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(54) Title: TRANS-CYCLOOCTENE CONJUGATES

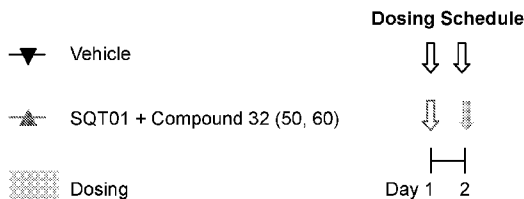
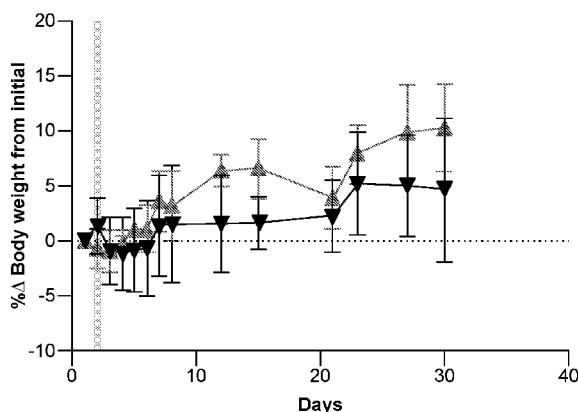


FIG. 1

(57) Abstract: The present disclosure relates generally trans-cyclooctene conjugates for bioorthogonal delivery of a payload to a targeted location in a subject. The compositions and methods have applications in the treatment of cancer, tumor growths, and immunotherapy.

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TRANS-CYCLOOCTENE CONJUGATES**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit under 35 U.S.C. §119(e) to U.S. Provisional Application Numbers 63/511,439, filed June 30, 2023, 63/511,436, filed June 30, 2023, and 63/486,233, filed February 21, 2023, each of which is hereby incorporated by reference in its entirety.

FIELD

[0002] The present disclosure relates generally trans-cyclooctene conjugates for bioorthogonal delivery of a payload to a targeted location in a subject, which conjugates have applications, e.g., in the treatment of cancer, tumor growth, and immunotherapy.

BACKGROUND

[0003] Bioorthogonal conjugation or click reactions are selective and orthogonal (non-interacting with) functionalities found in biological systems, and have found use in various applications in the fields of chemistry, chemical biology, molecular diagnostics, and medicine, where they can be used to facilitate the selective manipulation of molecules, cells, particles and surfaces, and the tagging and tracking of biomolecules *in vitro* and *in vivo*. These reactions include the Staudinger ligation, the azide-cyclooctyne cycloaddition, and the inverse-electron-demand Diels-Alder reaction.

SUMMARY

[0004] Provided herein are conjugates for use in bioorthogonal reactions, which conjugates comprise a payload covalently bonded to one or more optionally substituted trans-cyclooctene moieties via a linker. In some embodiments, the payload is a taxane, such as paclitaxel, a taxane, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof.

[0005] In some embodiments, provided is a method for delivering an effective amount of a payload (i.e., a taxane, such as paclitaxel, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof) to a target location in a subject, the method comprising administering to the subject at the target location a therapeutic support composition as described herein, and administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein. In some embodiments, the conjugate comprises one or more solubilizing groups.

[0006] In some embodiments, provided is a method for treating cancer, comprising administering to a subject in need thereof, a therapeutic support composition as described herein to a target location, and administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein.

[0007] In some embodiments, the cancer is metastatic. In some embodiments the cancer is melanoma, renal cancer, prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous

cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, Kaposi's sarcoma, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, malignant extrinsic or intrinsic airway compression, or cutaneous T-cell lymphoma.

[0008] In some embodiments, the cancer is a melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is a lymphoma or leukemia. In some embodiments, the cancer is a hematological malignancy.

BRIEF DESCRIPTION OF THE FIGURES

[0009] Figure 1 shows percent body weight changes after administering SQT01 combination with Compound 32 to CrTac:NCr-Foxn1nu mice bearing NCI-N87 Subcutaneous Xenografts. Error bars represent standard error of the mean (SEM).

[0010] Figure 2 shows tumor volume traces after administering SQT01 combination with Compound 32 to CrTac:NCr-Foxn1nu mice bearing NCI-N87 Subcutaneous Xenografts.

DETAILED DESCRIPTION

[0011] The following description sets forth exemplary embodiments of the present technology. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

[0012] It is appreciated that certain features, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace subject matter that are, for example, compounds that are stable compounds (i.e., compounds that can be made, isolated, characterized, and tested for biological activity). In addition, all sub-combinations of the various embodiments and elements thereof (e.g., elements of the chemical groups listed in the embodiments describing such variables) are also specifically embraced and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

A. Definitions

[0013] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of

the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0014] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0015] The modifier “about” used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). The modifier “about” should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression “from about 2 to about 4” also discloses the range “from 2 to 4.” The term “about” may refer to plus or minus 10% of the indicated number. For example, “about 10%” may indicate a range of 9% to 11%, and “about 1” may mean from 0.9-1.1. Other meanings of “about” may be apparent from the context, such as rounding off, so, for example “about 1” may also mean from 0.5 to 1.4.

[0016] The conjunctive term “or” includes any and all combinations of one or more listed elements associated by the conjunctive term. For example, the phrase “an apparatus comprising A or B” may refer to an apparatus including A where B is not present, an apparatus including B where A is not present, or an apparatus where both A and B are present. The phrases “at least one of A, B, . . . and N” or “at least one of A, B, . . . N, or combinations thereof” are defined in the broadest sense to mean one or more elements selected from the group comprising A, B, . . . and N, that is to say, any combination of one or more of the elements A, B, . . . or N including any one element alone or in combination with one or more of the other elements which may also include, in combination, additional elements not listed.

[0017] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, 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; Smith and March *March’s Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987; the entire contents of each of which are incorporated herein by reference.

[0018] The term “alkoxy” as used herein, refers to an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, and tert-butoxy.

[0019] The term “alkyl” as used herein, means a straight or branched, saturated hydrocarbon chain containing from 1 to 30 carbon atoms. The term “lower alkyl” or “C₁-C₆-alkyl” means a straight or branched chain hydrocarbon containing from 1 to 6 carbon atoms. The term “C₁-C₃- alkyl” means a straight or branched chain hydrocarbon containing from 1 to 3 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *n*-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, *n*-heptyl, *n*-octyl, *n*-nonyl, and *n*-decyl.

[0020] The term “alkenyl” as used herein, means a hydrocarbon chain containing from 2 to 30 carbon atoms with at least one carbon-carbon double bond. The alkenyl group may be substituted or unsubstituted. For example, the alkenyl group may be substituted with an aryl group, such as a phenyl.

[0021] The term “alkynyl,” as used herein, refers to straight or branched monovalent hydrocarbyl groups having from 2 to 30 carbon atoms, such as 2 to 20, or 2 to 10 carbon atoms and having at least 1 site of triple bond unsaturation. The term “alkyne” also includes non-aromatic cycloalkyl groups of from 5 to 20 carbon atoms, such as from 5 to 10 carbon atoms, having single or multiple rings and having at least one triple bond. Examples of such alkynyl groups include, but are not limited to acetylenyl (-C≡CH), and propargyl (-CH₂C≡CH), and cycloalkynyl moieties, such as, but not limited to, substituted or unsubstituted cyclooctyne moieties.

[0022] The term “alkoxyalkyl” as used herein, refers to an alkoxy group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein.

[0023] The term “alkylene” as used herein, refers to a divalent group derived from a straight or branched chain hydrocarbon of 1 to 30 carbon atoms, for example, of 2 to 10 carbon atoms. Representative examples of alkylene include, but are not limited to, -CH₂-, -CH(CH₃)-, -C(CH₃)₂-, -CH₂CH₂-, -CH(CH₃)CH₂-, -C(CH₃)₂CH₂-, -CH₂CH₂CH₂-, -CH(CH₃)CH₂CH₂-, -C(CH₃)₂CH₂CH₂-, -CH₂C(CH₃)₂CH₂-, -CH₂CH₂CH₂CH₂-, and -CH₂CH₂CH₂CH₂CH₂-.

[0024] The term “amino acid” refers to both natural and unnatural amino acids, protected natural and unnatural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids include 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine) and pyrrolidine and selenocysteine. Amino acid analogs refer to compounds having the same basic chemical structure as a naturally occurring amino acid, i.e., by way of example only, an α -carbon attached to a hydrogen, carboxyl group, amino group, and R group. Such analogs can have a modified R group (e.g., norleucine as an example) or retain a modified peptide backbone while retaining the same

basic chemical structure as a natural amino acid. Non-limiting examples of amino acid analogs include citrulline, homoserine, norleucine, methionine sulfoxide, methionine methylsulfonium, homophenylalanine, ornithine, formyl glycine, phenyl glycine, para-azidophenyl glycine, para-azidophenylalanine, para-acetophenylalanine, 4-(3-methyl-(1,2,4,5-tetrazine))-phenylglycine, and 4-(3-methyl-(1,2,4,5-tetrazine))-phenylalanine.

[0025] The term “aryl” as used herein, refers to a phenyl group, or bicyclic aryl or tricyclic aryl fused ring systems. Bicyclic fused ring systems are exemplified by a phenyl group appended to the parent molecular moiety and fused to a phenyl group. Tricyclic fused ring systems are exemplified by a phenyl group appended to the parent molecular moiety and fused to two other phenyl groups. Representative examples of bicyclic aryls include, but are not limited to, naphthyl. Representative examples of tricyclic aryls include, but are not limited to, anthracenyl. The monocyclic, bicyclic, and tricyclic aryls are connected to the parent molecular moiety through any carbon atom contained within the rings, and can be unsubstituted or substituted.

[0026] The term “azide” as used herein, refers to the functional group $-N_3$.

[0027] The term “cycloalkyl” as used herein, refers to a carbocyclic ring system containing three to ten carbon atoms, zero heteroatoms and zero double bonds. Representative examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl and cyclodecyl. “Cycloalkyl” also includes carbocyclic ring systems in which a cycloalkyl group is appended to the parent molecular moiety and is fused to an aryl group as defined herein, a heteroaryl group as defined herein, or a heterocycle as defined herein.

[0028] The term “cycloalkenyl” as used herein, means a non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and preferably having from 5-10 carbon atoms per ring. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl or cycloheptenyl.

[0029] The term “cyclooctene” as used herein, refers to a substituted or unsubstituted non-aromatic cyclic alkyl group of 8 carbon atoms, having a single ring with a double bond. Examples of such cyclooctene groups include, but are not limited to, substituted or unsubstituted trans-cyclooctene (TCO).

[0030] The term “fluoroalkyl” as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by fluorine. Representative examples of fluoroalkyl include, but are not limited to, 2-fluoroethyl, 2,2,2-trifluoroethyl, trifluoromethyl, difluoromethyl, pentafluoroethyl, and trifluoropropyl such as 3,3,3-trifluoropropyl.

[0031] The term “alkoxyfluoroalkyl” as used herein, refers to an alkoxy group, as defined herein, appended to the parent molecular moiety through a fluoroalkyl group, as defined herein.

[0032] The term “fluoroalkoxy” as used herein, means at least one fluoroalkyl group, as defined herein, is appended to the parent molecular moiety through an oxygen atom. Representative examples of

fluoroalkoxy include, but are not limited to, difluoromethoxy, trifluoromethoxy and 2,2,2-trifluoroethoxy.

[0033] The term “halogen” or “halo” as used herein, means Cl, Br, I, or F.

[0034] The term “haloalkyl” as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by a halogen.

[0035] The term “haloalkoxy” as used herein, means at least one haloalkyl group, as defined herein, is appended to the parent molecular moiety through an oxygen atom.

[0036] The term “heteroalkyl” as used herein, means an alkyl group, as defined herein, in which one or more of the carbon atoms has been replaced by a heteroatom selected from S, Si, O, P and N. The heteroatom may be oxidized. Representative examples of heteroalkyls include, but are not limited to, alkyl ethers, secondary and tertiary alkyl amines, and alkyl sulfides.

[0037] “Heteroalkylene” refers to a divalent heteroalkyl group. “Heteroalkylene” groups must have at least one carbon and at least one heteroatomic group within the chain. The term “heteroalkylene” includes unbranched or branched saturated chain having carbon and heteroatoms. By way of example, 1, 2 or 3 carbon atoms may be independently replaced with the same or different heteroatomic group. Heteroatomic groups include, but are not limited to, $-NR^y$ -, $-O$ -, $-S$ -, $-S(O)$ -, $-S(O)_2$ -, and the like, wherein R^y is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroalkyl or heteroaryl; each of which may be optionally substituted, as defined herein, such as with an oxo. Examples of heteroalkylene groups include, e.g., polyethers, $-CH_2OCH_2-$, $-CH(CH_3)OCH_2-$, $-CH_2CH_2OCH_2-$, $-OCH_2-$, $-CH(CH_3)O-$, $-CH_2CH_2O-$, $-CH_2CH_2OC(O)-$, $-CH_2CH_2OCH_2CH_2OCH_2-$, $-CH_2CH_2OCH_2CH_2O-$, $-CH_2SCH_2-$, $-CH(CH_3)SCH_2-$, $-CH_2CH_2SCH_2-$, $-CH_2CH_2SCH_2CH_2SCH_2-$, $-SCH_2-$, $-CH(CH_3)S-$, $-CH_2CH_2S-$, $-CH_2CH_2SCH_2CH_2S-$, $-CH_2S(O)_2CH_2-$, $-CH(CH_3)S(O)_2CH_2-$, $-CH_2CH_2S(O)_2CH_2-$, $-CH_2CH_2S(O)_2CH_2CH_2OCH_2-$, $-CH_2NR^yCH_2-$, $-CH_2CH_2NR^y-$, $-CH_2CH_2C(O)NR^y-$, $-CH(CH_3)NR^yCH_2-$, $-CH_2CH_2NR^yCH_2-$, $-CH_2CH_2NR^yCH_2CH_2NR^yCH_2-$, etc., where R^y is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroalkyl, or heteroaryl; each of which may be optionally substituted, as defined herein). As used herein, heteroalkylene includes 1 to 10 carbon atoms, 1 to 8 carbon atoms, or 1 to 4 carbon atoms; and 1 to 3 heteroatoms, 1 to 2 heteroatoms, or 1 heteroatom. As used herein, the term “heteroalkylene” includes groups such as amides or other functional groups having an oxo present on one or more carbon atoms.

[0038] The term “heteroaryl” as used herein, refers to an aromatic monocyclic ring or an aromatic bicyclic ring system or an aromatic tricyclic ring system. The aromatic monocyclic rings are five or six membered rings containing at least one heteroatom independently selected from the group consisting of N, O and S (e.g. 1, 2, 3, or 4 heteroatoms independently selected from O, S, and N). The five membered aromatic monocyclic rings have two double bonds and the six membered aromatic monocyclic rings have three double bonds. The bicyclic heteroaryl groups are exemplified by a monocyclic heteroaryl ring appended to the parent molecular moiety and fused to a monocyclic

cycloalkyl group, as defined herein, a monocyclic aryl group, as defined herein, a monocyclic heteroaryl group, as defined herein, or a monocyclic heterocycle, as defined herein. The tricyclic heteroaryl groups are exemplified by a monocyclic heteroaryl ring appended to the parent molecular moiety and fused to two of a monocyclic cycloalkyl group, as defined herein, a monocyclic aryl group, as defined herein, a monocyclic heteroaryl group, as defined herein, or a monocyclic heterocycle, as defined herein.

Representative examples of monocyclic heteroaryl include, but are not limited to, pyridinyl (including pyridin-2-yl, pyridin-3-yl, pyridin-4-yl), pyrimidinyl, pyrazinyl, thienyl, furyl, thiazolyl, thiadiazolyl, isoxazolyl, pyrazolyl, and 2-oxo-1,2-dihydropyridinyl. Representative examples of bicyclic heteroaryl include, but are not limited to, chromenyl, benzothienyl, benzodioxolyl, benzotriazolyl, quinolinyl, thienopyrrolyl, thienothienyl, imidazothiazolyl, benzothiazolyl, benzofuranyl, indolyl, quinolinyl, imidazopyridine, benzooxadiazolyl, and benzopyrazolyl. Representative examples of tricyclic heteroaryl include, but are not limited to, dibenzofuranyl and dibenzothienyl. The monocyclic, bicyclic, and tricyclic heteroaryls are connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the rings, and can be unsubstituted or substituted.

[0039] The term “heterocycle” or “heterocyclic” as used herein, means a monocyclic heterocycle, a bicyclic heterocycle, or a tricyclic heterocycle. The monocyclic heterocycle is a three-, four-, five-, six-, seven-, or eight-membered ring containing at least one heteroatom independently selected from the group consisting of O, N, and S. The three- or four-membered ring contains zero or one double bond, and one heteroatom selected from the group consisting of O, N, and S. The five-membered ring contains zero or one double bond and one, two or three heteroatoms selected from the group consisting of O, N and S. The six-membered ring contains zero, one or two double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. The seven- and eight-membered rings contains zero, one, two, or three double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. Representative examples of monocyclic heterocycles include, but are not limited to, azetidiny, azepanyl, aziridinyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, 1,3-dithiolanyl, 1,3-dithianyl, 1,3-dimethylpyrimidine-2,4(1H,3H)-dione, imidazolinyl, imidazolidinyl, isothiazolinyl, isothiazolidinyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolinyl, oxadiazolidinyl, oxazoliny, oxazolidinyl, oxetanyl, piperazinyl, piperidinyl, pyranyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydrothienyl, thiadiazolinyl, thiadiazolidinyl, 1,2-thiazinanyl, 1,3-thiazinanyl, thiazolinyl, thiazolidinyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl (thiomorpholine sulfone), thiopyranyl, and trithianyl. The bicyclic heterocycle is a monocyclic heterocycle fused to a phenyl group, or a monocyclic heterocycle fused to a monocyclic cycloalkyl, or a monocyclic heterocycle fused to a monocyclic cycloalkenyl, or a monocyclic heterocycle fused to a monocyclic heterocycle, or a spiro heterocycle group, or a bridged monocyclic heterocycle ring system in which two non-adjacent atoms of the ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Representative examples of bicyclic heterocycles include, but are not limited to, benzopyranyl, benzothiopyranyl, chromanyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzothienyl, 2,3-dihydroisoquinoline, 2-azaspiro[3.3]heptan-2-yl,

azabicyclo[2.2.1]heptyl (including 2-azabicyclo[2.2.1]hept-2-yl), 2,3-dihydro-1*H*-indolyl, isoindolyl, octahydrocyclopenta[*c*]pyrrolyl, octahydropyrrolopyridinyl, and tetrahydroisoquinolyl. Tricyclic heterocycles are exemplified by a bicyclic heterocycle fused to a phenyl group, or a bicyclic heterocycle fused to a monocyclic cycloalkyl, or a bicyclic heterocycle fused to a monocyclic cycloalkenyl, or a bicyclic heterocycle fused to a monocyclic heterocycle, or a bicyclic heterocycle in which two non-adjacent atoms of the bicyclic ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Examples of tricyclic heterocycles include, but are not limited to, octahydro-2,5-epoxypentalene, hexahydro-2*H*-2,5-methanocyclopenta[*b*]furan, hexahydro-1*H*-1,4-methanocyclopenta[*c*]furan, aza-adamantane (1-azatricyclo[3.3.1.1^{3,7}]decane), and oxa-adamantane (2-oxatricyclo[3.3.1.1^{3,7}]decane). The monocyclic, bicyclic, and tricyclic heterocycles are connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the rings, and can be unsubstituted or substituted.

[0040] The term “hydroxyl” as used herein, means an –OH group.

[0041] The term “hydroxyalkyl” as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by a hydroxyl group.

[0042] In some instances, the number of carbon atoms in a hydrocarbyl substituent (e.g., alkyl or cycloalkyl) is indicated by the prefix “C_x-C_y” or “C_{x-y},” wherein *x* is the minimum and *y* is the maximum number of carbon atoms in the substituent. Thus, for example, “C₁-C₃-alkyl” and “C₁₋₃alkyl” refer to an alkyl substituent containing from 1 to 3 carbon atoms. The two conventions “C_x-C_y” and “C_{x-y}” are used interchangeably and have the same meaning.

[0043] The term “substituted” refers to a group that may be further substituted with one or more non-hydrogen substituent groups. Substituent groups include, but are not limited to, halogen, =O, =S, cyano, nitro, fluoroalkyl, alkoxyfluoroalkyl, fluoroalkoxy, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, heteroalkyl, cycloalkyl, cycloalkenyl, aryl, heteroaryl, heterocycle, cycloalkylalkyl, heteroarylalkyl, arylalkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, alkylene, aryloxy, phenoxy, benzyloxy, amino, alkylamino, acylamino, aminoalkyl, arylamino, sulfonylamino, sulfinylamino, sulfonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, sulfinyl, -COOH, ketone, amide, carbamate, and acyl.

[0044] The term “tetrazine” refers to a substituted or unsubstituted aromatic cyclic group of 2 carbon atoms and 4 nitrogen atoms, having a single ring with three double bonds. Examples of tetrazine groups include 1,2,3,4-tetrazine and 1,2,4,5-tetrazine. As used herein, 1,2,4,5-tetrazine is referred to as a “Tz” group.

[0045] The term “selectively delivering” refers to delivering an agent (e.g., a payload) to an organ or tissue (or portion thereof) in need of treatment or diagnosis, without significant binding to other non-target organs or tissues (or portions thereof).

[0046] The term “payload” refers to an agent for delivery to a target site in a subject. Payloads include therapeutic agents.

[0047] The term “therapeutic agent” refers to an agent capable of treating and/or ameliorating a condition or disease, or one or more symptoms thereof, in a subject. Therapeutic agents of the present disclosure also include prodrug forms of therapeutic agents.

[0048] The term “diagnostic agent” refers to agents that assist in diagnosing conditions or diseases. Representative diagnostic agents include imaging agents such as paramagnetic agents, optical probes, radionuclides, and the like. Paramagnetic agents are imaging agents that are magnetic under an externally applied field. Examples of paramagnetic agents include, but are not limited to, iron particles including iron nanoparticles and iron microparticles. Optical probes are fluorescent compounds that can be detected by excitation at one wavelength of radiation and detection at a second, different, wavelength of radiation. Optical probes of the present disclosure include, but are not limited to, Cy5.5, Alexa 680, Cy5, DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate) and DiR (1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide). Other optical probes include quantum dots. Radionuclides are elements that undergo detectable radioactive decay. Radionuclides useful in embodiments of the present disclosure include, but are not limited to, ^3H , ^{11}C , ^{13}N , ^{18}F , ^{19}F , ^{60}Co , ^{64}Cu , ^{67}Cu , ^{68}Ga , ^{82}Rb , ^{89}Zr , ^{90}Sr , ^{90}Y , ^{99}Tc , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{129}I , ^{131}I , ^{137}Cs , ^{177}Lu , ^{186}Re , ^{188}Re , ^{211}At , Rn, Ra, Th, U, Pu, and ^{241}Am .

[0049] The term “targeting agent” refers to a chemical or biological agent that specifically binds to a target (e.g., a targeted organ or tissue), thereby forming a stable association between the targeting agent and the specific target. By “stably associated” or “stable association” is meant that a moiety is bound to or otherwise associated with another moiety or structure under standard physiological conditions. Bonds may include covalent bonds and non-covalent interactions, such as, but not limited to, ionic bonds, hydrophobic interactions, hydrogen bonds, van der Waals forces (e.g., London dispersion forces), dipole-dipole interactions, and the like. A targeting agent may be a member of a specific binding pair, such as, but are not limited to: a member of a receptor/ligand pair; a ligand-binding portion of a receptor; a member of an antibody/antigen pair; an antigen-binding fragment of an antibody; a hapten; a member of a lectin/carbohydrate pair; a member of an enzyme/substrate pair; biotin/avidin; biotin/streptavidin; digoxin/antidigoxin; a member of a DNA or RNA aptamer binding pair; a member of a peptide aptamer binding pair; and the like. Targeting agents include ligands that specifically bind (or substantially specifically bind) a particular clinically-relevant target receptor or cell surface target. The ligand can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or other molecule with a specific affinity for a target receptor or cell surface target. Examples of receptors and cell surface targets include, but are not limited to, PD-1, CTLA-4, HER2/neu, HER1/EGFR, VEGFR, 4-1BB, GITR, LT4 - human mAb directed against the inhibitory immune checkpoint receptor immunoglobulin-like transcript 4 (ILT4; leukocyte immunoglobulin-like receptor subfamily B member 2, LILRB2, lymphocyte immunoglobulin-like receptor 2, LIR2, monocyte/macrophage immunoglobulin-like receptor 10, MIR-10, CD85d, or other

cellular receptors or cell surface targets. Additional examples are included in various embodiments disclosed herein.

[0050] The term “targeted organ or tissue” refers to an organ or tissue that is being targeted for delivery of the payload. Representative organs and tissues for targeting include those that can be targeted by chemical or biological targeting agents, as well as those organs and tissues that cannot be targeted by chemical or biological targeting agents.

[0051] The term “implanting” refers to surgical implantation into a subject’s body.

[0052] The term “contacting” or “contact” refers to the process of bringing into contact at least two distinct species such that they can interact with each other, such as in a non-covalent or covalent binding interaction or binding reaction. It should be appreciated, however, the resulting complex or reaction product can be produced directly from an interaction or a reaction between the added reagents or from an intermediate from one or more of the added reagents or moieties, which can be produced in the contacting mixture.

[0053] The term “binding agent” refers to an agent having a functional group capable of forming a covalent bond to a complementary functional group of another binding agent in a biological environment. Binding between binding agents in a biological environment may also be referred to as bioconjugation. Binding agents include bioorthogonal binding agents, which are binding agents having bioorthogonal functional groups. Bioorthogonal functional groups of bioorthogonal binding agents selectively react with a complementary bioorthogonal functional group of another bioorthogonal binding partner. Selective reaction between bioorthogonal binding partners can minimize side reactions with other binding agents, biological compounds, or other non-complementary bioorthogonal binding agents or non-complementary bioorthogonal functional groups. Bioorthogonal moieties or functional groups of bioorthogonal binding agents include, but are not limited to, an azide and alkyne for formation of a triazole via Click-chemistry reactions, trans-cyclooctene (TCO) and tetrazine (Tz) (e.g., 1,2,4,5-tetrazine), and others. The binding agents useful in the present disclosure may have a high reactivity with the corresponding binding agent so that the reaction is rapid.

[0054] The term “functionalized” refers to a moiety having a functional group attached to the moiety, such as for example a moiety having a binding agent functional group (e.g., a bioorthogonal functional group) attached thereto.

[0055] The term “administering” refers to any suitable route of administration to a subject, such as, but not limited to, oral administration, administration as a suppository, topical contact, parenteral, intravenous, intraperitoneal, intramuscular, intralesional, intranasal or subcutaneous administration, intrathecal administration, or the implantation of a slow-release device e.g., a mini-osmotic pump, to the subject.

[0056] The term “pharmaceutically effective amount” and “therapeutically effective amount” refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent or reduce the risk of the occurrence or reoccurrence of the disease or disorder or symptom(s) thereof. In reference to tumorigenic proliferative disorders, a pharmaceutically or therapeutically effective amount comprises an amount sufficient to, among other things, cause the tumor to shrink or decrease the growth rate of the tumor.

[0057] As used herein, the term “subject,” “patient,” or “organism” includes humans and mammals (e.g., mice, rats, pigs, cats, dogs, and horses). Typical subjects to which an agent(s) of the present disclosure may be administered may include mammals, particularly primates, especially humans. For veterinary applications, suitable subjects may include, for example, livestock such as cattle, sheep, goats, cows, swine, and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, suitable subjects may include mammals, such as rodents (e.g., mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like.

[0058] The term “treating” or “treatment” as used herein means the treating or treatment of a disease or medical condition or symptom(s) thereof in a patient, such as a mammal (particularly a human) that includes: (a) ameliorating the disease or medical condition or symptom(s) thereof, such as, eliminating or causing regression of the disease or medical condition or symptom(s) thereof in a patient; (b) suppressing the disease or medical condition or symptom(s) thereof, for example by, slowing or arresting the development of the disease or medical condition or symptom(s) thereof in a patient; or (c) alleviating a symptom of the disease or medical condition or symptom(s) thereof in a patient.

[0059] The term “physiological conditions” is meant to encompass those conditions compatible with living cells, e.g., predominantly aqueous conditions of a temperature, pH, salinity, *etc.* that are compatible with living cells.

[0060] For compounds described herein, groups and substituents thereof may be selected in accordance with permitted valence of the atoms and the substituents, such that the selections and substitutions result in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, *etc.*

[0061] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0062] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0063] The compounds may exist as stereoisomers wherein asymmetric or chiral centers are present. The stereoisomers are “*R*” or “*S*” depending on the configuration of substituents around the chiral carbon atom. The terms “*R*” and “*S*” used herein are configurations as defined in IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, in Pure Appl. Chem., 1976, 45: 13-30. The disclosure contemplates various stereoisomers and mixtures thereof, and these are specifically included within the scope of this invention. Stereoisomers include enantiomers and diastereomers and mixtures of enantiomers or diastereomers. Individual stereoisomers of the compounds may be prepared synthetically from commercially available starting materials, which contain asymmetric or chiral centers or by preparation of racemic mixtures followed by methods of resolution well-known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography, and optional liberation of the optically pure product from the auxiliary as described in Furniss, Hannaford, Smith, and Tatchell, “Vogel’s Textbook of Practical Organic Chemistry,” 5th edition (1989), Longman Scientific & Technical, Essex CM20 2JE, England, or (2) direct separation of the mixture of optical enantiomers on chiral chromatographic columns, or (3) fractional recrystallization methods.

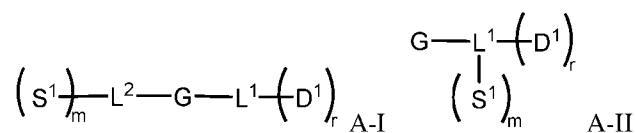
[0064] It should be understood that the compounds may possess tautomeric forms as well as geometric isomers, and that these also constitute an aspect of the invention.

[0065] The present disclosure also includes isotopically-labeled compounds, which are identical to those recited herein, but for the fact that 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 suitable for inclusion in the compounds of the invention are hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, and chlorine, such as, but not limited to, ²H, ³H, ¹³C, ¹⁴C, ¹⁵N, ¹⁸O, ¹⁷O, ³¹P, ³²P, ³⁵S, ¹⁸F, and ³⁶Cl, respectively. Substitution with heavier isotopes such as deuterium, i.e., ²H, 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. The compound may incorporate positron-emitting isotopes for medical imaging and positron-emitting tomography (PET) studies for determining the distribution of receptors. Suitable positron-emitting isotopes that can be incorporated are ¹¹C, ¹³N, ¹⁵O, and ¹⁸F. Isotopically-labeled compounds disclosed herein can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using appropriate isotopically-labeled reagent in place of non-isotopically-labeled reagent.

B. Conjugates

[0066] Provided herein are conjugates for use in bioorthogonal reactions. In some embodiments, the conjugates comprise a payload bonded to a trans-cyclooctene moiety. In some embodiments, the conjugates comprise a payload (i.e., a taxane, such as paclitaxel, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof) bonded to a trans-cyclooctene moiety comprising one or more solubilizing groups.

[0067] In some embodiments, provided is a conjugate of Formula A-I or Formula A-II, or a pharmaceutically acceptable salt thereof:



wherein:

m is an integer from 1-10;

r is 1 or 2;

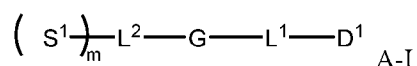
each D^1 is independently a taxane, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof;

L^1 and L^2 are each independently a linker;

G an optionally substituted trans-cyclooctene moiety; and

each S^1 is independently a solubilizing group.

[0068] In some embodiments, provided is a conjugate of Formula A-I, or a pharmaceutically acceptable salt thereof:



wherein:

m is an integer from 1-10;

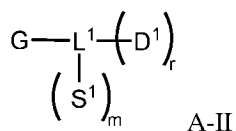
D^1 is a taxane, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof;

L^1 and L^2 are each independently a linker;

G an optionally substituted trans-cyclooctene moiety; and

each S^1 is independently a solubilizing group.

[0069] In some embodiments, provided is a conjugate of Formula A-II, or a pharmaceutically acceptable salt thereof:



wherein:

m is an integer from 1-10;

r is 1 or 2;

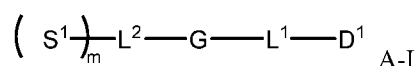
each D¹ is independently a taxane, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof;

L² is a linker;

G an optionally substituted trans-cyclooctene moiety; and

each S¹ is independently a solubilizing group.

[0070] In some embodiments, provided is a conjugate of Formula A-I, or a pharmaceutically acceptable salt thereof:



wherein:

m is an integer from 1-10;

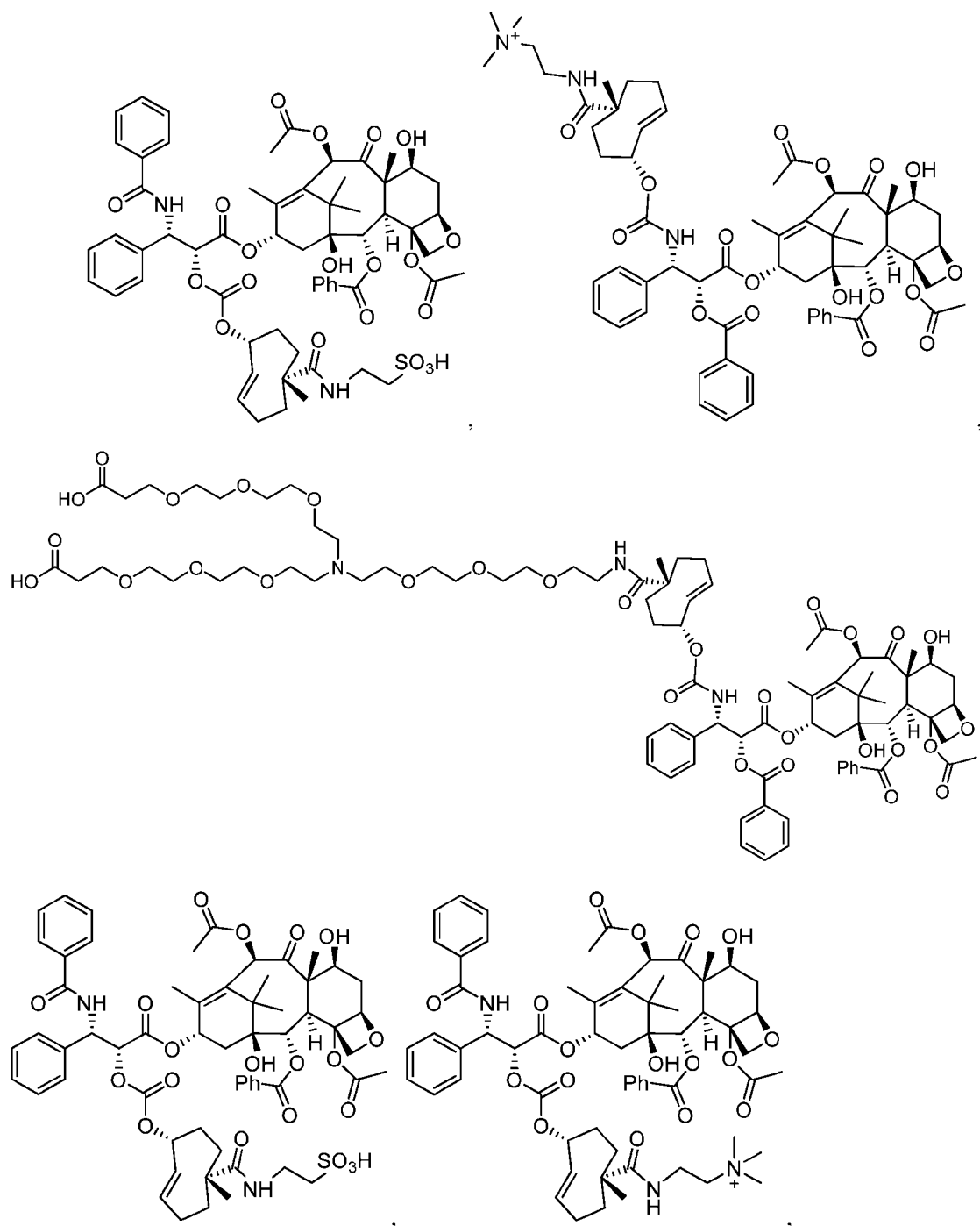
D¹ is a taxane payload, or a derivative, or analog thereof;

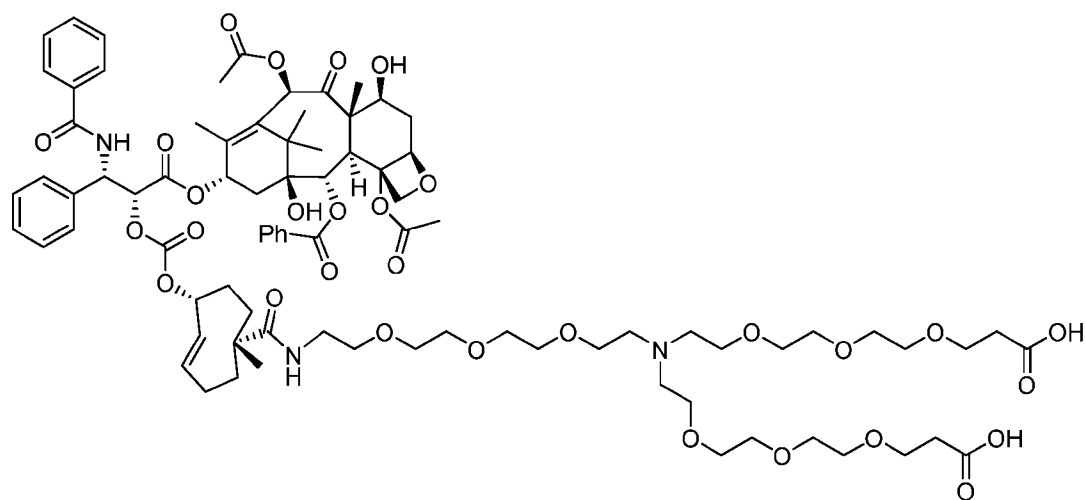
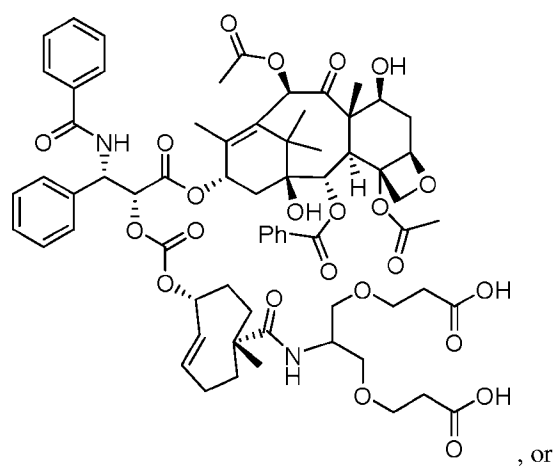
L¹ and L² are each independently a linker;

G an optionally substituted trans-cyclooctene moiety; and

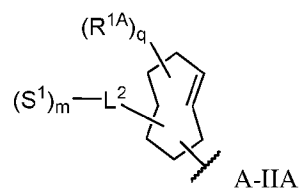
each S¹ is independently a solubilizing group.

[0071] In some embodiments, the conjugate is not:





[0072] In some embodiments, the moiety $(S^1)_m - L^2 - G$ is of Formula A-IIA:



wherein:

q is 0, 1, or 2;

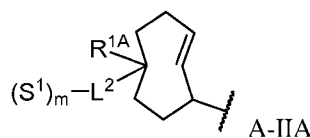
m is an integer from 1-10;

R^{1A} , at each occurrence, is independently selected from the group consisting of C_{1-4} alkyl, C_{1-4} haloalkyl, and C_{1-4} alkoxy;

L^2 is a linker; and

each S^1 is independently a solubilizing group.

[0073] In some embodiments, the moiety $(S^1)_m-L^2-G$ is of Formula A-IIA:



wherein:

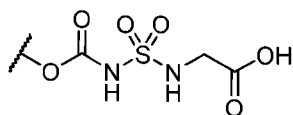
m is an integer from 1-10;

R^{1A} is selected from the group consisting of C_{1-4} alkyl, C_{1-4} haloalkyl, and C_{1-4} alkoxy;

L^2 is a linker; and

each S^1 is independently a solubilizing group.

[0074] In some embodiments, each S^1 is independently selected from the group consisting of -NHC(NH)NH₂, -P(O)(OH)₂, -S(O)₂OH, -(OCH₂CH₂)₃₀₋₈₅-OCH₃, -N(CH₂CH₂C(O)OH)₂, or



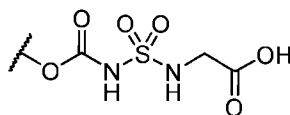
[0075] In some embodiments, one S^1 is -NHC(NH)NH₂.

[0076] In some embodiments, one S^1 is -P(O)(OH)₂.

[0077] In some embodiments, one S^1 is -S(O)₂OH.

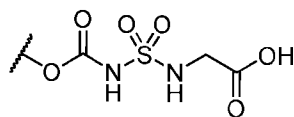
[0078] In some embodiments, one S^1 is -(OCH₂CH₂)₃₀₋₈₅-OCH₃.

[0079] In some embodiments, one S^1 is -N(CH₂CH₂C(O)OH)₂.



[0080] In some embodiments, one S^1 is

[0081] In some embodiments, each S^1 is independently selected from -NHC(NH)NH₂, -P(O)(OH)₂,



-S(O)₂OH, -N(CH₂CH₂C(O)OH)₂, or

[0082] In some embodiments, L^1 is -OC(O)-, -C(O)O-, -NR^{lf}C(O)-, or -C(O)NR^{lf}-; and

R^{lf} is hydrogen, C_{1-6} alkyl, or C_{0-4} alkylene-CO₂H.

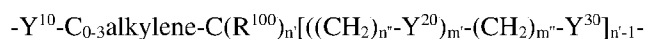
[0083] In some embodiments, L^1 is -OC(O)-_{aa} or -NHC(O)-_{aa}, where bond aa is attached to D¹.

[0084] In some embodiments, L^2 is a heteroalkylene linker. In some embodiments, L^2 is a linear or branched heteroalkylene linker.

[0085] In some embodiments, m is 1.

[0086] In some embodiments, m is 2.

[0087] In some embodiments, L^2 is:



wherein:

each Y^{10} , Y^{20} , and Y^{30} is independently a bond, $-NR^{110}-$, $-O-$, $-S(O)_{0-2}-$, $-NR^{110}C(O)-$, $-C(O)NR^{110}-$, $-NR^{110}S(O)_2-$, $-S(O)_2NR^{110}-$, $-CR^{120}=N-NR^{110}-$, $-NR^{110}-N=CR^{120}-$, $-C(O)-$, $-OC(O)-$, $-C(O)O-$, $-OC(O)O-$, alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene is independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl;

each R^{100} is independently hydrogen, $-C(O)OH$, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

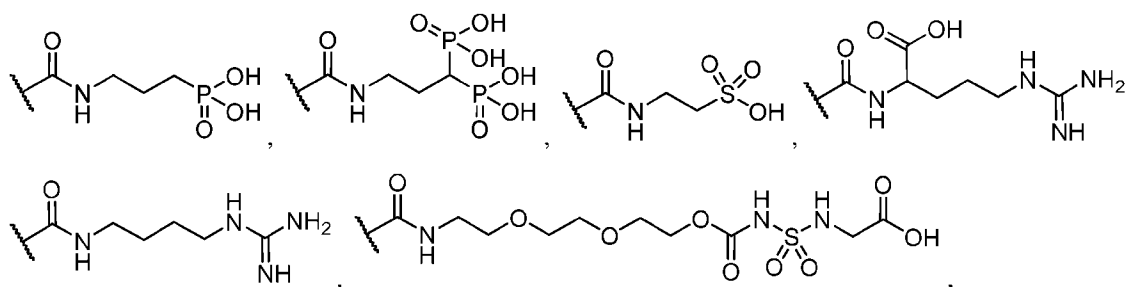
each R^{110} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

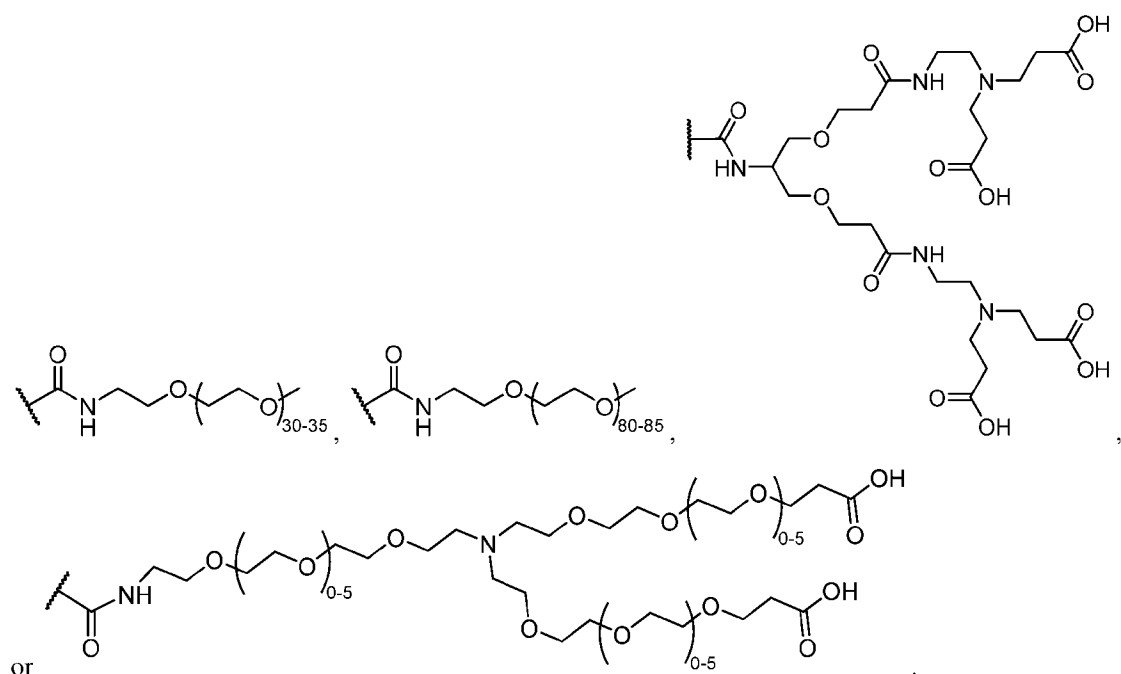
each R^{120} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl; and

n'' , m' , and m'' are each independently 0, 1, 2, 3, 4, 5, 6, 7, or 8; and

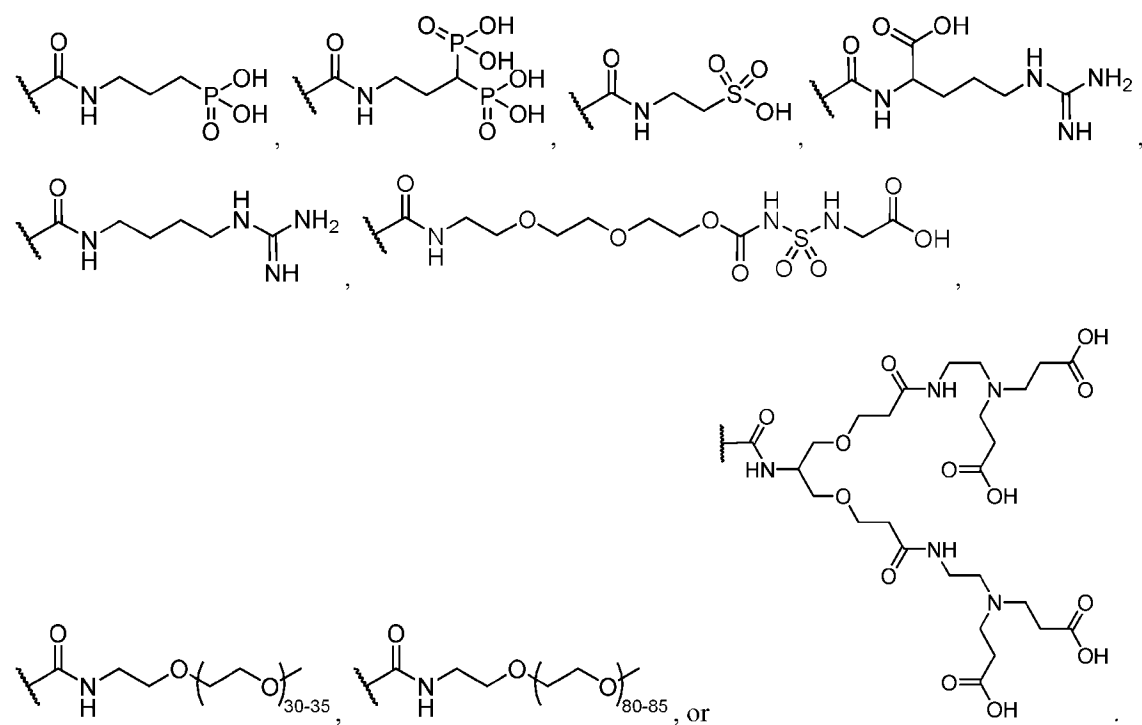
n' is 2.

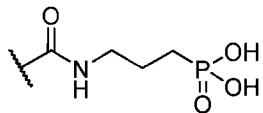
[0088] In some embodiments, the moiety $(S^1)_m-L^2-$ is:

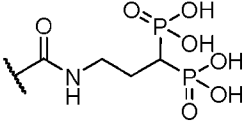


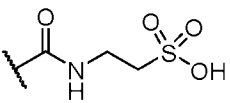


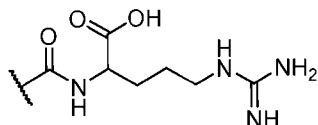
[0089] In some embodiments, the moiety $(S^1)_m - L^2 -$ is:

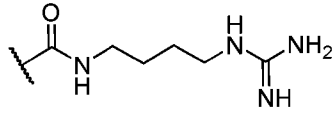


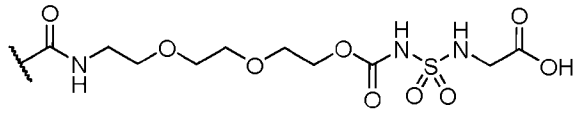
[0090] In some embodiments, the moiety $(S^1)_m-L^2-G$ is . In some

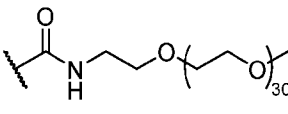
embodiments, the moiety $(S^1)_m-L^2-G$ is . In some embodiments, the

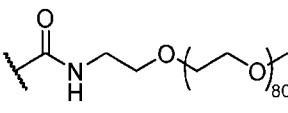
moiety $(S^1)_m-L^2-G$ is . In some embodiments, the moiety

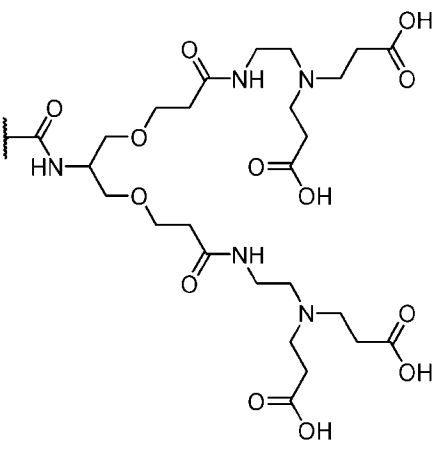
$(S^1)_m-L^2-G$ is . In some embodiments, the moiety

$(S^1)_m-L^2-G$ is . In some embodiments, the moiety

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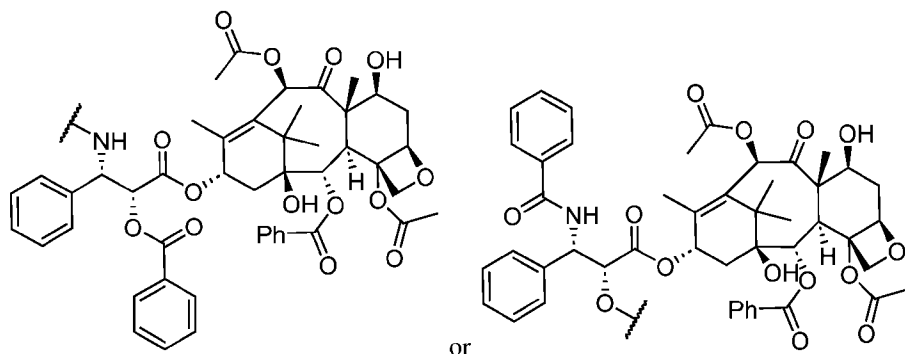
[0091] In certain embodiments, D^1 is a taxane, a topoisomerase inhibitor, or MMAE, or a derivative, or analog thereof. In some embodiments, D^1 is paclitaxel, or a derivative, or analog thereof. In some embodiments, D^1 is paclitaxel or isotaxel, or a derivative, or analog thereof. In some embodiments, D^1 is a topoisomerase inhibitor, or a derivative, or analog thereof. In some embodiments, D^1 is a camptothecin,

or a derivative, or analog thereof. In certain embodiments, D¹ is MMAE, or a derivative, or analog thereof.

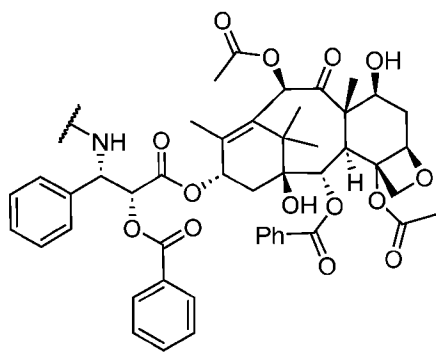
[0092] In certain embodiments, the terms “derivative” or “analog” or “derived from” as used in reference to a payload, means that one or more atoms, including hydrogen or non-hydrogen atoms, of the original, unmodified payload is replaced by a covalent bond to one or more linker L¹. The D¹ payloads are derived from the known payload and are modified to be covalently bonded to at least one optionally substituted trans-cyclooctene via a linker L¹. The D¹ payloads, even after modification to arrive at the compounds described herein, maintain biological activity which is comparable to that observed in the original, unmodified payload. In certain embodiments, the D¹ payloads exhibit a binding activity or inhibition which is at least about 98%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, or about 50% of that observed in the original, unmodified payload.

[0093] In certain embodiments, a hydrogen atom bound to a heteroatom (e.g., N, O, or S) of the original, unmodified payload is replaced by a covalent bond to a linker L¹. In certain embodiments, a halogen atom on a payload is replaced for attachment to the remainder of the compound. In certain embodiments, a hydrogen atom on a payload is replaced for attachment to the remainder of the compound. In certain embodiments, the hydrogen atom is on a heteroatom. In certain embodiments, the hydrogen atom is on a nitrogen. In certain embodiments, the hydrogen atom is on an oxygen. In certain embodiments, the hydrogen atom is on a carbon.

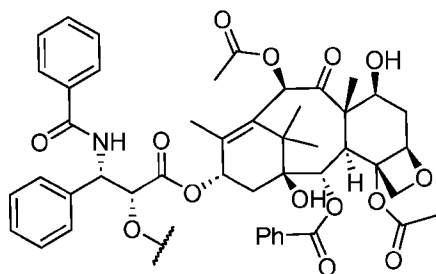
[0094] In some embodiments, D¹ is:



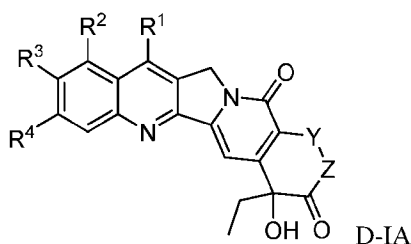
[0095] In some embodiments, D¹ is:



[0096] In some embodiments, D¹ is:



[0097] In some embodiments, D¹ is a compound of Formula D-IA:



wherein:

Y is a bond, -CH₂-, or -CH₂-CH₂-;

Z is a bond, -O-, or -CH₂-O-;

R¹, R², R³ and R⁴ are each independently hydrogen, halo, cyano, nitro, -OR⁵, -SR⁵, -NR⁵R⁶, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, C₃₋₁₀ cycloalkyl, heterocyclyl, aryl, heteroaryl, -C(=O)R⁵, -C(=O)OR⁵, -OC(=O)R⁵, -C(=O)NR⁵R⁶, -NR⁵C(=O)R⁶, -NR⁵C(=O)OR⁶, -S(=O)₁₋₂R⁵, -S(=O)₁₋₂NR⁵R⁶, -NR⁵S(=O)₁₋₂R⁶, -Si(R⁵)₃, or -C=NOR⁵, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl of R¹, R², R³, and R⁴ are independently optionally substituted with one or more R¹⁰ as valency permits;

or R¹ and R² are taken together with the atoms to which they are attached to form a C₃₋₁₀ cycloalkyl, heterocyclyl, aryl, or heteroaryl, optionally substituted with one or more R¹⁰ as valency permits;

or R² and R³ are taken together with the atoms to which they are attached to form a C₃₋₁₀ cycloalkyl, heterocyclyl, aryl, or heteroaryl, optionally substituted with one or more R¹⁰ as valency permits;

or R³ and R⁴ are taken together with the atoms to which they are attached to form a C₃₋₁₀ cycloalkyl, heterocyclyl, aryl, or heteroaryl, optionally substituted with one or more R¹⁰ as valency permits;

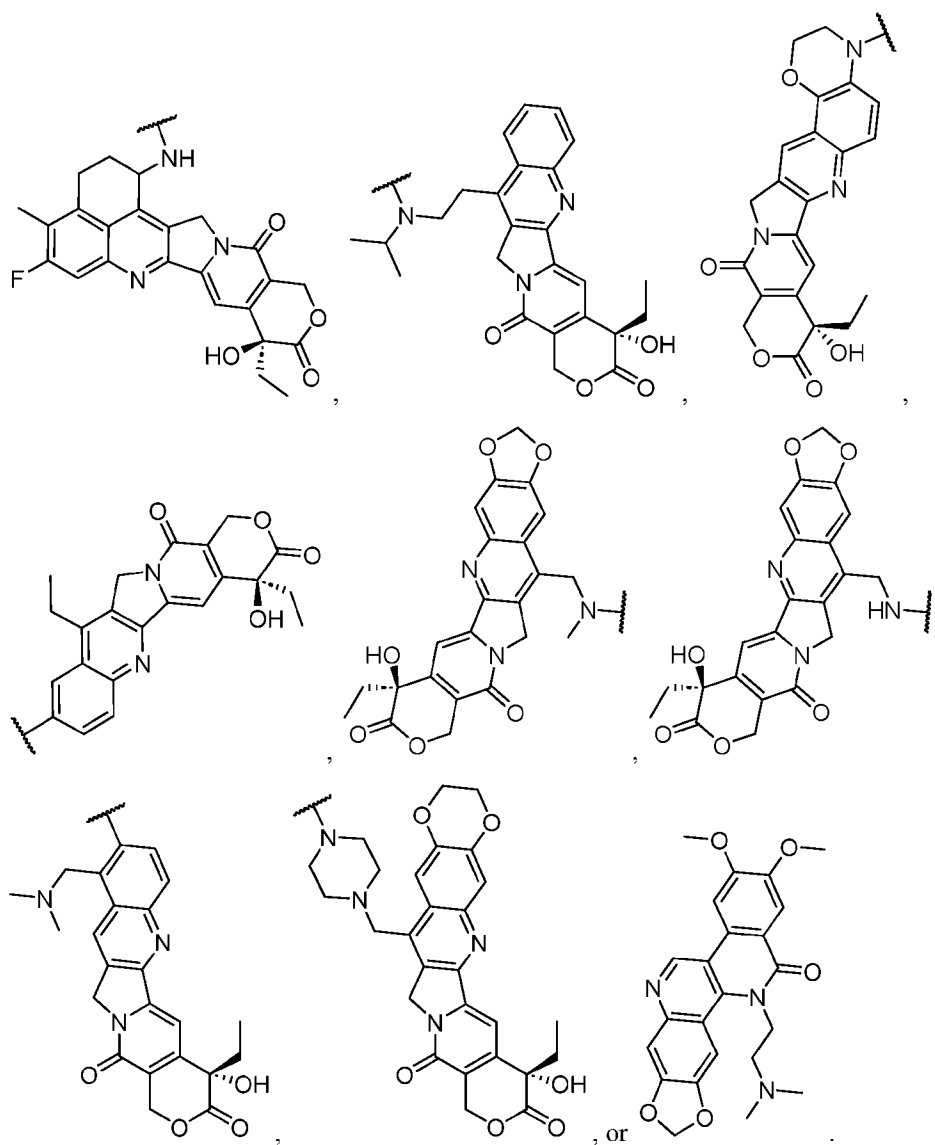
each R⁵ and R⁶ is independently hydrogen, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, or C₃₋₁₂ cycloalkyl, wherein each alkyl, alkenyl, alkynyl, or cycloalkyl is optionally independently substituted with oxo, halo, hydroxyl or amino as valency permits; or R⁵ and R⁶ are taken together with the atoms to which they are attached to form heterocyclyl optionally substituted by halo or C₁₋₁₂ alkyl optionally substituted by oxo, halo, hydroxyl or amino; and

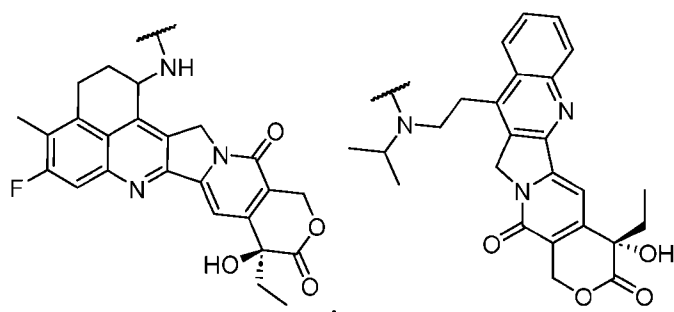
each R⁷ and R⁸ is independently hydrogen, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, or C₃₋₁₂ cycloalkyl, wherein each C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, or C₃₋₁₂ cycloalkyl is optionally substituted with oxo, halo, hydroxyl or amino as valency permits; or R⁷ and R⁸ are taken together with the atoms to which they are attached to form heterocyclyl optionally substituted by halo or C₁₋₁₂ alkyl optionally substituted by oxo, halo, hydroxyl or amino;

each R¹⁰ is independently halo, cyano, nitro, -OR⁷, -SR⁷, -SF₅, -NR⁷R⁸, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, C₃₋₁₀ cycloalkyl, heterocyclyl, aryl, heteroaryl, -C(=O)R⁷, -C(=O)OR⁷, -OC(=O)OR⁷, -OC(=O)R⁷, -C(=O)NR⁷R⁸, -OC(=O)NR⁷R⁸, -NR⁷C(=O)NR⁷R⁸, -S(=O)₁₋₂R⁷, -S(=O)₁₋₂NR⁷R⁸, -NR⁷S(=O)₁₋₂R⁸, -NR⁷S(=O)₁₋₂NR⁷R⁸, -NR⁷C(=O)R⁸, -NR⁷C(=O)OR⁸, -Si(R⁷)₃, or -C=NOR⁷, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl of R¹⁰ are independently optionally substituted with one or more halo or C₁₋₁₂ alkyl optionally substituted by oxo, halo, hydroxyl or amino as valency permits; and

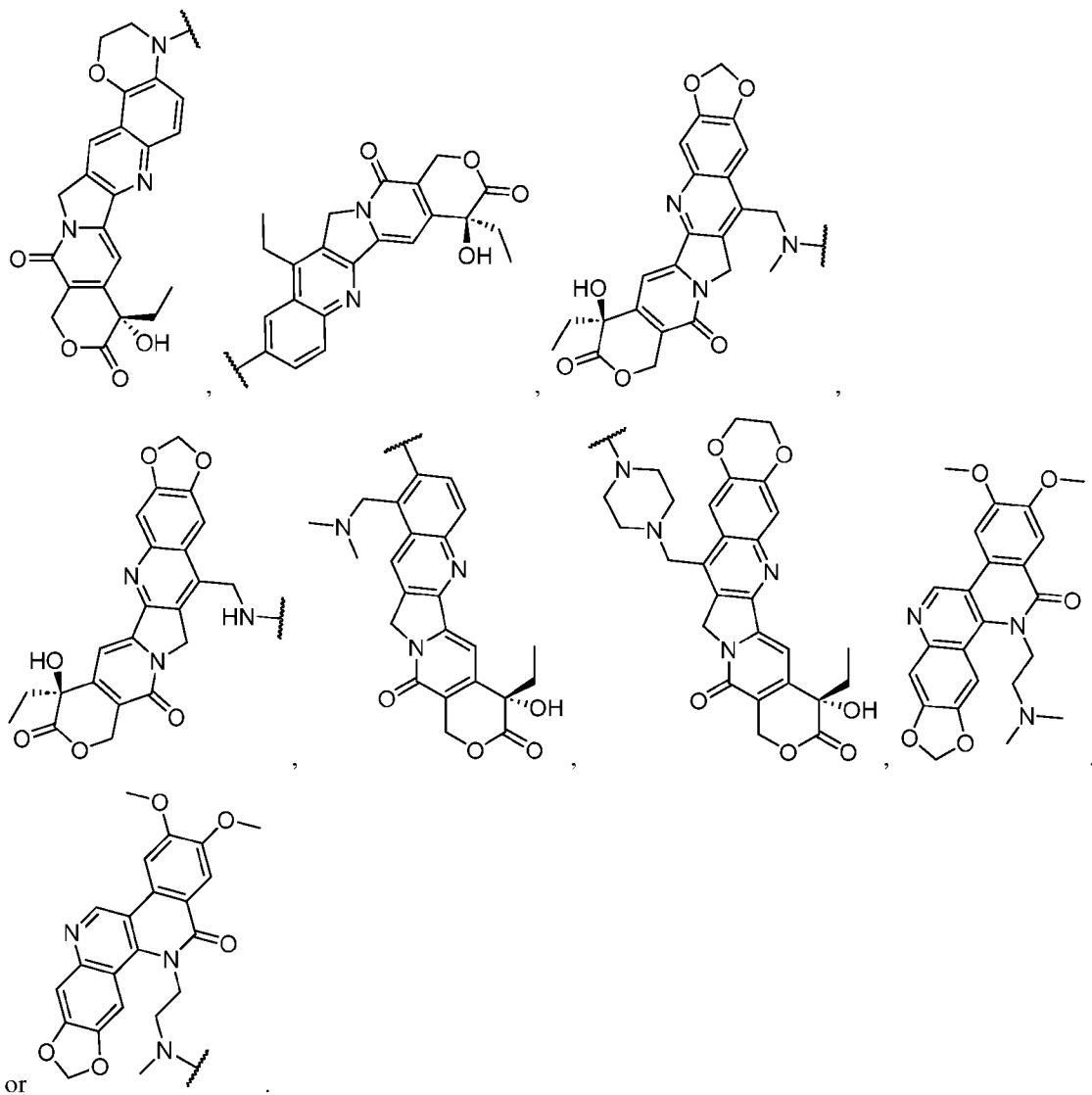
wherein one or more atoms of Formula D-IA (e.g., hydrogen atom) is replaced by a direct covalent bond to L¹.

[0098] In certain embodiments, D¹ is:



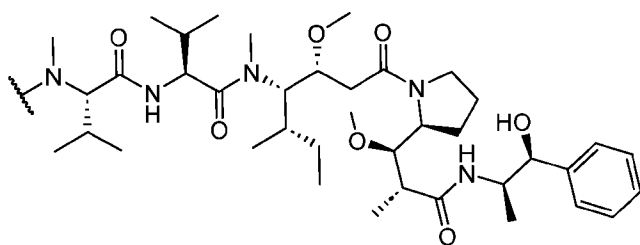


In certain embodiments, D¹ is:

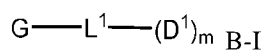


[0099] In certain embodiments, D¹ is MMAE, or a derivative, or analog thereof.

[0100] In certain embodiments, D¹ is:



[0101] In certain embodiments, provided herein are conjugates for use in bioorthogonal reactions, wherein the conjugates comprise a payload bonded to a trans-cyclooctene moiety. In some embodiments, provided is a conjugate of Formula B-I, or a pharmaceutically acceptable salt thereof:



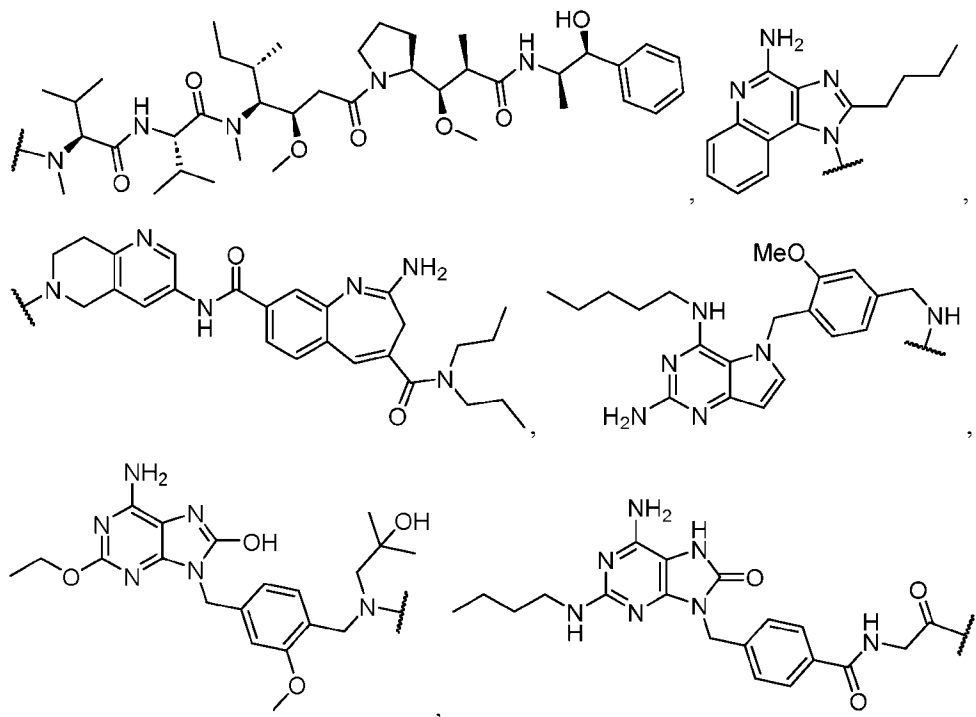
wherein:

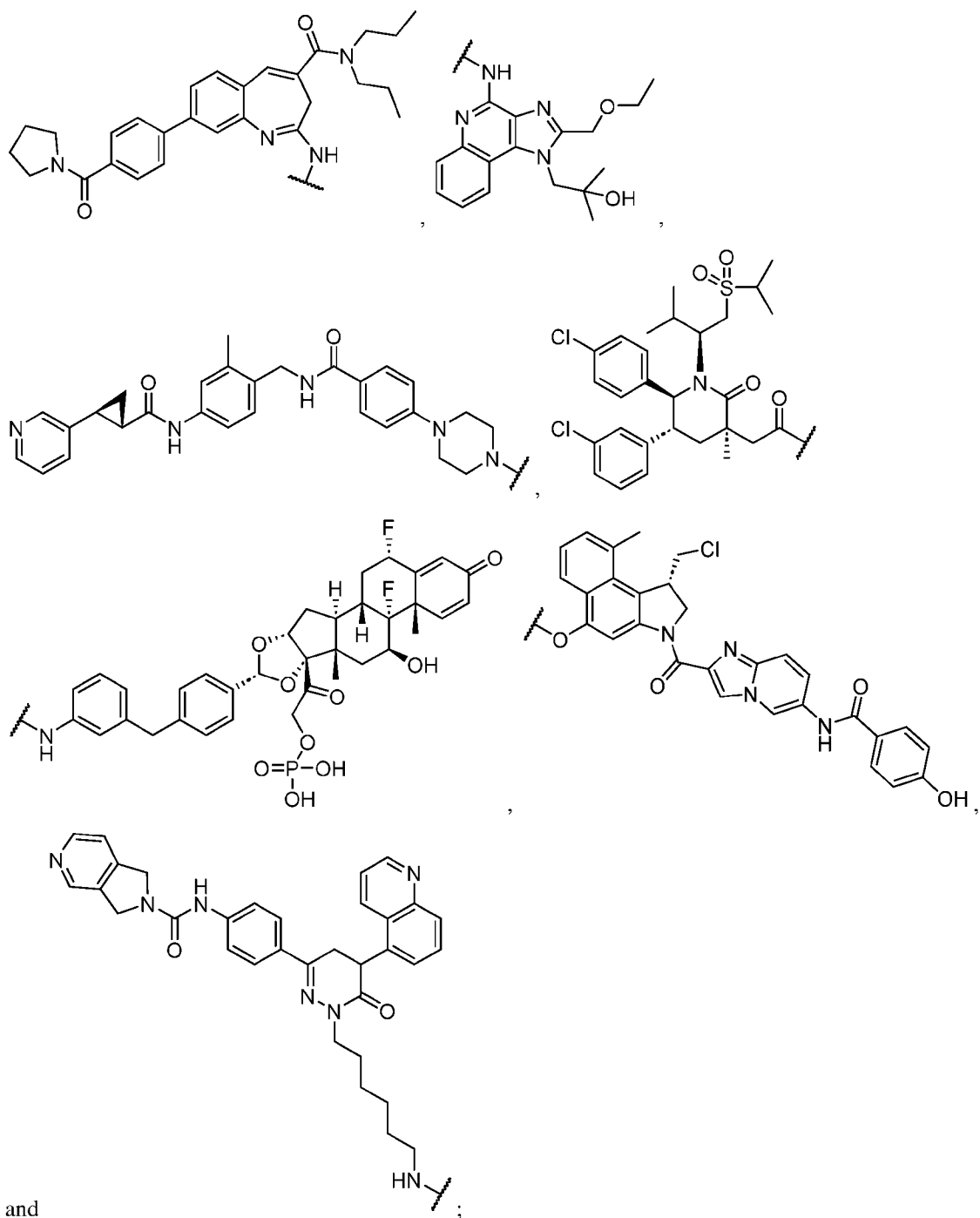
G an optionally substituted trans-cyclooctene moiety;

L^1 is a linker;

m is 1 or 2; and

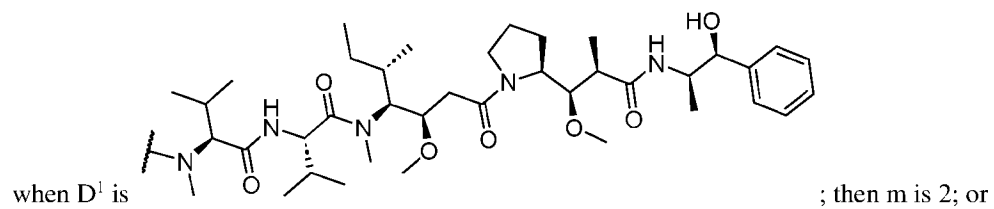
each D^1 is independently a payload selected from the group consisting of:

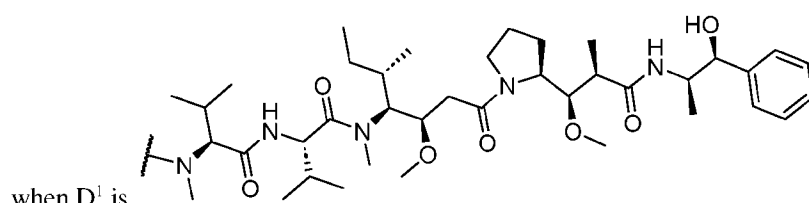




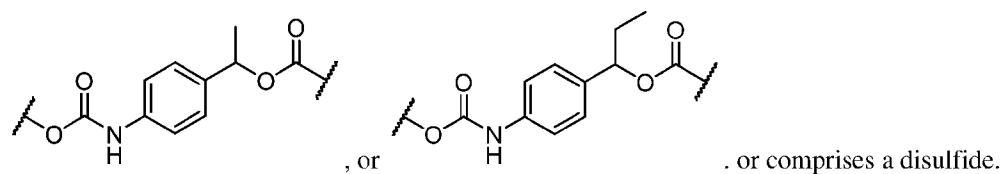
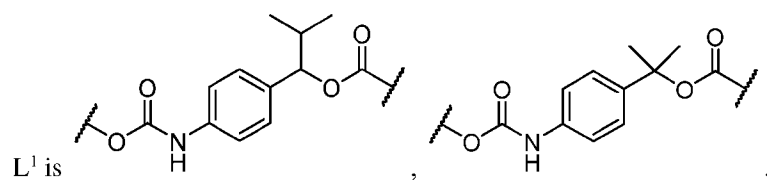
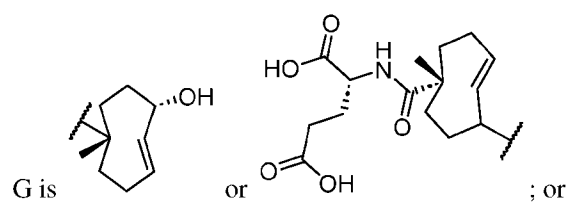
and

provided that:

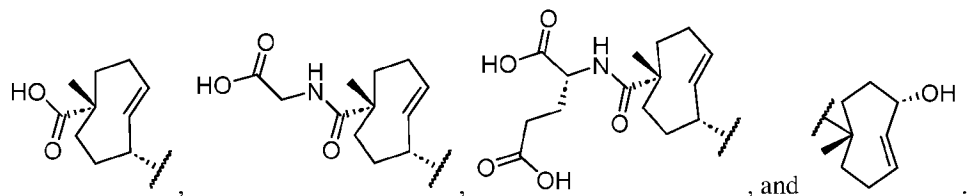


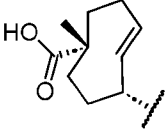


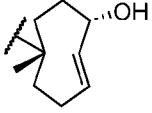
and m is 1, then:

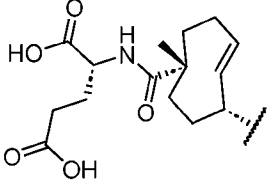


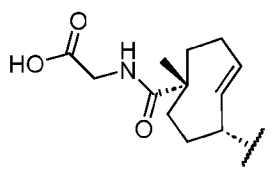
[0102] In some embodiments, G is selected from the group consisting of:



[0103] In some embodiments, G is  .

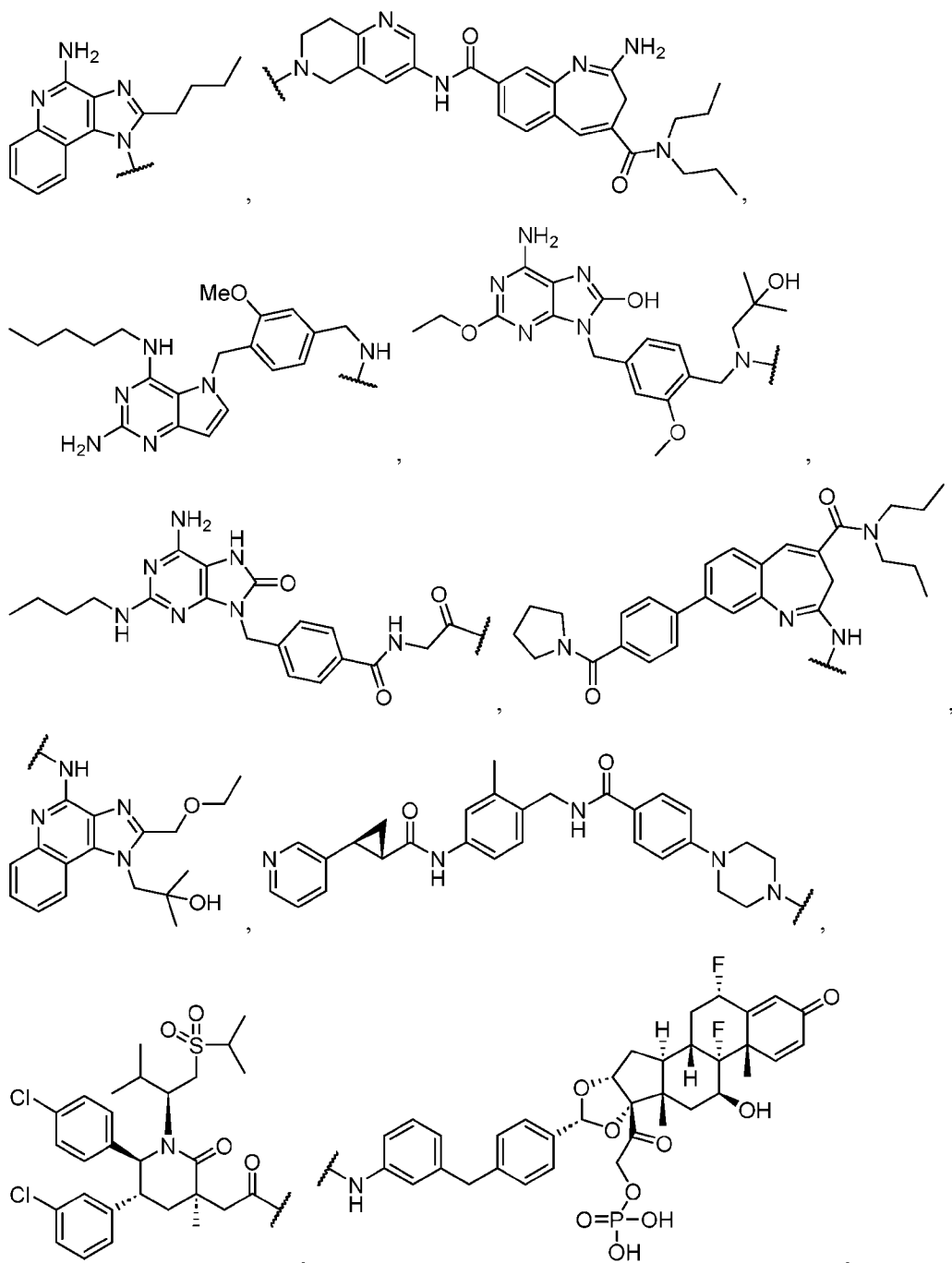
[0104] In some embodiments, G is  .

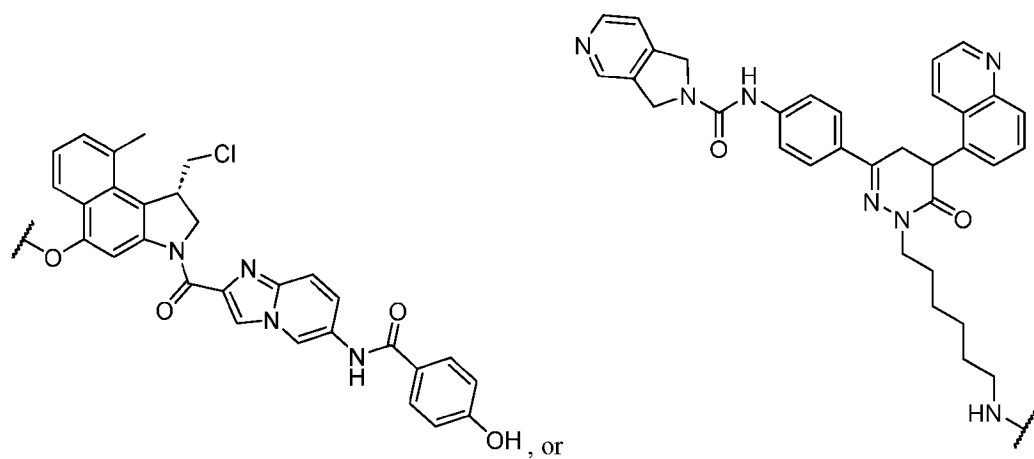
[0105] In some embodiments, G is  .



