega-G7573).

Data Analysis

[001077] Inhibition rate (IR) of the test articles were determined by the following formula: IR (%) = [1– (RLU test article – RLU blank) / (RLU control – RLU blank)]*100%. The inhibitions of different doses of test articles were calculated in Excel file, and then were used to plot inhibition curve and evaluate related parameters, such as Bottom, Top and IC50. The data were interpreted by GraphPad Prism.

(5). Dissolve compounds into 10mM stock with DMSO.

(6). Dilute compound stock into 0.1mM working stock and dilute other compounds' stocks into 1mM working stocks with DMSO.

(7). Add working stocks into plate with Tecan D300e Digital Dispenser. The final DMSO concentration was 0.25%.

(8). Return the assay plates into incubator and incubate for 7 days.

CellTiter-Glo Luminescent Cell Viability Assay

The procedures were performed according to the Promega CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega-G7573).

Data analysis

[001078] Inhibition rate (IR) of the test articles were determined by the following formula: IR (%) = [1– (RLU test article – RLU blank) / (RLU control – RLU blank)]*100%. The inhibitions of different doses of test articles were calculated in Excel file, and then were used to plot inhibition curve and evaluate related parameters, such as Bottom, Top and IC50. The data were interpreted by GraphPad Prism.

[001079] IC50 data of ADCs is shown in **Table 6.1**.

Table 6.1

Compound/ADC	NCI-N87 (nM)	Compound/ADC	NCI-N87 (nM)
SN38	D	ADC-7	D
4	D	ADC-9	D
10	C	ADC-10	D
11	D	ADC-11	C
13	D	ADC-13	C
15	D	ADC-14	D
Exatecan	D	ADC-15	D
Trastuzumab	D	ADC-16	D
MMAE	D	ADC-17	D
Deruxtecan (Dxd)	B	ADC-18	C
Trastuzumab	A		

A: >1000 nM; B: 50-1000 nM; C: 5-49 nM; D: <5 nM

Example 110. Anti-proliferation assay in NCI-N87 cell line (Method 2) and in JIMT-1

[001080] The Cell Titer-Glo (CTG) assay was used to evaluate the inhibitory effect of the ADC on tumor cell growth. The principle of the CTG assay is as follows:

[001081] The Cell Titer-Glo (CTG) assay is a bioluminescent technique based on ATP content, used to assess cell viability and proliferation. The core principle is to reflect cellular metabolic activity by quantifying the ATP concentration within living cells, thereby indirectly characterizing cell number and viability. ATP (adenosine triphosphate) is a central molecule in cellular energy metabolism, and its content is positively correlated with cell number and metabolic activity. When cells die or are damaged, ATP rapidly degrades, making ATP levels a reliable indicator of cell viability. The key components of the CTG reagent are luciferase and luciferin. In the reaction: luciferase catalyzes the oxidation of luciferin, requiring ATP as an energy source and relying on magnesium ions (Mg^{2+}) and oxygen (O_2) for participation. This reaction generates oxyluciferin and releases a light signal, with the luminescence intensity proportional to the ATP concentration. Detection wavelength: emission wavelength around 560 nm. The main peak of the luminescence reaction catalyzed by firefly luciferase is in the yellow-

green spectral range (approximately 550-570 nm), and the specific detection wavelength is usually set to 560 nm.

NCI-N87 Growth Inhibition Assay (Method 2)

[001082] In this study, NCI-N87 (HER2 overexpressing cells) were used as an *in vitro* pharmacodynamic research system. Cells were seeded at 2000 cells/well in 96-well plates and cultured in 89% RPMI1640 + 10% FBS + 1% P/S medium for 24 hours, after which the ADC was added. The concentration of ADC was 10 nM to 0.15 pM, with 4-fold dilutions. After 7 days of treatment, cell viability was measured by CTG assay.

JIMT-1 Growth Inhibition Assay

[001083] In this study, JIMT-1 (human breast cancer cells) were used as an *in vitro* pharmacodynamic research system. Cells were seeded at 2500 cells/well in 96-well plates. After 24 hours, the ADC was added. The concentration of ADC for JIMT-1 was 1000 nM to 2.56 pM, with 5-fold dilutions. After 120 hours of treatment, cell viability was measured by CTG assay. IC50 data of ADCs is shown in **Table 6.2**.

Table 6.2

LP	ADC	NCI-N87 (IC50, nM)	JIMT-1 (IC50, nM)
34	ADC-27	A	
37	ADC-30	A	
35	ADC-28	A	
39	ADC-31	C	
36	ADC-29	B	B
42	ADC-32	C	B
45	ADC-33	B	
48	ADC-34	B	
49	ADC-35	C	
58	ADC-39	C	
56	ADC-37	C	
57	ADC-38	C	
15	ADC-40	C	C

58	ADC-41	C	C
77	ADC-43	C	C
78	ADC-44	C	
80	ADC-46	C	C
79	ADC-47	C	
82	ADC-48	B	A
83	ADC-49	C	C
65	ADC-50	C	C
75	ADC-51	C	
89	ADC-52	C	C
90	ADC-53	C	C
87	ADC-54	B	C
95	ADC-55	B	B
94	ADC-56	C	B
	Enhertu	C	A
	RC48	C	C

NCI-N87: A: > 1 nM; B: 0.1-1 nM; C: < 0.1 nM; JIMT-1: A: > 100 nm, B: 1-100 nM, C: < 1 nM

Example 111. In Vivo Efficacy Study

[001084] NCI-N87 cells (human gastric cancer cells), purchased from ATCC, were cultured as a monolayer *in vitro*. The culture medium was RPMI-1640 supplemented with 10% fetal bovine serum and 0.01 mg/mL bovine insulin. The cells were cultured at 37 °C with 5% carbon dioxide. The passage ratio was 1:2 to 1:3, and the cells were passaged every 4-5 days. When the cell confluence reached 80%-90%, the cells were harvested, counted, and the cell concentration was adjusted to 5×10^7/mL (PBS:Matrigel=1:1). The prepared cells (0.1 mL/5×10^6 NCI-N87 cells) were subcutaneously injected into the right axilla of mice (BALB/c-Nude). Seven days after inoculation, the average tumor volume reached approximately 175 mm3. The mice were then randomly divided into groups of 6-8 mice per group based on tumor volume. The test article was diluted to the target concentration with 1xPBS. The required drug dose was calculated based on the mouse weight, and the drug was injected into the mice through the tail vein at a dosing volume of 10 mL/g.

[001085] The tumor diameter was measured twice a week or every other day using a vernier caliper. The tumor volume was calculated using the formula: V = 0.5a × b2, where a and b represent the long and short diameters of the tumor, respectively. The antitumor efficacy of the compound was evaluated by TGI (%) or relative tumor growth rate T/C (%). TGI (%) reflects the tumor growth inhibition rate. TGI (%) was calculated as follows: TGI (%) = [(1 - (average tumor volume at the end of dosing - average tumor volume at the start of dosing in the treated group)) / (average tumor volume at the end of treatment in the vehicle control group - average tumor volume at the start of treatment in the vehicle control group)] × 100%. The relative tumor volume (RTV) was calculated from the tumor measurement results. The formula for calculating RTV is RTV = Vt / V0, where V0 is the average tumor volume measured at the start of dosing (i.e., d0), and Vt is the average tumor volume at a certain measurement time. The TRTV and CRTV data were taken on the same day.

[001086] At the end of the experiment, the tumor weight was measured, and T/Cweight (%) was calculated. Tweight and Cweight represent the tumor weight of the treated group and the vehicle control group, respectively.

[001087] After single dosing of 3 mg/kg *iv* in the animal model at day 0, the following ADC's show efficacy greater than or equal to 100 % TGI at day 45: ADC-34, ADC-35, ADC-39, ADC-40, ADC-43, ADC-46, ADC-47, ADC-48, ADC-49, ADC-52.

[001088] Applicant's disclosure is described herein in preferred embodiments with reference to the Figures, in which like numbers represent the same or similar elements. Reference throughout this specification to "one embodiment," "an embodiment," or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment," "in an embodiment," and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

[001089] The described features, structures, or characteristics of Applicant's disclosure may be combined in any suitable manner in one or more embodiments. In the description, herein, numerous specific details are recited to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that Applicant's composition and/or method may be practiced without one or more of the specific details, or with other

methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the disclosure.

[001090] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[001091] 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

Incorporation by Reference

[001092] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

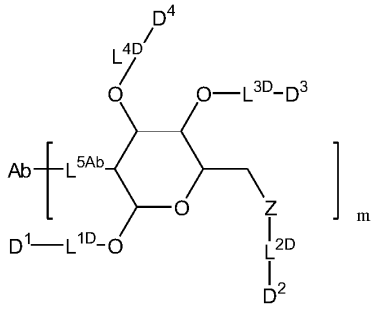
Equivalents

[001093] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is Claimed is:

CLAIMS

1. An antibody-drug conjugate having the structural formula (**I**):



(**I**)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3, 4, 5, 6, 7 or 8 hydrophilic groups;

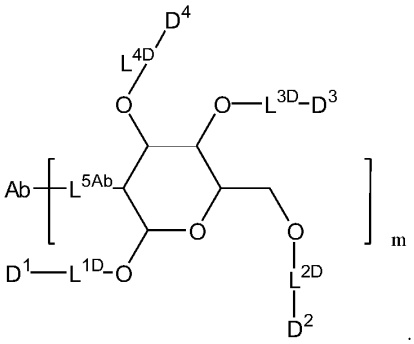
Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N; and

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

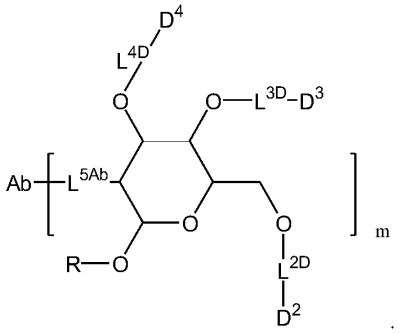
2. The antibody-drug conjugate of claim 1, wherein Z is O, having the structural formula (**I^A**):



(**I^{A}**)

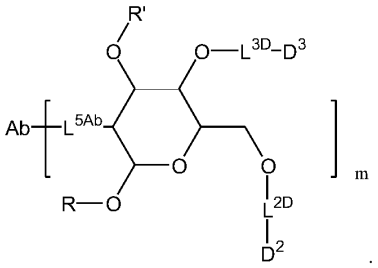
3. The antibody-drug conjugate of claim 1 or 2, wherein none of L^{1D}-D^{1}, L^{2D}-D^{2}, L^{3D}-D^{3} and L^{4D}-D^{4} is H or a C_{1-3} alkyl.

4. The antibody-drug conjugate of claim 1 or 2, wherein L^{1D}-D^{1} is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**I^{B}**):



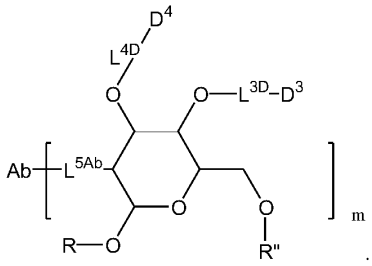
(**I^{B}**)

5. The antibody-drug conjugate of claim 4, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I^{C}**):



(**I^{C}**)

6. The antibody-drug conjugate of claim 4, wherein L^{2D}-D^2 is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**I^D**):



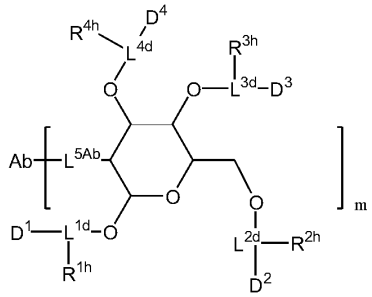
(**I^D**)

7. The antibody-drug conjugate of any one of claims 1-6, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

8. The antibody-drug conjugate of claim 7, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

9. The antibody-drug conjugate of claim 7, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

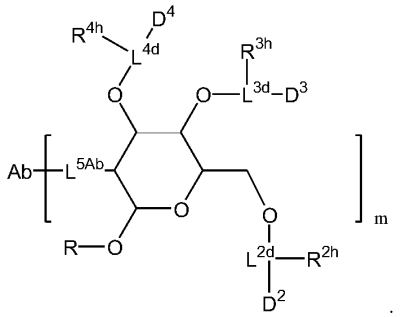
10. The antibody-drug conjugate of claim 1 or 2, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**II**):



(**II**)

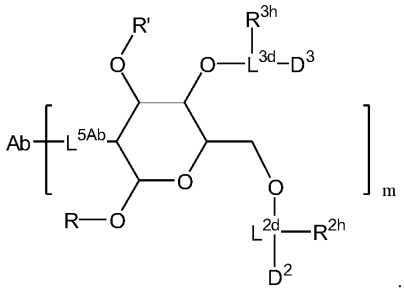
wherein L^{1d}, L^{2d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{2D}, L^{3D} and L^{4D}, respectively.

11. The antibody-drug conjugate of claim 10, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**II^A**):



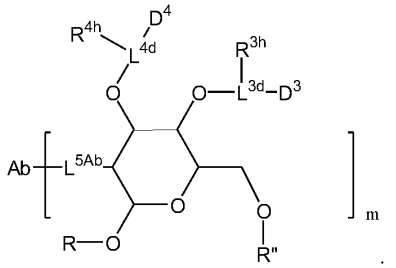
(**II^{A}**)

12. The antibody-drug conjugate of claim 11, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**II^{B}**):



(**II^{B}**)

13. The antibody-drug conjugate of claim 11, wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**II^{C}**):

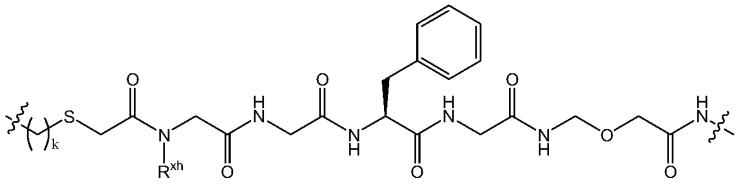


(**II^{C}**)

14. The antibody-drug conjugate of any one of claims 10-13, wherein each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups wherein each of which comprises *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

15. The antibody-drug conjugate of any one of claims 10-14, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

16. The antibody-drug conjugate of claim 15, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} is independently:



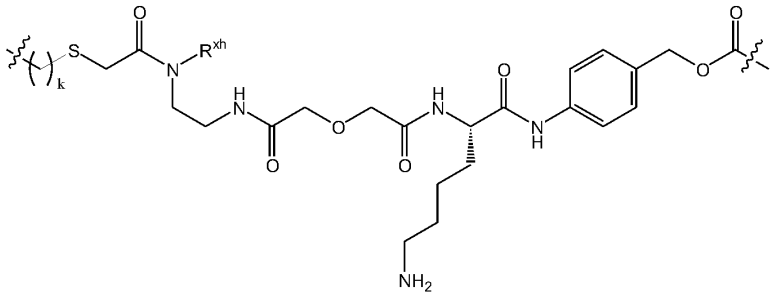
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

17. The antibody-drug conjugate of claim 15, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

18. The antibody-drug conjugate of claim 17, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

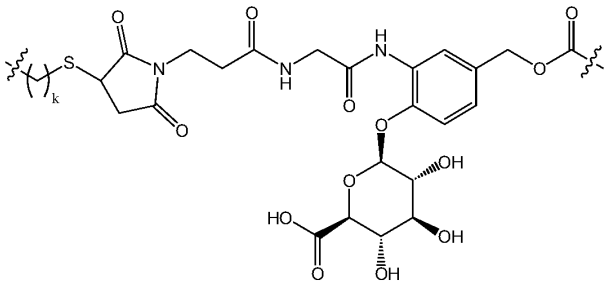


wherein

k is an integer selected from 1 and 4; and

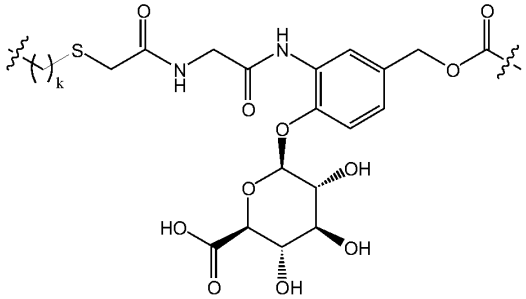
x is 1, 2, 3 or 4.

19. The antibody-drug conjugate of any one of claims 1-9, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



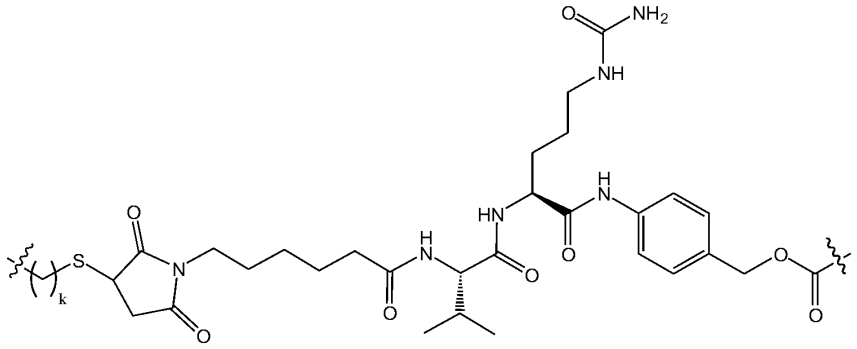
wherein *k* is an integer selected from 1 and 4.

20. The antibody-drug conjugate of any one of claims 1-9, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

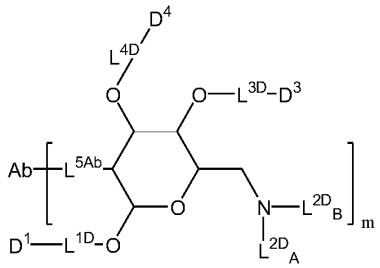
21. The antibody-drug conjugate of any one of claims 1-9, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

22. The antibody-drug conjugate of any one of claims 1-21, wherein each of D^1, D^2, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{2d}, L^{3d} and L^{4d} via a linkage selected from carbonate linkage, a carbamate linkage, and an amide linkage.

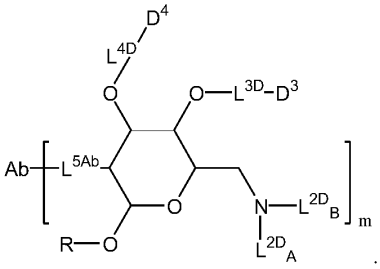
23. The antibody-drug conjugate of claim 1, wherein Z is N, D^2 is absent and L^{2D} represents L^{2D}_A and L^{2D}_B, having the structural formula (**III**):



(**III**)

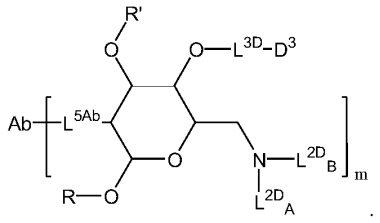
24. The antibody-drug conjugate of claim 1 or 23, wherein none of L^{1D}-D^1, L^{3D}-D^3 and L^{4D}-D^4 is H or a C_{1-3} alkyl.

25. The antibody-drug conjugate of claim 1 or 23, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**III^A**):



(**III^A**)

26. The antibody-drug conjugate of claim 25, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I^B**):



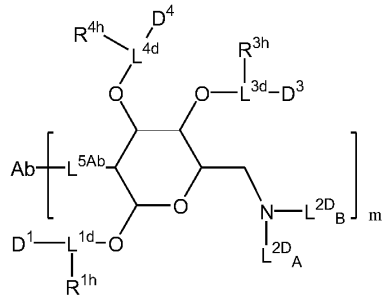
(**III^B**)

27. The antibody-drug conjugate of any one of claims 23-26, wherein each of L^{1D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

28. The antibody-drug conjugate of claim 27, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

29. The antibody-drug conjugate of claim 27, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

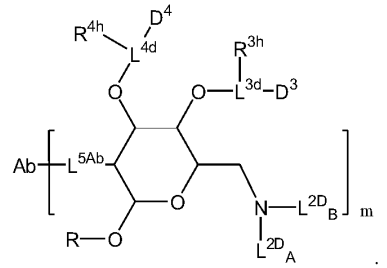
30. The antibody-drug conjugate of claim 1 or 23, wherein each of L^{1D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IV**):



(**IV**)

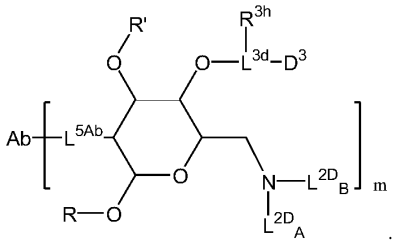
wherein L^{1d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{3D} and L^{4D}, respectively.

31. The antibody-drug conjugate of claim 30, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**IV^A**):



(**IV^A**)

32. The antibody-drug conjugate of claim 31, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**IV^B**):

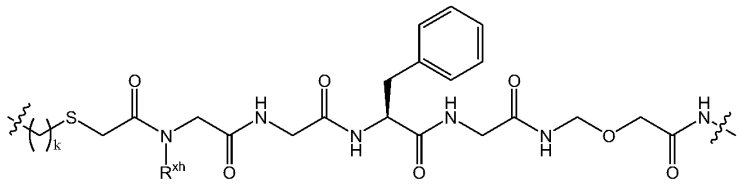


(IV^B)

33. The antibody-drug conjugate of any one of claims 30-32, wherein each of R^{1h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

34. The antibody-drug conjugate of any one of claims 30-33, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

35. The antibody-drug conjugate of claim 34, wherein one or more of L^{1d}, L^{3d} and L^{4d} is independently:



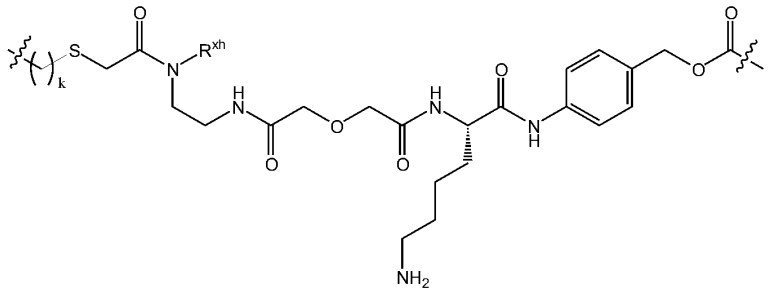
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

36. The antibody-drug conjugate of claim 34, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a lysine unit.

37. The antibody-drug conjugate of claim 36, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently is:

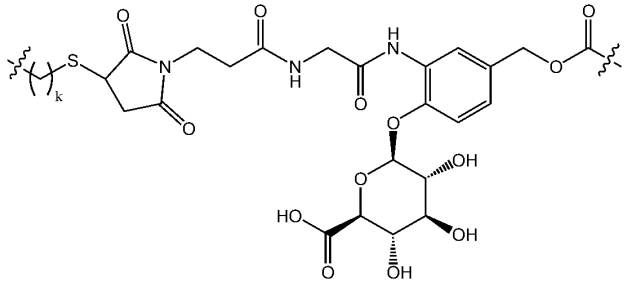


wherein

k is an integer selected from 1 and 4; and

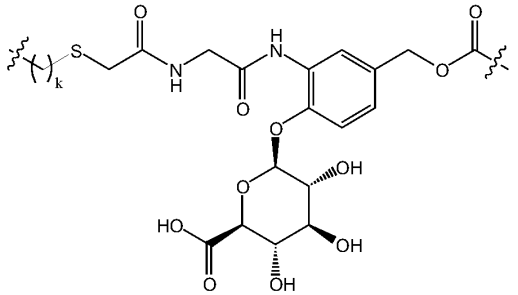
x is 1, 2, 3 or 4.

38. The antibody-drug conjugate of any one of claims 23-29, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



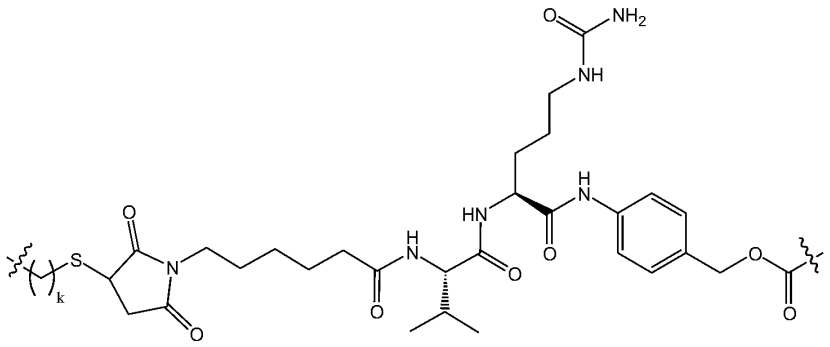
wherein *k* is an integer selected from 1 and 4.

39. The antibody-drug conjugate of any one of claims 23-29, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

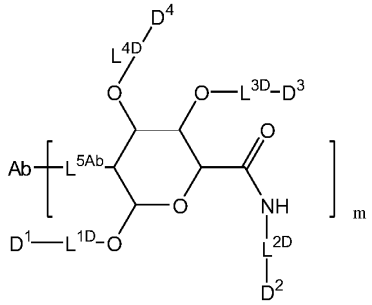
40. The antibody-drug conjugate of any one of claims 23-29, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

41. The antibody-drug conjugate of any one of claims 23-40, wherein each of D^1, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{3d} and L^{4d} via a linkage selected from a carbonate linkage, a carbamate linkage, and an amide linkage.