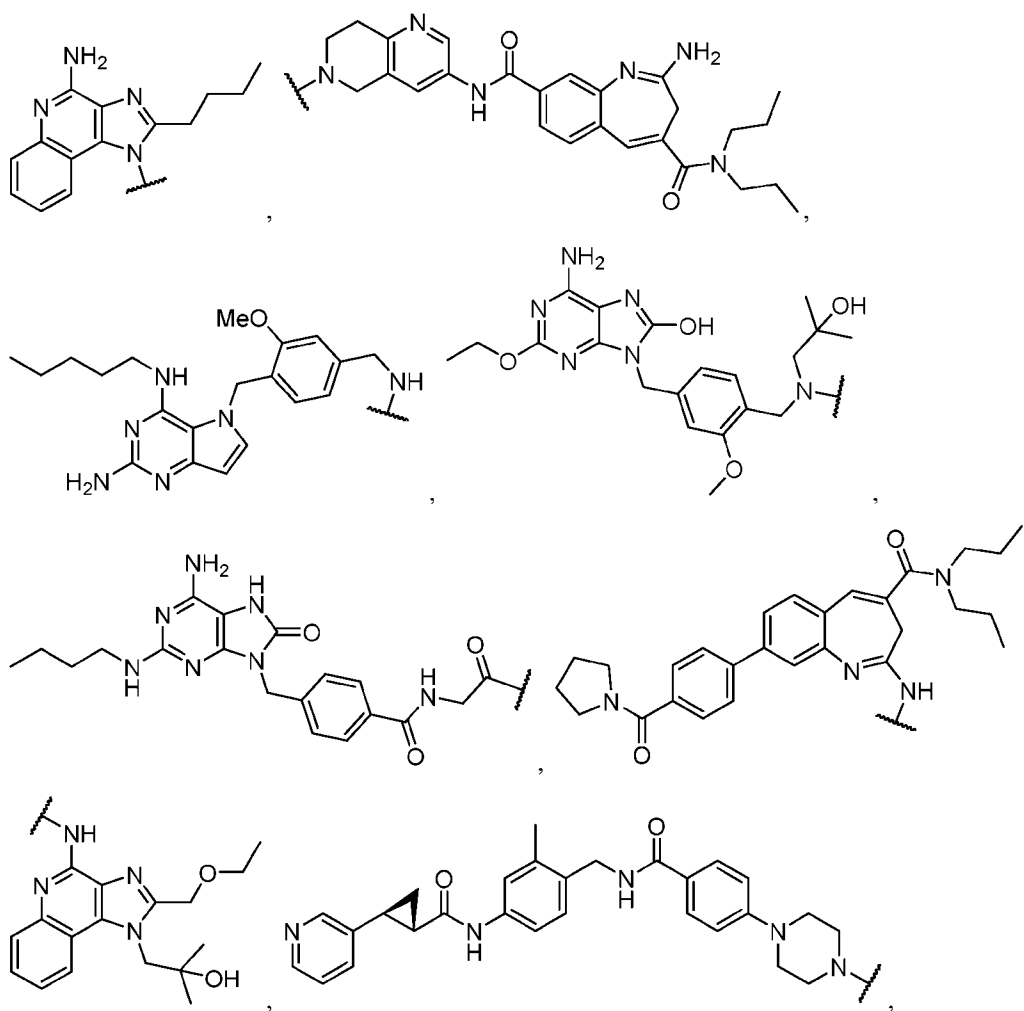
[0106] In some embodiments, G is

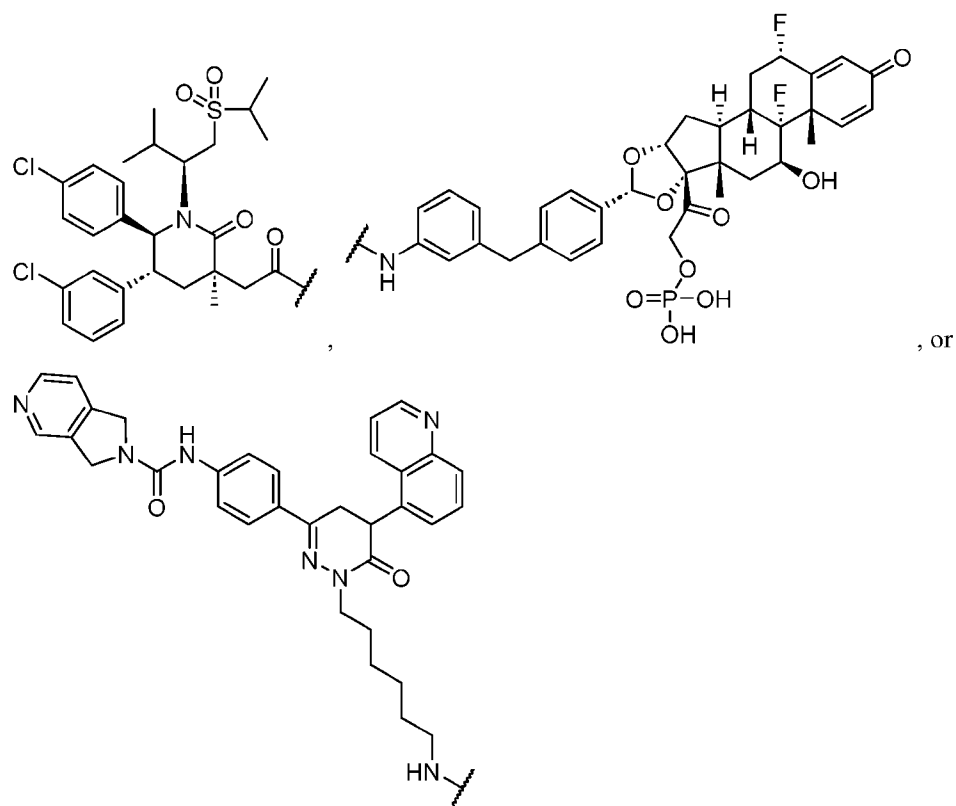
[0107] In some embodiments, each D¹ is independently:



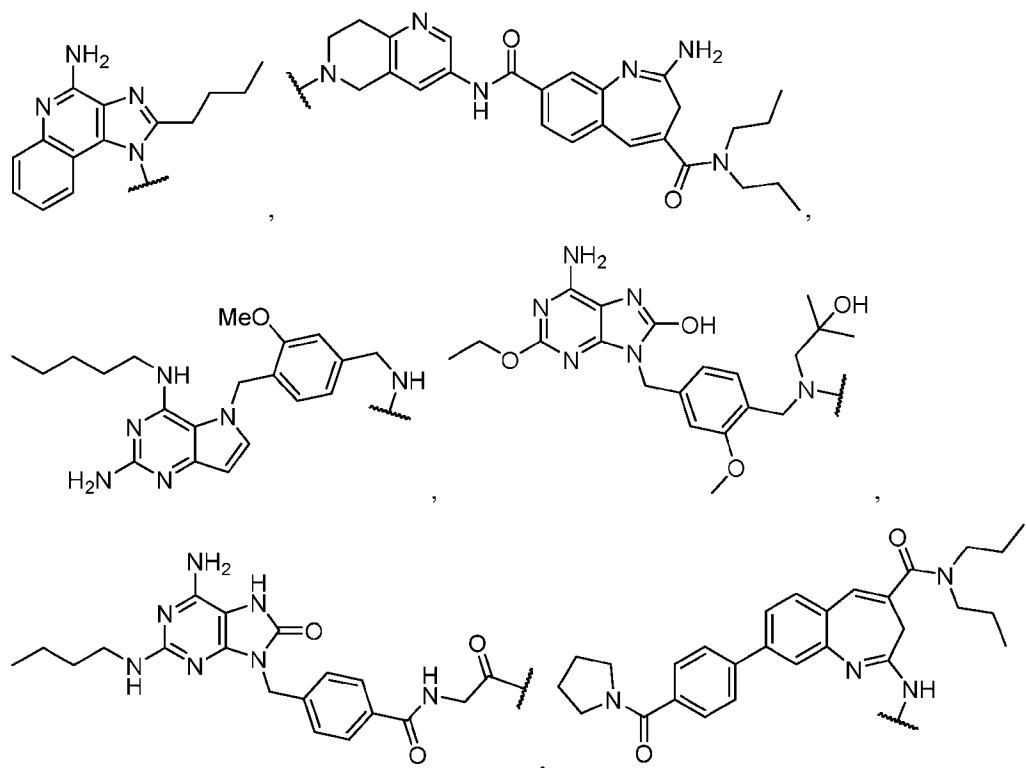


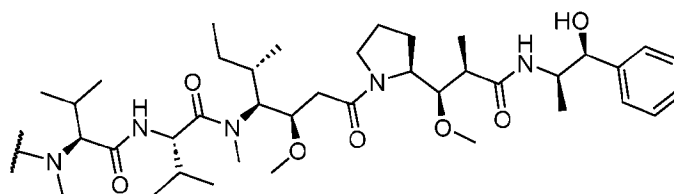
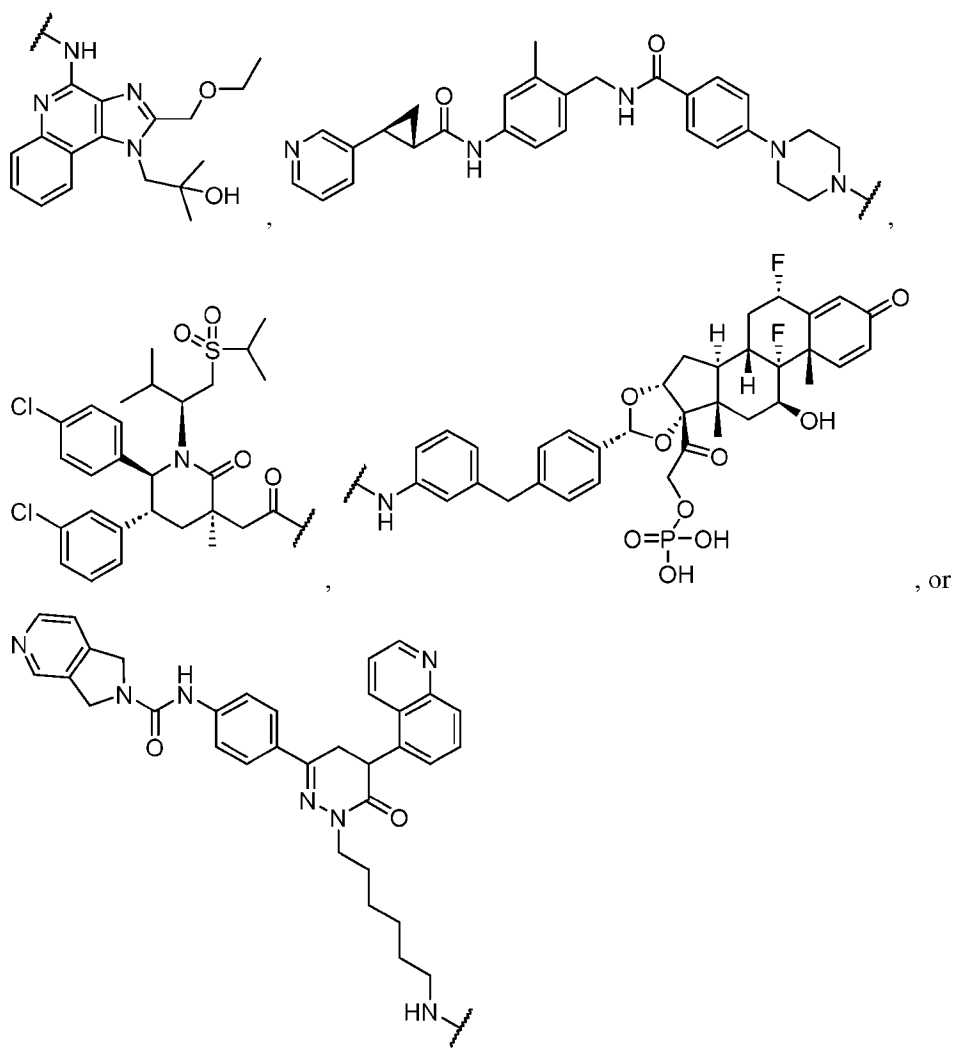
[0108] In some embodiments, each D¹ is independently:



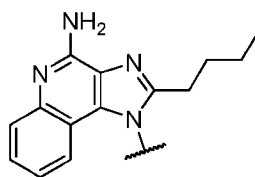


[0109] In some embodiments, m is 1 and D¹ is:

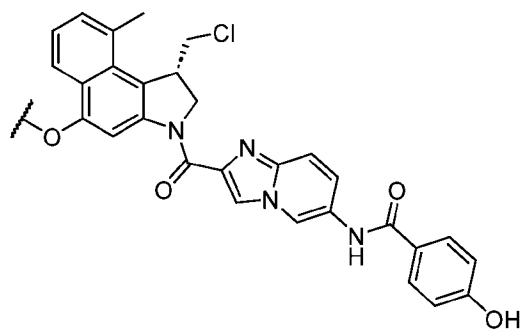




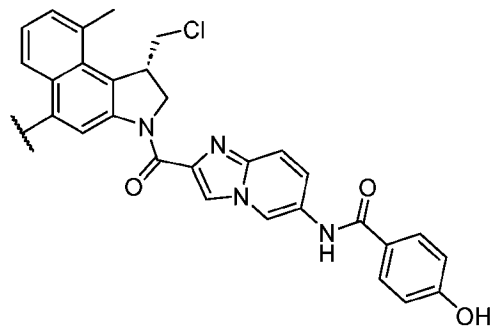
[0110] In some embodiments, D¹ is



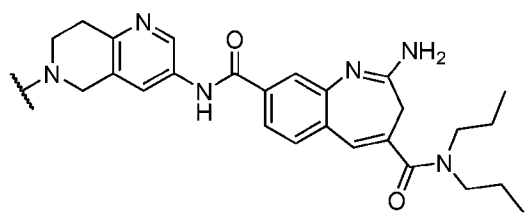
[0111] In some embodiments, D¹ is



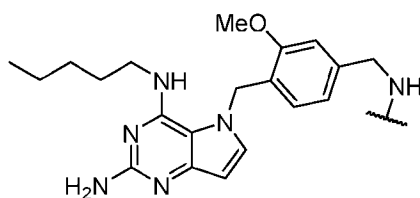
[0112] In some embodiments, D¹ is



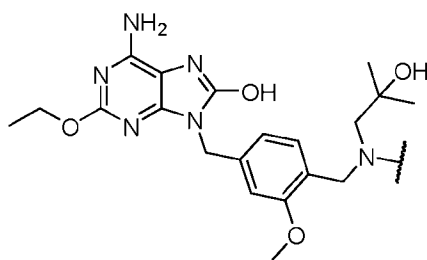
[0113] In some embodiments, D¹ is



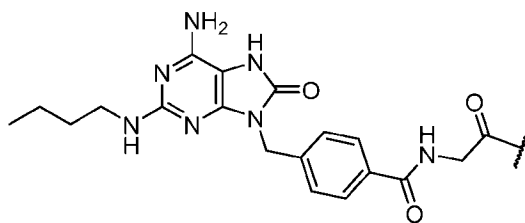
[0114] In some embodiments, D¹ is



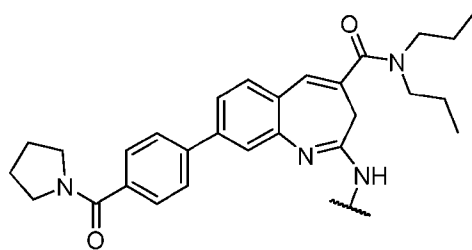
[0115] In some embodiments, D¹ is



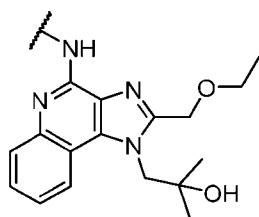
[0116] In some embodiments, D¹ is



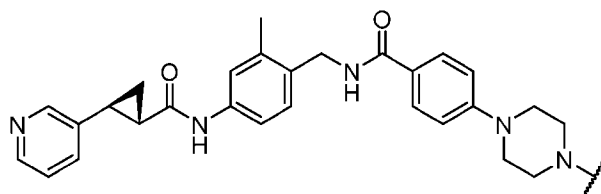
[0117] In some embodiments, D¹ is



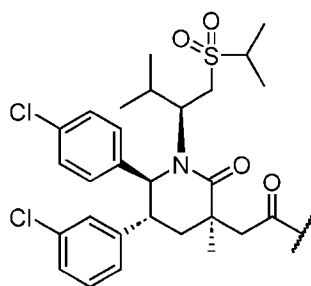
[0118] In some embodiments, D¹ is



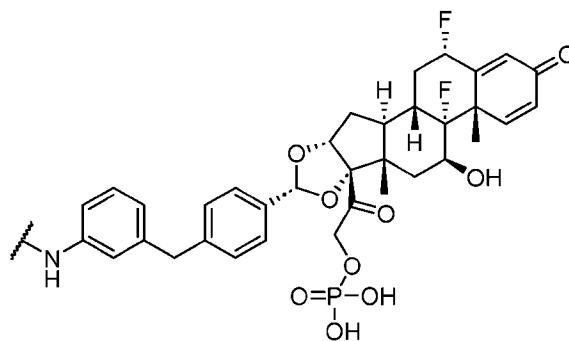
[0119] In some embodiments, D¹ is



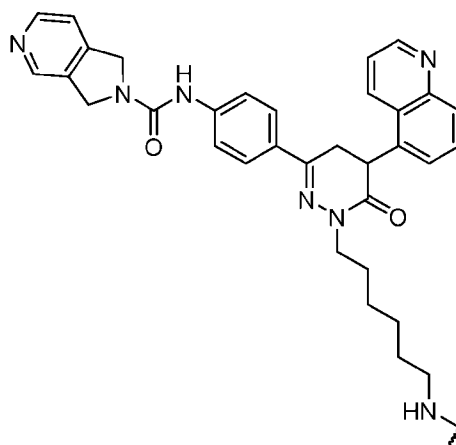
[0120] In some embodiments, D¹ is



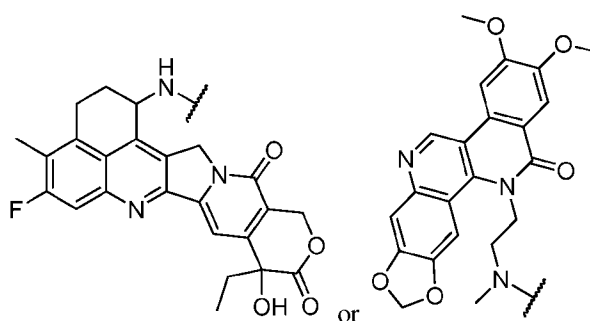
[0121] In some embodiments, D¹ is



[0122] In some embodiments, D¹ is



[0123] In some embodiments, D^1 is



[0124] In some embodiments, D^1 is

[0125] In some embodiments, m is 1. In some embodiments, L^1 is a linear linker.

[0126] In some embodiments, m is 2. In some embodiments, L^1 is a branched linker.

[0127] In some embodiments, L¹ is -C(O)O- or -O-.

[0128] Also provided is a pharmaceutical composition comprising the conjugate, or a pharmaceutically acceptable salt thereof, as disclosed herein and a pharmaceutically acceptable carrier.

Linkers

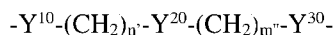
[0129] In some embodiments of the conjugates described herein, each linker L¹ or L² may independently have 1 to 100 linking atoms, and may include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms, or from 1 to 40 linking atoms, or from 1 to 30 linking atoms, or from 1 to 20 linking atoms, or from 1 to 10 linking atoms, or from 1 to 5 linking atoms, or from 5 to 30 linking atoms, or from 10 to 30 linking atoms, or from 5 to 40 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms.

[0130] In some embodiments of the conjugates described herein, linker L¹ or L² may be a bond.

[0131] In some embodiments of the conjugates described herein, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L¹ or L²) may comprise one or more (e.g., 1-10 or 1-5) chain heteroatoms (e.g., O, N, S) and one or more (e.g., 1-10 or 1-5) alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene moieties; wherein each alkylene,

alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene moiety, may be independently optionally substituted with one to five substituents independently selected from oxo, halo, C₁₋₄ alkyl, C₁₋₄ alkoxy, and C₁₋₄ haloalkyl.

[0132] In some embodiments of the conjugates described herein, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L¹ or L²) may be of the formula:



wherein:

each of Y¹⁰, Y²⁰, and Y³⁰ are independently a bond, -NR¹¹⁰-, -O-, -S(O)₀₋₂-, -NR¹¹⁰C(O)-, -C(O)NR¹¹⁰-, -NR¹¹⁰S(O)₂-, -S(O)₂NR¹¹⁰-, -CR¹²⁰=N-NR¹¹⁰-, -NR¹¹⁰-N=CR¹²⁰-, -C(O)-, -OC(O)-, -OC(O)O-, alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene is independently optionally substituted with one to five substituents independently selected from oxo, halo, C₁₋₄ alkyl, C₁₋₄ alkoxy, and C₁₋₄ haloalkyl;

each R¹¹⁰ is independently hydrogen, C₁₋₄ alkyl, C₁₋₄ haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

each R¹²⁰ is independently hydrogen, C₁₋₄ alkyl, C₁₋₄ haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl; and

n' and m'' are each independently 0, 1, 2, 3, 4, 5, 6, 7, or 8.

[0133] In certain embodiments, the linker L¹ is not a bond. In some embodiments, L¹ is a cleavable linker. In some embodiments, L¹ is a non-cleavable linker.

[0134] In certain embodiments, the linker L² is not a bond. In some embodiments, L² is a cleavable linker. In some embodiments, L² is a non-cleavable linker.

[0135] In certain embodiments, each R¹¹⁰ is independently hydrogen, C₁₋₄ alkyl, C₁₋₄ haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl; and each R¹²⁰ is independently hydrogen, C₁₋₄ alkyl, C₁₋₄ haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl.

[0136] In some embodiments, L¹ is a heteroalkylene linker. In some embodiments, L¹ is a linear or branched heteroalkylene linker. In some embodiments, L¹ is a linear heteroalkylene linker. In some embodiments, L¹ is a branched heteroalkylene linker.

[0137] In some embodiments, L¹ is -OC(O)-, -C(O)O-, -NR^{1f}C(O)-, or -C(O)NR^{1f}-; and

R^{1f} is hydrogen, C₁₋₆alkyl, or C₀₋₄alkylene-CO₂H.

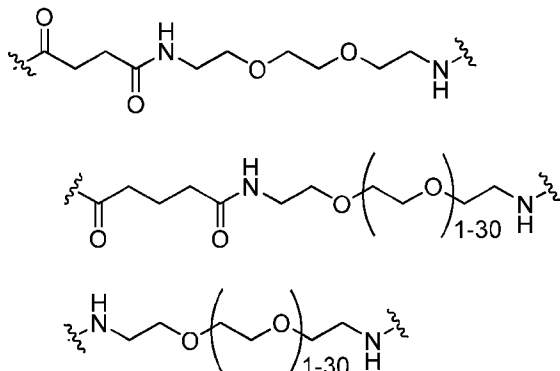
[0138] In some embodiments, L¹ is -OC(O)-_{aa} or -NHC(O)-_{aa}, where bond aa is attached to D¹.

[0139] Also provided is a pharmaceutical composition comprising the conjugate, or a pharmaceutically acceptable salt thereof, as disclosed herein and a pharmaceutically acceptable carrier.

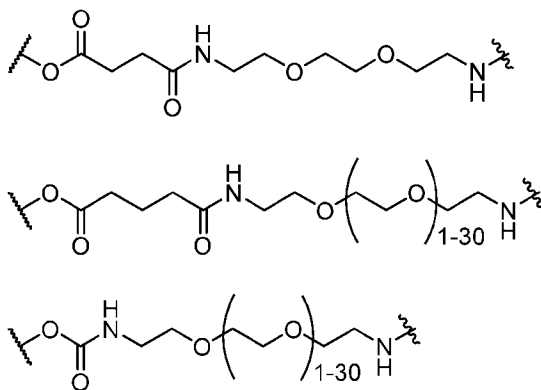
[0140] In some embodiments of the conjugates described herein, linker L^1 may be a bond.

[0141]

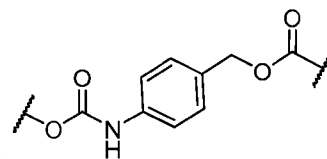
[0142] Representative linkers include, but are not limited to, those shown below:



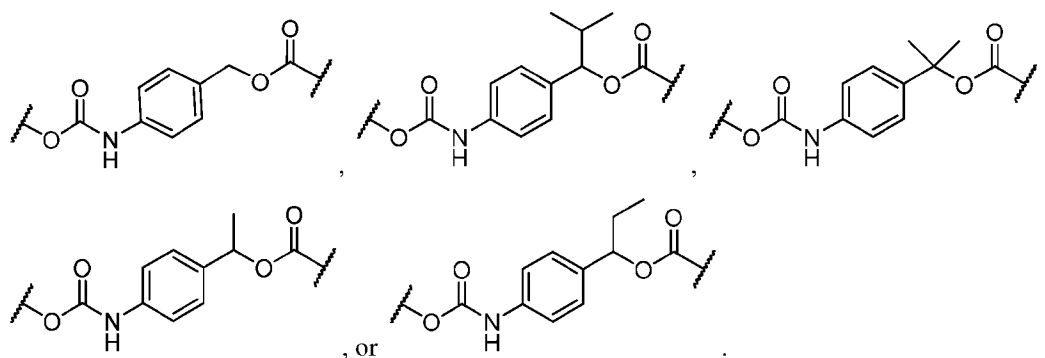
[0143] Representative linkers include, but are not limited to, those shown below:



[0144] In some embodiments of the conjugates described herein, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L^1 or L^2) L^1 or L^2) may comprise one or more of polyethylene glycol (e.g., PEG having an average molecular weight of from 200 g/mol to 10,000 g/mol), ethylene-1,2-diylbis(methylcarbamate), an arylene (e.e., phenylene), ethylene-oxy, amine, ester, amide, carbamate, ketone (i.e., formyl), or carbonate. The linker of a Formula as disclosed herein, e.g., in



Formula A-I, A-II, B-I, etc. (e.g., L^1 or L^2) L^1 or L^2) may comprise . The linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L^1 or L^2) may comprise



[0145] In some embodiments of the conjugates described herein, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L¹ or L²) may comprise one or more natural or unnatural amino acids, which may be referred to as a peptide linker. Where the drug (D¹) comprises an amino moiety, the linker may be bound thereto using a peptide linker made up of a carboxylic acyl unit, and one or more amino acids making up a protein or peptide sequence. The linker may also contain a self-immolating spacer which spaces the drug and the protein peptide sequence.

[0146] In some embodiments of the conjugates described herein, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II, B-I, etc. (e.g., L¹ or L²) may be a peptide linker represented by “A—Y—Z—X—W” in which “A” is the carboxylic acyl unit, “Y” and “Z” are each one or more natural or unnatural amino acids and together form a peptide sequence, and “X” and “W” are optional additional linkers having from 1 to 50 linking atoms, or from 5 to 10 linking atoms, or from 1 to 10 linking atoms which spaces the peptide and the drug, D¹, or the bioorthogonal moiety. In certain embodiments, one or more of the amino acids in the peptide linker is N-methylated.

[0147] In some embodiments, Y may be at least one amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan and proline. In some embodiments, Y may be at least one amino acid selected from the group consisting of phenylalanine, alanine, and valine.

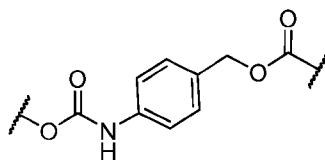
[0148] In some embodiments, Z may be at least one amino acid selected from the group consisting of alanine, lysine, lysine protected with acetyl or formyl, arginine, arginine protected with tosyl or nitro groups, histidine, ornithine, ornithine protected with acetyl or formyl, and citrulline. In some embodiments, Z may be at least one amino acid selected from the group consisting of alanine, lysine and citrulline.

[0149] In some embodiments, Y-Z combinations include Valine-Citrulline; Valine-Alanine; and Alanine-Alanine.

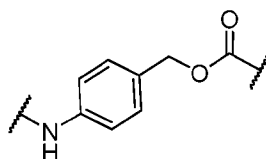
[0150] In certain embodiments, A is -OC(O)-.

[0151] In certain embodiments, X is -OC(O)-.

[0152] In certain embodiments, W is -OC(O)-. In certain embodiments, X is absent and W is -OC(O)-.



[0153] In certain embodiments, —X—W is



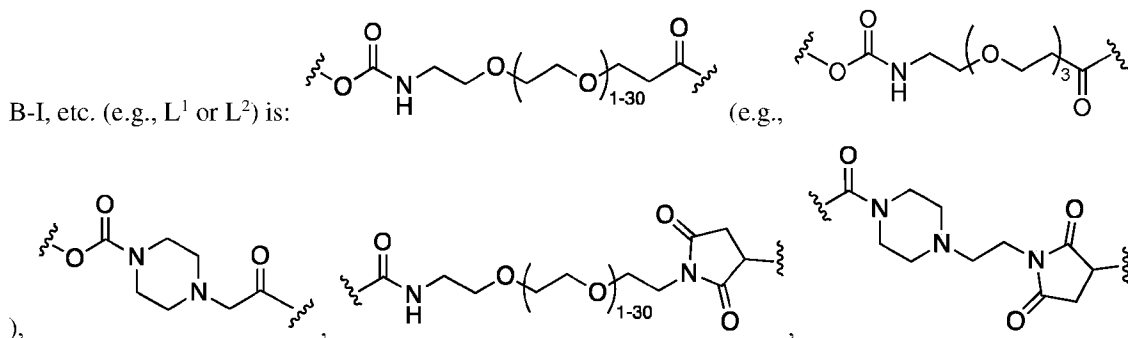
[0154] In certain embodiments, —X—W is

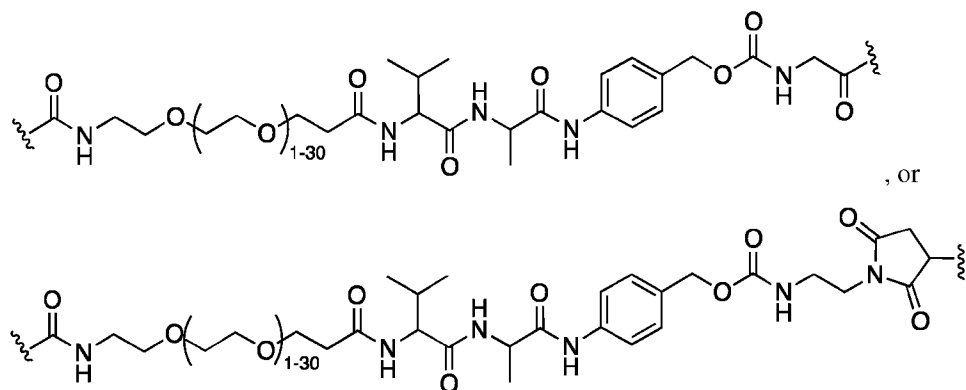
[0155] In certain embodiments, the peptide linker is specifically tailored so that it will be selectively cleaved (e.g., enzymatically cleaved) releasing the drug, such as by one or more of the tumor-associated proteases.

[0156] In certain embodiments, the peptide linker has a chain length of two to four amino acid residues (i.e., a di-, tri-, or tetra-peptide). It will be understood, however, that peptide linkers up to five, six, seven, or eight amino acid residues may also suitably be employed.

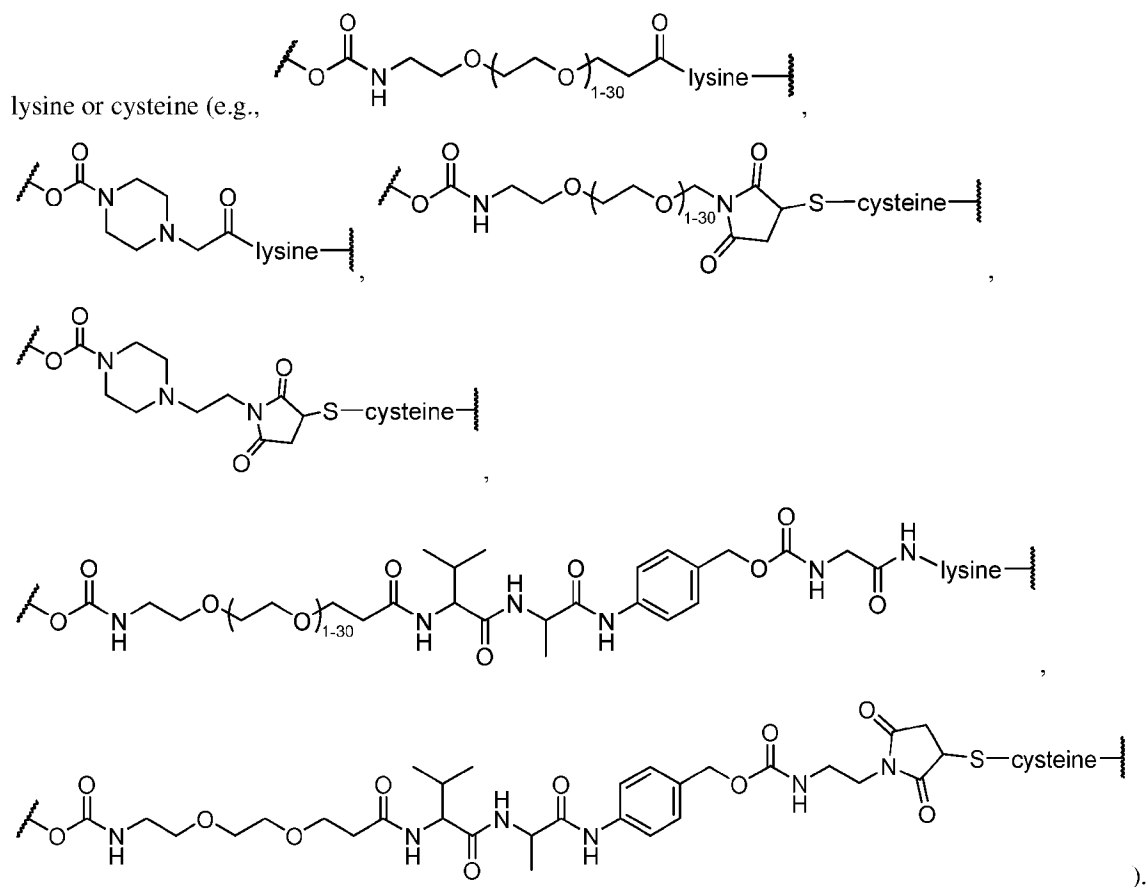
[0157] In certain embodiments, the peptide linker is Phe-Lys, Val-Lys, Val-Ala, Ala-Ala, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Ala-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Gly-Phe-Leu-Gly [SEQ ID NO: 1], Ala-Leu-Ala-Leu [SEQ ID NO:2], Phe-N⁹-tosyl-Arg, or Phe-N⁹-Nitro-Arg. In certain embodiments, the peptide linker is Phe-Lys, Val-Lys, Val-Ala, Ala-Ala, Val-Val, Val-Cit, or D-Phe-L-Phe-Lys. In certain embodiments, the peptide linker is Val-Cit, Val-Ala, or Ala-Ala.

[0158] In certain embodiments, the linker of a Formula as disclosed herein, e.g., in Formula A-I, A-II,



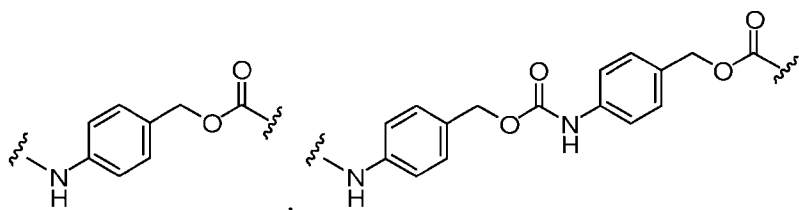


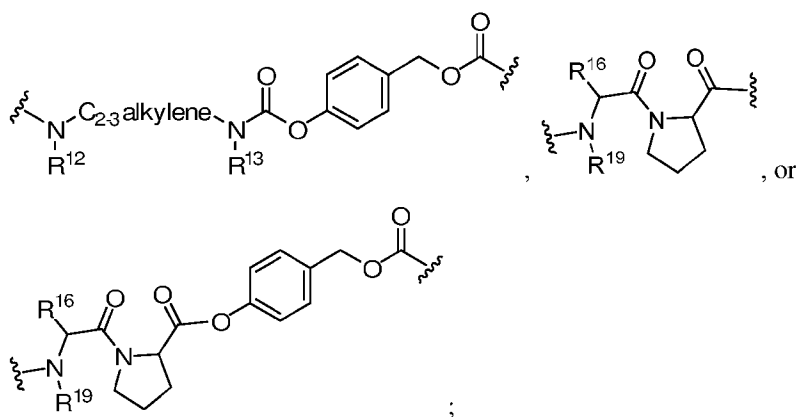
[0159] The foregoing linkers may attach on the right-hand side to amino acid side chains of D¹ such



[0160] In some embodiments, L¹ is -OC(O)L⁴- or -OC₁₋₆alkyleneC(O)L⁴-;

L^4 is a bond, $-N(R^{12})-C_{2-3}alkylene-N(R^{13})C(O)-$, $-CH(NHC(O)R^{14})C_{1-4}alkylene-S-S-C_{1-4}alkylene-$
 $OC(O)-$, $-NHNHC(O)CH(NHC(O)R^{15})CH_2C(O)-$, $-C_{1-6}alkylene-CH(G^x)OC(O)-$,





R^{12} , R^{13} , R^{14} , R^{15} , and R^{19} are each independently hydrogen or C_{1-4} alkyl;

R^{16} is hydrogen, C_{1-4} alkyl, $-C_{1-4}$ alkylene-OH, $-C_{1-4}$ alkylene- OC_{1-4} alkyl, $-C_{1-4}$ alkylene- CO_2H , or $-C_{1-4}$ alkylene- $CONH_2$; and

G^x is phenyl optionally substituted with 1-5 substituents independently selected from the group consisting of halogen, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, cyano, and nitro.

[0161] In some embodiments, R^{1B} is selected from the group consisting of $-NR^{1c}-CH_2CH_2-N(CH_3)_3^+$, $-N(R^{1c})-CH_2CH_2-SO_3H$, $-N(R^{1c})-(CH_2CH_2O)_3-CH_2CH_2N((CH_2CH_2O)_3-CH_2CH_2-CO_2H)_2$, and $-N(R^{1c})-CH(CH_2O-CH_2CH_2-CO_2H)_2$.

[0162] In some embodiments, R^{1A} is C_{1-4} alkyl.

[0163] In some embodiments, R^{1A} is CH_3 .

[0164] In some embodiments, R^{1c} is hydrogen.

[0165] In some embodiments, R^{1A} is C_{1-4} alkyl;

R^{1B} is selected from the group consisting of G^1 , OH, $-NR^{1c}-C_{1-4}$ alkylene- G^1 , $-NR^{1c}-C_{1-4}$ alkylene- $N(R^{1d})_2$, $-N(R^{1c})CHR^{1e}CO_2H$, $-N(R^{1c})CH_2CO_2H$, and $-N(R^{1f})-CH_2CH_2-(N(CH_2CO_2H)CH_2CH_2)_n-N(CH_2CO_2H)_2$;

R^{1e} is $-C_{1-4}$ alkylene- CO_2H ;

R^{1f} is hydrogen or C_{1-4} alkylene- CO_2H ;

G^1 is a 4- to 8-membered monocyclic heterocyclyl containing a first nitrogen and optionally one additional heteroatom selected from nitrogen, oxygen, and sulfur, G^1 being attached at the first nitrogen and optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4} alkyl, C_{1-4} haloalkyl, halo, cyano, OH, $-OC_{1-4}$ alkyl, and oxo; and

n is 0, 1, or 2.

[0166] In some embodiments, R^{1A} is CH_3 ;

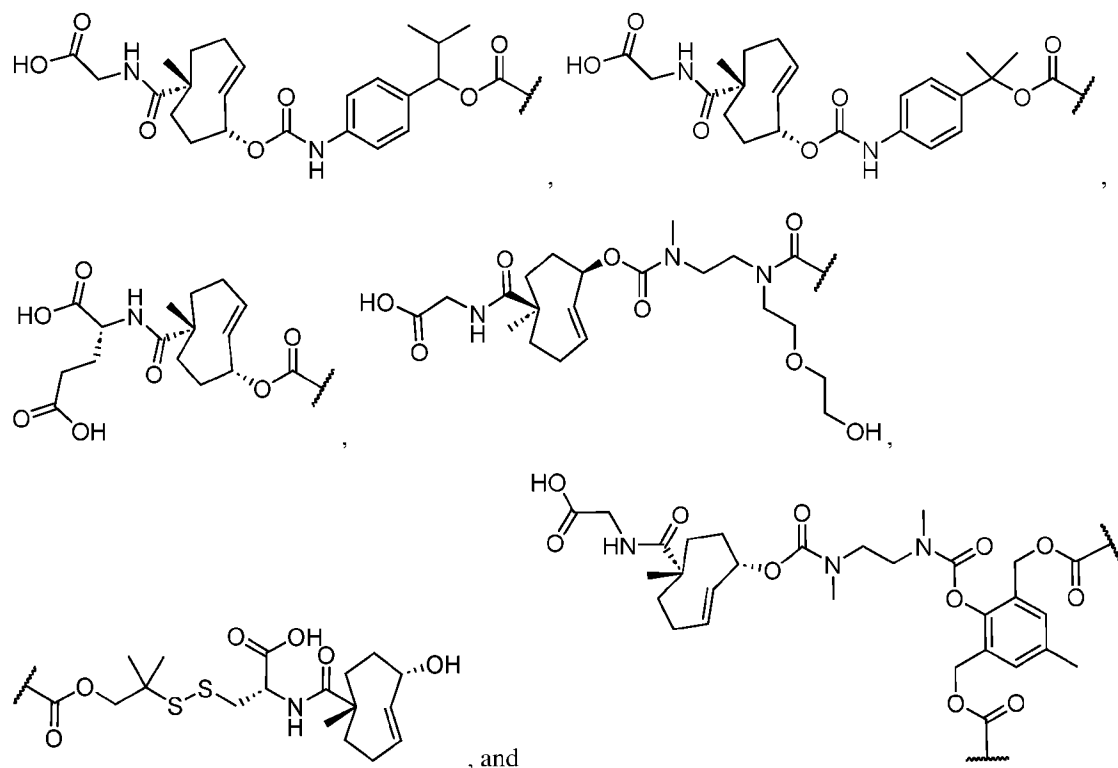
R^{1e} is $-CH_2CO_2H$;

R^{1f} is hydrogen or $\text{CH}_2\text{CO}_2\text{H}$; and

G^1 is a piperazinyl, morpholinyl, piperidinyl, azepanyl, or pyrrolidinyl, attached through a ring nitrogen atom and optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4} alkyl, C_{1-4} haloalkyl, halo, cyano, OH, $-\text{OC}_{1-4}$ alkyl, and oxo.

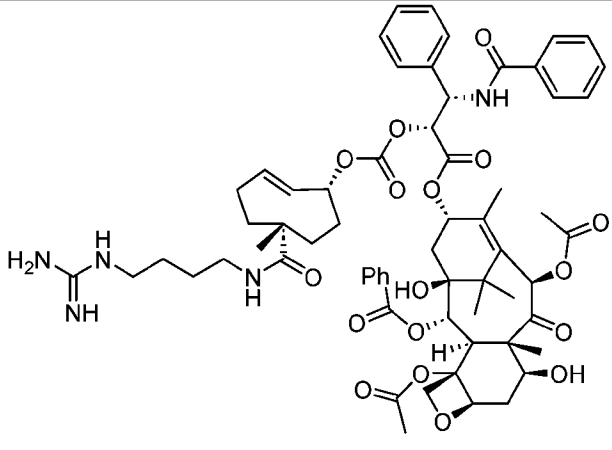
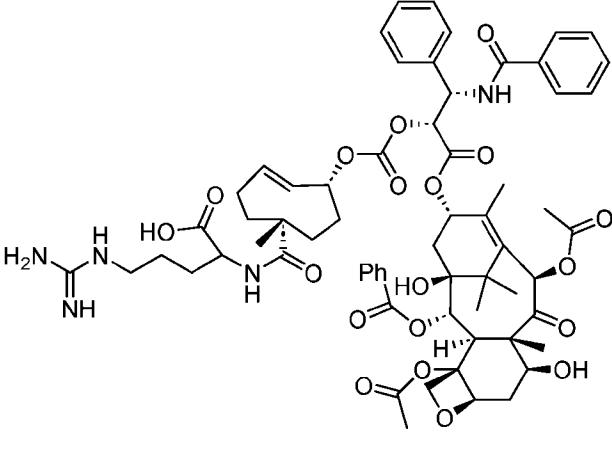
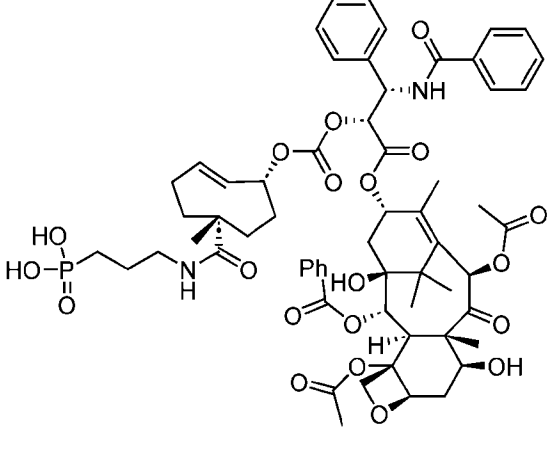
[0167] In some embodiments, R^{1B} is selected from the group consisting of OH, $\text{N(H)CH}_2\text{CO}_2\text{H}$, $-\text{N(H)CHR}^{1e}\text{CO}_2\text{H}$, $-\text{N(H)}-\text{CH}_2\text{CH}_2-(\text{N}(\text{CH}_2\text{CO}_2\text{H})\text{CH}_2\text{CH}_2)_n-\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$, and $-\text{N}(\text{CH}_2\text{CO}_2\text{H})-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$; and R^{1e} is $-\text{CH}_2\text{CO}_2\text{H}$.

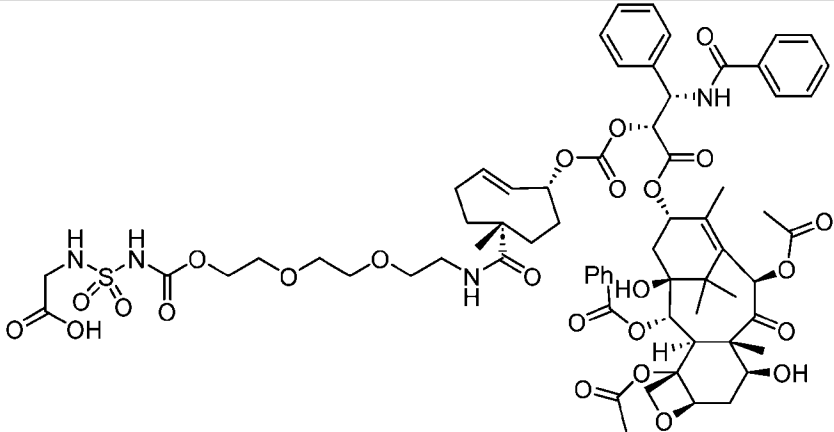
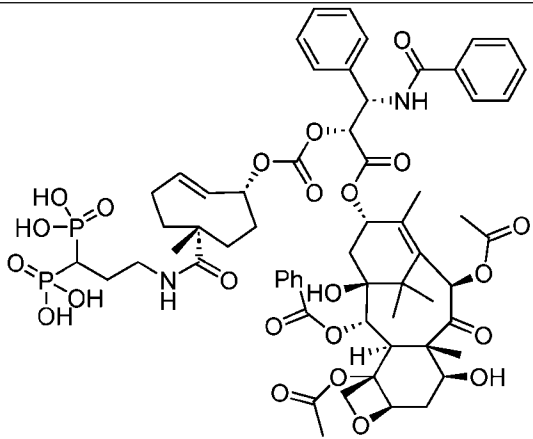
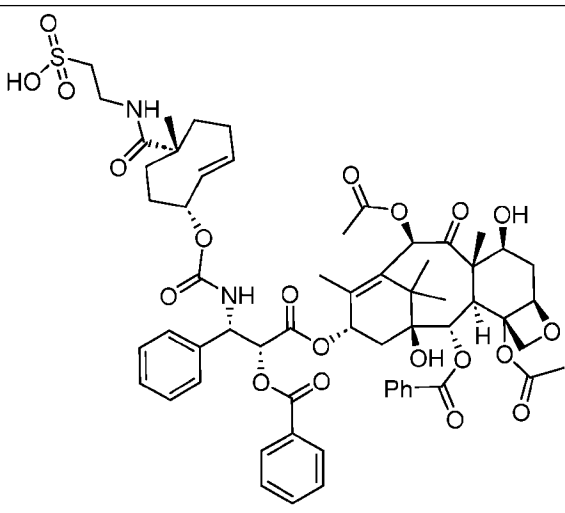
[0168] In some embodiments, the moiety $G-L^1$ is selected from the group consisting of:

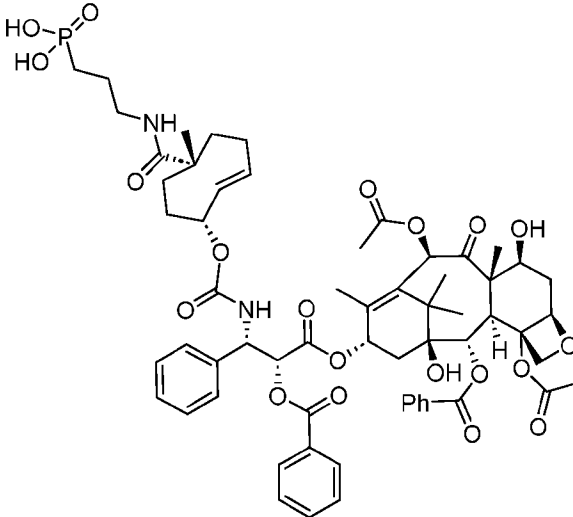
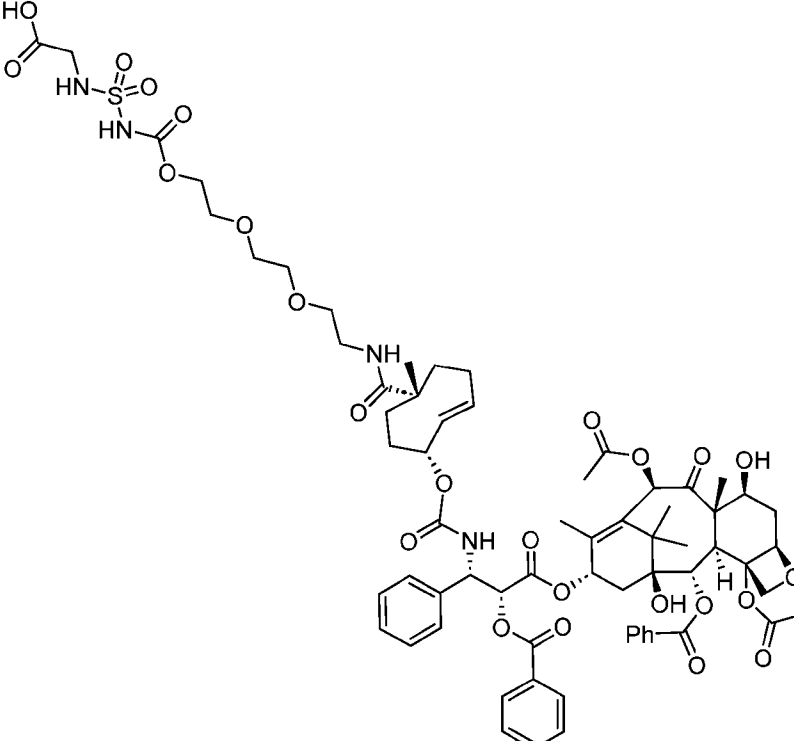


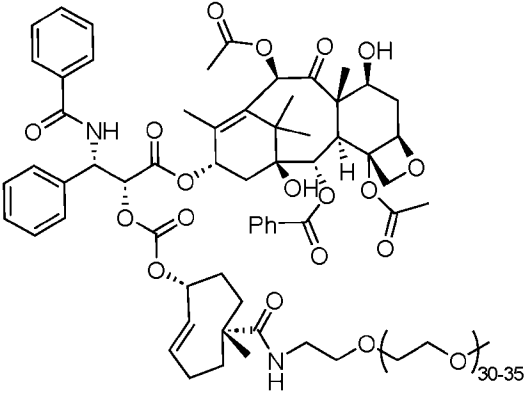
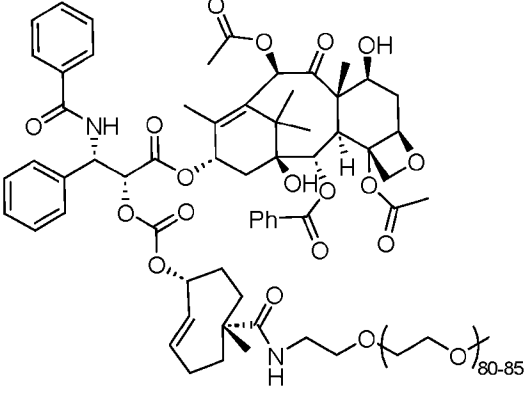
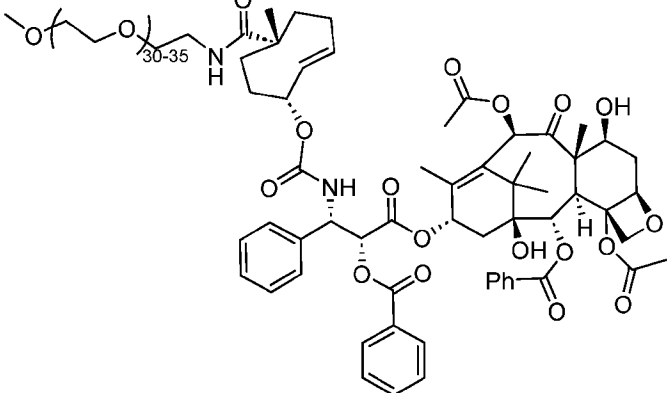
[0169] In certain embodiments, provided is a conjugate, or a pharmaceutically acceptable salt thereof, where the conjugate is selected from Table A-1.

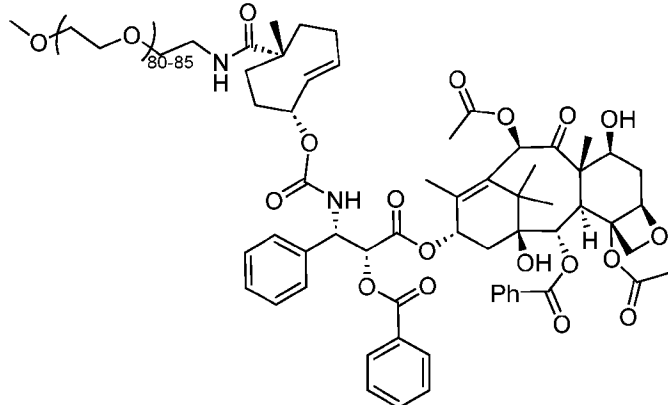
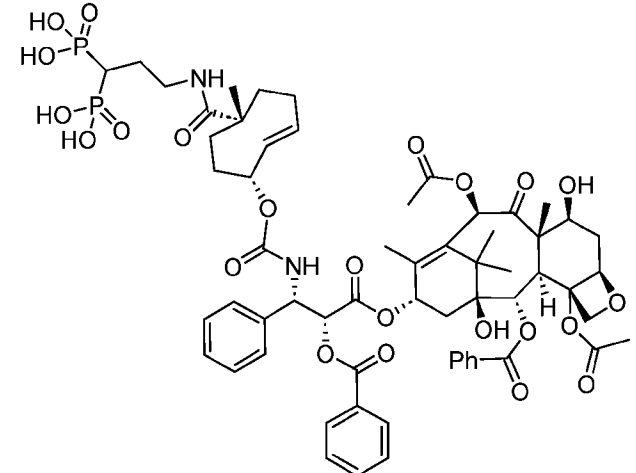
Table A-1

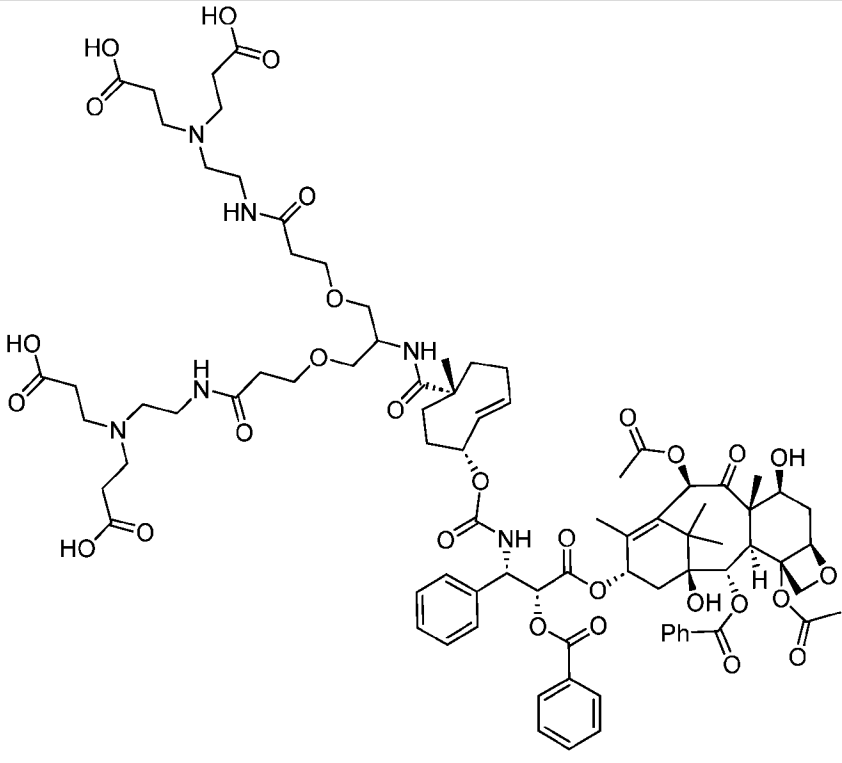
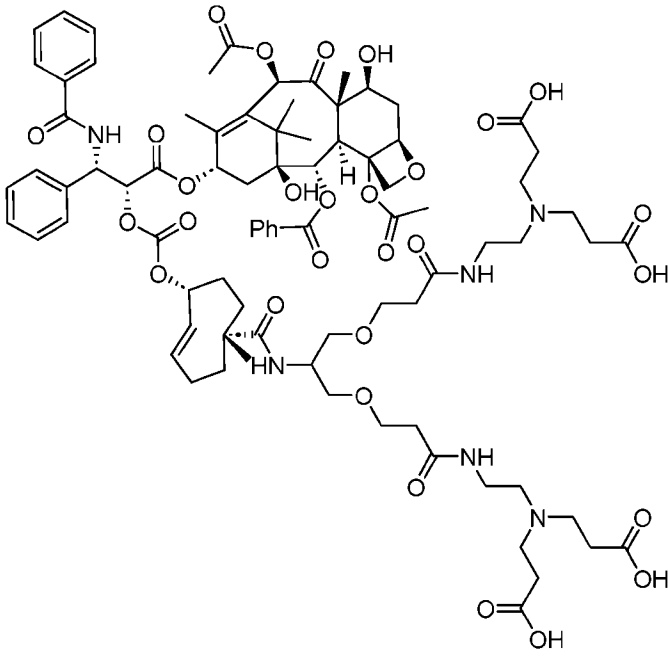
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2	
3	

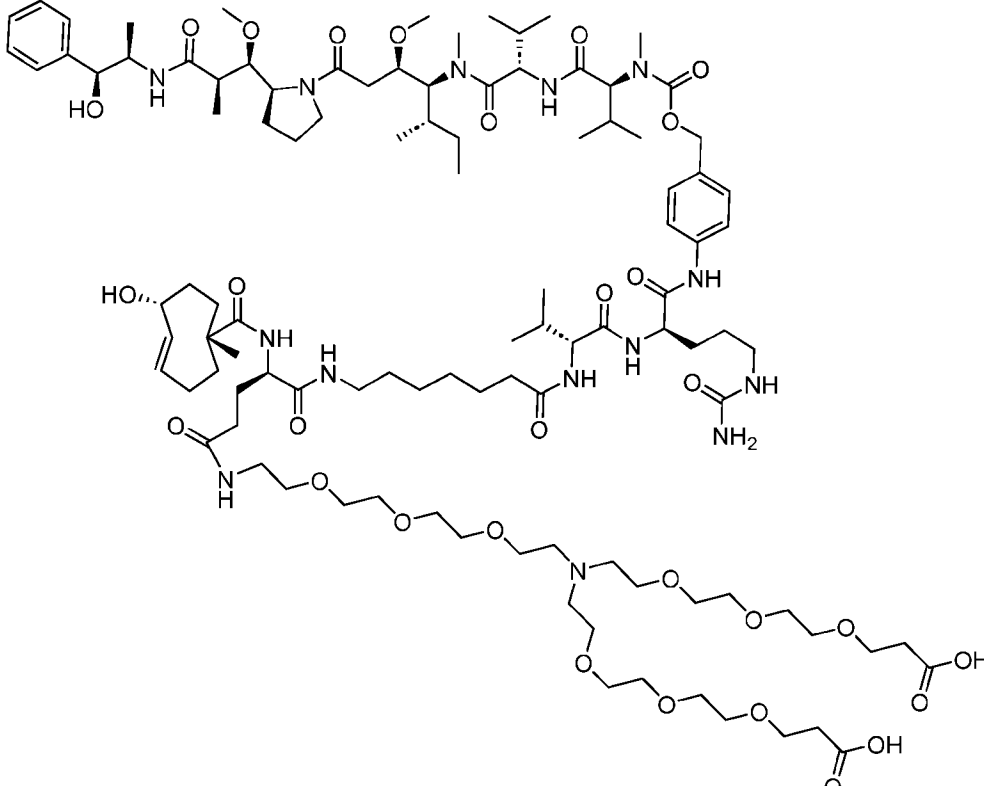
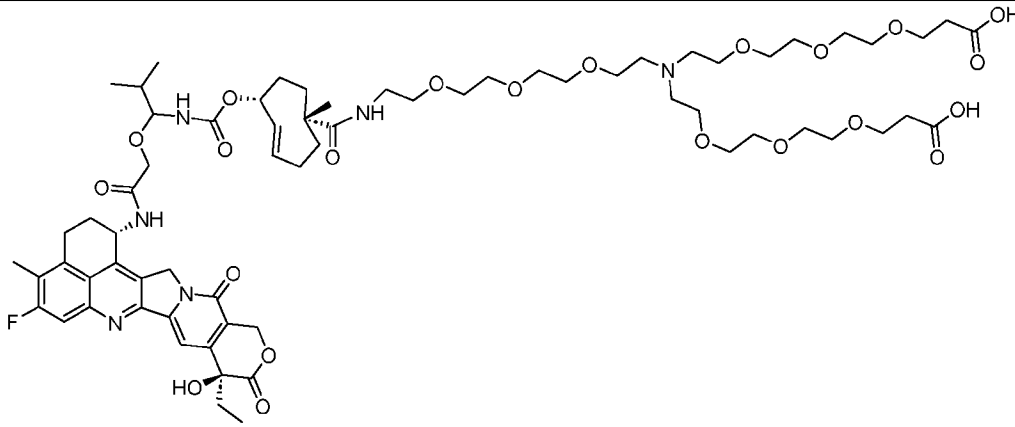
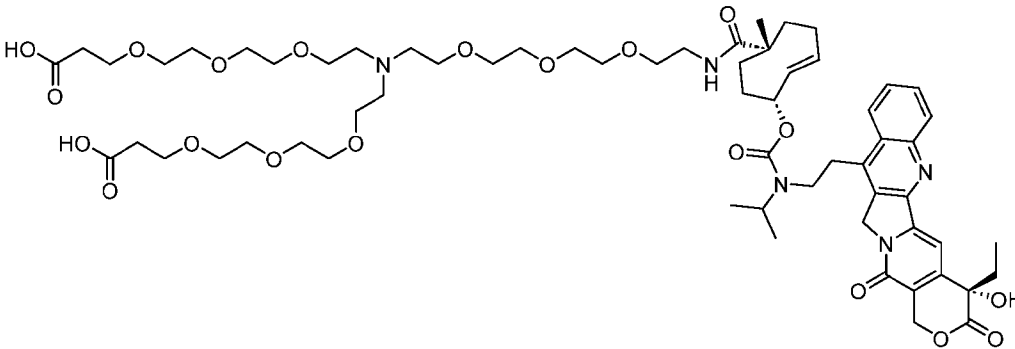
No.	Structure
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5	
6	

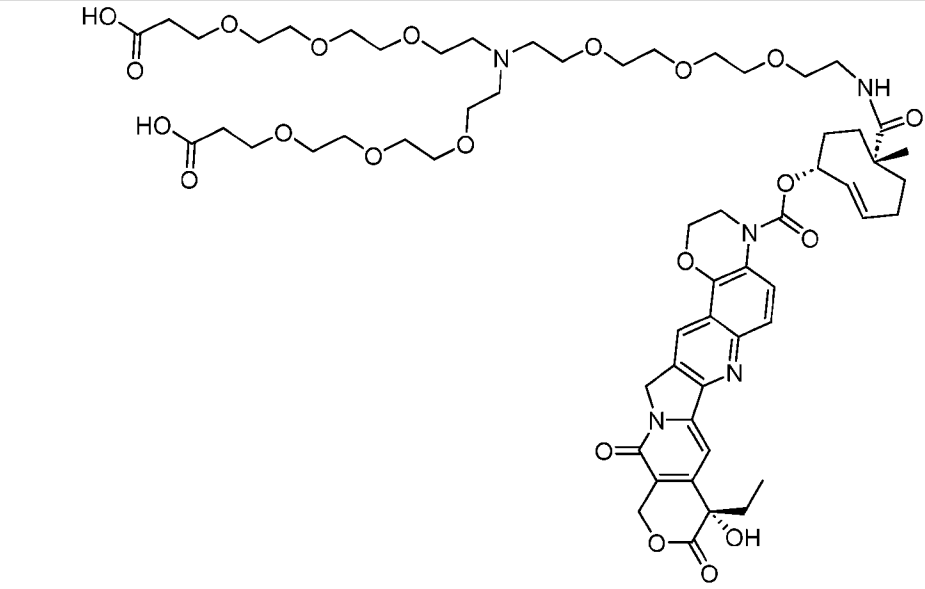
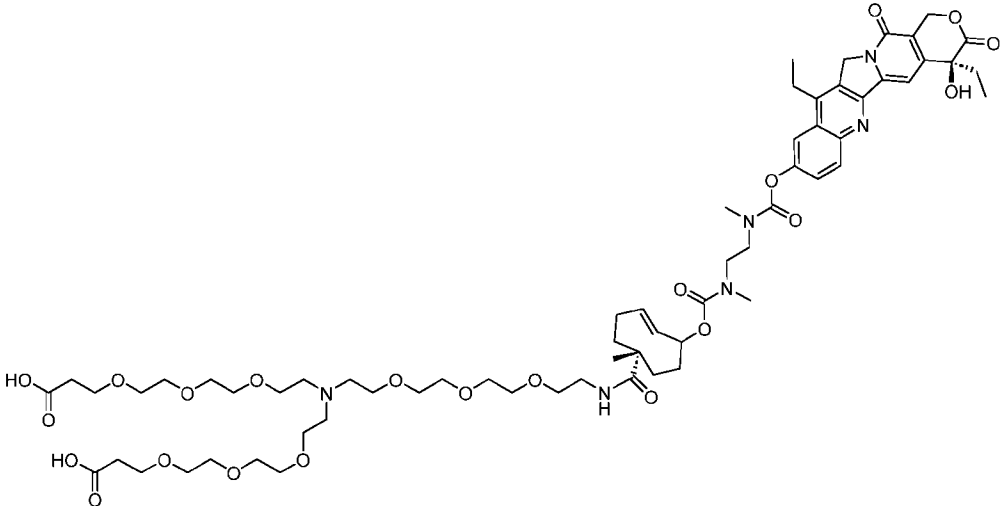
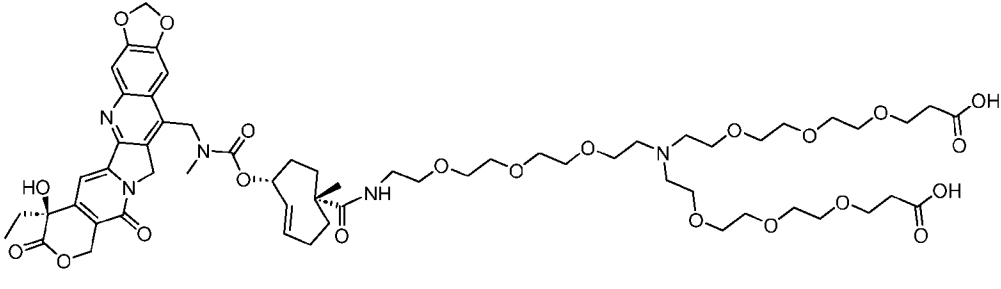
No.	Structure
7	
8	

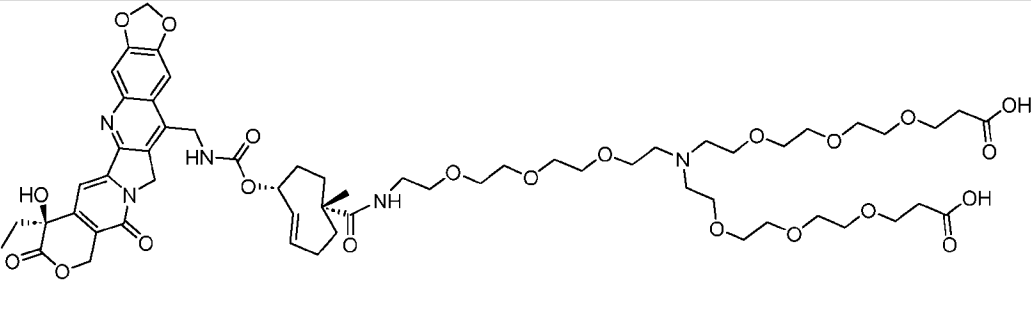
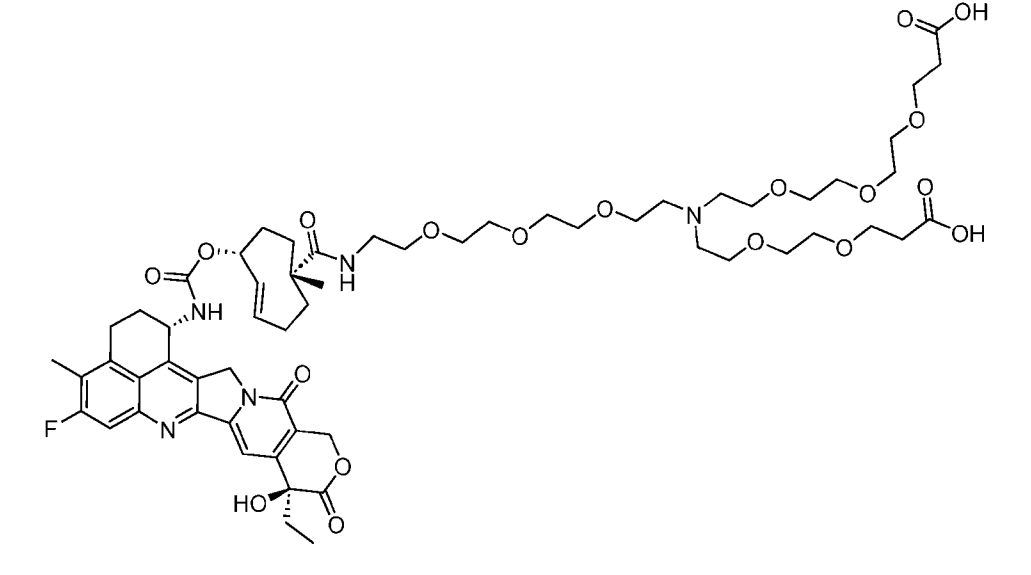
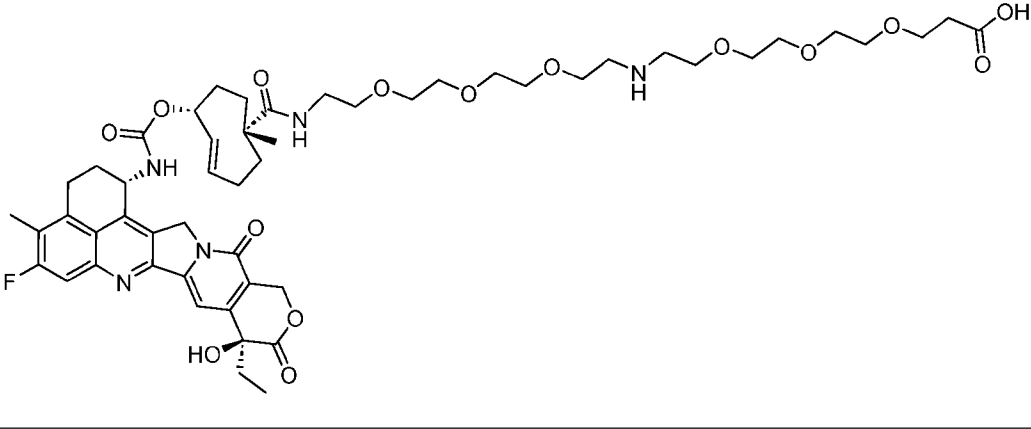
No.	Structure
9a	
9b	
10a	

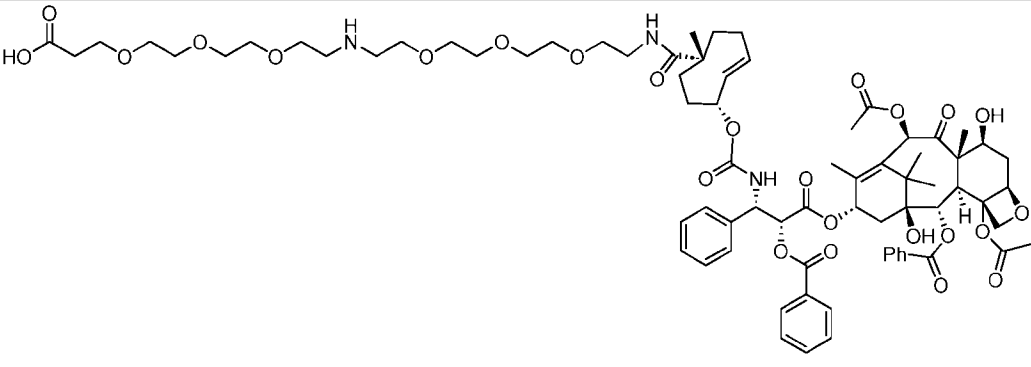
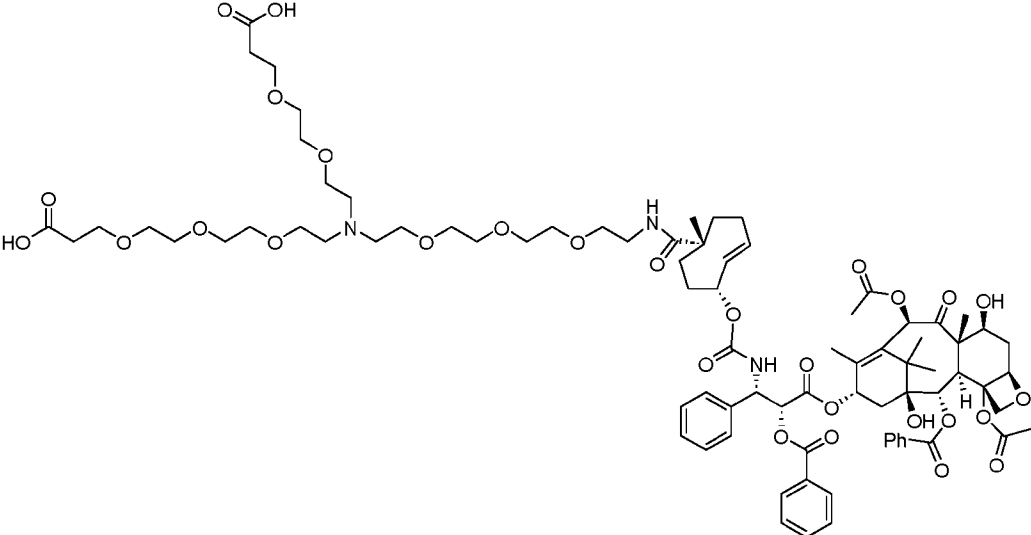
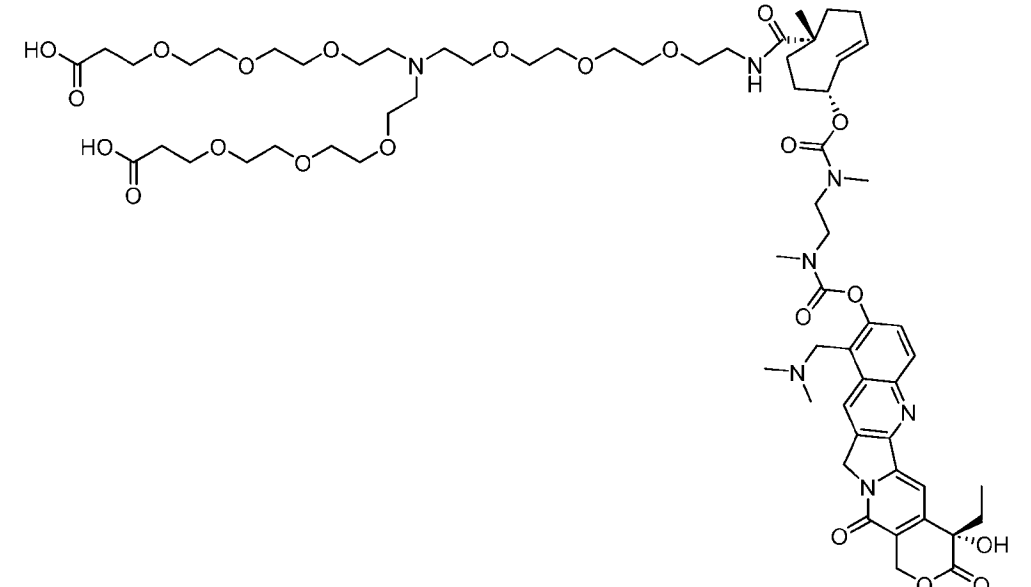
No.	Structure
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11	

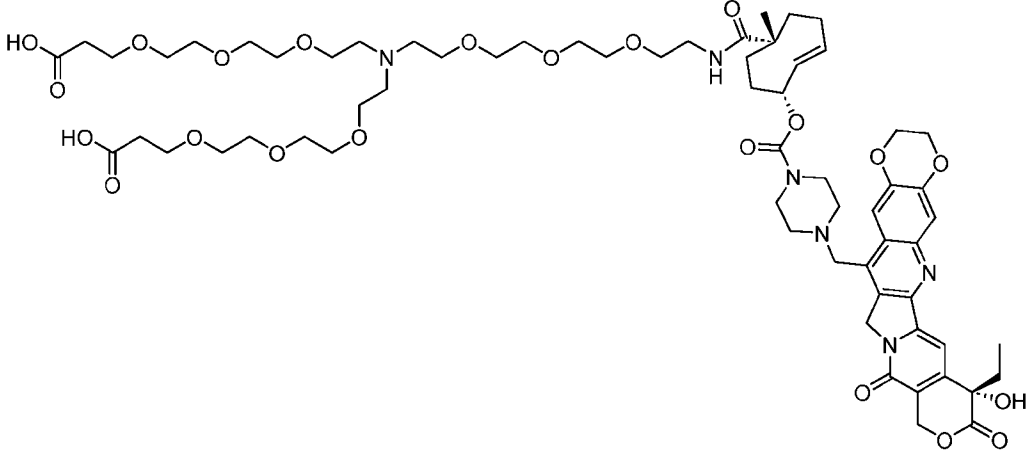
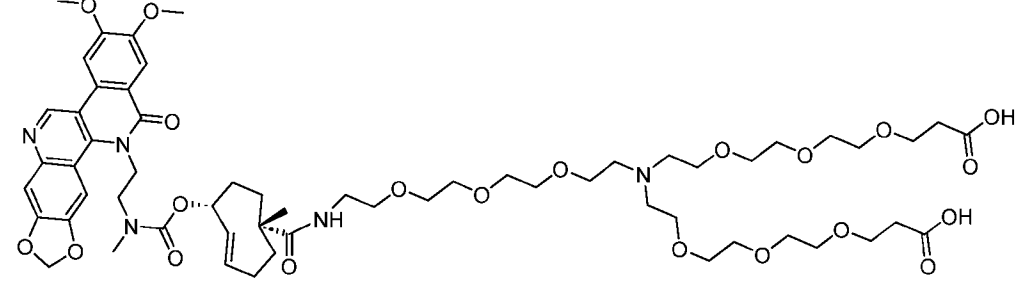
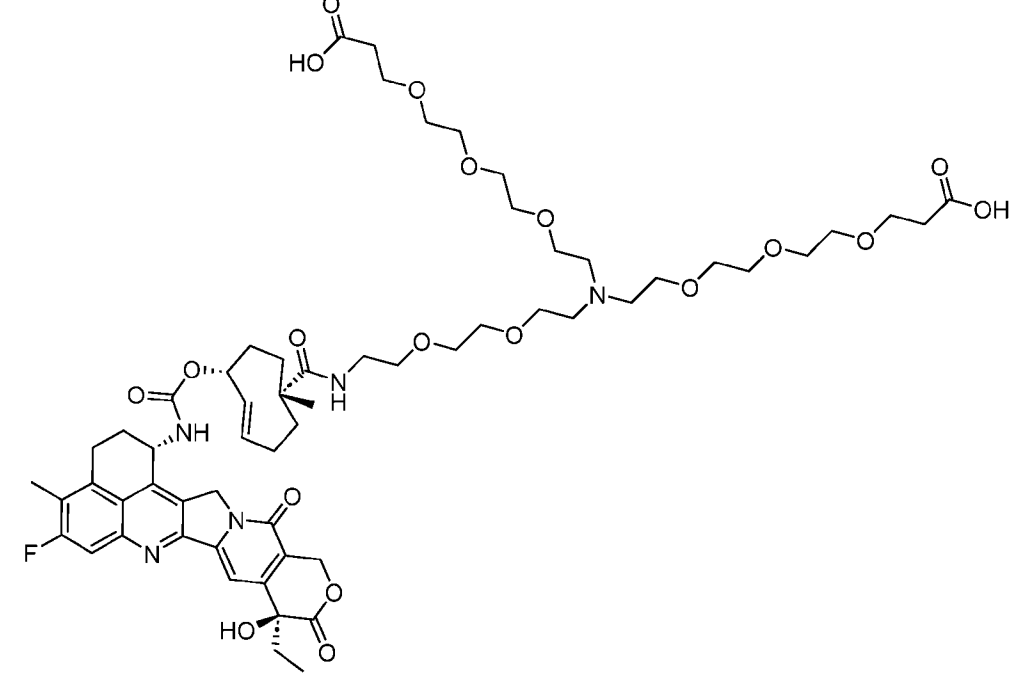
No.	Structure
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13	

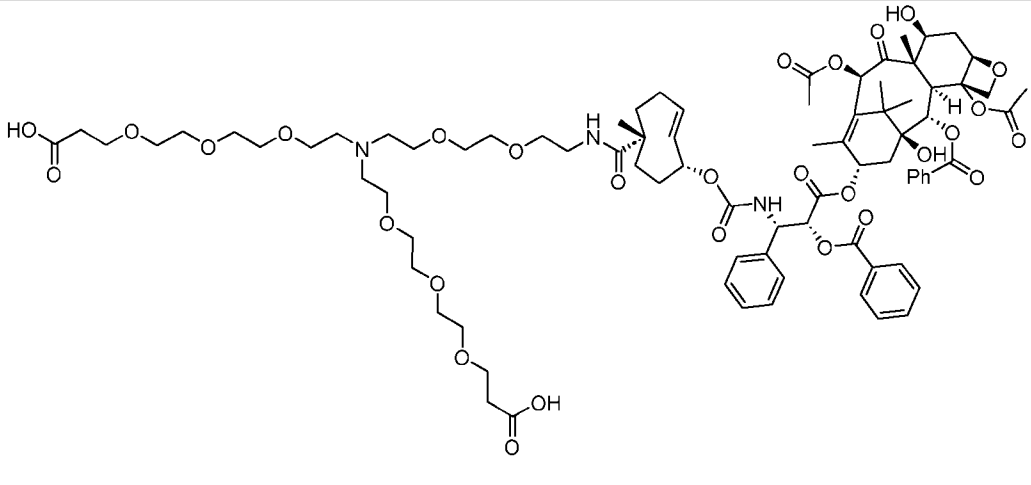
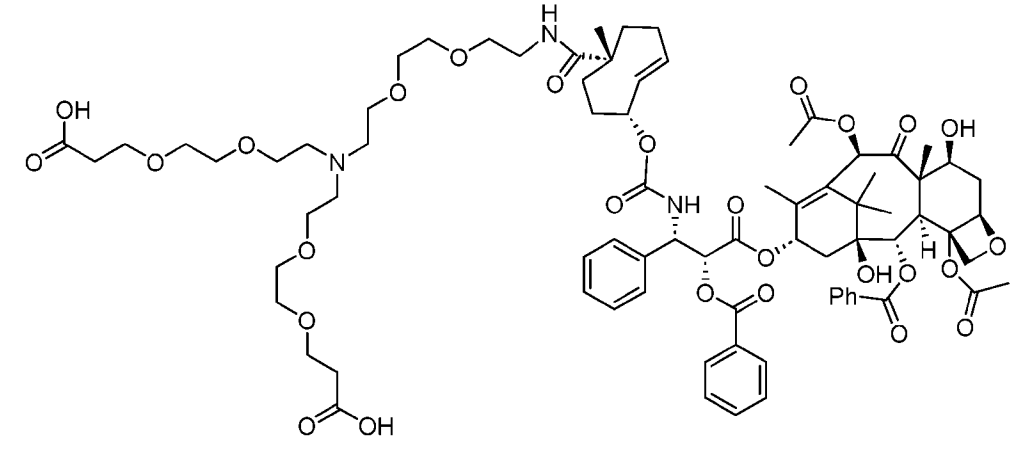
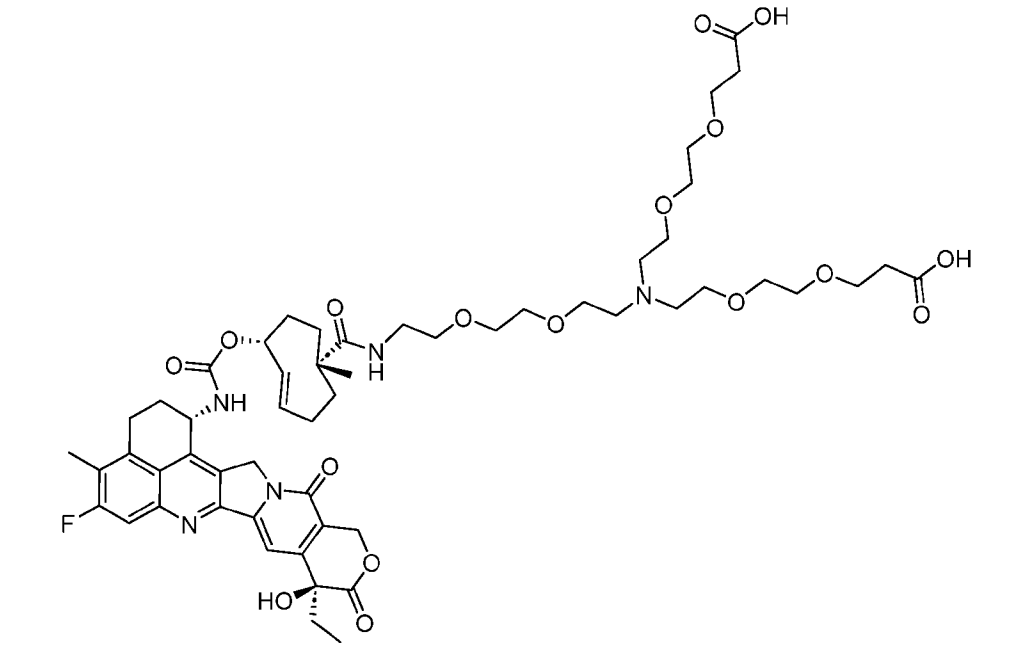
No.	Structure
14	
15	
16	

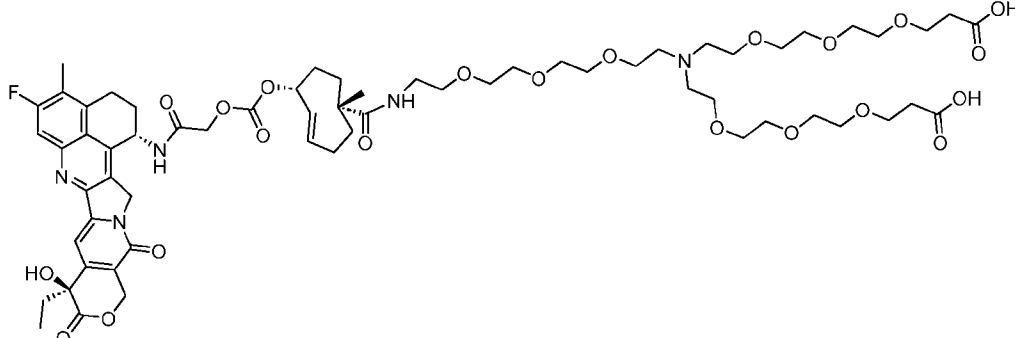
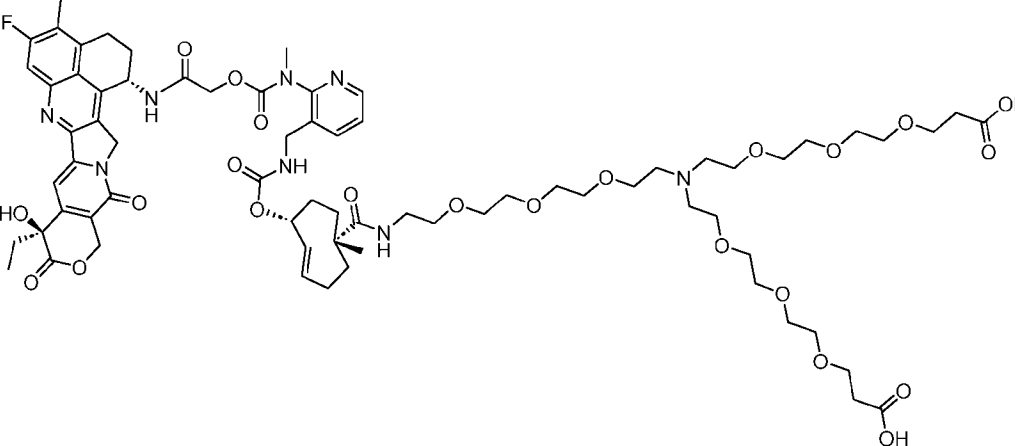
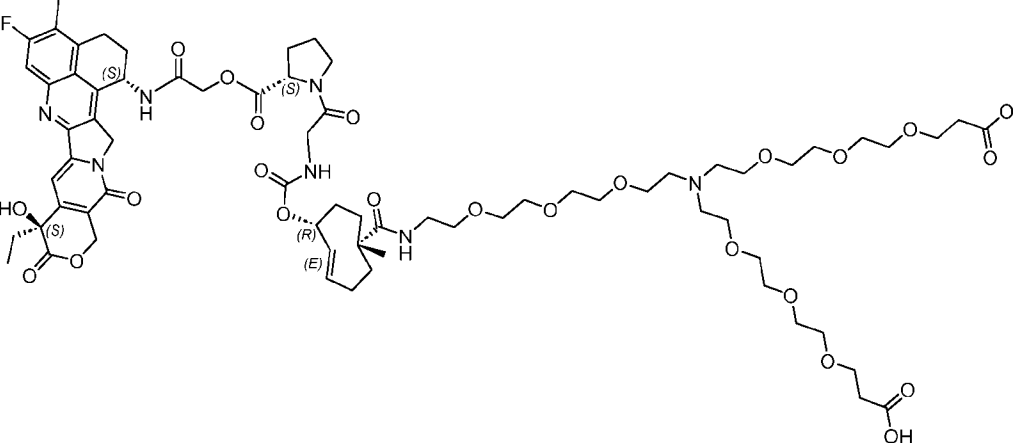
No.	Structure
17	
18	
19	

No.	Structure
20	
21	
22	

No.	Structure
23	
24	
25	

No.	Structure
26	
27	
28	

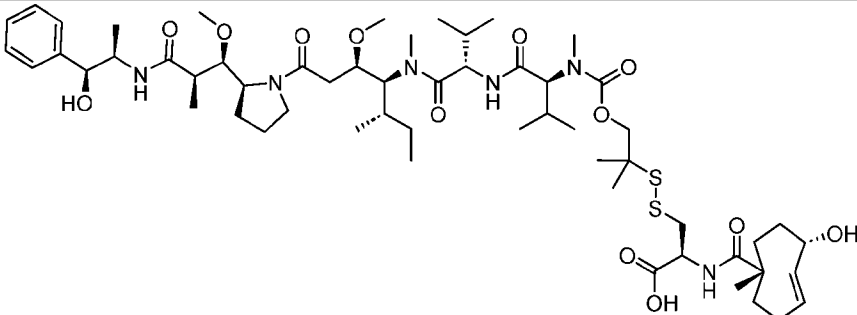
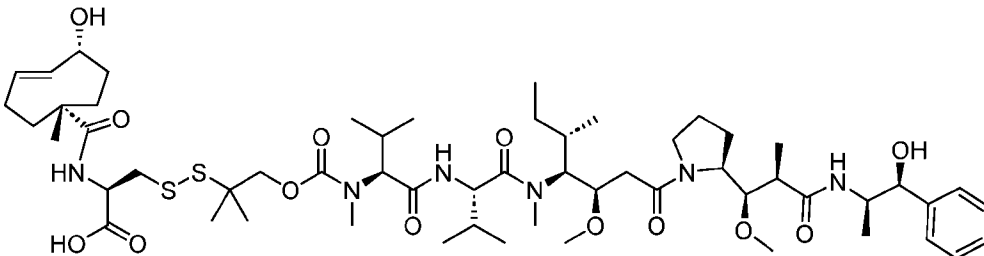
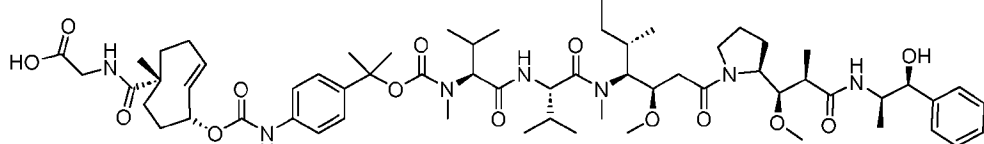
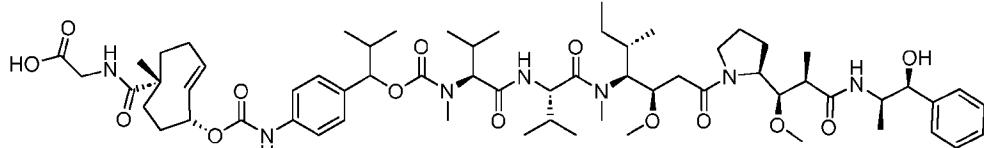
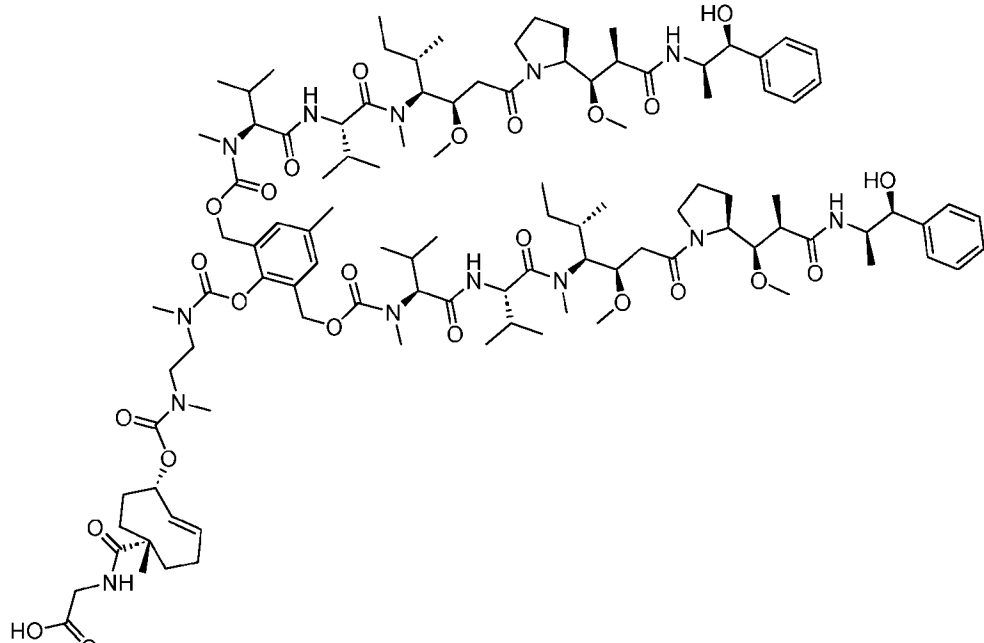
No.	Structure
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30	
31	

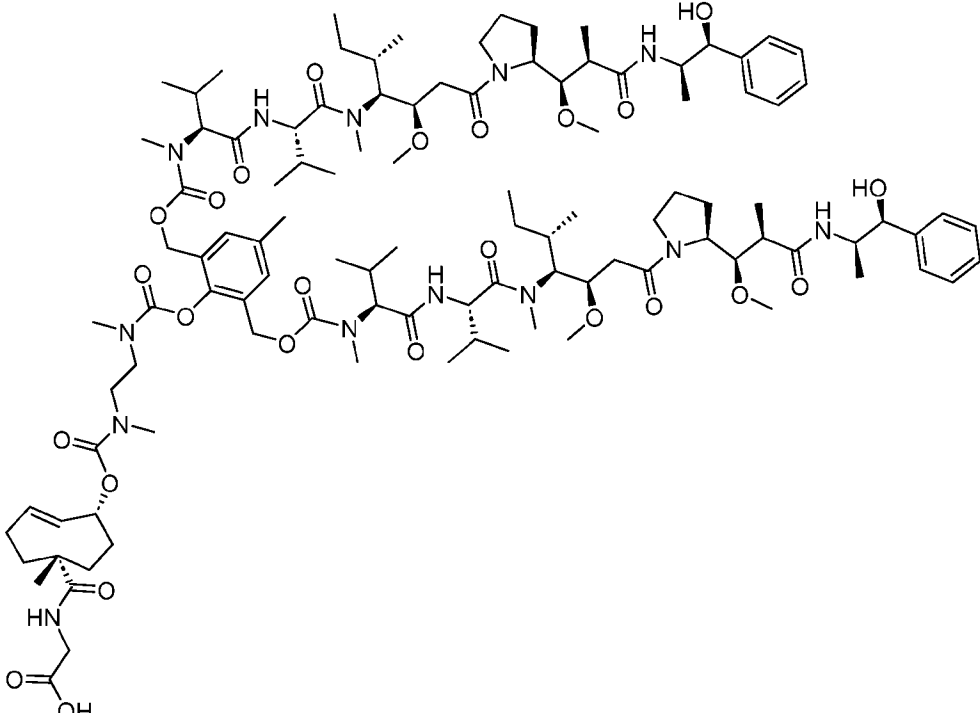
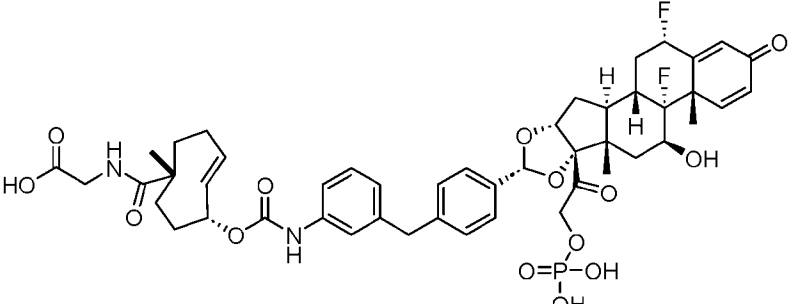
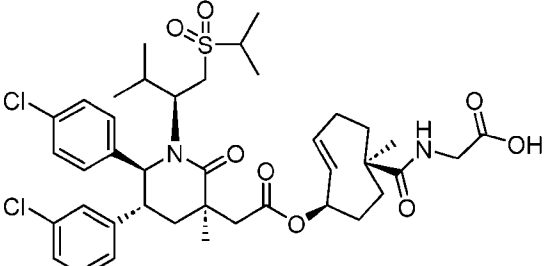
No.	Structure
32	
33	
34	

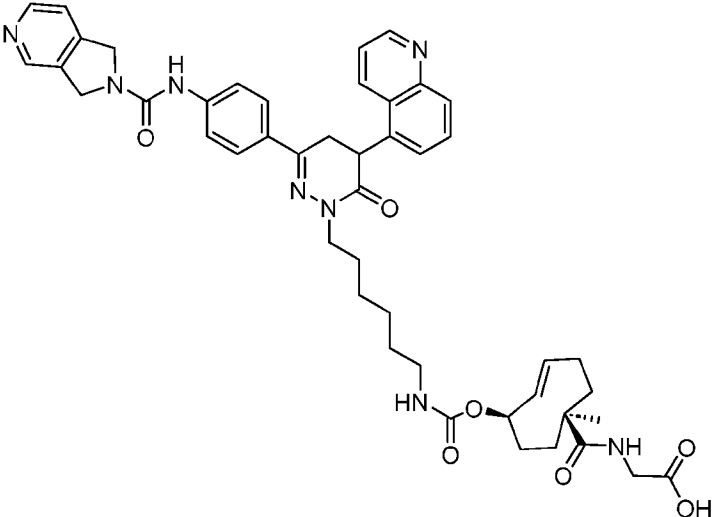
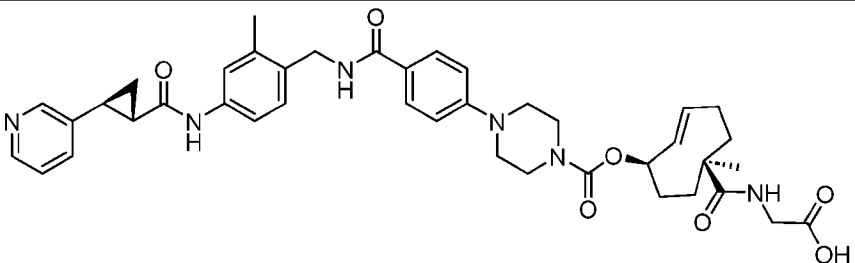
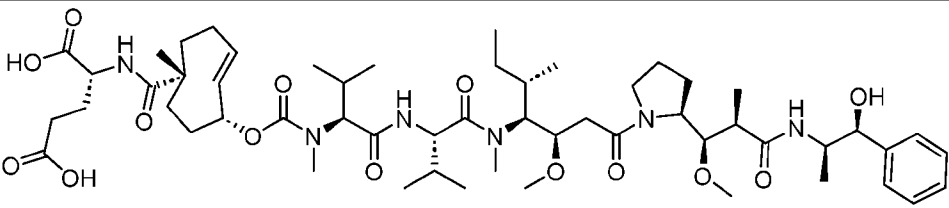
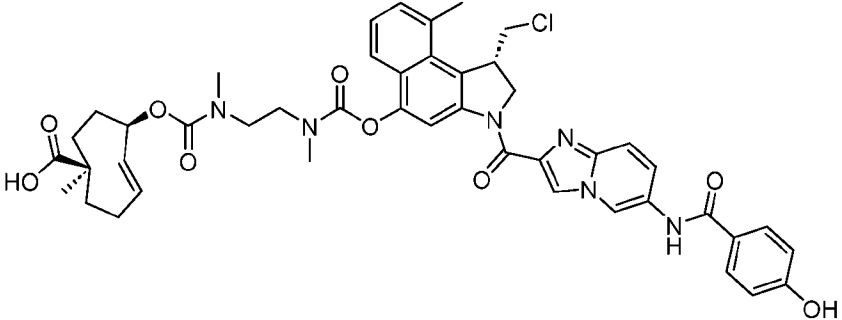
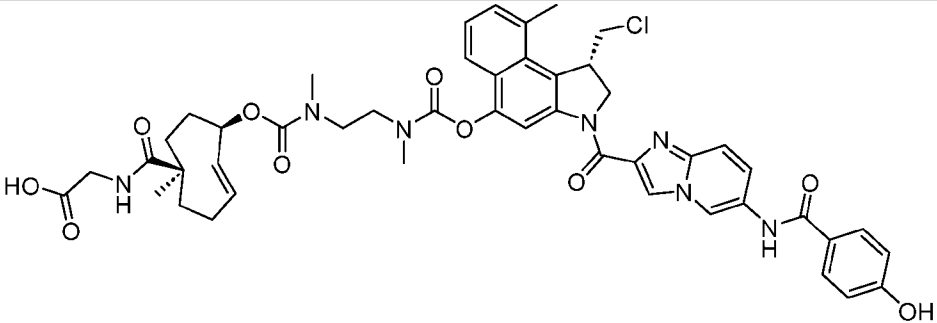
[0170] In certain embodiments, provided is a conjugate, or a pharmaceutically acceptable salt thereof, where the conjugate is selected from Table B-1.