42. An antibody-drug conjugate having the structural formula (**I'**):



(**I'**)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group covalently attached to D^1, D^2, D^3 and D^4, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

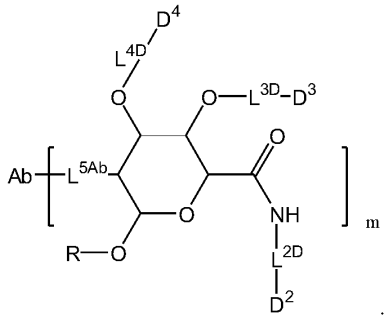
m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H, a C_{1-3} alkyl or a hydrophilic group.

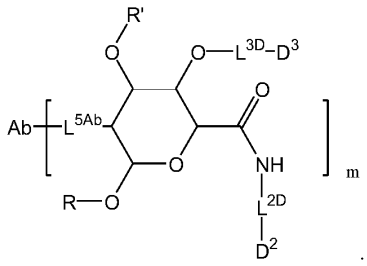
43. The antibody-drug conjugate of claim 42, wherein none of L^{1D}-D^1, L^{2D}-D^2, L^{3D}-D^3 and L^{4D}-D^4 is H or a C_{1-3} alkyl.

44. The antibody-drug conjugate of claim 42, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**I'^{B}**):



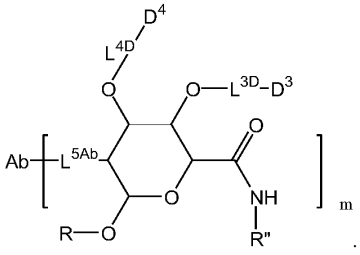
(**I'^{B}**)

45. The antibody-drug conjugate of claim 44, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**I'^{C}**):



(**I'^{C}**)

46. The antibody-drug conjugate of claim 44, wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**I'^{D}**):



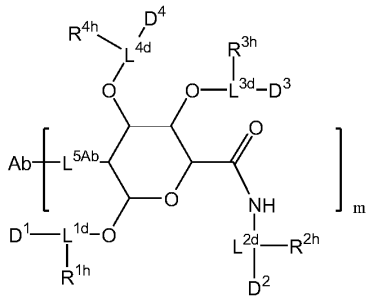
(**I'^{D}**)

47. The antibody-drug conjugate of any one of claims 42-46, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

48. The antibody-drug conjugate of claim 47, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

49. The antibody-drug conjugate of claim 47, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

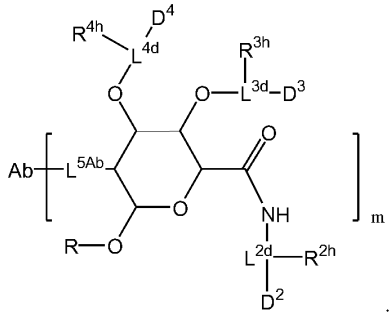
50. The antibody-drug conjugate of claim 42, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**II'**):



(**II'**)

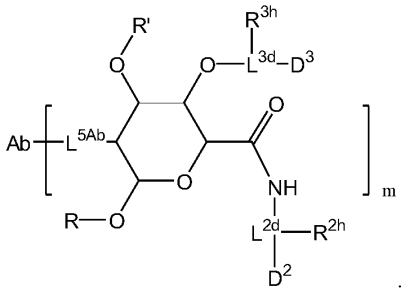
wherein L^{1d}, L^{2d}, L^{3d} and L^{4d} represents the remaining portion of L^{1D}, L^{2D}, L^{3D} and L^{4D}, respectively.

51. The antibody-drug conjugate of claim 50, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**II'^{A}**):



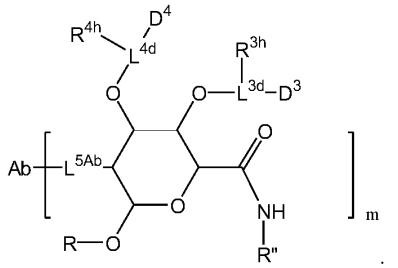
(**II'^{A}**)

52. The antibody-drug conjugate of claim 51, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**II'^{B}**):



(II'^{B})

53. The antibody-drug conjugate of claim 51, wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (II'^{C}):

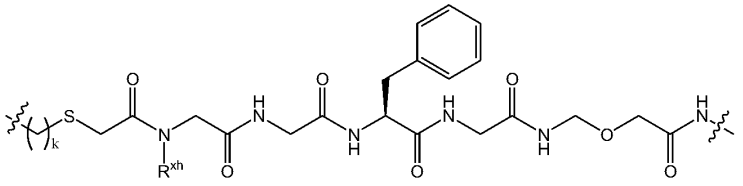


(II'^{C})

54. The antibody-drug conjugate of any one of claims 50-53, wherein each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

55. The antibody-drug conjugate of any one of claims 50-54, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

56. The antibody-drug conjugate of claim 55, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} is independently:



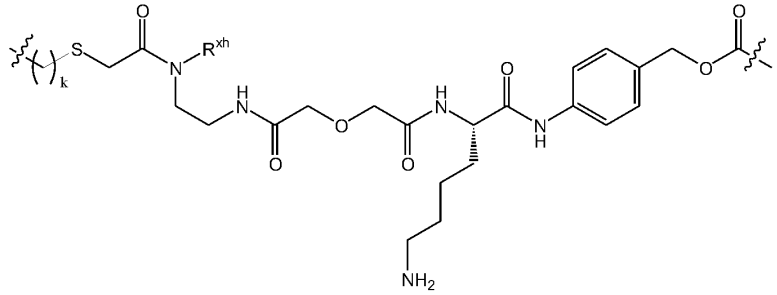
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

57. The antibody-drug conjugate of claim 55, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

58. The antibody-drug conjugate of claim 57, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

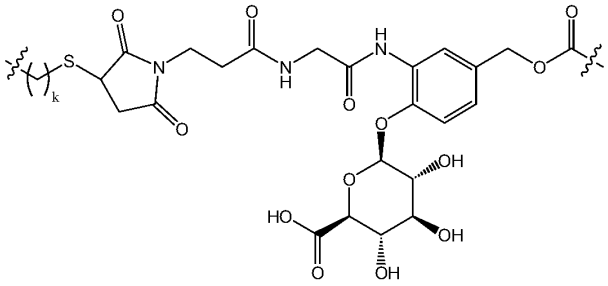


wherein

k is an integer selected from 1 and 4; and

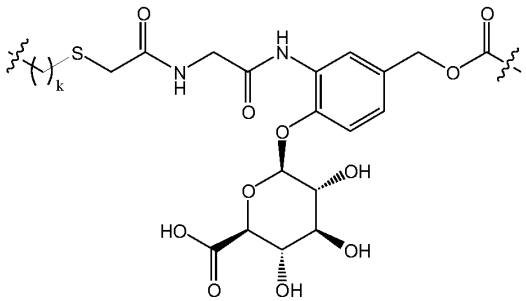
x is 1, 2, 3 or 4.

59. The antibody-drug conjugate of any one of claims 42-49, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



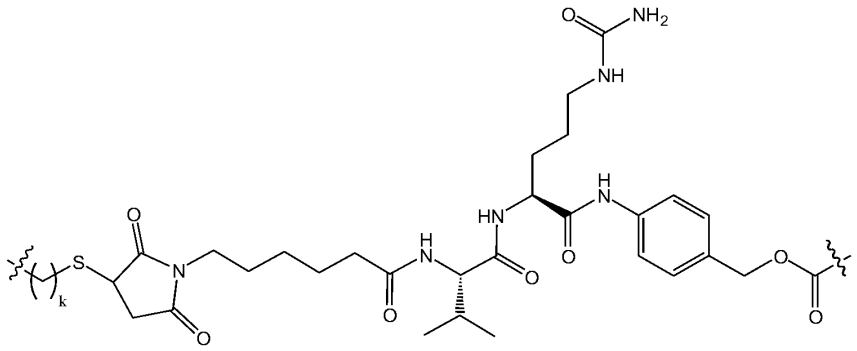
wherein *k* is an integer selected from 1 and 4.

60. The antibody-drug conjugate of any one of claims 42-49, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

61. The antibody-drug conjugate of any one of claims 42-49, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

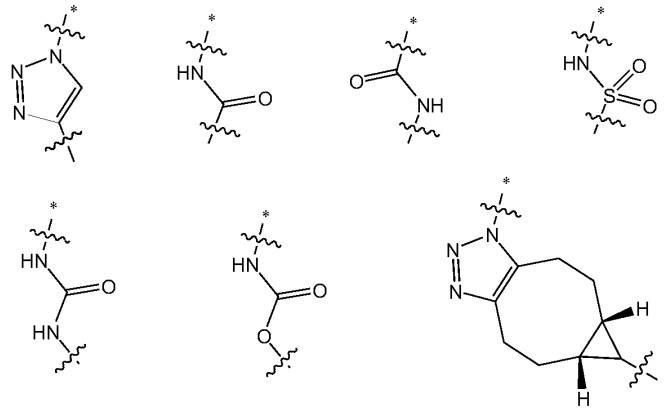
62. The antibody-drug conjugate of any one of claims 42-61, wherein each of D^1, D^2, D^3 and D^4, if present, is covalently attached respectively to L^{1d}, L^{2d}, L^{3d} and L^{4d} via a linkage selected from carbonate linkage, a carbamate linkage, and an amide linkage.

63. The antibody-drug conjugate of any one of claims 10-14, 30-33 and 50-54, wherein R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are the same.

64. The antibody-drug conjugate of any one of claims 10-14, 30-33 and 50-54, wherein R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, are not the same.

65. The antibody-drug conjugate of any one of claims 10-14, 30-33 and 50-54, wherein one or more of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, is independently $(CH_2\text{-}CH_2O)_pCH_3$, wherein *p* is an integer selected from about 4 to about 12.

66. The antibody-drug conjugate of any one of claims 1-65, wherein L^{5Ab} comprises a group selected from:



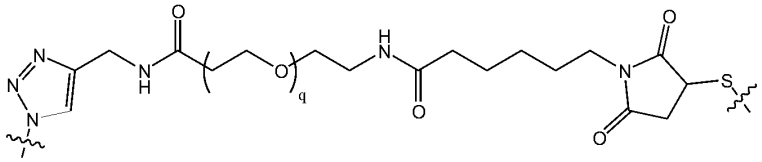
wherein * represents the attachment point to the central sugar ring of Formula (**I**).

67. The antibody-drug conjugate of any one of claims 1-66, wherein L^{5Ab} is covalently attached to the Ab via a thioether linkage.

68. The antibody-drug conjugate of claim 67, wherein L^{5Ab} comprises 1-8 hydrophilic groups.

69. The antibody-drug conjugate of claim 67 or 68, wherein the thioether linkage comprises maleimide thioether.

70. The antibody-drug conjugate of claim 69, wherein L^{5Ab} is:



wherein q is an integer selected from 1 to 6.

71. The antibody-drug conjugate of any one of claims 1-70, wherein each of D^1, D^2, D^3 and D^4, if present, is independently selected from chemotherapeutic agents and toxins.

72. The antibody-drug conjugate of any one of claims 1-71, wherein D^1, D^2, D^3 and D^4, if present, are the same.

73. The antibody-drug conjugate of any one of claims 1-71, wherein D^1, D^2, D^3 and D^4, if present, are not the same.

74. The antibody-drug conjugate of claim 73, wherein D^1, D^2, D^3 and D^4, if present, represent two distinct drug moieties.

75. The antibody-drug conjugate of any one of claims 1-74, wherein Ab is an antibody.

76. The antibody-drug conjugate of claim 75, wherein Ab is a monoclonal antibody.

77. The antibody-drug conjugate of claim 75, wherein Ab is a chimeric antibody.

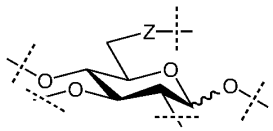
78. The antibody-drug conjugate of claim 75, wherein Ab is a humanized antibody.

79. The antibody-drug conjugate of claim 75, wherein Ab is an antibody fragment.

80. The antibody-drug conjugate of claim 75, wherein Ab is a peptide.

81. The antibody-drug conjugate of claim 75, wherein Ab binds to a receptor selected from: HER2, HER3, CD7, CD19, CD20, CD22, CD25, CD27, CD30, CD33, CD37, CD38, CD46, CD70, CD71, CD74, CD79b, CD123, CD138, CD142, CD166, CD205, CD228, CCR2, CA6, p-Cadherin, CEA, CEACAM5, C4.4a, DLL3, EGFR, EGFRVIII, ENPP3, PSMA, SLITRK6, STEAP1, TROP2, 5T4, SSEA4, GloboH, Gb5, STn, Tn, B7H3, BCMA, MUC1, cMet, ROR1 MSLN, FRa, CLDN18.2, CLDN6, EphA2, EphrinA, FLOR1, FGFR2, GCC, cKIT, LIV1, LY6E, MSLN, MUC16, NaPi2b, Nectin4, gpNMB, Axl and PTK7.

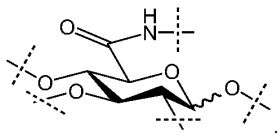
82. The antibody-drug conjugate of any one of claims 1-41, having the stereochemistry shown in Formula (**V**):



(**V**)

wherein Z is O or N.

83. The antibody-drug conjugate of any one of claims 42-62, having the stereochemistry shown in Formula (**V'**):



(**V'**)

84. An antibody-drug conjugate selected from **Table A.**

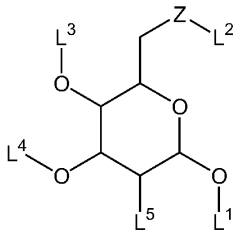
85. A pharmaceutical composition comprising an antibody-drug conjugate according to any of claims 1-84.

86. The pharmaceutical composition of claim 85, effective to treat, prevent, or reduce autoimmune disease, or a related disease or disorder.
87. The pharmaceutical composition of claim 85 or 86, effective to treat, prevent, or reduce graft rejection, or a related disease or disorder.
88. The pharmaceutical composition of claim 85 or 86, effective to treat, prevent, or reduce an allergy, or a related disease or disorder.
89. The pharmaceutical composition of claim 85 or 86, effective to treat, prevent, or reduce immunodeficiency, or a related disease or disorder.
90. The pharmaceutical composition of claim 85 or 86, effective to treat, prevent, or reduce infection, sepsis, or a related disease or disorder.
91. The pharmaceutical composition of claim 85 or 86, effective to treat, prevent, or reduce cancer, or a related disease or disorder.
92. A unit dosage form comprising a pharmaceutical composition according to any of claims 85-91.
93. A method for treating, reducing, or preventing a disease or disorder, comprising administering to a subject in need thereof an antibody-drug conjugate according to any of claims 1-84.
94. The method of claim 93, wherein the disease or disorder is selected from the group consisting of: autoimmune diseases, graft rejection, allergies, immunodeficiency, infection, sepsis, cancer, and related diseases or disorders.
95. The method of claim 94, wherein the disease or disorder is an autoimmune disease, or a related disease or disorder.
96. The method of claim 94, wherein the disease or disorder is graft rejection, or a related disease or disorder.
97. The method of claim 94, wherein the disease or disorder is allergy, or a related disease or disorder.
98. The method of claim 94, wherein the disease or disorder is immunodeficiency, or a related disease or disorder.
99. The method of claim 94, wherein the disease or disorder is infection and/or sepsis, or a related disease or disorder.

100. The method of claim 94, wherein the disease or disorder is cancer, or a related disease or disorder.
101. A method for modulating immune response, comprising administering to a subject in need thereof an antibody-drug conjugate according to any of claims 1-84.
102. A method for modulating TLR7- and/or TLR8-mediated signaling, comprising administering to a subject in need thereof an antibody-drug conjugate according to any of claims 1-84.
103. A method for of treating or reducing a condition or disorder treatable by modulation of TLR7- and/or TLR8-mediated cellular activities, comprising administering to a subject in need thereof an antibody-drug conjugate according to any of claims 1-84.
104. Use of an antibody-drug conjugate of any of claims 1-84 for treating or reducing a disease or disorder.
105. Use of an antibody-drug conjugate of any of claims 1-84, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating or reducing a disease or disorder.
106. The use of claim 104 or 105, wherein the disease or disorder is associated with TLR7- and/or TLR8-mediated signaling.
107. The use of claim 104 or 105, wherein the disease or disorder is selected from autoimmune diseases, graft rejection, allergies, immunodeficiency, infection, sepsis, cancer, or a related disease or disorder thereof.
108. The use of claim 104 or 105, to treat or reduce an autoimmune disease, or a related disease or disorder.
109. The use of claim 104 or 105, to treat or reduce graft rejection, or a related disease or disorder.
110. The use of claim 104 or 105, to treat or reduce allergy, or a related disease or disorder.
111. The use of claim 104 or 105, to treat or reduce immunodeficiency, or a related disease or disorder.
112. The use of claim 104 or 105, to treat or reduce infection and/or sepsis, or a related disease or disorder.
113. The use of claim 104 or 105, to treat or reduce cancer, or a related disease or disorder.
114. The use of claim 104 or 105, as a vaccine adjuvant.

115. A method for making an antibody-drug conjugate of any one of claims 1-84.

116. A compound having the structural formula (**VI**):



(**VI**)

wherein

each of L^1, L^2, L^3 and L^4 is independently selected from: H, C_{1-3} alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

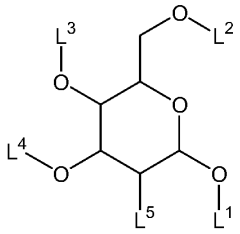
L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

Z is O or N, wherein when Z is N, L^2 represents $L^2{}_A$ and $L^2{}_B$, each is independently a hydrophilic group covalently bound to said N,

wherein

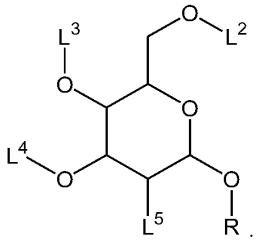
0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a hydrophilic group.

117. The compound of claim 116, wherein Z is O, having the structural formula (**VI^A**):



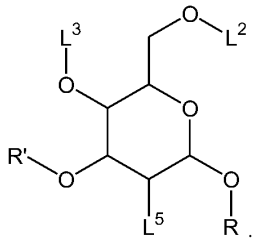
(**VI^A**)

118. The compound of claim 116 or 117, wherein L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural formula (**VI^B**):



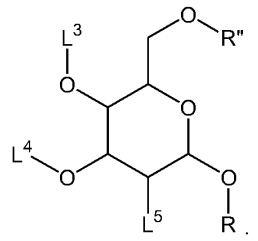
(**VI^{B}**)

119. The compound of claim 118, wherein L^4 is R', wherein R' is H or C_{1-3} alkyl, having the structural formula (**VI^{C}**):



(**VI^{C}**)

120. The compound of claim 118, wherein L^2 is R", wherein R" is H or C_{1-3} alkyl, having the structural formula (**VI^{D}**):



(**VI^{D}**)

121. The compound of any one of claims 116-120, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

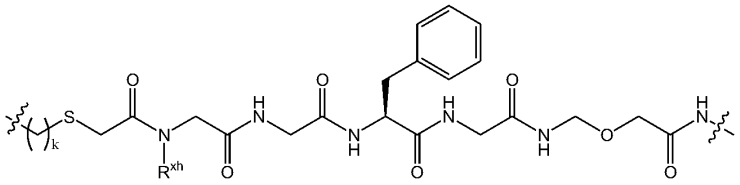
122. The compound of claim 121, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

123. The compound of claim 121, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

124. The compound of any one of claims 116-120, wherein L^4 comprises N_3.

125. The compound of any one of claims 116-120, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker.

126. The compound of claim 125, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprise:



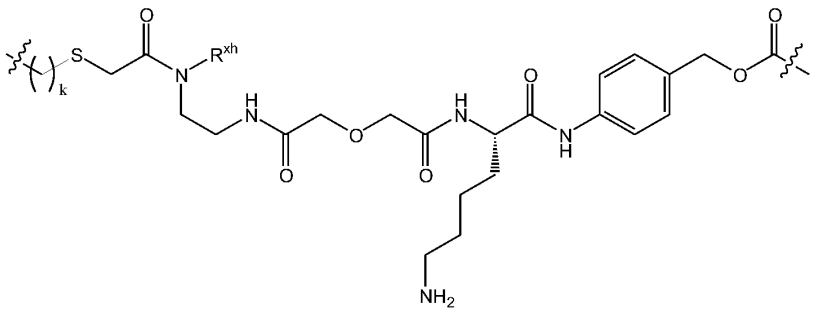
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

127. The compound of claim 125, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprises a lysine unit.

128. The compound of claim 127, wherein one or more of L^1, L^2, L^3 and L^4 independently comprises:

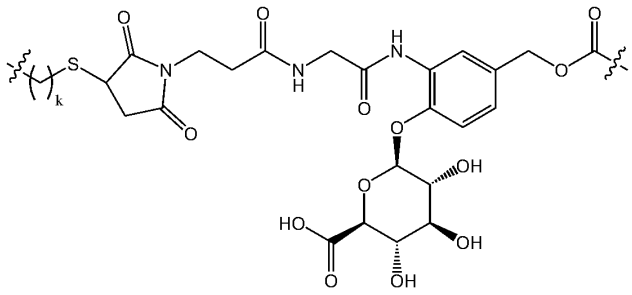


wherein

k is an integer selected from 1 and 4; and

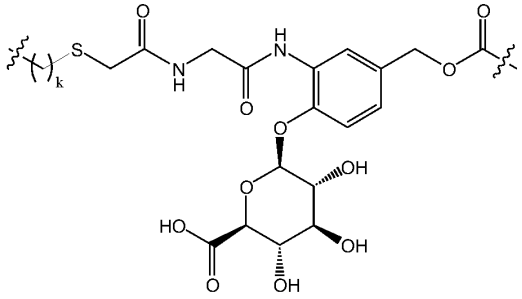
x is 1, 2, 3 or 4.

129. The compound of any one of claims 116-120, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



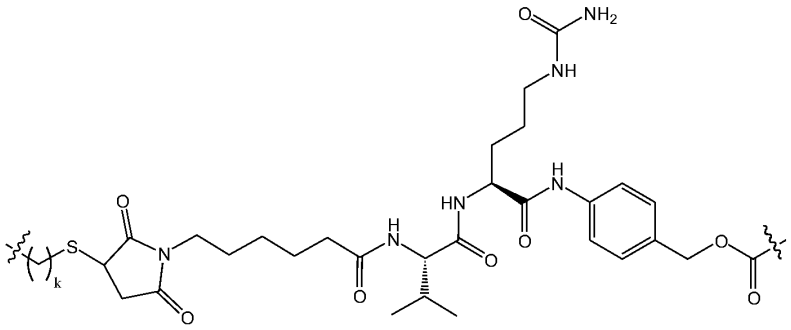
wherein *k* is an integer selected from 1 and 4.

130. The compound of any one of claims 116-120, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



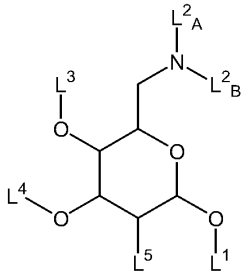
wherein *k* is an integer selected from 1 and 4.

131. The compound of any one of claims 116-120, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



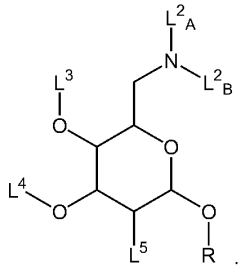
wherein *k* is an integer selected from 1 and 4.

132. The compound of claim 116, wherein Z is N, D^2 is absent and L^2 represents L^2_A and L^2_B, having the structural formula (**VII**):



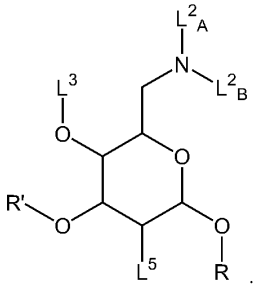
(VII)

133. The compound of claim 132, wherein L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural Formula (**VII^A**):



(VII^A)

134. The compound of claim 133, wherein L^4 is R', wherein R' is H or C_{1-3} alkyl, having the structural Formula (**VII^B**):



(VII^B)

135. The compound of any one of claims 132-134, wherein each of L^{1D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

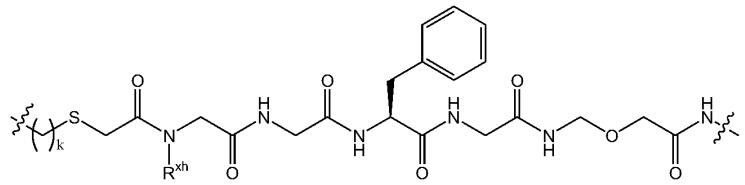
136. The compound of claim 135, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

137. The compound of claim 135, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

138. The compound of any one of claims 132-134, wherein L^4 comprises N_3.

139. The compound of any one of claims 132-138, wherein one or more of L^1, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker.

140. The compound of claim 139, wherein one or more of L^1, L^3 and L^4 if present independently comprise:



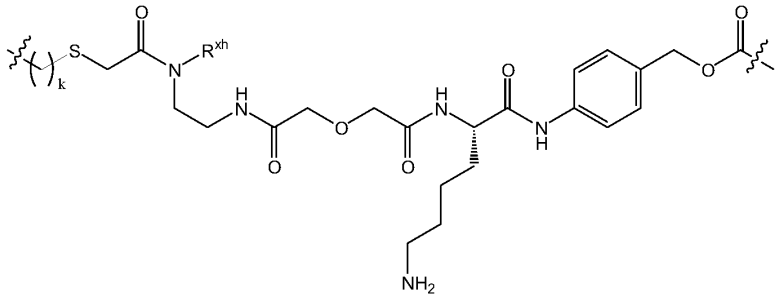
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

141. The compound of claim 139, wherein one or more of L^1, L^3 and L^4 if present independently comprises a lysine unit.