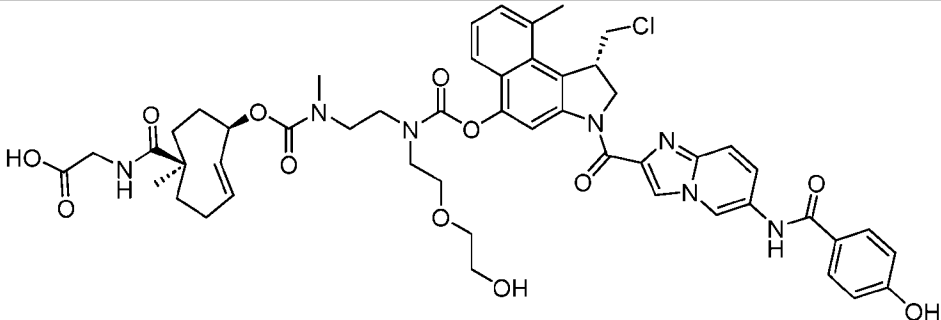
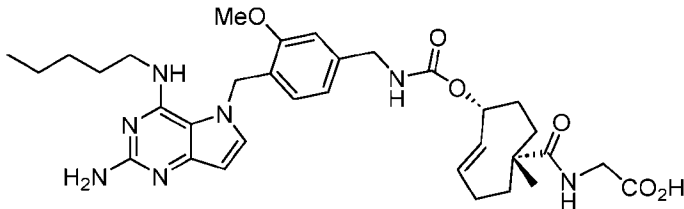
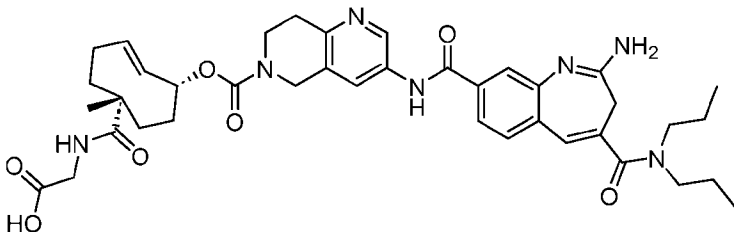
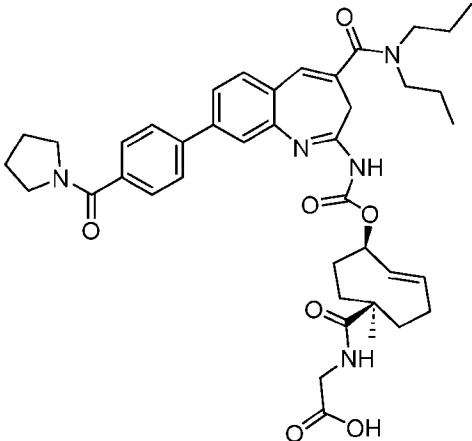
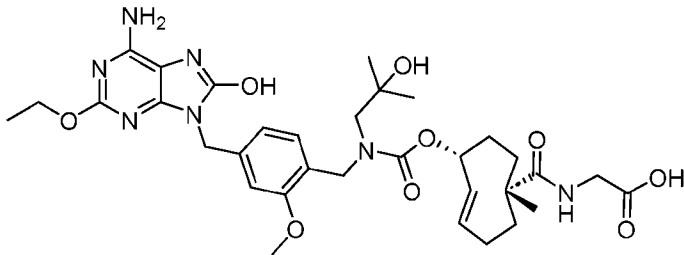
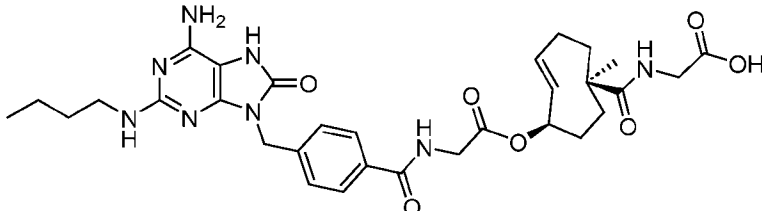
Table B-1

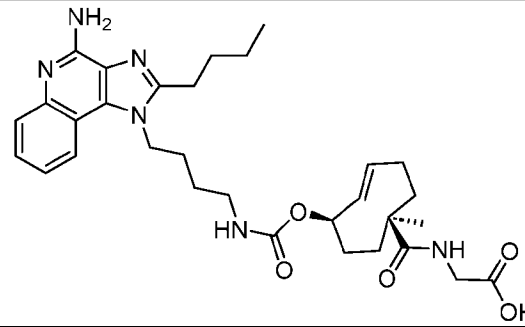
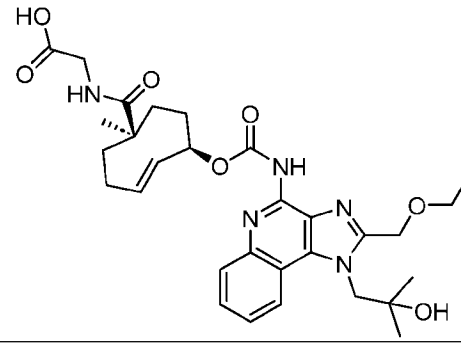
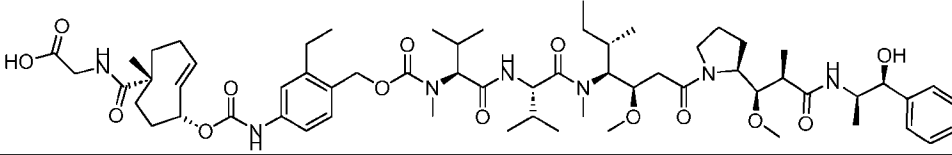
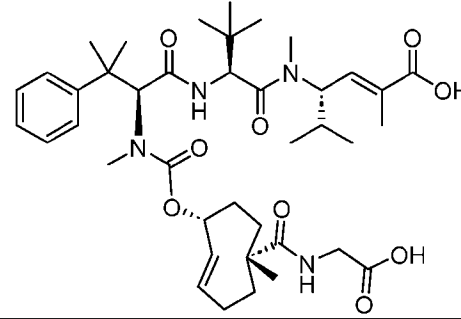
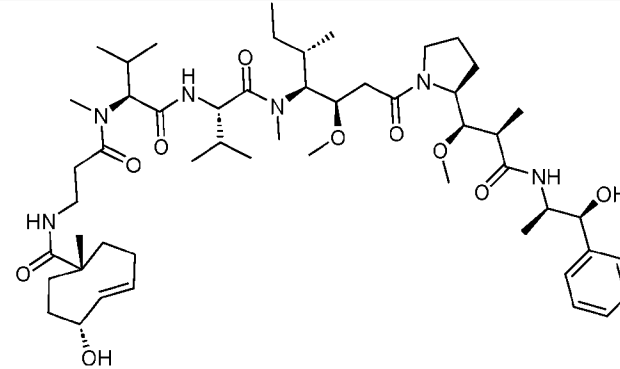
No.	Structure
-----	-----------

No.	Structure
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101	
102	
103	
104a	

No.	Structure
104	
105	
106	

No.	Structure
107	
108	
109	
110	
111	

No.	Structure
112	
113	
114	
115	
116	
117	

No.	Structure
118	
119	
120	
121	
122	

[0171] In some embodiments, provided is a method for delivering an effective amount of a payload (i.e., a taxane, such as paclitaxel, or a camptothecin, such as exatecan, or a derivative, or analog thereof) to a target location in a subject, the method comprising administering to the subject at the target location a

therapeutic support composition as described herein, and administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein.

C. Therapeutic Support Compositions

[0172] The therapeutic support composition comprises a support. Supports may be biocompatible supports compositions, i.e., compatible with the subject's body. In some instances, a support is non-toxic to the subject and does not substantially react with tissue or biological compounds in the subject. For example, the support can be a hydrogel, among others. A support is capable of implantation into a subject's body and supporting binding agents (e.g., tetrazine-containing group), as well as payloads after the binding agents conjugate. Representative supports include, but are not limited to polymers, viscous or non-viscous liquid materials, gels, hydrogels, polysaccharide hydrogels, a cross-linked polymer matrix, a metal, a ceramic, a plastic, a bone graft material, alginate, cellulose, chitosan, hyaluronic acid, chondroitin sulfate, heparin, and the like. Supports also include particles, such as nanoparticles, microparticles, and the like.

[0173] Hydrogels may be polysaccharide hydrogels, alginate, cellulose, hyaluronic acid, chitosan, chitosin, chitin, hyaluronic acid, chondroitin sulfate, heparin, and the like. Other suitable sugar-based biomaterials include those described in *Polymer Advanced Technology*, 2014, 25, 448-460. Polymers that may be used as the support can include, but are not limited to, polyphosphazenes, polyanhydrides, polyacetals, poly(ortho esters), polyphosphoesters, polycaprolactones, polyurethanes, polylactides, polycarbonates, polyamides, and polyethers, and blends/composites/co-polymers thereof. Representative polyethers include, but are not limited to, poly(ethylene glycol) (PEG), polypropylene glycol (PPG), triblock Pluronic ([PEG]_n-[PPG]_m-[PEG]_n), PEG diacrylate (PEGDA), and PEG dimethacrylate (PEGDMA), where n and m are each independently an integer from 1-100. The support can also include proteins and other poly(amino acids), such as collagen, gelatin, elastin and elastin-like polypeptides, albumin, fibrin, poly(gamma-glutamic acid), poly(L-lysine), poly(L-glutamic acid), poly(aspartic acid), and the like.

[0174] In some embodiments, the support is a hydrogel. In some embodiments, the support is an alginate. In some embodiments, the support is chitin. In some embodiments, the support is a hyaluronic acid (e.g., a non-hydrogel hyaluronic acid substantially without crosslinks). In some embodiments, the support is chitosin. In some embodiments, the support is chitosan.

[0175] In certain embodiments, the support is a particle. Particles of the present disclosure can have a diameter that is 2 cm or less, such as 1.5 cm or less, or 1 cm or less, or 0.5 cm or less. For example, the particles can be nanoparticles or microparticles. Nanoparticles include particles having average dimensions in the nanometer scale (e.g., 1000 nm or less). Microparticles are particles having average dimensions in the micrometer scale (e.g., 1000 μm or less). By "average" is meant the arithmetic mean. In some embodiments, the nanoparticles have a diameter ranging from 1 nm to 1 μm, such as from 10 nm to 1 μm, or 25 nm to 1 μm, or 50 nm to 1 μm, or 75 nm to 1 μm, or 100 nm to 1 μm, or 150 nm to 1 μm,

or 200 nm to 1 μ m, or 250 nm to 1 μ m, or 300 nm to 1 μ m, or 350 nm to 1 μ m, or 400 nm to 1 μ m, or 450 nm to 1 μ m, or 500 nm to 1 μ m. In other embodiments, the microparticles have a diameter ranging from 1 μ m to 1 mm, such as from 10 μ m to 1 mm, or 25 μ m to 1 mm, or 50 μ m to 1 mm, or 75 μ m to 1 mm, or 100 μ m to 1 mm, or 150 μ m to 1 mm, or 200 μ m to 1 mm, or 250 μ m to 1 mm, or 300 μ m to 1 mm, or 350 μ m to 1 mm, or 400 μ m to 1 mm, or 450 μ m to 1 mm, or 500 μ m to 1 mm. In further embodiments, small particles on the order of 10-100 nm in diameter may be assembled to form larger complexes, such as clusters or assemblies on the order of 1-10 μ m. Particles of the present disclosure may be substantially spherical, such that the particles have a substantially circular cross-section. Other particle shapes may also be used, such as, but not limited to, ellipsoid, cubic, cylindrical, conical, needle, or other irregular shapes.

[0176] A “particle” may take the form of any fabricated material, a molecule, cryptophan, a virus, a phage, etc. The particle may be composed of a material, such as, but not limited to, a metal, a ceramic, a plastic, a glass, a composite, a polymer, a hydrogel, and the like. For example, the particles may be made of an inert material, such as alginate or iron oxide. In some examples, the particles may be magnetic and can be formed from a paramagnetic, super-paramagnetic or ferromagnetic material, or other material that responds to a magnetic field. Further, a particle may be of any shape, for example, spheres, rods, non-symmetrical shapes, etc. The particles, or a group of several particles in a complex, may be functionalized with a receptor that has a specific affinity to bind to or interact with a clinically relevant substrate. The receptor may be inherent to the particle itself. For example, the particle itself may be a virus or a phage with an inherent affinity for certain substrates. Additionally or alternatively, the particles can be functionalized by covalently or otherwise attaching or associating a receptor that specifically binds or otherwise recognizes a particular clinically relevant substrate. The functionalized receptor can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or any other molecule with a defined affinity for a target substrate. Examples of material that may be used for the “particles” and/or “carrier” include polylactic acid, polyglycolic acid, PLGA polymers, alginates and alginate derivatives, gelatin, collagen, fibrin, hyaluronic acid, laminin rich gels, agarose, natural and synthetic polysaccharides, polyamino acids, polypeptides, polyesters, poly anhydrides, polyphosphazines, poly(vinyl alcohols), poly(alkylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers, pluronic polyols, polyoxamers, poly(uronic acids), poly(vinylpyrrolidone) and copolymers or graft copolymers of any of the above. These examples do not limit their concentration, their cross-linking with different agents, their method of administration, their tailored degradation profiles and other characteristics known to those skilled in the art.

[0177] The particles, or a group of several particles in a complex, may be functionalized with a targeting agent (e.g., a ligand or antibody) that specifically binds (or substantially specifically binds) to a target (e.g., a target receptor or a cell surface target, such as a clinically relevant receptor or cell surface target (e.g., antigen)). The targeting agent may be attached directly to the particle itself.

[0178] In some embodiments, the biocompatible support is a targeting agent.

[0179] The targeting agent can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or any other molecule with a specific affinity for a target receptor or cell surface target. In some instances, the receptor or cell surface target is PD-1, CTLA-4, HER2/neu, HER1/EGFR, VEGFR, 4-1BB, GITR, or other cellular receptors or cell surface targets.

[0180] In some embodiments, the targeting agent is a monoclonal antibody. A monoclonal antibody can be an entire monoclonal antibody, or a fragment thereof (e.g., antigen-binding fragment (Fab)). In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets one or more of CD25 (NCBI Gene ID 3559), CEA (NCBI Gene ID 634), CEACAM5 (NCBI Gene ID 1048), ASPH (NCBI Gene ID 444), EGFR (NCBI Gene ID 1956), EPCAM (NCBI Gene ID 4072), VEGFR (NCBI Gene ID 3791), PDGFR (NCBI Gene ID 5159), TROP2 (NCBI Gene ID 4070), Nectin4 (NCBI Gene ID 81607), PSMA (NCBI Gene ID 2346), BCMA (NCBI Gene ID 608), CD22 (NCBI Gene ID 933), CD20 (NCBI Gene ID 920), CD19 (NCBI Gene ID 930), CD79b (NCBI Gene ID 974), CD38 (NCBI Gene ID 952), CD45 (NCBI Gene ID 5788), Endoglin (NCBI Gene ID 2022), FGFR2 (NCBI Gene ID 14183), C4.4A (NCBI Gene ID 27076), Claudin-18.2 (NCBI Gene ID 51208), MMP9 (NCBI Gene ID 4318), Folate receptor (NCBI Gene ID 2348), DLL3 (NCBI Gene ID 10683), CD138 (NCBI Gene ID 6382), CD56 (NCBI Gene ID 4684), CD37 (NCBI Gene ID 951), CD74 (NCBI Gene ID 972), mesothelin (NCBI Gene ID 10232), IL-6R (NCBI Gene ID 3570), SLAMF7 (NCBI Gene ID 57823), BAFF (NCBI Gene ID 10673), MUC1 (NCBI Gene ID 4582), GPC3 (NCBI Gene ID 2719), HER2 (NCBI Gene ID 2064), HER3 (NCBI Gene ID 2065), CD30 (NCBI Gene ID 943), CD33 (NCBI Gene ID 945), CD123 (NCBI Gene ID 3563), GPNMB (NCBI Gene ID 10457), cMET (NCBI Gene ID 4233), CD142 (NCBI Gene ID 2152), NaPi2B (NCBI Gene ID 10568), GCC (NCBI Gene ID 2984), STEAP1 (NCBI Gene ID 26872), MUC16 (NCBI Gene ID 94025), CD70 (NCBI Gene ID 970), CD44 (NCBI Gene ID 960), (NCBI Gene ID), Antibody fragments (NCBI Gene ID), vWF (NCBI Gene ID 7450), TNF (NCBI Gene ID 7124), IL-6R (NCBI Gene ID 3570), BCMA (NCBI Gene ID 608), ADAMTS5 (NCBI Gene ID 11096), CX3CR1 (NCBI Gene ID 1524), CXCR4 (NCBI Gene ID 7852), Tlr1 (NCBI Gene ID 7037), VEGFR (NCBI Gene ID 3791), or PSMA (NCBI Gene ID 2346).

[0181] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD25, such as Daclizumab, RG6292, basiliximab, or HuMax-TAC, or an antibody fragment derived therefrom.

[0182] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CEA, such as Labetuzumab, 15-1-32, PR1A3, or cT84.66, or an antibody fragment derived therefrom.

[0183] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CEACAM5, such as tusamitamab or CC4, or an antibody fragment derived therefrom.

[0184] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets ASPH, such as PAN-622, or an antibody fragment derived therefrom.

[0185] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets EGFR, such as Cetuximab, necitumumab, nimotuzumab, matuzumab, AMG595, depatuxizumab, daptuxizumab, duligotuzumab, futuximab, GC1118, imgatuzumab, panitumumab, alutumumab, tomuzotuximab, or laprituximab, or an antibody fragment derived therefrom.

[0186] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets EPCAM, such as oportuzumab, citatuzumab, tucotuzumab, catumaxomab, edrecolomab, or adecatuzumab, or an antibody fragment derived therefrom.

[0187] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets VEGFR, such as ramucizumab, ramucirumab, or vulinacimab, or an antibody fragment derived therefrom.

[0188] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PDGFR, such as olaratumab or ramucirumab, or an antibody fragment derived therefrom.

[0189] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TROP2, such as sacituzumab or Pr1E11, or an antibody fragment derived therefrom.

[0190] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets Nectin4, such as enfortumab, or an antibody fragment derived therefrom.

[0191] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PSMA, such as J591 or MLN591, or an antibody fragment derived therefrom.

[0192] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BCMA, such as belantamab, or an antibody fragment derived therefrom.

[0193] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD22, such as moxetumomab, inotuzumab, epratuzumab, or pinatuzumab, or an antibody fragment derived therefrom.

[0194] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD20, such as ublituximab, ofatumumab, rituximab, obinutuzumab, tositumomab, or ibritumomab, or an antibody fragment derived therefrom.

[0195] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD19, such as loncastuximab, XMAB-5574, MOR208, coltuximab, denintuzumab, taplitumomab, or MDX-1342, or an antibody fragment derived therefrom.

[0196] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD79b, such as polatuzumab, or an antibody fragment derived therefrom.

[0197] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD38, such as isatuximab, daratumumab, MOR202, or TAK-079, or an antibody fragment derived therefrom.

[0198] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD45, such as I-131-BC8, or Iomab-B, or an antibody fragment derived therefrom.

[0199] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets endoglin, such as carotuximab, or an antibody fragment derived therefrom.

[0200] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets FGFR2, such as bemarituzumab or aprutumab, or an antibody fragment derived therefrom.

[0201] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets C4.4A, such as lupartumab, or an antibody fragment derived therefrom.

[0202] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets Claudin-18.2, such as zolbetuximab, or claudiximab, or an antibody fragment derived therefrom.

[0203] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MMP9, such as andecaliximab, or an antibody fragment derived therefrom.

[0204] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets folate receptor, such as mirvetuximab, farletuzumab, MORAb-202, MORAb-003, or SP8166, or an antibody fragment derived therefrom.

[0205] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets DLL3, such as rovalpituzumab, or an antibody fragment derived therefrom.

[0206] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD138, such as indatuximab, or an antibody fragment derived therefrom.

[0207] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD56, such as lorvotuzumab, promiximab, or an antibody fragment derived therefrom.

[0208] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD37, such as BI 836826, otlertuzumab, or naratuximab, or an antibody fragment derived therefrom.

[0209] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD74, such as milatuzumab, or an antibody fragment derived therefrom.

[0210] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets mesothelin, such as anetumab, amatuximab, or MMOT-0530A, or an antibody fragment derived therefrom.

[0211] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets IL-6R, such as tocilizumab or sarilumab, or an antibody fragment derived therefrom.

[0212] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets SLAMF7, such as clotuzumab, or an antibody fragment derived therefrom.

[0213] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BAFF, such as belimumab, or an antibody fragment therefrom.

[0214] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MUC1, such as KL-6, MY.1E12, hMUC1-1H7, TAB004, huC242, clivatuzumab, 8HuDS6, gatipotuzumab, AR20.5, or cantuzumab, or an antibody fragment derived therefrom.

[0215] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GPC3, such as codrituzumab, ECT204, or MDX-1414, or an antibody fragment derived therefrom.

[0216] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets HER2, such as pertuzumab, trastuzumab, or margetuximab, or an antibody fragment derived therefrom.

[0217] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets HER3, such as patritumab, seribantumab, lumretuzumab, elgemtumab, AV-203, CDX-3379, or GSK284933, or an antibody fragment derived therefrom.

[0218] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD30, such as brentuximab, or an antibody fragment derived therefrom.

[0219] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD33, such as gemtuzumab, BI 835858, vadastuximab, or lintuzumab, or an antibody fragment derived therefrom.

[0220] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD123, such as KHK2823, taclotuzumab, or G4723A, or an antibody fragment derived therefrom.

[0221] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GPNMB, such as glembatumumab, or an antibody fragment derived therefrom.

[0222] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets cMET, such as telisotuzumab, onartuzumab, or SAIT301, or an antibody fragment derived therefrom.

[0223] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD142, such as tisotumab, or an antibody fragment derived therefrom.

[0224] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets NaPi2B, such as lifastuzumab, or an antibody fragment derived therefrom.

[0225] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GCC, such as indusatumab, or an antibody fragment derived therefrom.

[0226] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets STEAP1, such as vandortuzumab, or an antibody fragment derived therefrom.

[0227] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MUC16, such as sofituzumab, or an antibody fragment derived therefrom.

[0228] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD70, such as vorsetuzumab, or an antibody fragment derived therefrom.

[0229] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD44, such as bivatuzumab, or an antibody fragment derived therefrom.

[0230] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets vWF, such as caplacizumab, or an antibody fragment derived therefrom.

[0231] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TNF, such as ozoralizumab, V565, or PF-05230905, or an antibody fragment derived therefrom.

[0232] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets IL-6R, such as vobarilizumab, or an antibody fragment derived therefrom.

[0233] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BCMA, such as LCAR-B38M, or an antibody fragment derived therefrom.

[0234] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets ADAMTS5, such as M6495, or an antibody fragment derived therefrom.

[0235] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CX3CR1, such as BI 655088, or an antibody fragment derived therefrom.

[0236] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CXCR4, such as AD-214 or ALX-0651, or an antibody fragment derived therefrom.

[0237] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TfR1, such as TXB4, or an antibody fragment derived therefrom.

[0238] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets VEGFR, such as CDP791, or an antibody fragment derived therefrom.

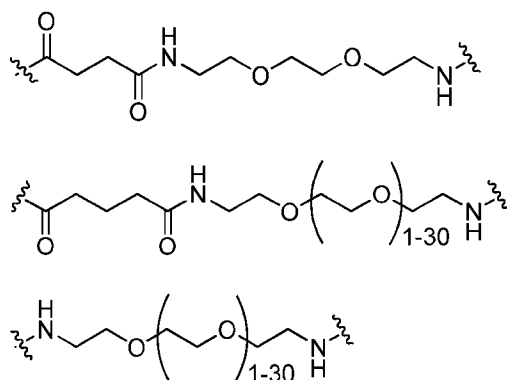
[0239] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PSMA, such as GY1, or an antibody fragment derived therefrom.

[0240] Other compounds or molecules, such as fluorophores or autofluorescent or luminescent markers, which may assist in detecting the particles (e.g., *in vivo* detection), may also be attached to the particles. The ligands and/or detectable labels may be attached directly to the particle or attached to the particle through bioorthogonal functional groups as described herein.

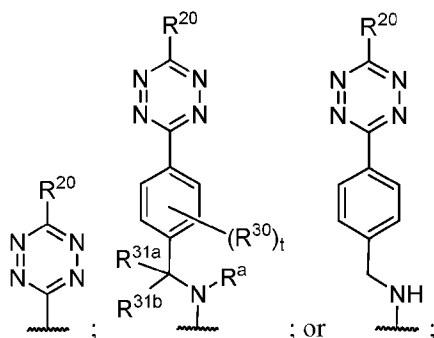
[0241] In certain embodiments, the support is a bone graft material, such as a bone graft substitute material. A bone graft substitute material is a material structurally similar to bone. In some instances, a bone graft substitute material is bioresorbable such that the bone graft substitute material can dissolve or

be absorbed in the body over time. A bone graft substitute material can be osteoconductive, such that it facilitates blood vessel and new bone formation into the bone graft substitute material. In some instances, the bone graft substitute material is osteoinductive, such that it facilitates the formation of new bone through active recruitment of mesenchymal stem cells from the surrounding tissue. For example, growth factors, such as bone morphogenetic proteins, may be included in the bone graft substitute material. Bone graft substitute materials include, but are not limited to, hydroxyapatite, tricalcium phosphate, demineralized bone matrix, bovine collagen, calcium sulfate, calcium phosphate, cancellous bone chips, and the like, and combinations thereof.

[0242] Therapeutic support compositions of the present disclosure include a support and a first binding agent covalently linked to the support. The binding agent may be attached to the support on a surface of the support, such as a solvent-accessible surface of the support (e.g., a surface of the support that is in contact with the surrounding solvent). In some cases, the binding agent is attached directly to the support. For example, the binding agent may be covalently attached to the surface of the support, e.g., through a covalent bond, such as an amide, amine, ester, carbamate, urea, thioether, thiocarbamate, thiocarbonate, thiourea, etc. In some instances, the binding agent is covalently attached to the support through an amide bond. In other instances, the binding agent may be linked to the support via a linker. Any suitable linker can be used to link the binding agent to the support. Representative linkers can have from 1 to 100 linking atoms, and can include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms. Representative linkers include, but are not limited to, those shown below:

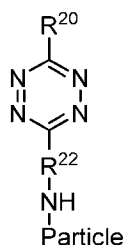


[0243] In certain embodiments, the therapeutic support compositions comprise a support and a tetrazine-containing group of formula:



wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R''$, $SC(=O)R''$, $OC(=S)R''$, $SC(=S)R''$, $S(=O)R'$, $S(=O)_2R''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R'R''$, $SC(=S)R'R''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$; R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl; and R''' at each occurrence is independently selected from aryl and alkyl; R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl; R^a , R^{31a} and R^{31b} are each independently hydrogen, C_1 - C_6 -alkyl, or C_1 - C_6 -haloalkyl; and t is 0, 1, 2, 3, or 4.

[0244] In certain embodiments, the therapeutic support compositions have formula:

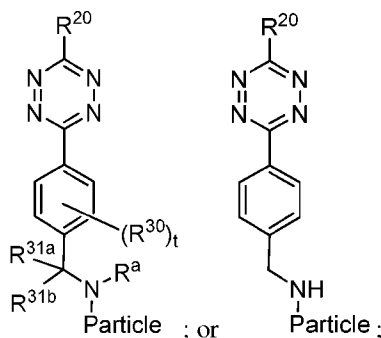


wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R''$, $SC(=O)R''$, $OC(=S)R''$, $SC(=S)R''$, $S(=O)R'$, $S(=O)_2R''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R'R''$, $SC(=S)R'R''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$; R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl; R''' at each occurrence is independently selected from aryl and alkyl; and R^{22} is a linker of 1 to 100 linking atoms, and can include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For

example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms.

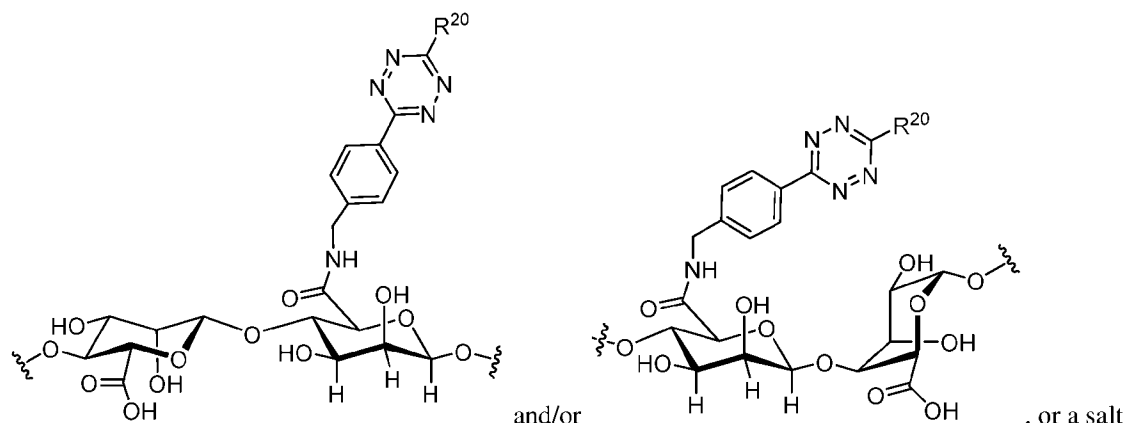
[0245] In certain embodiments, the therapeutic support compositions have formula:



wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R''$, $SC(=O)R'''$, $OC(=S)R'''$, $SC(=S)R'''$, $S(=O)R'$, $S(=O)_2R'''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R'R'''$, $SC(=S)R'R'''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$; R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl; R''' at each occurrence is independently selected from aryl and alkyl; R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl; R^a , R^{31a} and R^{31b} are each independently hydrogen, C_1 - C_6 -alkyl, or C_1 - C_6 -haloalkyl; and t is 0, 1, 2, 3, or 4.

[0246] In certain embodiments, the therapeutic support compositions comprise substituted alginate having units of formula:

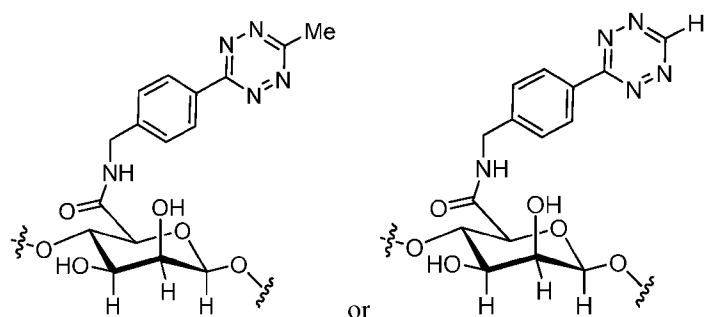


thereof,

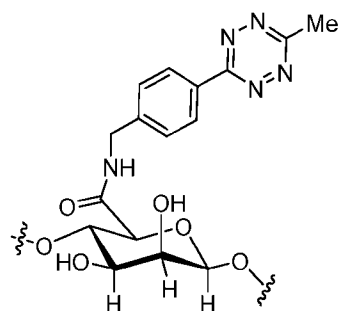
, or a salt

wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R''$, $SC(=O)R''$, $OC(=S)R''$, $SC(=S)R''$, $S(=O)R'$, $S(=O)_2R''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R'R''$, $SC(=S)R'R''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$; R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl; and R''' at each occurrence is independently selected from aryl and alkyl.

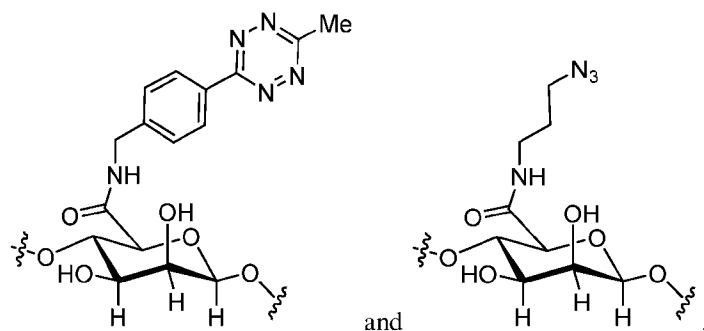
[0247] In certain embodiments, the therapeutic support composition comprises units of formula:



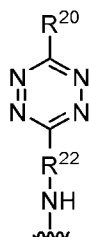
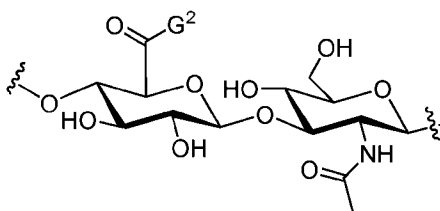
[0248] In some embodiments, the therapeutic support compositions comprise units of formula:



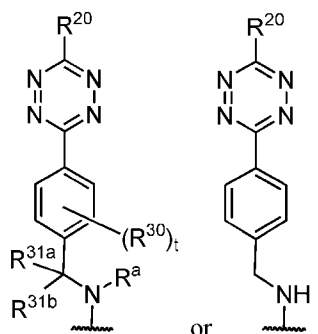
[0249] In some embodiments, the therapeutic support compositions comprise units of formula:



[0250] In some embodiments, the therapeutic support compositions comprise substituted hyaluronic acid having units of formula:

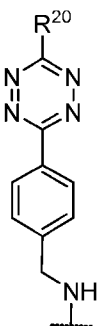


wherein G^2 is ; R^{22} is a linker of 1 to 100 linking atoms; and R^{20} is as defined herein.



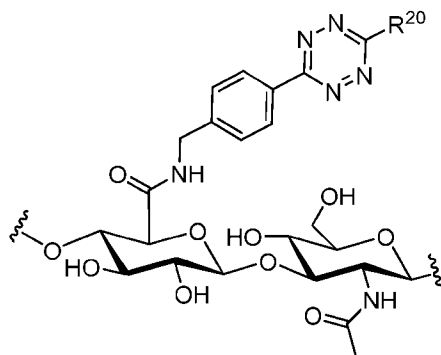
[0251] In further embodiments, G^2 is

or .



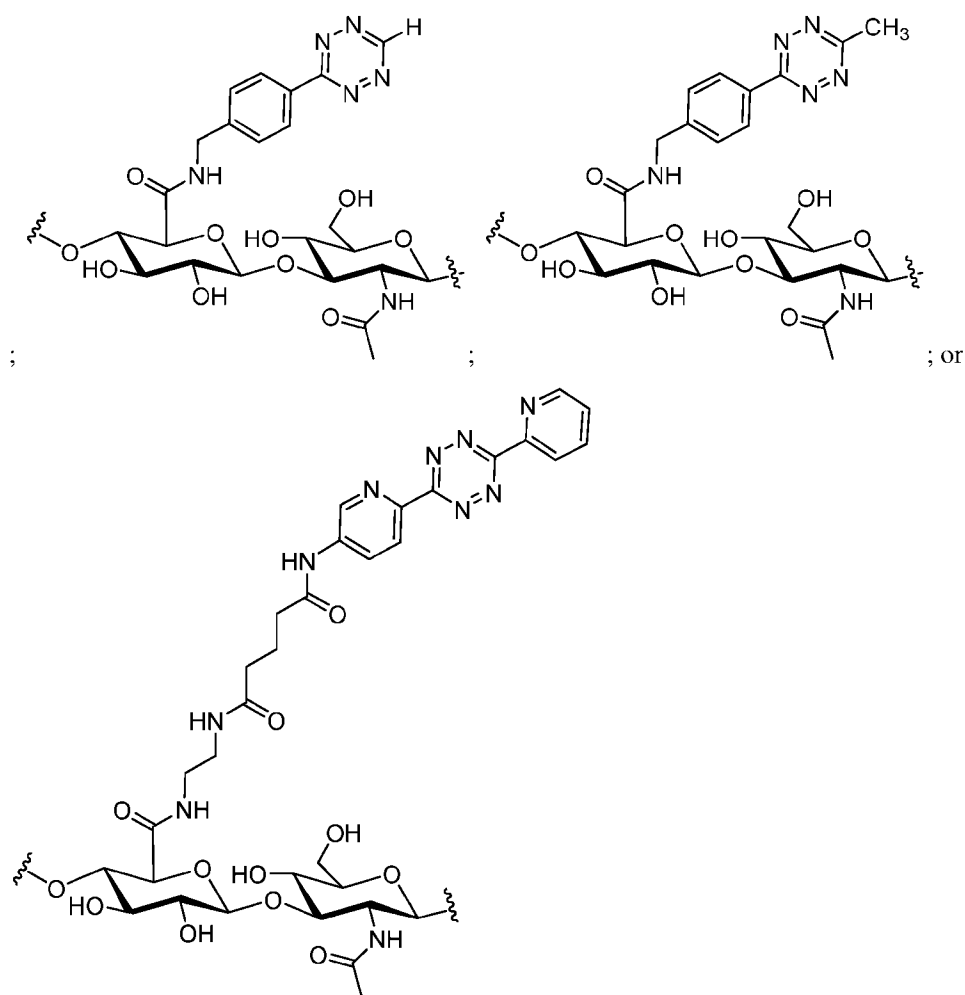
[0252] In still further embodiments, G^2 is ; and R^{20} is hydrogen or C_{1-4} alkyl.

[0253] Compounds of formula (II) include compounds of formula:



wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R''$, $SC(=O)R''$, $OC(=S)R''$, $SC(=S)R''$, $S(=O)R'$, $S(=O)_2R''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R''$, $SC(=S)R''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$; R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl; and R''' at each occurrence is independently selected from aryl and alkyl. In further embodiments according to formula (II-A), R^{20} is hydrogen or C_{1-4} alkyl.

[0254] In some embodiments, the therapeutic support compositions comprise units of formula:



[0255] Additional therapeutic support compositions are exemplified in WO2017/044983, WO/2015/139025A1, and WO/2014/205126A1, the entire contents of each of which is incorporated herein by reference in their entirety.

[0256] The hyaluronic acid derivative includes a hyaluronic acid having a plurality of glucuronic acid units and a tetrazine-containing group linked or directly bonded to a glucuronic acid unit of the

hyaluronic acid. The hyaluronic acid may also have a plurality of N-acetylglucosamine units. In certain embodiments, the N-acetylglucosamine units of the hyaluronic acid are not linked or conjugated to the tetrazine-containing group.

[0257] The tetrazine-containing group can be linked or directly bonded through a carboxylic acid of a glucuronic acid unit. The tetrazine-containing group can be incorporated into the hyaluronic acid from about 0.1% to about 80% as measured by the % of carboxylic acids being linked or conjugated to the tetrazine-containing group, such as about 1% to about 75%, about 5% to about 75%, about 10% to about 50%, or about 40% to about 75% as measured by the % of carboxylic acids being linked or conjugated to the tetrazine-containing group.

D. Methods of Treatment

[0258] Aspects of the present disclosure include methods for delivering a payload to a target location in a subject. In certain embodiments, the method includes selectively delivering a payload to the target location in a subject. Selective delivery of the payload includes delivering the payload to the target location (e.g., an organ or tissue, or portion thereof), without targeting other locations in the subject (e.g., other organs or tissues, or portions thereof) that do not need administration of the payload. Selective delivery of the payload may be achieved through use of the support compositions and the functionalized payloads described herein.

[0259] In some instances, a support composition of the present disclosure may be localized to a desired target location in a subject. For example, methods of the present disclosure may include administering to a subject a support composition as described herein. The support composition may be administered to the subject at a desired target location in the subject. In some instances, the support composition may be implanted into the subject at the desired target location in the subject. In some embodiments, the support composition may be attached to a targeting agent as described herein, and the method may include administering the support composition to the subject (e.g., administered systemically). In these embodiments, the support composition that is attached to a targeting agent may localize at a desired target location in the subject through specific binding of the targeting agent to its target (e.g., antibody-antigen interaction, and the like), or may localize on the surface of a desired target (e.g., a cell surface) through specific binding of the targeting agent to its target (e.g., antibody-antigen interaction, and the like).

[0260] As described herein, selective binding between bioorthogonal binding partners (e.g., between a tetrazine binding agent of the support composition and its complementary trans-cyclooctene binding agent of a functionalized payload) may occur. Due to the localized administration of the support composition to a desired location in the subject as described above, the selective binding between the binding agent of the support composition and its complementary binding agent of the functionalized payload will localize the payload to the desired target location. Accordingly, in certain embodiments, the method includes administering to the subject a functionalized payload such that the functionalized

payload binds to the support composition to form a support complex. For example, the functionalized payload may be administered systemically to the subject. Upon administration of the functionalized payload to the subject, contact between the binding agent of the support composition and the complementary binding agent of the functionalized payload may occur, such that the binding agent and its complementary binding agent bind to one another to form a support complex, thereby selectively delivering the payload to the target location in the subject. In some embodiments, selective delivery of the functionalized payload results in a concentration of the payload at the target location that is greater than the concentration of the payload elsewhere in the subject (e.g., at non-targeted areas in the subject).

[0261] Provided herein is a method of treating cancer comprising administering to a subject in need thereof, a therapeutically effective amount of a conjugate as described herein, or a pharmaceutically acceptable salt thereof, and a therapeutic support composition.

[0262] In some embodiments, the cancer is metastatic. In some embodiments the cancer is melanoma, renal cancer, prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, Kaposi's sarcoma, Non-Hodgkin lymphoma, Hodgkin's lymphoma, Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, malignant extrinsic or intrinsic airway compression, or cutaneous T-cell lymphoma.

[0263] In certain embodiments, the approach can be used for the treatment and/or diagnosis of hematological malignancies such as myelodysplastic syndromes, acute myeloid leukemia, myelodysplastic syndromes, chronic myelogenous leukemia, chronic myelomonocytic leukemia, primary myelofibrosis, diffuse large B-cell lymphoma, chronic lymphocytic leukemia, monoclonal gammopathy, plasma cell myeloma, follicular lymphoma, marginal zone lymphoma, classical Hodgkin lymphoma, monoclonal B-cell lymphocytosis, lymphoproliferative disorder NOS, T-cell lymphoma, precursor B-lymphoblastic leukemia, mantle cell lymphoma, plasmacytoma, Burkitt lymphoma, T-cell leukemia, hairy-cell leukemia, precursor T-lymphoblastic leukemia, nodular lymphocyte predominant Hodgkin lymphoma, as well as others.

[0264] In some embodiments, the cancer is a melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer.

[0265] In some embodiments, the cancer is a solid tumor.

[0266] In some embodiments, the cancer is a lung carcinoma.

[0267] In some embodiments, the cancer is a soft tissue sarcoma.

[0268] In some embodiments, the soft tissue sarcoma is a fibrosarcoma, rhabdomyosarcoma, or Ewing's sarcoma.

[0269] In some embodiments, the method also comprises enhancing or eliciting an immune response. In some embodiments the immune response is an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

[0270] In some embodiments, the method further comprising administering a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof. Anticancer agents, immunomodulatory agents, and their trans-cyclooctene prodrugs are known in the art.

[0271] Indications for this approach include cancer, both hematological and solid cancers. In certain embodiments, the approach can be used for the treatment and/or diagnosis of soft tissue sarcomas: rhabdomyosarcoma, fibrosarcoma, Ewing's sarcoma, and all the different subtypes of soft tissue sarcoma as well as osteosarcoma. The compositions can be for the treatment and/or diagnosis of pigmented villonodular synovitis.

[0272] The compositions of the present disclosure find use in treatment and/or diagnosis of a condition or disease in a subject that is amenable to treatment or diagnosis by administration of the payload (e.g., the parent drug (i.e., the drug prior to conjugation to the composition)). By "treatment" is meant that at least an amelioration of the symptoms associated with the condition afflicting the subject is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g., symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g., terminated, such that the subject no longer suffers from the condition, or at least the symptoms that characterize the condition. Treatment may include inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease. Treatment may include relief, that is, causing the regression of clinical symptoms. For example, in the context of cancer, the term "treating" includes any or all of: reducing growth of a solid tumor, inhibiting replication of cancer cells, reducing overall tumor burden, prolonged survival and ameliorating one or more symptoms associated with a cancer.

[0273] The subject to be treated can be one that is in need of therapy, where the subject to be treated is one amenable to treatment using the parent drug. Accordingly, a variety of subjects may be amenable to treatment using the compositions disclosed herein. Generally, such subjects are "mammals," with humans being of interest. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees, and monkeys).

[0274] In certain embodiments, the functionalized payloads, therapeutic support compositions, additional therapeutic agents, and methods can be used for the treatment, prevention, and/or diagnosis of

solid tumors, including but not limited to, melanoma (e.g., unresectable, metastatic melanoma), renal cancer (e.g., renal cell carcinoma), prostate cancer (e.g., metastatic castration resistant prostate cancer), ovarian cancer (e.g., epithelial ovarian cancer, such as metastatic epithelial ovarian cancer), endometrial carcinoma, breast cancer (e.g., triple negative breast cancer), glioblastoma (e.g., glioblastoma multiforme), and lung cancer (e.g., non-small cell lung cancer), soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, Kaposi's sarcoma, Non-Hodgkin lymphoma, Hodgkin lymphoma Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, malignant extrinsic or intrinsic airway compression, cutaneous T-cell lymphoma, among others. The disclosed approach lends itself well as an adjuvant / neoadjuvant system. For example, particles as disclosed herein could be placed during the biopsy, once the results from the study come back, the practitioner could deliver the appropriate cocktail to the desired site in the body. This would minimize the size of the tumor particularly in the context of a surgically resectable tumor. Then at the end of the surgery, the surgeon could place more particles around the surgical cavity and treat the patient with further doses of treatment (e.g. chemotherapy through the disclosed approach) to minimize the risk of any cancer cells that may have been missed in the surgical margins.

[0275] In certain embodiments, the disclosed methods provide the ability to place particles as disclosed herein at the time of the biopsy. When the results return, the practitioner can deliver through to the biopsy site immunomodulatory agents such as TLR agonists, STING agonists, chemokines (agents that attract cancerous cells and/or immune cells) and adjuvants to enhance the immune system with fewer side effects as well as the chemotherapeutics agents combined with immunotherapy agents. This combination approach would be beneficial to patients. The chemotherapy agent would treat the solid tumor or specific location, while the enhanced response of the immunotherapy would help with distant metastatic sites. For example, in certain embodiments, the disclosed compositions and methods could employ or be used with anthracyclines, taxanes, gemcitabine and other agents to enhance the efficacy of one or more immunomodulatory agents such as ipilimumab, nivolumab, pembrolizumab, avelumab (also known as MSB0010718C; Pfizer).

Cancer

[0276] The disclosed methods may be used to treat or prevent cancer, including metastatic cancer. Cancer is a group of related diseases that may include sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enablement of replicative immortality, induction of angiogenesis, and the activation of invasion and metastasis. The disclosed methods may enhance or elicits an immune response against a cancer in the subject. The immune response may lead to an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

[0277] Cancer that may be treated by the disclosed methods, includes, but is not limited to, astrocytoma, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain cancer, brain stem cancer, brain stem glioma, breast cancer, cervical cancer, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, diffuse intrinsic pontine glioma, ductal cancer, endometrial cancer, ependymoma, Ewing's sarcoma, esophageal cancer, eye cancer, fibrosarcoma, gallbladder cancer, gastric cancer, gastrointestinal cancer, germ cell tumor, glioma, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, macroglobulinemia, melanoma, mesothelioma, mouth cancer, multiple myeloma, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, skin cancer, small cell lung cancer, small intestine cancer, soft tissue carcinoma, soft tissue sarcoma, solid tumor, squamous cell carcinoma, stomach cancer, T-cell lymphoma, testicular cancer, throat cancer, thymoma, thyroid cancer, trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor.

[0278] In some embodiments, the cancer that may be treated by the disclosed methods is melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is a soft tissue carcinoma. In some embodiments, the cancer is a fibrosarcoma. In some embodiments, the cancer is diffuse intrinsic pontine glioma. In some embodiments, the cancer is a metastatic cancer.

[0279] Without being bound by a particular theory, local release of certain anti-cancer agents using the compounds and methods of the invention may produce or contribute to immunogenic cell death (ICD). For example, certain anti-cancer agents (e.g., anthracyclines, cyclophosphamide, oxaliplatin) have been reported to induce ICD. Kroemer et al. *Annu. Rev. Immunol.* 2013 (31), 51-72. Immunogenic apoptosis of cancer cells can induce an effective antitumor immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. ICD is characterized by secretion of damage-associated molecular patterns (DAMPs). Three important DAMPs which are exposed to the cell surface during ICD. Calreticulin (CRT), one of the DAMP molecules, which is normally in the lumen of endoplasmic reticulum (ER), is translocated after the induction of immunogenic apoptosis to the surface of dying cell where it functions as an "eat me" signal for professional phagocytes. Other important surface exposed DAMPs are heat-shock proteins (HSPs), namely HSP70 and HSP90, which are under stress condition also translocated to the plasma membrane. On the cell surface they have an immunostimulatory effect, based on their interaction with number of antigen-presenting cell (APC) surface receptors like CD91 and CD40 and also facilitate crosspresentation of antigens derived from tumor cells on MHC class I molecule, which then leads to the CD8+ T cell response. Other important DAMPs, characteristic for ICD are secreted amphotericin (HMGB1) and ATP. HMGB1 is considered to

be late apoptotic marker and its release to the extracellular space seems to be required for the optimal release and presentation of tumor antigens to dendritic cells. It binds to several pattern recognition receptors (PRRs) such as Toll-like receptor (TLR) 2 and 4, which are expressed on APCs. The most recently found DAMP released during immunogenic cell death is ATP, which functions as a "find-me" signal for monocytes when secreted and induces their attraction to the site of apoptosis. Kroemer et. al. Curr. Op. Immunol. 2008 (20), 504-511.

[0280] Thus, local release of ICD inducers using the compounds and methods of the invention may be beneficially combined with one or more immunomodulatory agents.

[0281] In certain embodiments, the functionalized payloads, therapeutic support compositions, and methods can be used for the treatment, prevention, and/or diagnosis of solid tumors, including but not limited to, melanoma (e.g., unresectable, metastatic melanoma), renal cancer (e.g., renal cell carcinoma), prostate cancer (e.g., metastatic castration resistant prostate cancer), ovarian cancer (e.g., epithelial ovarian cancer, such as metastatic epithelial ovarian cancer), breast cancer (e.g., triple negative breast cancer), glioblastoma (e.g., glioblastoma multiforme), and lung cancer (e.g., non-small cell lung cancer), soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, among others.

[0282] The disclosed approach lends itself well as an adjuvant / neoadjuvant system. For example, therapeutic support compositions as disclosed herein could be placed during the biopsy, once the results from the study come back, the practitioner could administer the appropriate cocktail to deliver treatment to the desired site in the body (compound of Formula A-I, A-2, or a subformula disclosed herein, and optional additional therapeutic agent(s)). The results of the biopsy may indicate the amount and type of treatment to deliver to the site of a tumor. For example, chemokines (agents that attract cancerous cells and/or immune cells) and adjuvants to enhance the immune system with fewer side effects as well as the chemotherapeutics agents could be delivered and combined with immunotherapy agents.

[0283] The disclosed compounds and compositions may be administered prior to surgical resection. The disclosed methods may minimize the size of the tumor prior to surgical resection. This would minimize the size of the tumor particularly in the context of a surgically resectable tumor. The disclosed conjugates, compounds and compositions may be administered during surgical resection. The disclosed conjugates, compounds and compositions may be administered after surgical resection. Therapeutic support composition may be placed around the surgical cavity at the end of surgical resection and the subject may then be treated with further doses of a treatment to minimize the risk of any cancer cells that may have been missed in the surgical margins.

[0284] The disclosed methods may include multiple systemic doses of functionalized payload that focus at one location. The disclosed methods may be used to deliver a second payload. The disclosed methods may be used to administer a second functionalized payload if the tumor is resistant to the first payload. A second payload may be a TCO-labeled payload of gemcitabine or docetaxel. The TCO-labeled payload of gemcitabine or docetaxel may be administered in combination with doxorubicin. The second

functionalized payload may be activated by the therapeutic support composition used for the first prodrug.

[0285] The functionalized payloads disclosed herein may function as adjuvants. This combination approach would be beneficial to patients. The chemotherapy agent would treat the solid tumor or specific location and may enhance or elicit an immune response, while the enhanced response of the immunotherapy of the functionalized payload and/or separate agent may help with distant metastatic sites. For example, in certain embodiments, the disclosed compositions and methods could employ or be used with anthracyclines, auristatins, vinca alkaloids, taxanes, gemcitabine, camptothecin analogues and other agents to enhance the efficacy of ipilimumab, nivolumab, pembrolizumab, avelumab (also known as MSB0010718C; Pfizer).

[0286] The disclosed methods may be used to treat diffuse intrinsic pontine gliomas. Diffuse intrinsic pontine gliomas (DIPG) are pediatric brainstem tumors that may be highly malignant and may be difficult to treat. There is no known curative treatment for DIPG, and survival odds have remained dismal over the past four decades. DIPG patients have a median overall survival of just 11 months, with a two-year survival rate below 10%. DIPG account for 75–80% of brainstem tumors in children, affecting an estimated 200–300 children in the U.S. each year. The rarity of this devastating disease and previous lack of experimental model systems has impeded research, and over the past four decades survival odds have remained the same. Diagnosis of DIPG may begin with clinical symptoms and may be confirmed by MRI. The disease may begin with several months of generalized symptoms, including behavioral changes and difficulties in school, double vision, abnormal or limited eye movements, an asymmetric smile, loss of balance, and weakness. Alternately, severe neurologic deterioration may happen more quickly, with symptoms present for less than a month prior to diagnosis. Clinical examination may reveal the triad of multiple cranial neuropathies, long tract signs such as hyperreflexia and clonus, as well as ataxia. Expansion of the pons section of the brainstem may cause obstructive hydrocephalus and increased intracranial pressure.

[0287] Nuclei critical for life-sustaining function such as breathing and heartbeat are located in the pons and without treatment, breathing and heartbeat may be damaged by DIPG.

[0288] The disclosed methods may be used to deliver molecular payloads to the site of a DIPG. The disclosed methods may include delivering drugs systemically that are only activated at the tumor site. The disclosed methods may be used as a neoadjuvant or adjuvant therapy. The biomaterial may be placed during a biopsy. The results of the biopsy may indicate the amount and type of treatment to deliver to the site of a tumor. The disclosed compounds and compositions may be administered prior to surgical resection. The disclosed methods may minimize the size of the tumor prior to surgical resection. The disclosed compounds and compositions may be administered during surgical resection. The disclosed compounds and compositions may be administered after surgical resection. Biomaterial may be placed around the surgical cavity at the end of surgical resection and the subject may then be treated

with further doses of a treatment. The disclosed biodegradable gel may be implanted at the time of biopsy or surgery. The disclosed methods may not require an additional invasive procedure to deliver additional doses of the disclosed compounds and compositions.

[0289] The disclosed methods may include multiple systemic doses of functionalized payload that focus at one location. The disclosed methods may be used to deliver a second payload. The disclosed methods may be used to administer a second functionalized payload if the tumor is resistant to the first payload. A second payload may be a TCO-labeled payload of paclitaxel, docetaxel, anthracyclines, auristatins, vinca alkaloids, taxanes, gemcitabine, camptothecin analogues, or other agents. The TCO-labeled payload of gemcitabine, paclitaxel, or docetaxel may be administered in combination with doxorubicin. The second functionalized payload may be activated by the therapeutic support composition used for the first prodrug.

Modes of Administration

[0290] Methods of treatment may include any number of modes of administering a disclosed conjugate, compound or composition. Modes of administration may include tablets, pills, dragees, hard and soft gel capsules, granules, pellets, skin patches, skin creams, skin gels, aqueous, lipid, oily or other solutions, emulsions such as oil-in-water emulsions, liposomes, aqueous or oily suspensions, syrups, elixirs, solid emulsions, solid dispersions or dispersible powders. In the pharmaceutical composition, the conjugate, compound or compositions disclosed herein may also be dispersed in a microparticle, e.g. a nanoparticulate composition.

[0291] For parenteral administration, the conjugates, compounds or compositions disclosed herein may be dissolved or suspended in a physiologically acceptable diluent, such as water, buffer, oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. Suitable oils may include, for example, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil. For parenteral administration, the conjugates, compounds or compositions disclosed herein may be administered in the form of an aqueous, lipid, oily, or other kind of solution or suspension, or even administered in the form of liposomes or nano-suspensions.

[0292] The term “parenterally,” as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0293] Therapeutic support compositions are preferably administered locally at the site of a tumor, such as by injection or implantation. Functionalized payloads, such as conjugates of a Formula as disclosed herein, e.g., Formula A-I or A-II, may be administered by any convenient route, in view of a subject's condition and judgment of medical professionals. Parenteral administration is a suitable means of administering conjugates of a Formula as disclosed herein, e.g., Formula A-I or A-II.

[0294] The amount of composition administered to a subject can be initially determined based on guidance of a dose and/or dosage regimen of the parent drug. In general, the compositions can provide for targeted delivery and/or enhanced serum half-life of the bound drug, thus providing for at least one of reduced dose or reduced administrations in a dosage regimen. Thus, the compositions can provide for reduced dose and/or reduced administration in a dosage regimen relative to the parent drug prior to being conjugated in a composition of the present disclosure.

[0295] The pharmaceutical formulation may be provided in unit dosage form. In such form the pharmaceutical formulation may be subdivided into unit doses containing appropriate quantities of the compositions of the present disclosure. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, such as packeted tablets, capsules, and powders in pouches, vials, or ampoules.

[0296] In some embodiments, provided is a kit comprising a conjugate, or a pharmaceutically acceptable salt thereof, as described herein, or the pharmaceutical composition comprising the same, and instructions for use thereof.

[0297] In some embodiments, the kit further comprising the therapeutic support composition.

[0298] Compositions of the present disclosure can be present in any suitable amount, and can depend on various factors including, but not limited to, weight and age of the subject, state of the disease, etc. Suitable dosage ranges for the composition of the present disclosure include from 0.1 mg to 10,000 mg, or 1 mg to 1000 mg, or 10 mg to 750 mg, or 25 mg to 500 mg, or 50 mg to 250 mg. For instance, suitable dosages for the composition of the present disclosure include 1 mg, 5 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg or 1000 mg.

[0299] In some embodiments, multiple doses of a composition are administered. The frequency of administration of a composition can vary depending on any of a variety of factors, e.g., severity of the symptoms, condition of the subject, etc. For example, in some embodiments, a composition is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

[0300] The compositions of the present disclosure can be administered at any suitable frequency, interval and duration. For example, the composition of the present disclosure can be administered once an hour, or two, three or more times an hour, once a day, or two, three, or more times per day, or once every 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days, so as to provide the desired dosage level to the subject. When the composition of the present disclosure is administered more than once a day, representative intervals include 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 minutes, as well as 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr, 20 hr, and 24 hours. The composition of the present

disclosure can be administered once, twice, or three or more times, for an hour, for 1 to 6 hours, for 1 to 12 hours, for 1 to 24 hours, for 6 to 12 hours, for 12 to 24 hours, for a single day, for 1 to 7 days, for a single week, for 1 to 4 weeks, for a month, for 1 to 12 months, for a year or more, or even indefinitely.

[0301] The compositions of the present disclosure can be co-administered with another active agent. Co-administration includes administering the composition of the present disclosure and active agent within 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr, 20 hr, or 24 hours of each other. Co-administration also includes administering the composition of the present disclosure and active agent simultaneously or approximately simultaneously (e.g., within about 1 min, 5 min, 10 min, 15 min, 20 min, or 30 minutes of each other), or sequentially in any order. In addition, the composition of the present disclosure and the active agent can each be administered once a day, or two, three, or more times per day so as to provide the desired dosage level per day.

[0302] Co-administration can be accomplished by coimplantation or coinjection.

[0303] In some embodiments, co-administration can be accomplished by co-formulation, e.g., preparing a single pharmaceutical formulation including both the composition of the present disclosure and the active agent. In other embodiments, the composition of the present disclosure and the active agent can be formulated separately and co-administered to the subject.

[0304] The composition of the present disclosure and the active agent can be present in a formulation in any suitable weight ratio, such as from 1:100 to 100:1 (w/w), or 1:50 to 50:1, or 1:25 to 25:1, or 1:10 to 10:1, or 1:5 to 5:1 (w/w). The composition of the present disclosure and the other active agent can be present in any suitable weight ratio, such as 1:100 (w/w), 1:75, 1:50, 1:25, 1:10, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 25:1, 50:1, 75:1, or 100:1 (w/w). Other dosages and dosage ratios of the composition of the present disclosure and the active agent are suitable in the formulations and methods described herein.

Combination Therapies

[0305] In one aspect, the invention provides a method of treating cancer or enhancing or eliciting an immune response comprising administering to a subject in need thereof: a therapeutically effective amount of a conjugate as disclosed herein (e.g., Formula A-I, A-II, etc.), or a pharmaceutically acceptable salt or composition thereof; a therapeutic support composition, as described herein; and a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof.

[0306] The invention also provides a pharmaceutical combination comprising a conjugate described herein, or a pharmaceutically acceptable salt, or composition thereof; a therapeutic support composition, as described herein; and an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof, for use in the treatment or prevention of a cancer or for use in enhancing or eliciting an immune response.

[0307] The invention also provides the use of a pharmaceutical combination comprising a conjugate described herein, or a pharmaceutically acceptable salt, or composition thereof; a therapeutic support composition; and a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof for the treatment or prevention of a cancer or for use in enhancing or eliciting an immune response.

[0308] In the methods and uses described herein, the components of the pharmaceutical combinations may be administered/used simultaneously, separately, or sequentially, and in any order, and the components may be administered separately or as a fixed combination. For example, the delay of progression or treatment of diseases according to the invention may comprise administration of the first active ingredient in free or pharmaceutically acceptable salt form and administration of the second active ingredient in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts or effective amounts, e.g. in daily dosages corresponding to the amounts described herein. The individual active ingredients of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single dosage forms. The present disclosure is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly. Thus, a pharmaceutical combination, as used herein, defines either a fixed combination in one dosage unit form or separate dosages forms for the combined administration where the combined administration may be independently at the same time or at different times. As a further example, the therapeutic support composition and conjugate may be administered/used simultaneously (e.g., through coinjection or coimplantation), separately, or sequentially, followed by administration of the additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof.

[0309] The methods and uses in treating cancer include administering/localizing the therapeutic support composition at a tumor. In the methods and uses disclosed herein, the administration of the conjugate, or a pharmaceutically acceptable salt, or composition thereof; the therapeutic support composition; and the additional therapeutic agent may inhibit the growth of the tumor.

[0310] Additional therapeutic agent(s) may be administered simultaneously or sequentially with the disclosed conjugates and compositions. Sequential administration includes administration before or after the disclosed conjugates and compositions. An additional therapeutic agent may be administered before the disclosed conjugates and compositions. An additional therapeutic agent may be administered after the disclosed conjugates and compositions. An additional therapeutic agent may be administered at the same time as the disclosed conjugates and compositions. In some embodiments, the additional therapeutic agent or agents may be administered in the same composition as the disclosed conjugates. In other embodiments, there may be an interval of time between administration of the additional therapeutic agent and the disclosed conjugates or compositions. In some embodiments, administration of an additional

therapeutic agent with a disclosed conjugate or composition may allow lower doses of the other therapeutic agents and/or administration at less frequent intervals. When used in combination with one or more other active ingredients, the conjugates or compositions of the present invention and the other active ingredients may be used in lower doses than when each is used singly. Accordingly, the pharmaceutical compositions of the present invention include those that contain one or more other active ingredients, in addition to a conjugates of the present disclosure.

Anticancer agents

[0311] Exemplary anti-cancer agents include, but are not limited to, Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin- stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil), Afatinib Dimaleate, Afinitor (Everolimus), Aldara (Imiquimod), Aldesleukin, Alemtuzumab, Alimta (Pemetrexed Disodium), Aloxi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase *Erwinia chrysanthemi*, Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and I 131 Iodine Tositumomab), Bicalutamide, Bleomycin, Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Busulfan, Busulfex (Busulfan), Cabazitaxel, Cabozantinib- S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carboplatin, Carboplatin-Taxol, Carfilzomib, Casodex (Bicalutamide), CeeNU (Lomustine), Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, Chlorambucil, Chlorambucil-Prednisone, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cometriq (Cabozantinib-S-Malate), COPP, COPP-ABV, Cosmegen (Dactinomycin), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytarabine, Cytarabine liposomal, Cytosar-U (Cytarabine), Cytosan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Efudex (Fluorouracil), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase *Erwinia chrysanthemi*), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, Fareston (Toremifene), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil),

Fluorouracil, Folex (Methotrexate), Folex PFS (Methotrexate), Folfiri, Folfiri- Bevacizumab, Folfiri-Cetuximab, Folfirinox, Folfox (Leucovorin, Fluorouracil, Oxaliplatin), Folutyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gazyva (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, Gemcitabine-Cisplatin, Gemcitabine-Oxaliplatin, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hyper-CVAD, Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Ifex (Ifosfamide), Ifosf amide, Ifosfamidum (Ifosfamide), Imatinib Mesylate, Imbruvica (Ibrutinib), Imiquimod, Inlyta (Axitinib), Intron A (Recombinant Interferon Alfa- 2b), Iodine 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Istodax (Romidepsin), Ixabepilone, Ixempria (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Kadcyla (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Kyprolis (Carfilzomib), Lapatinib Ditosylate, Lenalidomide, Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfofizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Lomustine, Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron Depot- 3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megace (Megestrol Acetate), Megestrol Acetate, Mekinist (Trametinib), Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mitomycin C, Mitozytrex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin- stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Nelarabine, Neosar (Cyclophosphamide), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilotinib, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Ofatumumab, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Ontak (Denileukin Diftitox), OEPA, OPPA, Oxaliplatin, Paclitaxel, Paclitaxel Albumin- stabilized Nanoparticle Formulation, Palifermin, Palonosetron Hydrochloride, Pamidronate Disodium, Panitumumab, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, Pegaspargase, Peginterferon Alfa-2b, PEG-Intron (Peginterferon Alfa-2b), Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Provenge (Sipuleucel-T), Purinethol (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Rasburicase, R-CHOP, R-CVP, Recombinant HPV Bivalent Vaccine, Recombinant HPV Quadrivalent Vaccine, Recombinant Interferon Alfa- 2b, Regorafenib, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Rituxan (Rituximab), Rituximab, Romidepsin,

Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc), Sipuleucel-T, Sorafenib Tosylate, Sprycel (Dasatinib), Stanford V, Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa- 2b), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), Tafenlar (Dabrafenib), Talc, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tassigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirrolimus), Tositumomab and 1 131 Iodine Tositumomab, Totect (Dexrazoxane Hydrochloride), Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride), Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Vandetanib, VAMP, Vectibix (Panitumumab), Velp, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, Vismodegib, Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), Xelox, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Zaltrap (Ziv-Aflibercept), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), and Zytiga (Abiraterone Acetate).

[0312] The anticancer agent may be a PBD dimer, calicheamicin, speromycin, tubulysin B, rhizoxin, dolastatin, didemnin B, camptothecin, CBI, temsirolimus, actinomycin D, epothilone B, taxol, cryptophycin, SN38, velcade, bruceantin, DAVLBH, DM1, Phyllanthoside, Alimta, T2 Toxin, MMC, vantalanib, vinorelbine, brefeldin, sunitinib, daunomycin, semaxanib, tarceva, iressa, irinotecan, LY-541503, geldanamycin, gemcitabine, methotrexate, gleevec, topotecan, bleomycin, doxorubicin, cisplatin, N-mustards, etoposide, or 5-FU.

[0313] In certain embodiments, an anticancer agent is an anthracycline. In certain embodiments, anticancer agent is a taxane. In certain embodiments, anticancer agent is gemcitabine. In certain embodiments, anticancer agent is doxorubicin. In certain embodiments, anticancer agent is docetaxel. In certain embodiments, anticancer agent is paclitaxel. In certain embodiments, anticancer agent is SN38. In certain embodiments, anticancer agent is monomethyl auristatin E. In certain embodiments, an anticancer agent is an alkylating agent, antimetabolite (folate antagonist, purine antagonist, pyrimidine antagonist), antibiotic, taxane, vinca alkaloid, or camptothecin analogue.

E. Synthesis of the Compounds

[0314] The conjugates may be prepared using the methods disclosed herein and routine modifications thereof, which will be apparent given the disclosure herein and methods well known in the art. Conventional and well-known synthetic methods may be used in addition to the teachings herein. The synthesis of typical compounds described herein may be accomplished as described in the following

examples. If available, reagents and starting materials may be purchased commercially, e.g., from Sigma Aldrich or other chemical suppliers.

[0315] It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[0316] Additionally, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art. For example, numerous protecting groups are described in Wuts, P. G. M., Greene, T. W., & Greene, T. W. (2006). *Greene's protective groups in organic synthesis*. Hoboken, N.J., Wiley-Interscience, and references cited therein.

[0317] Furthermore, the conjugates of this disclosure may contain one or more chiral centers. Accordingly, if desired, such conjugates can be prepared or isolated as pure stereoisomers, i.e., as individual enantiomers or diastereomers or as stereoisomer-enriched mixtures. All such stereoisomers (and enriched mixtures) are included within the scope of this disclosure, unless otherwise indicated. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well-known in the art. Alternatively, racemic mixtures of such conjugates can be separated using, for example, chiral column chromatography, chiral resolving agents, and the like.

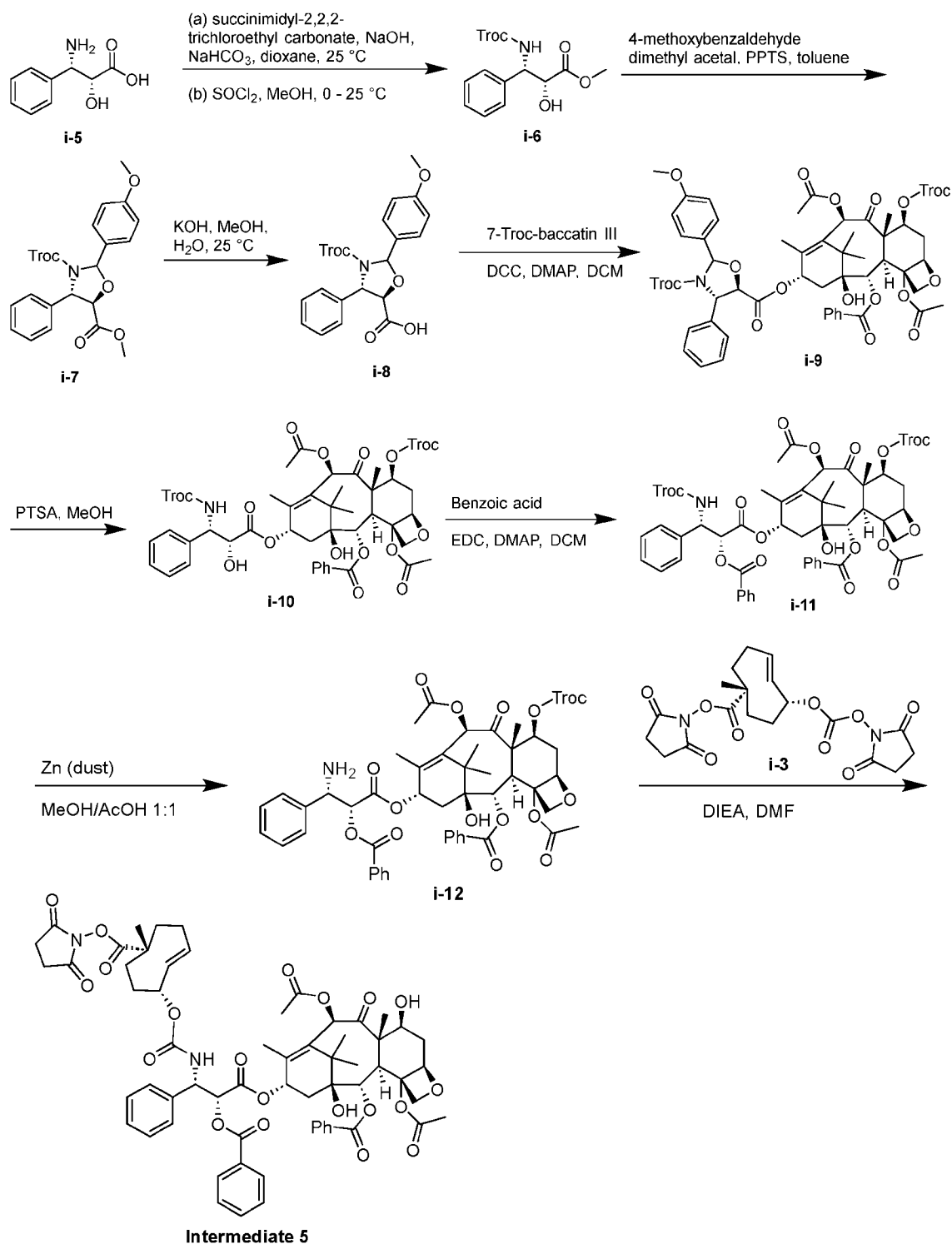
[0318] The starting materials for the following reactions are generally known compounds or can be prepared by known procedures or obvious modifications thereof. For example, many of the starting materials are available from commercial suppliers such as Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemce or Sigma (St. Louis, Missouri, USA). Others may be prepared by procedures or obvious modifications thereof, described in standard reference texts such as Fieser and Fieser's *Reagents for Organic Synthesis*, Volumes 1-15 (John Wiley, and Sons, 1991), *Rodd's Chemistry of Carbon Compounds*, Volumes 1-5, and Supplementals (Elsevier Science Publishers, 1989) *organic Reactions*, Volumes 1-40 (John Wiley, and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley, and Sons, 5th Edition, 2001), and *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

EXAMPLES

[0319] The following examples are included to demonstrate specific embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques to function well in the practice of the disclosure, and thus can be considered to constitute specific modes for its practice. However, those of skill in the art should, in light of the

present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

[0320] General procedure for preparation of Intermediate 5



General procedure for preparation of intermediate i-6

[0321] To a solution of intermediate **i-5** (150 g, 689 mmol, HCl) in NaOH (1 M, 1.38 L) and NaHCO₃ (1 M, 1.38 L) was added (2, 5-dioxopyrrolidin-1-yl) 2, 2, 2-trichloroethyl carbonate (210 g, 723 mmol) in dioxane (1 L). The mixture was stirred at 25 °C for 2 hrs. The reaction mixture was concentrated under reduced pressure to remove dioxane. The residue was extracted with MTBE (5 L), then the aqueous phase was adjusted pH~4 with *Sat.* KHSO₄ aq. and extracted with EtOAc (5 L). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. To a solution of above crude in MeOH (2 L) was added SOCl₂ (90.2 g, 758 mmol) and the mixture was stirred at 25 °C for 2 hrs. LC-MS showed reaction was completed and one main peak with desired mass was detected. The reaction mixture was adjusted pH~9-10 with *Sat.* NaHCO₃ aq., then extracted with EtOAc (5 L). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give crude. The crude was precipitated by PE (10 Vol) to give intermediate **i-6** (190 g, 74.4% yield).

[0322] ¹H NMR: (400 MHz, CDCl₃): δ 3.25 (br s, 1 H) 3.85 (s, 3 H) 4.64 - 4.83 (m, 2 H) 5.30 (dd, *J* = 9.51, 1.13 Hz, 1 H) 5.92 (br d, *J* = 9.38 Hz, 1 H) 7.30 - 7.45 (m, 5 H).

[0323] LCMS (*m/z*): 391.9/393.9 (*M*+H)⁺.

General procedure for preparation of intermediate i-7

[0324] To a solution of intermediate **i-6** (185 g, 499 mmol) in toluene (1.9 L) was added 4-methylbenzenesulfonic acid pyridine (3.90 g, 15.4 mmol) and 4-methoxybenzaldehyde dimethyl acetal (121 g, 666 mmol). The mixture was stirred at 110 °C for 4 hrs. LC-MS showed one main peak with desired mass was detected. Then reaction mixture was allowed to cool to 25 °C, The reaction mixture was concentrated under reduced pressure to remove toluene. The residue was diluted with H₂O (500 mL), then extracted with EtOAc (500 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give intermediate **i-7** (285 g, crude) which was carried forward as is.

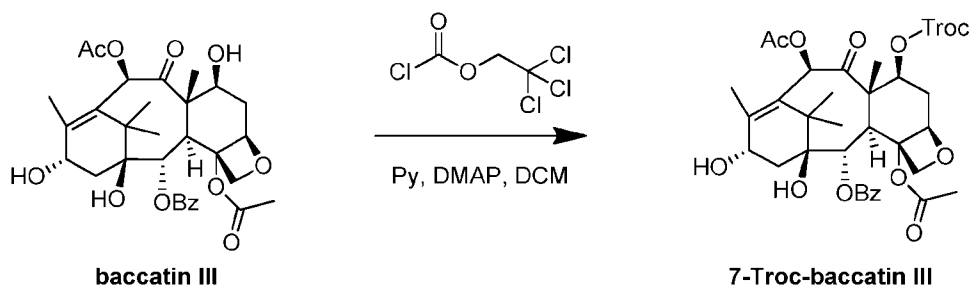
General procedure for preparation of intermediate i-8

[0325] To a solution of intermediate **i-7** (285 g, crude) in MeOH (2000 mL) was added KOH (42.5 g, 758 mmol) in H₂O (1000 mL). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate **i-7** was consumed completely and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove MeOH. The residue was extracted with MTBE (5 L). The aqueous phase layers were diluted with *sat.* KHSO₄ (1L) aq. extracted with EtOAc (5 L), the combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give crude. The crude was precipitated by PE (10 Vol) to give intermediate **i-8** (95.0 g, 34.3% yield).

[0326] ¹H NMR (400 MHz, MeOD): δ 3.82 (s, 3 H) 4.41 - 4.47 (m, 1 H) 4.50 - 4.56 (m, 1 H) 4.60 (d, *J* = 4.88 Hz, 1 H) 5.47 (d, *J* = 4.75 Hz, 1 H) 6.46 (s, 1 H) 6.86 - 6.94 (m, 2 H) 7.34 - 7.46 (m, 7 H).

[0327] LCMS (m/z): 495.9 (M+Na)⁺.

General procedure for preparation of 7-Troc-baccatin III



[0328] To a solution of baccatin III (30.0 g, 51.1 mmol) in DCM (300 mL) was added DMAP (625 mg, 5.11 mmol) and pyridine (14.2 g, 179 mmol) and 2,2,2-trichloroethyl carbonochloridate (15.2 g, 71.6 mmol). The mixture was stirred at 25 °C for 0.5 hrs. LC-MS showed baccatin III was consumed completely and one main peak with desired mass was detected. The residue was diluted with water (300 mL) and extracted with DCM (300 mL) and washed with water (200 mL) and brine (200 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give **7-Troc-baccatin III** (45.0 g, 34.3% yield).

[0329] LCMS (m/z): 761.5/763.5 (M+Na)⁺.

General procedure for preparation of intermediate i-9

[0330] To a solution of 7-Troc-baccatin III (26.0 g, 34.1 mmol) and intermediate **8** (32.4 g, 68.2 mmol) in DCM (1000 mL) was added DMAP (4.20 g, 34.1 mmol) and DCC (21.1 g, 102 mmol). The mixture was stirred at 0 °C for 1 hrs. LC-MS showed intermediate **i-8** was consumed completely and one main peak with desired mass was detected. The reaction mixture filtered. The crude was washed by *sat.* NH₄Cl aq. (100 mL) and water (1000 mL) dried over Na₂SO₄, filtered and concentrated under reduced pressure to give intermediate **i-9** (35.0 g, crude).

[0331] LCMS (m/z): 1240.0/1242.0 (M+Na)⁺.

General procedure for preparation of intermediate i-10

[0332] To a solution of intermediate **i-9** (80.0 g, 65.6 mmol) in MeOH (350 mL) was added 4-methylbenzenesulfonic acid; hydrate (24.9 g, 131 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed ~50% of intermediate **i-9** was remained and one main peak with desired mass was detected. The reaction mixture filtered, concentrated and the residue was purified by prep-HPLC (Water (0.1% TFA)-ACN). The elution was concentrated under reduced pressure to remove solvent, then, extracted with EtOAc (500 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give intermediate **i-10** (13.0 g, 17.9% yield).

[0333] LCMS (m/z): 1120.2 (M+Na)⁺.

General procedure for preparation of intermediate i-11

[0334] To a solution of intermediate **i-10** (13.0 g, 11.8 mmol) and DMAP (722 mg, 5.90 mmol) and EDCI (2.70 g, 14.2 mmol) and benzoic acid (1.70 g, 14.2 mmol) in DCM (260 mL). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate **i-10** was consumed completely and one main peak with desired mass was detected. The reaction mixture was washed with sat. citric acid aq. (100 mL), sat. NaHCO₃ aq. (100 mL) and water (200 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give intermediate **i-11** (11.0 g, 77.3% yield).

[0335] LCMS (m/z): 1204.1 (M+H)⁺.

General procedure for preparation of intermediate i-12

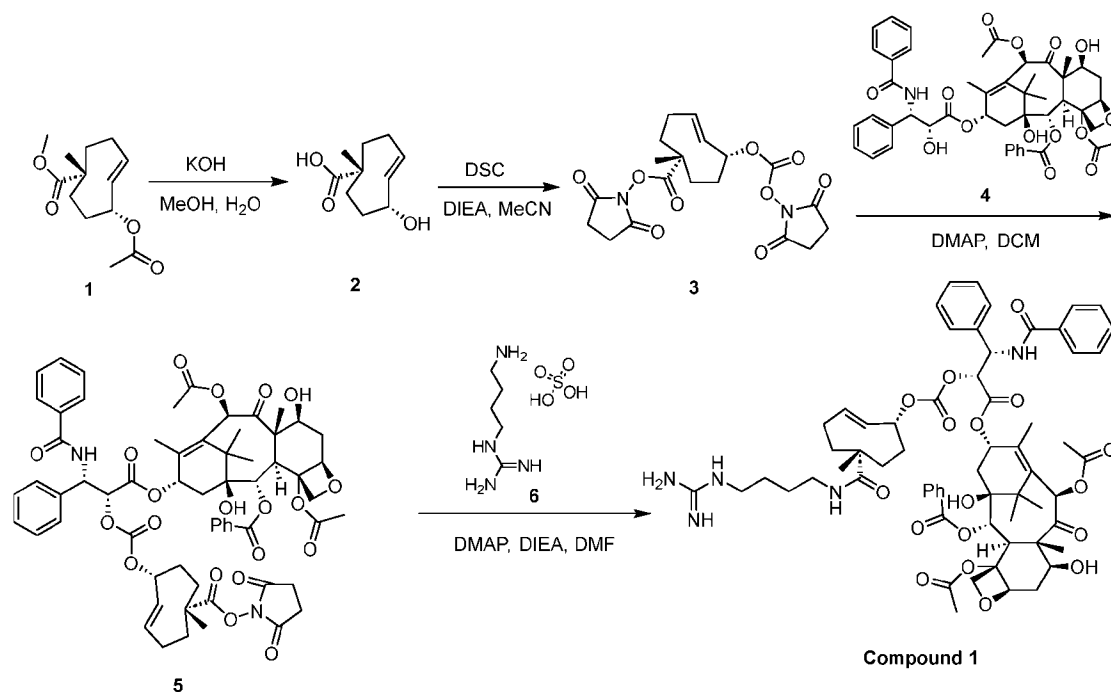
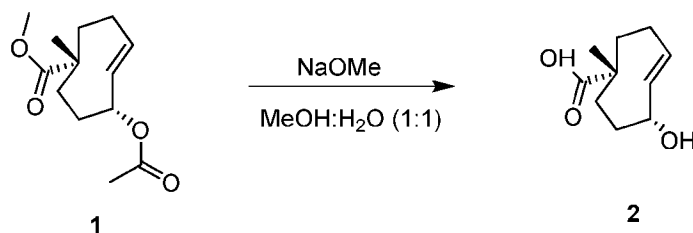
[0336] To a solution of intermediate **i-11** (20.0 g, 16.6 mmol) in MeOH (200 mL) and AcOH (200 mL) was added Zn dust (21.6 g, 331 mmol). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate **i-11** was consumed completely and one main peak with desired mass was detected. The reaction mixture was filtered and diluted with H₂O (500 mL), then extracted with EtOAc (100 mL * 3). The combined organic layers were washed with sat. NaHCO₃ aq. (200 mL) and brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a crude product. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give intermediate **i-12** (5.0 g, 21% yield).

[0337] LCMS (m/z): 854.3 (M+H)⁺.