142. The compound of claim 141, wherein one or more of L^1, L^3 and L^4 independently comprises:

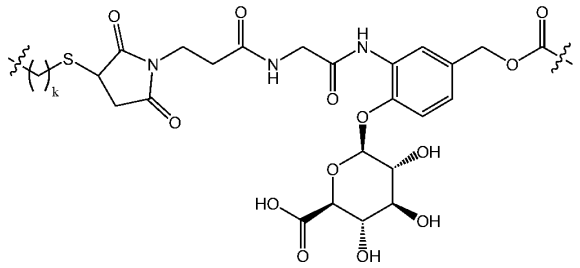


wherein

k is an integer selected from 1 and 4; and

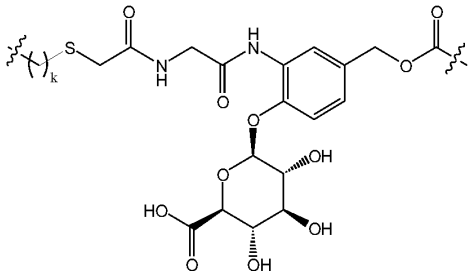
x is 1, 2, 3 or 4.

143. The compound of any one of claims 132-138, wherein one or more of L^1, L^3 and L^4, if present, comprises:



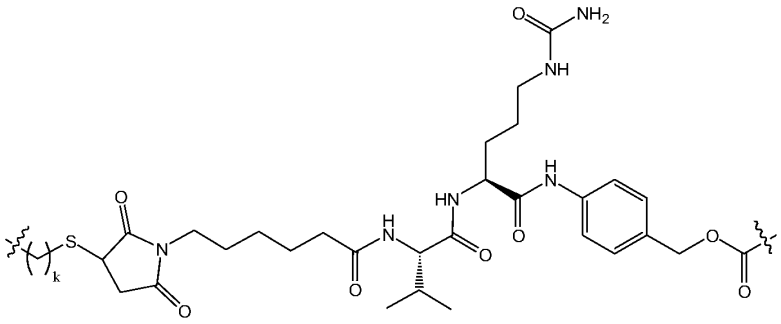
wherein *k* is an integer selected from 1 and 4.

144. The compound of any one of claims 132-138, wherein one or more of L^1, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

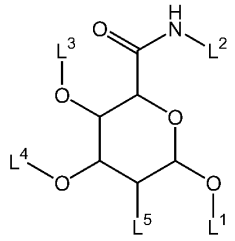
145. The compound of any one of claims 132-138, wherein one or more of L^1, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

146. The compound of any one of claims 132-145, wherein if present each of L^1, L^2, L^3 and L^4 comprises a functional group capable of forming a carbonate linkage, a carbamate linkage, or an amide linkage.

147. A compound having the structural formula (**VI'**):



(VI')

wherein

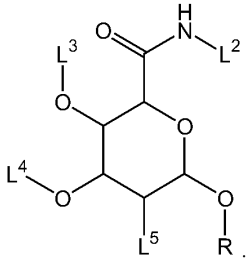
each of L^1, L^2, L^3 and L^4 is independently selected from: H, C_{1-3} alkyl, or $(CH_2)_k$-S-R_L^A, wherein R_L^A is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

L^5 is selected from: N_3 or R_L^B, wherein R_L^B is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group;

wherein

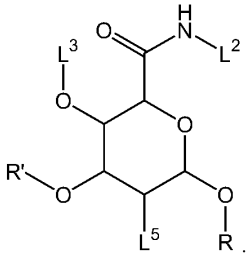
0, 1, 2 or 3 of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a hydrophilic group.

148. The compound of claim 147, wherein L^1 is R, wherein R is H or C_{1-3} alkyl, having the structural formula (**VI'^{B}**):



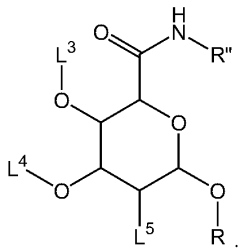
(VI'^{B})

149. The compound of claim 148, wherein L^4 is R', wherein R' is H or C_{1-3} alkyl, , having the structural formula (**VI'^{C}**):



(**VI'^{C}**)

150. The compound of claim 148, wherein L^2 is R", wherein R" is H or C_{1-3} alkyl, having the structural formula (**VI'^{D}**):



(**VI'^{D}**)

151. The compound of any one of claims 147-150, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

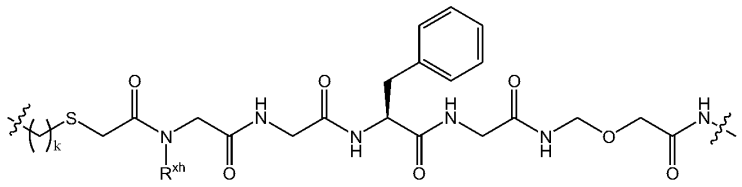
152. The compound of claim 151, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

153. The compound of claim 151, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

154. The compound of any one of claims 147-153, wherein L^4 comprises N_3

155. The compound of any one of claims 147-153, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprises a di-, tri- or tetra-peptide linker

156. The compound of claim 155, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprise:



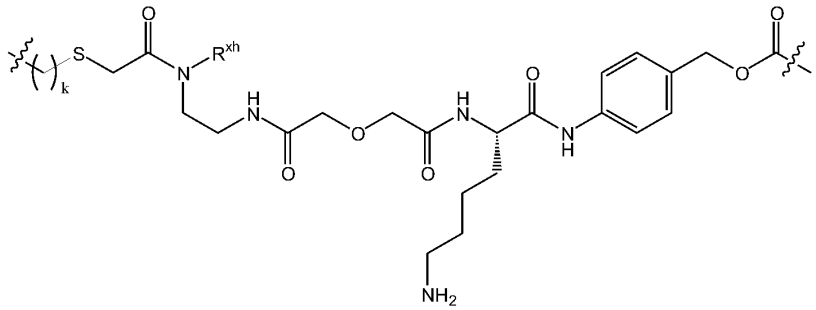
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

157. The compound of claim 155, wherein one or more of L^1, L^2, L^3 and L^4 if present independently comprises a lysine unit.

158. The compound of claim 157, wherein one or more of L^1, L^2, L^3 and L^4 independently comprises:

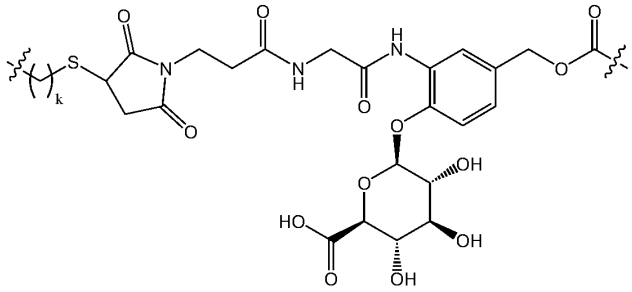


wherein

k is an integer selected from 1 and 4; and

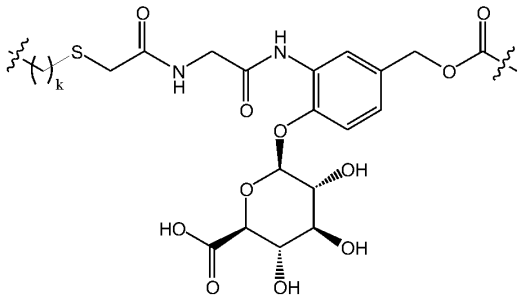
x is 1, 2, 3 or 4.

159. The compound of any one of claims 147-153, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



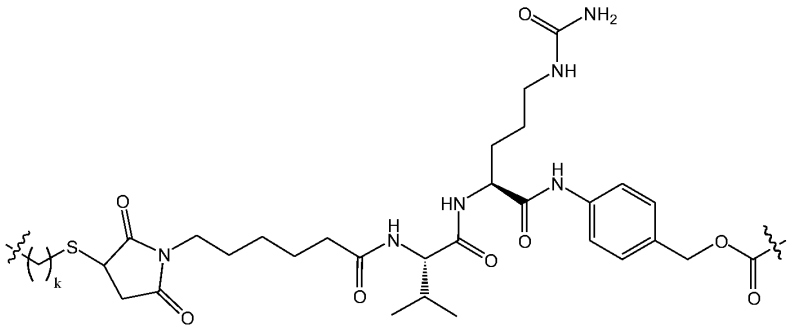
wherein *k* is an integer selected from 1 and 4.

160. The compound of any one of claims 147-153, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



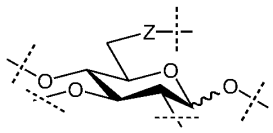
wherein *k* is an integer selected from 1 and 4.

161. The compound of any one of claims 147-153, wherein one or more of L^1, L^2, L^3 and L^4, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

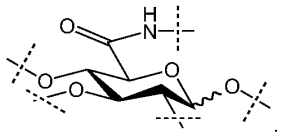
162. The compound of any one of claims 116-146, having the stereochemistry shown in Formula (**V**):



(**V**)

wherein Z is O or N.

163. The compound of any one of claims 147-161, having the stereochemistry shown in Formula (**V'**):

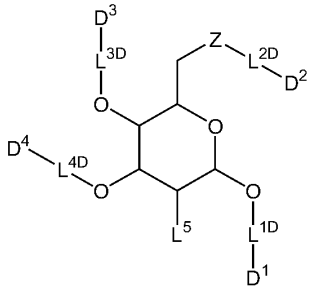


(**V'**)

165. A compound selected from **Table B**.

166. A method for making a compound of any one of claims 116-165.

167. A linker-drug conjugate having the structural formula (**VIII**):



(**VIII**)

wherein

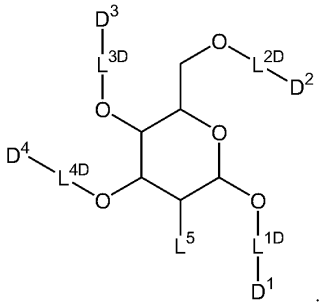
each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

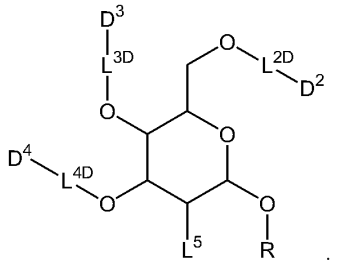
Z is O or N, wherein when Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N.

168. The linker-drug conjugate of claim 167, wherein Z is O, having the structural formula (**VIII^A**):



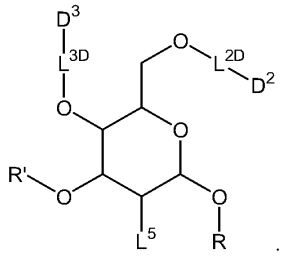
(**VIII^A**)

169. The linker-drug conjugate of claim 167 or 168, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**VIII^B**):



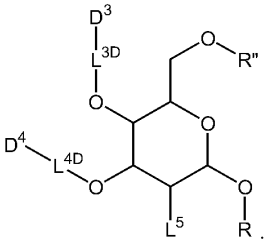
(**VIII^{B}**)

170. The linker-drug conjugate of claim 169, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**VIII^{C}**):



(**VIII^{C}**)

171. The linker-drug conjugate of claim 169, wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**VIII^{D}**):



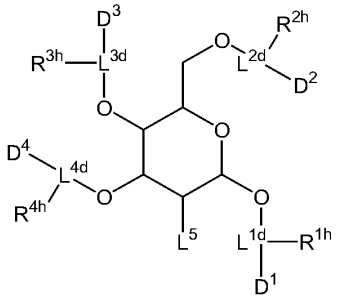
(**VIII^{D}**)

172. The linker-drug of any one of claims 167-171, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

173. The linker-drug of claim 172, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

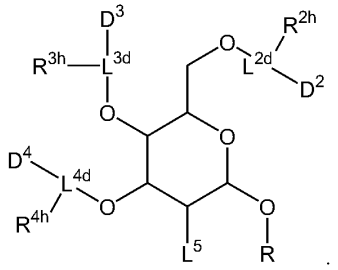
174. The linker-drug of claim 172, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

175. The linker-drug conjugate of claim 167 or 168, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IX**):



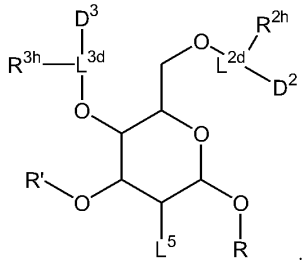
(**IX**)

176. The linker-drug conjugate of claim 175, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**IX^A**):



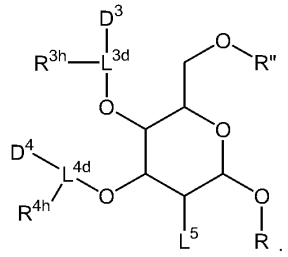
(**IX^A**)