General procedure for preparation of intermediate 5

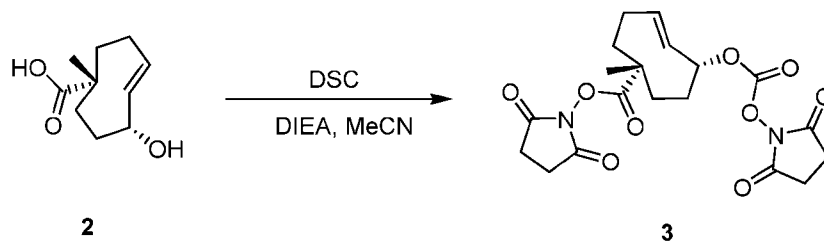
[0338] To a solution of intermediate **12** (5.00 g, 5.90 mmol), DIEA (1.50 g, 11.7 mmol) and intermediate **i-3** (3.90 g, 8.80 mmol) in DMF (50 mL). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed ~50% intermediate **12** was remained and one main peak with desired mass was detected. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give intermediate **5** (505 mg, 7.4% yield).

[0339] LCMS (m/z): 1161.4 (M+H)⁺.

[0340] General procedure for preparation of Compound 1**General procedure for preparation of Intermediate 2**

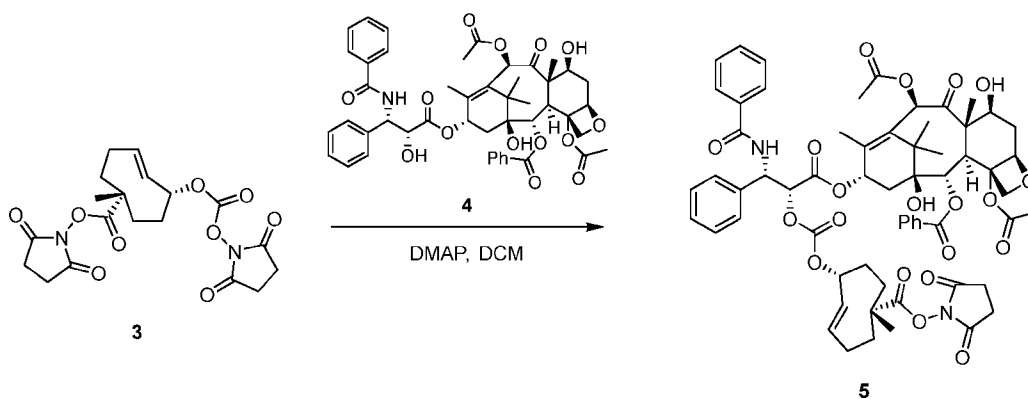
[0341] To a solution of intermediate **1** (10.0 g, 41.6 mmol) in MeOH (50 mL) was added NaOMe (5.40 M, 46.2 mL, 249 mmol) in H₂O (50 mL). The mixture was stirred at 25 °C for 16 hrs. TLC indicated compound **1** was consumed completely and two new spots were detected. The reaction mixture was diluted with H₂O (50 mL) and extracted with MTBE (3 × 100 mL). The aqueous layer was acidified with 1 M HCl until pH = 4 while cooling in an ice-water bath (T < 7 °C). The aqueous layer was extracted with MTBE (3 × 100 mL). The combined MTBE layers were dried with Na₂SO₄, filtered and concentrated under reduced pressure to give intermediate **2** (3.50 g, 45.6% yield).

[0342] ¹H NMR: 400 MHz, CDCl₃ δ 1.11 (s, 3 H), 1.16 - 1.29 (m, 1 H), 1.65 (dd, J = 15.82, 6.19 Hz, 1 H), 1.83 - 2.03 (m, 4 H), 2.11 - 2.37 (m, 4 H), 4.49 (br s, 1 H), 5.64 (dd, J = 16.63, 2.25 Hz, 1 H), 6.02 - 6.12 (m, 1 H).

General procedure for preparation of Intermediate 3

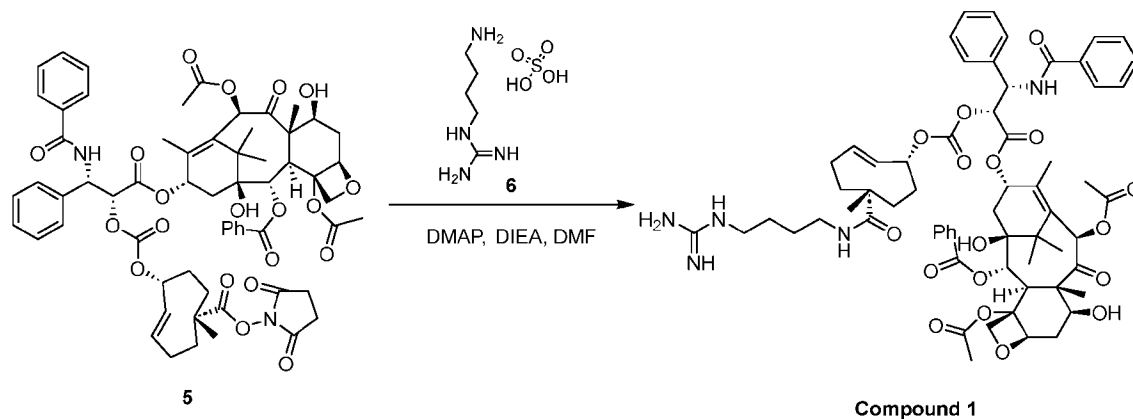
[0343] To a solution of intermediate **2** (3.50 g, 19.0 mmol) in MeCN (60 mL) was added DIEA (17.2 g, 133 mmol) and DSC (20.9 g, 81.7 mmol). The resulting mixture was stirred at 25 °C for 16 hrs. HPLC showed compound **2** was consumed completely and one main peak with desired product was detected. The reaction mixture was purified by re-crystallization from H₂O (600 mL*4) at 25 °C to give intermediate **3** (6.90 g, 89.5% yield).

[0344] ¹HNMR: 400MHz, CDCl₃ δ 1.28 (s, 3 H), 1.97 - 2.16 (m, 4 H), 2.26 - 2.47 (m, 4 H), 2.84 (s, 8 H), 5.29 (br s, 1 H), 5.62 (dd, *J* = 16.70, 2.31 Hz, 1 H), 6.02 - 6.15 (m, 1 H).

General procedure for preparation of Compound 5

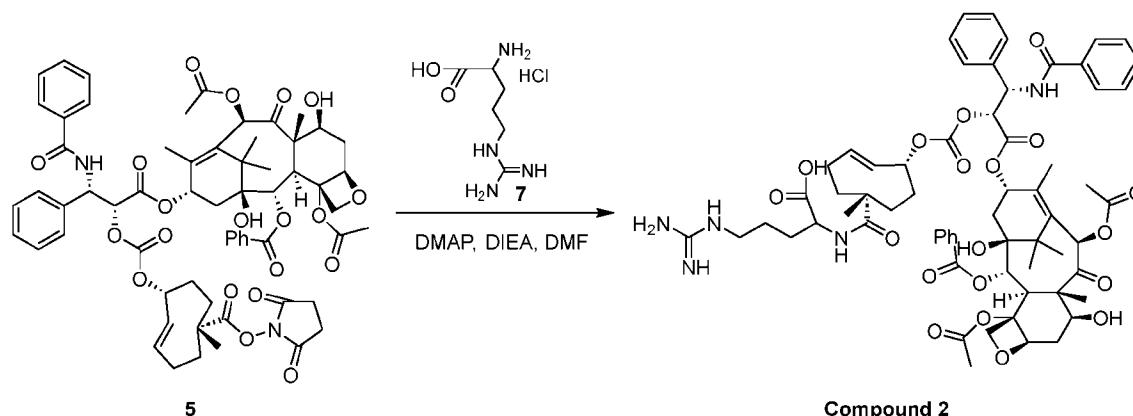
[0345] To a solution of intermediate **4** (1.50 g, 1.76 mmol) in DCM (30 mL) was added DMAP (472 mg, 3.86 mmol) and intermediate **3** (816 mg, 1.93 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed intermediate **4** was consumed completely and one main peak with desired mass was detected. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, DCM: MeOH = 50/1 to 10/1) to give intermediate **5** (1.10 g, 51.7% yield).

[0346] LCMS: ESI-LCMS: MH⁺ calculated 1161.4; observed 1161.7

General procedure for preparation of Compound 1

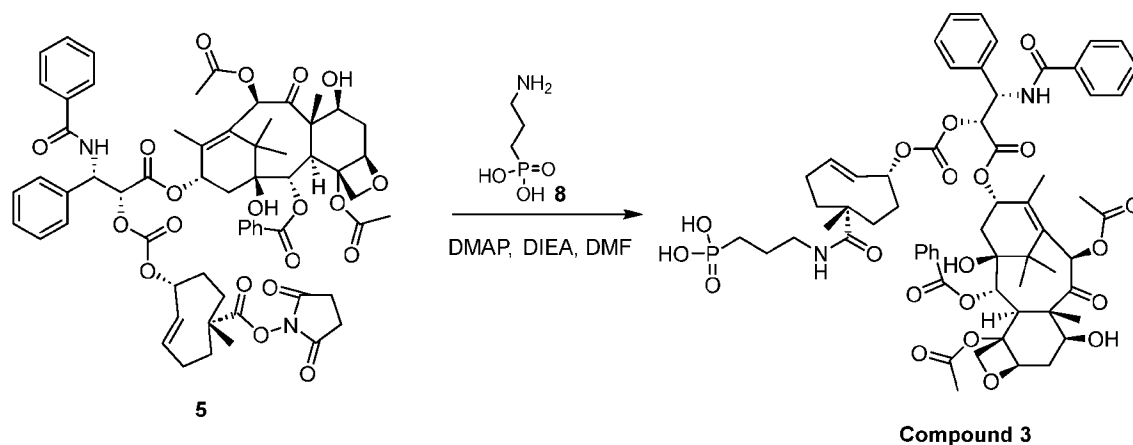
[0347] To a solution of intermediate **6** (314 mg, 1.38 mmol) in DMF (1.0 mL) was added DMAP (168 mg, 1.38 mmol) and intermediate **5** (200 mg, 172 μ mol) and DIEA (178 mg, 1.38 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed one main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (TFA condition) to give Compound 1 (20 mg, 8.31% yield).

[0348] LCMS: ESI-LCMS: MH^+ calculated 1176.5; observed 1176.7

General procedure for preparation of Compound 2

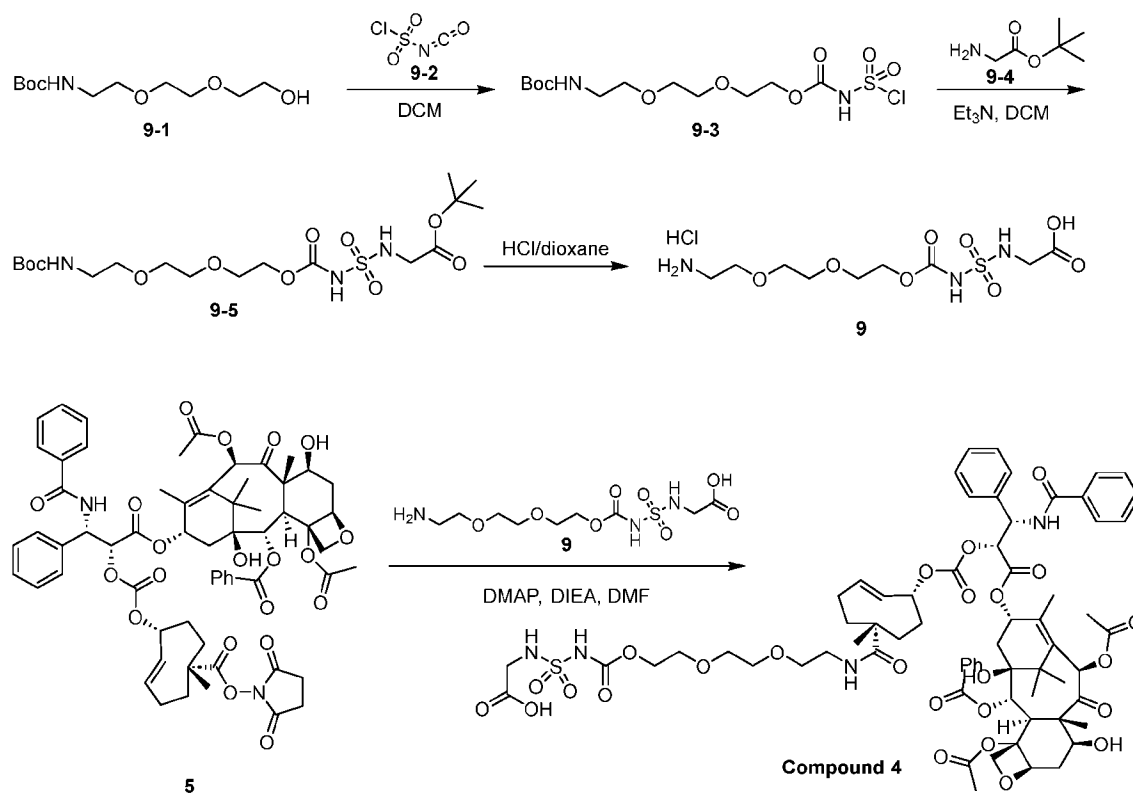
[0349] To a solution of intermediate **7** (145 mg, 689 μ mol) in DMF (0.5 mL) was added DMAP (84.2 mg, 689 μ mol) and intermediate **5** (100 mg, 86.1 μ mol), DIEA (89.0 mg, 689 μ mol). The mixture was stirred at 35 °C for 40 hrs. LC-MS showed intermediate **5** was consumed completely and one main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (TFA condition) to give **Compound 2** (30 mg, 28.1% yield).

[0350] LCMS: ESI-LCMS: MH^+ calculated 1220.5; observed 1220.8

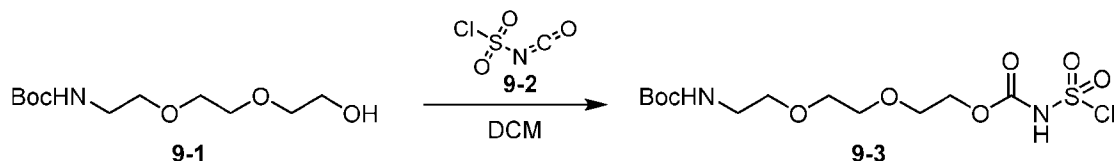
General procedure for preparation of Compound 3

[0351] To a solution of intermediate **8** (192 mg, 1.38 mmol) in DMF (1.0 mL) was added DMAP (168 mg, 1.38 mmol) and intermediate **5** (200 mg, 172 μ mol) and DIEA (178 mg, 1.38 mmol). The mixture was stirred at 35 °C for 60 hrs. LC-MS showed one main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (TFA condition) to give **Compound 3** (19 mg, 7.71% yield).

[0352] LCMS: ESI-LCMS: MH^+ calculated 1185.5; observed 1185.6.

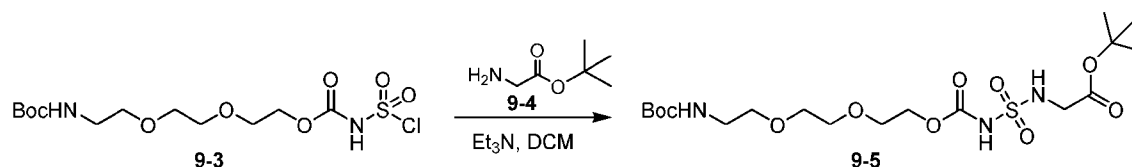
General procedure for preparation of Compound 4

General procedure for preparation of intermediate 9-3



[0353] To a solution of intermediate **9-1** (1.0 g, 4.01 mmol) in DCM (10 mL) was added intermediate **9-2** (568 mg, 4.01 mmol). The mixture was stirred at 0 °C for 1 hr. TLC indicated intermediate **9-1** was consumed completely and one new spot formed. The mixture was used into next step without any work-up.

General procedure for preparation of intermediate 9-5

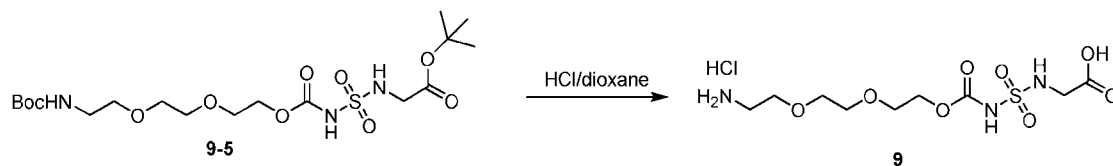


[0354] To a solution of intermediate **9-4** (500 mg, 3.81 mmol) in DCM (5.0 mL) was added Et₃N (771 mg, 7.62 mmol) and intermediate **9-3** (1.49 g, 3.81 mmol). The mixture was stirred at 0 °C for 2 hrs. TLC indicated intermediate **9-3** was consumed completely and one new spot formed. The reaction mixture was partitioned between DCM 20 mL and H₂O 20 mL. The organic phase was separated, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, DCM: MeOH=0/1 to 100/1) to give intermediate **9-5** (1.0 g, 51.6% yield).

[0355] ¹H NMR: 400 MHz, DMSO-*d*₆ δ 11.28 (s, 1 H), 8.12 (t, *J* = 6.07 Hz, 1 H), 6.67 - 6.81 (m, 1 H), 4.15 (dd, *J* = 5.44, 3.81 Hz, 2 H), 3.70 (d, *J* = 6.00 Hz, 2 H), 3.58 - 3.63 (m, 2 H), 3.48 - 3.54 (m, 4 H), 3.37 (t, *J* = 6.07 Hz, 2 H), 3.06 (q, *J* = 5.88 Hz, 2 H), 1.41 (s, 9 H), 1.37 (s, 9 H).

[0356] LCMS: ESI-LCMS: MH^+ calculated 486.2; observed 486.2

General procedure for preparation of intermediate 9

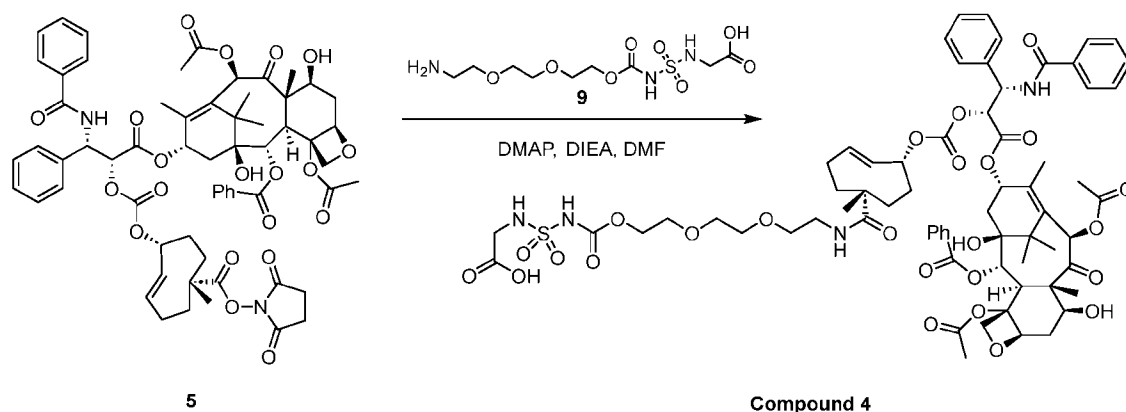


[0357] To a solution of intermediate **9-5** (700 mg, 1.38 mmol) in dioxane (7.0 mL) was added HCl/dioxane (4 M, 7.0 mL). The mixture was stirred at 0-25 °C for 48 hrs. LC-MS showed intermediate **9-5** was consumed completely and one main peak with desired mass was detected. The reaction solution was precipitated with isopropyl ether 200 mL (100 mL*2), filtered and concentrated under reduced pressure to give intermediate **9** (500 mg, crude).

[0358] ¹H NMR: 400 MHz, DMSO-*d*₆ δ 11.25 (s, 1 H), 8.02 - 8.16 (m, 3 H), 4.16 (br dd, *J* = 5.32, 3.69 Hz, 2 H), 3.72 (br d, *J* = 5.88 Hz, 2 H), 3.62 (br t, *J* = 4.88 Hz, 4 H), 3.56 (s, 4 H), 2.86 - 3.01 (m, 2 H).

[0359] LCMS: ESI-LCMS: MH⁺ calculated 330.1; observed 330.1

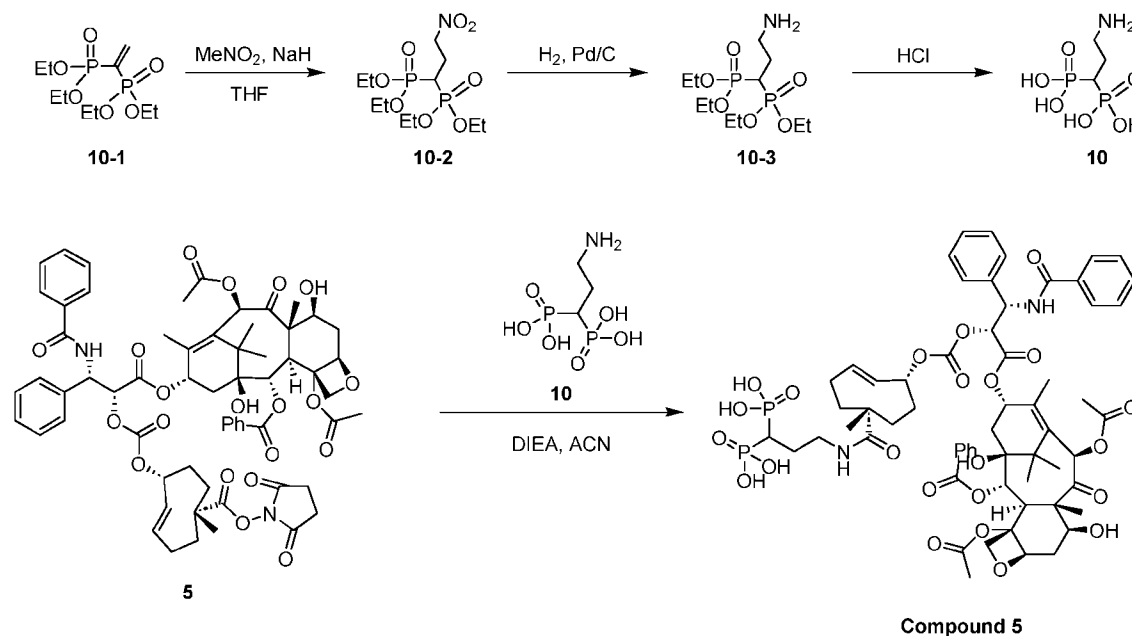
General procedure for preparation of Compound 4

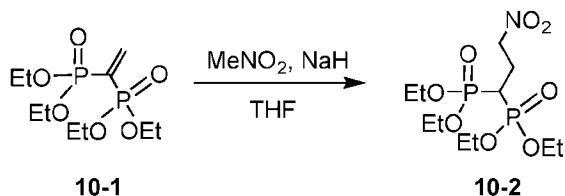


[0360] To a solution of intermediate **9** (252 mg, 689 μmol) in DMF (0.5 mL) was added DMAP (84.2 mg, 689 μmol) and intermediate **5** (100 mg, 86.1 μmol) and DIEA (89.0 mg, 689 μmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed compound **5** was consumed completely and one main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (TFA condition) to give **Compound 4** (20.5 mg, 16.5% yield).

[0361] LCMS: ESI-LCMS: MH⁺ calculated 1375.5; observed 1375.7

General procedure for preparation of Compound 5

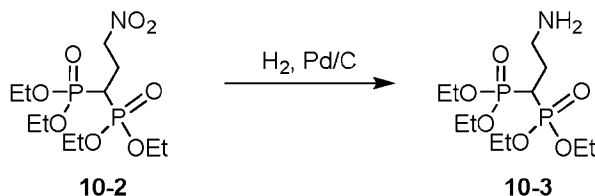


General procedure for preparation of intermediate 10-2

[0362] To a stirred THF (50 mL) was added NaH (153 mg, 3.83 mmol, 60% purity) in batches at 0 °C under N₂ atmosphere, MeNO₂ (35.6 g, 583 mmol) was added dropwise to the stirred suspension. After 1 h, intermediate **10-1** (5.0 g, 16.6 mmol) was added in one portion and the mixture was stirred for 16 hrs at 25 °C. LC-MS showed intermediate **10-1** was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with Sat. NH₄Cl aq. (100 mL) and then extracted with DCM (200 mL*2), the combined organic phase was washed with brine (400 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, DCM:MeOH=100/1 to 50/1) to give intermediate **10-2** (3.0 g, 48.2% yield).

[0363] ¹HNMR: 400 MHz, CDCl₃-d δ 4.70 (t, *J* = 6.82 Hz, 2 H), 4.14 - 4.26 (m, 8 H), 2.42 - 2.65 (m, 3 H), 1.35 (t, *J* = 7.07 Hz, 12 H).

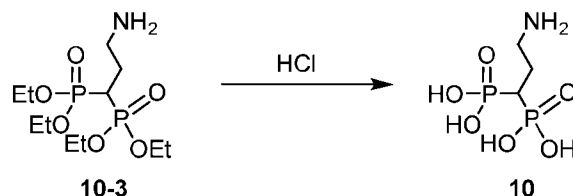
[0364] LCMS: ESI-LCMS: MH⁺ calculated 362.1; observed 362.1

General procedure for preparation of intermediate 10-3

[0365] The 100 mL round-bottom flask was purged with Ar for 3 times and added Pd/C (300 mg, 10% purity) carefully. Then THF (30 mL) was added to infiltrate the Pd/C completely, followed by the solution intermediate **10-2** (3.0 g, 8.30 mmol) in THF slowly under Ar atmosphere. The resulting mixture was degassed and purged with H₂ for 3 times, and then the mixture was stirred at 70 °C for 16 hrs under H₂ atmosphere. LC-MS showed intermediate **10-2** was consumed completely and one main peak with desired mass was detected. The reaction mixture was filtered under reduced pressure carefully and concentrated under reduced pressure to give a crude. The residue was purified by column chromatography (SiO₂, DCM:MeOH=50/1 to 5/1) to give intermediate **10-3** (1.1 g, 39.5% yield).

[0366] ¹HNMR: 400 MHz, CDCl₃-d δ 4.09 - 4.29 (m, 8 H), 2.85 - 3.03 (m, 2 H), 2.45 - 2.65 (m, 1 H), 2.02 - 2.11 (m, 2 H), 1.34 (br t, *J* = 5.69 Hz, 12 H).

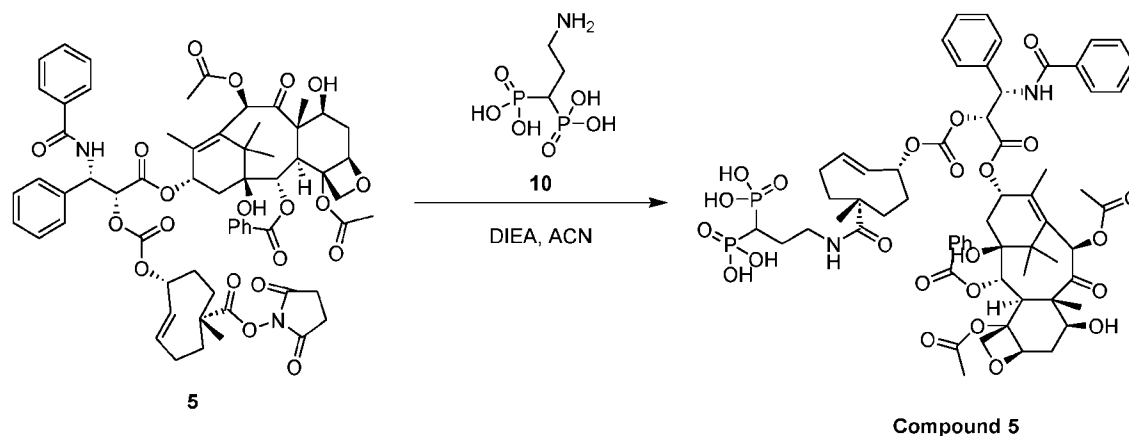
[0367] LCMS: ESI-LCMS: MH⁺ calculated 332.1; observed 332.1

General procedure for preparation of intermediate 10

[0368] A solution of intermediate **10-3** (1.1 g, 3.32 mmol) in HCl (6 M, 22 mL) was stirred at 100 °C for 40 hrs. LC-MS showed intermediate **10-3** was consumed completely and one main peak with desired mass was detected. The reaction mixture was added H₂O and washed with DCM (5 mL * 3) for three times, the aqueous layer was lyophilized to give intermediate **10** (0.65 g, 78.5% yield).

[0369] ¹HNMR: 400 MHz, D₂O δ 3.16 (br t, *J* = 7.19 Hz, 2 H), 2.05 - 2.26 (m, 3 H).

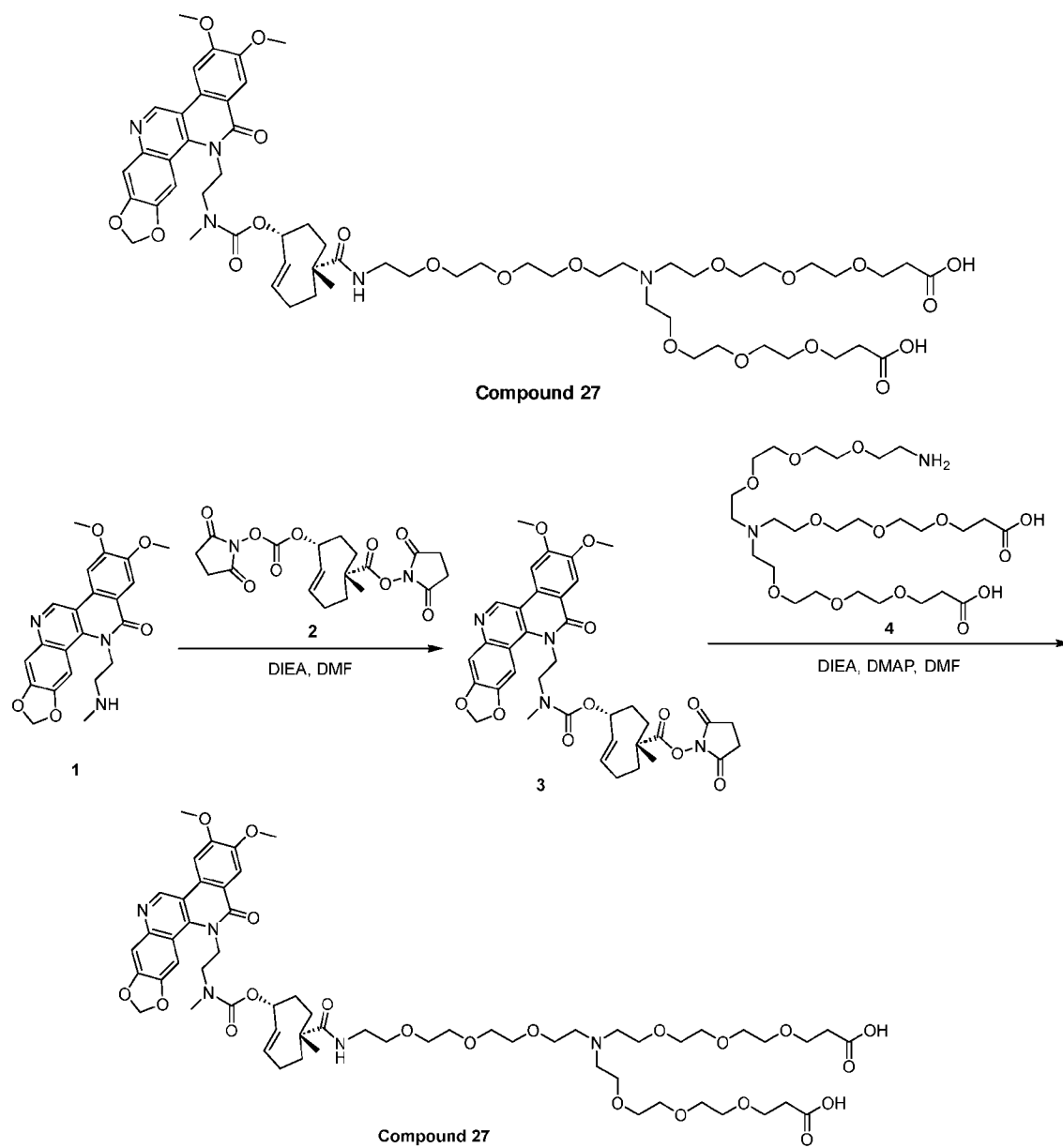
[0370] LCMS: ESI-LCMS: MH⁺ calculated 220.0; observed 220.0

General procedure for preparation of Compound 5

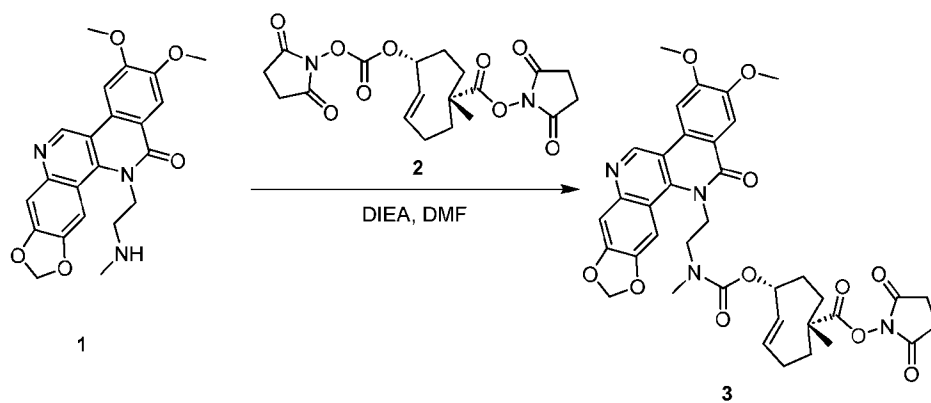
[0371] To a solution of intermediate **10** (302 mg, 1.38 mmol) in ACN (1.0 mL) was added DIEA (178 mg, 1.38 mmol) and intermediate **5** (200 mg, 172 μmol). The mixture was stirred at 50 °C for 60 hrs. LC-MS showed one main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (TFA condition) to give **Compound 5** (22 mg, 10.1% yield).

[0372] LCMS: ESI-LCMS: MH⁺ calculated 1265.4; observed 1265.8.

[0373] General procedure for preparation of Compound 27

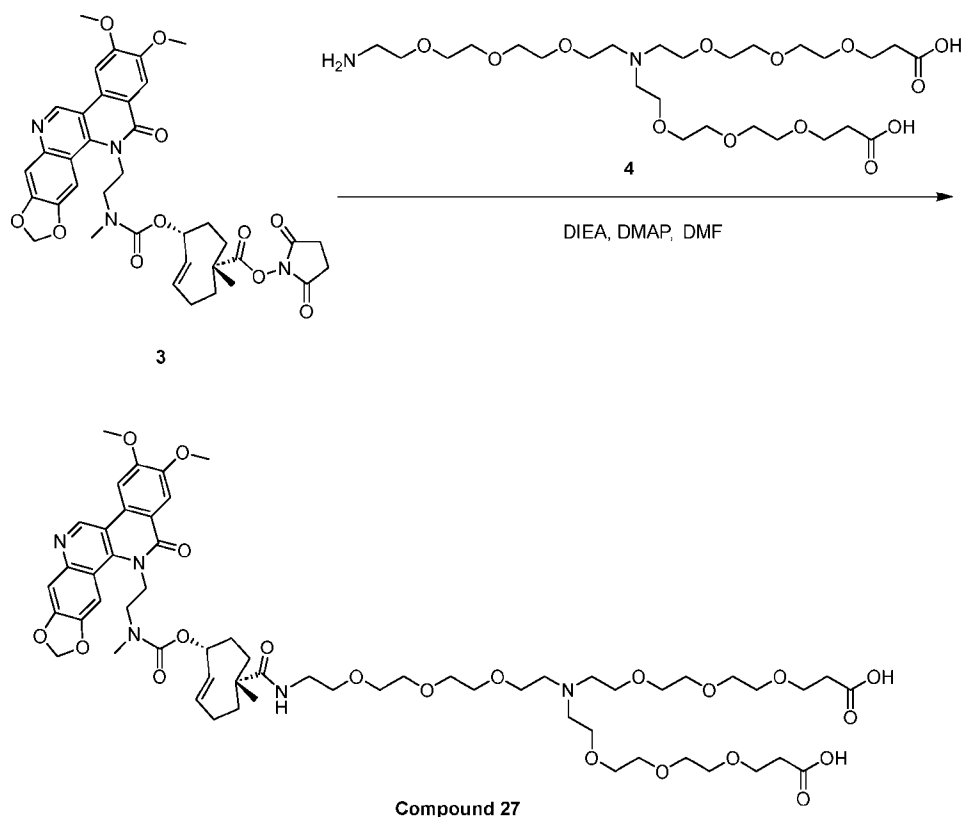


[0374] Procedure for preparation of compound 3:



[0375] To a solution of compound **1** (200 mg, 490 μ mol, 1.0 equiv.) in DMF (2.0 mL) was added compound **2** (248 mg, 589 μ mol, 1.2 equiv.) and DIEA (253 mg, 1.96 mmol, 4.0 equiv.). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed one peak (R_t = 0.38 min) with desired mass (MS cal.: 714.2, MS observed: $[M+H]^+$ = 715.3) was detected. The reaction mixture was added to the 40 mL isopropyl ether and filtered to give compound **3** (320 mg, 87.9% yield, 96.4% purity) as a brown solid. LCMS: R_t = 0.38 min, MS cal.: 714.2, MS observed: $[M+H]^+$ = 715.3. HPLC: R_t = 2.26 min, purity: 96.4%

[0376] Procedure for preparation of Compound 27

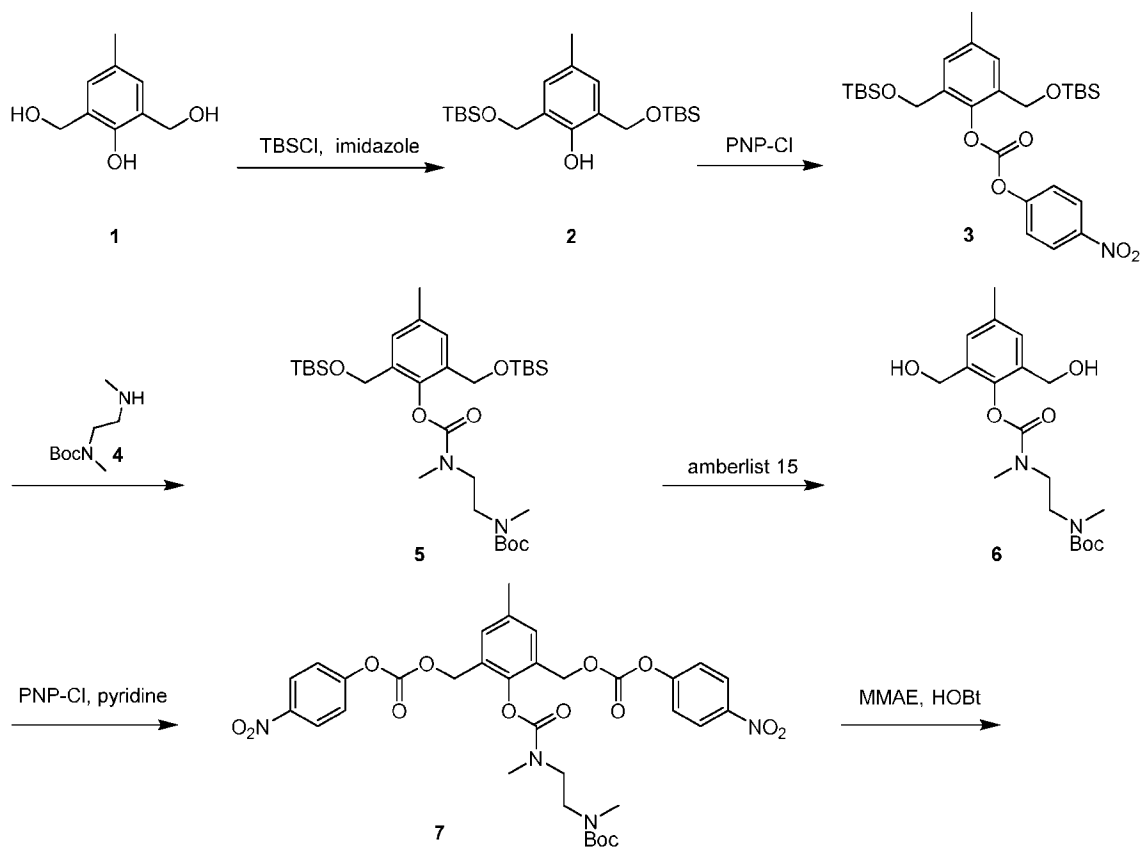


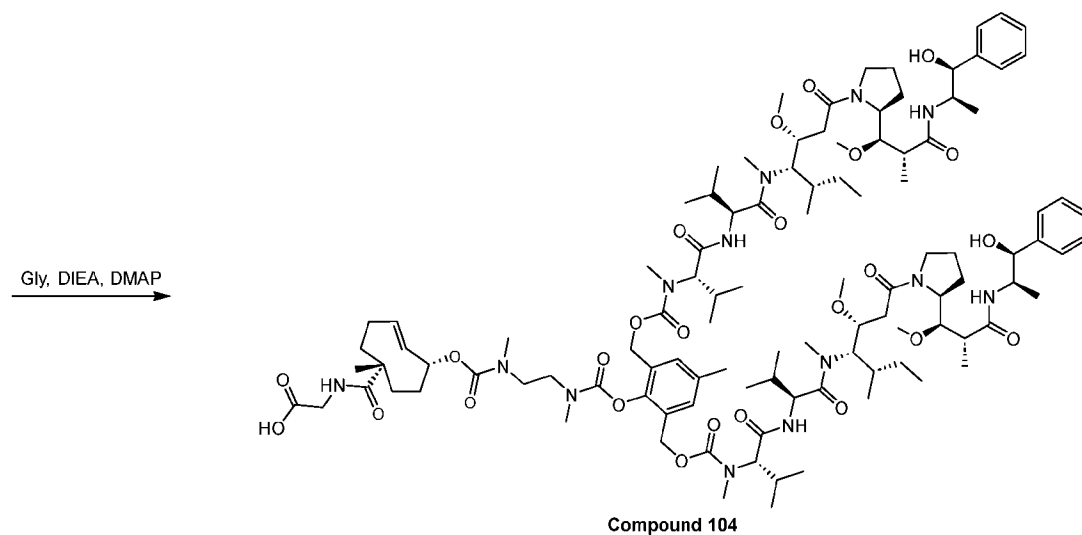
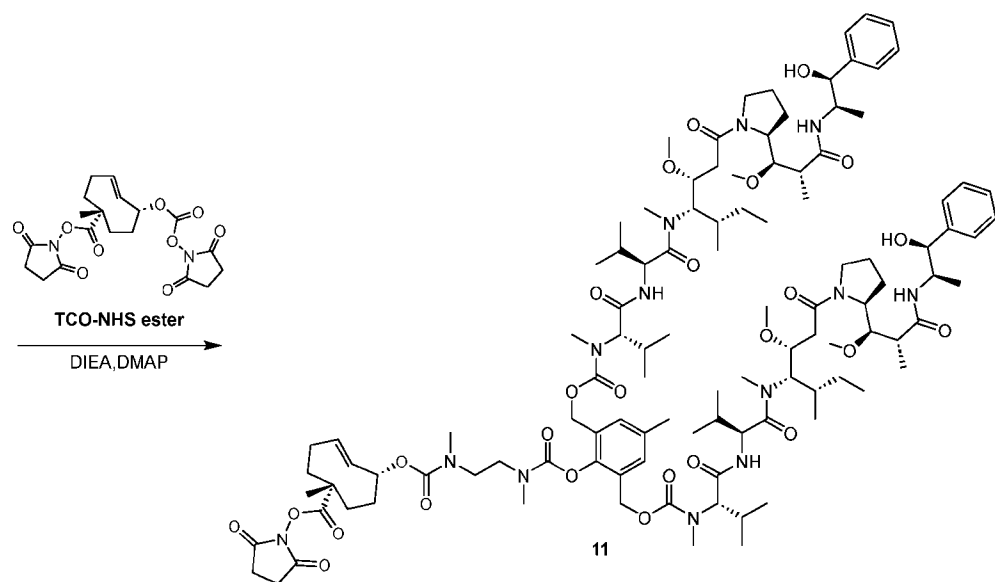
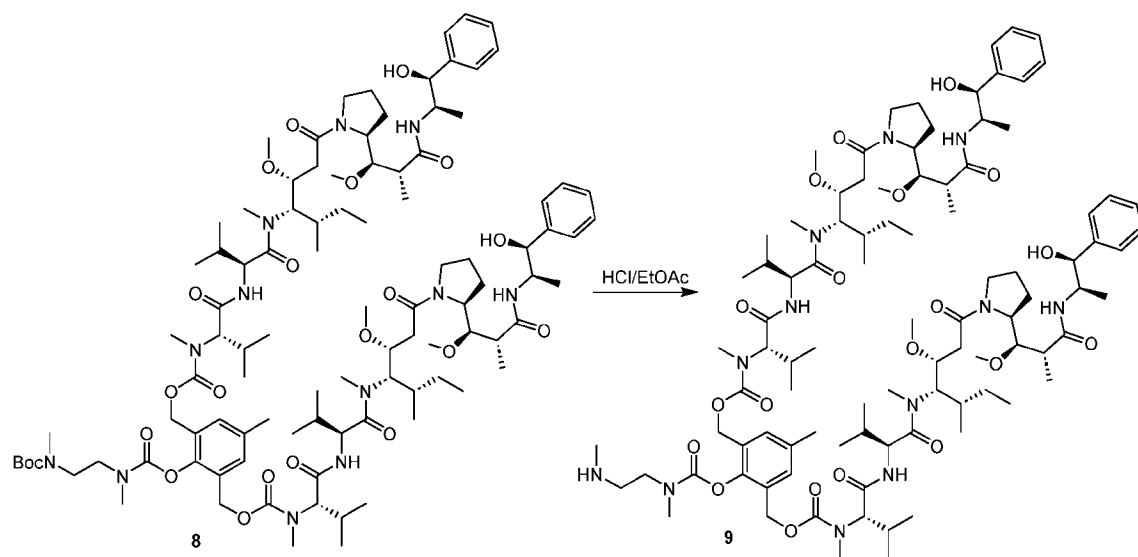
[0377] To a solution of compound **3** (630 mg, 881 μ mol, 1.0 equiv.) in DMF (6.0 mL) was added compound **4** (1.58 g, 2.21 mmol, 2.50 equiv.), DMAP (215 mg, 1.76 mmol, 2.0 equiv.) and DIEA (683 mg, 5.29 mmol, 6.0 equiv.). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed 34.0% of **Target 152** was formed, 62.6% of compound **3** was remained. Then compound **4** (1.58 g, 2.21 mmol, 2.5 equiv.) and DIEA (228 mg, 1.76 mmol, 2.0 equiv.) was added to the solution. The mixture was stirred at 30 °C for 48 hrs. LC-MS showed 64.4% of **Compound 27** was formed, 30.0% of compound **3** was remained. The reaction mixture was added to the 150 mL isopropyl ether and filtered to give the crude product. The residue was purified by prep-HPLC (0.1% TFA condition) to give **Compound 27** (200 mg, 17.9% yield, 94.7% purity).

[0378] LCMS: R_t = 0.349 min, MS cal.: 1199.6, MS observed: $[M+2H]^{2+}$ = 601.1; $[M+H]^+$ = 1200.5.

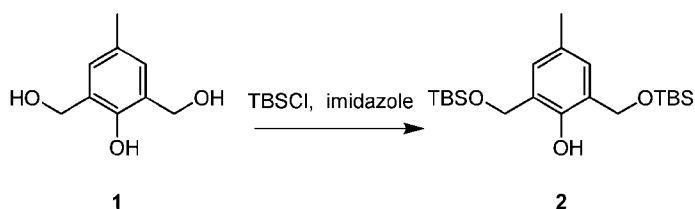
[0379] HPLC: $R_t = 1.837$ min, purity: 94.7%.

Example 1: Synthesis of Compound 104





General procedure for preparation of compound 2



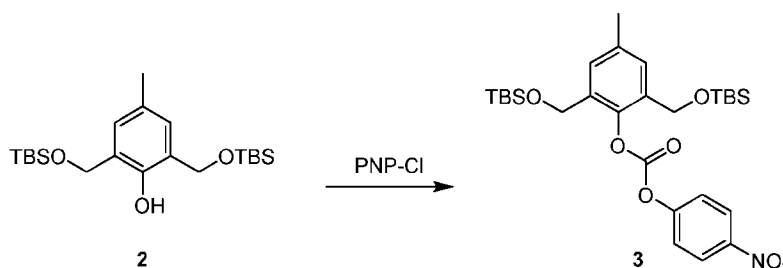
[0380] To a solution of compound **1** (5.0 g, 29.7 mmol, 1 *eq.*) in DMF (50 mL) was added imidazole (4.65 g, 68.4 mmol, 2.3 *eq.*) and TBSCl (9.86 g, 65.4 mmol, 8.05 mL, 2.2 *eq.*). The mixture was stirred at 0-25 °C for 2 hrs. LCMS showed one peak ($R_t = 0.703$ min) with desired mass (MS cal.: 396.2, MS observed: $[M-H]^+ = 395.1$) detected. The reaction mixture was partitioned between MTBE 100 mL and H₂O 100 mL*3. The organic phase was separated, washed with brine (100 mL), dried over Na₂SO₄ filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 100/0 to 95 /5). (Petroleum ether/Ethyl acetate = 10:1, $R_f = 0.84$ min) LCMS showed Compound **2** (9.2 g, 23.1 mmol, 78.0% yield, 100% purity) as a colorless oil.

[0381] LCMS: $R_t = 0.703$ min, (MS cal.: 396.2, MS observed: $[M-H]^+ = 395.1$).

[0382] HPLC: $R_t = 4.769$ min, purity: 100%

[0383] ¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 8.08 (s, 1 H), 6.97 (s, 2 H), 4.69 (s, 4 H), 2.20 (s, 3 H), 0.90 (s, 18 H), 0.07 (s, 12 H)

General procedure for preparation of compound 3



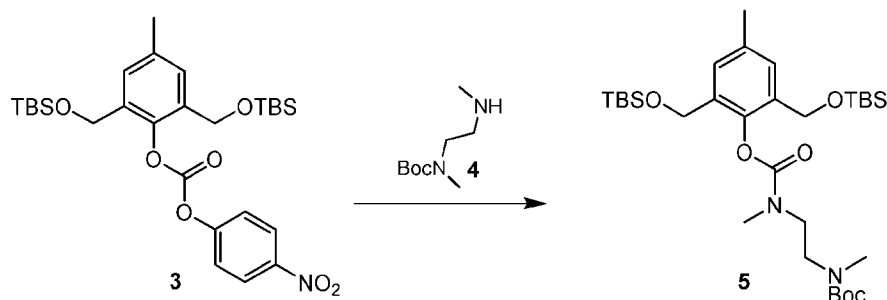
[0384] To a solution of compound **2** (8.0 g, 20.2 mmol, 1.0 *eq.*) in THF (80 mL) was added TEA (6.12 g, 60.5 mmol, 8.42 mL, 3.0 *eq.*) and **PNP-Cl** (6.10 g, 30.2 mmol, 1.5 *eq.*). The mixture was stirred at 0-25 °C for 2 hrs. LCMS showed one peak ($R_t = 0.576$ min) with desired mass (MS cal.: 561.2, MS observed: $[M+18]^+ = 579.1$) detected. Filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 1/0 to 100/1) (Petroleum ether/Ethyl acetate: 20:1, $R_f = 0.65$). LCMS showed Compound **3** (10.1 g, 17.9 mmol, 89.1% yield, 100% purity) as a white solid.

[0385] LCMS: $R_t = 0.578$ min, MS cal.: 561.2, MS observed: $[M+Na]^+ = 584.3$.

[0386] HPLC: $R_t = 4.363$ min, purity: 100%

[0387] ¹H NMR (400 MHz, CDCl₃): δ ppm 8.27 - 8.36 (m, 2 H), 7.43 - 7.54 (m, 2 H), 7.23 (s, 2 H), 4.73 (s, 4 H), 2.39 (s, 3 H), 0.93 (s, 17 H), 0.10 (s, 12 H)

General procedure for preparation of compound 5



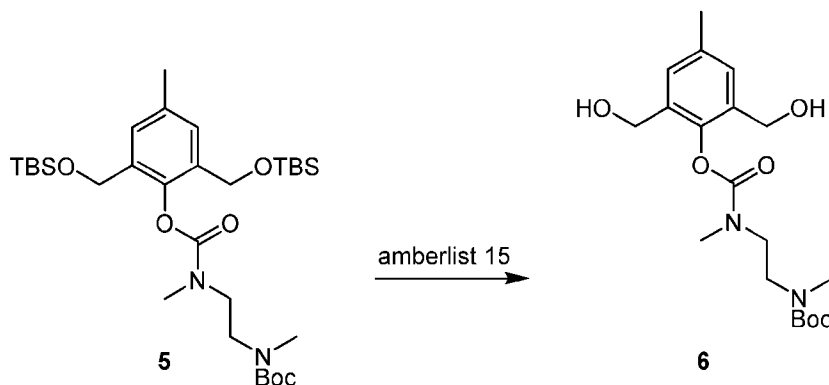
[0388] To a solution of compound **3** (5.0 g, 8.90 mmol, 1.0 *eq.*) in DMF (50 mL) was added compound **4** (2.51 g, 13.3 mmol, 1.5 *eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS showed one peak (*R*_t = 0.577 min) with desired mass (MS cal.: 610.3, MS observed: [M+Na]⁺ = 633.3) was detected. The reaction mixture was partitioned between MTBE 100 mL and H₂O 100 mL. The organic phase was separated, washed with brine (100 mL * 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=100/0 to 20/1), (Petroleum ether: Ethyl acetate: 5:1, *R*_f = 0.56). LCMS showed Compound **5** (3.4 g, 5.23 mmol, 58.7% yield, 94.0% purity) as a colorless oil.

[0389] LCMS: *R*_t = 0.577 min, (MS cal.: 610.3, MS observed: [M+Na]⁺ = 633.3)

[0390] HPLC: *R*_t = 4.389min, purity: 94.0%

[0391] ¹H NMR (400 MHz, DMSO-*d*₆): δppm 7.14 (s, 2 H), 4.55 (br s, 4 H), 3.52 - 3.60 (m, 1 H), 3.36 - 3.50 (m, 3 H), 3.07 (br s, 1 H), 2.74 - 2.97 (m, 5 H), 2.30 (s, 3 H), 1.31 - 1.42 (m, 9 H), 0.89 (d, *J* = 2.1 Hz, 18 H), 0.06 (d, *J* = 3.4 Hz, 12 H)

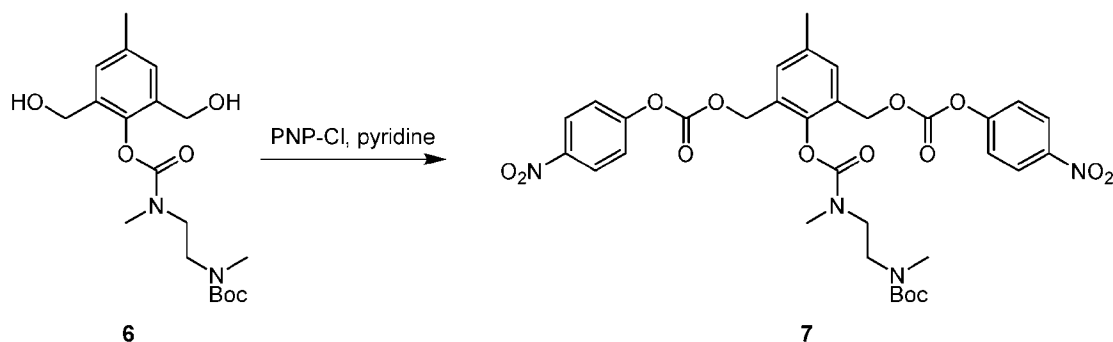
General procedure for preparation of compound 6



[0392] To a solution of compound **5** (3.4 g, 5.56 mmol, 1.0 *eq.*) in MeOH (75 mL) was added Amberlist 15 (75 mg, 5.56 mmol, 1.0 *eq.*). The mixture was stirred at 25 °C for 12 hrs. LCMS showed one peak (*R*_t = 0.355 min) with desired mass (MS cal.: 382.2, MS observed: [M+Na]⁺ = 405.0) detected. Filtered and

concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. LCMS showed compound **6** (2.1 g, 5.38 mmol, 96.6% yield, 97.9% purity) as a colorless gum. LCMS: $R_t = 0.355$ min, MS cal.: 382.2, MS observed: $[M+Na]^+ = 405$. HPLC: $R_t = 2.143$ min, purity: 97.9%. 1H NMR (400 MHz, DMSO- d_6): δ ppm 7.15 (s, 2 H), 5.02 - 5.11 (m, 2 H), 4.35 (br s, 4 H), 3.54 (br t, $J = 5.9$ Hz, 1 H), 3.40 (br d, $J = 5.0$ Hz, 3 H), 3.05 (br d, $J = 7.1$ Hz, 1 H), 2.91 (br s, 2 H), 2.77 - 2.88 (m, 3 H), 2.30 (s, 3 H), 1.34 - 1.44 (m, 9 H)

General procedure for preparation of compound 7



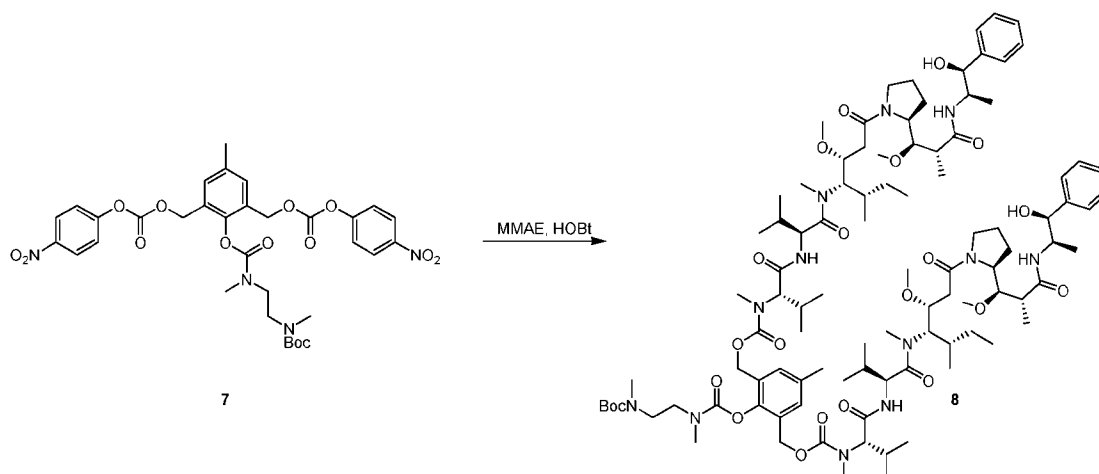
[0393] To a solution of compound **6** (1.0 g, 2.61 mmol, 1.0 *eq.*) in THF (10 mL) was added DIEA (2.70 g, 20.9 mmol, 3.64 mL, 8.0 *eq.*) and pyridine (103 mg, 1.31 mmol, 105 μ L, 0.5 *eq.*), then was PNP-Cl (3.16 g, 15.6 mmol, 6.0 *eq.*) under the ice bath. The mixture was stirred at 0-25 °C for 2 hrs. LCMS showed one peak ($R_t = 0.556$ min) with desired mass (MS cal.: 712.2, MS observed: $[M+Na]^+ = 735.2$) detected. The residue was diluted with DCM (20.0 mL) and extracted with H₂O (20.0 mL * 2). The combined organic layers were washed with brine (20.0 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 100/1 to 1/1, Petroleum ether: Ethyl acetate = 2:1, $R_f = 0.29$). LCMS showed compound **7** (1.1 g, 1.45 mmol, 55.4% yield, 94.0% purity) as a white solid.

[0394] LCMS: $R_t = 0.556$ min, MS cal.: 712.2, MS observed: $[M+Na]^+ = 735.2$.

[0395] HPLC: $R_t = 4.387$ min, purity: 94.0%

[0396] 1H NMR: (400 MHz, DMSO- d_6): δ ppm 8.32 (d, $J = 8.8$ Hz, 4 H), 7.54 (br d, $J = 8.9$ Hz, 4 H), 7.40 (s, 2 H), 5.23 (br d, $J = 9.8$ Hz, 4 H), 3.59 (br s, 1 H), 3.35 - 3.50 (m, 3 H), 3.09 (s, 1 H), 2.69 - 2.97 (m, 5 H), 2.36 (s, 3 H), 1.28 - 1.39 (m, 9 H)

General procedure for preparation of compound 8

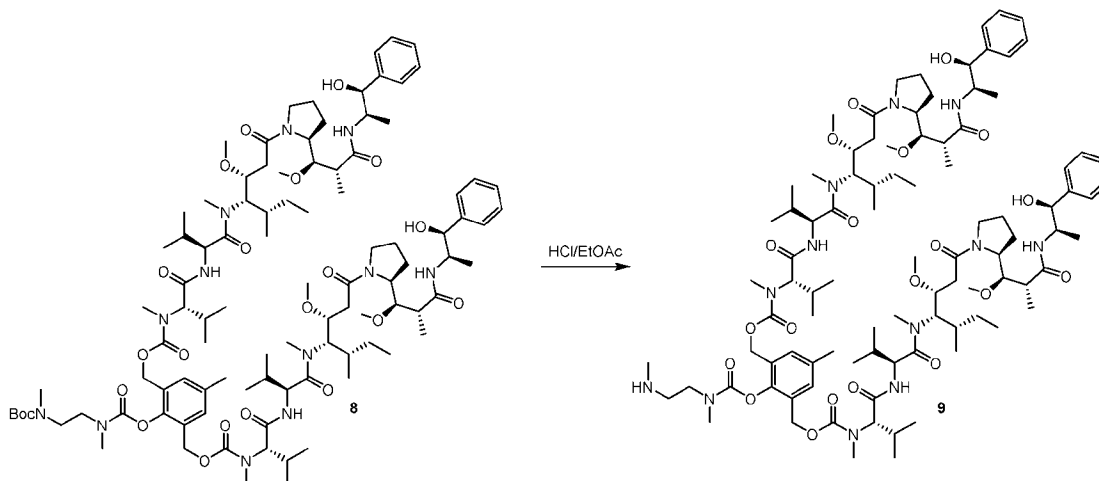


[0397] To a solution of compound **7** (500 mg, 701 μ mol, 1.0 *eq.*), MMAE (1.06 g, 1.47 mmol, 2.1 *eq.*) in DMF (10 mL) was added HOBt (227 mg, 1.68 mmol, 2.4 *eq.*) and DIEA (362 mg, 2.81 mmol, 488 μ L, 4.0 *eq.*). The mixture was stirred at 25 °C for 16 hrs. LCMS showed one peak (R_t = 0.470 min) with desired mass (MS cal.: 1869.1, MS observed: $[M+H]^+$ = 1871.5) detected. The reaction mixture was quenched by addition HCl (4 M, 1.0 mL) at 0-5 °C until pH = 5-6. The residue was purified by prep-HPLC (TFA condition). LCMS showed compound **8** (900 mg, 457 μ mol, 65.1% yield, 95.0% purity) as a white solid.

[0398] LCMS: R_t = 0.470 min, MS cal.: 1869.1, MS observed: $[M+H]^+$ = 1871.5).

[0399] HPLC: R_t = 3.664 min, purity: 95.0%

General procedure for preparation of compound 9

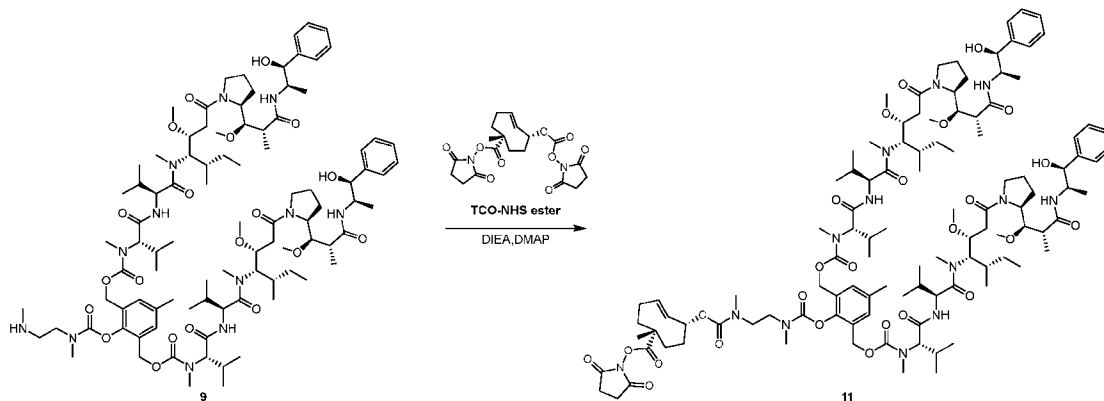


[0400] To a solution of compound **8** (700 mg, 374 μ mol, 1.0 *eq.*) in DCM (8.0 mL) was added HCl/EtOAc (4 M, 2.0 mL). The mixture was stirred at 0-5 °C for 1 hr. LCMS showed one peak (R_t = 0.486 min) with desired mass (MS cal.: 1769.1, MS observed: $[M+Na]^+$ = 1771.3) detected. The mixture concentrated under reduced pressure to give a residue. LCMS showed compound **9** (600 mg, 305 μ mol, 81.5% yield, 90.0% purity) as a white solid.

[0401] LCMS: $R_t = 0.486$ min, MS cal.: 1869.1, MS observed: $[M+H]^+ = 1871.5$).

[0402] HPLC: $R_t = 3.610$ min, purity: 90.0%

General procedure for preparation of compound 11

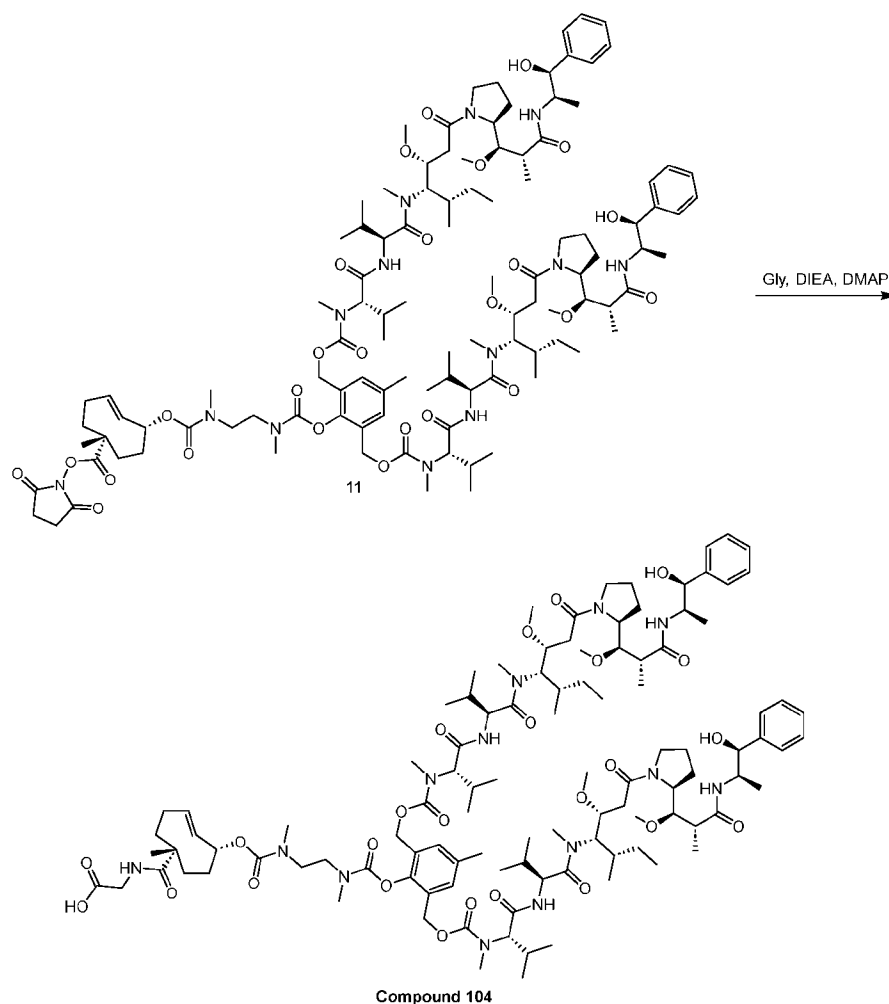


[0403] To a solution of compound **9** (600 mg, 339 μ mol, 1.0 *eq.*) in DMF (6.0 mL) was added DIEA (87 mg, 678 μ mol, 118 μ L, 2.0 *eq.*) and DMAP (41.4 mg, 339 μ mol, 1.0 *eq.*). Then was added TCO-NHS ester (186 mg, 440 μ mol, 1.3 *eq.*). The mixture was stirred at 25 °C for 3 hrs. LCMS showed one peak ($R_t = 0.590$ min) with desired mass (MS cal.: 2076.2, MS observed: $[M+H/2]^+ = 1039.7$) detected. The mixture concentrated under reduced pressure to give a residue. LCMS showed compound **11** (610 mg, 251 μ mol, 74.3% yield, 85.8% purity) as a white solid.

[0404] LCMS: $R_t = 0.486$ min, MS cal.: 2076.2, MS observed: $[M+2H]^{2+} = 1039.7$.

[0405] HPLC: $R_t = 4.484$ min, purity: 85.8%

General procedure for preparation of Compound 104



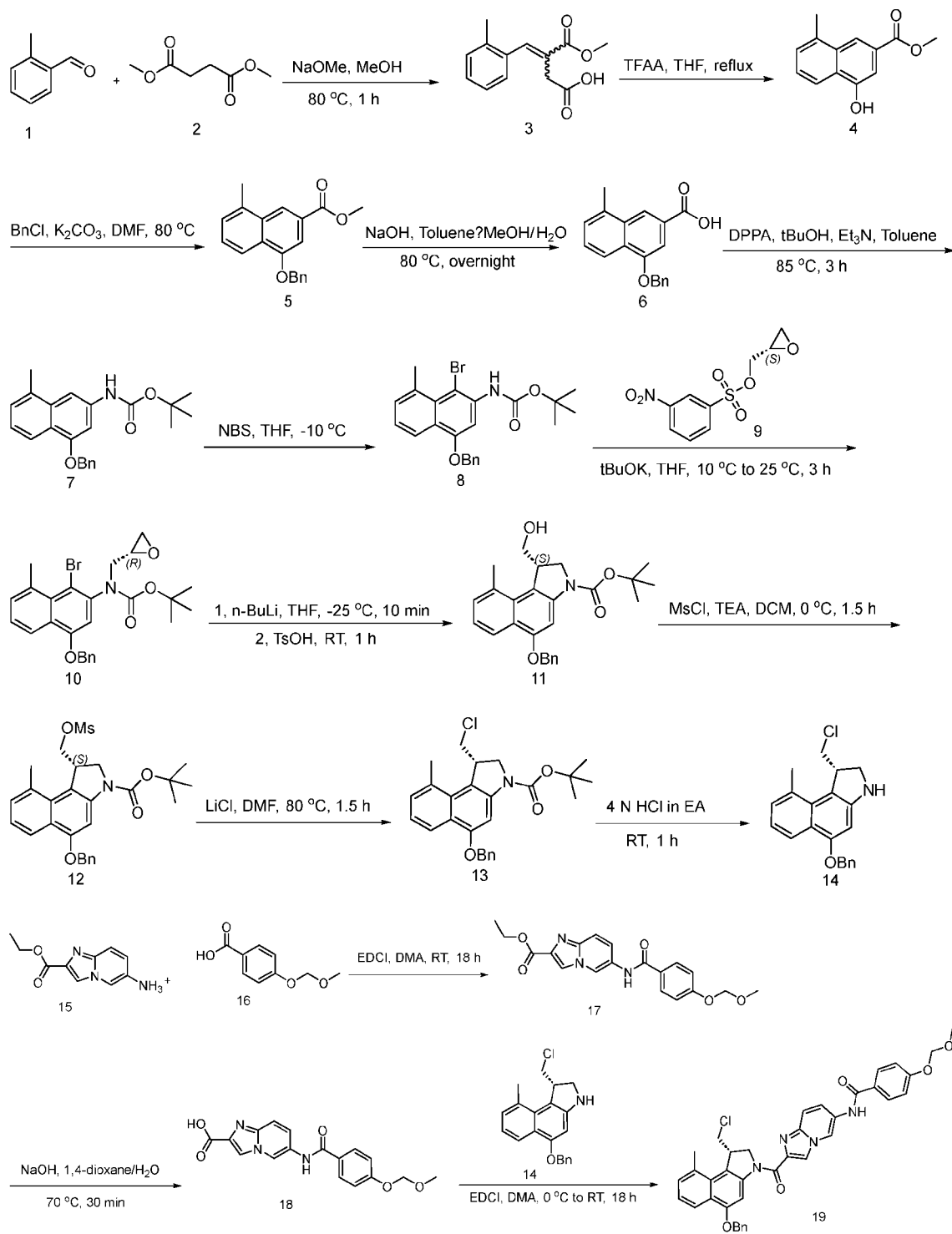
[0406] To a solution of glycine (144 mg, 1.93 mmol, 8.0 *eq.*) in DMF (5.0 mL) and H₂O (1.0 mL) was added DIEA (186 mg, 1.44 mmol, 251 μ L, 6.0 *eq.*) and DMAP (58.8 mg, 481 μ mol, 2.0 *eq.*). Then was added compound **11** (500 mg, 240 μ mol, 1.0 *eq.*). The mixture was stirred at 25 °C for 16 hrs. LCMS showed one peak (R_t = 0.561 min) with desired mass (MS cal.: 2036.2, MS observed: $[M+2H]^{2+}$ = 1019.5) detected. The reaction mixture was filtered and the filtrate was purified by prep HPLC (TFA condition). LCMS showed **Compound 104** (210 mg, 103 μ mol, 42.8% yield, 100% purity)) as a white solid.

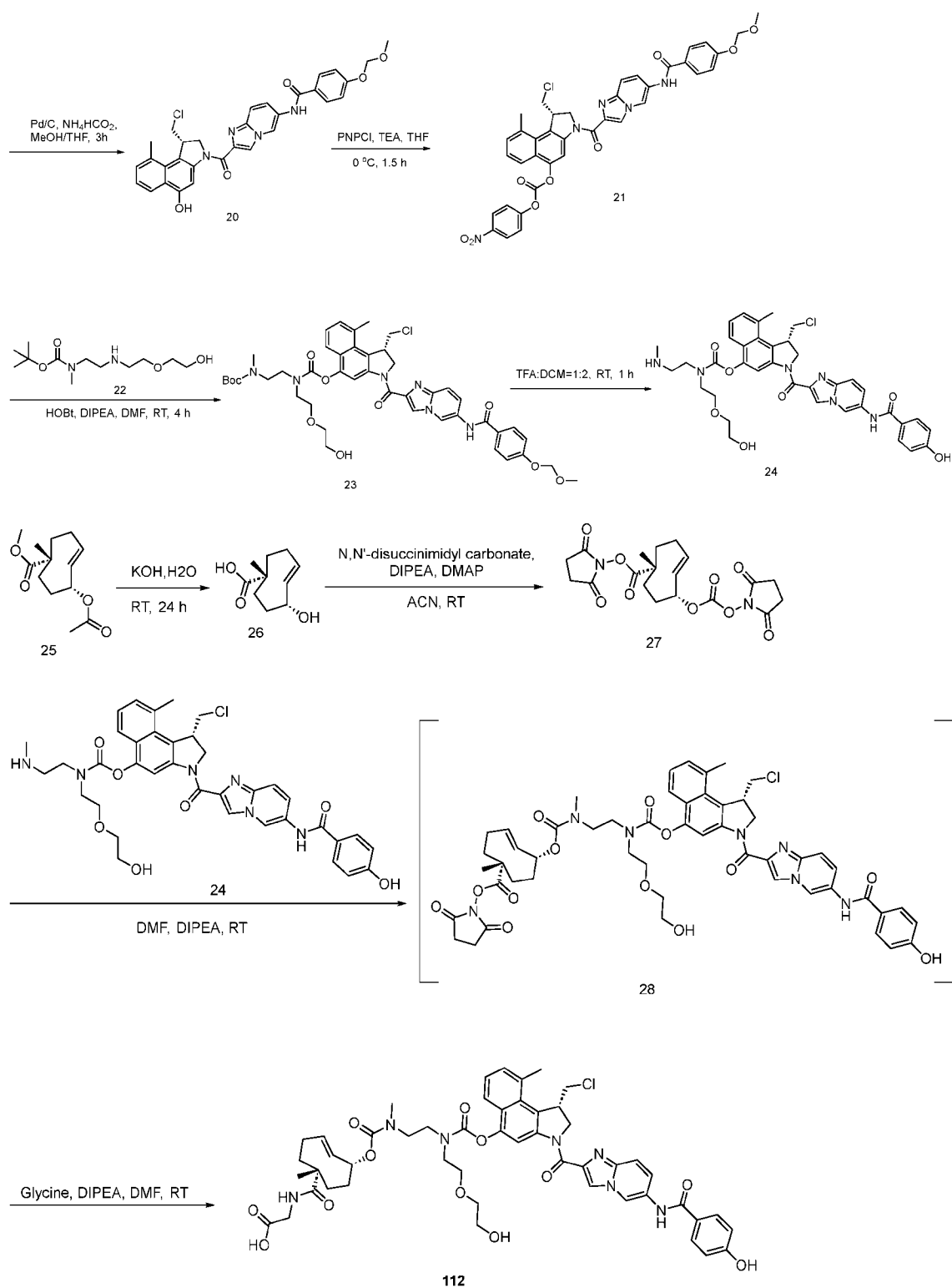
[0407] LCMS: R_t = 0.561 min, MS cal.: 2036.2, MS observed: $[M+2H]^{2+}$ = 1019.5.

[0408] HRMS: R_t = 2.374 min, MS cal.: $[M+H]^+$ = 2037.2425, MS observed: $[M+H]^+$ = 2037.2447

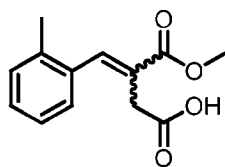
[0409] HPLC: R_t = 3.107 min, purity: 100%

Example 2: Synthesis of Compound 112



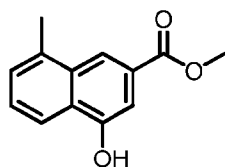


[0410] 3-(methoxycarbonyl)-4-(2-methylphenyl)but-3-enoic acid (3)



[0411] 2-Methylbenzaldehyde (**1**) (50 g, 0.42 mol) and dimethyl succinate (**2**) (103 g, 0.71 mol) in methanol (130 mL) was treated with NaOMe (29.5 g, 0.55 mol) at 80 °C for 1 h. The mixture was cooled to 20 °C, neutralized with 3 M hydrochloric acid, and diluted with water. Unreacted **2** was removed by extraction with CH₂Cl₂. The mixture was acidified with 3 M hydrochloric acid, and the product was extracted into CH₂Cl₂. The extract was dried with MgSO₄, filtered, and concentrated, providing a 9:1 mixture of (E) isomer **3a** and (Z) isomer **3b** (93 g, 95% yield). ESI m/z: 235.1 (M+1)⁺.

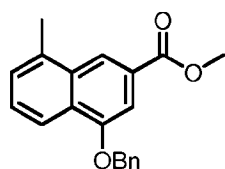
[0412] methyl 4-hydroxy-8-methylnaphthalene-2-carboxylate (**4**)



[0413] The crude product **3** (93 g, 0.40 mol) was dissolved in THF (350 mL), and the solution was treated with TFAA (55 mL, 0.40 mol) at reflux temperature until complete conversion to **4**. The reaction mixture was neutralized with an aqueous K₂CO₃ solution. The product was extracted with EtOAc. The organic layer was dried with MgSO₄, filtered, and concentrated, and the residue was crystallized by cooling to 5 °C. The white-yellow crystals were filtered, washed with acetonitrile, and dried, providing alcohol **4** (23.4 g, 27%). ESI m/z: 217.1 (M+1)⁺.

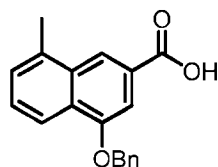
[0414] ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.53 (s, 1H), 8.15-8.11 (m, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.51 – 7.42 (m, 2H), 7.40 (d, *J* = 1.4 Hz, 1H), 3.90 (s, 3H), 2.67 (s, 3H).

[0415] methyl 4-(benzyloxy)-8-methylnaphthalene-2-carboxylate (**5**)



[0416] Alcohol **4** (23.4 g, 0.11 mol) was treated with benzyl chloride (13.4 mL, 117 mmol) in DMF (95 mL) at 80 °C in the presence of K₂CO₃ (21.5 g, 155 mmol). When the reaction was complete, the mixture was cooled and diluted with CH₂Cl₂ and water. The layers were separated, and the organic layer was concentrated to get the crude **5** (33.1 g, 100% yield). ESI m/z: 307.1 (M+1)⁺.

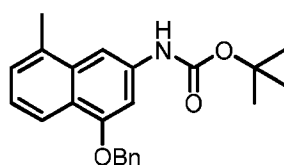
[0417] 4-(benzyloxy)-8-methylnaphthalene-2-carboxylic acid (**6**)



[0418] The residue, comprising methyl 4-(benzyloxy)-8-methyl-2-naphthoate (**5**) (33.1 g, 0.11 mol), was dissolved in toluene (150 mL) and methanol (200 mL), and the resultant solution was treated with a 4 M aqueous NaOH solution (200 mL) under reflux for 2 h. Water (800 mL) was added in several portions, and methanol and toluene were removed by distillation. Crude **6** was precipitated by addition of 4 M hydrochloric acid (100 mL), the suspension was cooled to 10 °C, and the precipitate was filtered off, washed with cold water, collected, and dried. Crude **6** was crystallized from toluene to give off-white crystals. Crystals were filtered, washed with toluene, and dried to give **6** (30.2 g, 95%). ESI m/z: 293.1 (M+1)⁺.

[0419] ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.14 (s, 1H), 8.30 (s, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 7.62 – 7.34 (m, 8H), 5.37 (s, 2H), 2.70 (s, 3H).

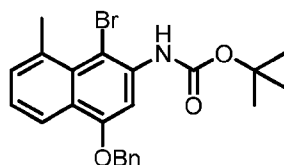
[0420] *tert*-butyl N-[4-(benzyloxy)-8-methylnaphthalen-2-yl]carbamate (**7**)



[0421] Acid **6** (28 g, 96 mmol) was reacted with diphenylphosphoryl azide (30.6 g, 111 mmol), *tert*-butyl alcohol (18.5 g, 249 mmol), and Et₃N (14.7 mL, 106 mmol) in toluene (140 mL) at 85 °C for 3 h. After the mixture had been cooled to 30 °C, EtOAc and water were added, and the layers were separated. The organic layer was washed with an aqueous Na₂CO₃ solution and saturated aqueous NaCl, dried with MgSO₄, filtered, and concentrated. The residue was triturated with isopropyl alcohol. The solid was filtered off, washed with isopropyl alcohol, and dried to provide **7** (28.8 g, 83%). ESI m/z: 364.1 (M+1)⁺.

[0422] ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.52 (s, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.77 (s, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 1H), 5.22 (s, 2H), 2.53 (s, 3H), 1.51 (s, 9H).

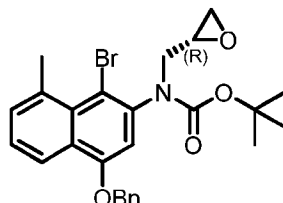
[0423] *tert*-butyl N-[4-(benzyloxy)-1-bromo-8-methylnaphthalen-2-yl]carbamate (**8**)



[0424] Carbamate **7** (34.1 g, 94 mmol) was treated with NBS (17.6 g, 98.6 mmol) in THF (550 mL) at 10 °C. After completion of the reaction, the reaction was quenched by addition of an aqueous Na₂SO₃

solution followed by a 1 M NaOH solution. EtOAc was added, and the layers were separated. The organic layer was washed with saturated aqueous NaCl, dried with MgSO₄, and concentrated to give crude **8** (41.4 g, 100% yield) as a yellow solid. ESI m/z: 442.0 (M+1)⁺.

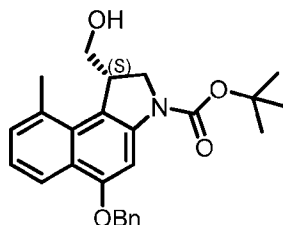
[0425] *tert*-butyl N-[4-(benzyloxy)-1-bromo-8-methylnaphthalen-2-yl]-N-[(2R)-oxiran-2-ylmethyl]carbamate (**10**)



[0426] Crude **8** (41.4 g, 94 mmol) was dissolved in THF (100 mL), deprotonated with KOt-Bu (13.7 g, 122 mmol) at 10 °C, and alkylated with (S)-glycidyl nosylate (29.2 g, 113 mmol) at 25 °C for 3 h. The reaction was quenched by addition of an aqueous NH₄Cl solution, and the mixture was extracted with EtOAc. The organic layer was washed with water and saturated aqueous NaCl. Crude **10** was obtained after evaporation of the solvents. Crystallization from heptane gave **10** (37.3 g, 80%). ESI m/z: 498.0 (M+1)⁺.

[0427] ¹H NMR (400 MHz, CDCl₃) δ 8.38 – 8.27 (m, 1H), 7.51 (t, *J* = 6.8 Hz, 2H), 7.45 – 7.34 (m, 5H), 6.92 (s, 1H), 6.80 (s, 1H), 5.31 – 5.21 (m, 2H), 4.23 – 4.09 (m, 1H), 3.47 – 3.35 (m, 1H), 3.12 (d, *J* = 4.6 Hz, 4H), 2.75 (t, *J* = 4.5 Hz, 1H), 2.68 – 2.63 (m, 1H), 2.45 – 2.40 (m, 1H), 1.60 – 1.56 (m, 3H), 1.34 – 1.31 (m, 6H).

[0428] *tert*-butyl (1S)-5-(benzyloxy)-1-(hydroxymethyl)-9-methyl-1H,2H,3H-benzo[*e*]indole-3-carboxylate (**11**)

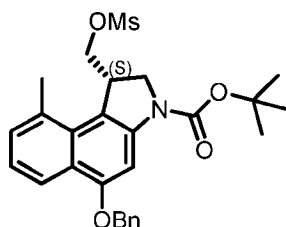


[0429] Epoxide **10** (37.3 g, 83 mmol) was dissolved in THF (400 mL). The solution was cooled to -25 °C under an atmosphere of nitrogen. Then *n*-BuLi (40 mL, 2.5 M in hexanes) was added gradually, while the temperature was kept at -25 to -20 °C. The mixture was stirred for an additional 10 min and then quenched with a saturated aqueous NH₄Cl solution. The mixture was extracted twice with EtOAc (2 x 200 mL). An aqueous solution of *p*-toluenesulfonic acid (4.8 g of monohydrate in 20 mL of water) was added to the combined organic layers, and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of a 1 M aqueous Na₂CO₃ solution. Layers were separated and the organic layer was washed with saturated aqueous NaCl, dried with MgSO₄, and concentrated. Crude **11** was dissolved in CH₂Cl₂

and filtered over silica gel (0.063-0.1 mm, 60 Å). Elution was carried out with CH₂Cl₂ (1 L) followed by CH₂Cl₂/EtOAc (1 L, 9:1, v/v) and CH₂Cl₂/EtOAc (1 L, 4:1, v/v). The fractions containing **11** were combined, the resultant solution was concentrated and dried, and the residue was crystallized from CH₂Cl₂/pentane. Crystals were collected and dried to give alcohol **11** (9.75 g, 31%). ESI m/z: 420.1 (M+1)⁺.

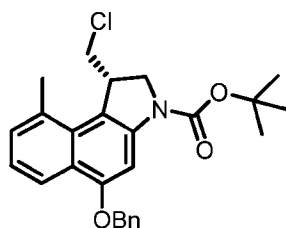
[0430] ¹H NMR (400 MHz, CDCl₃) δ 8.22 (d, *J* = 8.0 Hz, 1H), 8.07 – 7.88 (m, 1H), 7.58 – 7.50 (m, 2H), 7.46 – 7.29 (m, 4H), 7.23 – 7.17 (m, 1H), 5.26 (s, 2H), 4.25-4.21 (m, 1H), 4.08 – 3.96 (m, 2H), 3.81 – 3.73 (m, 1H), 3.49 – 3.42 (m, 1H), 2.81 (s, 3H), 2.70 – 2.54 (m, 1H), 1.60 (s, 9H).

[0431] *tert*-butyl (1*S*)-5-(benzyloxy)-1-[(methanesulfonyloxy)methyl]-9-methyl-1*H*,2*H*,3*H*-benzo[*e*]indole-3-carboxylate (**12**)



[0432] Alcohol **11** (5.0 g, 11.9 mmol) was treated with methanesulfonyl chloride (1.2 mL, 15.5 mmol) and Et₃N (4.3 mL, 30.9 mmol) in CH₂Cl₂ (40 mL) for 90 min at 0-5 °C. The reaction mixture was washed with hydrochloric acid, water, and saturated aqueous NaCl, dried with MgSO₄, and concentrated to give **12** (5.93 g, 100%). ESI m/z: 442.0 (M-tBu)⁺.

[0433] *tert*-butyl (1*S*)-5-(benzyloxy)-1-(chloromethyl)-9-methyl-1*H*,2*H*,3*H*-benzo[*e*]indole-3-carboxylate (**13**)

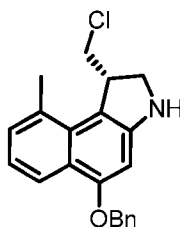


[0434] The residue **12** was dissolved in DMF (35 mL), and the solution was treated with LiCl (2.53 g, 59.7 mmol) at 80 °C for 90 min. After evaporation of DMF, the residue was partitioned between CH₂Cl₂ and water. The layers were separated, and the organic layer was washed with saturated aqueous NaCl, dried with MgSO₄, and concentrated. The residue was dissolved in hot heptane (100 mL), and the mixture was treated with activated carbon and filtered. The activated carbon was washed with another portion of heptane. The combined filtrate was cooled to approximately 50 °C, seeded, and kept at this temperature for 1 h. The suspension was cooled to 7 °C over 2 h and stirred for another hour at this temperature. Crystals were filtered, washed with heptane, collected, and dried. Dried crystals were

recrystallized from heptane using the procedure described above, giving chloride **13** (3.88 g, 74%). ESI m/z : 438.1 ($M+H$)⁺.

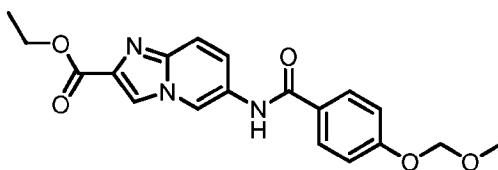
[0435] ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.08 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 7.4 Hz, 2H), 7.45 (t, J = 7.4 Hz, 2H), 7.41 – 7.32 (m, 2H), 7.29 – 7.20 (m, 1H), 5.31–5.24 (m, 2H), 4.28 – 4.20 (m, 1H), 4.14 – 4.07 (m, 1H), 4.06 – 3.97 (m, 1H), 3.75 – 3.69 (m, 1H), 3.45 – 3.37 (m, 1H), 2.75 (s, 3H), 1.56 (s, 9H).

[0436] (1S)-5-(benzyloxy)-1-(chloromethyl)-9-methyl-1H,2H,3H-benzo[e]indole (14)



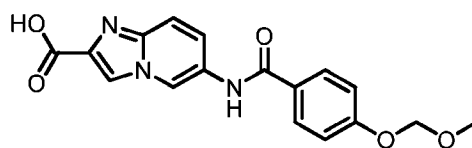
[0437] Compound **13** (3.88 g, 8.8 mmol) was dissolved in 4 M HCl in dioxane (30 mL), and the solution was stirred for 4 h. A suspension was formed, which was concentrated and dried to yield amine **14** (2.99 g, 100%) as a yellow solid. ESI m/z : 302.1 ($M-Cl$)⁺.

[0438] ethyl 6-[4-(methoxymethoxy)benzamido]imidazo[1,2-a]pyridine-2-carboxylate (17)



[0439] To a solution of amine **15** (5 g, 24.4 mmol) in DMA (75 mL) were added 4-(methoxymethoxy)benzoic acid **16** (4.45 g, 24.4 mmol) and EDC·HCl (5.62 g, 29.3 mmol). The resulting mixture was stirred for 18 h at room temperature. Subsequently, the reaction mixture was concentrated. The residue was dissolved in water (100 mL) and CH₂Cl₂ (100 mL), and the layers were separated. The organic layer was washed with water, dried with MgSO₄, and concentrated. The residue was transferred to a filter and rinsed with EtOAc. The residue was dried under vacuum to afford **17** (7.74 g, 86%). ESI m/z : 370.2 ($M+H$)⁺.

[0440] 6-[4-(methoxymethoxy)benzamido]imidazo[1,2-a]pyridine-2-carboxylic acid (18)

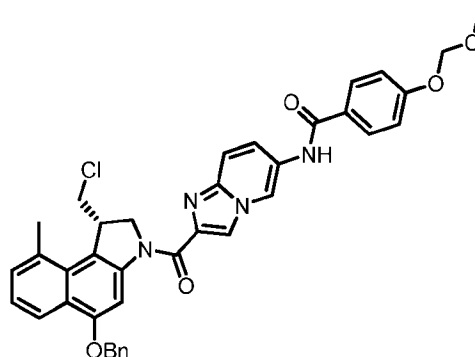


[0441] Compound **17** (7.74 g, 21.0 mmol) was dissolved in 1,4-dioxane (20 mL) and water (20 mL), and an aqueous 2 M NaOH solution (40 mL) was added. The mixture was stirred at 70 °C for 30 min. Next, the mixture was cooled to room temperature, water was added, and the mixture was acidified with a 4 M

hydrochloric acid solution. The resulting suspension was filtered, and the residue was dried to give acid **18** (6.37 g, 89%). ESI m/z: 342.1 (M+H)⁺.

[0442] ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.37 (s, 1H), 9.47 (s, 1H), 8.66 (s, 1H), 7.99 (d, *J* = 8.9 Hz, 2H), 7.67 (s, 2H), 7.18 (d, *J* = 8.9 Hz, 2H), 5.30 (s, 2H), 3.40 (s, 3H).

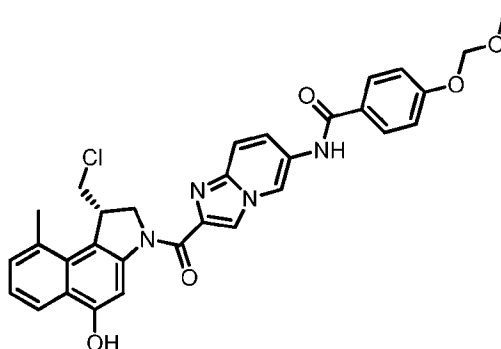
[0443] N-{2-[(1S)-5-(benzyloxy)-1-(chloromethyl)-9-methyl-1H,2H,3H-benzo[*c*]indole-3-carbonyl]imidazo[1,2-*a*]pyridin-6-yl}-4-(methoxymethoxy)benzamide (19)



[0444] Compound **14** (2.99 g, 8.8 mmol) was then dissolved in DMA (80 mL). The solution was cooled to 0 °C, and compound **18** (3.30 g, 9.7 mmol) and EDC·HCl (5.05 g, 26.3 mmol) were added. The mixture was stirred for 18 h, the temperature slowly being increased to 20 °C. Subsequently, the reaction mixture was concentrated, the crude product was dissolved in CH₂Cl₂/water (1 L, 1:1, v/v), and the layers were separated. The organic layer was dried with MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (CH₂Cl₂/methanol, 1:0 to 39:1, v/v). Compound **19** was obtained (5.27 g, 90%). ESI m/z: 661.3 (M+H)⁺.

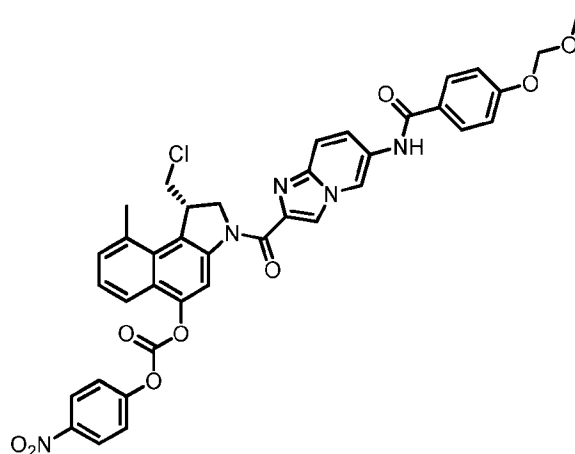
[0445] ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 9.50 (s, 1H), 8.70 (s, 1H), 8.31 (s, 1H), 8.14 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 8.7 Hz, 2H), 7.75 (d, *J* = 9.7 Hz, 1H), 7.63 – 7.54 (m, 3H), 7.50 – 7.27 (m, 6H), 7.19 (d, *J* = 8.7 Hz, 2H), 5.31 (s, 4H), 5.19 – 5.11 (m, 1H), 4.67 – 4.57 (m, 1H), 4.41 – 4.34 (m, 1H), 3.82 – 3.75 (m, 1H), 3.52 – 3.45 (m, 1H), 3.41 (s, 3H), 2.82 (s, 3H).

[0446] N-{2-[(1S)-1-(chloromethyl)-5-hydroxy-9-methyl-1H,2H,3H-benzo[*c*]indole-3-carbonyl]imidazo[1,2-*a*]pyridin-6-yl}-4-(methoxymethoxy)benzamide (20)



[0447] A suspension of Pd/C (10 wt %, 0.527 g, 0.476 mmol) and ammonium formate (5.03 g, 79.8 mmol) in methanol (20 mL) was heated at 95 °C for 5 min. The mixture was then allowed to cool to room temperature. Subsequently, additional ammonium formate (5.03 g, 79.8 mmol) was added followed by a suspension of compound **19** (5.27 g, 7.98 mmol) in THF (100 mL). The resulting mixture was stirred for 3 h at room temperature. When the reaction was complete, the mixture was filtered over Hyflo celite. The Hyflo celite pad was rinsed with THF, and the combined filtrate was concentrated. The crude product was purified by column chromatography (CH₂Cl₂/methanol, 39:1 to 9:1, v/v) to yield compound **20** (3.34 g, 73%). ESI m/z: 571.0 (M+H)⁺.

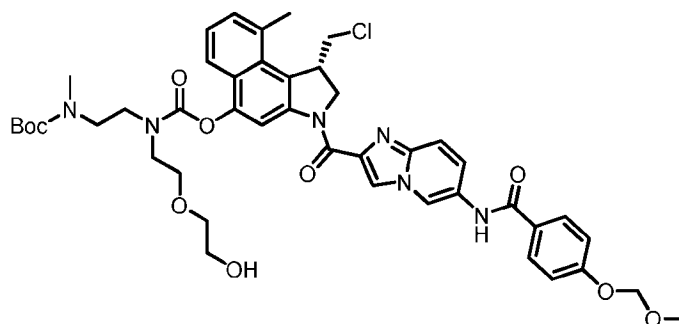
[0448] (1S)-1-(chloromethyl)-3-{6-[4-(methoxymethoxy)benzamido]imidazo[1,2-a]pyridine-2-carbonyl}-9-methyl-1H,2H,3H-benzo[e]indol-5-yl 4-nitrophenyl carbonate (21)



[0449] A solution of compound **20** (2.69 g, 4.72 mmol) in anhydrous THF (250 mL) was cooled to 0 °C under a nitrogen atmosphere, after which 4-nitrophenyl chloroformate (1.24 g, 6.18 mmol) and Et₃N (3.23 mL, 23.7 mmol) were added. The mixture was stirred at 0 °C for 1.5 h. Subsequently, the reaction mixture was concentrated, the crude product was dissolved in CH₂Cl₂/water (1 L, 1:1, v/v), and the layers were separated. The organic layer was dried with MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (CH₂Cl₂/methanol, 1:0 to 39:1, v/v). Compound **21** was obtained (2.24 g, 64%). ESI m/z: 736.3 (M+H)⁺.

[0450] ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 9.49 (s, 1H), 8.77 – 8.65 (m, 2H), 8.44 – 8.35 (m, 2H), 8.06 – 7.94 (m, 3H), 7.85 – 7.78 (m, 2H), 7.75 (d, *J* = 9.7 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.53 – 7.41 (m, 2H), 7.18 (d, *J* = 8.8 Hz, 2H), 5.31 (s, 2H), 5.21 (d, *J* = 11.9 Hz, 1H), 4.76 – 4.66 (m, 1H), 4.56 – 4.45 (m, 1H), 3.90 – 3.83 (m, 1H), 3.65 – 3.56 (m, 1H), 3.41 (s, 3H), 2.87 (s, 3H).

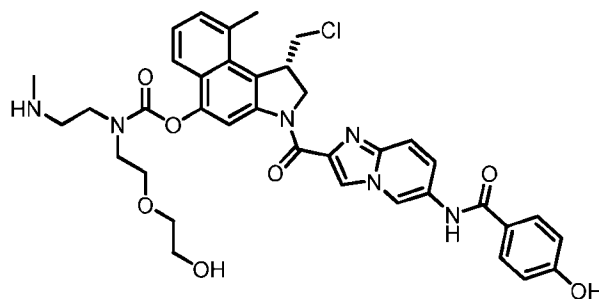
[0451] tert-butyl N-{2-[(1S)-1-(chloromethyl)-3-{6-[4-(methoxymethoxy)benzamido]imidazo[1,2-a]pyridine-2-carbonyl}-9-methyl-1H,2H,3H-benzo[e]indol-5-yl]oxy}carbonyl[2-(2-hydroxyethoxy)ethyl]amino]ethyl}-N-methylcarbamate (23)



[0452] Compound **21** (2.24 g, 3.05 mmol) was dissolved in THF (100 mL), then compound **22** (1.20 g, 4.57 mmol) and DIPEA (0.79 g, 6.10 mmol) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then concentrated, and the crude product was purified by column chromatography (CH_2Cl_2 /methanol, 1:0 to 97:3, v/v) to yield compound **23** (2.12 g, 81%). ESI m/z : 859.2 ($\text{M}+\text{H}$)⁺.

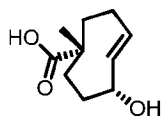
[0453] ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.33 (s, 1H), 9.48 (s, 1H), 8.71 (s, 1H), 8.34 (s, 1H), 8.00 (d, $J = 8.8$ Hz, 2H), 7.75 (d, $J = 9.8$ Hz, 1H), 7.61 – 7.56 (m, 1H), 7.45 – 7.41 (m, 1H), 7.39 – 7.34 (m, 1H), 7.18 (d, $J = 8.8$ Hz, 2H), 5.31 (s, 2H), 5.22 – 5.15 (m, 1H), 4.71 – 4.63 (m, 2H), 4.49 – 4.44 (m, 1H), 3.84 (d, $J = 11.0$ Hz, 1H), 3.78 – 3.73 (m, 2H), 3.66 – 3.61 (m, 1H), 3.57 – 3.48 (m, 6H), 3.41 (s, 3H), 2.88 – 2.82 (m, 4H), 2.03 – 1.95 (m, 1H), 1.45 – 1.23 (m, 11H).

[0454] (1S)-1-(chloromethyl)-3-[6-(4-hydroxybenzamido)imidazo[1,2-a]pyridine-2-carbonyl]-9-methyl-1H,2H,3H-benzo[e]indol-5-yl N-[2-(2-hydroxyethoxy)ethyl]-N-[2-(methyldamino)ethyl]carbamate (**24**)



[0455] A solution of **23** (2.12 g, 2.47 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C, after which TFA (25 mL) was added. The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2 (200 mL), and concentrated. The residue was dissolved in CH_2Cl_2 /toluene, and the mixture was concentrated to yield compound **24** (1.53 g, 87%). ESI m/z : 715.2 ($\text{M}+\text{H}$)⁺.

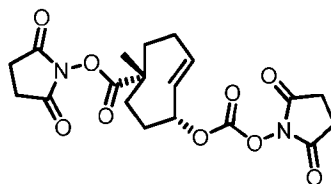
[0456] (1R,4E,6R)-6-hydroxy-1-methylcyclooct-4-ene-1-carboxylic acid (**26**)



[0457] A three necked flask (100 mL) was filled with methyl (1R,E)-6-acetoxy-1-methylcyclooct-4-ene-1-carboxylate (2 g, 1eq, 8.33 mmol). To the material was added water (8 mL). A heterogeneous (oil/water) mixture was obtained. To reaction mixture was added a solution of potassium hydroxide (0.8 g, 1.5 eq, 12.5 mmol) in water (8 mL). The flask containing the reaction mixture was covered with aluminium foil to avoid light exposure and was stirred at room temperature for 25 hours. The progress of the reaction was monitored by ¹H-NMR analysis. The reaction mixture was extracted with TBME (3 x 100 mL). The combined organic layers were washed with water (100 mL), dried with anhydrous sodium sulfate, filtered and concentrated in vacuo to provide the undesired ester (0.87 g, 53% yield) as a colorless / slightly pale-yellow colored oil. The aqueous layer was acidified with 1 M HCl until pH = 2 while cooling in an ice/water bath (T < 7 °C). The aqueous layer was extracted with TBME (3 x 100 mL). The combined TBME layers were washed with water (100 mL) and brine (100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and concentrated in vacuo to provide the desired acid **26** (1.02 g, 66% yield). The product was stored in the freezer. ESI m/z: 207.3 (M+H)⁺.

[0458] ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.93 – 5.81 (m, 1H), 5.62 – 5.53 (m, 1H), 4.66 (d, *J* = 3.1 Hz, 1H), 4.24 (s, 1H), 2.21 – 2.09 (m, 2H), 2.02 – 1.95 (m, 1H), 1.85 – 1.66 (m, 4H), 1.46 – 1.37 (m, 1H), 0.97 (s, 3H).

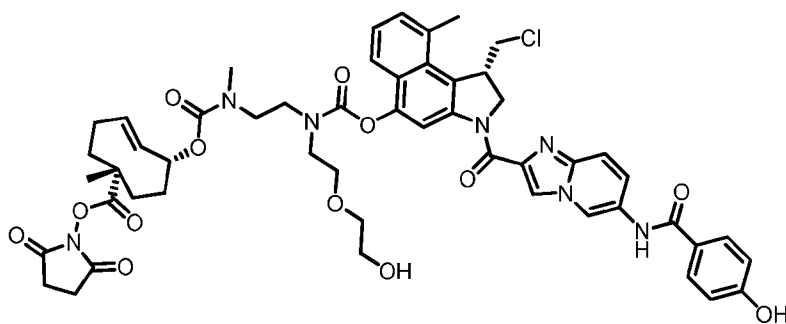
[0459] 2,5-dioxopyrrolidin-1-yl (1R,4E,6R)-6-({[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}oxy)-1-methylcyclooct-4-ene-1-carboxylate (**27**)



[0460] A mixture of compound **26** (1.02 g, 5.54 mmol), N,N'-Disuccinimidyl carbonate (DSC) (6.1 g, 23.84 mmol), DIPEA (5.29 g, 41.00 mmol) in dry acetonitrile (20 mL) was stirred at 25 °C for 24 h. LCMS showed the reaction was completed and **26** was consumed. The reaction mixture was concentrated under reduced pressure and the residue was purified by reverse phase column chromatography (C18 column (40 g), eluting with 0-65% acetonitrile in water with 0.01% TFA in 15 min) to give compound **27** (1.90 g, 81% yield). ESI m/z: 445.1 (M+Na)⁺.

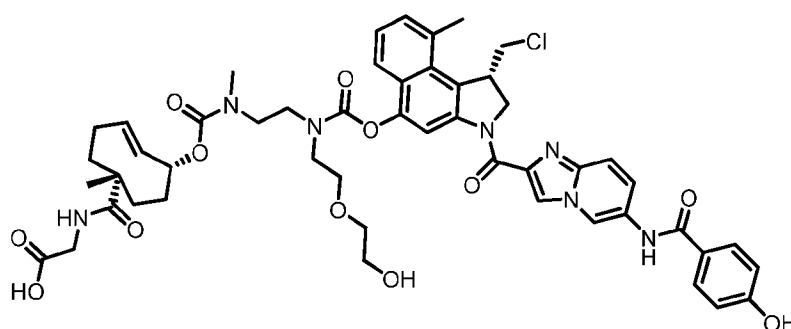
[0461] ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.94 – 5.78 (m, 2H), 5.30 – 5.24 (m, 1H), 2.81 (d, *J* = 4.6 Hz, 8H), 2.39 – 1.85 (m, 8H), 1.30 – 1.12 (m, 4H).

[0462] 2,5-dioxopyrrolidin-1-yl (1R,4E,6R)-6-[(2-[(1S)-1-(chloromethyl)-3-[6-(4-hydroxybenzamido)imidazo[1,2-a]pyridine-2-carbonyl]-9-methyl-1H,2H,3H-benzo[e]indol-5-yl]oxy]carbonyl)[2-(2-hydroxyethoxy)ethyl]amino]ethyl(methyl)carbamoyloxy]-1-methylcyclooct-4-ene-1-carboxylate (**28**)



[0463] To a DMF (10 mL) solution of compound **24** (479 mg, 0.67 mmol, 1.0 eq.) was added compound **27** (340 mg, 0.81 mmol, 1.2 eq.) followed by adding DIPEA (259 mg, 2.01 mmol, 3.0 eq.). The mixture was stirred at RT for 1.5 h. LCMS showed the reaction was completed and **24** was consumed. The reaction mixture was used in next step directly without further purification. ESI m/z : 1022.4 (M+H)⁺.

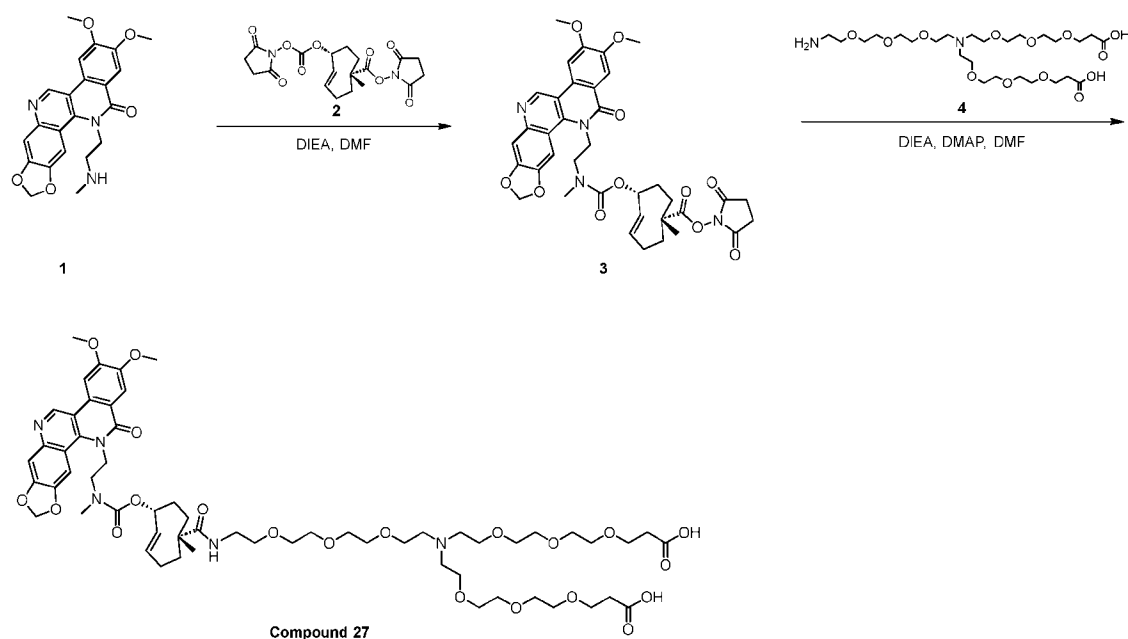
[0464] 2-[(1R,4E,6R)-6-[(2-[(1S)-1-(chloromethyl)-3-[6-(4-hydroxybenzamido)imidazo[1,2-a]pyridine-2-carbonyl]-9-methyl-1H,2H,3H-benzo[e]indol-5-yl]oxy)carbonyl][2-(2-hydroxyethoxy)ethyl]amino]ethyl(methyl)carbamoyloxy]-1-methylcyclooct-4-en-1-yl]formamido}acetic acid (**Compound 112**)



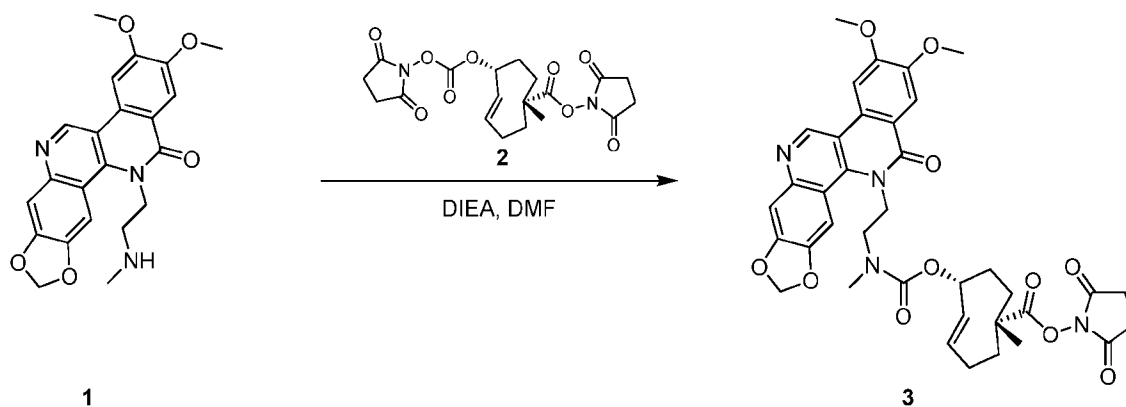
[0465] To the reaction mixture was added a solution of glycine (502 mg, 6.70 mmol, 10.0 eq.) and DIPEA (1.73 g, 13.40 mmol, 20.0 eq.) in H₂O (10 mL). The resulting mixture was stirred at RT for 1 h. LCMS showed the reaction was completed and no intermediate **28** remained. The reaction mixture was purified by reverse phase column chromatography (C18 column (120 g), eluting with 0-65% acetonitrile in water (NH₄HCO₃) in 15 min to give **Compound 112** (210 mg, 32% yield). ESI m/z : 982.3 (M+H)⁺.

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.28 – 10.19 (m, 1H), 9.46 (s, 1H), 8.70 (s, 1H), 8.32 (s, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.80 – 7.68 (m, 2H), 7.59 (d, J = 9.7 Hz, 1H), 7.47 – 7.40 (m, 1H), 7.38 – 7.33 (m, 1H), 6.90 (d, J = 8.7 Hz, 2H), 5.87 – 5.54 (m, 3H), 5.22 – 5.07 (m, 2H), 4.71 – 4.63 (m, 1H), 4.50 – 4.42 (m, 1H), 3.89 – 3.50 (m, 16H), 3.08-2.94 (m, 3H), 2.84 (s, 3H), 2.17 – 2.05 (m, 2H), 1.88 – 1.70 (m, 3H), 1.65 – 1.42 (m, 3H), 1.23 (s, 2H), 0.98 – 0.87 (m, 3H).

Example 3: Synthesis of Compound 27



General procedure for preparation of compound 3

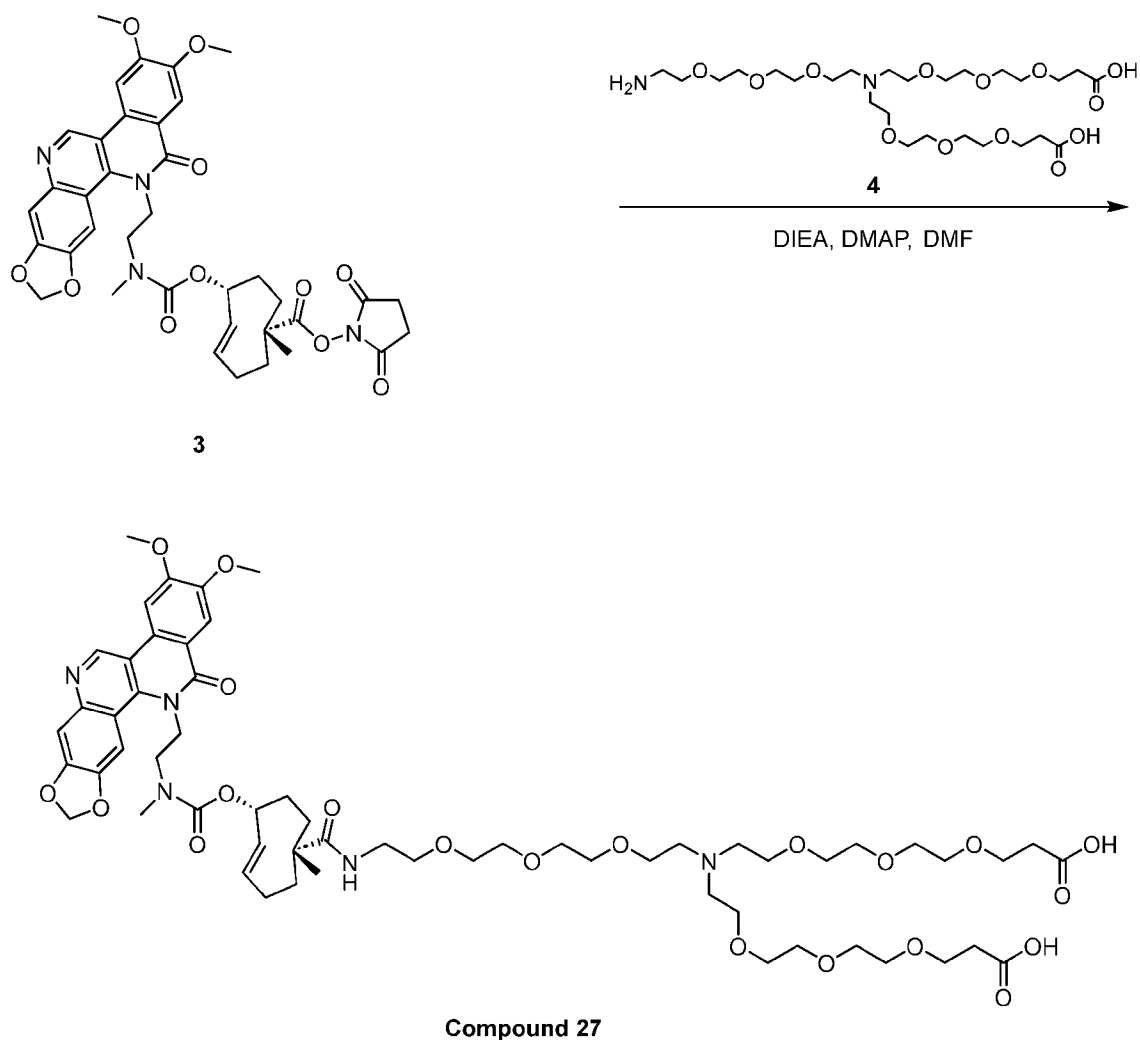


[0466] To a solution of compound **1** (200 mg, 490 μmol , 1.00 equiv.) in DMF (2.00 mL) was added compound **2** (248 mg, 589 μmol , 1.20 equiv.) and DIEA (253 mg, 1.96 mmol, 4.00 equiv.). The mixture was stirred at 25 $^{\circ}\text{C}$ for 16 hrs. LC-MS showed one peak ($R_t = 0.38$ min) with desired mass (MS cal.: 714.2, MS observed: $[\text{M}+\text{H}]^+ = 715.3$) was detected. The reaction mixture was added to the 40 mL isopropyl ether and filtered to give compound **3** (320 mg, 87.9% yield, 96.4% purity) as a brown solid.

[0467] LCMS: $R_t = 0.38$ min, MS cal.: 714.2, MS observed: $[\text{M}+\text{H}]^+ = 715.3$.

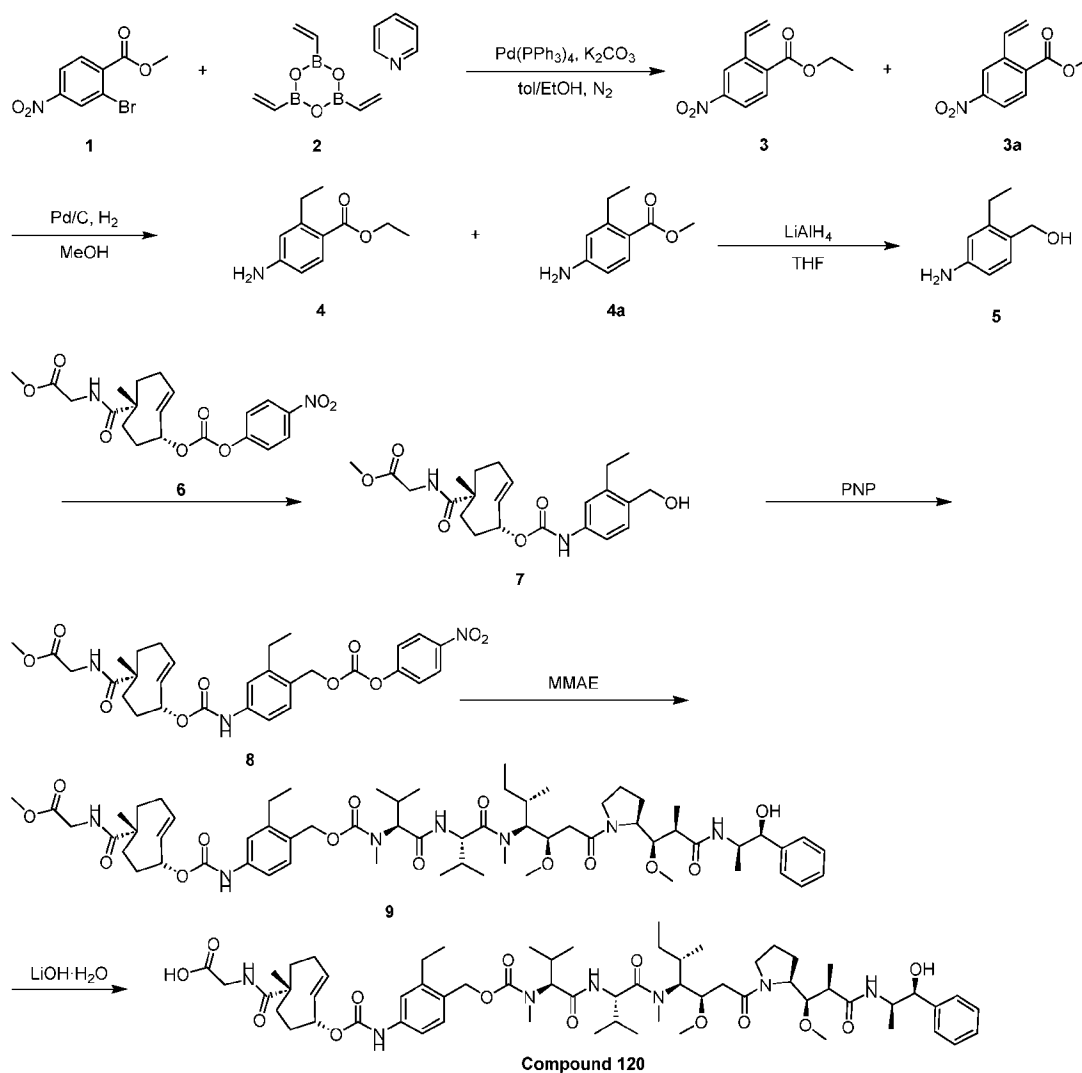
[0468] HPLC: $R_t = 2.26$ min, purity: 96.4%

General procedure for preparation of Compound 27

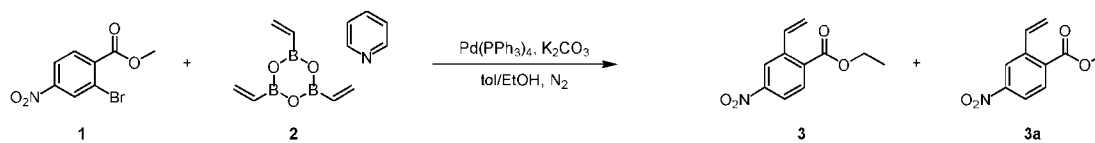


[0469] To a solution of compound **3** (630 mg, 881 μ mol, 1.00 equiv.) in DMF (6.00 mL) was added compound **4** (1.58 g, 2.21 mmol, 2.50 equiv.), DMAP (215 mg, 1.76 mmol, 2.00 equiv.) and DIEA (683mg, 5.29 mmol, 6.00 equiv.). The mixture was stirred at 25 °C for 16 hrs. LC-MS 1 (EC16907-6-P1A3) showed 34.0% of **Compound 27** was formed, 62.6% of compound **3** was remained. Then compound **4** (1.58 g, 2.21 mmol, 2.50 equiv.) and DIEA (228 mg, 1.76 mmol, 2.00 equiv.) was added to the solution. The mixture was stirred at 30 °C for 48 hrs. LC-MS showed 64.4% of **Compound 27** was formed, 30.0% of compound **3** was remained. The reaction mixture was added to the 150 mL isopropyl ether and filtered to give the crude product. The residue was purified by prep-HPLC (0.1% TFA condition) to give **Compound 27** (200 mg, 17.9% yield, 94.7% purity) was obtained as a light yellow solid. LCMS R_t = 0.331 min, MS cal.: 1199.6, MS observed: $[M+H]^+$ = 1200.5. HPLC: R_t = 1.837 min, purity: 94.7%.

Example 4: Synthesis of Compound 120



General procedure for preparation of compound 3



[0470] To a solution of compound 1 (14.1 g, 54.0 mmol, 1.0 eq.) and compound 2 (13.0 g, 54.0 mmol, 1.0 eq.) in toluene (300 mL) and EtOH (300 mL) was added Pd(PPh₃)₄ (3.12 g, 2.70 mmol, 0.05 eq.) and K₂CO₃ (29.8 g, 216 mmol, 4.0 eq.). The mixture was stirred at 90 °C for 16.0 hrs. TLC (Petroleum ether/EtOAc = 10:1, product R_f = 0.50 and 0.57) indicated compound 1 was consumed completely and two new spots formed. The reaction mixture was concentrated under reduced pressure to

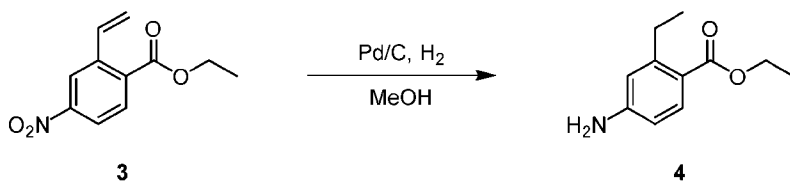
remove solvent. The residue was diluted with water (500 mL) and extracted with EtOAc (500 mL *3). The combined organic layers were washed with brine (500 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=100/1 to 0/1) to give compound **3** and **3a** (9.50 g, 79.5% yield) as a yellow solid.

[0471] Note: 1.8 g of compound **3**, 2.8 g of compound **3a**, 4.9 g mixture of compound **3** and **3a** were obtained respectively.

[0472] ¹H NMR of Compound **3** (400 MHz, CDCl₃) δ : 8.41 (d, J = 2.25 Hz, 1 H), 8.12 (dd, J = 8.57, 2.31 Hz, 1 H), 8.00 (d, J = 8.63 Hz, 1 H), 7.43 (dd, J = 17.51, 11.01 Hz, 1 H), 5.82 (d, J = 17.13 Hz, 1 H), 5.53 (d, J = 11.26 Hz, 1 H), 4.42 (q, J = 7.13 Hz, 2 H), 1.42 (t, J = 7.13 Hz, 3 H).

[0473] ¹H NMR of Compound **3a** (400 MHz, CDCl₃) δ : 8.40 (d, J = 2.13 Hz, 1 H), 8.11 (dd, J = 8.63, 2.25 Hz, 1 H), 8.00 (d, J = 8.51 Hz, 1 H), 7.42 (dd, J = 17.39, 11.01 Hz, 1 H), 5.82 (d, J = 17.51 Hz, 1 H), 5.52 (d, J = 11.01 Hz, 1 H), 3.95 (s, 3 H).

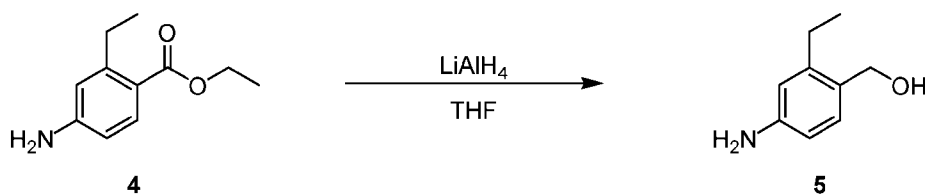
General procedure for preparation of compound 4



[0474] To a solution of Pd/C (220 mg, 206 μ mol, 10% purity, 0.02 eq.) in MeOH (2.00 mL) was purged with N₂ for 3 times. Then compound **3** (1.8 g, 8.13 mmol, 1.0 eq.) in MeOH (25 mL) was added slowly and purged with H₂ for 3 times. The reaction mixture was stirred at 25 °C for 4.0 hrs under H₂ atmosphere (15 psi). TLC (Petroleum ether/EtOAc = 10:1, product R_f = 0.15) indicated compound **3** was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was filtered, the solvent was removed by rotary evaporation to give compound **4** (1.50 g, crude) as a yellow solid.

[0475] ¹H NMR (400 MHz, CDCl₃) δ : 7.80 (d, J = 8.13 Hz, 1 H), 6.45 - 6.50 (m, 2 H), 4.29 (q, J = 7.13 Hz, 2 H), 3.60 - 3.95 (m, 2 H), 2.94 (q, J = 7.42 Hz, 2 H), 1.36 (t, J = 7.13 Hz, 3 H), 1.21 (t, J = 7.44 Hz, 3 H).

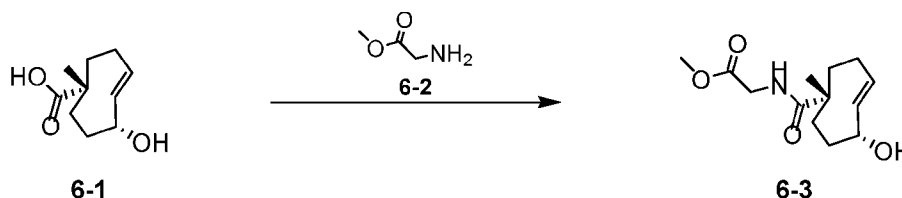
General procedure for preparation of compound 5



[0476] To a solution of ethyl compound 4 (1.50 g, 7.76 mmol, 1.0 eq.) in THF (30.0 mL) was added LiAlH₄ (2.5 M, 9.31 mL, 3.0 eq.) at 0 °C under N₂ atmosphere. Then the mixture was stirred at 25 °C for 4.0 hrs. TLC (Petroleum ether/EtOAc = 1:1, product R_f = 0.20) indicated compound 4 was consumed completely and one new spot formed. The reaction mixture was cooled to 0 °C, and worked up by addition of water (900 µL), 15% NaOH solution (900 µL) and water (2.70 mL) again under N₂ atmosphere. The resulting slurry was stirred at 25 °C for 15 mins, dried over Na₂SO₄, filtered off and washed with THF (50.0 mL). The filtrate was concentrated under reduced pressure to give the residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=100/1 to 0/1) to give compound 5 (0.60 g, 3.97 mmol, 48.8% yield over two steps, 90.0% purity) as a yellow oil.

[0477] ¹H NMR (400 MHz, CDCl₃) δ: 7.06 - 7.13 (m, 1 H), 6.55 - 6.59 (m, 1 H), 6.51 (br d, *J* = 7.75 Hz, 1 H), 4.59 (s, 2 H), 2.67 (q, *J* = 7.67 Hz, 2 H), 1.22 (t, *J* = 7.57 Hz, 3 H).

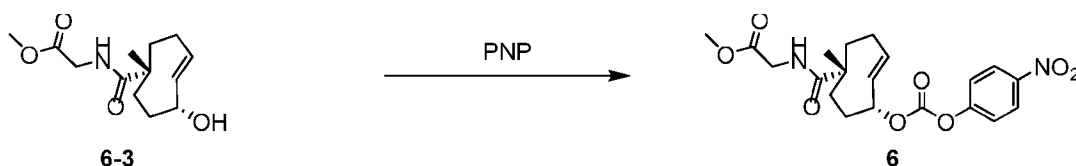
General procedure for preparation of compound 6-3



[0478] To a solution of compound 6-1 (4.00 g, 21.7 mmol, 1.0 eq.) and compound 6-2 (5.45 g, 43.4 mmol, HCl salt, 2.0 eq.) in DCM (40.0 mL) was added DIEA (11.2 g, 86.8 mmol, 15.1 mL, 4.0 eq.), HOBt (7.33 g, 54.2 mmol, 2.5 eq.) and EDCI (10.4 g, 54.2 mmol, 2.5 eq.). The mixture was stirred at 25 °C for 2.0 hrs. TLC (Petroleum ether/EtOAc = 1:1, product R_f = 0.20) indicated compound 6-1 was consumed completely and one new spot formed. The reaction mixture was partitioned between HCl solution (1.0 M, 100 mL) and DCM (100 mL). The organic phase was separated, washed with sat. NaHCO₃ (100 mL) and brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give compound 6-3 (3.20 g, crude) as a white solid.

[0479] ¹H NMR (400 MHz, CDCl₃) δ: 6.08 - 6.15 (m, 1 H), 6.00 - 6.08 (m, 1 H), 5.63 (dd, *J* = 16.57, 2.31 Hz, 1 H), 4.45 (br s, 1 H), 3.96 (d, *J* = 5.25 Hz, 2 H), 3.73 (s, 3 H), 2.20 - 2.33 (m, 2 H), 2.05 - 2.12 (m, 2 H), 1.87 - 1.99 (m, 2 H), 1.78 - 1.86 (m, 2 H), 1.57 (dd, *J* = 15.51, 6.25 Hz, 1 H), 1.11 (s, 3 H).

General procedure for preparation of compound 6



[0480] To a solution of compound 6-3 (3.2 g, 12.5 mmol, 1.0 eq.) in DMF (30.0 mL) was added bis(4-nitrophenyl) carbonate (7.63 g, 25.0 mmol, 2.0 eq.) and DIEA (4.86 g, 37.6 mmol, 6.55 mL, 3.0 eq.). The mixture was stirred at 25 °C for 2.0 hr. TLC (Petroleum ether/EtOAc = 1:2, product R_f = 0.20) indicated

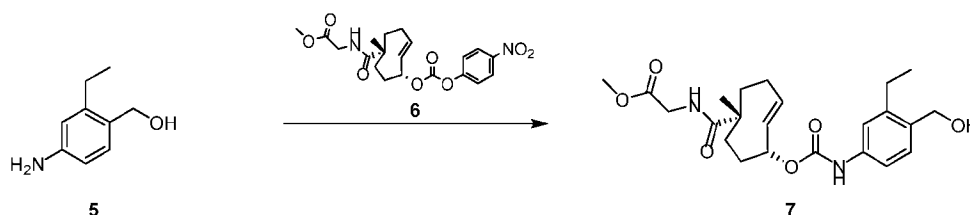
compound 6-3 was consumed completely and one new spot formed. The reaction mixture was quenched with sat. NH_4Cl (50 mL) and extracted with EtOAc (50.0 mL * 3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 100/1 to 0/1) to give compound **6** (3.60 g, 8.56 mmol, 39.4% yield over two steps, 98.6% purity) as a yellow oil.

[0481] LCMS: $R_t = 0.41$ min, MS cal.: 420.1, MS observed: $[\text{M} + \text{Na}]^+ = 443.1$.

[0482] HPLC: $R_t = 2.55$ min, purity: 98.6%.

[0483] ^1H NMR (400 MHz, CDCl_3) δ : 7.26 - 7.30 (m, 2 H), 7.19 - 7.24 (m, 1 H), 6.99 (br s, 1 H), 6.10 (br t, $J = 4.69$ Hz, 1 H), 5.89 - 5.99 (m, 1 H), 5.64 (dd, $J = 16.63, 2.38$ Hz, 1 H), 5.26 (br s, 1 H), 4.66 (s, 2 H), 3.98 (dd, $J = 5.00, 2.25$ Hz, 2 H), 3.75 (s, 3 H), 2.69 (q, $J = 7.50$ Hz, 2 H), 2.22 - 2.32 (m, 2 H), 2.06 - 2.15 (m, 2 H), 1.82 - 1.99 (m, 4 H), 1.22 (t, $J = 7.57$ Hz, 3 H), 1.16 (s, 3 H).

General procedure for preparation of compound 7

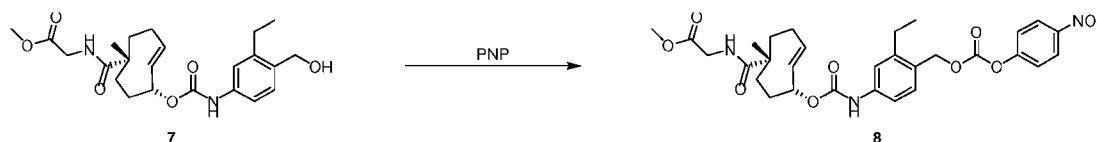


[0484] To a solution of compound **5** (1.17 g, 7.73 mmol, 1.3 eq.) and compound **6** (2.50 g, 5.95 mmol, 1.0 eq.) in DMF (25.0 mL) was added HOAt (1.21 g, 8.92 mmol, 1.25 mL, 1.5 eq.) and DIEA (1.54 g, 11.8 mmol, 2.07 mL, 2.0 eq.). The mixture was stirred at 50 °C for 2.0 hr. LC-MS showed compound **6** was consumed completely and one main peak ($R_t = 0.37$ min) with desired mass (MS cal.: 432.2, MS observed: $[\text{M} + \text{Na}]^+ = 455.1$) was detected. The reaction mixture was quenched with sat. NH_4Cl (100 mL) and extracted with EtOAc (100 mL * 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 100/1 to 0/1) to give compound **7** (2.20 g, 4.86 mmol, 81.7% yield, 95.6% purity) as a yellow solid.

[0485] LCMS: $R_t = 0.38$ min, MS cal.: 432.2, MS observed: $[\text{M} + \text{Na}]^+ = 455.2$.

[0486] HPLC: $R_t = 2.06$ min, purity: 95.6%.

General procedure for preparation of compound 8

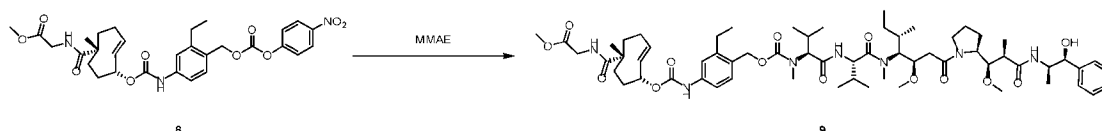


[0487] To a solution of compound **7** (2.20 g, 5.09 mmol, 1 eq.) in DMF (20.0 mL) was added bis(4-nitrophenyl) carbonate (4.64 g, 15.2 mmol, 3 eq.) and DIEA (1.97 g, 15.26 mmol, 2.66 mL, 3.0 eq.). The mixture was stirred at 25 °C for 2.0 hrs. LC-MS showed compound **7** was consumed completely and one main peak ($R_t = 0.49$ min) with desired mass (MS cal.: 597.2, MS observed: $[M + Na]^+ = 620.3$) was detected. The reaction mixture was extracted with water and EtOAc (100 mL * 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 100/1 to 0/1) to give compound **8** (2.60 g, 2.82 mmol, 55.4% yield, 64.8% purity) as a yellow solid.

[0488] LCMS: $R_t = 0.49$ min, MS cal.: 597.2, MS observed: $[M + Na]^+ = 620.2$.

[0489] HPLC: $R_t = 3.30$ min, purity: 64.8%.

General procedure for preparation of compound 9

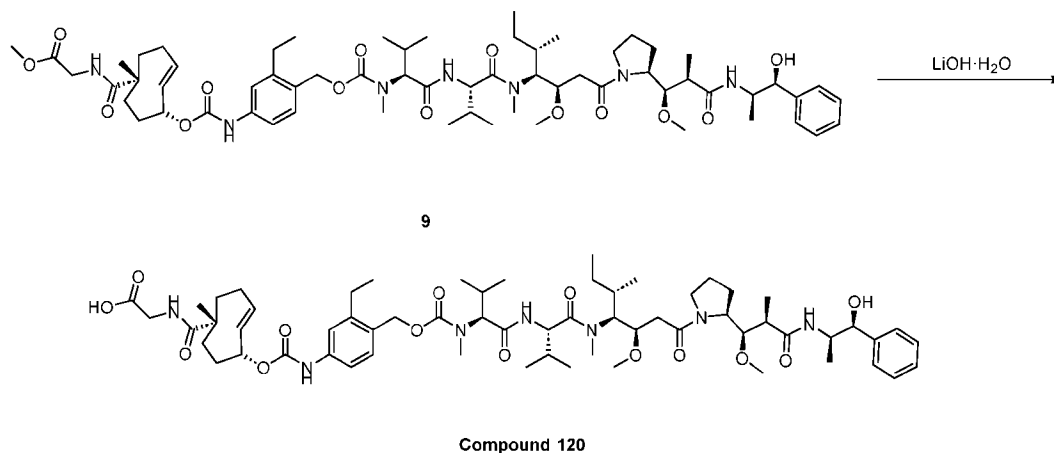


[0490] To a solution of compound **8** (720 mg, 1.21 mmol, 1.0 eq.) and MMAE (517 mg, 722 μ mol, 0.6 eq.) in DMF (7.00 mL) was added HOAt (245 mg, 1.81 mmol, 252 μ L, 1.5 eq.) and DIEA (311 mg, 2.40 mmol, 419 μ L, 2.0 eq.). The mixture was stirred at 25 °C for 4.0 hrs. LC-MS showed compound **8** was consumed completely and one peak ($R_t = 0.52$ min) with desired mass (MS cal.: 1175.7, MS observed: $[M + H]^+ = 1176.8$) was detected. The reaction mixture was purified by prep-HPLC (TFA condition) to give compound **9** (300 mg, 255 μ mol, 19.7% yield, 99.2% purity) as a white solid.

[0491] LCMS: $R_t = 0.52$ min, MS cal.: 1175.7, MS observed: $[M + H]^+ = 1176.8$.

[0492] HPLC: $R_t = 2.38$ min, purity: 99.2%.

General procedure for preparation of Compound 120

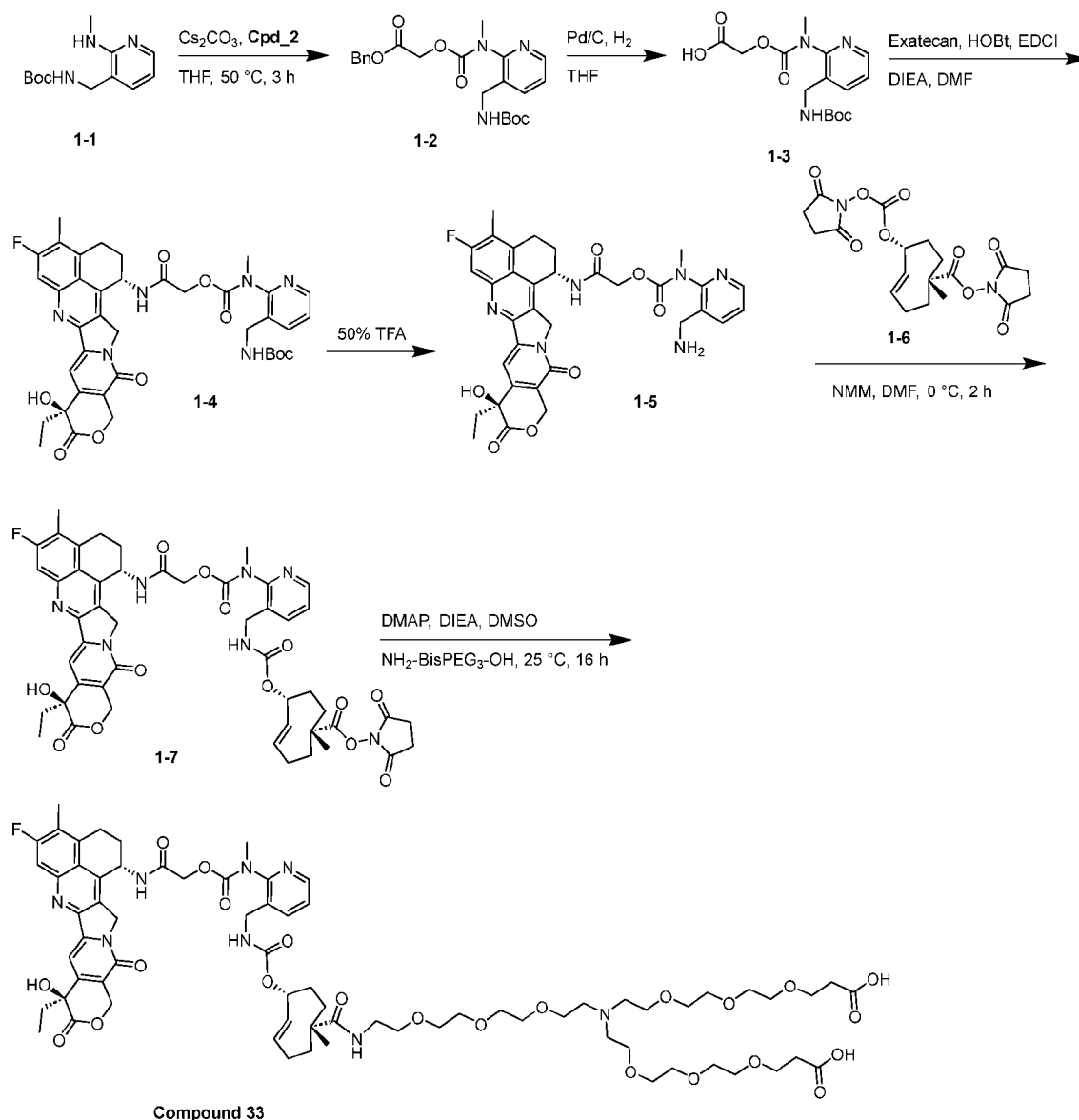


[0493] To a solution of methyl compound **9** (400 mg, 339 μmol , 1.0 eq.) in THF (4.00 mL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (1.0 M, 1.36 mL, 4.0 eq.). The mixture was stirred at 0 °C for 2.0 hrs. LC-MS showed compound **9** was consumed completely and one peak ($R_t = 0.51$ min) with desired mass (MS cal.: 1161.6, MS observed: $[\text{M} + \text{Na}]^+ = 1184.7$) was detected. The reaction mixture was acidified with 10% TFA aqueous solution at 0 °C under stirring and then concentrated under reduced pressure to give the residue. The residue was purified by prep-HPLC (TFA condition) to give **Compound 120** (323 mg, 278 μmol , 82.0% yield, 99.8% purity, TFA salt) as a white solid.

[0494] LCMS: $R_t = 0.36$ min, MS cal.: 1161.6, MS observed: $[\text{M} + \text{Na}]^+ = 1184.8$.

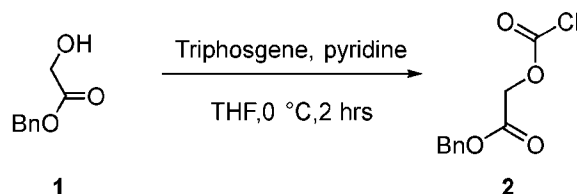
[0495] HPLC: $R_t = 2.76$ min, purity: 99.8%.

Example 5: Synthesis of Compound 33



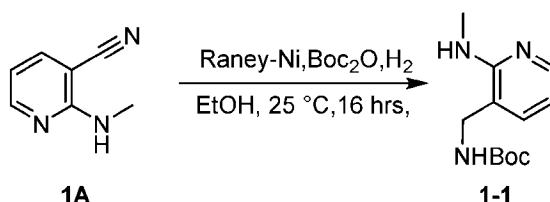


General procedure for preparation of compound 2



[0496] To a solution of triphosgene (25.8 g, 87.2 mmol, 1.45 *eq.*) in THF (150 mL) was added pyridine (6.47 g, 81.7 mmol, 6.60 mL, 1.36 *eq.*) at 0 °C under N₂ atmosphere. After addition, compound **1** (10.0 g, 60.1 mmol, 8.54 mL, 1.00 *eq.*) in THF (150 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 2 hrs. TLC (petroleum ether: ethyl acetate = 3: 1) indicated compound **1** (R_f = 0.35) was consumed completely and many new spots (R_f = 0.01, R_f = 0.3, R_f = 0.59, R_f = 0.75, R_f = 0.85). The reaction mixture was diluted with 1.0 M HCl (150 mL) at 0 °C and extracted with DCM (150 mL * 3), the combined organic layers were washed with brine (150 mL * 2), dried over Na₂SO₄. After filtration, the filtrate was concentrated under reduced pressure to give a residue. The residue was treated to column chromatography (SiO₂, petroleum ether: ethyl acetate = 1: 0 to 0: 1, petroleum ether: ethyl acetate = 3: 1, R_f = 0.75, by plate 2) to give compound **2** (6.10 g, crude) as yellow oil, which was used without further purification.

General procedure for preparation of compound 1-1

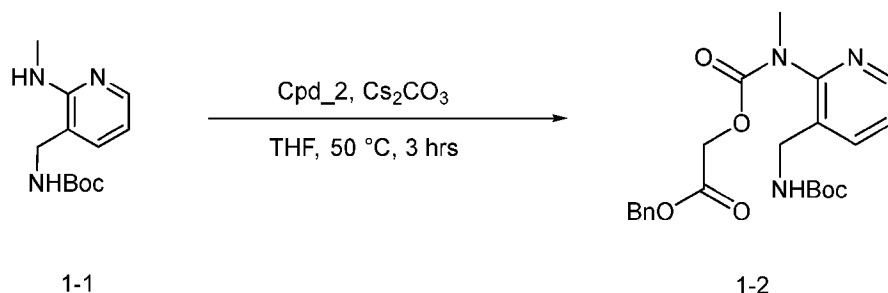


[0497] To a solution of compound **1A** (7.10 g, 53.3 mmol, 1.00 *eq.*) and Boc₂O (17.5 g, 80.0 mmol, 18.4 mL, 1.50 *eq.*) in EtOH (112 mL) was added **Raney-Ni** (710 mg, 8.29 mmol) under N₂ atmosphere. The suspension was degassed and purged with H₂ for 3 times. The mixture was stirred under H₂ (50 Psi) at 25 °C for 16 hrs. LCMS showed compound **1A** consumed completely and desired mass was detected. (R_t =0.23 min). The reaction mixture was filtered and concentrated under reduced pressure to give crude product. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=1/0 to 0/1, Petroleum ether: Ethyl acetate=1:1, R_f =0.30). HNMR and HPLC showed compound **1-1** (8.20 g, 32.0 mmol, 60.0% yield, 92.6%) as a white solid.

[0498] LCMS: R_t = 0.22 min, MS cal.: 237.2, MS observed: $[M+H]^+$ =238.0.

[0499] HPLC: $R_t = 0.78$ min, purity: 92.6%. ^1H NMR (400 MHz, CDCl_3) δ : ppm 8.10 (dd, $J = 5.1, 1.8$ Hz, 1 H), 7.18 (dd, $J = 7.1, 1.3$ Hz, 1 H), 6.47 (dd, $J = 7.0, 5.3$ Hz, 1 H), 5.75 (br s, 1 H), 4.79 (br s, 1 H), 4.17 (d, $J = 6.5$ Hz, 2 H), 3.00 (d, $J = 4.8$ Hz, 3 H), 1.46 (s, 9 H).

General procedure for preparation of compound 1-2



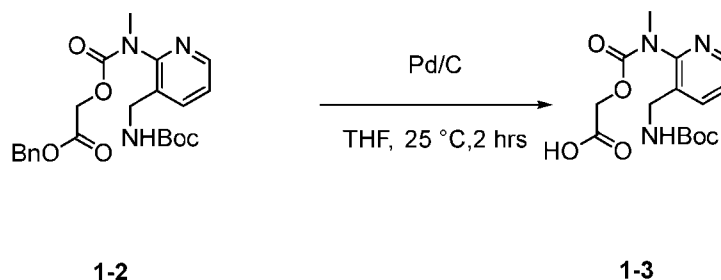
[0500] To a solution of compound 1-1 (1.30 g, 5.07 mmol, 1.00 eq.) in THF (40 mL) was added Cs_2CO_3 (1.98 g, 6.09 mmol, 1.20 eq.) and compound 2 (5.80 g, 25.3 mmol, 5.00 eq.). The mixture was stirred at 50°C for 3 hrs. LCMS showed compound 1-1 was remained ($R_t = 0.29$ min). Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.50$ min, MS cal.: 429.1, MS observed: $[\text{M}+\text{H}]^+ = 452.1$). The reaction mixture was diluted with ice water (60.0 mL) and extracted with DCM (80.0 mL * 3). The combined organic layers were washed with brine (150 mL * 2), dried over Na_2SO_4 . After filtration, the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , petroleum ether: ethyl acetate = 1: 0 to 0: 1, petroleum ether: ethyl acetate = 2: 1, $R_f = 0.26$). Compound 1-2 (1.39 g, 3.19 mmol, 62.9% yield, 98.5% purity) was obtained as yellow oil, confirmed by HNMR, LCMS ($R_t = 0.48$ min), and HPLC ($R_t = 3.46$ min).

[0501] LCMS: $R_t = 0.48$ min, MS cal.: 429.1, MS observed: $[\text{M}+\text{H}]^+ = 452.2$.

[0502] HPLC: $R_t = 3.46$ min, purity: 98.5%.

[0503] ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : ppm 8.38 - 8.36 (m, 1 H), 7.72 (d, $J = 6$ Hz, 1 H), 7.40 - 7.34 (m, 7 H), 5.16 (s, 2 H), 4.80 - 5.54 (m, 2 H), 4.12 (s, 2 H), 3.18 (s, 3 H), 1.38 (s, 9 H).

General procedure for preparation of compound 1-3



[0504] A 50 mL round-bottom flask was purged with Ar for 3 times and added Pd/C (120 mg, 113 μmol , 10% purity) carefully. Then THF (4.00 mL) was added to infiltrate the Pd/C completely, followed

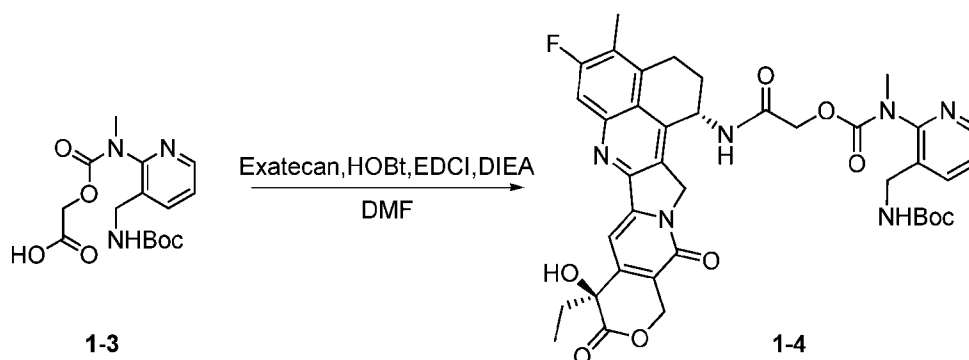
by the compound **1-2** (1.20 g, 2.79 mmol, 1.00 *eq.*) in THF (8.00 mL) slowly under Ar atmosphere. The resulting mixture was degassed and purged with H₂ for 3 times, and then the mixture was stirred at 25 °C for 2 hrs under H₂ atmosphere (15 psi). LCMS showed compound **1-2** was consumed completely (MS cal.: 339.1, MS observed: [M+Na]⁺= 362.0) was detected. (R_t=0.348 min). The reaction mixture was dissolved in THF (10.0 mL) and filtered through siliceous earth, the cake was washed with THF (10.0 mL *2) and the filtrate was concentrated under reduced pressure. The crude product was used into the next step without further purification. Compound **1-3** (1.00 g, crude) was obtained as a colourless oil, confirmed by LCMS (R_t=0.34 min), HNMR, and HPLC (R_t= 1.43 min).

[0505] LCMS: R_t=0.34 min, MS cal.: 339.1, MS observed:[M+Na]⁺= 362.0.

[0506] HPLC: R_t= 1.43 min, purity: 96.2%.

[0507] ¹H NMR (400 MHz, DMSO-*d*₆) δ: ppm 8.37 (br d, *J* = 3.4 Hz, 1 H), 7.72 (br s, 1 H), 7.23 - 7.50 (m, 2 H), 4.33 - 4.67 (m, 2 H), 3.98 - 4.21 (m, 2 H), 3.14 - 3.24 (m, 3 H), 1.30 - 1.45 (m, 9 H).

General procedure for preparation of compound 1-4

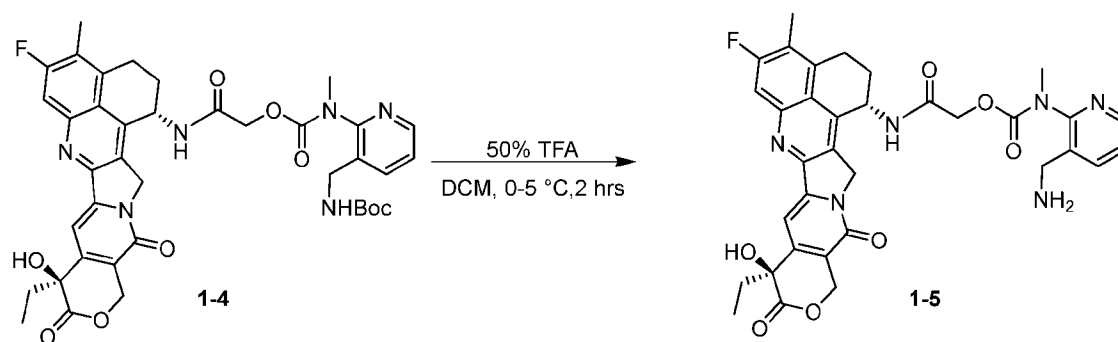


[0508] To a solution of compound **1-3** (766 mg, 2.26 mmol, 1.20 *eq.*) in DMF (10.0 mL) was added HOBt (305 mg, 2.26 mmol, 1.20 *eq.*) and DIEA (486 mg, 3.76 mmol, 655 μL, 2.00 *eq.*), Exatecan (1.00 g, 1.88 mmol, 1.00 *eq.*), EDCI (541 mg, 2.82 mmol, 1.50 *eq.*). The mixture was stirred at 25 °C for 2 hrs. LCMS showed compound **1-3** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected (R_t= 0.47 min, MS cal.: 756.2, MS observed: [M+Na]⁺= 757.3). The reaction mixture was added to isopropyl ether (100 mL*2) and the crude product was precipitated, then centrifuged to afford the crude product as a residue. The liquid supernatant was discarded. The crude product was used into the next step without further purification. Compound **1-4** (2.0 g, crude) was obtained as a brown solid, confirmed by LCMS (R_t=0.47 min), and HPLC (R_t= 2.84 min).

[0509] LCMS: R_t=0.47 min, MS cal.: 756.2, MS observed: [M+Na]⁺= 757.3

[0510] HPLC: R_t= 2.84 min, purity: 84.3%

General procedure for preparation of compound 1-5

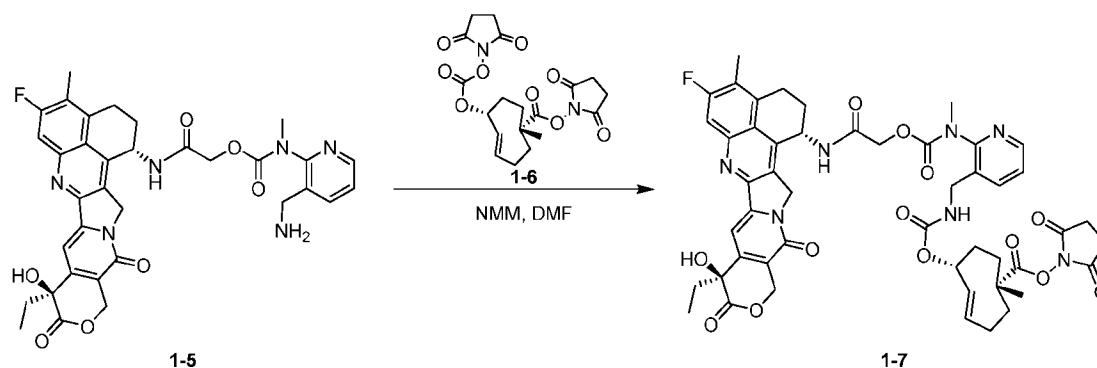


[0511] To a solution of compound **1-4** (2.00 g, 2.64 mmol, 1.00 *eq.*) in DCM (10.0 mL) was added TFA (15.3 g, 135 mmol, 10.0 mL, 50.9 *eq.*). The mixture was stirred at 0-25 °C for 1 hr. LCMS showed compound **1-4** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t=0.34$ min, MS cal.: 656.2, MS observed: $[M+H]^+=657.3$). The reaction mixture was added to isopropyl ether (200 mL*2) and the crude product was precipitated, then centrifuged to afford the crude product as a residue. The liquid supernatant was discarded. The crude product was used into the next step without further purification. Compound **1-5** (2.00 g, crude, TFA salt) was obtained as a gray solid, confirmed by LCMS ($R_t=0.35$ min), and HPLC ($R_t=1.64$ min).

[0512] LCMS: $R_t=0.35$ min, 656.2, MS observed: $[M+Na]^+=657.3$

[0513] HPLC: $R_t=2.84$ min, purity: 92.7%.

General procedure for preparation of compound 1-7

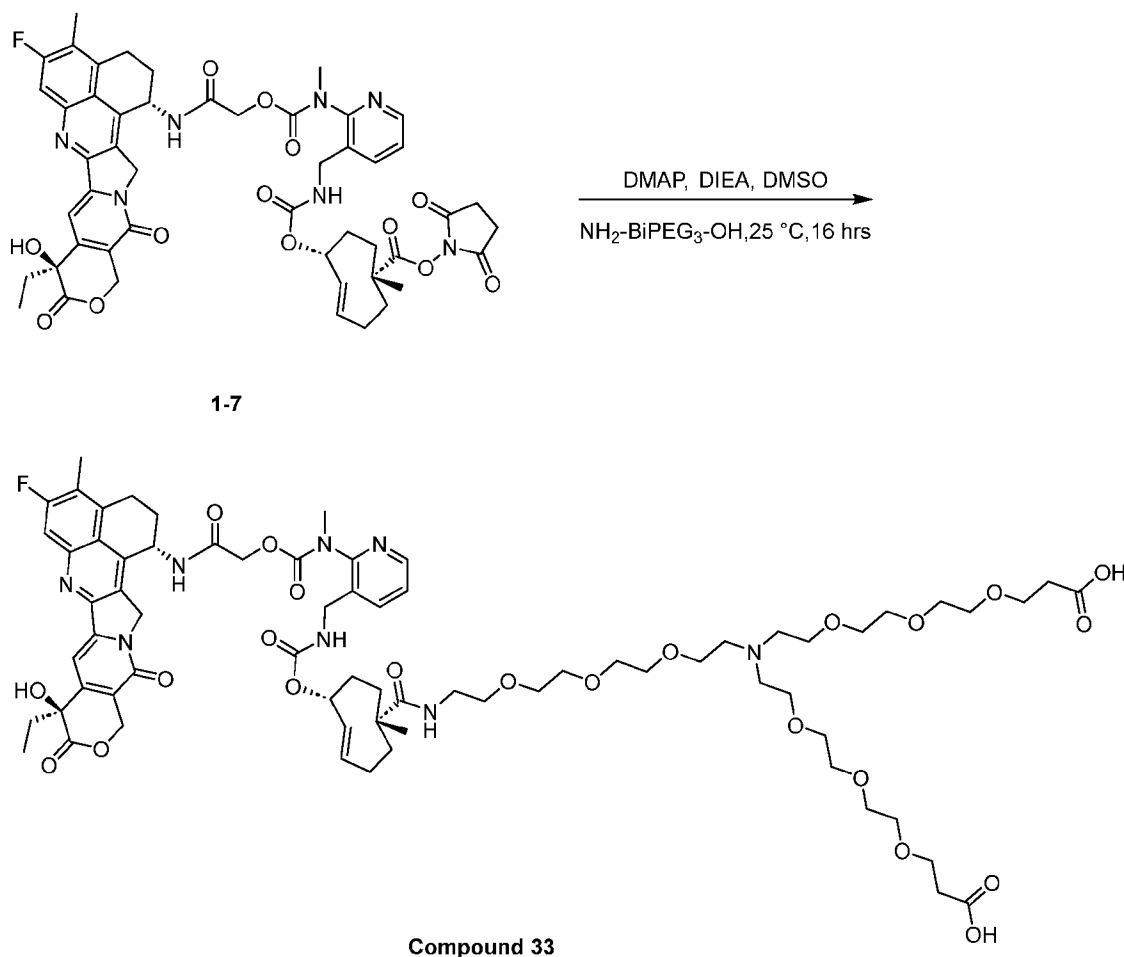


[0514] To a solution of compound **1-5** (1.50 g, 1.95 mmol, 1.00 *eq.*, TFA) in DMF (15.0 mL) was added N-methyl morpholine (394 mg, 3.89 mmol, 428 μL , 2.00 *eq.*) and compound **1-6** (411 mg, 973 μmol , 0.50 *eq.*). The mixture was stirred at 0-5 °C for 4 hrs. LCMS showed compound **1-5** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t=0.49$ min, MS cal.: 963.3, MS observed: $[M+Na]^+=964.5$). The reaction mixture was added to isopropyl ether (150 mL *2) and the crude product was precipitated, then centrifuged to afford the crude product as a residue. The liquid supernatant was discarded. The residue was purified by prep-HPLC (**TFA condition**). Compound **1-7** (330 mg, 338 μmol , 17.40% yield, 98.9% purity) was obtained as a yellow solid. LCMS ($R_t=0.47$ min) and HPLC ($R_t=2.88$ min) confirmed the desired product.

[0515] LCMS: $R_t=0.47$ min, MS cal.: 963.3, MS observed: $[M+H]^+=964.5$.

[0516] HPLC: $R_t=2.88$ min, purity: 98.9%.

General procedure for preparation of Compound 33

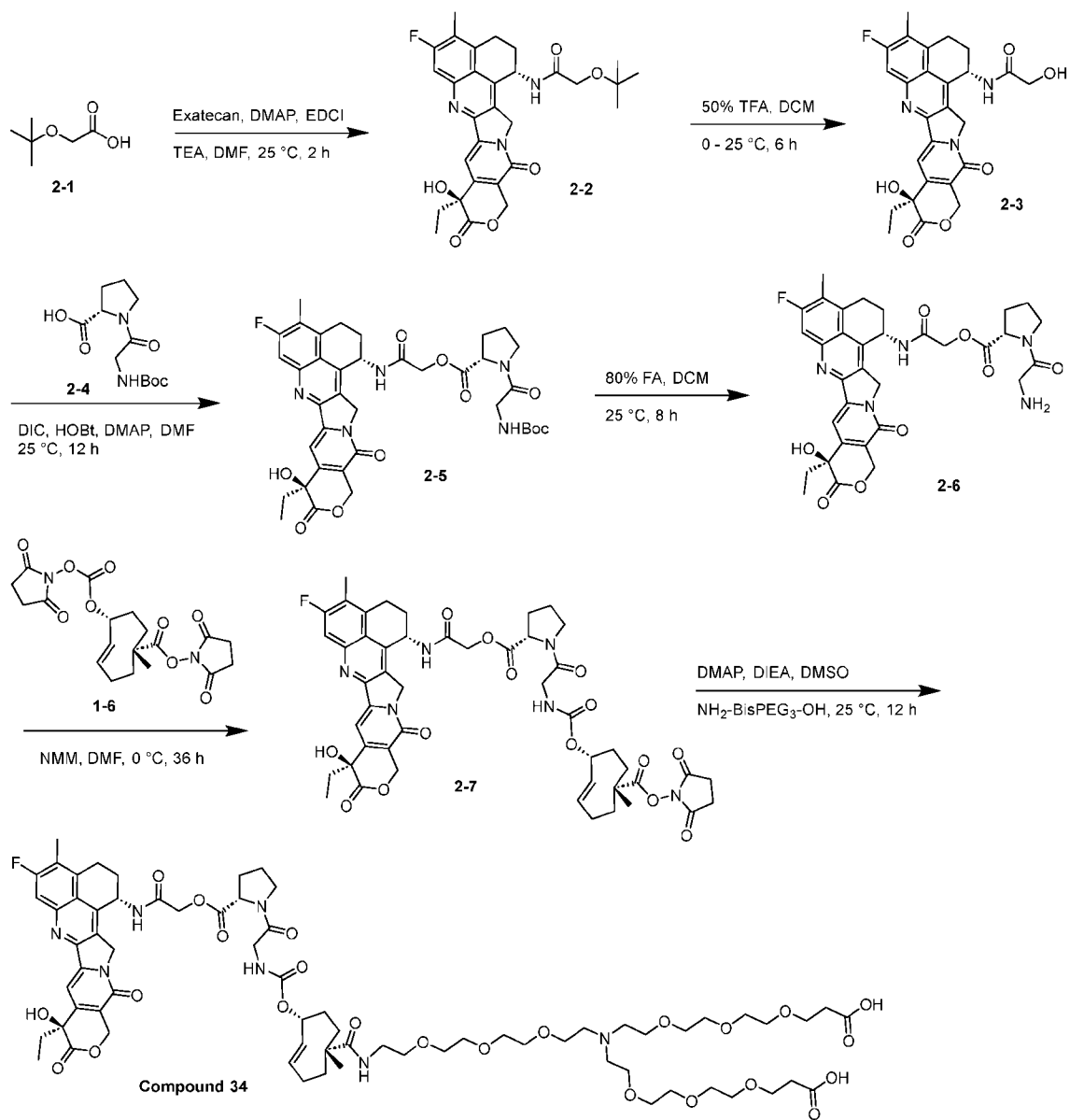


[0517] To a solution of compound **1-7** (350 mg, 363 μ mol, 1.00 *eq.*) and NH_2 -BiPEG3-OH in DMSO (3.50 mL) was added DIEA (187 mg, 1.45 mmol, 253 μ L, 4.00 *eq.*) and DMAP (4.44 mg, 36 μ mol, 0.10 *eq.*). The mixture was stirred at 25 °C for 16 hrs. LCMS showed compound **1-7** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.39$ min, MS cal.: 1448.6, MS observed: $[M+Na]^+=1450.0$). The reaction filter liquid is directly without work-up. The residue was purified by prep-HPLC (0.25% AcOH condition). **Target 245** (310 mg, 205.35 μ mol, 56.5% yield, 100% purity, AcOH salt.) was obtained as a white solid, confirmed by LCMS ($R_t=0.39$ min) and HPLC ($R_t=2.26$ min).

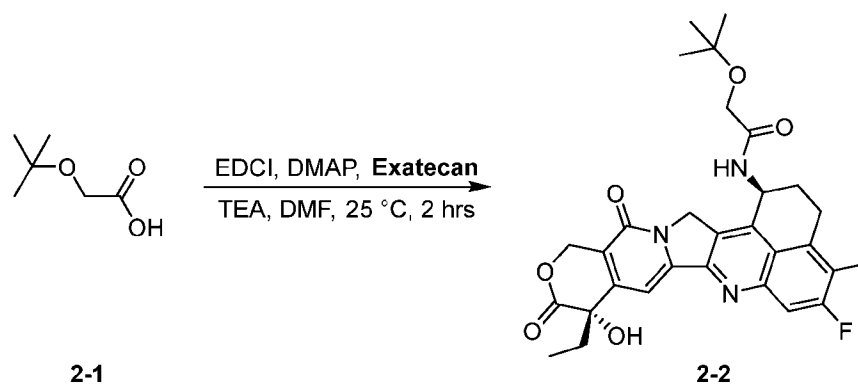
[0518] LCMS: $R_t=0.39$ min, MS cal.: 1448.6, MS observed: $[M+H]^+=1449.9$.

[0519] HPLC: $R_t=2.26$ min, purity: 100%.

Example 6: Synthesis of Compound 34



General procedure for preparation of compound 2-2

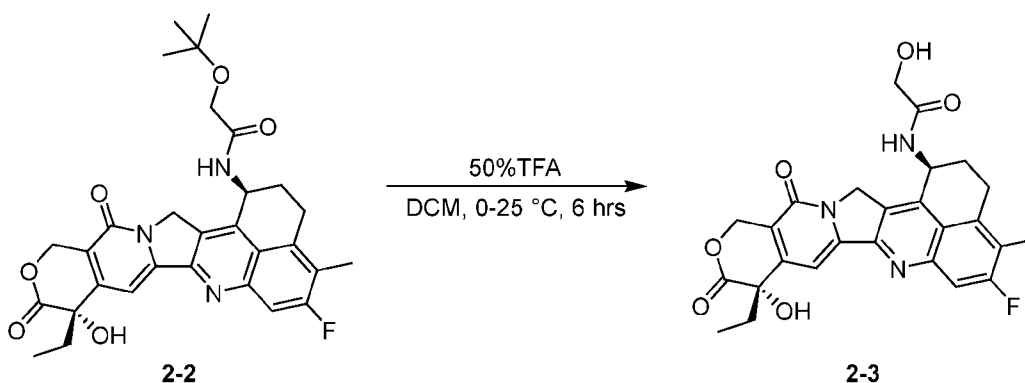


[0520] To a solution of Exatecan (5.00 g, 9.41 mmol, 1.00 eq.), compound 2-1 (1.62 g, 12.2 mmol, 1.30 eq.) in DMF (50.0 mL) was added DMAP (574 mg, 4.70 mmol, 0.50 eq.) and TEA (1.43 g, 14.1 mmol, 1.96 mL, 1.50 eq.), EDCI (3.61 g, 18.8 mmol, 2.00 eq.). The mixture was stirred at 25 °C for 2 hrs. LCMS showed **Exatecan** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.43$ min, MS cal.: 549.2, MS observed: $[M+H]^+ = 550.2$). The reaction mixture was addition H₂O (150 mL), and extracted with DCM (50.0 mL * 3). The combined organic layers were washed with brine (80.0 mL), dried over Na₂SO₄, filtered and concentrated to give a residue. The mixture reaction was poured into isopropyl ether (210 mL) and triturated with petroleum ether (210 mL) at 25 °C for 3 hrs. Compound **2** (5.10 g, 8.89 mmol, 94.5% yield, 95.8% purity) was obtained as a gray solid, confirmed by LCMS ($R_t = 0.44$ min), HPLC ($R_t = 2.79$ min).

[0521] LCMS: $R_t = 0.44$ min, MS cal.: 549.2, MS observed: $[M+H]^+ = 550.2$.

[0522] HPLC: $R_t = 2.79$ min, purity: 95.8%

General procedure for preparation of compound 2-3

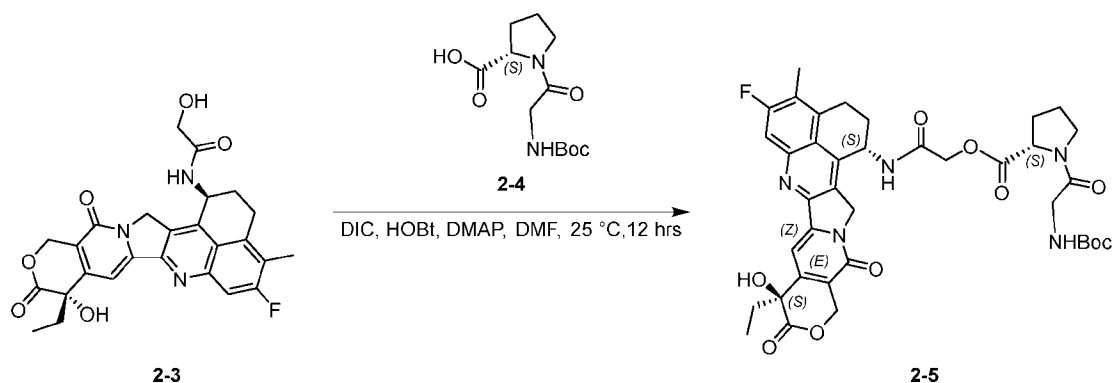


[0523] To a solution of compound **2-2** (5.00 g, 8.72 mmol, 1.00 eq.) in DCM (25 mL) was added TFA (38.3 g, 336 mmol, 25.0 mL, 38.6 eq.) at 0 °C and stirred for 1 hr. The mixture was stirred at 25 °C for 5 hrs. LCMS showed compound **2-2** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.38$ min, MS cal.: 493.1, MS observed: $[M+H]^+ = 494.2$). The mixture reaction was poured into isopropyl ether (500 mL) and triturated with petroleum ether (150 mL) at 25 °C for 2 hrs. Compound **3** (5.00 g, 8.07 mmol, 92.5% yield, 98.0% purity) was obtained as a yellow solid, confirmed by LCMS ($R_t = 0.35$ min), and HPLC ($R_t = 1.92$ min).

[0524] LCMS: $R_t = 0.35$ min, MS cal.: 549.2, MS observed: $[M+H]^+ = 494.1$.

[0525] HPLC: $R_t = 1.92$ min, purity: 98.0%

General procedure for preparation of compound 2-5

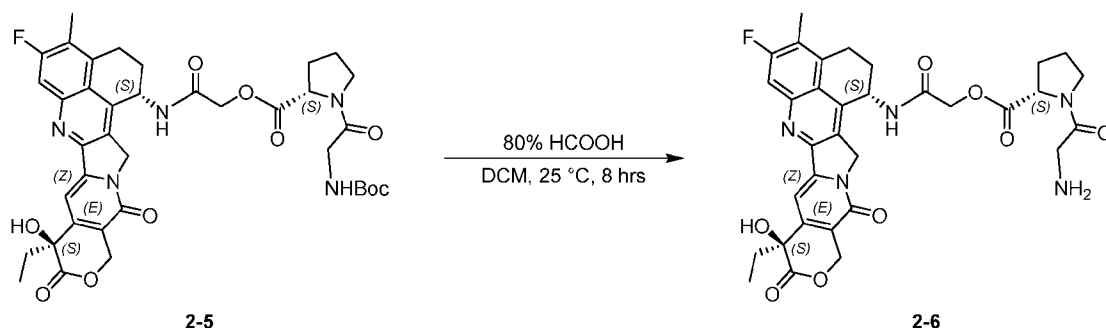


[0526] To a solution of compound **2-3** (500 mg, 806.5 μmol , 1.00 *eq.*, TFA) in DMF (5.00 mL) was added compound **4** (395 mg, 1.45 mmol, 1.80 *eq.*), HOBT (218 mg, 1.61 mmol, 2.00 *eq.*) and DMAP (98.5 mg, 806 μmol , 1.00 *eq.*) and DIC (203 mg, 1.61 mmol, 249 μL , 2.00 *eq.*). The mixture was stirred at 25 °C for 12 hrs. LCMS showed compound **2-3** was consumed completely. Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.43$ min, MS cal.: 747.2, MS observed: $[\text{M}+\text{H}]^+ = 748.3$). The mixture reaction was poured into isopropyl ether (100 mL * 2) and crystallized. The crude product compound **2-5** (2.00 g, crude) was obtained as a gray solid and used into the next step.

[0527] LCMS: $R_t = 0.43$ min, MS cal.: 747.2, MS observed: $[\text{M}+\text{H}]^+ = 748.3$

[0528] HPLC: $R_t = 2.73$ min, purity: 83.0%

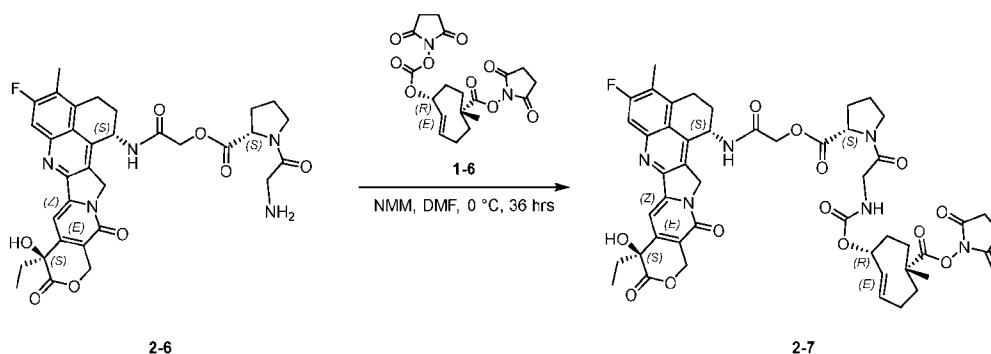
General procedure for preparation of compound 2-6



[0529] To a solution of compound **2-5** (2.00 g, 2.67 mmol, 1.00 *eq.*) in DCM (4.00 mL) was added formic acid (19.5 g, 424 mmol, 16.0 mL). The mixture was stirred at 25 °C for 8 hrs. LCMS showed compound **2-5** was remained ($R_t = 0.42$ min). Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.32$ min, MS cal.: 647.2, MS observed: $[\text{M}+\text{H}]^+ = 648.2$). The mixture reaction was poured into isopropyl ether (200 mL) and crystallized. The residue was purified by prep-HPLC (TFA condition). Compound **2-6** (556 mg, 849 μmol , 31.7% yield, 98.9% purity) was obtained as a yellow solid, confirmed by LCMS ($R_t = 0.32$ min) and HPLC ($R_t = 1.60$ min).

[0530] LCMS: $R_t = 0.32$ min, MS cal.: 647.2, MS observed: $[\text{M}+\text{H}]^+ = 648.2$.