177. The linker-drug conjugate of claim 176, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**IX^B**):



(**IX^B**)

178. The linker-drug conjugate of claim 176, wherein L^{2D}-D^2 is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**IX^C**):

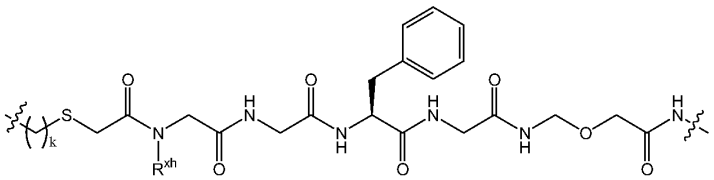


(**IX^C**)

179. The linker-drug conjugate of any one of claims 175-178, wherein each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

180. The linker-drug conjugate of any one of claims 175-178, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

181. The linker-drug conjugate of claim 180, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} if present independently is:



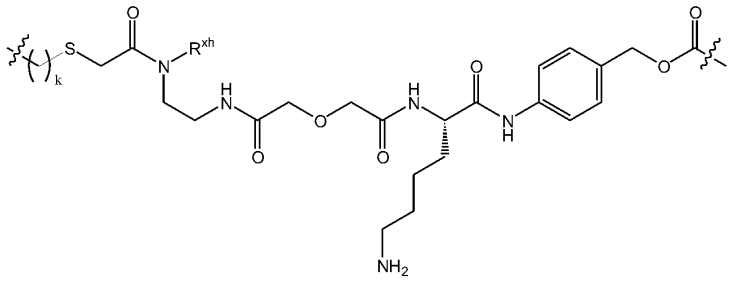
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

182. The linker-drug conjugate of claim 181, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit

183. The linker-drug conjugate of claim 182, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

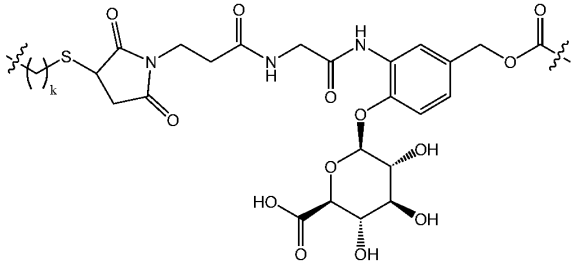


wherein

k is an integer selected from 1 and 4; and

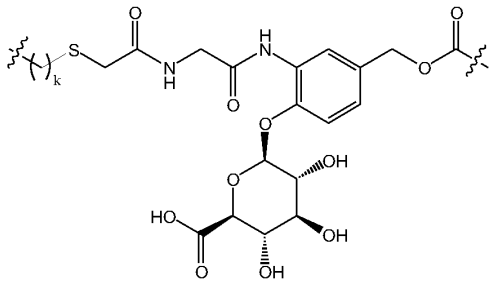
x is 1, 2, 3 or 4.

184. The linker-drug conjugate of any one of claims 175-178, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



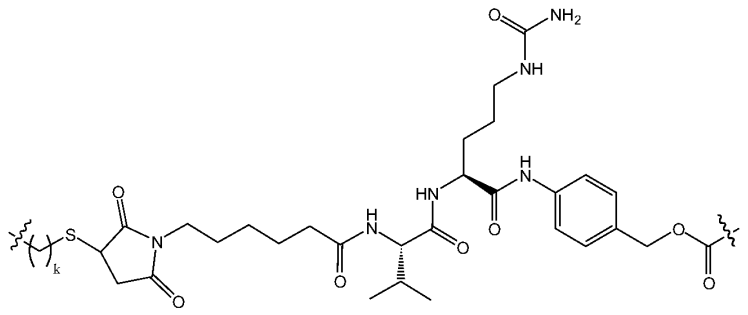
wherein *k* is an integer selected from 1 and 4.

185. The linker-drug conjugate of any one of claims 175-178, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



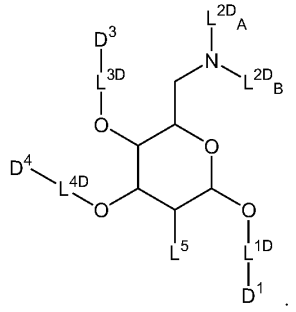
wherein *k* is an integer selected from 1 and 4.

186. The linker-drug conjugate of any one of claims 175-178, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



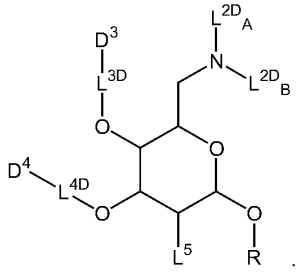
wherein *k* is an integer selected from 1 and 4.

184. The linker-drug conjugate of claim 167, wherein Z is N, D^2 is absent and L^{2D} represents $L^{2D}{}_A$ and $L^{2D}{}_B$, each is independently a hydrophilic group covalently bound to said N, having the structural formula (**X**):



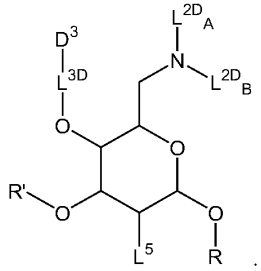
(**X**)

185. The linker-drug conjugate of claim 184, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula ($\mathbf{X^A}$):



($\mathbf{X^A}$)

186. The linker-drug conjugate of claim 185, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula ($\mathbf{X^B}$):



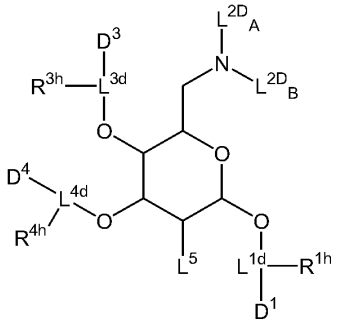
(**X^{B}**)

187. The linker-drug of any one of claims 184-186, wherein each of L^{1D}, L^{3D} and L^{4D}, if present, comprises a hydrophilic group.

188. The linker-drug of claim 187, wherein the hydrophilic group comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

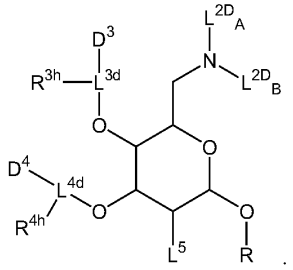
189. The linker-drug of claim 187, wherein the hydrophilic group comprises one or more polyol groups, each comprising *j* units of OH, wherein *j* is an integer in the range of about 2 to about 6.

190. The linker-drug conjugate of claim 184, wherein each of L^{1D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{3h} or R^{4h}, having the structural formula (**XI**):



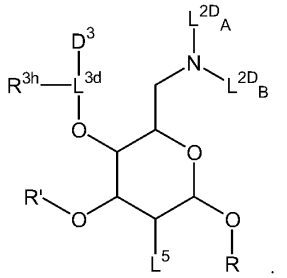
(**XI**)

191. The linker-drug conjugate of claim 190, wherein L^{1D}-D^{1} is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**XI^{A}**):



(**XI^{A}**)

192. The linker-drug conjugate of claim 191, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**XI^{B}**):

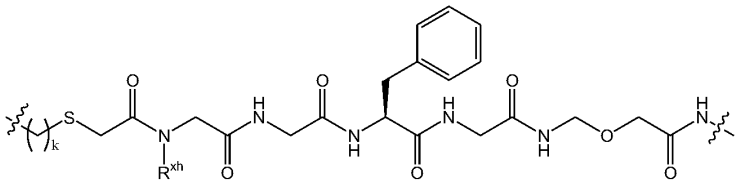


(**XI^{B}**)

193. The linker-drug conjugate of any one of claims 190-192, wherein each of R^{1h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

194. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

195. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1d}, L^{3d} and L^{4d} if present independently is:



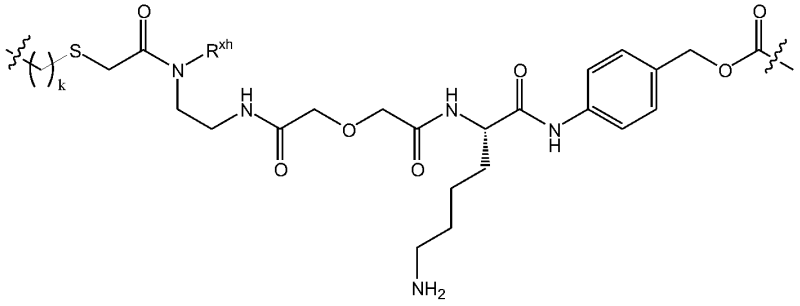
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

196. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently comprises a lysine unit.

197. The linker-drug conjugate of claim 196, wherein one or more of L^{1d}, L^{3d} and L^{4d} independently is:

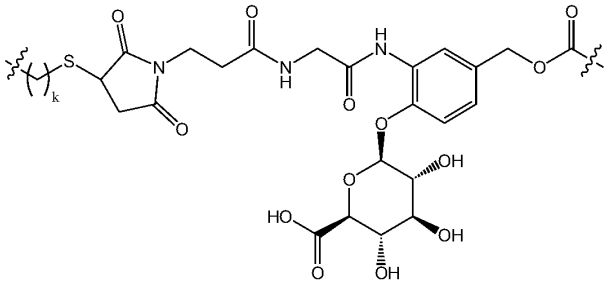


wherein

k is an integer selected from 1 and 4; and

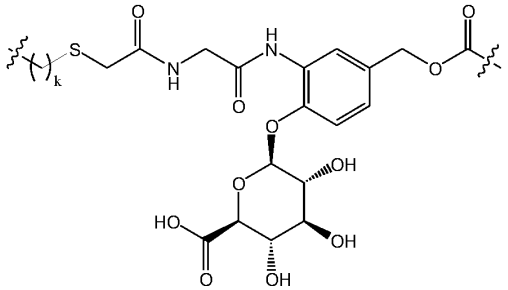
x is 1, 2, 3 or 4.

198. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



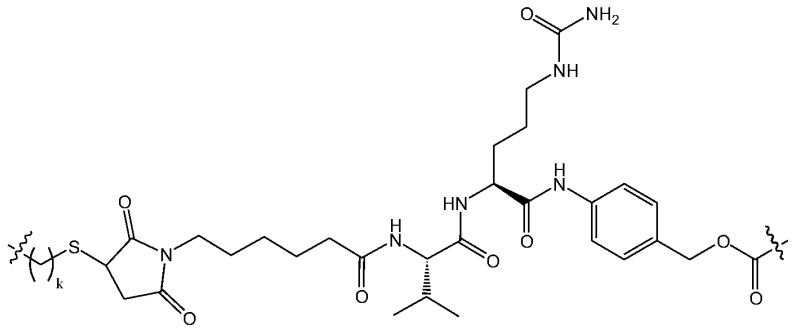
wherein *k* is an integer selected from 1 and 4.

199. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

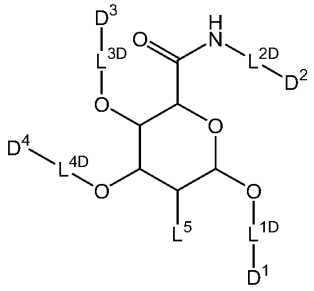
200. The linker-drug conjugate of any one of claims 190-192, wherein one or more of L^{1D}, L^{3D} and L^{4D}, if present, comprises:



wherein *k* is an integer selected from 1 and 4.

201. The linker-drug conjugate of any one of claims 190-200, wherein if present each of D^1, D^3 and D^4 is covalently attached respectively to L^{1d}, L^{3d} and L^{4d} via a carbonate linkage, a carbamate linkage, or an amide linkage.

202. A linker-drug conjugate having the structural formula (**VIII'**):



(**VIII'**)

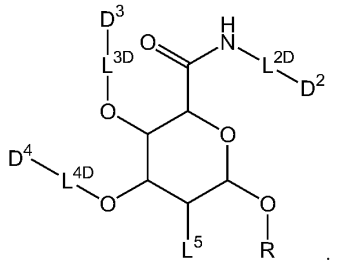
wherein

each of D^1, D^2, D^3 and D^4 is independently a drug moiety or absent;

each of L^{1D}, L^{2D}, L^{3D} and L^{4D} is independently a linker group; and

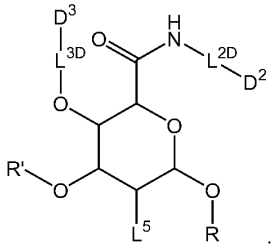
L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group.