[0531] HPLC: $R_t = 1.60$ min, purity: 98.9%

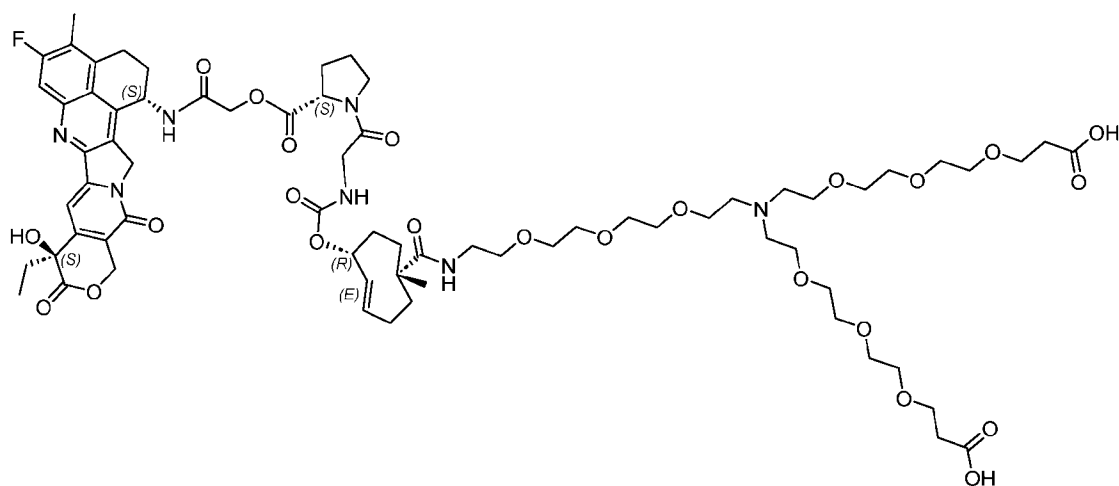
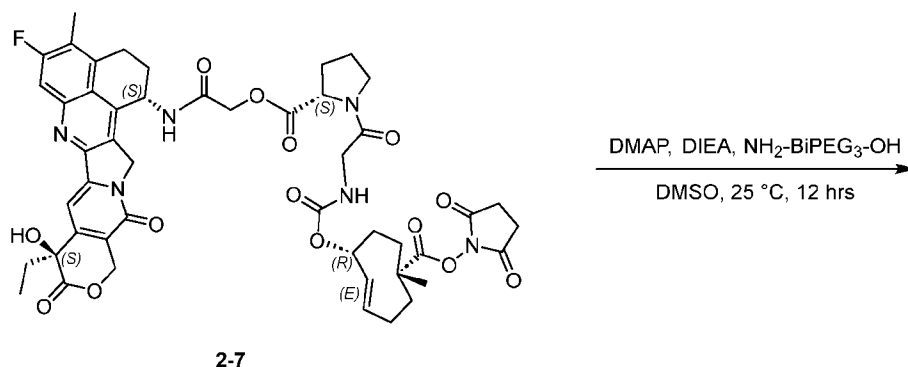
General procedure for preparation of compound 2-7

[0532] To a solution of compound **2-6** (556 mg, 849 μmol , 1.00 *eq.*) in DMF (6.00 mL) was added N-methyl morpholine (42.9 mg, 424 μmol , 46.6 μL , 0.50 *eq.*) and compound **7** (437 mg, 1.02 mmol, 1.20 *eq.*). The mixture was stirred at 0 °C for 36 hrs. LCMS showed compound **2-6** was remained (R_t = 0.33 min). Several new peaks were shown on LCMS and desired mass was detected (R_t = 0.45 min, MS cal.: 954.3, MS observed: $[\text{M}+\text{H}]^+ = 955.4$). The mixture reaction was poured into isopropyl ether (60.0 mL * 1) and crystallized. The residue was purified by prep-HPLC (TFA condition). Compound **2-7** (445 mg, 454.8 μmol , 53.5% yield, 97.6% purity) was obtained as a yellow solid, confirmed by LCMS (R_t = 0.47 min) and HPLC (R_t = 2.79 min).

[0533] LCMS: R_t = 0.47 min, MS cal.: 954.3, MS observed: $[\text{M}+\text{H}]^+ = 955.3$.

[0534] HPLC: R_t = 2.79 min, purity: 97.6%.

General procedure for preparation of Compound 34

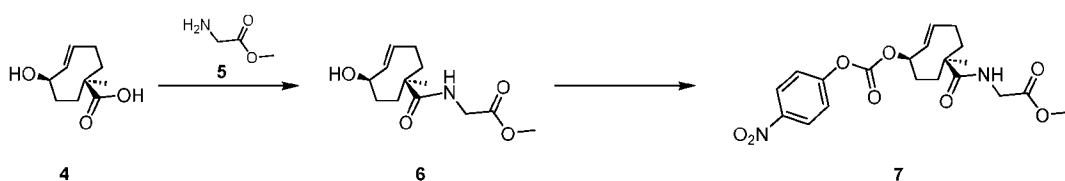
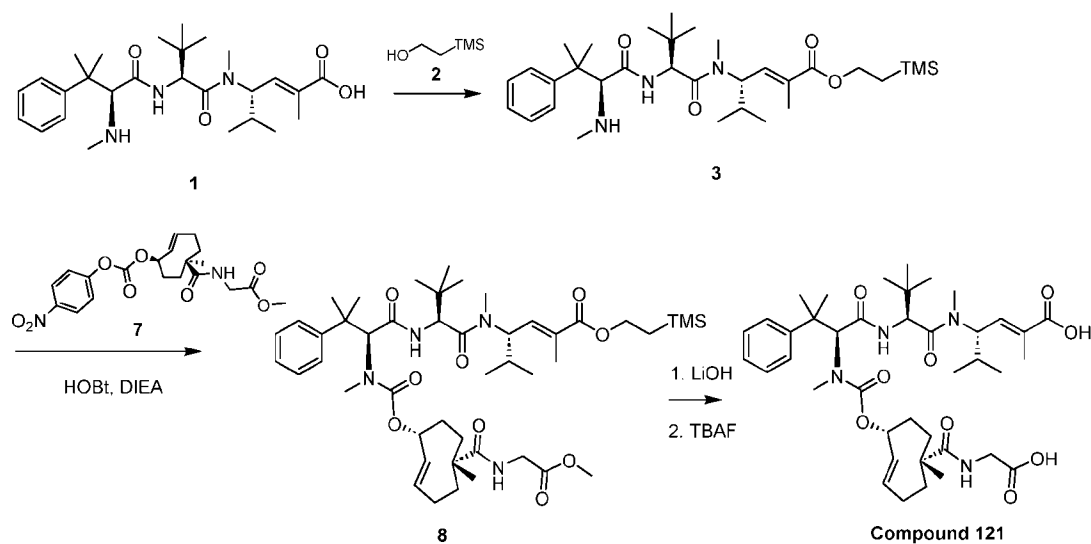


[0535] To a solution of $\text{NH}_2\text{-BiPEG}_3\text{-OH}$ (386 mg, 613 μmol , 2.00 *eq.*) in DMSO (3.00 mL) was added DIEA (79.2 mg, 613 μmol , 107 μL , 2.00 *eq.*) and DMAP (3.75 mg, 30.6 μmol , 0.10 *eq.*), compound **2-7** (300 mg, 306 μmol , 1.0 *eq.*). The mixture was stirred at 25 °C for 12 hrs. LCMS showed compound **8** was remained ($R_t = 0.46$ min). Several new peaks were shown on LCMS and desired mass was detected ($R_t = 0.39$ min, MS cal.: 1439.6, MS observed: $[\text{M}+\text{H}]^+ = 1440.7$). The residue was purified by prep-HPLC (0.25% AcOH condition). Compound **34** (151 mg, 104 μmol , 34.0% yield, 99.6% purity) was obtained as a white solid, confirmed by LCMS ($R_t = 0.40$ min) and HPLC ($R_t = 2.23$ min).

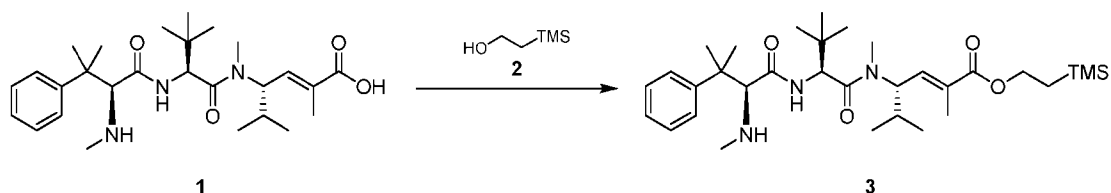
[0536] LCMS: $R_t = 0.40$ min, MS cal.: 1439.6, MS observed: $[\text{M}+\text{H}]^+ = 1440.6$.

[0537] HPLC: $R_t = 2.23$ min, purity: 99.6%.

Example 7: Synthesis of Compound 121



General procedure for preparation of compound 3

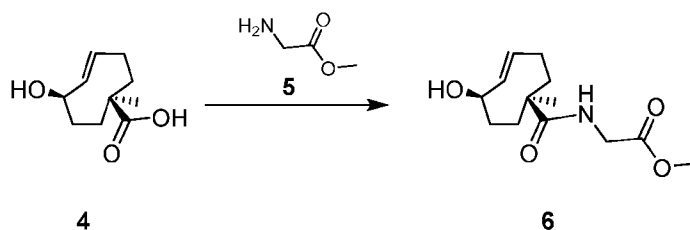


[0538] To a solution of compound **1** (500 mg, 1.06 mmol) in DCM (5.0 mL) was added compound **2** (374 mg, 3.17 mmol), DIEA (272 mg, 2.11 mmol, 367 μ L), EDCI (303 mg, 1.58 mmol) and DMAP (193 mg, 1.58 mmol). The mixture was stirred at 20-25°C for 2 hrs. LCMS showed compound **1** was consumed completely and one main peak with desired m/z was detected. The reaction mixture was partitioned between DCM (10.0 mL) and HCl (1M) (10.0 mL). The organic phase was separated, washed with NaHCO_3 (10.0 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give compound **3** (550 mg, 90.2% yield) as a white solid.

[0539] LCMS: R_t = 0.474 min, MS cal.: 573.9, MS observed: $[M + H]^+ = 574.4$.

[0540] HPLC: R_t = 3.389 min, purity: 99.4%.

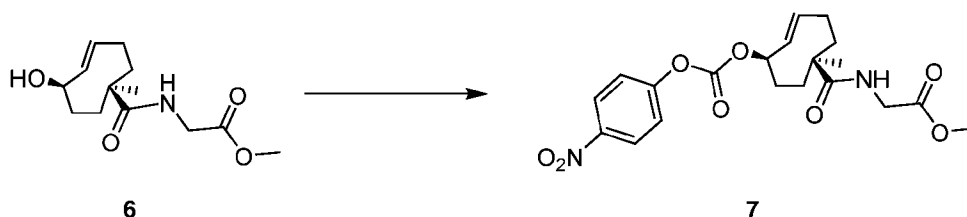
General procedure for preparation of compound 6



[0541] To a solution of compound **4** (2.00 g, 10.8 mmol) in DCM (20.0 mL) was added compound **5** (2.73 g, 21.7 mmol, HCl), DIEA (5.61 g, 43.4 mmol, 7.56 mL), HOBT (3.67 g, 27.1 mmol) and EDCI (5.20 g, 27.1 mmol). The mixture was stirred at 20-25 °C for 2 hrs. TLC (Petroleum ether/EtOAc = 1:1, product R_f = 0.32) indicated compound **4** was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was partitioned between HCl (1 M) (50.0 mL) and DCM (50.0 mL). The organic phase was separated, washed with NaHCO_3 (50.0 mL) and brine (50.0 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give compound **6** (2.00 g, 71.1% yield, crude) as a white solid.

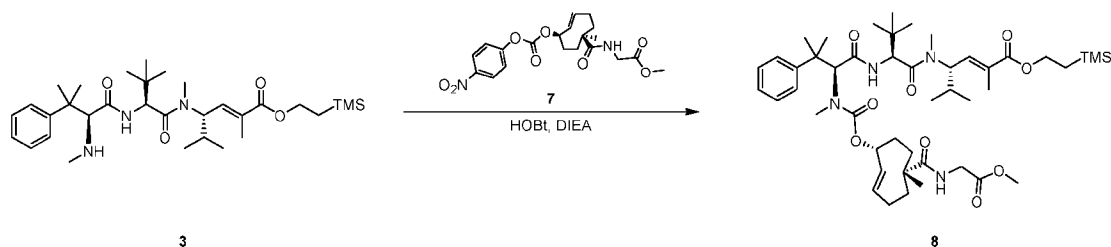
[0542] ^1H NMR (400 MHz, CDCl_3) δ : 5.98 - 6.15 (m, 2 H), 5.65 (br d, J = 16.5 Hz, 1 H), 4.48 (br s, 1 H), 3.98 (d, J = 5.1 Hz, 2 H), 3.75 (s, 3 H), 2.23 - 2.38 (m, 2 H), 1.82 - 2.15 (m, 6 H), 1.59 (br dd, J = 15.5, 6.1 Hz, 1 H), 1.13 (s, 3 H).

General procedure for preparation of compound 7



[0543] To a solution of compound **6** (2.00 g, 7.83 mmol) in DMF (20.0 mL) was added bis(4-nitrophenyl) carbonate (4.77 g, 15.6 mmol) and DIEA (3.04 g, 23.5 mmol, 4.09 mL). The mixture was stirred at 20-25 °C for 16 hrs. TLC (Petroleum ether/EtOAc = 1:1, product R_f = 0.63) indicated compound **6** was consumed completely and one new spot formed. The reaction mixture was partitioned between HCl (1M) (50.0 mL) and EtOAc (50.0 mL). The organic phase was separated, washed with brine (50.0 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/ EtOAc = 10/1 to 1/1) to give compound **7** (3.00 g, 90.0% yield) as a yellow oil. LCMS: R_t = 0.422 min, MS cal.: 420.4, MS observed: $[\text{M} + \text{H}]^+$ = 443.0. HPLC: R_t = 2.826 min, purity: 98.9%. ^1H NMR (400 MHz, CDCl_3) δ : 8.29 (br d, J = 9.1 Hz, 2 H), 7.41 (br d, J = 9.1 Hz, 2 H), 5.99 - 6.15 (m, 2 H), 5.66 (dd, J = 16.6, 1.8 Hz, 1 H), 4.00 (dd, J = 4.9, 1.6 Hz, 2 H), 3.77 (s, 3 H), 2.28 - 2.43 (m, 2 H), 2.16 - 2.25 (m, 2 H), 1.89 - 2.04 (m, 3 H), 1.76 (br dd, J = 14.9, 6.3 Hz, 1 H), 1.19 (s, 3 H).

General procedure for preparation of compound 8

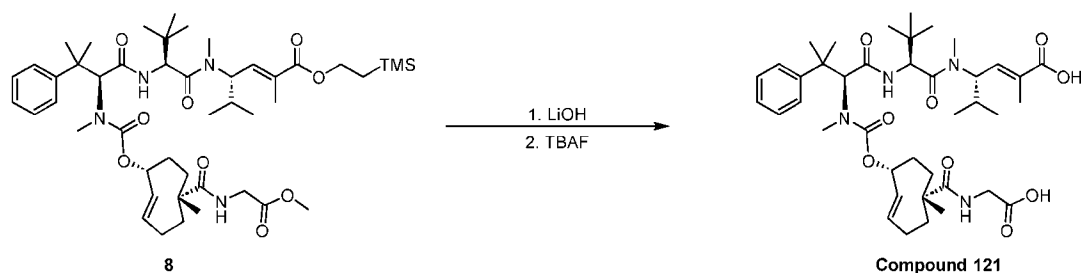


[0544] To a solution of compound **3** (280 mg, 407 μ mol) in DMF (3.00 mL) was added DIEA (210 mg, 1.63 mmol, 283 μ L), compound **7** (342 mg, 814 μ mol) and HOAt (110 mg, 814 μ mol). The mixture was stirred at 40 °C for 16 hrs. LCMS showed 28.7% of compound **3** remained. Several new peaks were shown on LC-MS and 22.7% of desired compound was detected. Three parallel reactions were combined and work-up. The mixture was purified by prep-HPLC (0.1% HCOOH condition) to give compound **8** (450 mg, 41.5% yield) as a white solid.

[0545] LCMS: R_t = 0.626 min, MS cal.: 855.2, MS observed: $[M + H]^+ = 855.6$.

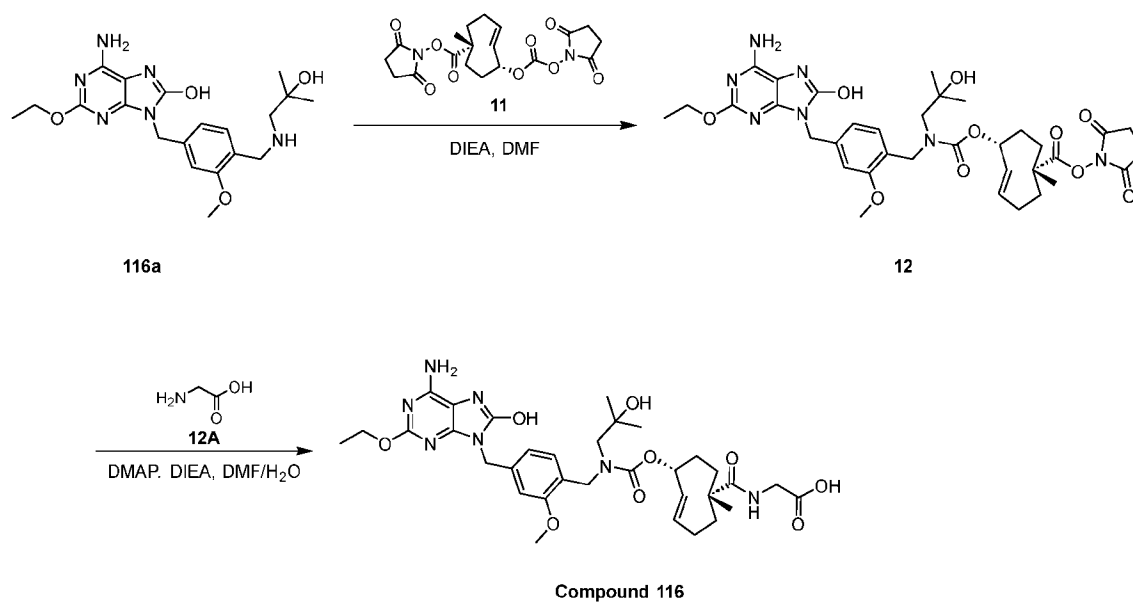
[0546] HPLC: R_t = 5.081 min, purity: 96.5%.

General procedure for preparation of Compound 121

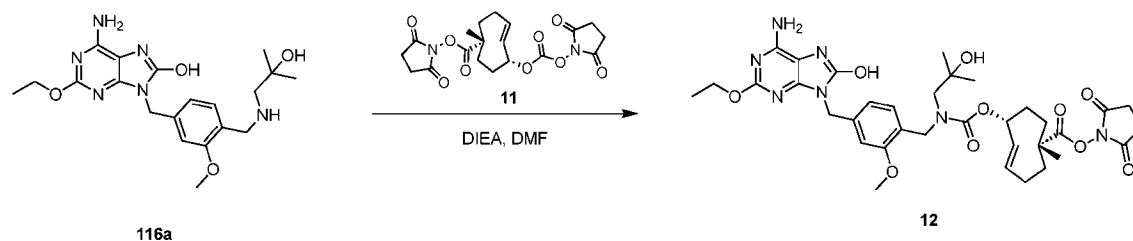


[0547] To a solution of compound **8** (450 mg, 526 μ mol) in THF (3.0 mL) was added LiOH·H₂O (110 mg, 2.63 mmol) in H₂O (3.0 mL). The mixture was stirred at 20-25 °C for 2 hrs. The mixture was added TBAF (1 M, 2.10 mL) and stirred at 16 hrs. LCMS showed compound **8** was consumed completely and one main peak with desired m/z. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (0.1% AcOH condition) to give **Compound 121** (200 mg, 44.9% yield) as a colorless oil. LCMS: R_t = 0.487 min, MS cal.: 740.9, MS observed: $[M + H]^+ = 741.4$. HPLC: R_t = 3.282 min, purity: 99.5%.

Example 8: Synthesis of Compound 116



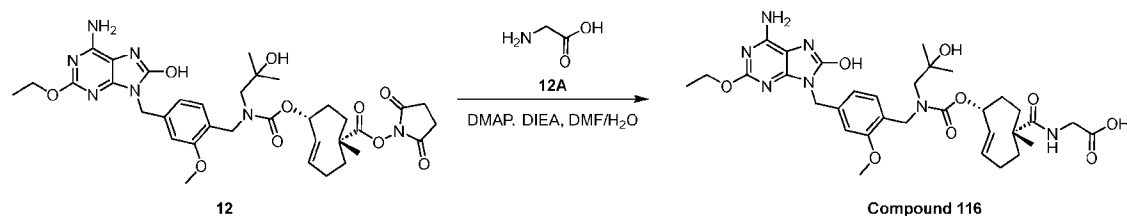
General procedure for preparation of compound 12



[0548] To a solution of **116a** (80 mg, 191 μ mol, 1.0 eq.) in DMF (1.0 mL) was added compound **11** (118 mg, 248 μ mol, 1.3 eq.) and DIEA (55.8 mg, 382 μ mol, 2.0 eq.). The mixture was stirred at 25 °C for 6.0 hrs. LCMS showed **116a** was consumed completely and one main peak (R_t = 0.41 min) with desired mass (MS cal.: 723.3, MS observed: $[M+H]^+$ = 724.4) was detected. The reaction mixture was triturated with isopropyl ether (10 mL) to give compound **12** (117 mg, crude) as a white solid, confirmed by LCMS.

[0549] LCMS: R_t = 0.41 min, MS cal.: 723.3, MS observed: $[M+H]^+$ = 724.4.

General procedure for preparation of Compound 116

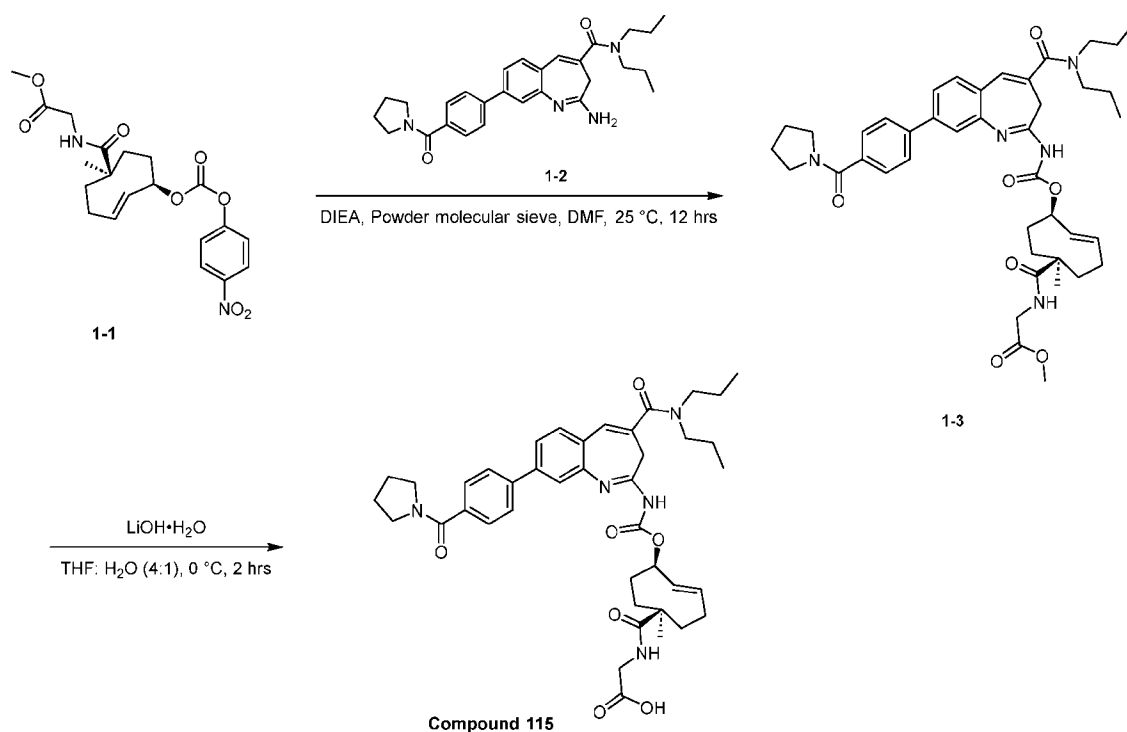


[0550] To a solution of compound **12** (117 mg, 207 μ mol, 1.0 eq.) in DMF (1.0 mL) and H₂O (0.2 mL) was added **12A** (77.8 mg, 1.04 mmol, 5.0 eq.), DMAP (75.9 mg, 622 μ mol, 3.0 eq.) and DIEA (1334 mg, 1.04 mmol, 5.0 eq.). The mixture was stirred at 25 °C for 2.0 hrs. LCMS showed compound **12** was consumed completely and one main peak (R_t = 0.37 min) with desired mass (MS cal.: 683.3, MS observed: $[M+H]^+$ = 684.4) was detected. The reaction mixture was filtered and purified by prep-HPLC (TFA condition), then exchanged to AcOH salt with ion exchange resin and to give **Compound 116** (42 mg, 58.9 μ mol, 28.4% yield, 95.9% purity) as a white solid, confirmed by LCMS and HPLC.

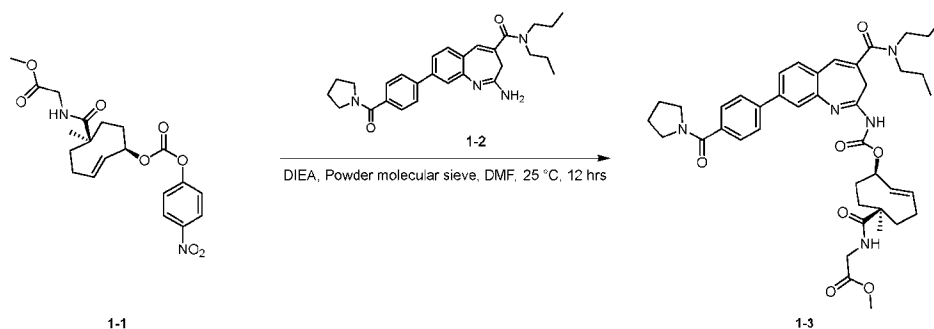
[0551] LCMS: R_t = 0.37 min, MS cal.: MS cal.: 683.3, MS observed: $[M+H]^+$ = 684.4.

[0552] HPLC: R_t = 1.99 min, purity: 95.9%.

Example 9: Synthesis of Compound 115



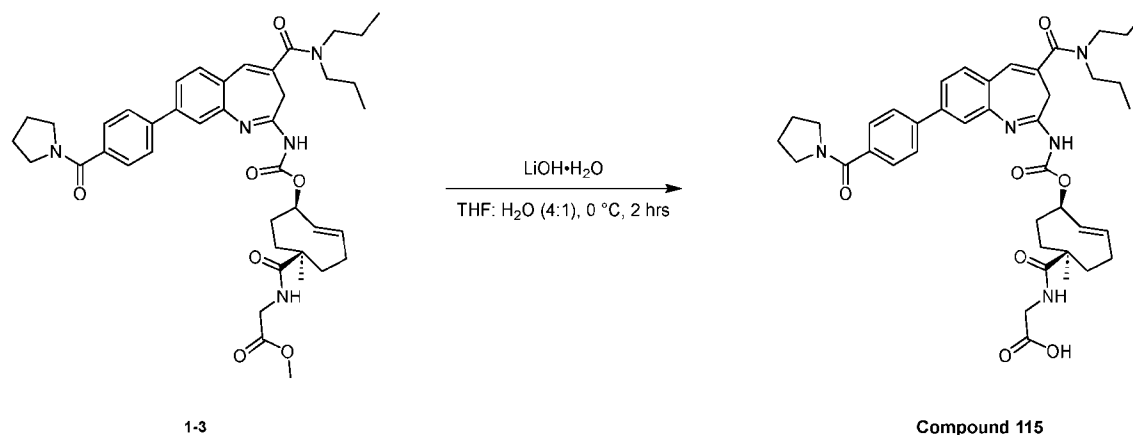
General procedure for preparation of compound 1-3



[0553] To a solution of compound **1-2** (116 mg, 235 μ mol, 0.50 eq., HCl salt) in DMF (3.00 mL) was added DIEA (121 mg, 940 μ mol, 163 μ L, 2.0 eq.), powder molecular sieve and compound **1-1** (200 mg, 470 μ mol, 1.0 eq.). The mixture was stirred at 25 °C for 12 hrs. LCMS showed the compound **1-2** was consumed (R_t = 0.46 min) and desired mass was detected (R_t = 0.47 min). The reaction mixture was filtered to remove the powder molecular sieve. The filtrate was triturated with isopropyl ether (60 mL) to give compound **1-3** (400 mg, crude) as yellow oil.

[0554] LCMS: R_t = 0.473 min, MS cal.: 739.3, MS observed: $[M+H]^+$ = 740.4.

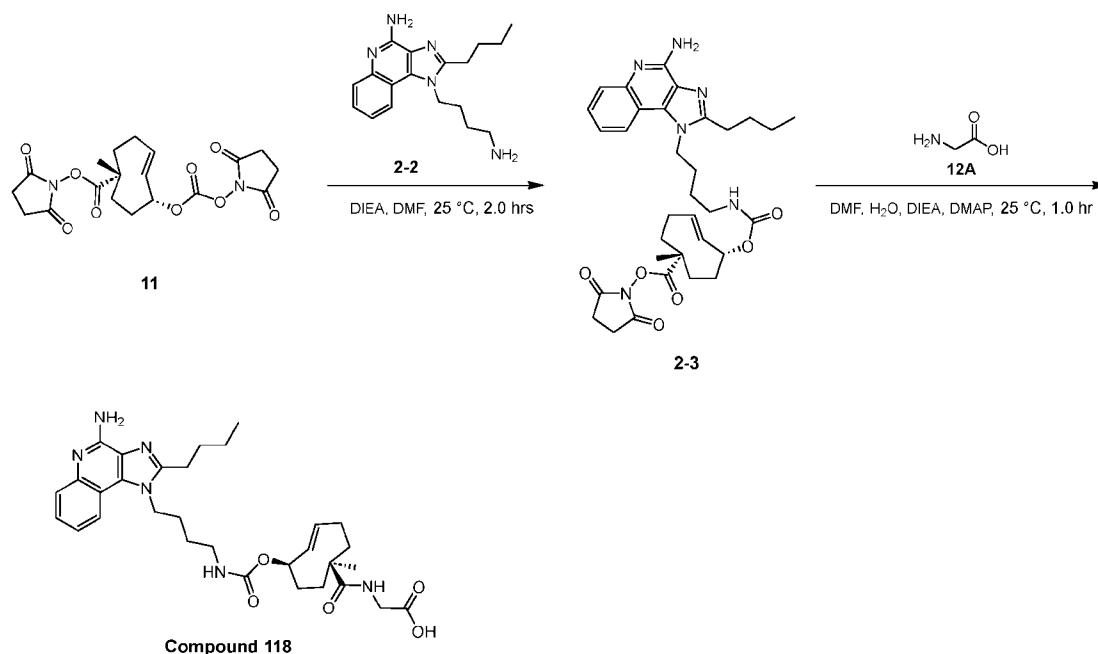
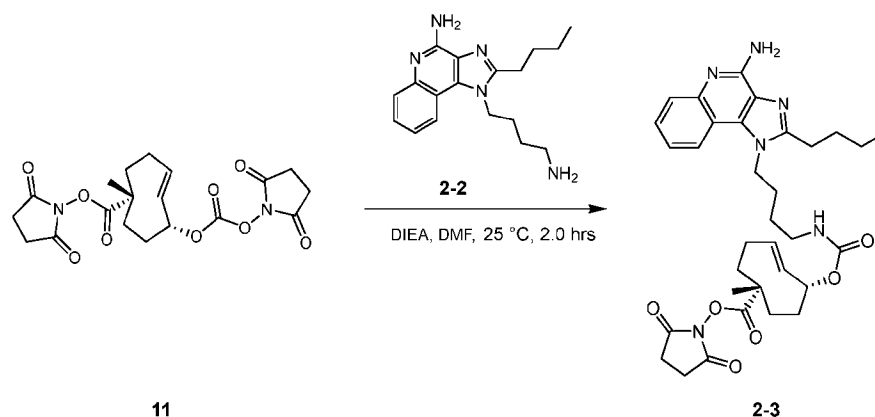
General procedure for preparation of Compound 115



[0555] To a solution of compound **1-3** (400 mg, 540 μ mol, 1.0 eq.) in THF (4.0 mL) was added LiOH·H₂O (45.3 mg, 1.08 mmol, 2.0 eq.) in H₂O (1.0 mL) at 0 °C. The mixture was stirred at 0 °C for 2.0 hrs. LCMS showed compound **1-3** was consumed completely and desired mass was detected (R_t = 0.45 min). The reaction mixture was adjusted pH to 6 with AcOH, then purified by prep-HPLC (AcOH condition) to give **Compound 115** (40.0 mg, 48.5 μ mol, 9.43% yield, 95.3% purity, AcOH salt) as a light yellow solid, confirmed by LCMS (R_t = 0.446 min) and HPLC (R_t = 3.21 min).

[0556] LCMS: R_t = 0.45 min, MS cal.: 725.3, MS observed: $[M+H]^+$ = 726.4.

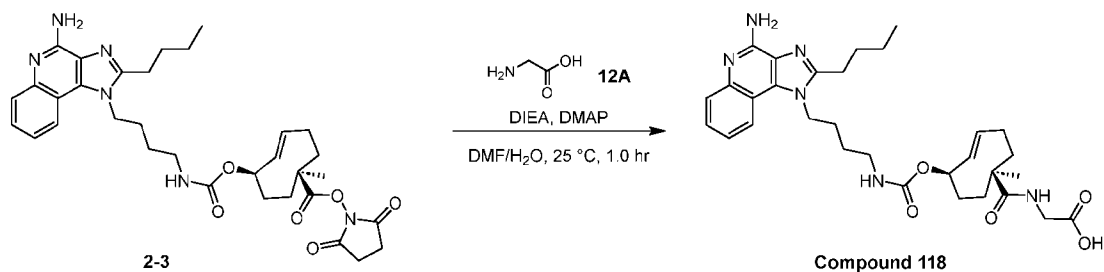
[0557] HPLC: R_t = 3.21 min, purity: 95.3%.

Example 10: Synthesis of Compound 118**General procedure for preparation of compound 2-3**

[0558] To a solution of compound **11** (209 mg, 488 μmol , 1.0 eq.) and compound **2-2** (170 mg, 488 μmol , 1.0 eq., HCl) in DMF (2.00 mL) was added DIEA (126 mg, 977 μmol , 170 μL , 2.0 eq.). The mixture was stirred at 25 $^\circ\text{C}$ for 2.0 hrs. LCMS showed compound **2-2** was consumed, and the desired mass was detected (R_t = 0.39 min). The reaction mixture was triturated with isopropyl ether (20.0 mL) and concentrated under reduce pressure to give compound **2-3** as yellow oil, confirmed by LCMS (R_t = 0.39 min).

[0559] LCMS: R_t = 0.391 min, MS cal.: 618.3 MS observed: $[\text{M}+\text{H}]^+ = 619.4$.

General procedure for preparation of Compound 118



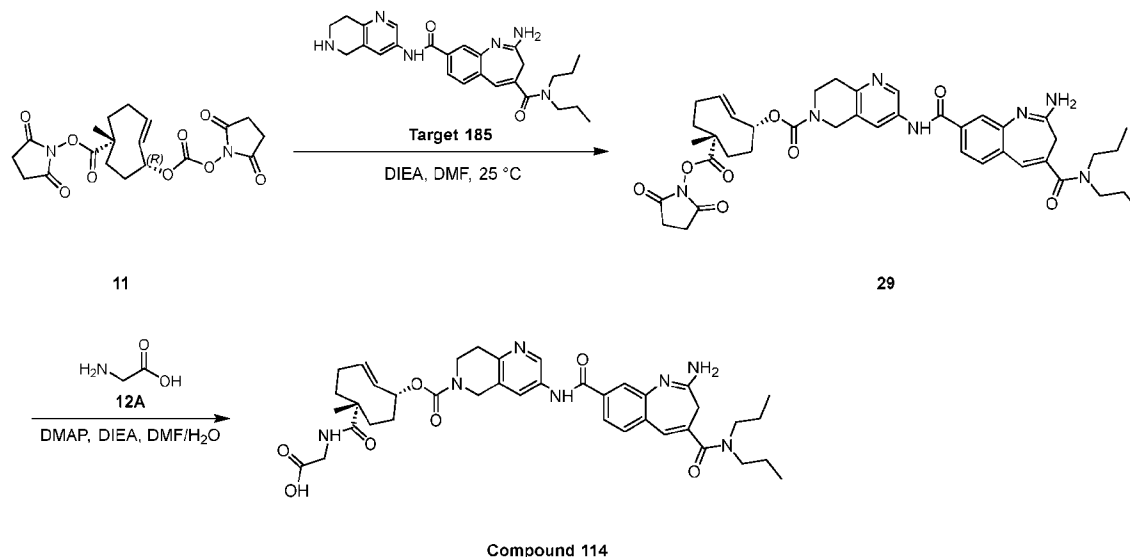
[0560] To a solution of compound **2-3** (300 mg, 480 μmol , 1.0 eq.) in DMF (2.40 mL) and H₂O (0.60 mL) was added compound **12A** (216 mg, 2.89 mmol, 6.0 eq.), DIEA (62.1 mg, 480 μmol , 83 μL , 1.0 eq.) and DMAP (58.7 mg, 480 μmol , 1.0 eq.). The mixture was stirred at 25 °C for 1.0 hr. LCMS showed compound **2-3** was remained (R_t = 0.41 min), desired mass was detected (R_t = 0.37 min). The reaction solution was purified by reversed-phase HPLC (AcOH condition) to give **Target 187** (120 mg, 181 μmol , 37.6% yield, 96.5% purity, AcOH salt) as a white solid, confirmed by LCMS, HPLC and ¹H NMR.

[0561] LCMS: R_t = 0.368 min, MS cal.: 578.3 MS observed: $[\text{M}+\text{H}]^+ = 579.4$.

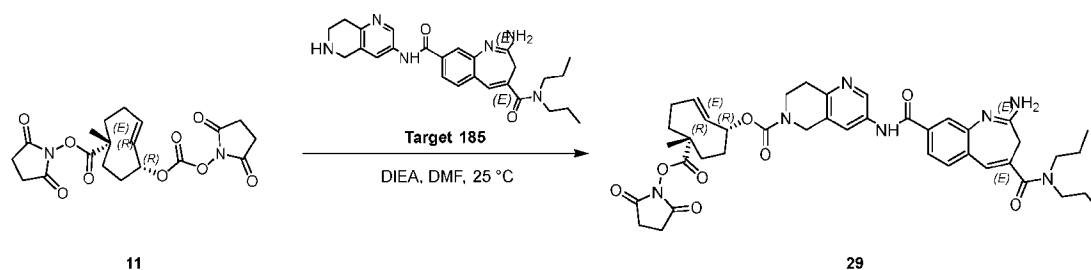
[0562] HPLC: R_t = 1.82 min, purity: 96.5%.

[0563] ¹H NMR (400 MHz, DMSO-*d*₆): δ : 7.99 (d, J = 8.38 Hz, 1 H), 7.60 (d, J = 8.25 Hz, 1 H), 7.37 - 7.50 (m, 2 H), 7.11 - 7.27 (m, 2 H), 6.48 - 6.59 (m, 2 H), 5.56 - 5.79 (m, 2 H), 4.94 - 5.07 (m, 1 H), 4.44 - 4.55 (m, 2 H), 3.57 (d, J = 5.00 Hz, 2 H), 3.00 - 3.07 (m, 2 H), 2.88 - 2.94 (m, 2 H), 2.09 - 2.18 (m, 2 H), 1.33 - 1.86 (m, 14 H), 0.90 - 1.01 (m, 6 H).

Example 11: Synthesis of Compound 114



General procedure for preparation of compound 29

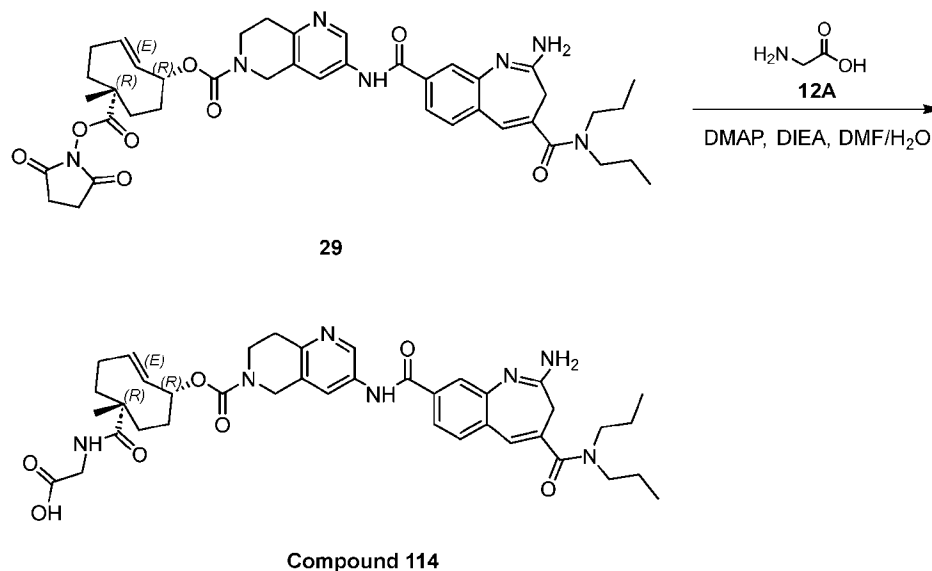


[0564] To a solution of compound **11** (100 mg, 236 μmol , 1.1 eq.) and **Target 185** (123 mg, 214 μmol , 1.0 eq., TFA salt) in DMF (2 mL) was added DIEA (55.3 mg, 428 μmol , 74.5 μL , 2.0 eq.). The mixture was stirred at 25 °C for 1.0 hr. LC-MS showed **Target 185** was consumed completely and one main peak with desired mass ($R_t = 0.37$ min) was detected. The mixture was used into the next step without work-up and purification. Compound **29** was confirmed by LCMS ($R_t = 0.37$ min) and HPLC ($R_t = 1.97$ min).

[0565] LCMS: $R_t = 0.37$ min, MS cal.: 767.3, MS observed: $[\text{M}+\text{H}]^+ = 768.5$.

[0566] HPLC: $R_t = 1.97$ min, purity: 89.8%.

General procedure for preparation of Compound 114

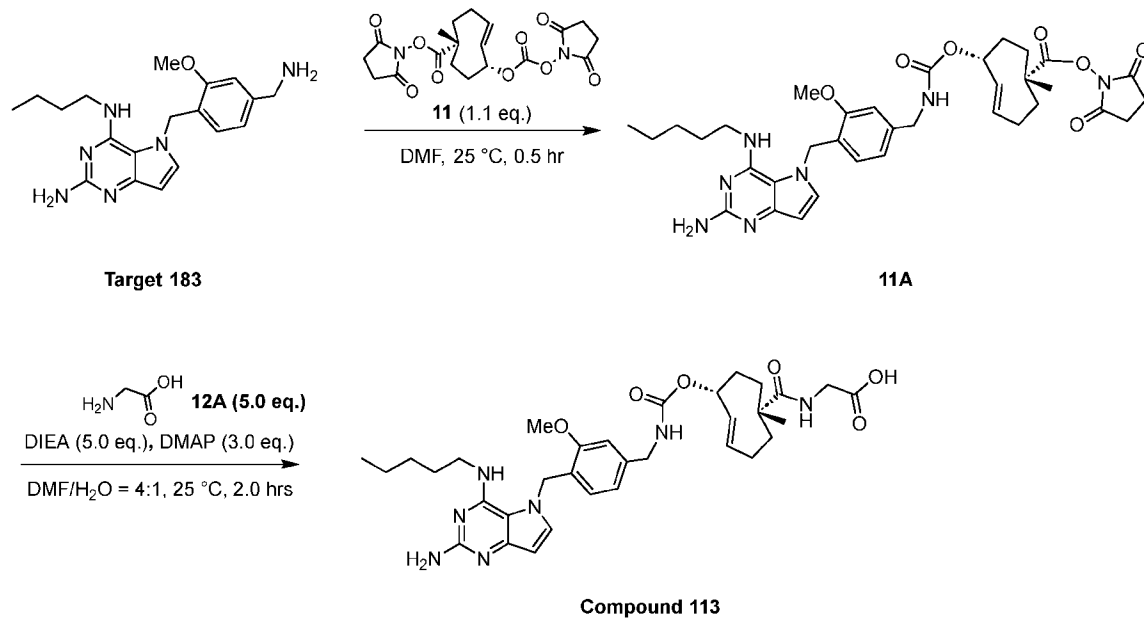


[0567] To the above solution of compound **29** (164 mg (theory amount), 213 μmol , 1.0 eq.) in DMF (2.0 mL) was added H_2O (0.5 mL), compound **12A** (79.7 mg, 1.06 mmol, 5.0 eq.), DMAP (78.1 mg, 639 μmol , 3.0 eq.) and DIEA (82.6 mg, 639 μmol , 111 μL , 3.0 eq.). The mixture was stirred at 25 °C for 1.0 hr. LC-MS showed compound **29** was consumed completely and one main peak with desired mass ($R_t = 0.34$ min) was detected. The mixture was purified by prep-HPLC (AcOH condition) to give **Compound 114** (67 mg, 92.0 μmol , 43.2% yield, 96.6% purity, AcOH salt) as a white solid, confirmed by LCMS ($R_t = 0.34$ min) and HPLC ($R_t = 2.65$ min).

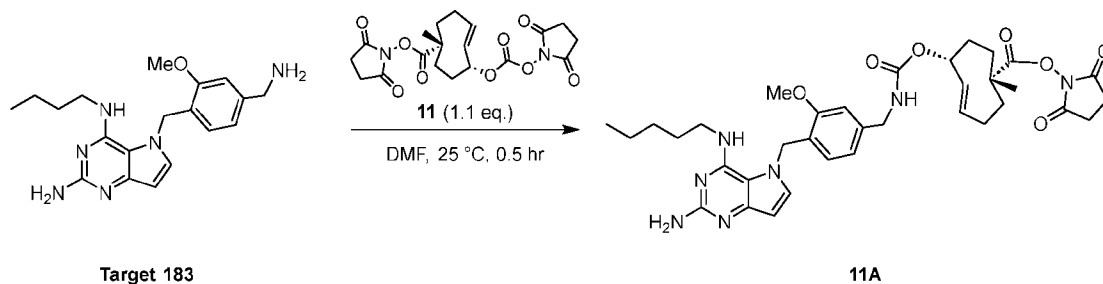
[0568] LCMS: $R_t = 0.34$ min, MS cal.: 727.37, MS observed: $[\text{M}+\text{H}]^+ = 728.5$.

[0569] HPLC: $R_t = 2.65$ min, purity: 96.6%.

Example 12: Synthesis of Compound 113



General procedure for preparation of compound 11A

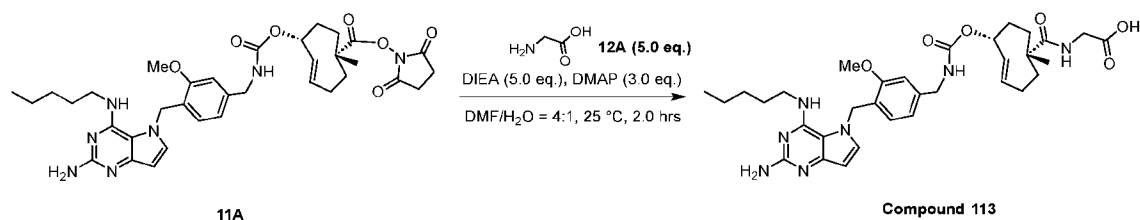


[0570] To a solution of **Target 183** (120 mg, 256 μmol , 1.0 eq., AcOH salt) and **11** (119 mg, 282 μmol , 1.1 eq.) in DMF (1.0 mL) was added DMAP (31.3 mg, 256 μmol , 1.0 eq.) and DIEA (66.2 mg, 512 μmol , 89.2 μL , 2.0 eq.), the mixture was stirred at 25 $^\circ\text{C}$ for 1.0 hr. LC-MS showed **Target 183** was consumed completely, several new peaks were shown on LC-MS and ~97.3% of desired compound was detected. The resulting reaction mixture was triturated with isopropyl ether (10 mL *2) for two times, the precipitated solid was filtered and dried *in vacuum* to give compound **11A** (115 mg, crude) as a brown solid, confirmed by LCMS ($R_t = 0.41$ min) and HPLC ($R_t = 3.09$ min).

[0571] LCMS: $R_t = 0.41$ min, MS cal.: 675.3, MS observed: $[\text{M}+\text{H}]^+ = 676.4$.

[0572] HPLC: $R_t = 3.09$ min, purity: 94.7%.

General procedure for preparation of Compound 113

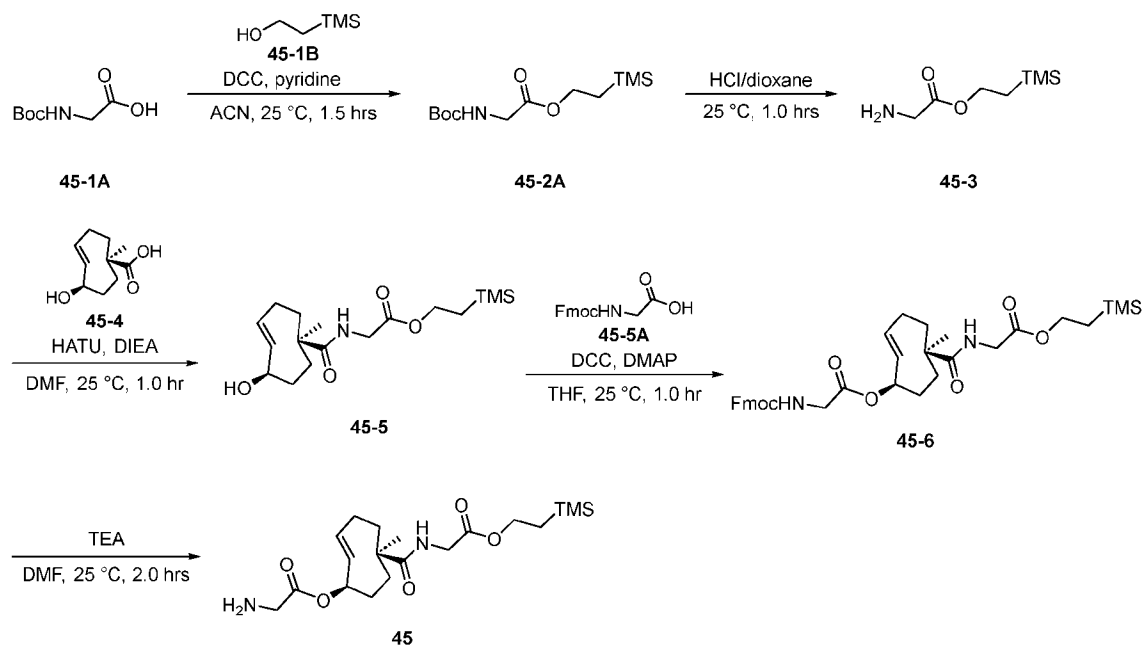


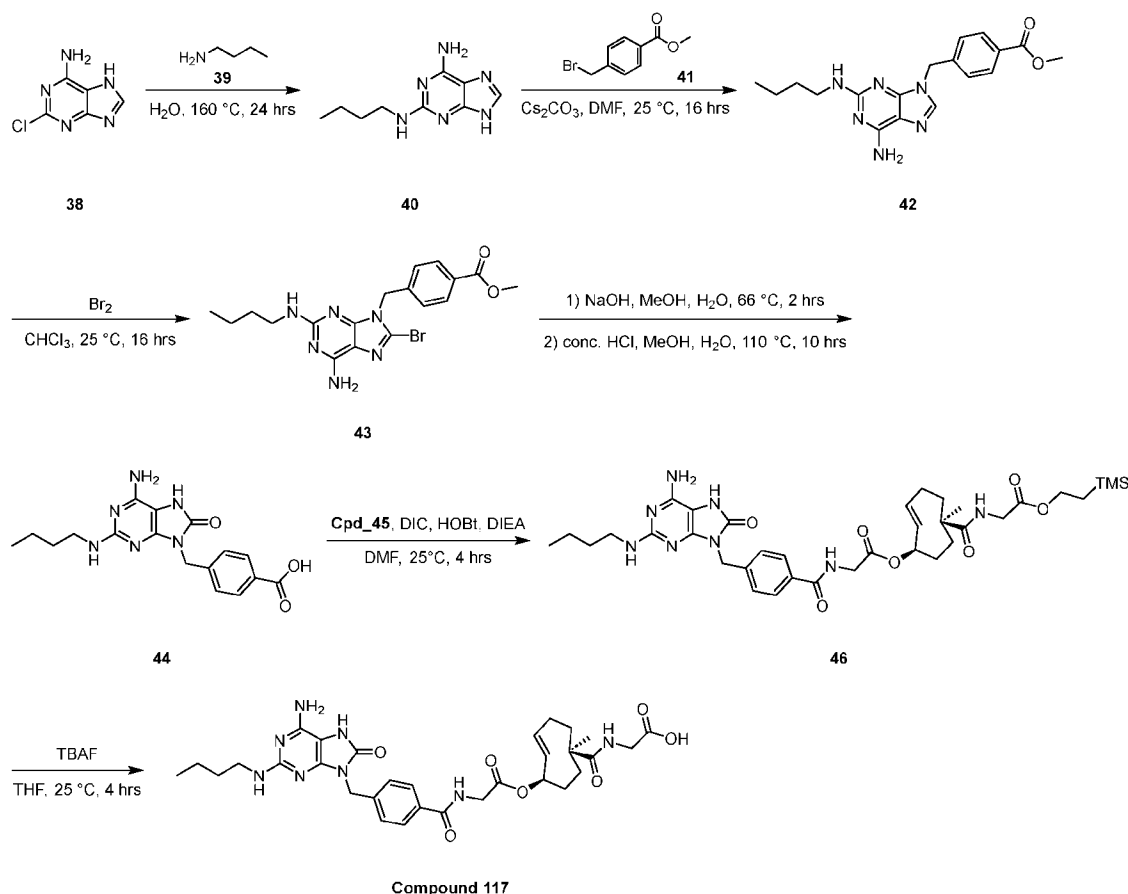
[0573] To a solution of compound **11A** (115 mg, 170 μ mol, 1.0 eq.) and compound **12A** (63.9 mg, 851 μ mol, 5.0 eq.) in DMF (1.0 mL) and H₂O (250 μ L) was added DIEA (66.0 mg, 511 μ mol, 88.9 μ L, 3.0 eq.) and DMAP (62.4 mg, 510 μ mol, 3.0 eq.), the mixture was stirred at 25 °C for 1.0 hr. LC-MS showed ~0% of compound **11A** remained. Several new peaks were shown on LC-MS and ~96.4% of desired compound was detected. The residue was purified by prep-HPLC (AcOH condition) to give **Compound 113** (45 mg, 64.7 μ mol, 38.0% yield, AcOH salt) as a white solid, confirmed by LCMS (R_t = 0.38 min) and HPLC (R_t = 2.19 min).

[0574] LCMS: R_t = 0.38 min, MS cal.: 635.3, MS observed: $[M+H]^+$ = 636.2.

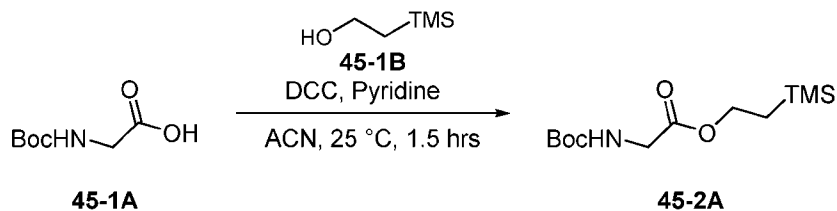
[0575] HPLC: R_t = 2.19 min, purity: 96.3%.

Example 13: Synthesis of Compound 117





General procedure for preparation of compound 45-2A

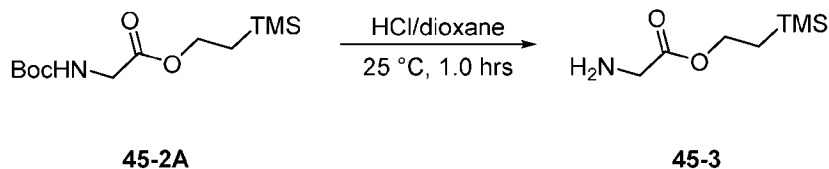


[0576] To a solution of compound **45-1A** (20.0 g, 114 mmol, 1.0 eq.) in ACN (120 mL) was added DCC (25.9 g, 125 mmol, 25.4 mL, 1.1 eq.), pyridine (27.1 g, 342 mmol, 27.6 mL, 3.0 eq.), and compound **45-1B** (14.8 g, 125 mmol, 18.0 mL, 1.1 eq.). The mixture was stirred at 25°C for 3.0 hrs. TLC (Petroleum ether/Ethyl acetate = 3:1) indicated compound **45-1A** ($R_f = 0.20$) was consumed completely and one new spot ($R_f = 0.57$) was formed. The reaction mixture was diluted with H_2O (400 mL) and extracted with EtOAc (200 mL * 3). The combined organic layers were washed with sat. NaHCO_3 solution (200 mL), brine (200 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 35°C to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 20: 1 to 1: 3) to give compound **45-2A** (20.0 g, 72.6 mmol, 60.0% yield, 94.1% purity) as a yellow oil, confirmed by LCMS ($R_t = 0.48$ min), HPLC ($R_t = 3.80$ min).

[0577] LCMS: $R_t = 0.48$ min, MS cal.: 275.42, MS observed: $[\text{M}+\text{Na}]^+ = 298.0$.

[0578] HPLC: $R_t = 3.80$ min, purity: 94.1%.

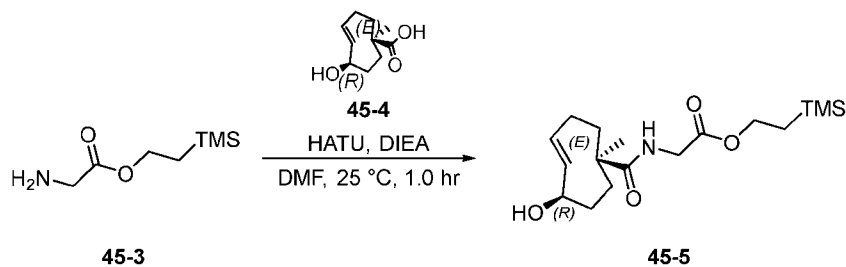
General procedure for preparation of compound 45-3



[0579] To a solution of compound **45-2A** (5.0 g, 18.1 mmol, 1.0 eq.) in HCl/dioxane (2.0 M, 10 mL). The mixture was stirred at 25 °C for 3.0 hrs. TLC (Petroleum ether/Ethyl acetate = 3:1) indicated compound **45-2A** ($R_f = 0.57$) was consumed completely and one new spot ($R_f = 0.21$) was formed. The reaction mixture was concentrated under vacuum to give compound **45-3** (4.0 g, crude) as a yellow oil, confirmed by LCMS ($R_t = 0.22$ min).

[0580] LCMS: $R_t = 0.22$ min, MS cal.: 175.10, MS observed: $[M+Na]^+ = 198.3$.

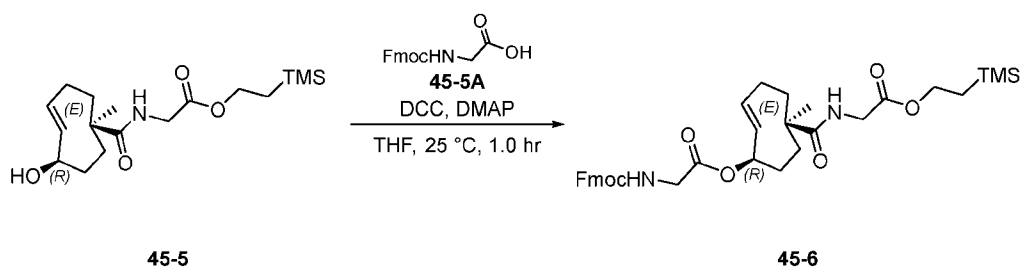
General procedure for preparation of compound 45-5



[0581] To a solution of compound **45-4** (1.7 g, 9.23 mmol, 1.0 eq.) in DMF (4.0 mL) was added DIEA (3.58 g, 27.7 mmol, 4.82 mL, 3.0 eq.) and HATU (4.21 g, 11.1 mmol, 1.2 eq.), finally added compound **45-3** (2.93 g, 13.8 mmol, 1.50 eq., HCl salt). The mixture was stirred at 25 °C for 2.0 hrs. LCMS showed one main peak with desired mass ($R_t = 0.42$ min) detected. The residue was purified by prep-HPLC (TFA condition) to give compound **45-5** (2.76 g, 7.73 mmol, 83.82% yield, 95.7% purity) as a white solid, confirmed by LCMS ($R_t = 0.42$ min) and HPLC ($R_t = 3.06$ min). LCMS: $R_t = 0.42$ min, MS cal.: 341.20, MS observed: $[M+Na]^+ = 364.1$.

[0582] HPLC: $R_t = 3.06$ min, purity: 99.5%.

General procedure for preparation of compound 45-6

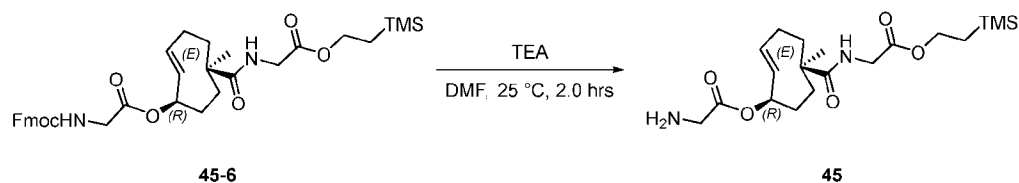


[0583] To a solution of compound **45-5A** (670 mg, 2.26 mmol, 1.1 eq.) in THF (7.0 mL) was added DCC (507 mg, 2.46 mmol, 497 μ L, 1.20 eq.) and DMAP (25.0 mg, 205 μ mol, 0.1 eq.), compound **45-5** (700 mg, 2.05 mmol, 1.0 eq.). The mixture was stirred at 25 °C for 1.0 hr. LCMS showed one peak with desired mass (R_t = 0.55 min) detected. The reaction mixture was filtered and dried under vacuum to give a residue, the residue was purified by prep-HPLC (TFA condition) to give compound **45-6** (790 mg, 1.21 mmol, 59.1% yield, 95.2% purity) as a white solid, confirmed by LCMS (R_t = 0.55 min), HPLC (R_t = 4.58 min).

[0584] LCMS: R_t = 0.55 min, MS cal.: 620, MS observed: $[M+Na]^+$ = 643.3.

[0585] HPLC: R_t = 4.58 min, purity: 91.3%.

General procedure for preparation of compound 45

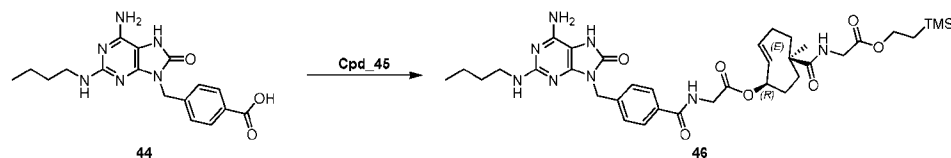


[0586] To a solution of compound **45-6** (600 mg, 966 μ mol, 1.0 eq.) in DMF (6.0 mL) was added TEA (97.8 mg, 966 μ mol, 134 μ L, 1.0 eq.). The mixture was stirred at 25 °C for 1.0 hr. LCMS showed compound **45-6** was consumed completely and one main peak with desired mass (R_t = 0.36 min). The reaction mixture was purified by prep-HPLC (FA condition) to give compound **45** (500 mg, 827 μ mol, 85.7% yield, 66.1% purity) as a yellow oil, confirmed by LCMS (R_t = 0.36 min), HPLC (R_t = 1.94 min).

[0587] LCMS: R_t = 0.36 min, MS cal.: 398.22, MS observed: $[M+Na]^+$ = 421.2.

[0588] HPLC: R_t = 1.94 min, purity: 66.1%.

General procedure for preparation of compound 46



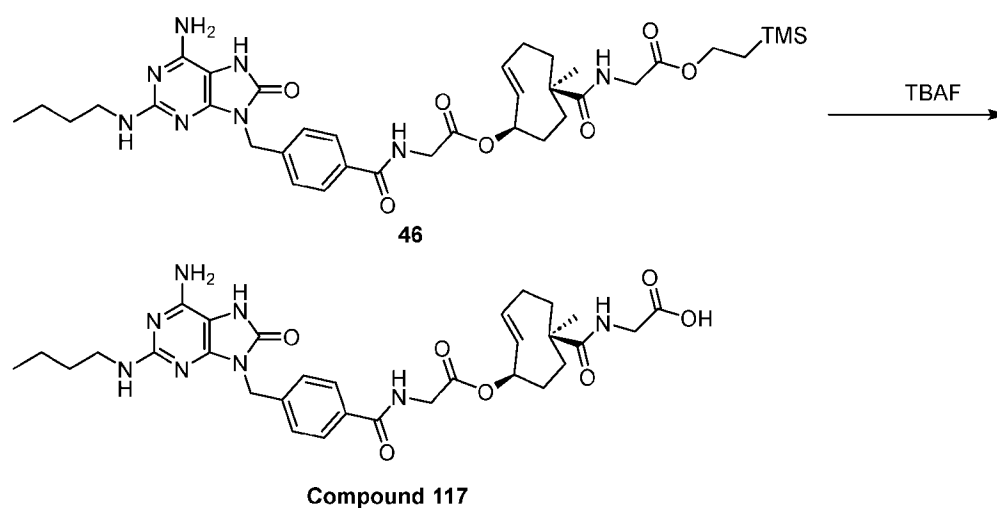
[0589] To a solution of compound **44** (210 mg, 589 μ mol, 1.0 eq.) and compound **45** (392 mg, 766 μ mol, 1.3 eq., TFA salt) in DMF (3.0 mL) was added DIC (112 mg, 883 μ mol, 137 μ L, 1.5 eq.), HOBt

(120 mg, 883 μ mol, 1.5 eq.) and DIEA (190 mg, 1.47 mmol, 256 μ L, 2.5 eq.). The mixture was stirred at 25 $^{\circ}$ C for 4.0 hrs. LCMS showed compound **44** was consumed completely and one main peak with desired mass (R_t = 0.43 min) was detected. The reaction mixture was triturated with isopropyl ether (30 mL * 2), the precipitate solid was centrifuged and purified by prep-HPLC (TFA condition) to give compound **46** (150 mg, 176 μ mol, 29.9% yield, 93.7% purity) as a white solid, confirmed by LCMS (R_t = 0.42 min) and HPLC (R_t = 2.77 min).

[0590] LCMS: $R_t = 0.42$ min, MS cal.: 736.37, MS observed: $[M+H]^+ = 737.4$.

[0591] HPLC: $R_t = 2.77$ min, purity: 93.7%.

General procedure for preparation of Compound 117

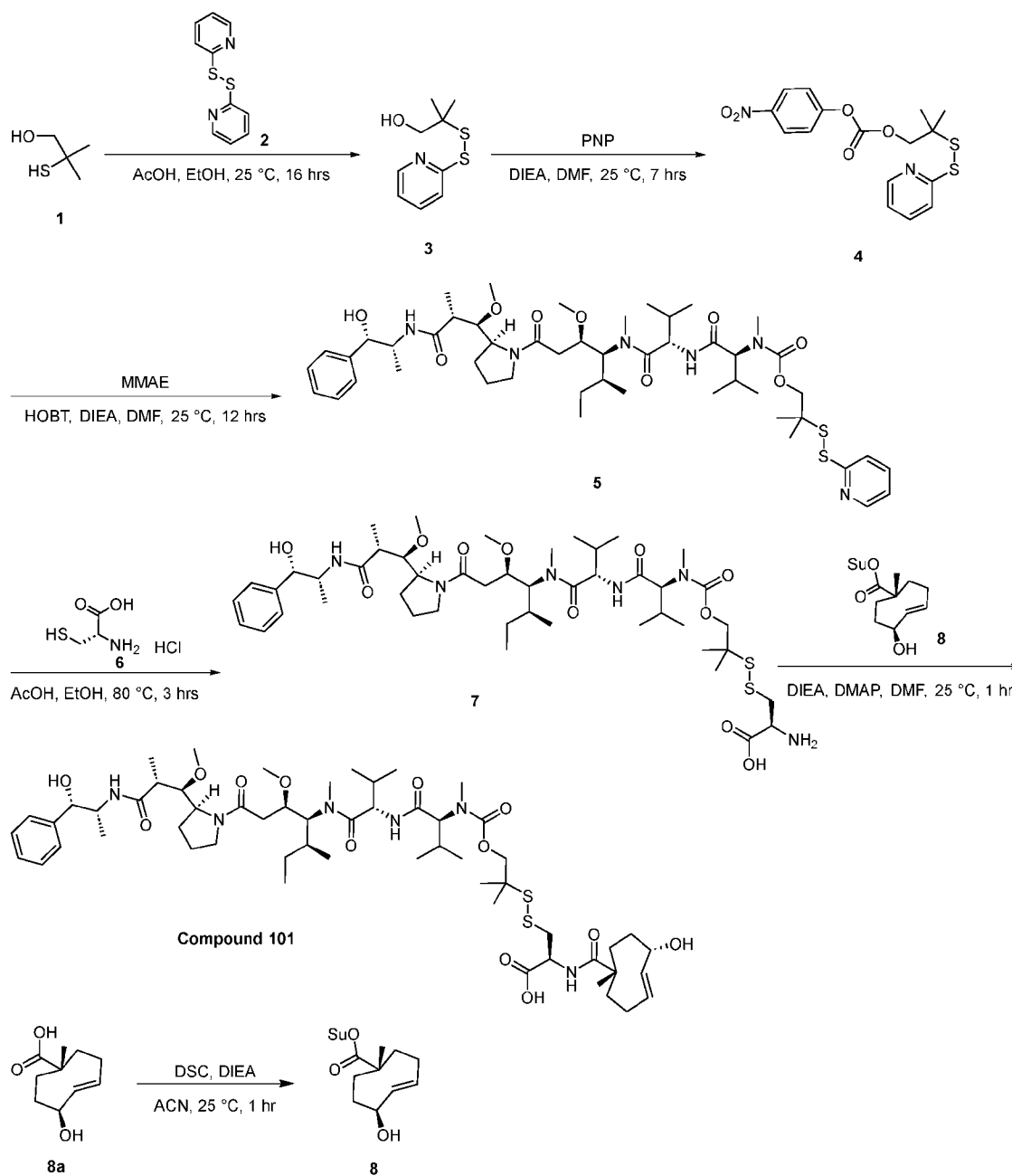


[0592] To a solution of compound **46** (150 mg, 203 μ mol, 1.0 eq.) in THF (1.0 mL) was added TBAF (1 M, 1.02 mL, 5 eq.). The mixture was stirred at 25 $^{\circ}$ C for 4.0 hrs. LC-MS showed compound **46** was consumed completely and one main peak with desired mass (R_t = 0.35 min) was detected. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was purified by prep-HPLC (AcOH condition) to give **Compound 117** (42 mg, 60.3 μ mol, 29.7% yield, 99.3% purity, AcOH salt) as a white solid.

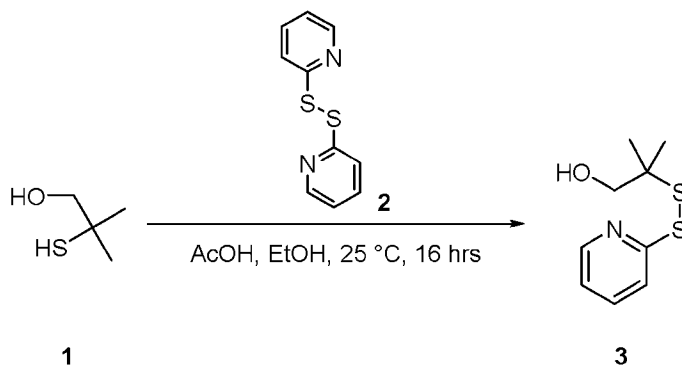
[0593] LCMS: R_t = 0.35 min, MS cal.: 636.30, MS observed: [M+H]⁺ = 637.4.

[0594] HPLC: $R_t = 2.70$ min, purity: 99.3%.

Example 14: Synthesis of Compound 101



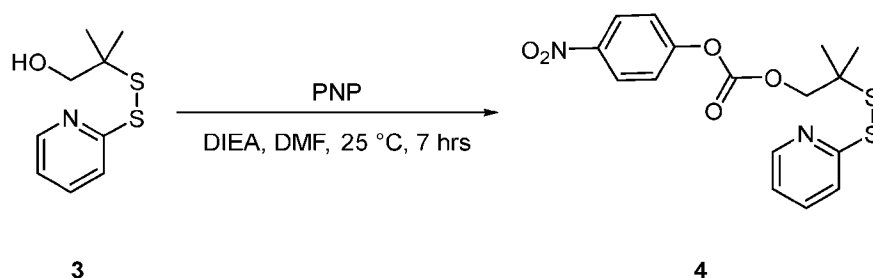
General procedure for preparation of compound 3



[0595] To a solution of compound **1** (1.22 g, 11.4 mmol, 1.00 *eq.*) in EtOH (12.0 mL) was added AcOH (614 mg, 10.2 mmol, 585 μ L, 0.89 *eq.*) and compound **2** (3.77 g, 17.1 mmol, 1.49 *eq.*). The mixture was stirred at 25 °C for 16 hrs. LCMS showed compound **1** was consumed completely and desired mass (MS cal.: 215.0, MS observed: $[M+H]^+ = 216.0$) was detected. The reaction mixture was concentrated under reduced pressure to compound **3** (5.67 g, crude) as yellow oil.

[0596] LCMS-1: R_t=0.36 min, MS cal.: 215.0, MS observed: [M+H]⁺ = 216.0

General procedure for preparation of compound 4



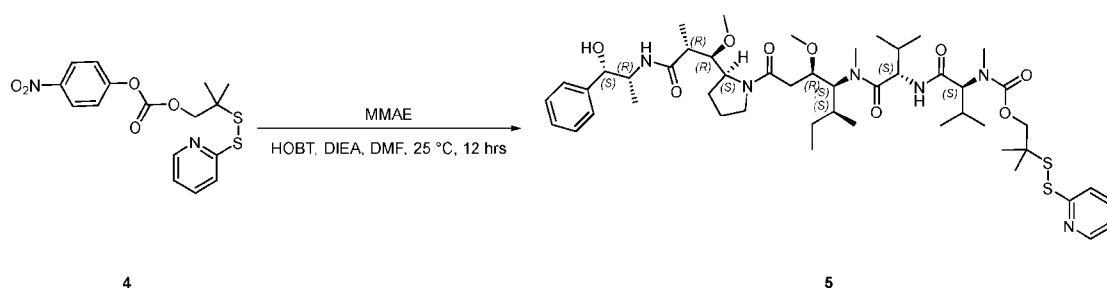
[0597] To a solution of compound **3** (5.67 g, 26.3 mmol, 1.00 *eq.*) in DMF (25.0 mL) was added DIEA (10.2 g, 78.9 mmol, 13.7 mL, 3.00 *eq.*) and PNP (16.0 g, 52.6 mmol, 2.00 *eq.*). The mixture was stirred at 25 °C for 7 hrs. LCMS showed compound **3** was consumed completely and desired mass (MS cal.: 380.05, MS observed: [M+H]⁺ = 381.0) was detected. The reaction mixture was diluted with H₂O (100 mL) and extracted with EtOAc (50 mL * 3). The combined organic layers were washed with brine (50 mL * 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **4** (1.40 g, 3.66 mmol, 31.8% yield for 2 steps, 99.4% purity) as a yellow solid which was confirmed via LCMS, HPLC and HNMR.

[0598] LCMS: R_t = 0.48 min, MS cal.: 380.0, MS observed: [M+H]⁺ = 381.1.

[0599] HPLC: $R_t = 3.53$ min, purity: 99.4%.

[0600] ¹H NMR (400 MHz, CDCl₃) δ: 8.47 - 8.45 (m, 1H), 8.30 - 8.26 (m, 2H), 7.76 - 7.74 (m, 1H), 7.65 - 7.60 (m, 2H), 7.39 - 7.35 (m, 2H), 7.11 - 7.07 (m, 1H).

General procedure for preparation of compound 5

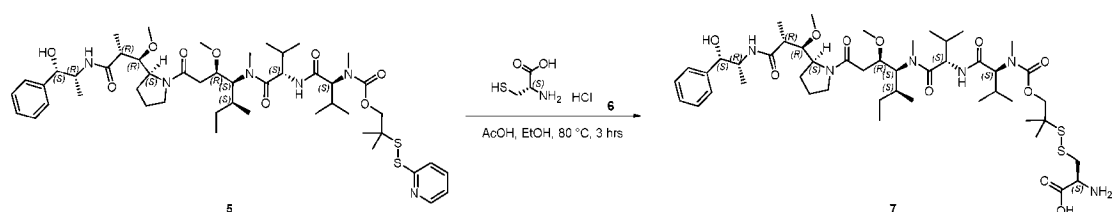


[0601] To a solution of compound **4** (1.40 g, 3.66 mmol, 1.00 *eq.*) in DMF (14.0 mL) was added MMAE (2.63 g, 3.66 mmol, 1.00 *eq.*), HOBt (741 mg, 5.49 mmol, 1.50 *eq.*) and DIEA (945 mg, 7.32 mmol, 1.27 mL, 2.00 *eq.*). The mixture was stirred at 25 °C for 12 hrs. LCMS showed compound **4** was consumed completely and desired mass (MS cal.: 958.53, MS observed: $[M+H]^+ = 959.5$) was detected. The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **5** (2.50 g, 2.54 mmol, 69.3% yield, 97.4% purity) as a white solid which was confirmed via LCMS and HPLC.

[0602] LCMS: $R_t = 0.55$ min, MS cal.: 958.5, MS observed: $[M+H]^+ = 959.7$.

[0603] HPLC: $R_t = 3.93$ min, purity: 97.4%.

General procedure for preparation of compound 7

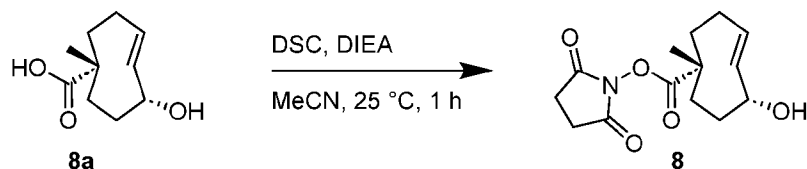


[0604] To a solution of compound **6** (2.40 g, 15.2 mmol, 10.0 *eq.*, HCl) in EtOH (15.0 mL) was added AcOH (81.4 mg, 1.36 mmol, 77.6 μ L, 0.89 *eq.*) and compound **5** (1.50 g, 1.52 mmol, 1.00 *eq.*). The mixture was stirred at 80 °C for 3 hrs. LCMS showed compound **5** was consumed completely and desired mass (MS cal.: 968.5, MS observed: $[M+H]^+ = 969.5$) was detected. The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **7** (780 mg, 767 μ mol, 50.4% yield, 99.0% purity, HCl) as a white solid which was confirmed via LCMS and HPLC.

[0605] LCMS: $R_t = 0.41$ min, MS cal.: 968.5, MS observed: $[M+H]^+ = 969.6$.

[0606] HPLC: $R_t = 2.62$ min, purity: 99.0%.

General procedure for preparation of compound 8



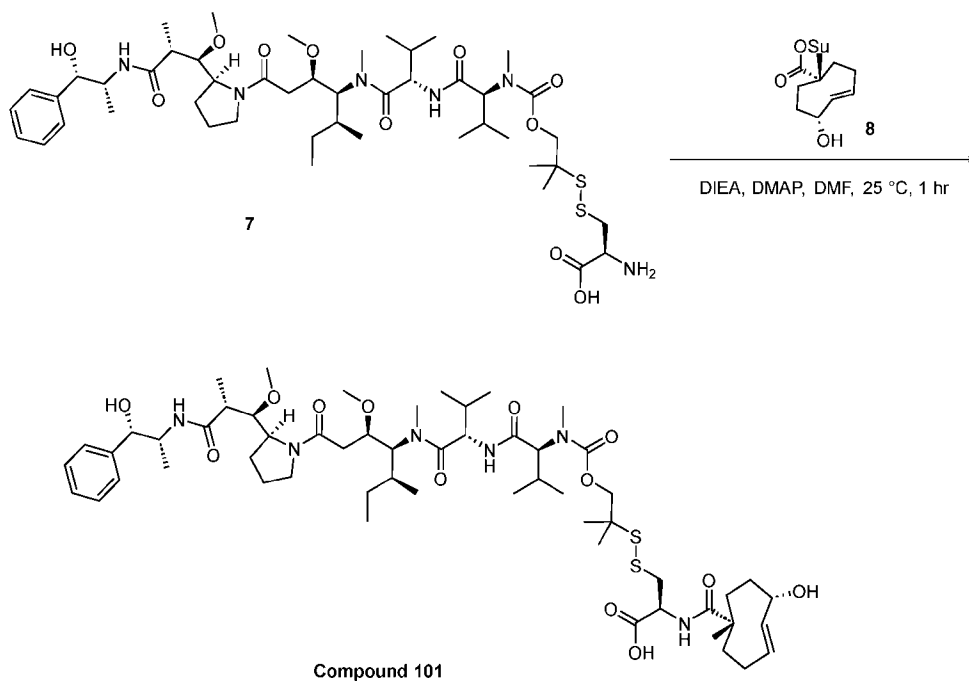
[0607] To a solution of compound **8a** (2.00 g, 10.8 mmol, 1.00 *eq.*) in ACN (10.0 mL) was added DIEA (4.21 g, 32.5 mmol, 5.67 mL, 3.00 *eq.*) and DSC (2.78 g, 10.8 mmol, 1.00 *eq.*) in ACN (10.0 mL). The mixture was stirred at 25 °C for 1 hr. Analysis by LCMS showed the desired mass (MS cal.: 281.1, MS observed: $[M+Na]^+ = 304.1$) was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether: EtOAc = 1: 1) to give compound **8** (1.82 g, 6.18 mmol, 56.9% yield, 95.5% purity) as a light yellow solid which was confirmed via LCMS, HPLC and HNMR.

[0608] LCMS: R_t = 0.44 min, MS cal.: 281.1, MS observed: $[M+H]^+$ = 304.0.

[0609] HPLC: R_t = 1.77 min, purity: 95.5%.

[0610] ^1H NMR (400 MHz, CDCl_3) δ : 6.13 - 6.06 (m, 1H), 5.68 - 5.64 (m, 2H), 4.51 (s, 1H), 2.82 - 2.81 (m, 4H), 2.41 - 2.25 (m, 3H), 2.17 - 1.98 (m, 3H), 1.93 - 1.83 (m, 2H), 1.25 (s, 3H).

General procedure for preparation of Compound 101

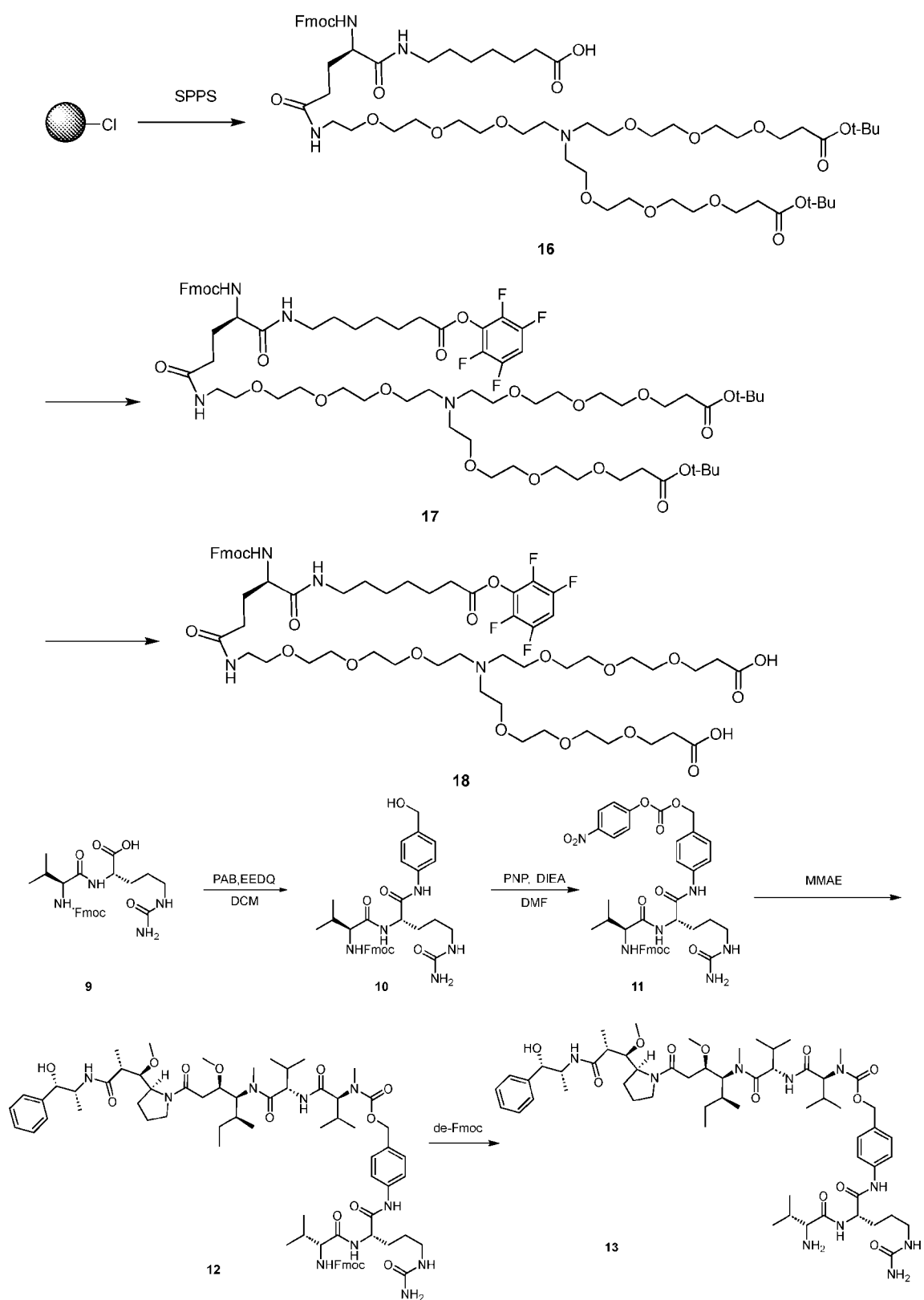


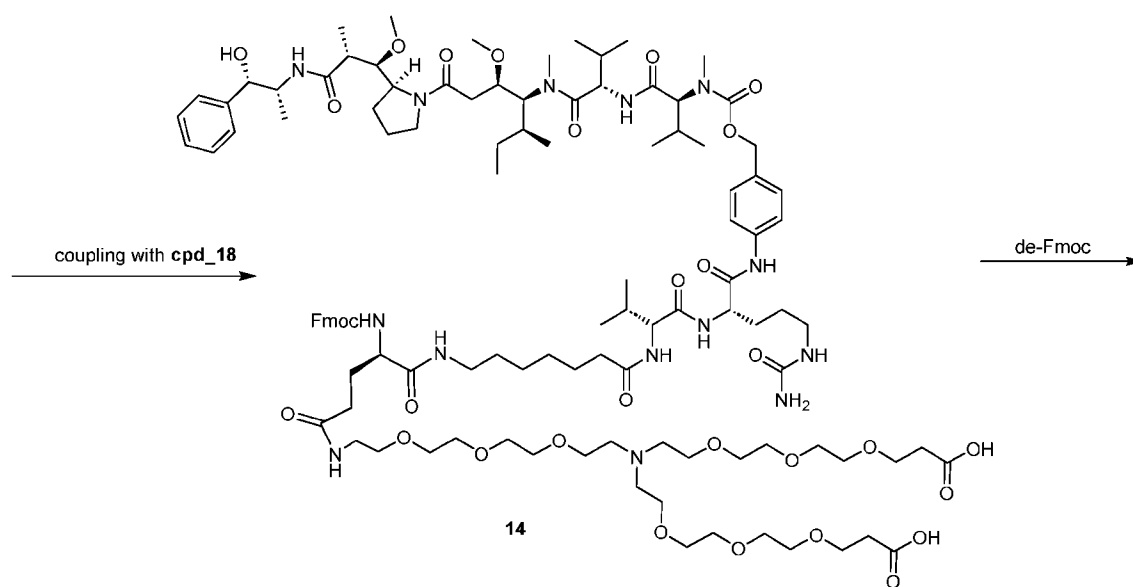
[0611] To a solution of compound **7** (280 mg, 275 μmol , 1.00 *eq.*, HCl) in DMF (1.00 mL) was added DMAP (269 mg, 2.20 mmol, 8.00 *eq.*) and compound **8** (89.3 mg, 303 μmol , 1.10 *eq.*), DIEA (284 mg, 2.20 mmol, 384 μL , 8.00 *eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS showed compound **7** (R_t = 0.43 min) was remained and desired mass (MS cal.: 1134.6, MS observed: $[M+H]^+$ = 1135.8) was detected. The reaction mixture was concentrated under reduced pressure to give a residue-1. Another residue-2 was obtained from 80 mg scale. The two batches were purified by prep-HPLC (AcOH condition) to give **Compound 101** (47.0 mg, 37.7 μmol , 13.6% yield, 95.9% purity, AcOH) as a white solid which was confirmed via LCMS and HPLC.

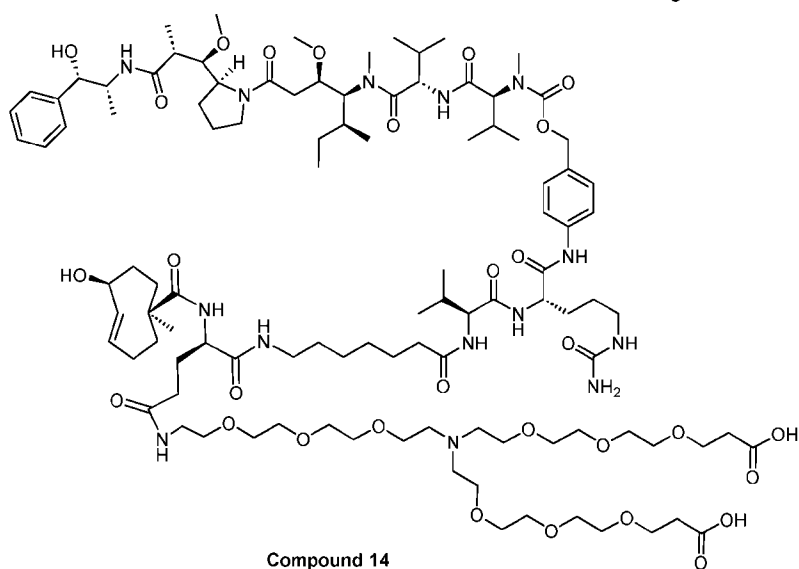
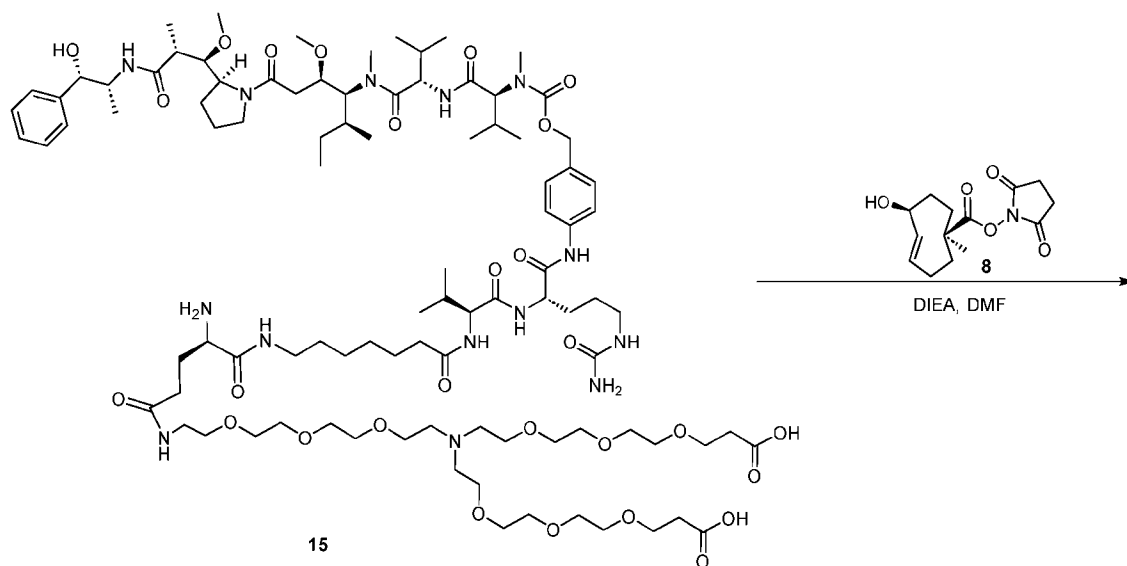
[0612] LCMS: R_t = 0.50 min, MS cal.: 1134.6, MS observed: $[M+H]^+$ = 1135.7.