203. The linker-drug conjugate of claim 202, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{B}**):



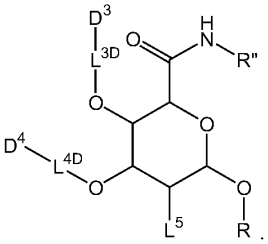
(VIII'^{B})

204. The linker-drug conjugate of claim 203, wherein L^{4D}-D^{4} is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{C}**):



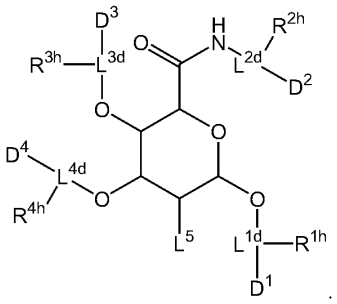
(VIII'^{C})

205. The linker-drug conjugate of claim 203, wherein L^{2D}-D^{2} is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**VIII'^{D}**):



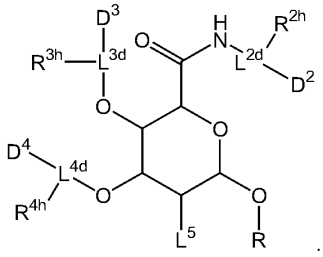
(VIII'^{D})

206. The linker-drug conjugate of claim 202, wherein each of L^{1D}, L^{2D}, L^{3D} and L^{4D} comprises a branching point and, respectively, a hydrophilic group R^{1h}, R^{2h}, R^{3h} or R^{4h}, having the structural formula (**IX'**):



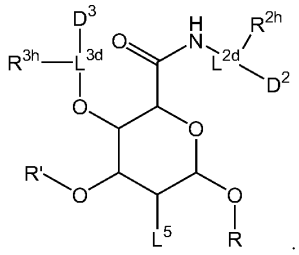
(**IX'**)

207. The linker-drug conjugate of claim 206, wherein L^{1D}-D^1 is R, wherein R is H or a C_{1-3} alkyl, having the structural formula (**IX'^{A}**):



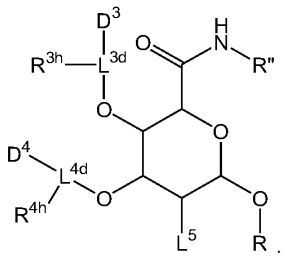
(**IX'^{A}**)

208. The linker-drug conjugate of claim 207, wherein L^{4D}-D^4 is R', wherein R' is H or a C_{1-3} alkyl, having the structural formula (**IX'^{B}**):



(**IX'^{B}**)

209. The linker-drug conjugate of claim 208, wherein L^{2D}-D^2 is R", wherein R" is H or a C_{1-3} alkyl, having the structural formula (**IX'^{C}**):

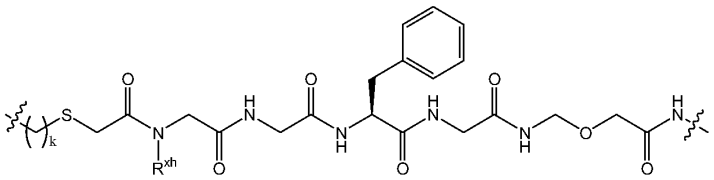


(IX'^{C})

210. The linker-drug conjugate of any one of claims 206-209, wherein each of R^{1h}, R^{2h}, R^{3h} and R^{4h}, if present, comprises one or more polyethylene glycol groups each having *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

211. The linker-drug conjugate of any one of claims 206-209, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a di-, tri- or tetra-peptide linker.

212. The linker-drug conjugate of claim 211, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} if present independently is:



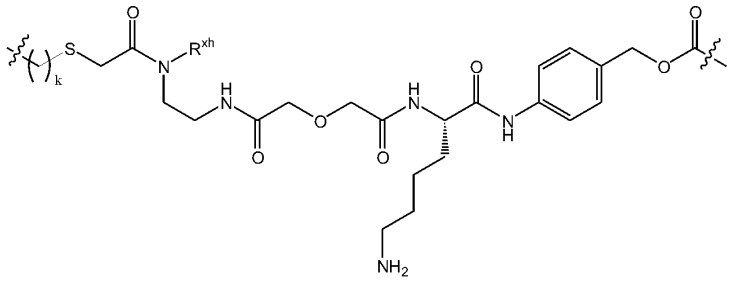
wherein

k is an integer selected from 1 and 4; and

x is 1, 2, 3 or 4.

213. The linker-drug conjugate of claim 211, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently comprises a lysine unit.

214. The linker-drug conjugate of claim 213, wherein one or more of L^{1d}, L^{2d}, L^{3d} and L^{4d} independently is:

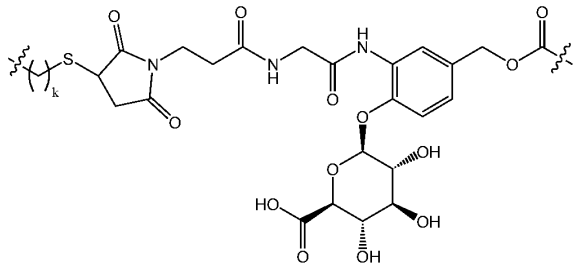


wherein

k is an integer selected from 1 and 4; and

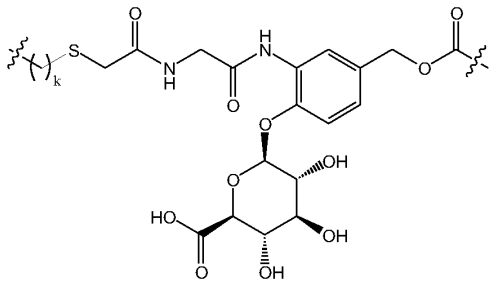
x is 1, 2, 3 or 4.

215. The linker-drug conjugate of any one of claims 206-209, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



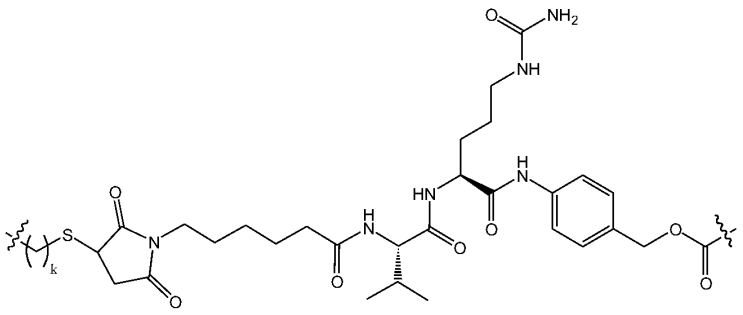
wherein *k* is an integer selected from 1 and 4.

216. The linker-drug conjugate of any one of claims 206-209, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



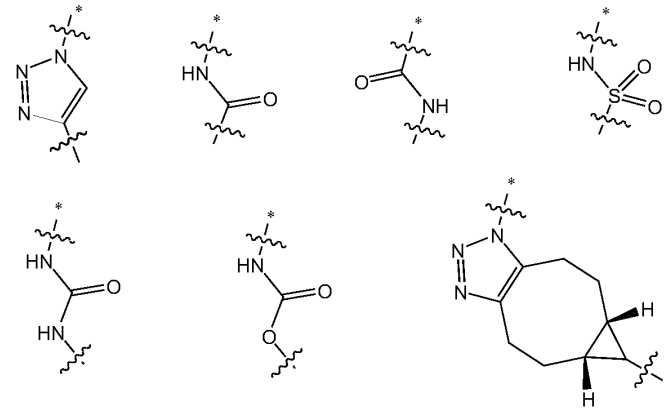
wherein *k* is an integer selected from 1 and 4.

217. The linker-drug conjugate of any one of claims 206-209, wherein one or more of L^{1D}, L^{2D}, L^{3D} and L^{4D}, if present, comprises:



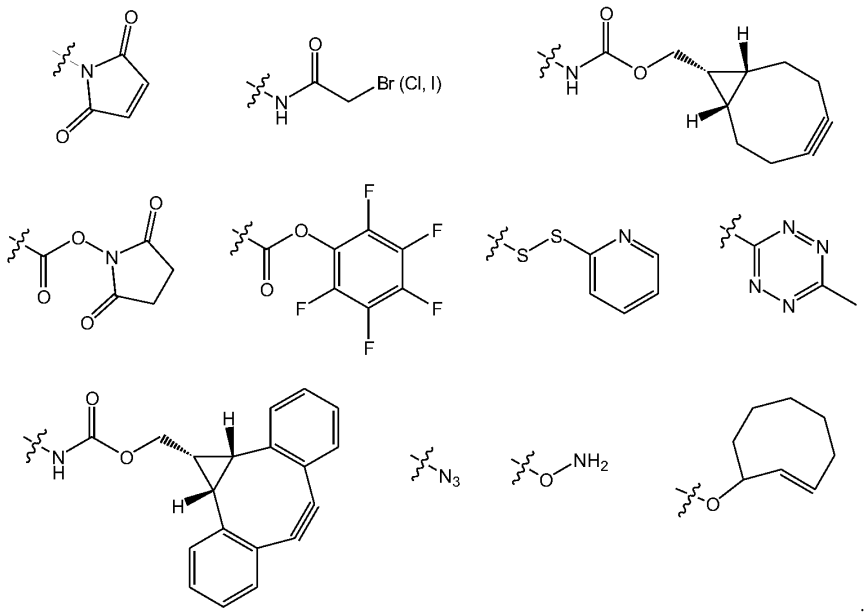
wherein *k* is an integer selected from 1 and 4.

218. The linker-drug conjugate of any one of claims 167-217, wherein L^5 comprises a group selected from:



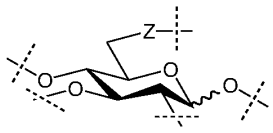
wherein * represents the attachment point to the central sugar ring of Formula (**I**).

219. The linker-drug conjugate of any one of claims 167-218, wherein L^5 comprises a group selected from:



220. The linker-drug conjugate of any one of claims 167-219, wherein L^5 comprises 1-4 hydrophilic groups.

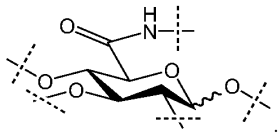
221. The linker-drug conjugate of any one of claims 167-201 and 218-220, having the stereochemistry shown in Formula (**V**):



(V)

wherein Z is O or N.

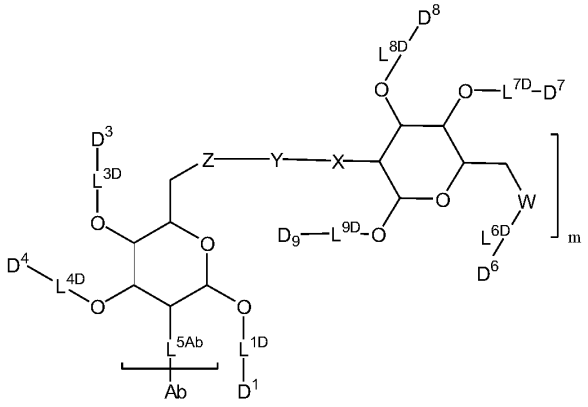
222. The linker-drug conjugate of any one of claims 202-220, having the stereochemistry shown in Formula (**V'**):



(V')

223. A linker-drug conjugate selected from **Table C**.

224. An antibody-drug conjugate having the structural formula (**XII**):



(XII)

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups;

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents L^{6D}_A and L^{6D}_B, each is independently a hydrophilic group covalently bound to said N;

each of X and Z is independently a linking group;

Y is a hydrophilic spacer group; and

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

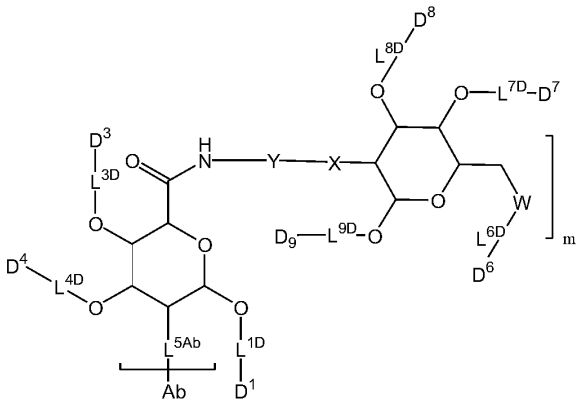
225. The antibody-drug conjugate of claim 224, wherein W is O.

226. The antibody-drug conjugate of claim 224, wherein W is N.

227. The antibody-drug conjugate of any one of claims 224-226, wherein each of X and Z is independently comprises a triazolyl group.

228. The antibody-drug conjugate of any one of claims 224-227, wherein Y comprises one or more polyethylene glycol groups each comprising *i* units of ethylene glycol, wherein *i* is an integer in the range of about 2 to about 16.