[0613] HPLC: R_t = 3.57 min, purity: 95.9%.

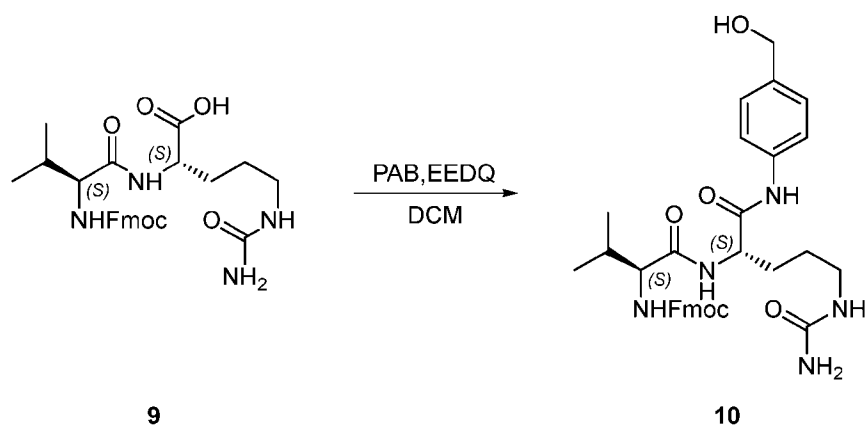
Example 15: Synthesis of Compound 14







General procedure for preparation of compound 10



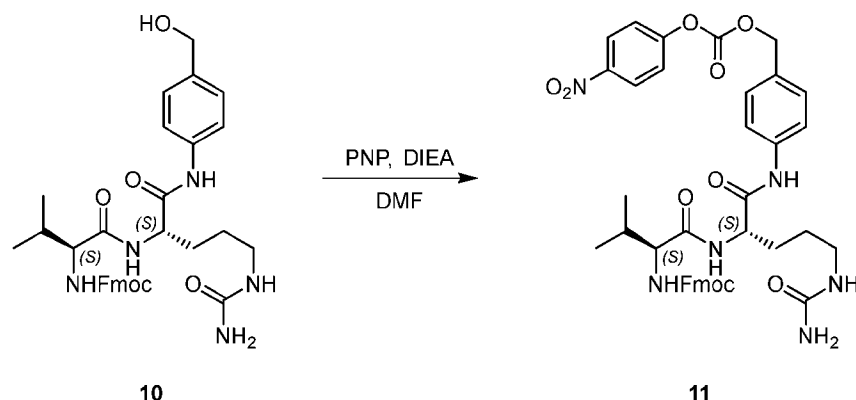
[0614] To a solution of compound 9 (2.00 g, 4.03 mmol, 1.00 eq.) in DCM (20 mL) and MeOH (5 mL) was added 4-aminobenzyl alcohol (992 mg, 8.06 mmol, 2.00 eq.) and EEDQ (1.99 g, 8.06 mmol, 2.00

eq.). The mixture was stirred at 35 °C for 16 hrs in the dark. TLC (DCM/MeOH = 10:1, product R_f = 0.32) indicated compound **9** was consumed completely and one new spot formed. The reaction mixture was filtered and concentrated under reduced pressure to give compound **10** (2.00 g, 3.02 mmol, 75.1% yield, 91.0% purity) as a yellow solid which was confirmed via LCMS and HPLC.

[0615] LCMS: R_t = 0.42 min, MS cal.: 601.3, MS observed: $[M+H]^+ = 602.3$.

[0616] HPLC: R_t = 2.72 min, purity: 91.0%.

General procedure for preparation of compound 11

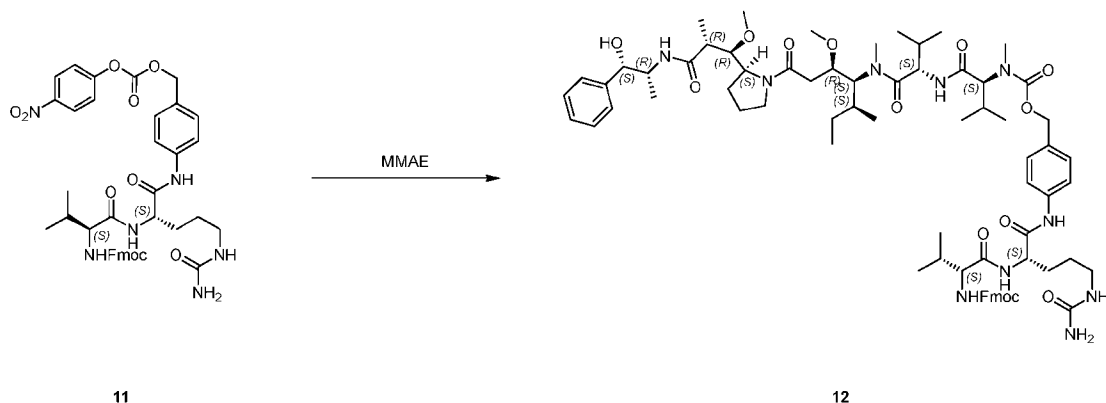


[0617] To a solution of compound **10** (2.00 g, 3.32 mmol, 1.00 *eq.*) in DMF (20 mL) was added DIEA (429 mg, 3.32 mmol, 578 μL , 1.00 *eq.*) and PNP (1.01 g, 3.32 mmol, 1.00 *eq.*). The mixture was stirred at 20-25 °C for 16 hrs. TLC (DCM/MeOH = 10:1, product R_f = 0.42) indicated compound **10** was consumed completely and one new spot formed. The crude product was triturated with isopropyl ether (200 mL) to give compound **11** (1.80 g, 2.35 mmol, 70.6% yield) as a yellow solid which was confirmed via LCMS and HPLC.

[0618] LCMS: R_t = 0.51 min, MS cal.: 766.3, MS observed: $[M+H]^+ = 767.5$.

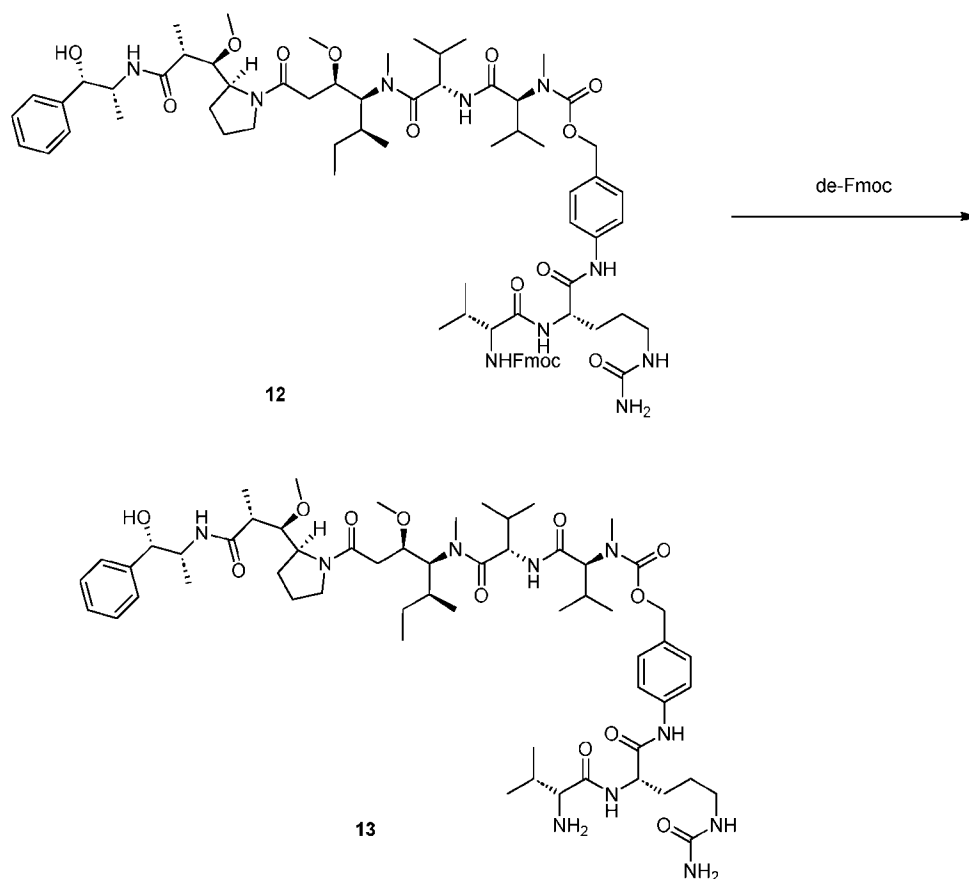
[0619] HPLC: R_t = 3.65 min, purity: 88.4%.

General procedure for preparation of compound 12



[0620] To a solution of compound **11** (1.80 g, 2.35 mmol, 1.00 *eq.*) in DMF (18.0 mL) was added MMAE (1.69 g, 2.35 mmol, 1.00 *eq.*) and DIEA (910 mg, 7.04 mmol, 1.23 mL, 3.00 *eq.*). The mixture was stirred at 20-25 °C for 16 hrs. The reaction was monitored by TLC (DCM/MeOH = 10:1, product R_f = 0.27), TLC indicated the reactant was consumed. The crude product was triturated with isopropyl ether (200 mL) to give compound **12** (3.0 g, crude) as a yellow solid.

General procedure for preparation of compound 13

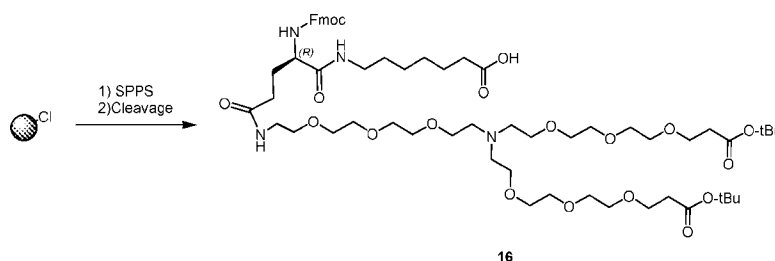


[0621] A solution of compound **12** (3.00 g, 2.23 mmol, 1.00 *eq.*) in TEA (4.19 g, 41.4 mmol, 6.00 mL, 18.5 *eq.*) and DMF (24.0 mL) was stirred at 20-25 °C for 16 hrs. The reaction was monitored by TLC, TLC (DCM: MeOH = 10:1, product R_f = 0.19) indicated the reactant was consumed. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was triturated with isopropyl ether (200 mL). The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **13** (620 mg, 551 μ mol, 24.7% yield for 2 steps) as a white solid which was confirmed via LCMS and HPLC.

[0622] LCMS: R_t = 0.40 min, MS cal.: 1122.7, MS observed: $[M+H]^+$ = 1123.7.

[0623] HPLC: R_t = 2.58 min, purity: 96.7%.

General procedure for preparation of compound 16



Peptide Synthesis:

[0624] The peptide was synthesized using standard Fmoc chemistry.

[0625] Resin preparation: To the vessel containing 2-CTC resin (1.50 mmol, Sub: 0.81 mmol/g, 1.85 g) and Fmoc-7-Ahp-OH (1.50 mmol, 0.55 g, 1.00 *eq.*), in DCM (30.0 mL) and then DIEA (4.00 *eq.*) was added with N₂ bubbling for 2 hrs at 15 °C. Add MeOH (15.0 mL) and mix for 30 mins. Then 20% piperidine in DMF (50.0 mL) was added and the mixture was bubbled with N₂ for 30 mins at 15 °C. Then the mixture was filtered to obtain the resin. The resin was washed with DMF (50.0 mL) *5 before proceeding to next step.

[0626] Coupling: A solution of Fmoc-D-Glu(OAll)-OH (4.50 mmol, 1.84 g, 3.00 *eq.*), HBTU (4.27 mmol, 1.62 g, 2.85 *eq.*) in DMF (30.0 mL) was added to the resin with N₂ bubbling. Then DIEA (9.00 mmol, 6.00 *eq.*) was added to the mixture dropwise and bubbled with N₂ for 30 min at 20 °C. The coupling reaction was monitored by ninhydrin test, if it showed colorless, the coupling was completed. The resin was then washed with DMF (50.0 mL) *5.

[0627] De-protection: A solution of PhSiH₃ (10.0 *eq.*) and Pd(PPh₃)₄ (0.10 *eq.*) in DCM (20 mL) was added to the resin and the mixture was bubbled with N₂ for 30 min at 15 °C. The resin was then washed with DCM (50.0 mL) *5, DMF (50.0 mL) *5.

[0628] Coupling: A solution of TFP (15.0 mmol, 2.49 g, 10.0 equiv) in DMF (30.0 mL) was added to the resin with N₂ bubbling. Then DIC (10.0 equiv.) was added to the mixture dropwise and bubbled with N₂ for 16h at 20 °C. The resin was then washed with DMF (50.0 mL) *5. A solution of Bis PEG₂-OtBu (3.00 mmol, 2.14 g, 2.00 *eq.*) in DMF (30.0 mL) was added to the resin with N₂ bubbling. Then DIEA (4.00 *eq.*) was added to the mixture dropwise and bubbled with N₂ for 2 hrs at 20 °C.

[0629] The resin was then washed with DMF (50.0 mL) *5, MeOH (50.0 mL) *5 and then dried under vacuum.

Peptide Cleavage:

[0630] Add cleavage buffer (HFIP/DCM, 2/8, v/v, 100 ml) to the flask containing the side chain protected peptide at room temperature and stir for 0.5 h for 2 times.

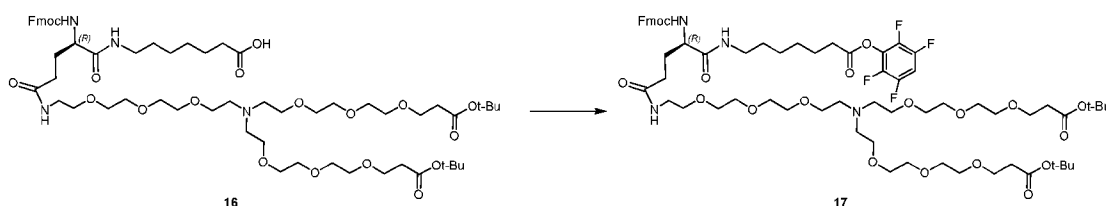
[0631] Filter and collect the filtrate. The mixture was concentrated under reduced pressure.

[0632] The residue was dissolved in ACN (100 ml) and H₂O (150 ml), then lyophilized to give the compound **16** (520 mg, 93.2% purity, 27.3% yield) which was confirmed via LCMS and HPLC.

[0633] LCMS: R_t=1.71 min MS cal: 1190.7, MS observed: [M+H]⁺=1191.4

[0634] HPLC: R_t=15.3 min, purity: 95.6%

General procedure for preparation of compound 17

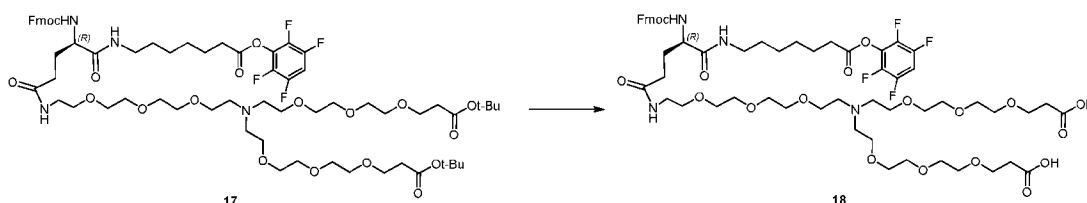


[0635] To a solution of compound **16** (370 mg, 310 μmol, 1.00 eq.) in DCM (4.00 mL) was added TFP (103 mg, 621 μmol, 2.00 eq.) and DIC (117 mg, 931 μmol, 144 μL, 3.00 eq.). The mixture was stirred at 20-25 °C for 16 hrs. LC-MS showed compound **16** was consumed completely and one main peak (R_t = 0.51 min) with desired m/z (MS cal.: 1338.7, MS observed: [M+H]⁺ = 1339.6). The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **17** (350 mg, 257 μmol, 82.9% yield, 98.6% purity) as a colorless oil which was confirmed via LCMS and HPLC.

[0636] LCMS: R_t=0.51 min, MS cal.: 1338.7, MS observed: [M+H]⁺ = 1339.6.

[0637] HPLC: R_t = 3.87 min, purity: 98.6%

General procedure for preparation of compound 18

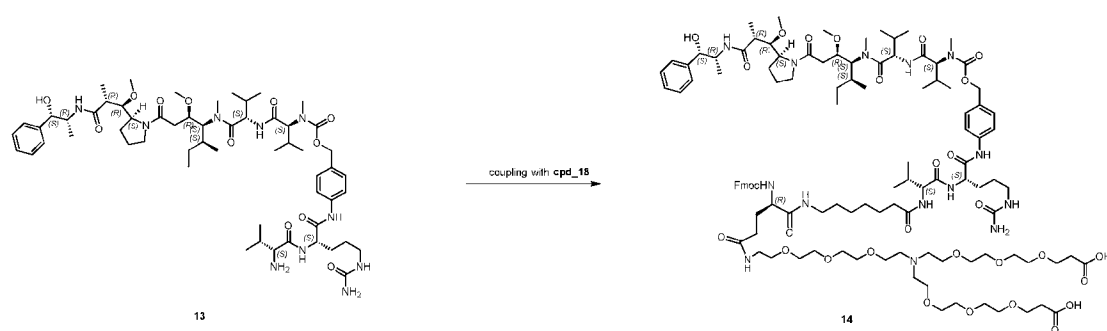


[0638] A solution of compound **17** (350 mg, 261 μmol, 1.00 eq.) in HCOOH (10 mL) was stirred at 20-25 °C for 16 hrs. LC-MS showed compound **17** was consumed completely and one main peak (R_t=0.45 min) with desired m/z (MS cal.: 1226.6, MS observed: [M+H]⁺ = 1227.6). The reaction mixture was concentrated under reduced pressure to give compound **18** (300 mg, 150 μmol, 57.5% yield, 61.5% purity) as a colorless oil which was confirmed via LCMS and HPLC.

[0639] LCMS: R_t=0.45 min, MS cal.: 1226.6, MS observed: [M+H]⁺ = 1227.6.

[0640] HPLC: R_t = 3.09 min, purity: 61.5%.

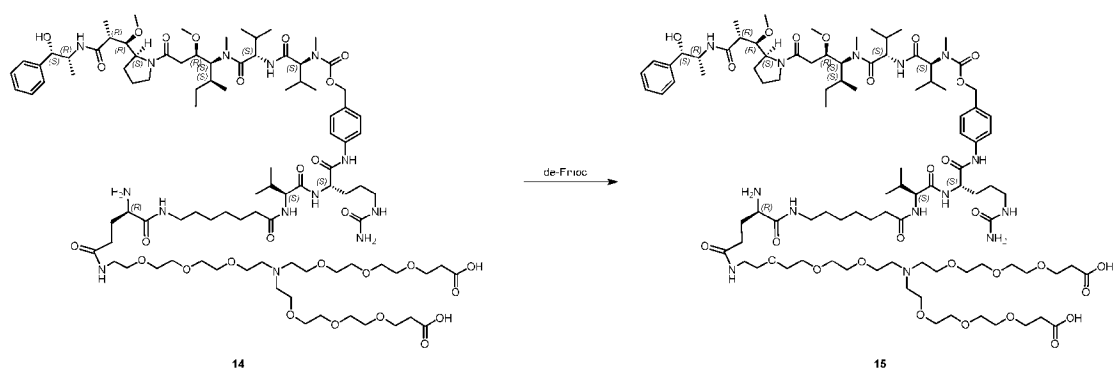
General procedure for preparation of compound 14



[0641] To a solution of compound **13** (300 mg, 242 μmol , 1.00 *eq.*, TFA) in DMF (3.00 mL) was added compound **18** (297 mg, 242 μmol , 1.00 *eq.*) and DIEA (94.0 mg, 727 μmol , 126 μL , 3.00 *eq.*). The mixture was stirred at 20-25 °C for 16 hrs. LCMS showed compound **13** was consumed completely and one main peak ($R_t=0.46$ min) with desired m/z (MS cal.: 2183.3, MS observed: $(M+2H)^{2+} = 1093.2$). The crude product was triturated with isopropyl ether (30 mL) to give compound **14** (610 mg, crude) as a colorless oil which was confirmed via LCMS.

[0642] LCMS: $R_t=0.46$ min, MS cal.: 2183.3, MS observed: $(M+2H)^{2+} = 1093.1$.

General procedure for preparation of compound 15

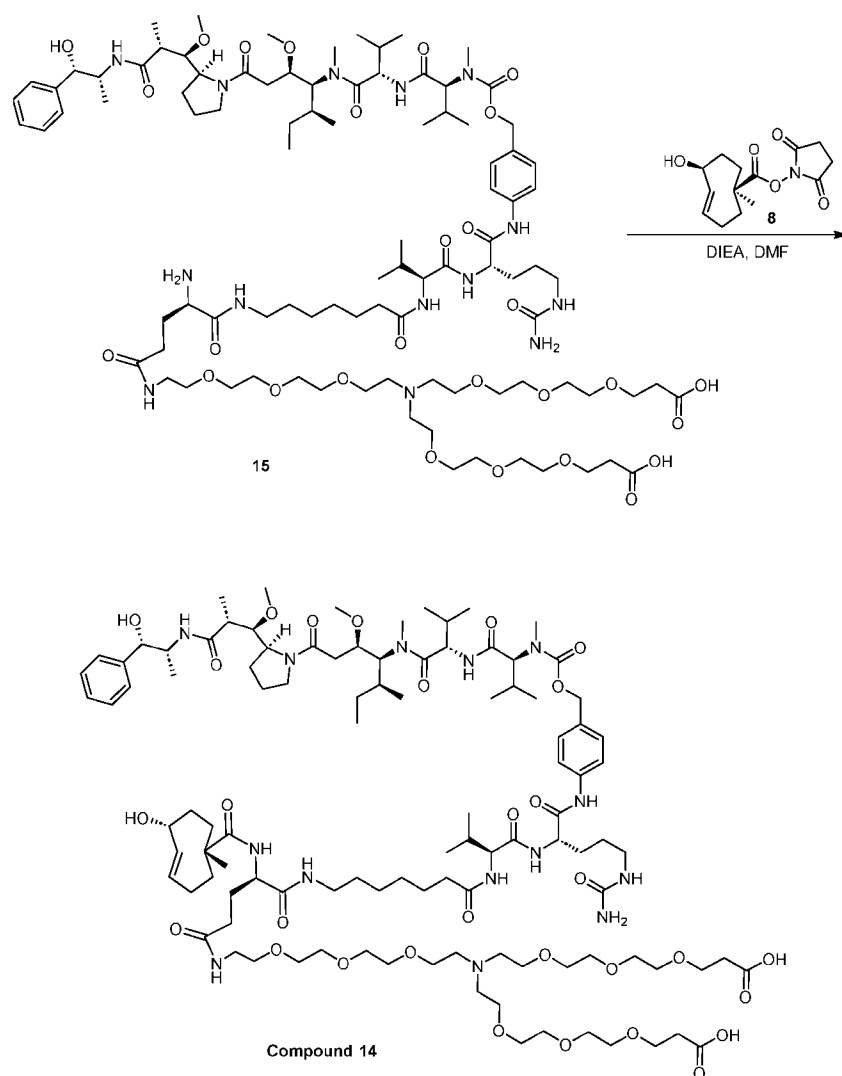


[0643] A solution of compound **14** (610 mg, 279 μmol , 1.00 *eq.*) in DMF (4 mL) and TEA (727 mg, 7.18 mmol, 1.00 mL, 25.7.00 *eq.*) was stirred at 20-25 °C for 16 hrs. LC-MS showed compound **14** was consumed completely and one main peak ($R_t = 0.39$ min) with desired m/z (MS cal.: 1961.2, MS observed: $(M+2H)^{2+} = 982.0$). The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (0.1% TFA condition) to give compound **15** (200 mg, 94.9 μmol , 34.0% yield for 2 steps, 98.6% purity, TFA) as a white solid which was confirmed via LCMS and HPLC.

[0644] LCMS: $R_t=0.40$ min, MS cal.: 1961.2, MS observed: $(M+2H)^{2+} = 982.0$.

[0645] HPLC: $R_t = 2.46$ min, purity: 98.6%.

General procedure for preparation of target Compound 14

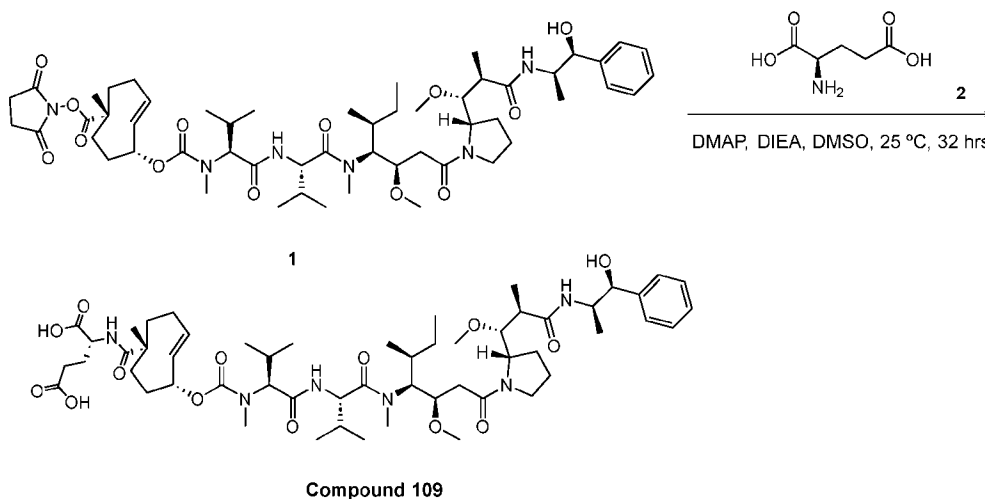


[0646] To a solution of compound **15** (200 mg, 96.3 μmol , 1.00 *eq.*, TFA) in THF (0.3 mL) and H₂O (0.3 mL) was added compound **8** (81.2 mg, 288 μmol , 3.00 *eq.*) and DIEA (37.3 mg, 288 μmol , 50.3 μL , 3.00 *eq.*) and DMAP (35.3 mg, 288 μmol , 3.00 *eq.*). The mixture was stirred at 20-25 °C for 2 hrs. LC-MS-1 showed ~14.1% of compound **15** remained and one main peak with desired *m/z*. The reaction mixture was concentrated under reduced pressure to give a residue (portion 1). Another residue (portion 2) was obtained from 120 mg scale. The combined residues were purified by prep-HPLC (0.1% TFA condition) and then salt exchanged *via* prep-HPLC (0.1% CH₃COOH condition) to give **Compound 14** (41.0 mg, 19.0 μmol , 12.4% yield, AcOH salt) as a white solid.

[0647] LCMS: *R*_t = 0.45 min, MS cal.: 2127.3, MS observed: (M+2H)²⁺ = 1065.1.

[0648] HPLC: *R*_t = 2.90 min, purity: >99.0%.

Example 16: Synthesis of Compound 109

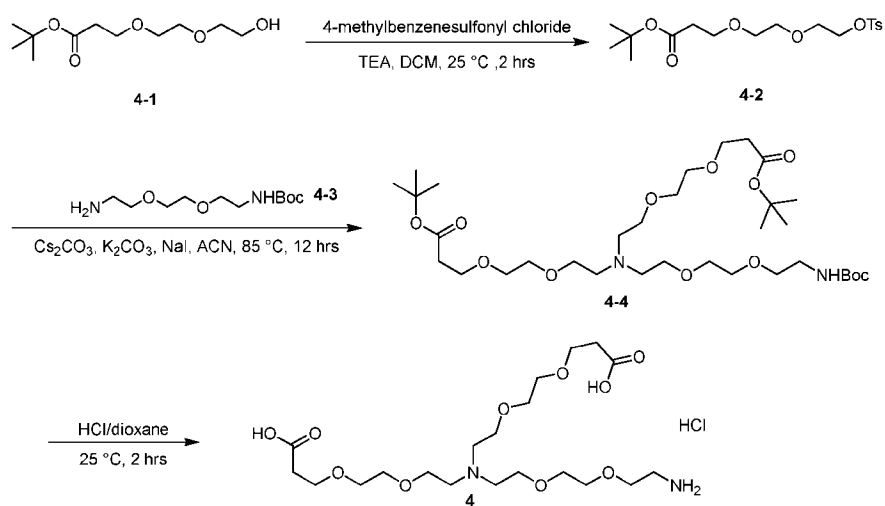
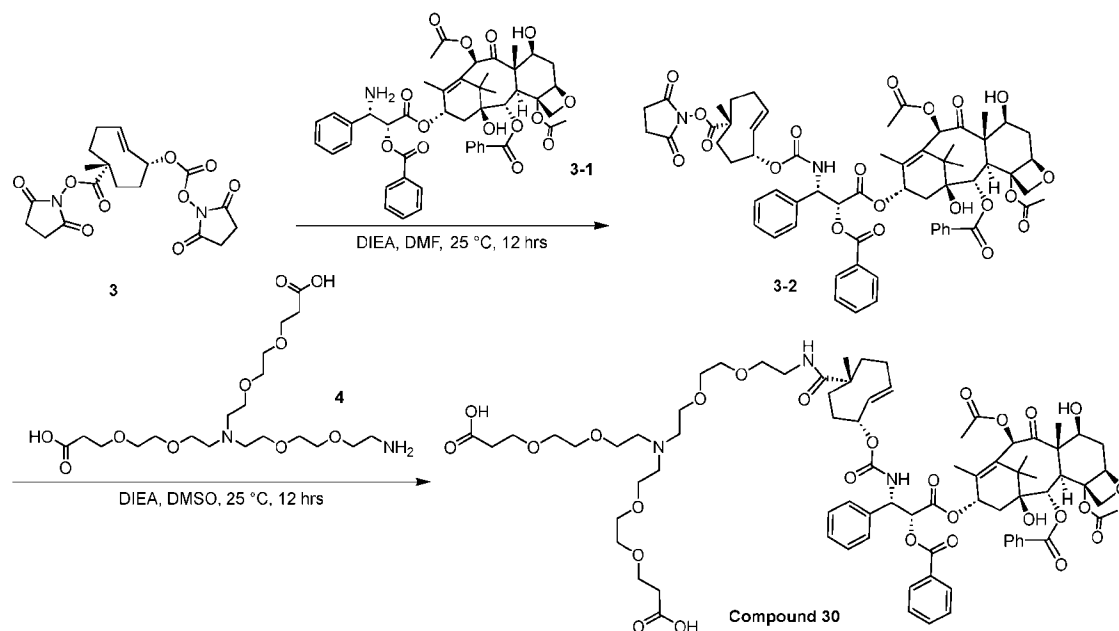


[0649] To a solution of compound **1** (500 mg, 487 μ mol, 1.00 eq.) and compound **2** (1.07 g, 7.31 mmol, 15 eq) in DMSO (5.0 mL) was added DIEA (0.501 g, 3.90 mmol, 0.68 mL, 8.00 eq.) and DMAP (476 mg, 3.90 mmol, 8.00 eq.). The mixture was stirred at 25 °C for 32 hrs. LCMS showed compound **1** was consumed completely and desired mass (R_t = 1.54 min) was detected. The mixed layers were filtered and the organic filtrate was separated. The crude product was purified by reversed-phase HPLC (FA condition). **Compound 109** (200 mg, 188 μ mol, 38.6% yield, 98.4% purity, ^1H NMR showed no HCOOH residue contained in **Compound 109**) as a white solid which was confirmed via LCMS (R_t = 1.54 min) and HPLC (R_t = 3.02 min).

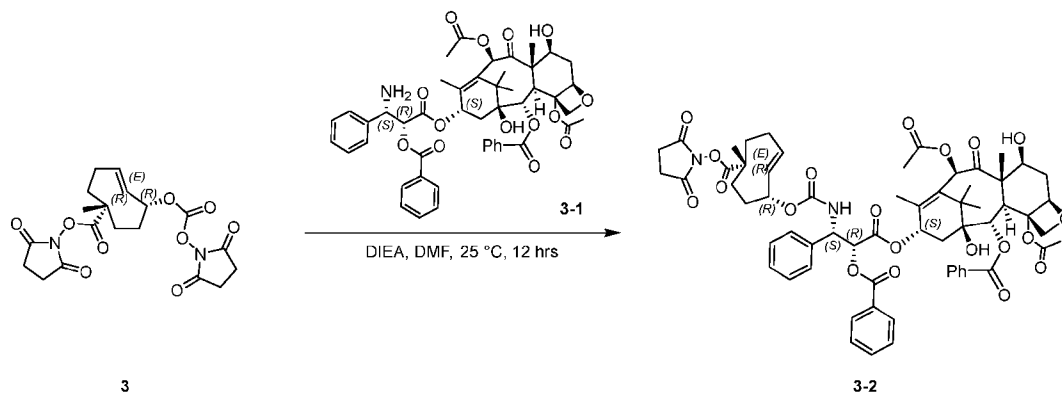
[0650] LCMS: R_t = 1.54 min, MS cal.: 1056.6 MS observed: $[\text{M}+\text{Na}]^+ = 1079.7$

[0651] HPLC: R_t = 3.02 min, purity: 98.4%

Example 17: Synthesis of Compound 30



General procedure for preparation of Compound 3-2

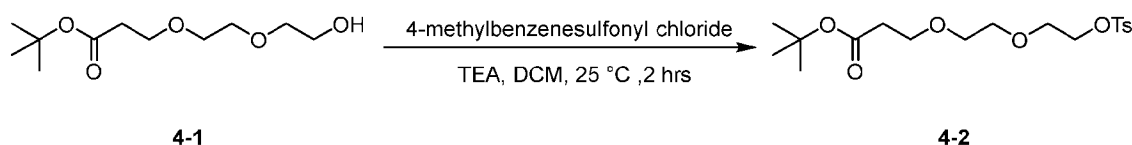


[0652] To a solution of compound 3 (500 mg, 555 μ mol, 1.00 eq) in DMF (10.0 mL) was added DIEA (143 mg, 1.11 mmol, 193 μ L, 2.00 eq) and compound 3-1 (375 mg, 888 μ mol, 1.60 eq). The mixture was stirred at 25 °C for 12hrs. LC-MS showed compound 3-1 remained (R_t = 0.46 min) and compound 3 consumed completely and desired mass was detected (R_t = 0.53 min). The reaction mixture was used to purification directly. The residue was purified by prep-HPLC (FA condition). Compound 3-2 (252 mg, 213 μ mol, 38.4% yield, 98.3% purity) was obtained as a brown solid, confirmed by LC-MS and HPLC.

[0653] LCMS: R_t = 0.52 min, MS cal.: 1160.44, MS observed: $[M+H]^+$ = 1161.5.

[0654] HPLC: R_t = 4.30 min, purity: 98.3%.

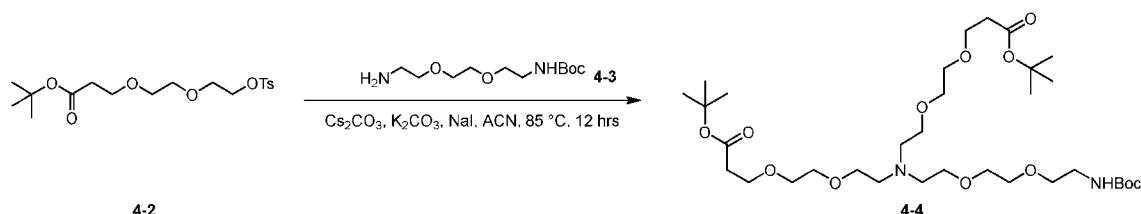
General procedure for preparation of compound 4-2



[0655] To a solution of compound 4-1 (20.0 g, 85.3 mmol, 1.00 eq.) in DCM (200 mL) at 0 °C was added TEA (12.9 g, 128 mmol, 17.8 mL, 1.50 eq.) and 4-methylbenzenesulfonyl chloride (17.9 g, 93.9 mmol, 1.10 eq.) under N_2 . The mixture was stirred at 25 °C for 2 hrs. LCMS showed compound 4-1 was consumed completely and one main peak with desired mass (R_t = 0.50 min) was detected. The reaction mixture was diluted with H_2O (300 mL) and extracted with DCM (120 mL $\times$ 2). The combined organic layers were washed with brine (150 mL $\times$ 2), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. Compound 4-2 (36.0 g, 82.3 mmol, 96.5% yield, 88.9% purity) was obtained as white oil, confirmed by LCMS (R_t = 0.47 min).

[0656] LCMS: R_t = 0.47 min, MS cal.: 388.1, MS observed: $[M+Na]^+$ = 411.1.

General procedure for preparation of compound 4-4

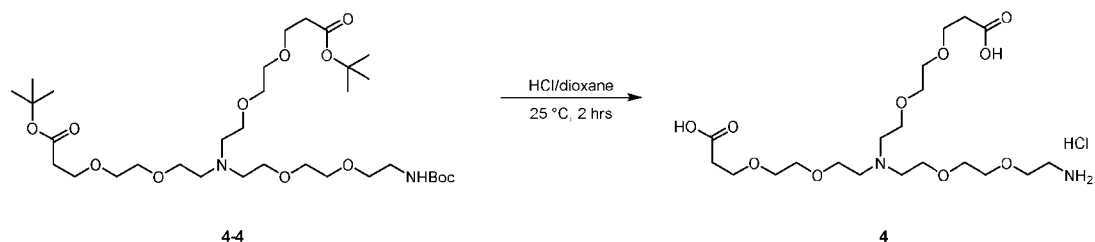


[0657] To a solution of compound 4-2 (35.3 g, 80.9 mmol, 3.00 eq.) and compound 4-3 (6.70 g, 26.9 mmol, 1.00 eq.) in ACN (350 mL) was added Cs_2CO_3 (2.64 g, 8.09 mmol, 0.30 eq.) and K_2CO_3 (11.1 g, 80.9 mmol, 3.00 eq.) and NaI (404 mg, 2.70 mmol, 0.10 eq.). The mixture was stirred at 85 °C for 12 hrs. LCMS showed compound 4-3 was consumed completely and two main peaks with desired mass (R_{t1} = 0.42 min) were detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , petroleum ether: ethyl acetate

= 1: 0 to 0: 1). Compound **4-4** (12.2 g, 17.9 mmol, 65.9% yield, 99.3% purity) was obtained as yellow oil, confirmed by LCMS ($R_t = 0.42$ min).

[0658] LCMS: $R_t = 0.42$ min, MS cal.: 680.4, MS observed: $[M+H]^+ = 681.4$.

General procedure for preparation of compound 4

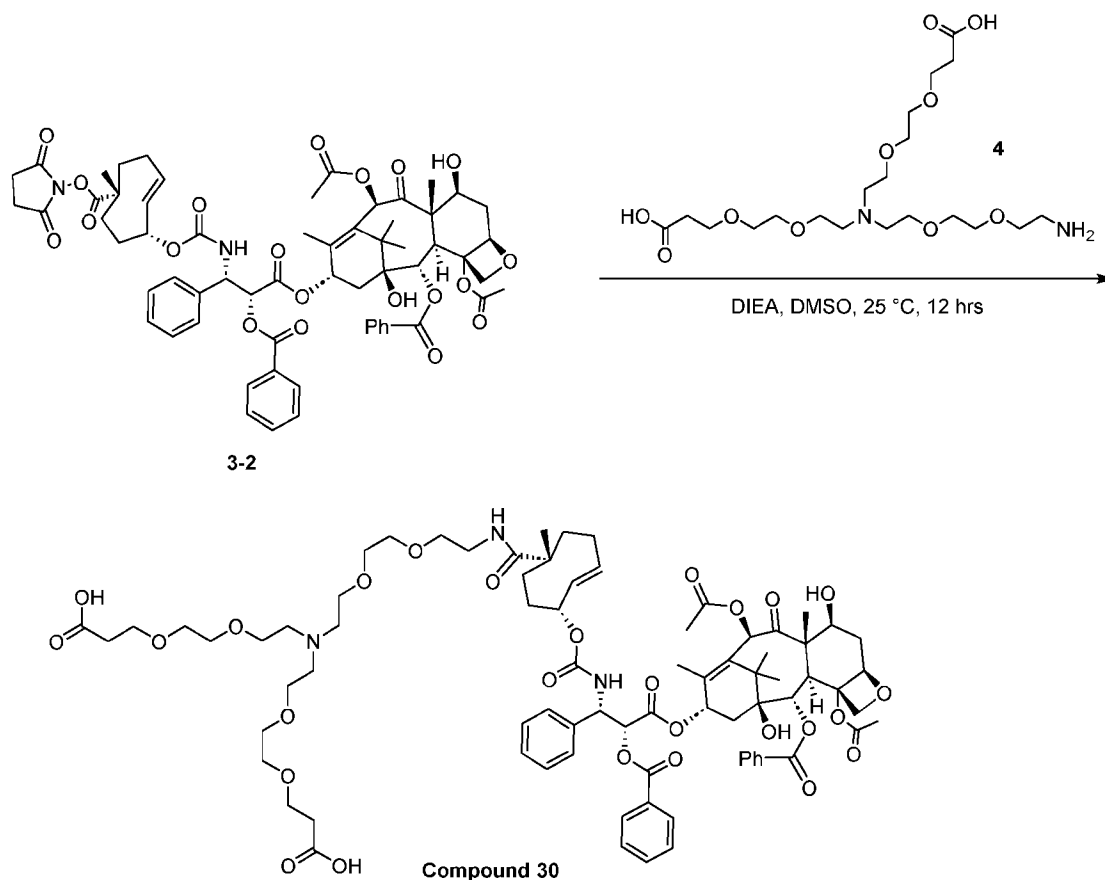


[0659] To a solution of compound **4-4** (6.00 g, 8.75 mmol, 1.00 eq.) in HCl/dioxane (60.0 mL). The mixture was stirred at 25 °C for 2hrs. LC-MS showed compound **4-4** was consumed completely and desired mass ($R_{t1} = 0.07$ min) was detected. The reaction mixture was concentrated under reduced pressure to give a residue. Compound **4** (4.50 g, crude, HCl) was obtained as yellow oil, confirmed by HNMR.

[0660] LCMS: $R_t = 0.07$ min, MS cal.: 468.2, MS observed: $[M+H]^+ = 469.3$.

[0661] ^1H NMR (400 MHz, CDCl_3) δ : 8.19 - 8.10 (m, 2 H), 4.56 - 3.88 (m, 10 H), 3.77 - 3.71 (m, 12 H), 3.66 - 3.62 (m, 12 H), 3.31 - 3.23 (m, 2 H), 2.60 - 2.57 (m, 2 H).

General procedure for preparation of Compound 30

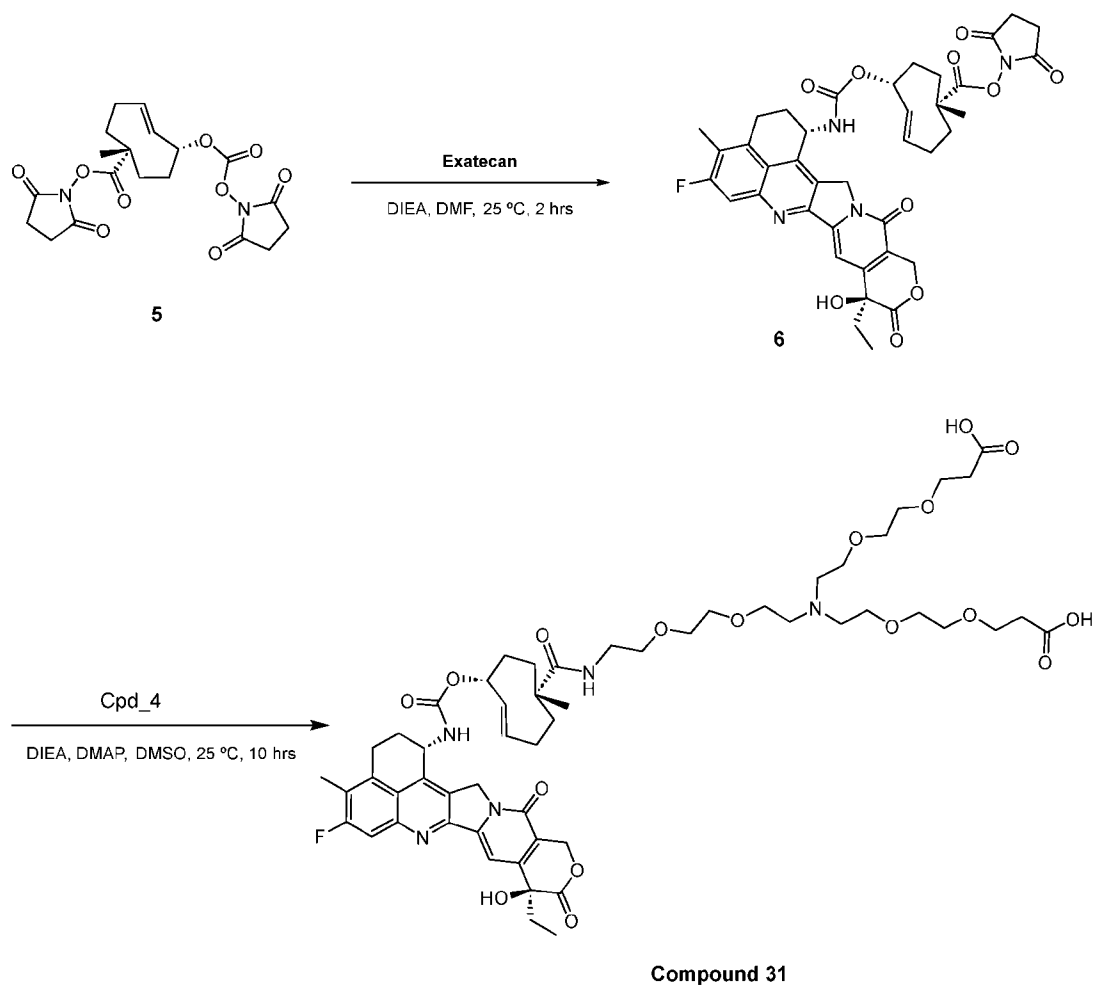


[0662] To a solution of compound 3-2 (310 mg, 266 μmol , 1.00 eq) in DMSO (0.50 mL) was added DIEA (69.0 mg, 533 μmol , 93.0 μL , 2.00 eq), DMAP (65.2 mg, 533 μmol , 2.00 eq) and compound 4 (250 mg, 533 μmol , 2.0 eq). The mixture was stirred at 25 °C for 12hrs. LC-MS showed 19.9% of compound 3-2 (R_t = 0.53 min) was remained and desired mass was detected (R_t = 0.43 min). The reaction mixture was used to purification directly. The residue was purified by prep-HPLC (AcOH condition). **Compound 30** (248 mg, 163 μmol , 47.2% yield, 95.5% purity) was obtained as a white solid, confirmed by LC-MS and HPLC.

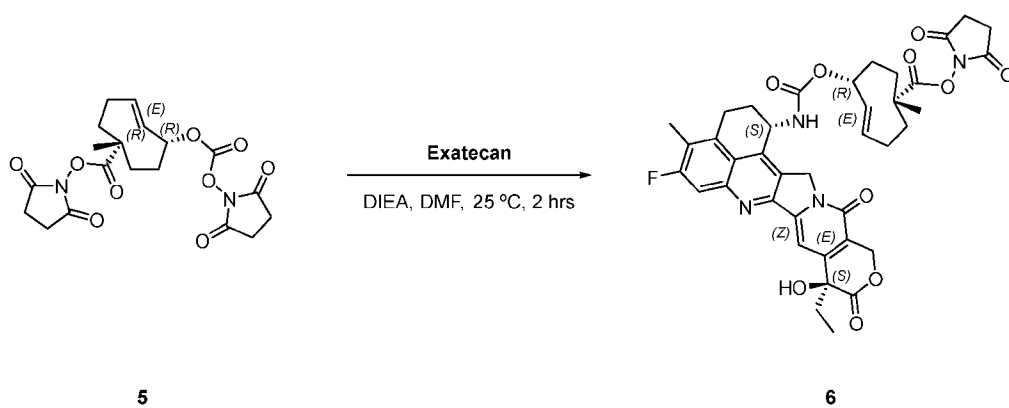
[0663] LCMS: R_t = 0.43 min, MS cal.: 1513.68, MS observed: $[M+H]^+$ = 1514.9.

[0664] HPLC: R_t = 3.47 min, purity: 95.5%.

Example 18: Synthesis of Compound 31



General procedure for preparation of compound 6

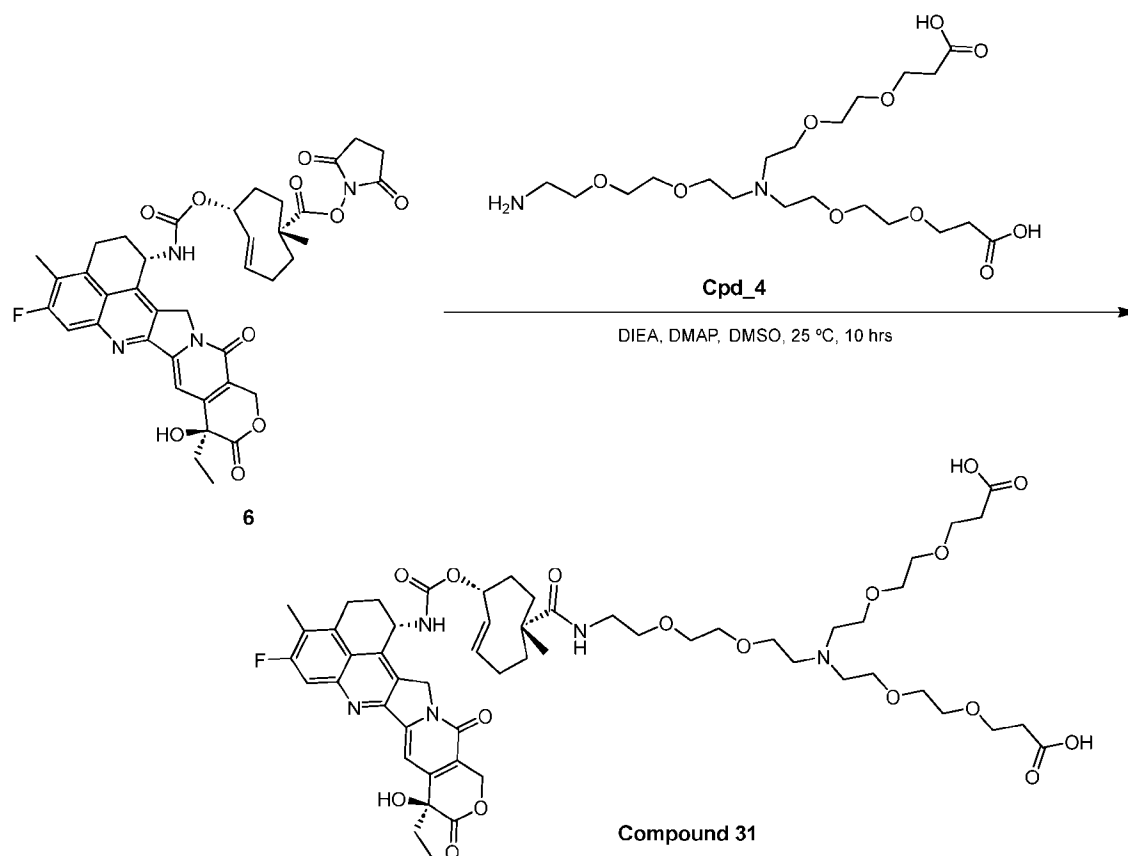


[0665] To a solution of compound 5 (2.00 g, 4.66 mmol, 1.00 eq.) in DMF (20.0 mL) was added DIEA (1.20 g, 9.32 mmol, 1.62 mL, 2.00 eq.) and Exatecan (1.86 g, 3.49 mmol, 0.75 eq.). The mixture was stirred at 25 °C for 2 hrs. LCMS showed compound **Exatecan** was consumed completely and desired mass ($R_t = 0.46$ min) was detected. The crude product was purified by reversed-phase HPLC (FA

condition). Compound **6** (2.1 g, 2.81 mmol, 60.4% yield, 99.5% purity) as a yellow solid which was confirmed via LCMS.

[0666] LCMS: $R_t = 0.46$ min, MS cal.: 742.3; MS observed: $[M+H]^+ = 743.4$

General procedure for preparation of Compound 31

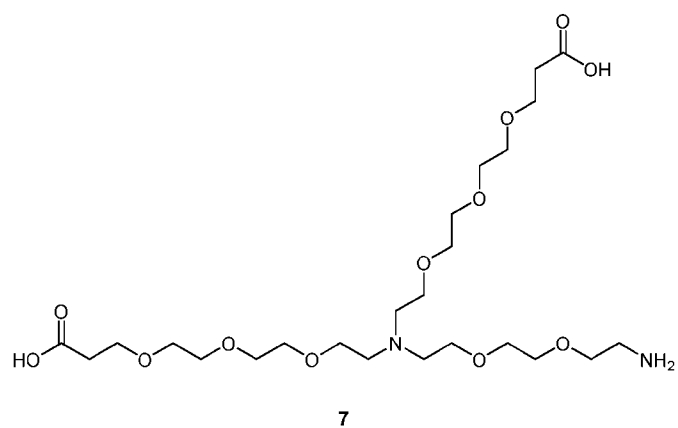
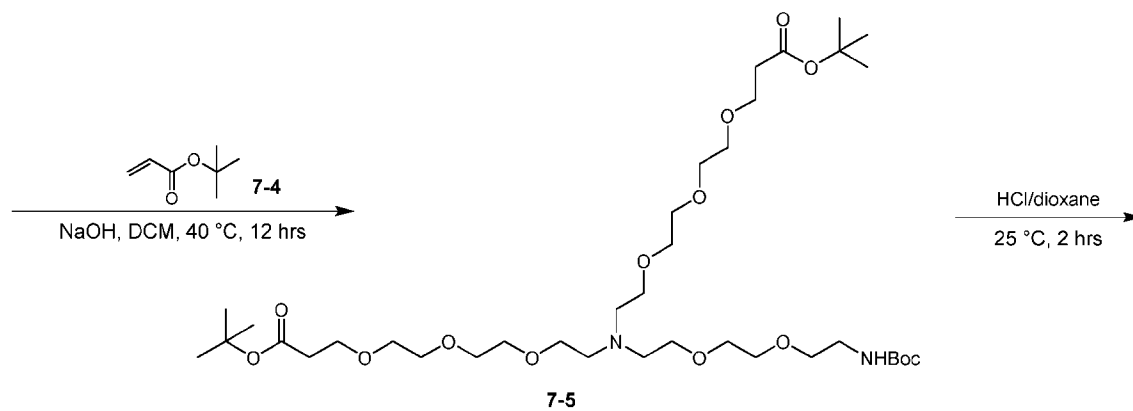
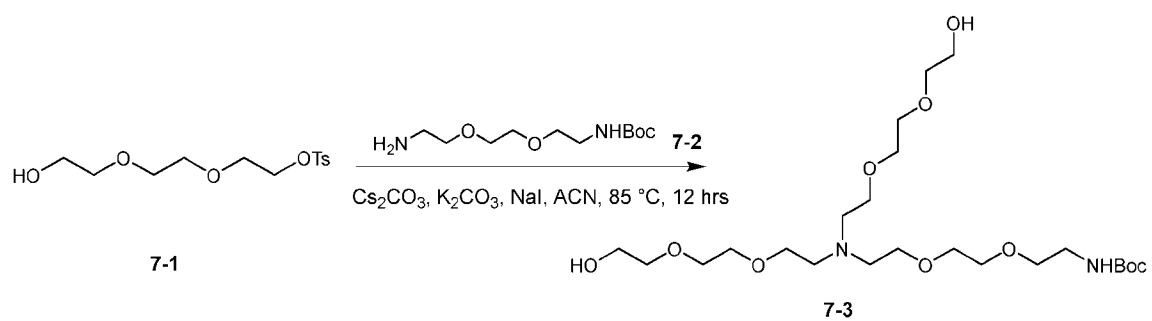


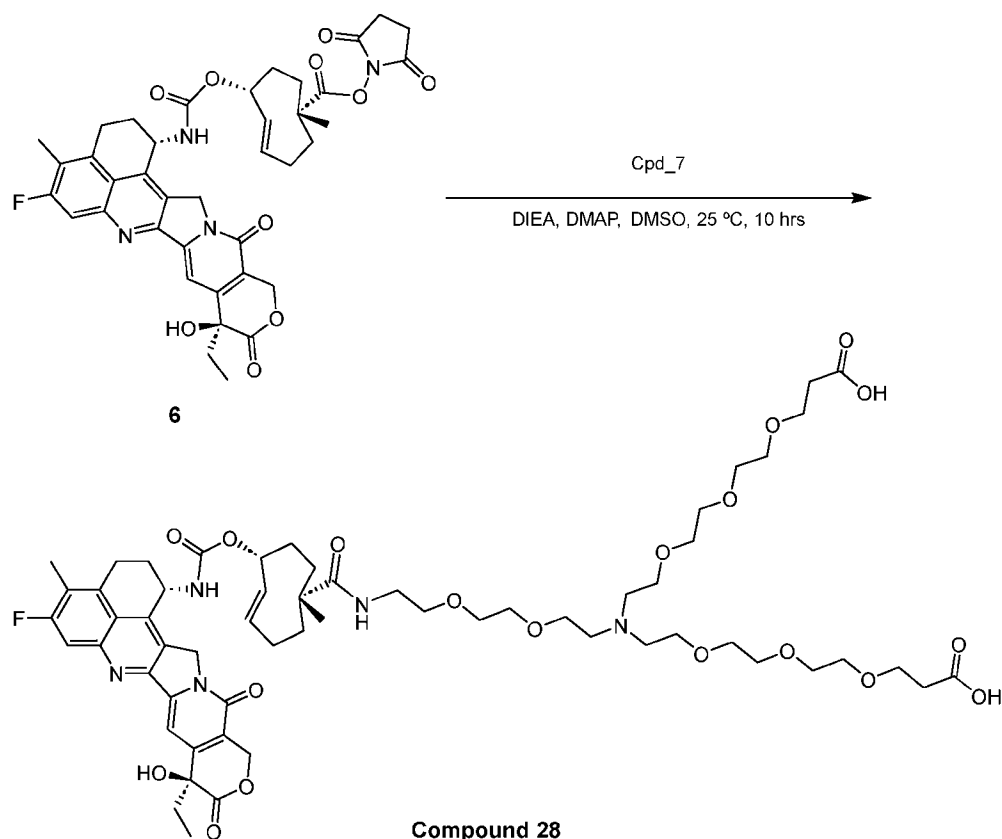
[0667] To a solution of compound **4** (as prepared in the above example) (1.37 g, 2.68 mmol, 2.00 eq.) and compound **6** (1.00 g, 1.34 mmol, 1.00 eq.) DMSO (10.0 mL) was added DIEA (346.3 mg, 2.68 mmol, 466.7 μ L, 2.00 eq.) and DMAP (327 mg, 2.68 mmol, 2.00 eq.). The mixture was stirred at 25 °C for 10 hrs. LCMS showed compound **6** was consumed completely and desired mass ($R_t = 0.39$ min) was detected. The mixed layers were filtered and the organic filtrate was separated. The crude product was purified by reversed-phase HPLC (AcOH condition). The crude product was purified by reversed-phase HPLC (FA condition). **Compound 31** (300 mg, 264 μ mol, 19.7% yield, 96.5% purity) as a yellow solid, confirmed via LCMS and HPLC.

[0668] LCMS: $R_t = 0.39$ min, MS cal.: 1095.5 MS observed: $[M+H]^+ = 1096.7$

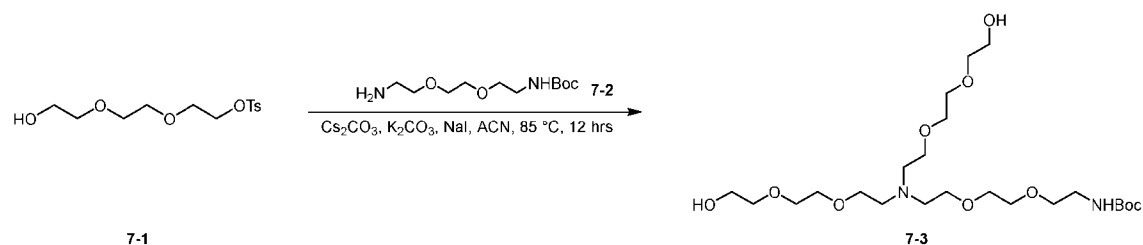
[0669] HPLC: $R_t = 2.50$ min purity: 96.5%

Example 19: Synthesis of Compound 28





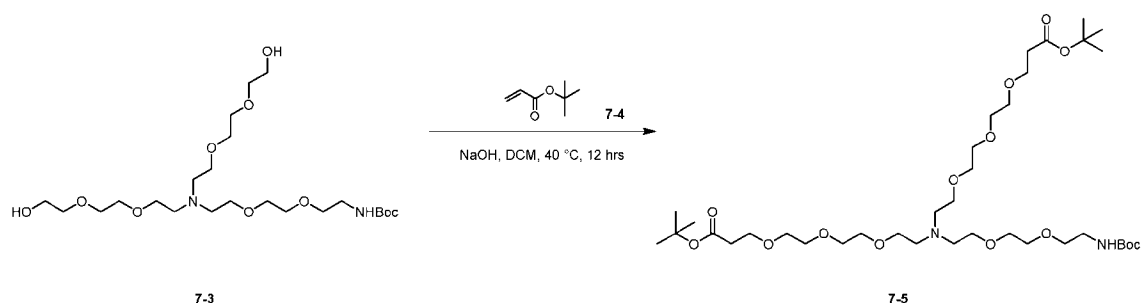
General procedure for preparation of compound 7-3



[0670] To a solution of compound **7-1** (11.0 g, 36.2 mmol, 3.00 eq.) and compound **7-2** (3.00 g, 12.0 mmol, 1.00 eq.) in ACN (120 mL) was added Cs_2CO_3 (1.18 g, 3.62 mmol, 0.30 eq.) and K_2CO_3 (5.01 g, 36.2 mmol, 3.00 eq.) and NaI (181 mg, 1.21 mmol, 0.10 eq.). The mixture was stirred at 85 °C for 12 hrs. LCMS showed compound **7-2** was consumed completely and one main peak with desired mass ($R_t = 0.28$ min) was detected. The residue was purified by column chromatography (SiO_2 , dichloromethane: methanol = 1: 0 to 0: 1, Dichloromethane: Methanol = 10: 1, $R_f = 0.20$). Compound **7-3** (6.60 g, 10.0 mmol, 82.8% yield, 77.7% purity) was obtained as yellow oil, confirmed by LCMS.

[0671] LCMS: $R_t = 0.27$ min, MS cal.: 512.3, MS observed: $[\text{M}+\text{H}]^+ = 513.3$

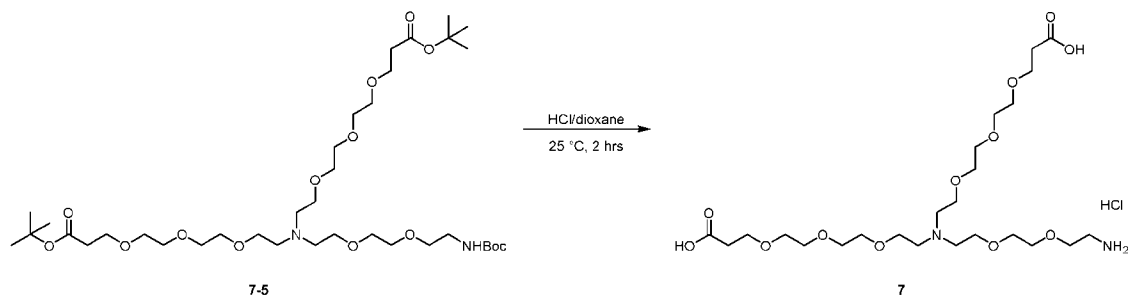
General procedure for preparation of compound 7-5



[0672] To a solution of compound **7-3** (6.60 g, 10.0 mmol, 1.00 eq.) in DCM (70.0 mL) was added NaOH (120 mg, 3.00 mmol, 0.30 eq.) and compound **7-4** (12.8 g, 100 mmol, 14.5 mL, 10.0 eq.). The mixture was stirred at 40 °C for 12 hrs. LCMS showed compound **7-3** was consumed completely and one main peak with desired mass ($R_t = 0.44$ min) was detected. The reaction mixture was diluted with NH_4Cl (20.0 mL) and extracted with DCM (30.0 mL $\times 2$). The combined organic layers were washed with brine (30.0 mL $\times 2$), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , dichloromethane: methanol = 1: 0 to 0: 1, dichloromethane: methanol = 10: 1, $R_f = 0.60$). Compound **7-5** (4.37 g, 5.58 mmol, 55.7% yield, 98.2% purity) was obtained as yellow oil, confirmed by LCMS.

[0673] LCMS: $R_t = 0.45$ min, MS cal.: 768.5, MS observed: $[\text{M}+\text{H}]^+ = 769.2$.

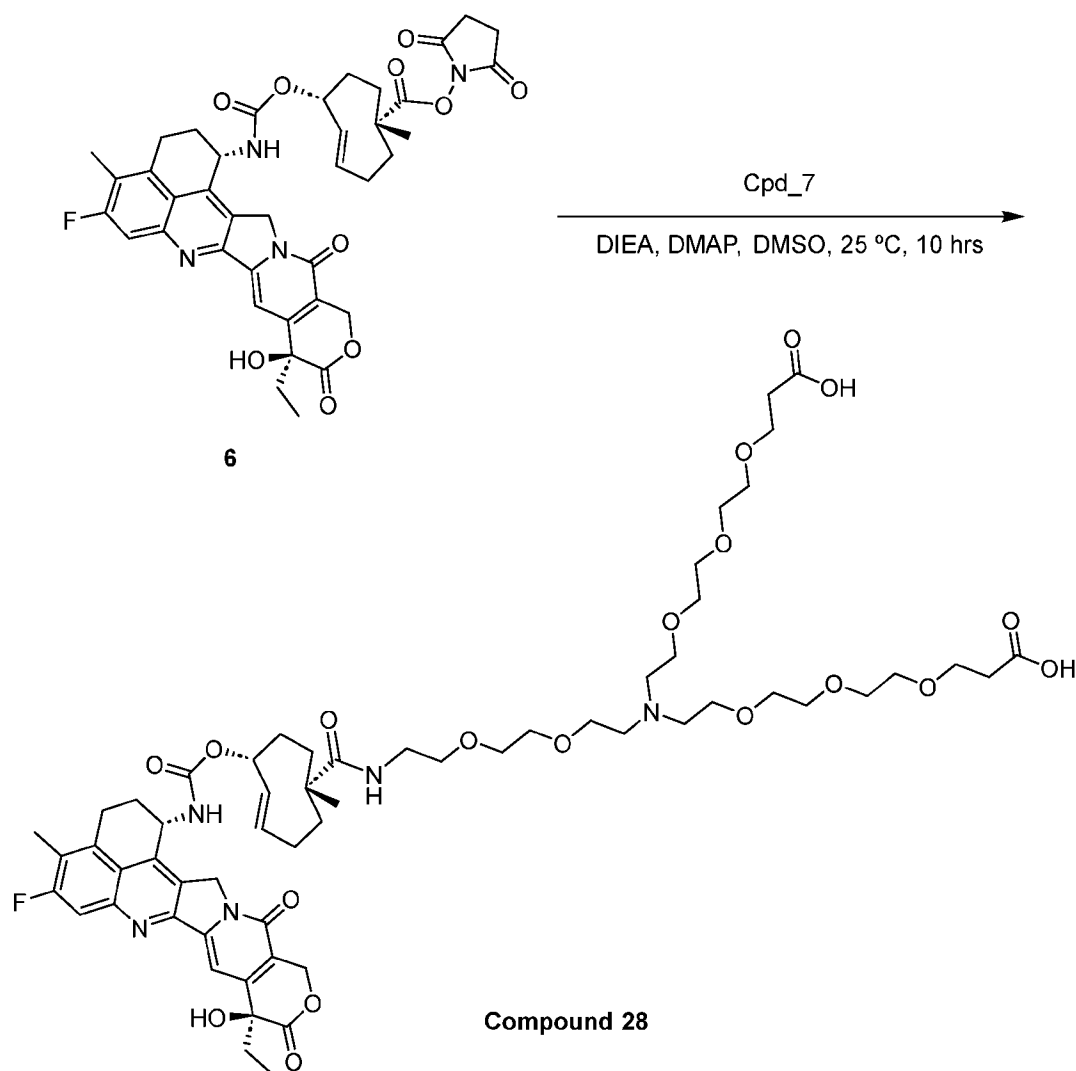
General procedure for preparation of compound 7



[0674] To a solution of compound **7-5** (2.04 g, 2.60 mmol, 1.00 eq.) in HCl/dioxane (20.0 mL). The mixture was stirred at 25 °C for 2 hrs. LC-MS showed compound **7-5** was consumed completely and three main peaks with desired mass ($R_{t1} = 0.13$ min) were detected. The reaction mixture was concentrated under reduced pressure to give a residue. Compound **7** (1.90 g, crude, HCl) was obtained as yellow oil, confirmed by HNMR.

[0675] LCMS: $R_t = 0.13$ min, MS cal.: 556.3, MS observed: $[\text{M}+\text{H}]^+ = 557.3$.

[0676] ^1H NMR (400 MHz, CDCl_3) δ : 7.96 (s, 2H), 4.03 - 3.88 (m, 8H), 3.76 - 3.50 (m, 32H), 3.26 - 3.25 (m, 2H), 2.65 - 2.59 (m, 4H).

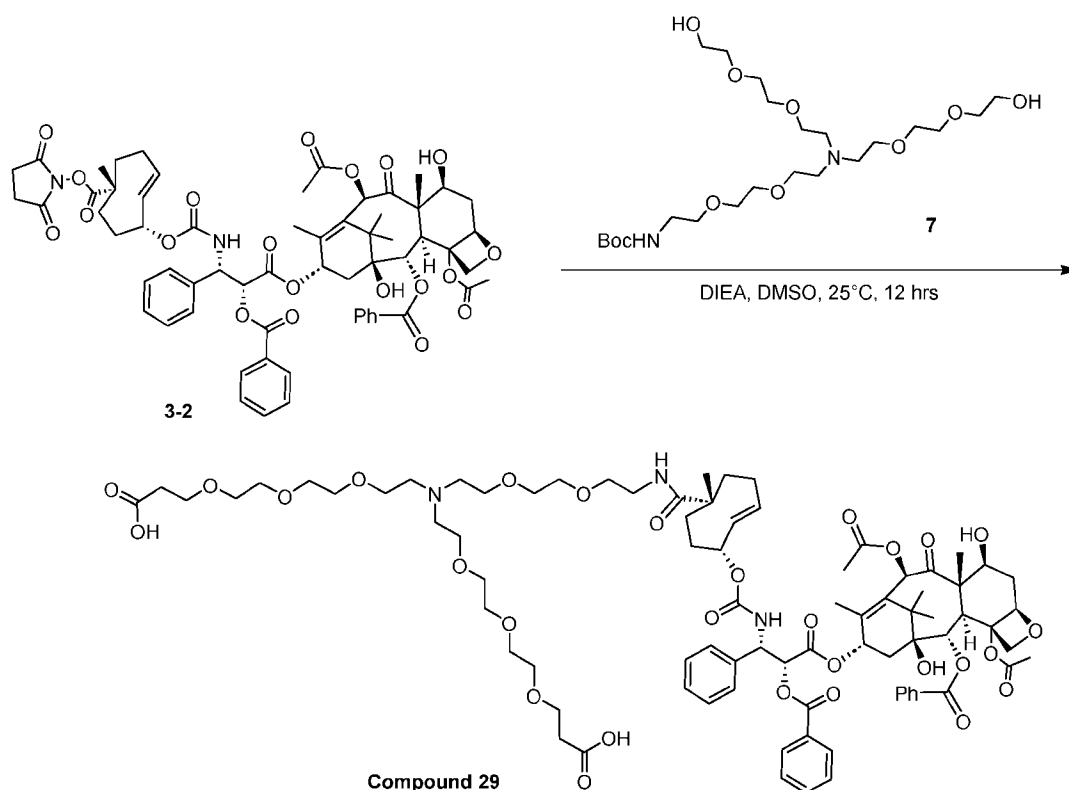


[0677] To a solution of compound **6** (500 mg, 669 μ mol, 1.00 eq.) and compound **7** (932 mg, 1.67 mmol, 2.50 eq.) in DMSO (5.00 mL) was added DIEA (173 mg, 1.34 mmol, 233.3 μ L, 2.00 eq.) and DMAP (163 mg, 1.34 mmol, 2.00 eq.). The mixture was stirred at 25 °C for 10 hrs. LCMS showed compound **6** was consumed completely and desired mass (R_t = 0.394 min) was detected. The mixed layers were filtered and the organic filtrate was separated. The crude product was purified by reversed-phase HPLC (AcOH condition). **Compound 28** (200 mg, 184 μ mol, 15.5% yield, 97.3% purity) as a yellow solid, confirmed via LCMS and HPLC.

[0678] LCMS: R_t = 0.39 min, MS cal.: 1083.6 MS observed: [M+H]⁺ = 1084.8

[0679] HPLC: $R_t = 2.55$ min, purity: 97.3%;

Example 20: Synthesis of Compound 29

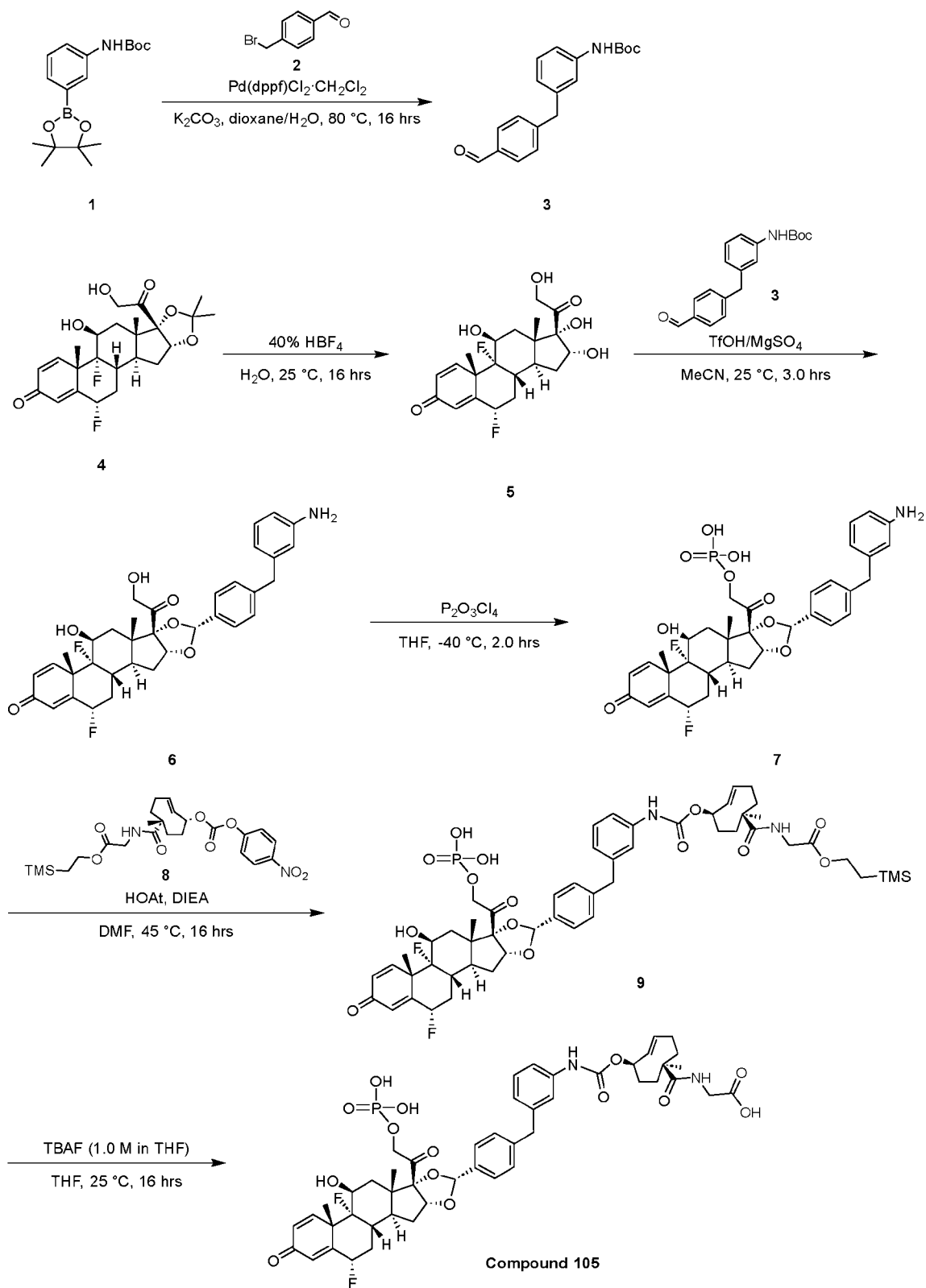


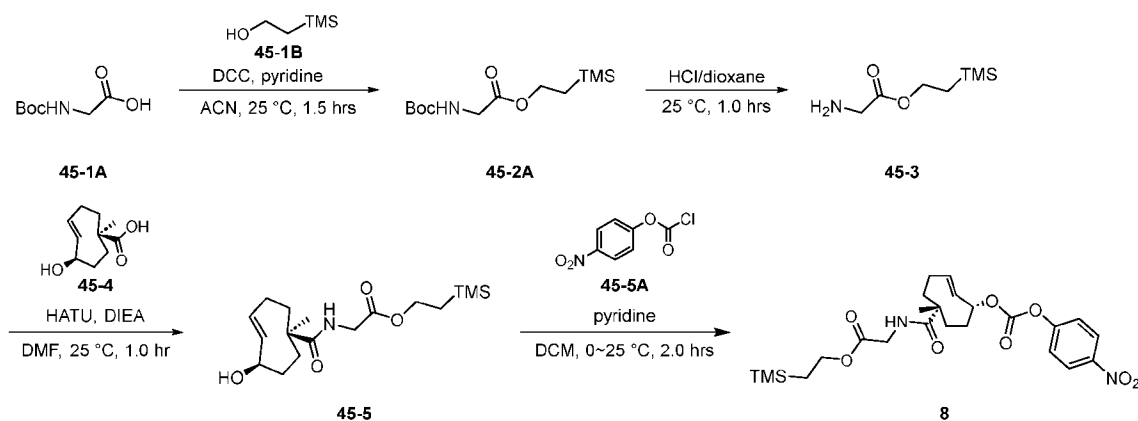
[0680] To a solution of compound **3-2** (398 mg, 342 μmol , 1.00 eq) in DMSO (0.50 mL) was added DIEA (88.5 mg, 685 μmol , 119 μL , 2.00 eq), DMAP (83.7 mg, 685 μmol , 2.00 eq) and compound **7** (381 mg, 685 μmol , 2.00 eq). The mixture was stirred at 25 °C for 12hrs. LC-MS showed compound **3-2** consumed completely and desired mass was detected ($R_t = 0.43$ min). The reaction mixture was used to purification directly. The residue was purified by prep-HPLC (AcOH condition). **Compound 29** (200 mg, 124 μmol , 35.7% yield, 95.3% purity) was obtained as a white solid, confirmed by LC-MS and HPLC.

[0681] LCMS: $R_t = 0.43$ min, MS cal.: 1601.73, MS observed: $[\text{M}+\text{H}]^+ = 1602.8$.

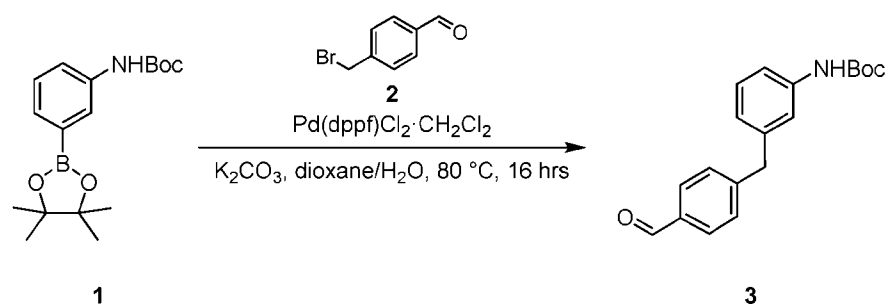
[0682] HPLC: $R_t = 3.50$ min, purity: 95.3%.

Example 21: Synthesis of Compound 105





General procedure for preparation of compound 3



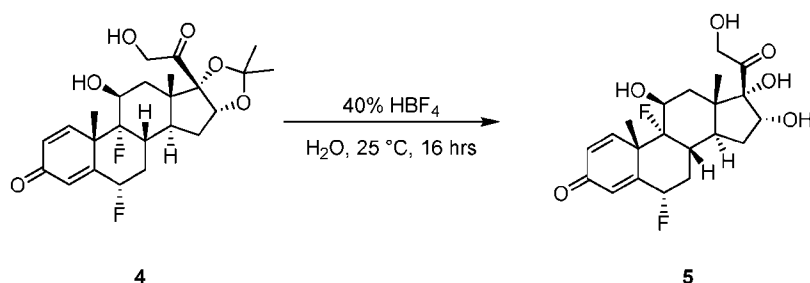
[0683] A mixture of compound **1** (22.0 g, 68.9 mmol, 1.0 eq.), compound **2** (15.0 g, 75.8 mmol, 1.1 eq.) and K₂CO₃ (28.5 g, 206 mmol, 3.0 eq.) in dioxane (220 mL) and H₂O (15 mL) was degassed and purged with N₂ for 3 times, and then Pd(dppf)Cl₂·CH₂Cl₂ (2.81 g, 3.45 mmol, 0.05 eq.) was added under N₂ atmosphere, purged with N₂ for 3 times, the mixture was stirred at 80 °C for 8.0 hrs under N₂ atmosphere. LCMS showed compound **1** was consumed completely and one main peak (*R*_t = 0.48 min) with desired mass (MS cal.: 311.1, MS observed: [M-tBu]⁺ = 256.1) was detected. The mixture was filtered and concentrated under reduced pressure to give the residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 30/1 to 10/1) to give compound **3** (19.6 g, 57.4 mmol, 83.2% yield, 90.7% purity) as a white solid, confirmed by HNMR, LCMS and HPLC.

[0684] LCMS: *R*_t = 0.48 min, MS cal.: 311.1, MS observed: [M-tBu]⁺ = 256.0.

[0685] HPLC: *R*_t = 3.21 min, purity: 90.7%.

[0686] ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.96 (s, 1 H), 9.28 (s, 1 H), 7.83 (d, *J* = 8.25 Hz, 2 H), 7.37 - 7.44 (m, 3 H), 7.26 (br d, *J* = 8.25 Hz, 1 H), 7.17 (t, *J* = 7.82 Hz, 1 H), 6.84 (d, *J* = 7.50 Hz, 1 H), 3.98 (s, 2 H), 1.45 (s, 9 H).

General procedure for preparation of compound 5

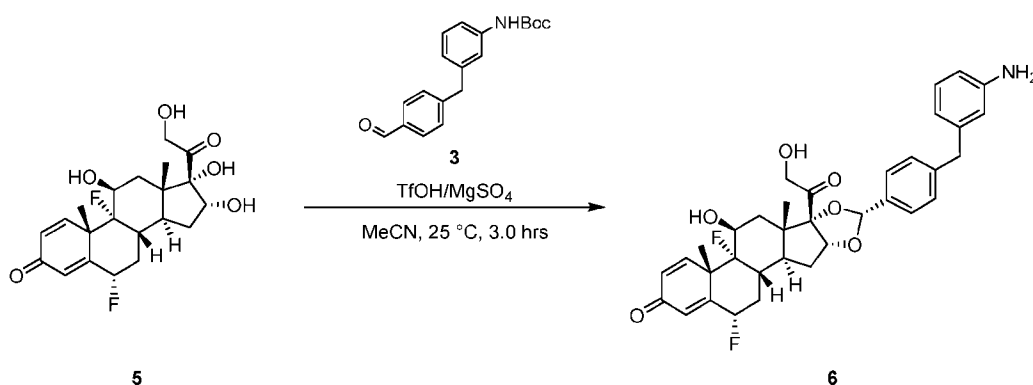


[0687] A mixture of compound **4** (45.0 g, 99.4 mmol, 1.0 eq.) in HBF_4 (654 g, 2.98 mol, 464 mL, 40% in H_2O , 30 eq.) was stirred at 25 °C for 16 hrs. LCMS showed compound **4** was consumed completely and main peak ($R_t = 0.31$ min) with desired mass (MS cal.: 412.1, MS observed: $[\text{M}+\text{H}]^+ = 413.0$) was detected. The reaction mixture was filtered, the filter was dried under reduced pressure to give compound **5** (39.0 g, crude) as a white solid, confirmed by LCMS and HPLC.

[0688] LCMS: $R_t = 0.31$ min, MS cal.: 412.1, MS observed: $[\text{M}+\text{H}]^+ = 413.0$.

[0689] HPLC: $R_t = 1.40$ min, purity: 97.4%.

General procedure for preparation of compound 6

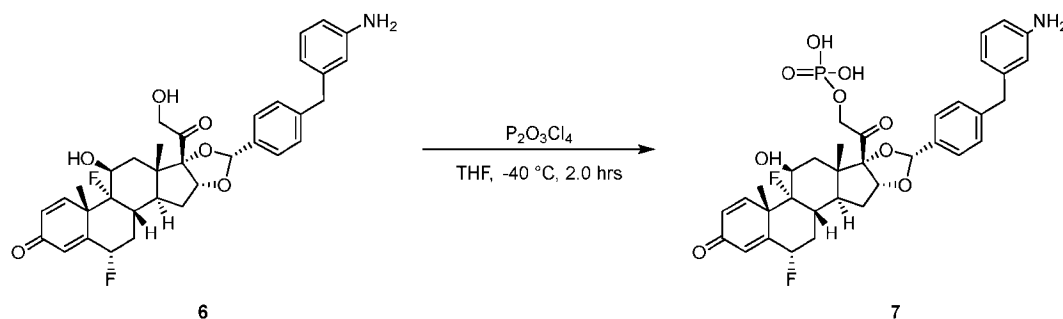


[0690] To a solution of compound **6** (5.00 g, 12.1 mmol, 1.0 eq.) and MgSO_4 (7.30 g, 60.6 mmol, 5.0 eq.) in ACN (100 mL) was stirred at 25 °C for 1.0 hr. Then a solution of compound **3** (4.15 g, 13.3 mmol, 1.1 eq.) in ACN (100 mL) was added, and then TfOH (9.10 g, 60.6 mmol, 5.36 mL, 5.0 eq.) was added dropwise to the mixture and keep temperature under 25 °C. The mixture was stirred at 25 °C for 3.0 hrs. LCMS showed compound **5** was consumed completely and main peak ($R_t = 0.37$ min) with desired mass (MS cal.: 605.2, MS observed: $[\text{M}+\text{H}]^+ = 606.3$) was detected. The mixture was filtered and concentrated under reduced pressure to give the residue. The residue was purified by prep-HPLC (TFA condition) to give compound **6** (3.74 g, 6.18 mmol, 50.9% yield, 91.6% purity) as a white solid, confirmed by LCMS and HPLC.

[0691] LCMS: $R_t = 0.36$ min, MS cal.: 605.2, MS observed: $[\text{M}+\text{H}]^+ = 606.3$.

[0692] HPLC: $R_t = 2.31$ min, purity: 91.9%.

General procedure for preparation of compound 7

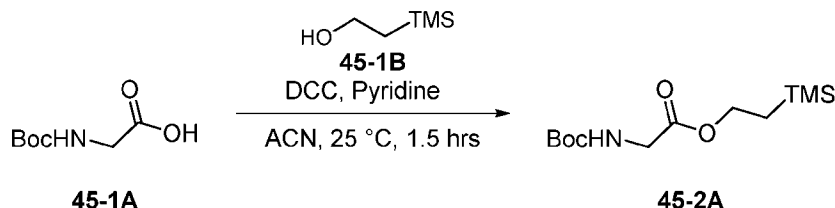


[0693] To a solution of compound **6** (2.40 g, 4.13 mmol, 1.0 eq.) in THF (12.5 mL) was cooled to -40 °C, and then added dropwise $\text{P}_2\text{O}_3\text{Cl}_4$ (11.3 g, 45.1 mmol, 6.25 mL, 10.9 eq.). The mixture was stirred at -40 °C for 2.0 hrs. LCMS showed compound **6** was consumed completely and one main peak ($R_t = 0.49$ min) with desired mass (MS cal.: 685.2, MS observed: $[\text{M}+\text{H}]^+ = 686.4$) was detected. The mixture was quenched with sat. NaHCO_3 under 0 °C and then lyophilized to give the crude product. The crude product was purified by prep-HPLC (NH_4HCO_3 condition) to give compound **7** (750 mg, 1.09 mmol, 26.5% yield, 99.1% purity) as a white solid, confirmed by LCMS and HPLC.

[0694] LCMS: $R_t = 0.50$ min, MS cal.: 685.2, MS observed: $[\text{M}-\text{H}]^+ = 684.3$.

[0695] HPLC: $R_t = 2.46$ min, purity: 99.1%.

General procedure for preparation of compound 45-2A

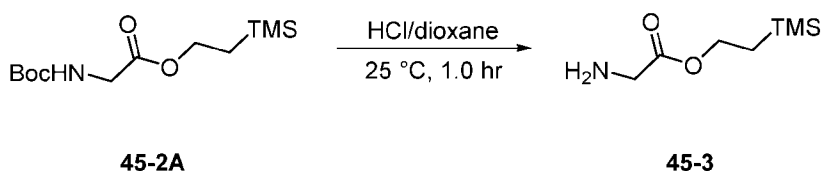


[0696] To a solution of compound **45-1A** (20.0 g, 114 mmol, 1.0 eq.) in ACN (120 mL) was added DCC (25.9 g, 125 mmol, 25.4 mL, 1.1 eq.), pyridine (27.1 g, 342 mmol, 27.6 mL, 3.0 eq.), and compound **45-1B** (14.8 g, 125 mmol, 18.0 mL, 1.1 eq.). The mixture was stirred at 25 °C for 3.0 hrs. TLC (Petroleum ether/Ethyl acetate = 3:1) indicated compound **45-1A** ($R_f = 0.20$) was consumed completely and one new spot ($R_f = 0.57$) was formed. The reaction mixture was diluted with H_2O (400 mL) and extracted with EtOAc (200 mL * 3). The combined organic layers were washed with sat. NaHCO_3 solution (200 mL) and brine (200 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 35 °C to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 20: 1 to 1: 3) to give compound **45-2A** (20.0 g, 72.6 mmol, 60.0% yield, 94.1% purity) as a yellow oil, confirmed by LCMS and HPLC.