229. An antibody-drug conjugate having the structural formula (**XII'**):



(XII')

or a pharmaceutically acceptable salt thereof,

wherein

Ab is an antigen binding moiety;

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^{5Ab} is a linker group attached to Ab, wherein L^{5Ab} optionally comprises 1, 2, 3 or 4 hydrophilic groups; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

X is a linking group;

Y is a hydrophilic spacer group;

m is an integer selected from 1, 2, 3, 4, 5, 6, 7 and 8,

wherein

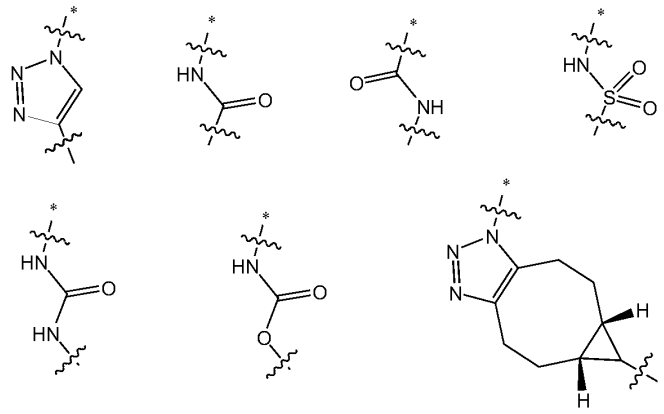
0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

230. The antibody-drug conjugate of claim 229, wherein W is O.

231. The antibody-drug conjugate of claim 229, wherein W is N.

232. The antibody-drug conjugate of any one of claims 229-231, wherein each of X and Z is independently comprises a triazolyl group.

233. The antibody-drug conjugate of any one of claims 224-232, wherein L^{5Ab} comprises a group selected from:



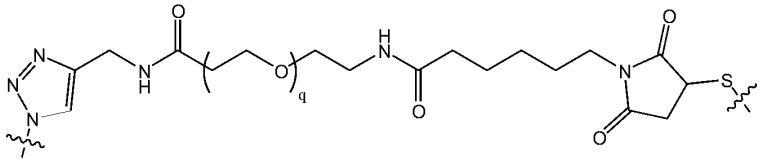
wherein * represents the attachment point to the central sugar ring of Formula (**I**).

234. The antibody-drug conjugate of any one of claims 224-232, wherein L^{5Ab} is covalently attached to the Ab via a thioether linkage.

235. The antibody-drug conjugate of claim 234, wherein L^{5Ab} comprises 1-4 hydrophilic groups.

236. The antibody-drug conjugate of claim 234 or 235, wherein the thioether linkage comprises maleimide thioether.

237. The antibody-drug conjugate of claim 236, wherein L^{5Ab} is:



wherein q is an integer selected from 1 to 6.

238. The antibody-drug conjugate of any one of claims 224-237, wherein each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, is independently selected from chemotherapeutic agents and toxins.

239. The antibody-drug conjugate of any one of claims 224-238, wherein D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, are the same.

240. The antibody-drug conjugate of any one of claims 224-238, wherein D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, are not the same.

241. The antibody-drug conjugate of claim 240, wherein D^1, D^3, D^4, D^6, D^7, D^8 and D^9, if present, represent two distinct drug moieties.

242. The antibody-drug conjugate of any one of claims 224-241, wherein Ab is an antibody.

243. The antibody-drug conjugate of claim 242, wherein Ab is a monoclonal antibody.

244. The antibody-drug conjugate of claim 242, wherein Ab is a chimeric antibody.

245. The antibody-drug conjugate of claim 242, wherein Ab is a humanized antibody.

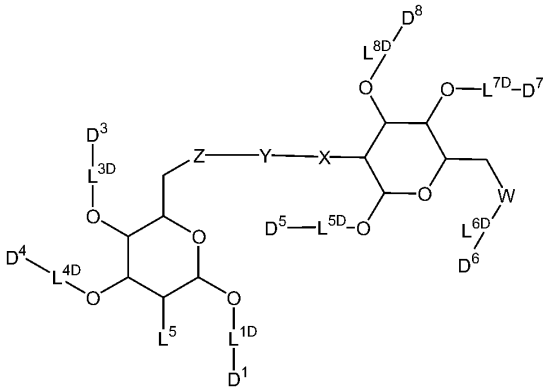
246. The antibody-drug conjugate of claim 242, wherein Ab is an antibody fragment.

247. The antibody-drug conjugate of claim 242, wherein Ab is a peptide.

248. The antibody-drug conjugate of claim 242, wherein Ab binds to a receptor selected from: HER2, HER3, CD7, CD19, CD20, CD22, CD25, CD27, CD30, CD33, CD37, CD38, CD46, CD70, CD71, CD74, CD79b, CD123, CD138, CD142, CD166, CD205, CD228, CCR2, CA6, p-Cadherin, CEA, CEACAM5, C4.4a, DLL3, EGFR, EGFRVIII, ENPP3,

PSMA, SLITRK6, STEAP1, TROP2, 5T4, SSEA4, GloboH, Gb5, STn, Tn, B7H3, BCMA, MUC1, cMet, ROR1 MSLN, FRa, CLDN18.2, CLDN6, EphA2, EphrinA, FLOR1, FGFR2, GCC, cKIT, LIV1, LY6E, MSLN, MUC16, NaPi2b, Nectin4, gpNMB, Axl and PTK7.

249. A linker-drug conjugate having the structural formula (**XIII**):



(XIII)

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

each of X and Z is independently a linking group; and

Y is a hydrophilic spacer group,

wherein

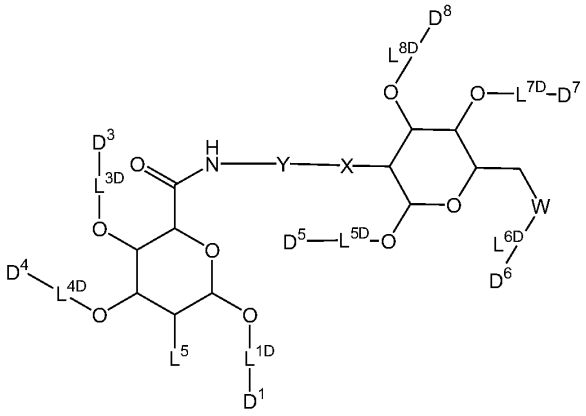
0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a $C_{1\text{-}3}$ alkyl or a hydrophilic group.

250. The linker-drug conjugate of claim 249, wherein W is O.

251. The linker-drug conjugate of claim 249, wherein W is Z.

252. The linker-drug conjugate of any one of claims 249-251, wherein each of X and Z is independently comprises a triazolyl group.

253. A linker-drug conjugate having the structural formula (**XIII'**):



(XIII')

wherein

each of D^1, D^3, D^4, D^6, D^7, D^8 and D^9 is independently a drug moiety or absent;

each of L^{1D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D} is independently a linker group covalently attached to D^1, D^3, D^4, D^6, D^7, D^8 and D^9, respectively, wherein optionally each of L^{1D}, L^{2D}, L^{3D} and L^{4D} independently comprises a hydrophilic group;

L^5 is selected from: N_3 or $R_L{}^B$, wherein $R_L{}^B$ is a linker group comprising a reactive functional group and optionally comprises a hydrophilic group; and

W is O or N, wherein when W is N, D^6 is absent and L^{6D} represents $L^{6D}{}_A$ and $L^{6D}{}_B$, each is independently a hydrophilic group covalently bound to said N;

X is a linking group; and

Y is a hydrophilic spacer group,

wherein

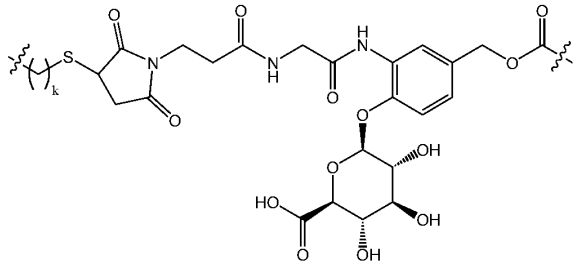
0, 1, 2 or 3 of L^{1D}-D^1, L^{3D}-D^3, L^{4D}-D^4, L^{6D}-D^6, L^{7D}-D^7, L^{8D}-D^8 and L^{9D}-D^9 is H, a C_{1-3} alkyl or a hydrophilic group.

254. The linker-drug conjugate of claim 253, wherein W is O.

255. The linker-drug conjugate of claim 153, wherein W is Z.

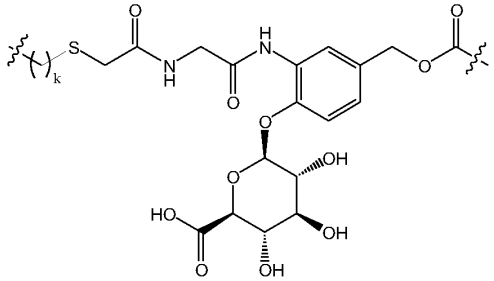
256. The linker-drug conjugate of any one of claims 253-255, wherein each of X and Z is independently comprises a triazolyl group.

257. The linker-drug conjugate of any one of claims 249-256, wherein one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



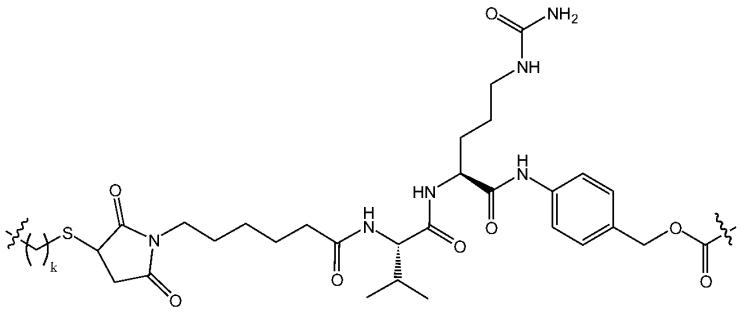
wherein *k* is an integer selected from 1 and 4.

258. The linker-drug conjugate of any one of claims 249-256, wherein one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



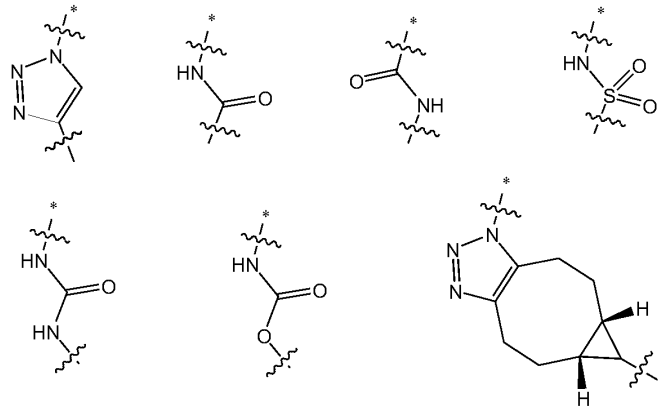
wherein *k* is an integer selected from 1 and 4.

259. The linker-drug conjugate of any one of claims 249-256, wherein one or more of L^{1D}, L^{2D}, L^{3D}, L^{4D}, L^{6D}, L^{7D}, L^{8D} and L^{9D}, if present, comprises:



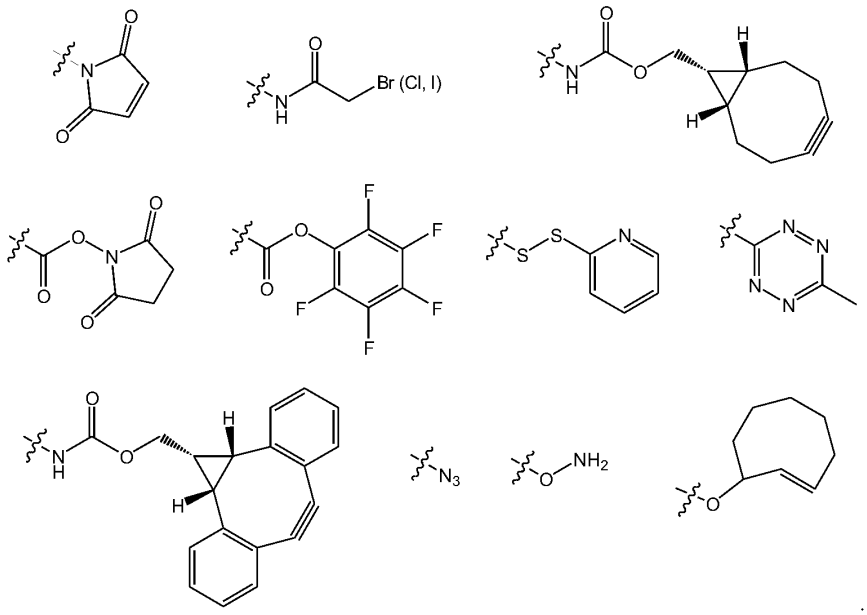
wherein *k* is an integer selected from 1 and 4.

260. The linker-drug conjugate of any one of claims 249-259, wherein L^5 comprises a group selected from:



wherein * represents the attachment point to the central sugar ring of Formula (**I**).

261. The linker-drug conjugate of any one of claims 249-259, wherein L^5 comprises a group selected from:



262. The linker-drug conjugate of any one of claims 249-261, wherein L^5 comprises 1-4 hydrophilic groups.

263. A method for making a linker-drug conjugate or antibody-drug conjugate of any one of claims 224-262.