[0697] LCMS: $R_t = 0.48$ min, MS cal.: 275.42, MS observed: $[\text{M}+\text{Na}]^+ = 298.0$.

[0698] HPLC: $R_t = 3.80$ min, purity: 94.1%.

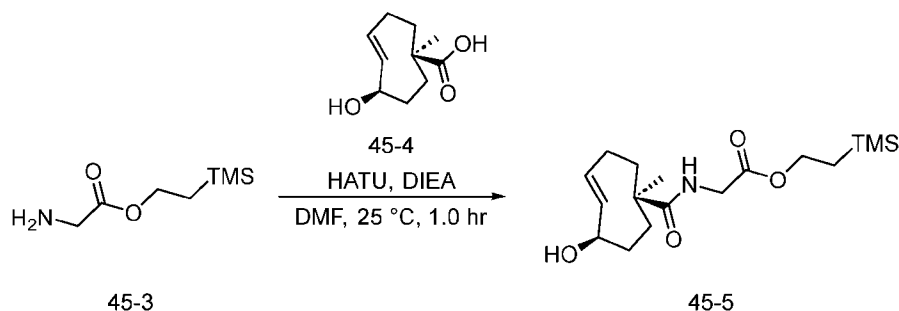
General procedure for preparation of compound 45-3



[0699] To a solution of compound **45-2A** (5.0 g, 18.1 mmol, 1.0 eq.) in HCl/dioxane (2.0 M, 10 mL). The mixture was stirred at 25 °C for 3.0 hrs. TLC (Petroleum ether/Ethyl acetate = 3:1) indicated compound **45-2A** ($R_f = 0.57$) was consumed completely and one new spot ($R_f = 0.21$) was formed. The reaction mixture was concentrated under vacuum to give compound **45-3** (4.0 g, crude) as a yellow oil, confirmed by LCMS.

[0700] LCMS: $R_t = 0.22$ min, MS cal.: 175.10, MS observed: $[M+Na]^+ = 198.3$.

General procedure for preparation of compound 45-5

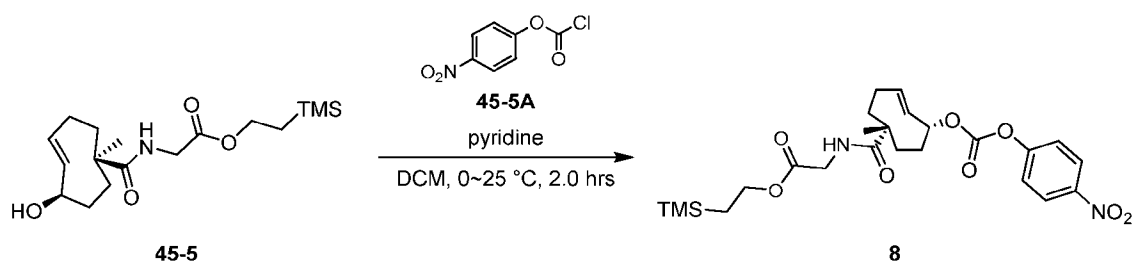


[0701] To a solution of compound **45-4** (1.7 g, 9.23 mmol, 1.0 eq.) in DMF (4.0 mL) was added DIEA (3.58 g, 27.7 mmol, 4.82 mL, 3.0 eq.) and HATU (4.21 g, 11.1 mmol, 1.2 eq.), finally added compound **45-3** (2.93 g, 13.8 mmol, 1.5 eq., HCl salt). The mixture was stirred at 25 °C for 2.0 hrs. LCMS showed one main peak with desired mass ($R_t = 0.42$ min) detected. The residue was purified by prep-HPLC (TFA condition) to give compound **45-5** (2.76 g, 7.73 mmol, 83.8% yield, 95.7% purity) as a white solid, confirmed by LCMS, HPLC.

[0702] LCMS: $R_t = 0.42$ min, MS cal.: 341.20, MS observed: $[M+Na]^+ = 364.1$.

[0703] HPLC: $R_t = 3.06$ min, purity: 99.5%.

General procedure for preparation of compound 8



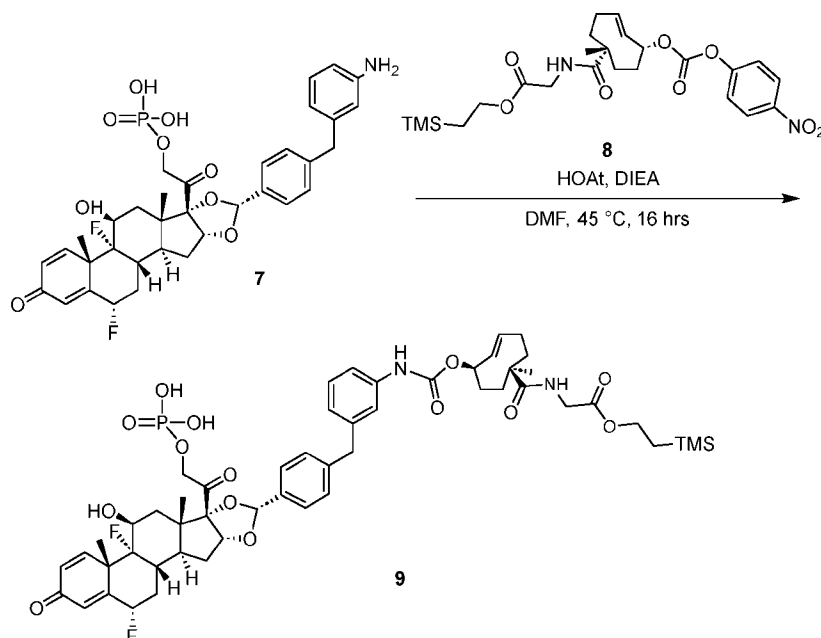
[0704] To a solution of compound 45-5 (1.30 g, 4.39 mmol, 1.0 eq.) in DCM (4.0 mL) was added pyridine (1.04 g, 13.1 mmol, 1.06 mL, 3.0 eq.) and cooled to 0 °C, then compound 45-4A (1.33 g, 6.59 mmol, 1.5 eq.) in DCM (4.0 mL) was added. The mixture was stirred at 25 °C for 4.0 hrs. LCMS showed one peak (R_t = 0.53 min) with desired mass (MS cal.: 506.2, MS observed: $[M+Na]^+$ = 529.0) was detected. The solvent was removed under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 30/1 to 1/1) to give compound **8** (1.7 g, 2.40 mmol, 54.5% yield, 94.4% purity) as a light yellow oil, confirmed by LCMS, HPLC, and HNMR.

[0705] LCMS: R_t = 0.53 min, MS cal.: 506.2, MS observed: $[M+Na]^+$ = 529.2.

[0706] HPLC: R_t = 4.27 min, purity: 94.4%.

[0707] 1H NMR (400 MHz, CHLOROFORM- d) δ : 8.25 - 8.32 (m, 2 H), 7.36 - 7.44 (m, 2 H), 6.00 - 6.14 (m, 2 H), 5.65 (dd, J =16.70, 2.06 Hz, 1 H), 4.21 - 4.29 (m, 2 H), 3.96 (dd, J =4.94, 1.06 Hz, 2 H), 2.28 - 2.42 (m, 2 H), 2.14 - 2.27 (m, 2 H), 1.94 - 2.03 (m, 2 H), 1.89 (br s, 1 H), 1.75 (dd, J =15.13, 6.25 Hz, 1 H), 1.18 (s, 3 H), 0.98 - 1.05 (m, 2 H), 0.04 (s, 9 H).

General procedure for preparation of compound 9

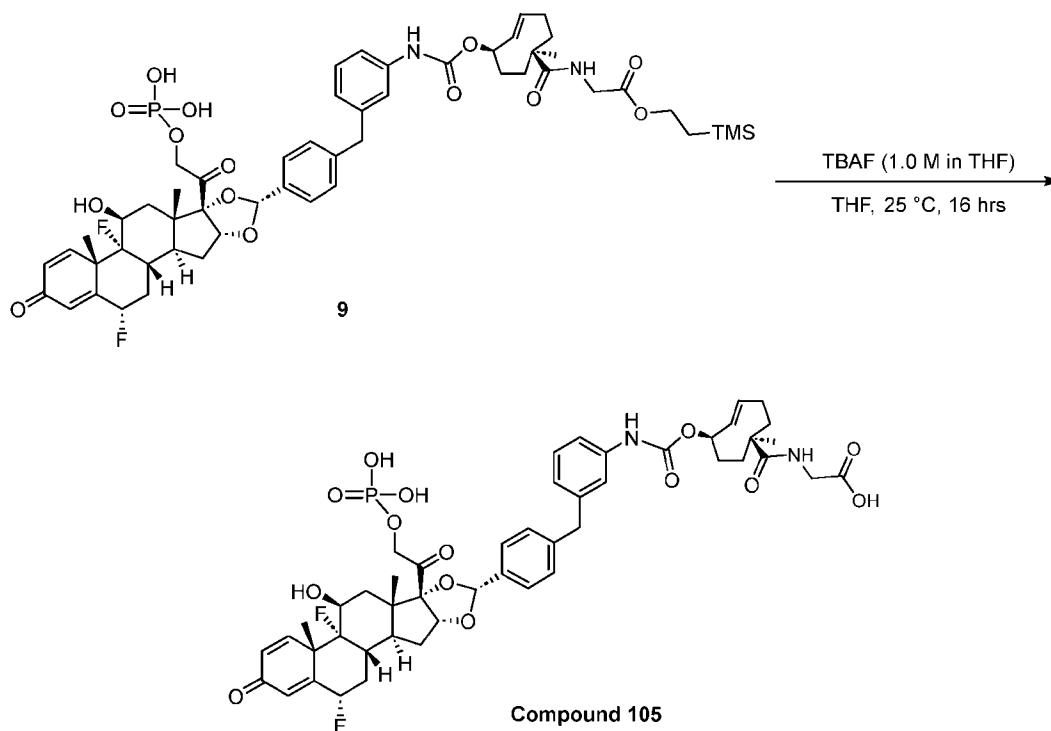


[0708] To a solution of compound **8** (1.62 g, 3.21 mmol, 2.0 eq.) and compound **7** (1.14 g, 1.60 mmol, 1.0 eq.) in DMF (15 mL) was added HOAt (655 mg, 4.81 mmol, 673 μ L, 3.0 eq.) and DIEA (829 mg, 6.42 mmol, 1.12 mL, 4.0 eq.). The mixture was stirred at 25 °C for 16 hrs. LCMS showed compound **8** was consumed completely and one main peak (R_t = 0.63 min) with desired mass (MS cal.: 1052.4, MS observed: $[M-H]^+$ = 1051.3) was detected. The mixture purified by prep-HPLC (NH_4HCO_3 condition) to give compound **9** (829 mg, 787 μ mol, 49.0% yield, 99.3% purity) as a white solid, confirmed by LCMS and HPLC.

[0709] LCMS: $R_t = 0.63$ min, MS cal.: 1052.4, MS observed: $[M-H]^+ = 1051.2$.

[0710] HPLC: $R_t = 3.72$ min, purity: 99.3%.

General procedure for preparation of Compound 105



[0711] To a solution of compound **9** (410 mg, 389 μ mol, 1.0 eq.) in THF (3.0 mL) was added TBAF (1.0 M, 1.95 mL, 5.0 eq.) under 0 °C. The mixture was stirred at 25 °C for 16 hrs. LCMS showed compound **9** was consumed completely and one main peak ($R_t = 0.48$ min) with desired mass (MS cal.: 952.3, MS observed: $[M-H]^+ = 951.5$) was detected. The second batch of compound **9** (455 mg) was carried out in parallel and combined to work-up and purification. The reaction mixture was diluted with DMF (5.0 mL) and purified by prep-HPLC (AcOH condition) to give **Compound 105** (210 mg, 220 μ mol, 26.8% yield, 99.3% purity) as a white solid, confirmed by LCMS, HPLC and FNMR.

Separation condition	
Dissolution condition	Dissolve in DMF
Instrument	Auno LC-2000
Mobile Phase	A: H ₂ O (0.5% AcOH in H ₂ O)
	B: CH ₃ CN
Gradient	30-60-40 min , Retention time: 27.7 min

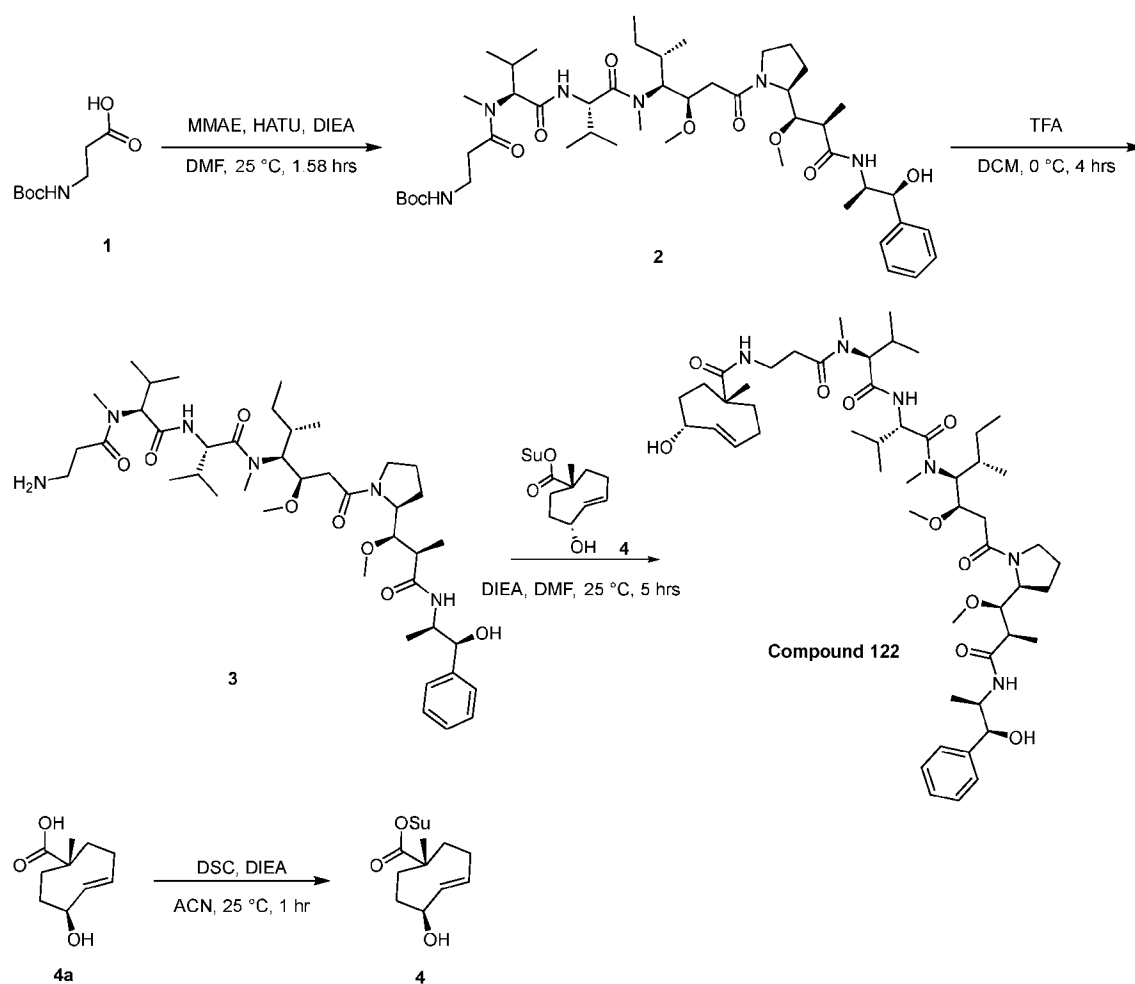
Column	Welch Ultimate® XB-C18, 250*50 mm, 7 μm, 120 Å
Flow Rate	60 mL/Min
Wavelength	220/254 nm
Oven Tem.	25 °C

[0712] LCMS: $R_t = 0.48$ min, MS cal.: 952.3, MS observed: $[M-H]^+ = 951.5$.

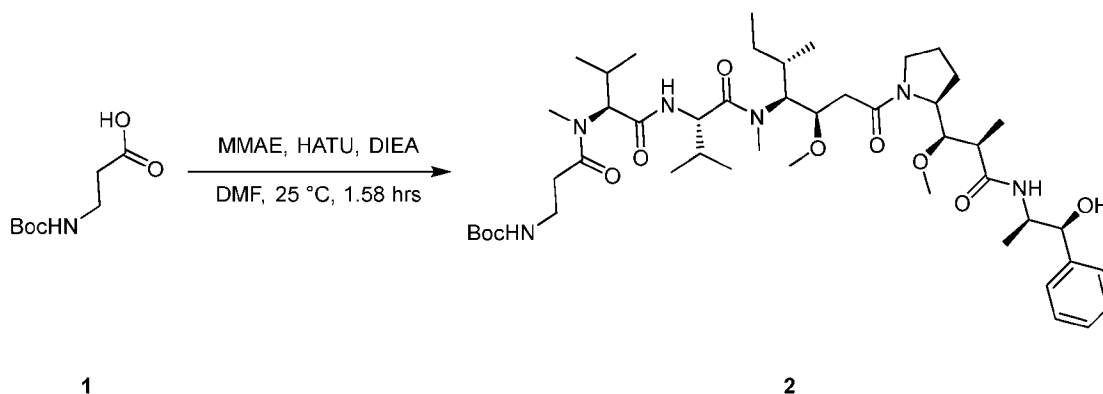
[0713] HPLC: $R_t = 2.34$ min, purity: 99.3%.

[0714] ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ : -164.95 (br s, 1 F), -186.36 (s, 1 F).

Example 22: Synthesis of Compound 122



General procedure for preparation of compound 2

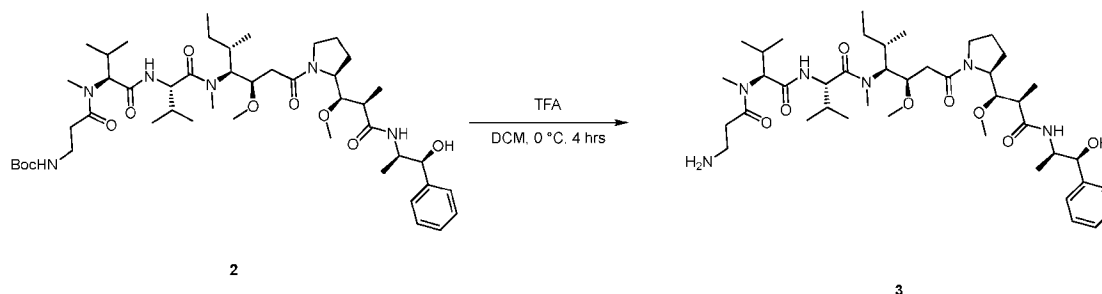


[0715] To a solution of compound **1** (289 mg, 1.53 mmol, 1.10 *eq.*) in DMF (10.0 mL) was added HATU (794 mg, 2.09 mmol, 1.50 *eq.*). The mixture was stirred at 25 °C for 0.08 hr. DIEA (540 mg, 4.18 mmol, 727 μ L, 3.00 *eq.*) and MMAE (1.00 g, 1.39 mmol, 1.00 *eq.*) was added. The mixture was stirred at 25 °C for 1.5 hrs. LCMS showed MMAE was consumed completely and the desired mass ($R_t = 0.50$ min) was detected. The reaction mixture was purified by prep-HPLC (TFA condition). Compound **2** (880 mg, 960 μ mol, 68.9% yield, 97.0% purity) was obtained as a white solid, confirmed by LMCS and HPLC.

[0716] LMCS: $R_t = 0.47$ min, MS cal.: 888.59, MS observed: $[M+H]^+ = 889.5$.

[0717] HPLC: $R_t = 3.39$ min, purity: 97.0%.

General procedure for preparation of compound 3

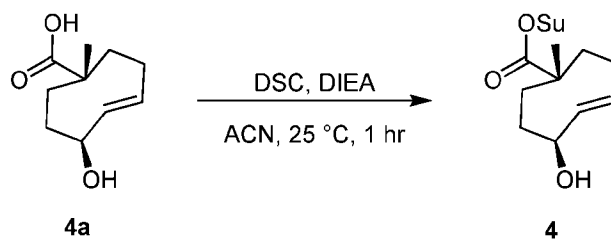


[0718] To a solution of compound **2** (0.88 g, 960 μ mol, 1.00 *eq.*) was added TFA (2.70 g, 23.6 mmol, 1.76 mL, 24.6 *eq.*) in DCM (7.04 mL). The mixture was stirred at 0 °C for 4 hrs. LCMS showed compound **2** was consumed completely and desired mass ($R_t = 0.35$ min) was detected. The reaction mixture was settled with isopropyl ether 140 mL* 3. Compound **3** (0.80 g, 701 μ mol, 73.0% yield, 79.2% purity, TFA) was obtained as a white solid, confirmed by LCMS and HPLC.

[0719] LCMS: $R_t = 0.37$ min, MS cal.: 788.54, MS observed: $[M+H]^+ = 789.7$.

[0720] HPLC: $R_t = 1.53$ min, purity: 79.2%.

General procedure for preparation of compound 4



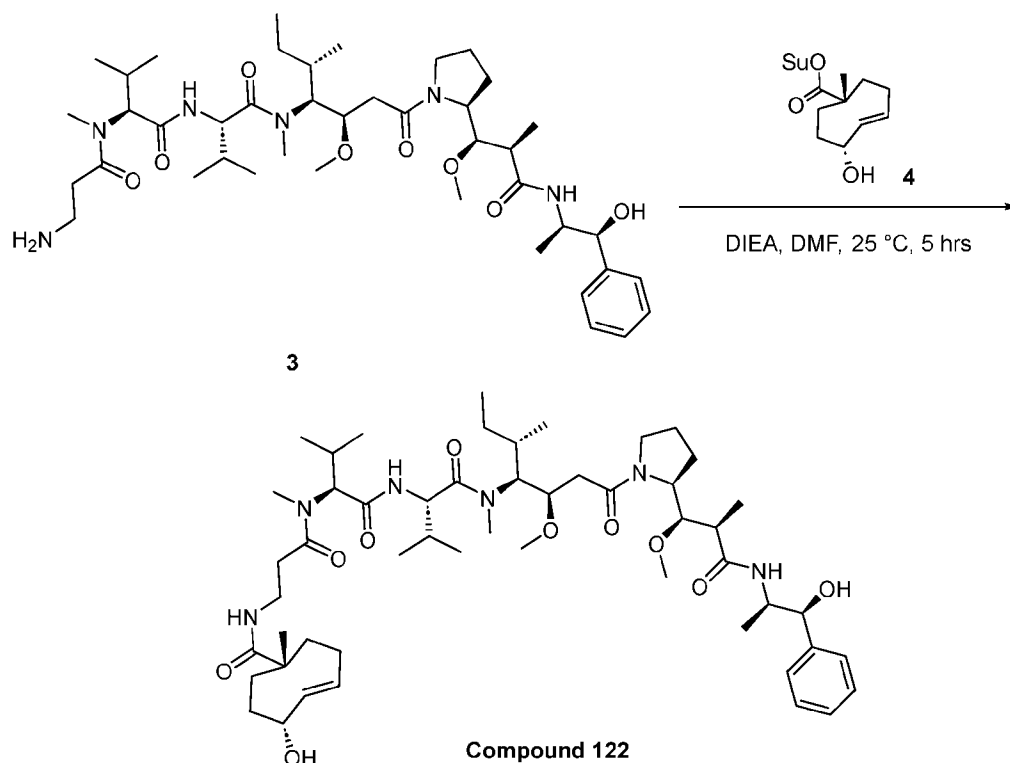
[0721] To a solution of compound **4a** (2.00 g, 10.8 mmol, 1.00 *eq.*) in ACN (10.0 mL) was added DIEA (4.21 g, 32.5 mmol, 5.67 mL, 3.00 *eq.*) and DSC (2.78 g, 10.8 mmol, 1.00 *eq.*) in ACN (10.0 mL). The mixture was stirred at 25 °C for 1 hr. TLC (dichloromethane: methanol = 10:1) indicated compound **4a** ($R_f = 0.35$) was consumed completely and two new spots ($R_f = 0.00, 0.50$) were formed. LCMS showed desired mass ($R_t = 0.32$ min) was detected. The reaction mixture was concentrated under reduced pressure to give a residue at 45 °C. The residue was purified by column chromatography (SiO₂, petroleum ether: ethyl acetate = 1: 0 to 1: 1, petroleum ether: ethyl acetate = 1:1, $R_f = 0.35$). Compound **4** (1.82 g, 6.18 mmol, 56.9% yield, 95.5% purity) was obtained as a light-yellow solid, confirmed by LCMS, HPLC and HNMR.

[0722] LCMS: $R_t = 0.44$ min, MS cal.: 281.13, MS observed: $[M+H]^+ = 304.0$.

[0723] HPLC: $R_t = 1.77$ min, purity: 95.5%.

[0724] ¹H NMR (400 MHz, CDCl₃) δ : 6.13 - 6.06 (m, 1H), 5.68 - 5.64 (m, 2H), 4.51 (s, 1H), 2.82 - 2.81 (m, 4H), 2.41 - 2.25 (m, 3H), 2.17 -1.98 (m, 3H), 1.93 - 1.83 (m, 2H), 1.25 (s, 3H)

General procedure for preparation of Compound 122



[0725] To a solution of compound **3** (0.80 g, 701 μmol , 1.00 *eq.*, TFA) in DMF (8.00 mL) was added compound **4** (206 mg, 701 μmol , 1.00 *eq.*) and DIEA (181 mg, 1.40 mmol, 244 μL , 2.00 *eq.*). The mixture was stirred at 25 °C for 5 hrs. LCMS showed compound **3** was consumed completely and desired mass ($R_t = 0.45$ min) was detected. The reaction mixture was purified by prep-HPLC (AcOH condition). **Compound 122** (peak 1, 210 mg, 202 μmol , 28.9% yield, 98.1% purity, AcOH) was obtained as a white solid, confirmed by LCMS, HPLC, and HRMS.

[0726] Note: Two peaks of **Compound 122** were obtained after prep HPLC.

Compound ID	Quantity	Purity (@220 nm)	Salt
122	210 mg peak 1	98.1%	AcOH
122	10 mg peak 2	85.6% (10.3% peak 1)	AcOH
	19 mg peak 2	64.8% (32.8% peak 1)	AcOH

[0727] The spectra of **Compound 122**_peak 1 was showed as below.

[0728] LCMS: $R_t = 0.46$ min, MS cal.: 954.64, MS observed: $[\text{M}+\text{H}]^+ = 955.8$. HRMS: $R_t = 1.80$ min, MS cal.: $[\text{M}+\text{H}]^+ 955.6478$, MS observed: $[\text{M}+\text{H}]^+ = 955.6491$. HPLC: $R_t = 351$ min, purity: 98.1%.

[0729] The compounds of Table A-1 and B-1 can be or were prepared according to the procedures described herein using the appropriate starting materials.

[0730] Therapeutic support compositions as described herein can be prepared as described in WO2018/187740. Methods for testing and using the conjugates in combination with the support compositions can likewise be found in WO2018/187740.

Example 23: Efficacy Evaluation of Compound 32 as a Single Agent and in Combination with SQT01 in the NCI-N87 Human Gastric Tumor Xenograft Model

[0731] Study Objective: The purpose of this study was to evaluate the anti-tumor activity of Compound 32 as a single agent and in combination with SQT01 in the NCI-N87 human gastric tumor xenograft model. A significant endpoint was tumor growth inhibition.

Experimental Design

Table 4.1 Groups and Treatments for Tumor Efficacy Study

Group	N	First IV treatment=0hr	Schedule (Days)	Second IV treatment=18hr	Schedule (Days)
1	6	Vehicle (PBS)	QDx1 (D1)	Vehicle (10% HPβCD) (Day 2)	QDx1 (D2)
2	6	50 mg/kg SQT01	QDx1 (D1)	60 mg/kg Compound 32	QDx1 (D2)

Note: For the group 2, First IV time point is 0hr, second IV first time point is 8hr post first IV

[0732] Materials: Animals and Housing Condition

Animals

Species/strains:	Athymic Nude (Athymic nudes (CrI:NU(NCr)-Foxn1 ^{nu}))
Physiological state:	Normal
Age range at time of inoculation:	7 weeks
Animal supplier:	Charles River (Wilmington, MA)
Number/sex of animals:	12/Female
Identification:	Transponder

Test Articles

Test Article – SQT01

Identity and lot number ^P :	SQT01 (McFarland, et al. <i>ACS Central Science</i> 2023 , 9 (7), 1400-1408)
Physical description:	Pink liquid
Source:	Shasqi, Inc. (San Francisco, CA)
Storage conditions:	4°C, protected from light
Stability/expiration date:	Not provided

Disposition of unused test article:	Unused test article was retained at TD2 and stored at 4°C (protected from light) until shipment to Sponsor.
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Test Article/Vehicle Mixture – SQT01

Dosage form:	Light pink solution
Dosage preparation/storage:	Stock test article (55.53 mg/mL) was diluted with the appropriate amount of sterile PBS (Corning, Inc.; Manassas, VA) and thoroughly mixed to obtain a final concentration of 5 mg/mL to deliver a dose of 50 mg/kg in a 10 mL/kg dose volume. Stock test article and dosing mixture were protected from light.
Stability/expiration date:	Made fresh prior to dosing
Disposition of unused dosing mixture:	Unused dosing mixture was disposed of at TD2

Administration of Test Agent – SQT01

Route and method of administration:	Intravenous Administration (IV)
Frequency and duration of dosing:	Single Dose (Day 1)
Administered doses:	50 mg/kg
Administered volume(s):	10 mL/kg

Test Article – Compound 32

Identity	Compound 32
Physical description:	Powder
Source:	Shasqi, Inc. (San Francisco, CA)
Storage conditions:	-20°C, protected from light
Stability/expiration date:	Not provided
Disposition of unused test article:	Unused test article was retained at TD2 and stored at -20°C (protected from light) until shipment to Sponsor.

Test Article/Vehicle Mixture – Compound 32

Dosage form:	Light yellow solution
Dosage preparation/storage:	The appropriate amount of test article was dissolved into 10% HPβCD (Sigma Aldrich; St. Louis, MO) to obtain final concentrations of 3 mg/mL and 6 mg/mL to deliver doses of 30 mg/kg and 60 mg/kg, respectively, in a 10 mL/kg dose volume. Dosing mixture was protected from light and vortexed until the solution was free of particulates. Solution was then filtered through a 0.22 mm filter (Merck Millipore; Burlington, MA) and dosed

	immediately after formulating. Prior to study start, TD2 performed a test formulation of Compound 32 at 6 mg/mL to ensure solubility. The test preparation resulted in a slightly yellow solution free of particulates, and a picture of the dosing mixture was taken and sent to Sponsor. Sponsor approved proceeding with the formulation described above.
Stability/expiration date:	Prepared fresh prior to dosing
Disposition of unused dosing mixture:	Unused dosing mixture was disposed of at TD2

Administration of Test Agent – Compound 32

Route and method of administration:	Intravenous Administration (IV)
Frequency and duration of dosing:	Single dose administered on Day 2, approximately 18 hours after Day 1 dosing
Administered doses:	60mg/kg
Administered volume(s):	10 mL/kg

Vehicle Control 1 - PBS

Identity and lot number:	Sterile phosphate-buffered saline (PBS), Lot # 14223006
Physical description:	Liquid
Source:	Corning (Manassas, VA)
Storage conditions:	Room temperature
Disposition of unused test article:	Unused test article was disposed of at TD2

Administration of Vehicle Control 1 -PBS

Route and method of administration:	Intravenous Administration (IV)
Frequency and duration of dosing:	Single Dose (Day 1)
Administered doses:	0 mg/kg
Administered volume(s):	10 mL/kg

Vehicle Control 2 - 10% HPβCD

Identity and lot number:	10% HPβCD Lot #: BCCG1707
Physical description:	10% 2-Hydroxypropyl-beta-cyclodextrin (HPβCD) in Sterile Water for Injection (SWFI)

Source:	Sigma Aldrich; St. Louis, MO (HPβCD) Braun Medical; Bethlehem, PA (SWFI)
Storage conditions:	Room temperature
Disposition of unused test article:	Unused test article was disposed of at TD2

Administration of Vehicle Control 2 - 10% HPβCD

Route and method of administration:	Intravenous Administration (IV)
Frequency and duration of dosing:	Single dose administered on Day 2, approximately 18 hours after Day 1 dosing
Administered doses:	0 mg/kg
Administered volume(s):	10 mL/kg

Experimental Methods and Procedures

Cell line:	NCI-N87
Number of cells injected:	1x10 ⁷ cells/mouse
Cell growth media:	Roswell Park Memorial Institute Media (RPMI 1640; Life Technologies; Grand Island, NY) + 5% Fetal Bovine Serum (FBS; Omega Scientific; Tarzana, CA) + 1% Penicillin-Streptomycin (Life Technologies; Grand Island, NY)
% CO ₂	5%
Seeding Density:	8.75x10 ⁶ cells/Flask
Inoculation Site:	Subcutaneous Right Flank
Inoculation Vehicle:	50% Matrigel (Corning; Manassa, VA) / 50% Media in 0.1mL
Subcutaneous tumor size calculation:	Tumor volume (mm ³) = (a x b ² /2) where 'b' is the smallest diameter and 'a' is the largest diameter
Study initiation:	Group mean tumor size of 135 mm ³
Randomization method:	Random equilibration of tumor volume
Treatment initiation:	On Day 1, According to Table 1 below
Data Collection:	Tumor volume measured twice weekly; body weights measured daily for the first week, then twice weekly thereafter; gross observations daily
Study Endpoint(s) ^P :	The study ended on Day 37 and all remaining mice were euthanized.

[0733] Results: Body Weight change and Tumor Growth Curve: Body weight changes and tumor growth curves are shown in Figure 1 and Figure 2. During this experiment, no obvious body weight loss was observed among all groups.

[0734] Results Summary: Mice body weight and body weight changes were summarized in Figure 1 and Figure 2. All mice were in good condition, and all treatments were well tolerated by tumor-bearing CrTac:NCr-Foxn1nu mice with no obvious body weight loss observed in each group during treatment days, which indicated the drug can be well tolerated by animals.

[0735] Tumor volumes in different groups are shown in Figure 2 Group 2 (SQT01+ Compound 32) shows anti-tumor effects when compared to the vehicle group.

Example 24: In-Vitro Cell Viability Assessment

Study Objective

[0736] The objective of this study was to evaluate the anti-proliferation effect of test compounds, Compound 32 and Compound 33, in 5 cancer cell lines (Calu-3, HCC827, MDA-MB-468, NCI-N87, and T47D) with or without a tetrazine reagent. Deruxtecan, Exatecan, and Staurosporine were included as positive controls. This study was performed based on internal standards.

Study Design

[0737] The plating of cells and compound treatments are shown below in the plate map.

Table 5.1. Plate map for 3 compounds tested:

	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, culture medium only	Compound 32 plus DMSO, top concentration: 10 μM, 9 points, 3-fold serial dilution, triplicate									Vehicle control, cells with DMSO only	PBS
C												PBS
D												PBS
E		Compound 32 plus Tetrazine reagent, top concentration: 1 μM, 9 points, 3-fold serial dilution, triplicate										PBS
F												PBS
G												PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

	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, culture medium only	Compound 33 plus DMSO, top concentration: 10 μM, 9 points, 3-fold serial dilution, triplicate									Vehicle control, cells with DMSO only	PBS
C												PBS
D												PBS
E		Compound 33 plus Tetrazine reagent, top concentration: 1 μM, 9 points, 3-fold serial dilution, triplicate										PBS
F												PBS
G												PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

Table 5.2. Plate map for Positive controls and Tetrazine only:

	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, culture medium only	Dxd, top concentration: 1 μ M, 9 points, 3-fold serial dilution, triplicate									Vehicle control, cells with	PBS
C												PBS
D												PBS
E		Exacetan, top concentration: 1 μ M, 9 points, 3-fold serial dilution, triplicate									DMSO only	PBS
F												PBS
G												PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

	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, culture medium only	Staurosporine, top concentration: 10 μ M, 9 points, 3-fold serial dilution, triplicate									Vehicle control, cells with	PBS
C												PBS
D												PBS
E		Tetrazine only, top concentration: 1 μ M, 9 points, 3-fold serial dilution, triplicate									DMSO only	PBS
F												PBS
G												PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

[0738] Cancer cells were maintained in culture conditions at 37°C in an atmosphere with 0% or 5% CO₂ in air. The tumor cells were routinely subcultured. The cells growing in an exponential growth phase were counted by haemocytometer with Trypan blue staining. After counting cell concentration was adjusted to proper cell density.

Cell line	Seeding density (96-well plate)
Calu-3	4000
HCC827	2500
MDA-MB-468	6000
NCI-N87	8000
T47D	4000

[0739] 90 μ L of cell suspension was plated into the assay plates according to the plate map as well as 90 μ L of assay medium into the Blank wells. The plate was incubated at 37°C, 0% or 5% CO₂, 95% air and 100% relative humidity overnight.

Compound Stock Plate Preparation

[0740] For each test compound, equal volumes of 20 mM compound solutions in DMSO were mixed with DMSO or 20 mM tetrazine in DMSO. The solutions were aged at ambient temperature protected from light for 15-20 minutes. This solution was designated as 10 mM compound stock. Preparation of compound stock plate (1000X stock plates): The stock solutions were Serially diluted from highest concentration down to lowest in DMSO according to the plate map (table 4). These were prepared fresh for use.

Table 5.4. Plate layout (μ M) of 1000X stock.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10000	3333. 3	1111.1	370. 3	123.4	41.1	13.7	4.57	1.52	Test compounds;		
B	1000	333.3	111.1	37.0	12.3	4.12	1.37	0.46	0.15	Test compounds plus Tetrazine		
C	10000	3333. 3	1111.1	370. 3	123.4	41.1	13.7	4.57	1.52	Staurosporine		
D	1000	333.3	111.1	37.0	12.3	4.12	1.37	0.46	0.15	Exatecan and Dxd		
E	1000	333.3	111.1	37.0	12.3	4.12	1.37	0.46	0.15	Tetrazine only		

[0741] For 10X concentrate compound plate preparation, 198 μ L of assay medium was added into each well of the V-bottom plate; then 2 μ L of the stock compound solution of each concentration from the 500X stock plate was transferred. This is followed by the addition of 2 μ L of DMSO into the Blank and Control wells.

[0742] For the Compound Treatment, 10 μ L of the compound-medium of each well from the 10X concentrate compound plate was added into the cells in 96-well assay plate according to the plate map. 10 μ L of the DMSO-medium was added into the Blank and Control wells. The final DMSO concentration was 0.1%. The assay plate into incubator and incubated for 3 days.

[0743] From this point, the procedures were performed according to the Promega CellTiter-Glo Luminescent Cell Viability Assay Kit manual (Promega-G7573).

[0744] The test articles are summarized in the table below.

Table 5.5. Summary of Test Articles

Compound ID	Exact Mass/MW	Purity/%	Amount/mg
Compound 32	1286	97.3	7

Compound 33	1449.5884	100	5
Dxd (Deruxtecan)	493.4914	98.0	5
Exatecan	435.45	99.53	5
Staurosporine	466.53	98.50	50

[0745] All compounds were stored at -20 C. A summary of the cell viability assay results are shown in Table 5.6.

Table 5.6. Summary of Cell Viability in Each Group

		IC50 (μM)				
		Calu-3	HCC827	MDA-MB-468	NCI-N87	T47D
Compound 32	+DMSO	0.350	0.429	0.130	0.069	0.023
	+Tz	0.056	0.115	0.023	0.013	0.005
	Fold	6.3	3.73	5.7	5.3	5
Compound 33	+DMSO	>10	>10	9.154	4.388	1.545
	+Tz	0.047	0.072	0.021	0.009	0.004
	Fold	>213	>139	436	488	386
DXd		0.053	0.077	0.027	0.010	0.004
Exatecan		0.010	0.014	0.007	0.002	0.001
Staurosporine		0.007	0.008	0.021	0.026	0.208
Tetrazine		>1	>1	>1	>1	>1

[0746] Overall, the data indicated that Compound 32 and Compound 33 pre-treated with tetrazine reagent inhibited the cell proliferation more effectively than the compound groups without tetrazine reagent pre-treatment. IC50 values of Compound 32 pre-treated with tetrazine reagent ranged from 0.005 to 0.115 μM and IC50 values of Compound 32 without tetrazine reagent pre-treatment ranged from 0.023 to 0.429 μM in all 5 cell lines; IC50 values of Compound 33 pre-treated with tetrazine reagent ranged from 0.004 to 0.072 μM in all 5 cell lines, and IC50 values of Compound 33 without tetrazine reagent pre-treatment were 9.154, 4.388 and 1.545 μM respectively in MDA-MB-468, NCI-N87 and T47D cells and more than 10 μM both in Calu-3 and HCC827 cells.

[0747] Unless otherwise defined, 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.

[0748] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the

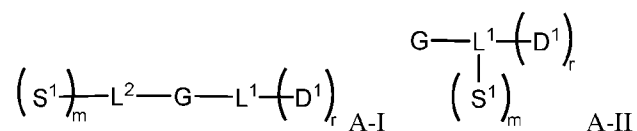
terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[0749] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0750] It is to be understood that while the disclosure has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the disclosure. Other aspects, advantages and modifications within the scope of the disclosure will be apparent to those skilled in the art to which the disclosure pertains.

What is claimed is:

1. A conjugate of Formula A-I or Formula A-II, or a pharmaceutically acceptable salt thereof:



wherein:

m is an integer from 1-10;

r is 1 or 2;

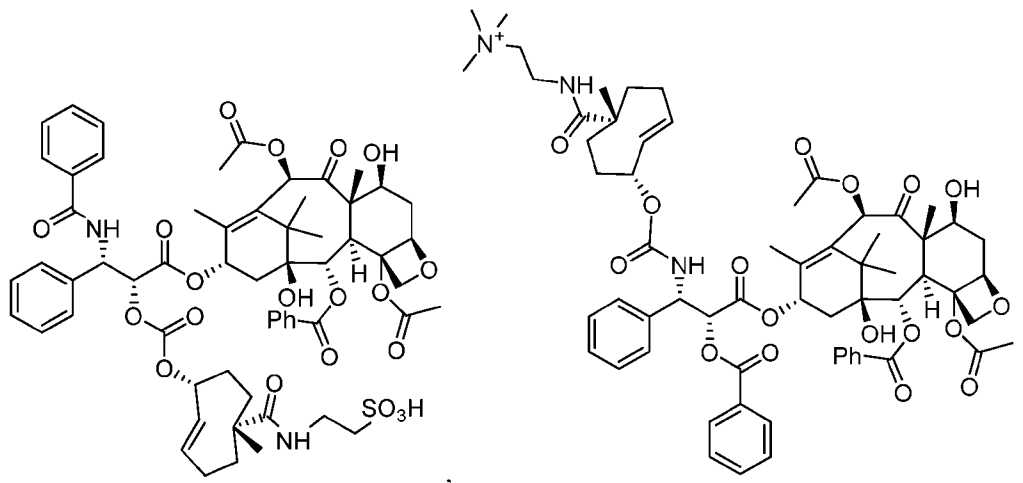
each D^1 is independently a taxane, a topoisomerase inhibitor, or a MMAE payload, or a derivative, or analog thereof;

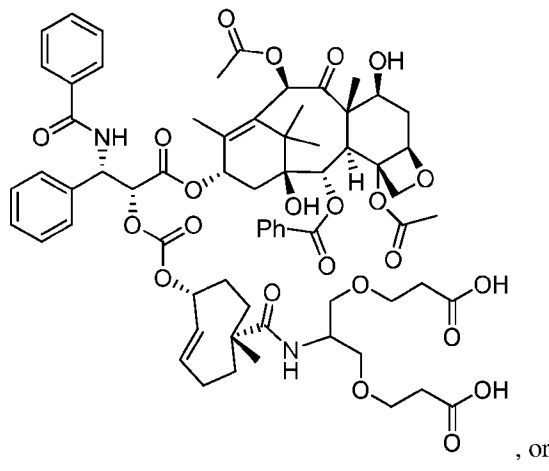
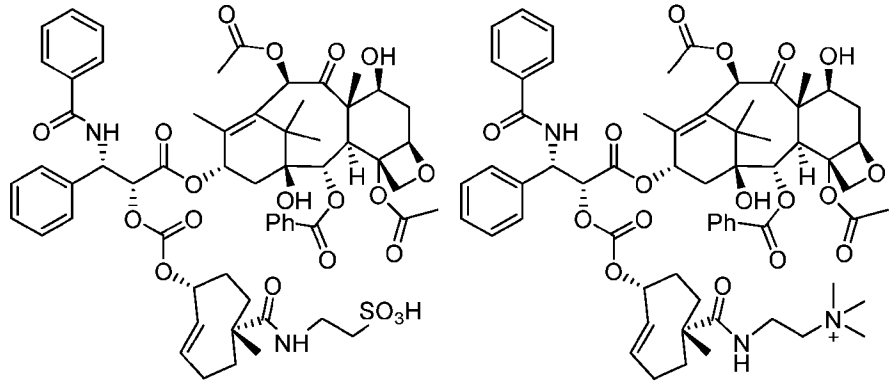
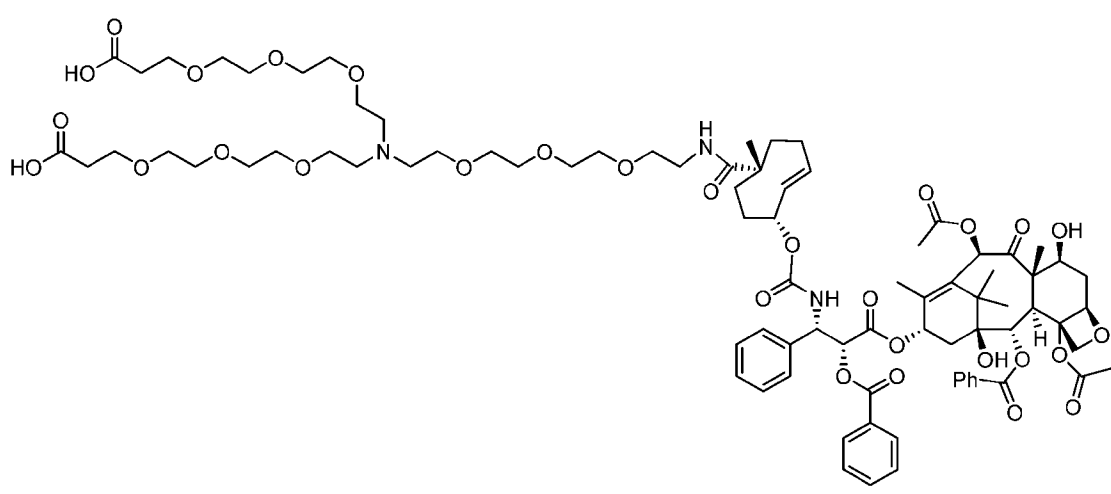
L^1 and L^2 are each independently a linker;

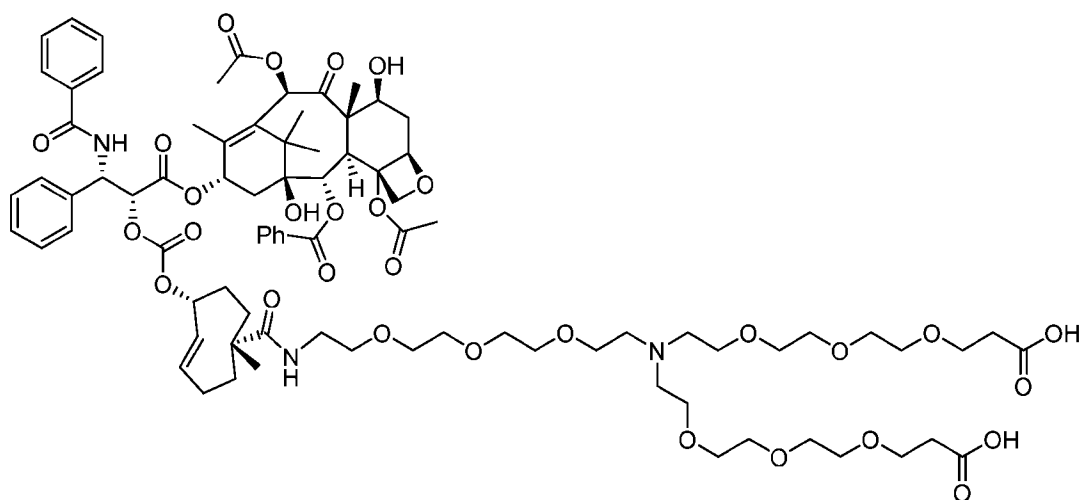
G an optionally substituted trans-cyclooctene moiety; and

each S^1 is independently a solubilizing group;

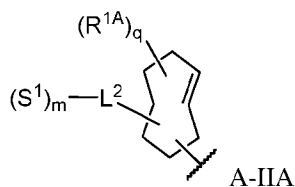
provided the compound is not:







2. The conjugate of claim 1, wherein the moiety $\left(\text{S}^1 \right)_m \text{L}^2 - \text{G} -$ is of Formula A-IIA:



wherein:

q is 0, 1, or 2;

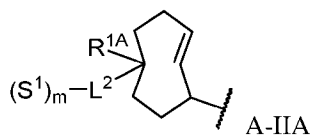
m is an integer from 1-10;

R^{1A}, at each occurrence, is independently selected from the group consisting of C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy;

L^2 is a linker; and

each S^1 is independently a solubilizing group.

3. The conjugate of claim 1 or 2, wherein the moiety $(S^1)_m-L^2-G$ is of Formula A-IIA:



wherein:

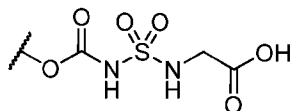
m is an integer from 1-10;

R^{1A} is selected from the group consisting of C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy;

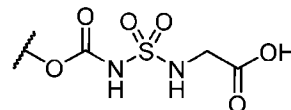
L^2 is a linker; and

each S^1 is independently a solubilizing group.

4. The conjugate of any preceding claim, wherein each S^1 is independently selected from the group consisting of $-\text{NHC}(\text{NH})\text{NH}_2$, $-\text{P}(\text{O})(\text{OH})_2$, $-\text{S}(\text{O})_2\text{OH}$, $-(\text{OCH}_2\text{CH}_2)_{30-85}-\text{OCH}_3$, $-\text{N}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{OH})_2$, or



5. The conjugate of any preceding claim, wherein each S^1 is independently selected from



$-\text{NHC}(\text{NH})\text{NH}_2$, $-\text{P}(\text{O})(\text{OH})_2$, $-\text{S}(\text{O})_2\text{OH}$, $-\text{N}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{OH})_2$, or

6. The conjugate of any preceding claim, wherein L^1 is $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $-\text{NR}^{1f}\text{C}(\text{O})-$, or $-\text{C}(\text{O})\text{NR}^{1f}-$; and

R^{1f} is hydrogen, C_{1-6} alkyl, or C_{0-4} alkylene- CO_2H .

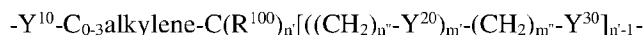
7. The conjugate of any preceding claim, wherein L^1 is $-\text{OC}(\text{O})-_{aa}$ or $-\text{NHC}(\text{O})-_{aa}$, where bond aa is attached to D^1 .

8. The conjugate of any preceding claim, wherein L^2 is a linear or branched heteroalkylene linker.

9. The conjugate of any preceding claim, wherein m is 1.

10. The conjugate of any preceding claim, wherein m is 2.

11. The conjugate of any preceding claim, wherein L^2 is:



wherein:

each Y^{10} , Y^{20} , and Y^{30} is independently a bond, $-\text{NR}^{110}-$, $-\text{O}-$, $-\text{S}(\text{O})_{0-2}-$, $-\text{NR}^{110}\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{NR}^{110}-$, $-\text{NR}^{110}\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{NR}^{110}-$, $-\text{CR}^{120}=\text{N}-\text{NR}^{110}-$, $-\text{NR}^{110}-\text{N}=\text{CR}^{120}-$, $-\text{C}(\text{O})-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{O}-$, alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene is independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl;

each R^{100} is independently hydrogen, $-\text{C}(\text{O})\text{OH}$, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

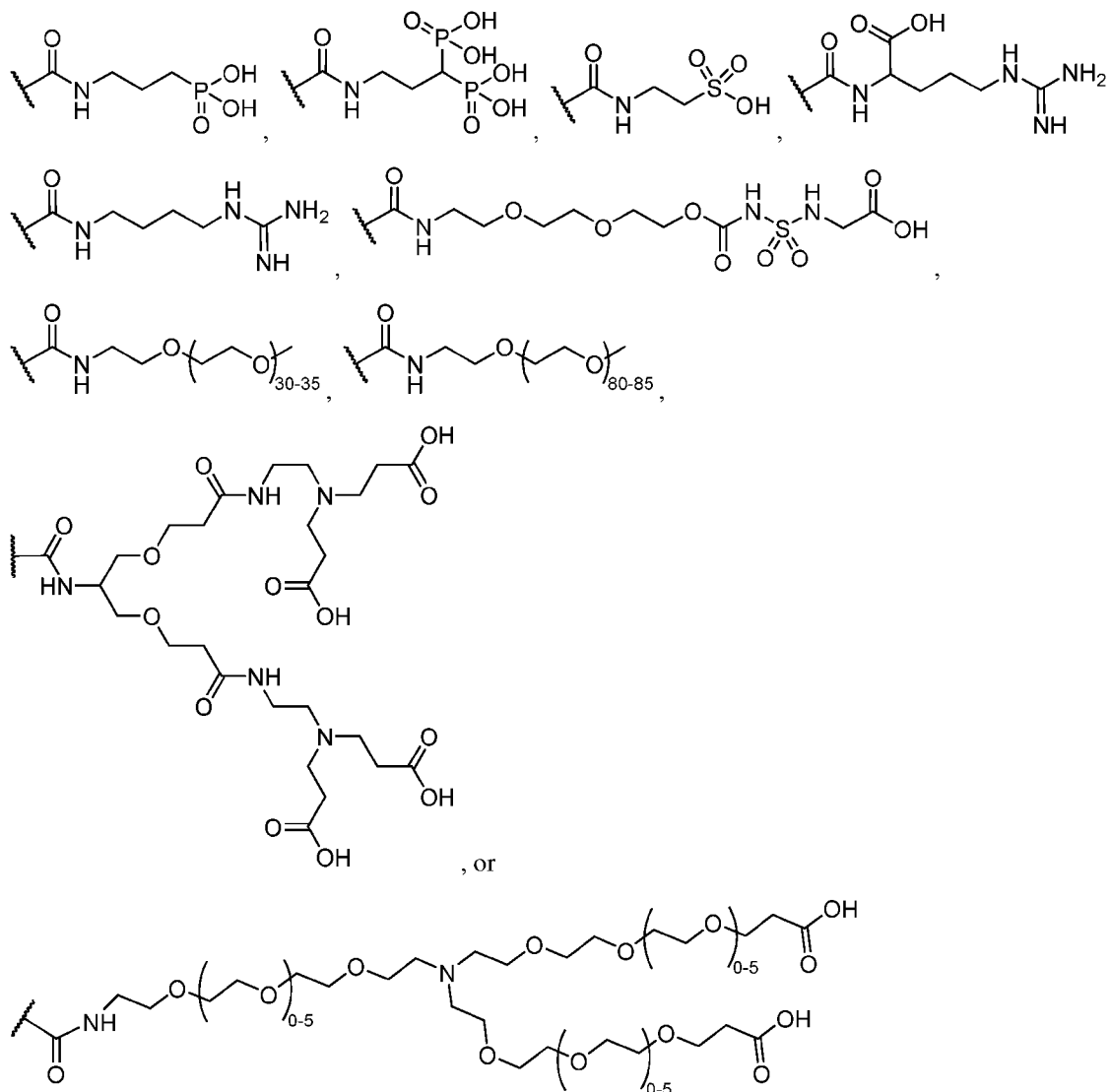
each R^{110} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

each R^{120} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl; and

n' , m' , and m'' are each independently 0, 1, 2, 3, 4, 5, 6, 7, or 8; and

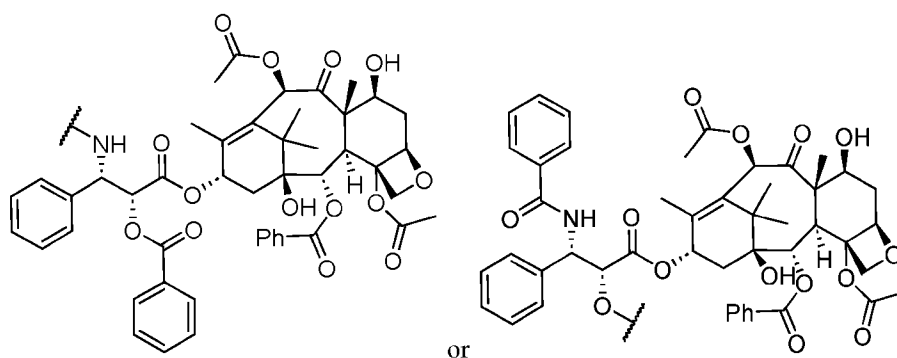
n' is 2.

12. The conjugate of any preceding claim, wherein the moiety $(S^1)_m-L^2-\text{wavy line}$ is:



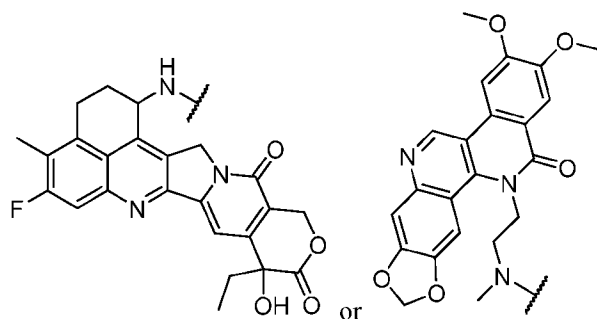
13. The conjugate of any preceding claim, wherein D¹ is paclitaxel or isotaxel, or a derivative, or analog thereof.

14. The conjugate of any preceding claim, wherein D¹ is:



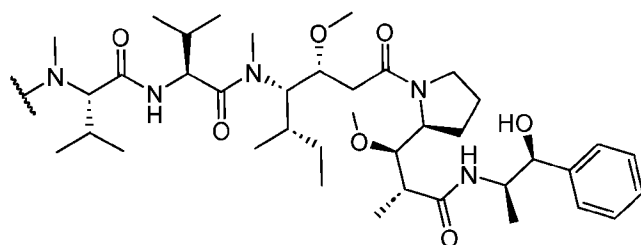
15. The conjugate of any one of claims 1-12, wherein D^1 is exatecan, or a derivative, or analog thereof.

16. The conjugate of any one of claims 1-12 or 15, wherein D^1 is:



17. The conjugate of any one of claims 1-12, wherein D^1 is MMAE, or a derivative, or analog thereof.

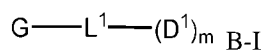
18. The conjugate of any one of claims 1-12 or 17, wherein D^1 is:



19. The conjugate of any preceding claim, wherein r is 1.

20. The conjugate of any preceding claim, wherein r is 2.

21. A conjugate of Formula B-I, or a pharmaceutically acceptable salt thereof:



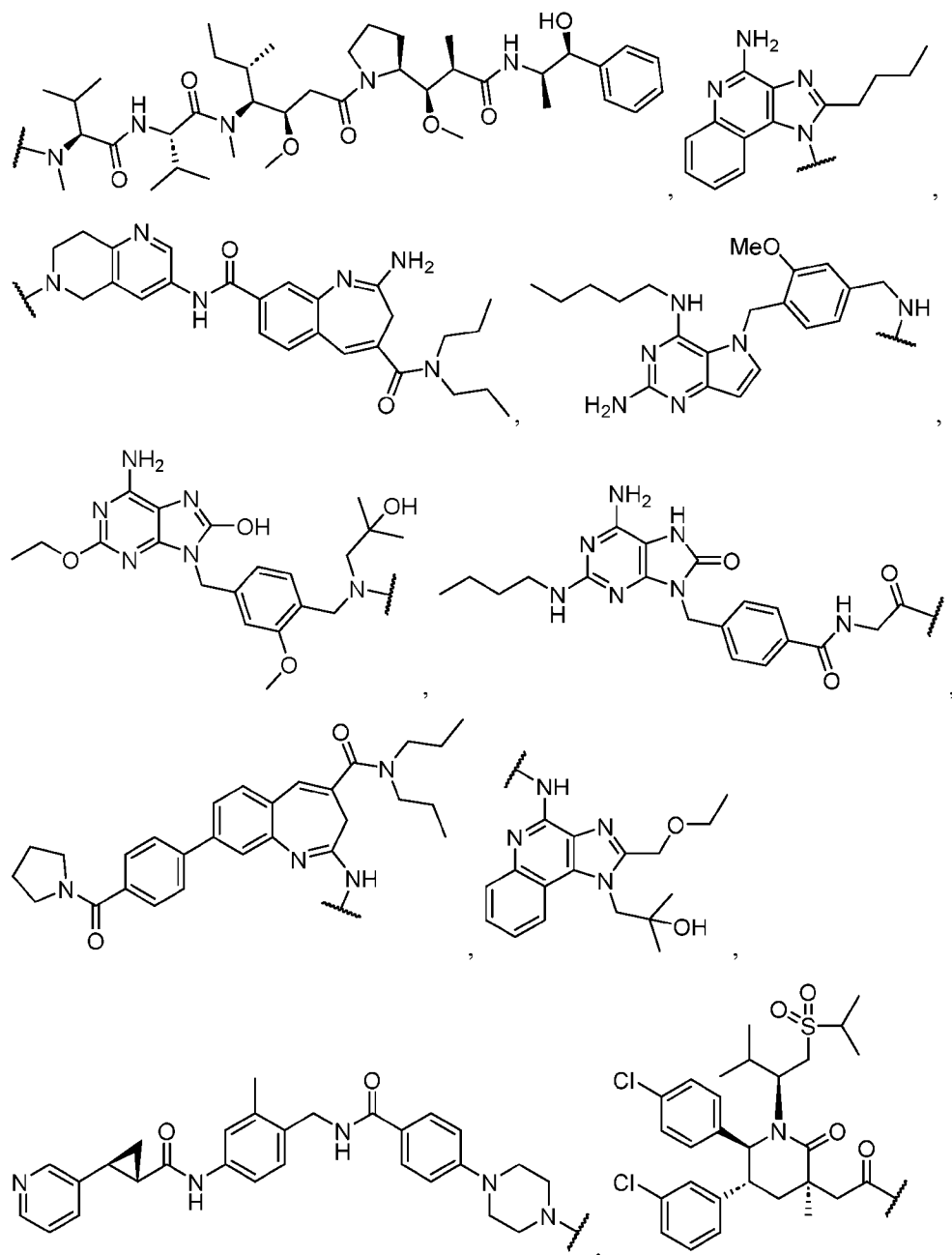
wherein:

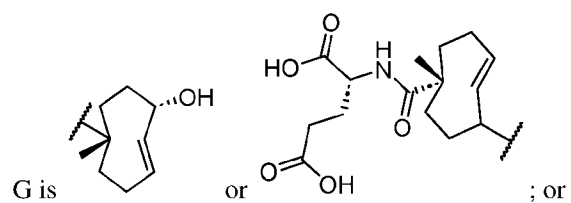
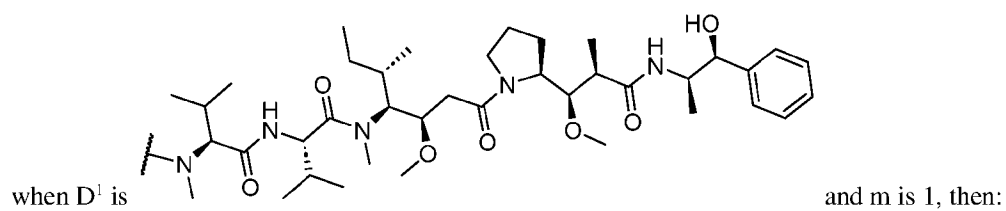
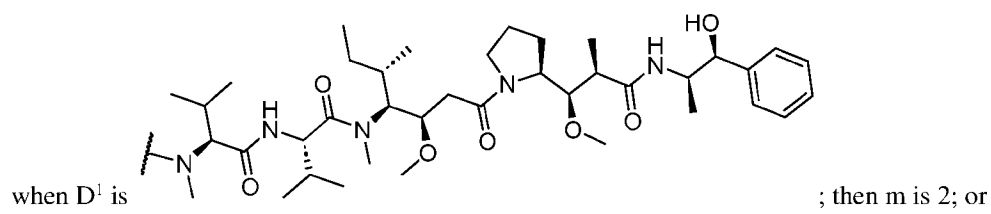
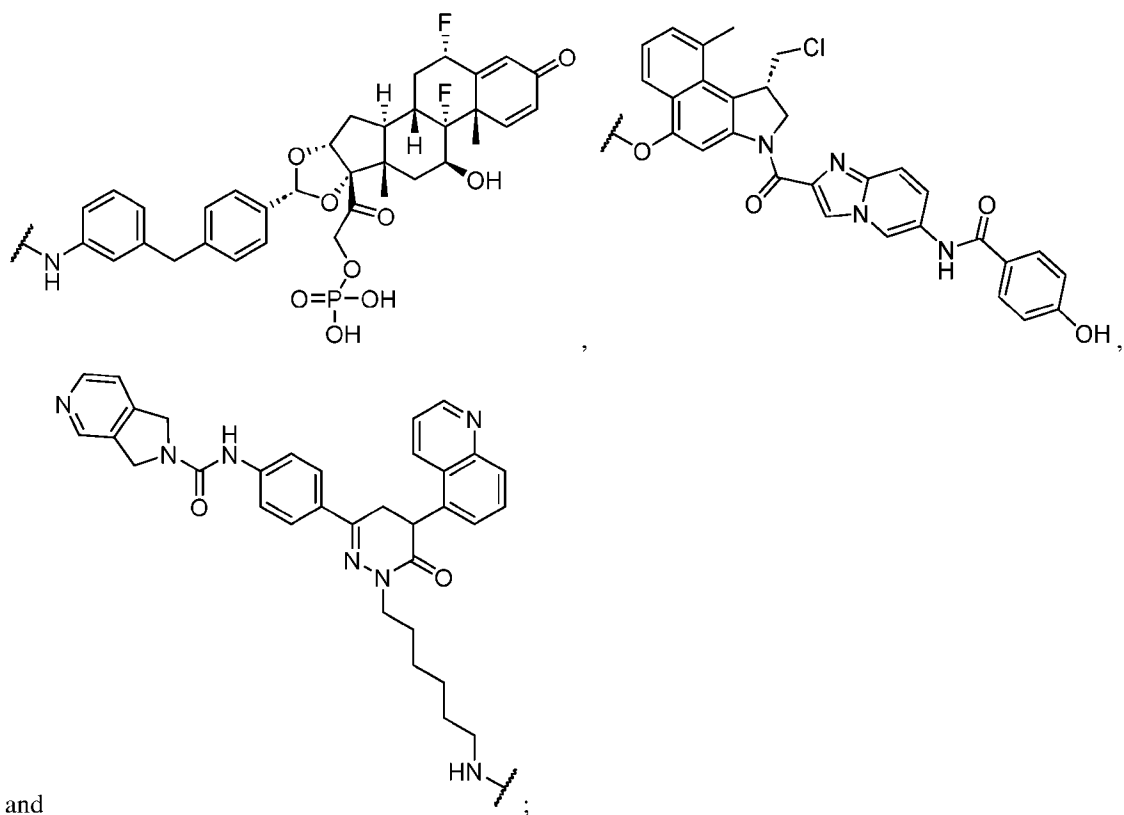
G an optionally substituted trans-cyclooctene moiety;

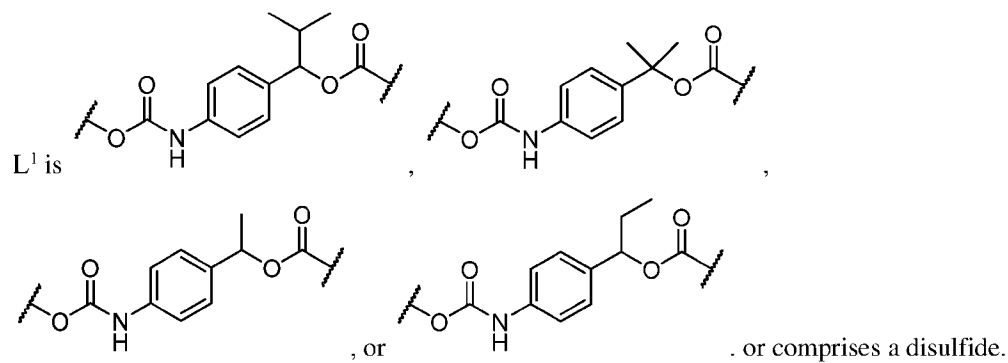
L^1 is a linker;

m is 1 or 2; and

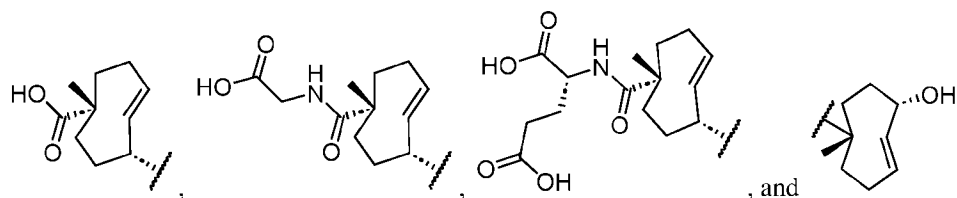
each D¹ is independently a payload selected from the group consisting of:



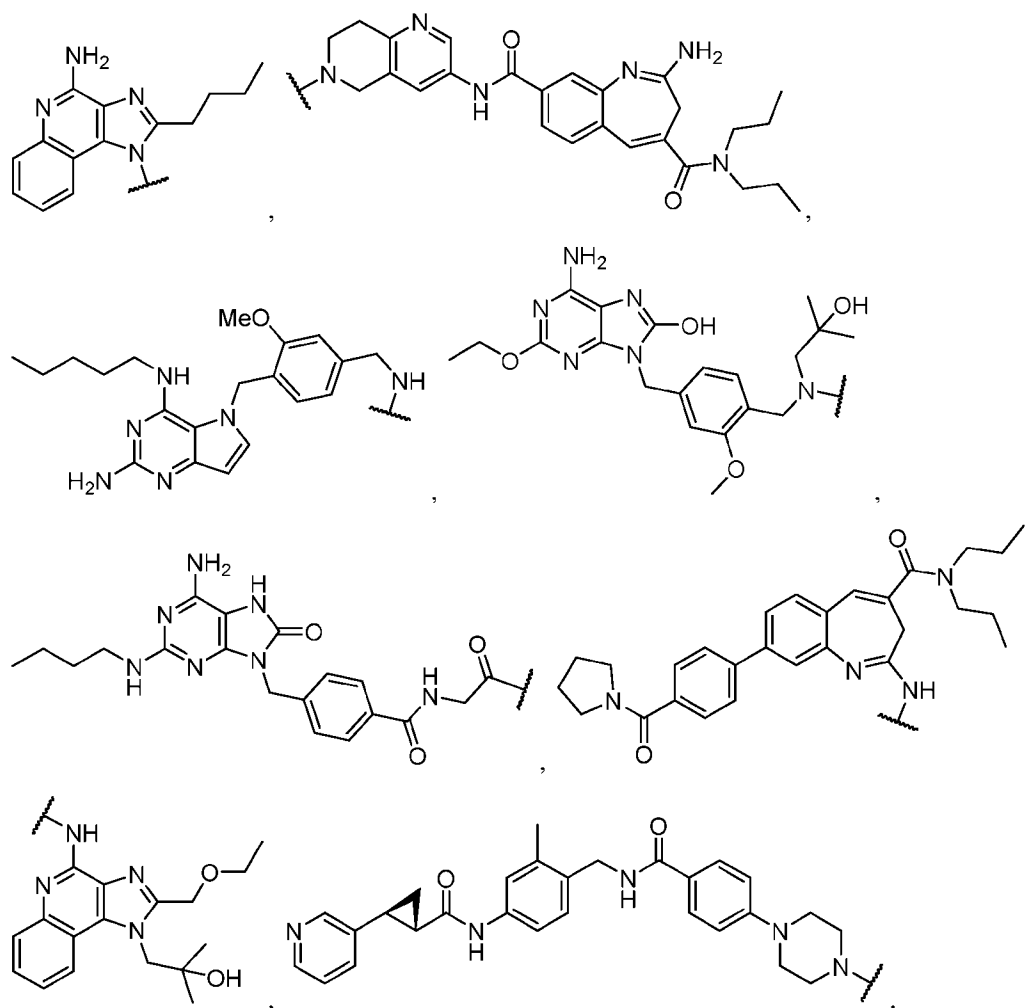


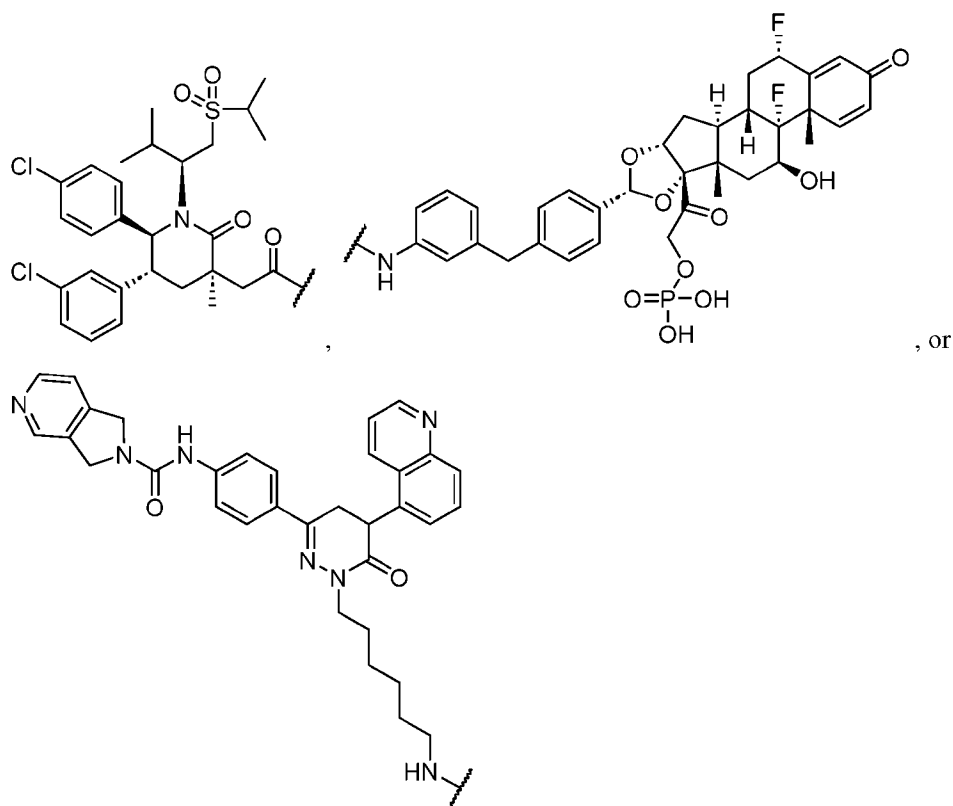


22. The conjugate of claim 21, wherein G is selected from the group consisting of:

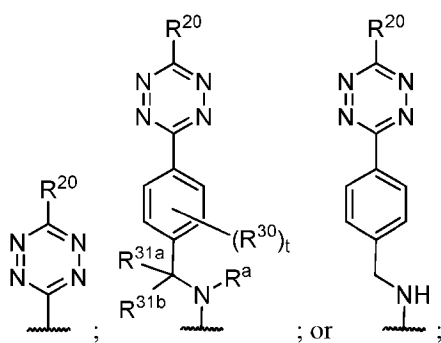


23. The conjugate of claim 21 or 22, wherein D¹ is:





24. A conjugate, or a pharmaceutically acceptable salt thereof, selected from Table A-1.
25. A conjugate, or a pharmaceutically acceptable salt thereof, selected from Table B-1.
26. A pharmaceutical composition comprising the conjugate of any of claims 1-25, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
27. A method of treating cancer, the method comprising administering to a subject in need thereof, a therapeutically effective amount of the conjugate of any of claims 1-25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 26, wherein a therapeutic support composition has been administered to the patient, the therapeutic support composition comprising a biocompatible support and a tetrazine-containing group of formula:



wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R'''$, $SC(=O)R'''$, $OC(=S)R'''$, $SC(=S)R'''$, $S(=O)R'$, $S(=O)_2R'''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$, $NR'C(=S)SR''$, $OC(=O)NR'R''$, $SC(=O)NR'R''$, $OC(=S)R'R'''$, $SC(=S)R'R'''$, $NR'C(=O)NR''R''$, and $NR'C(=S)NR''R''$;

R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl;

R''' at each occurrence is independently selected from aryl and alkyl;

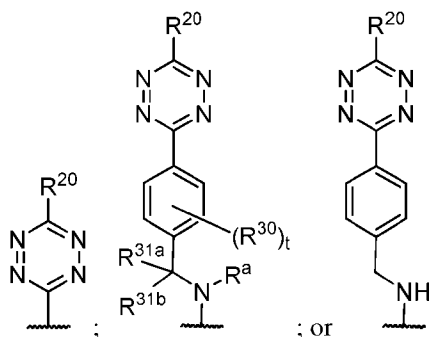
R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl;

R^a , R^{31a} and R^{31b} are each independently hydrogen, C_1 - C_6 -alkyl, or C_1 - C_6 -haloalkyl; and

t is 0, 1, 2, 3, or 4.

28. The method of claim 27, wherein the tetrazine-containing group is linked or directly bonded to an antibody biocompatible support.

29. A method of treating cancer, the method comprising administering to a subject in need thereof, a therapeutically effective amount of the conjugate of any of claims 1-25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 26, and a therapeutic support composition, the therapeutic support composition comprising a biocompatible support and a tetrazine-containing group of formula:



wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3 , CF_2-R' , NO_2 , OR' , SR' , $C(=O)R'$, $C(=S)R'$, $OC(=O)R'''$, $SC(=O)R'''$, $OC(=S)R'''$, $SC(=S)R'''$, $S(=O)R'$, $S(=O)_2R'''$, $S(=O)_2NR'R''$, $C(=O)O-R'$, $C(=O)S-R'$, $C(=S)O-R'$, $C(=S)S-R'$, $C(=O)NR'R''$, $C(=S)NR'R''$, $NR'R''$, $NR'C(=O)R''$, $NR'C(=S)R''$, $NR'C(=O)OR''$, $NR'C(=S)OR''$, $NR'C(=O)SR''$,

$\text{NR}'\text{C}(=\text{S})\text{SR}''$, $\text{OC}(=\text{O})\text{NR}'\text{R}''$, $\text{SC}(=\text{O})\text{NR}'\text{R}''$, $\text{OC}(=\text{S})\text{R}'\text{R}'''$, $\text{SC}(=\text{S})\text{R}'\text{R}''$, $\text{NR}'\text{C}(=\text{O})\text{NR}''\text{R}''$,
and $\text{NR}'\text{C}(=\text{S})\text{NR}''\text{R}''$;

R' and R'' at each occurrence are independently selected from hydrogen, aryl and alkyl;

R''' at each occurrence is independently selected from aryl and alkyl;

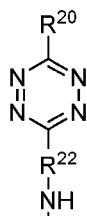
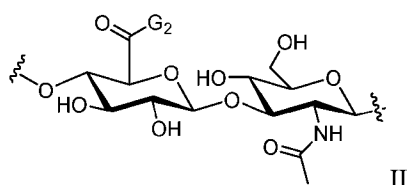
R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl;

R^a , R^{31a} and R^{31b} are each independently hydrogen, C_1 - C_6 -alkyl, or C_1 - C_6 -haloalkyl; and

t is 0, 1, 2, 3, or 4.

30. The method of any one of claims 27-29, wherein the tetrazine-containing group is linked or directly bonded to a hyaluronic acid biocompatible support.

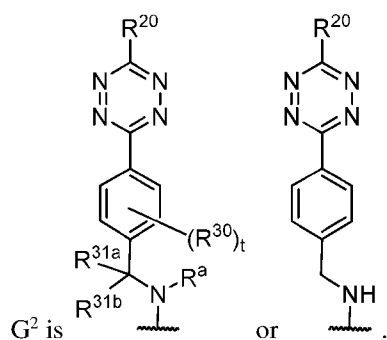
31. The method of any one of claims 27-30, wherein the therapeutic support composition comprises substituted hyaluronic acid units of formula (II),



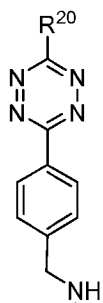
wherein G^2 is ; and

R^{22} is a linker of 1 to 100 linking atoms.

32. The method of claim 31, wherein:



33. The method of claim 32, wherein



G² is ; and R²⁰ is hydrogen or C₁₋₄alkyl.

34. The method of any of claims 27-33, wherein the method is a method of treating cancer.
35. The method of claim 34, wherein the cancer is a melanoma, renal cancer, prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, Kaposi's sarcoma, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, Wilms' tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, malignant extrinsic or intrinsic airway compression, or cutaneous T-cell lymphoma.
36. The method of claim 34 or 35, wherein the cancer is a solid tumor.
37. The method of claim 34 or 35, wherein the cancer is a soft tissue sarcoma.
38. The method of claim 34 or 35, wherein the cancer is lung cancer.
39. The method of claim 34 or 35, wherein the cancer is malignant extrinsic or intrinsic airway compression.
40. The method of claim 34, wherein the cancer is a hematological malignancy such as myelodysplastic syndrome, acute myeloid leukaemia, myelodysplastic syndromes, chronic myelogenous leukaemia, chronic myelomonocytic leukaemia, primary myelofibrosis, diffuse large B-cell lymphoma, chronic lymphocytic leukaemia, monoclonal gammopathy, plasma cell myeloma, follicular lymphoma, marginal zone lymphoma, classical Hodgkin lymphoma, monoclonal B-cell lymphocytosis, lymphoproliferative disorder NOS, T-cell lymphoma, precursor B-lymphoblastic leukaemia, mantle cell lymphoma, plasmacytoma, Burkitt lymphoma, T-cell leukaemia, hairy-cell leukaemia, precursor T-lymphoblastic leukaemia, or nodular lymphocyte predominant Hodgkin lymphoma.
41. The method of any of claims 27-40, wherein the method is a method of enhancing or eliciting an immune response.
42. The method of claim 41, wherein the immune response is an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

43. The method of any of claims 27-42, further comprising administering a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an, or a trans-cyclooctene prodrug thereof.

44. A kit comprising the conjugate of any of claims 1-25, or a pharmaceutically acceptable salt thereof, or the therapeutic support composition of any of claims 27-33, and instructions for use thereof.

45. The kit of claim 44, further comprising the therapeutic support composition as defined in any of claims 27-33.

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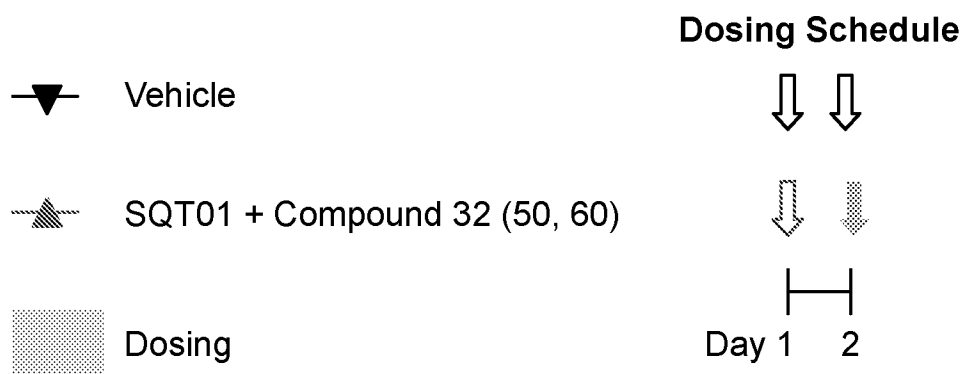
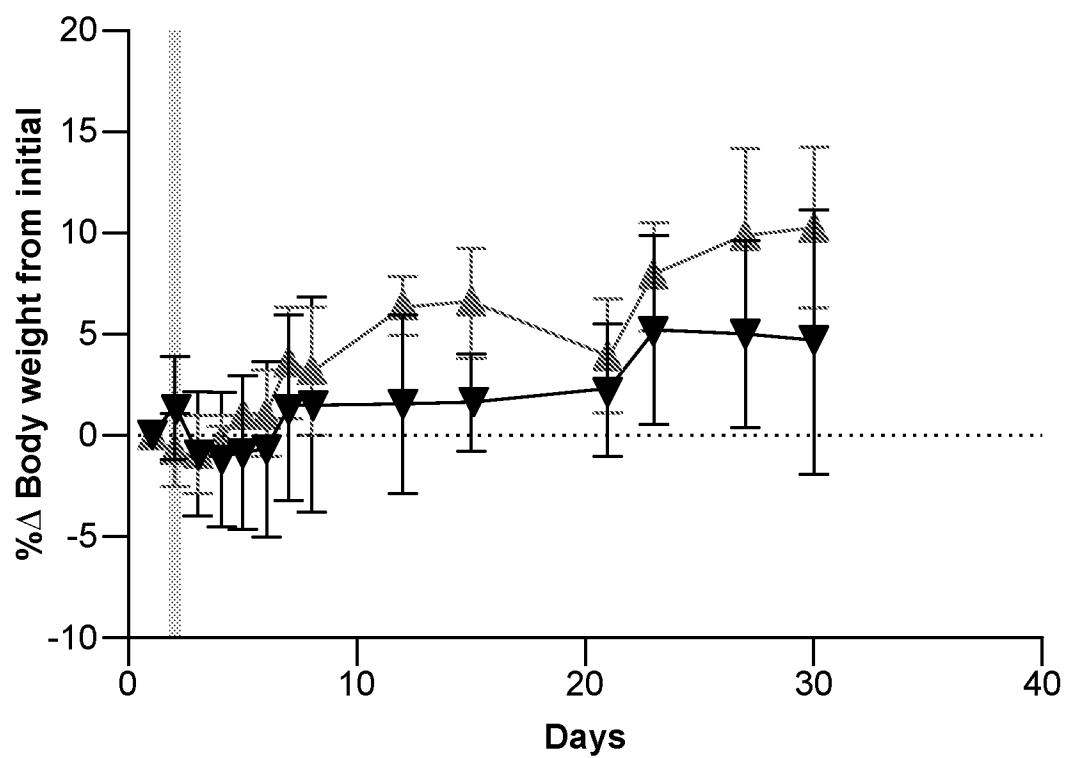


FIG. 1

2 / 2

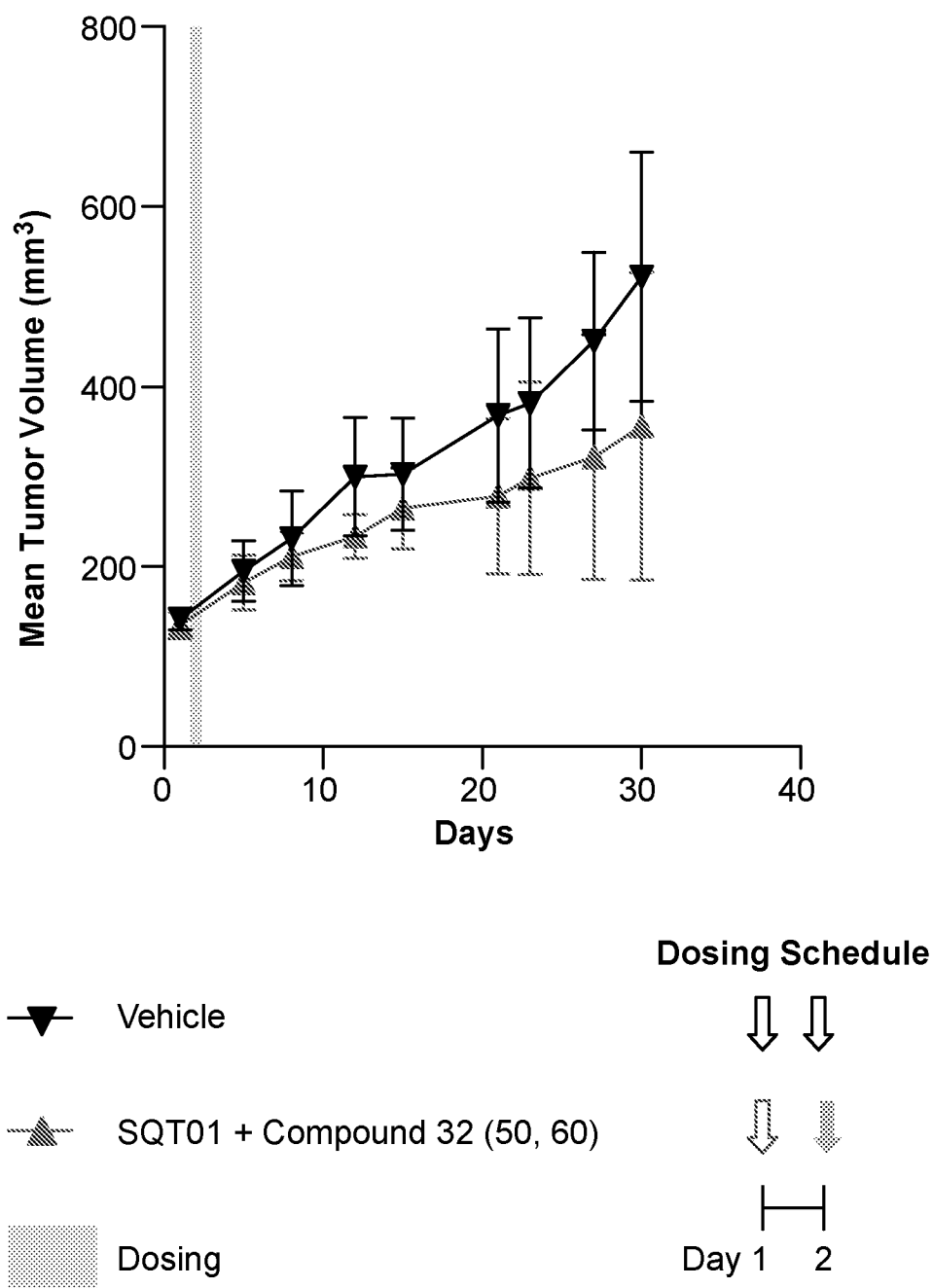


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/016775

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K47/68	A61K47/61
	A61P35/00	C07D491/22
	A61K45/06	
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
A61K C07D A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/032191 A1 (TAMBO INC [US]) 10 February 2022 (2022-02-10)	1 - 12, 15 - 20, 24 - 27, 29 - 45
Y	examples 2-6, 9, 10, 12-14 page 122 page 126 - page 130 paragraphs [0071], [0080], [0081] paragraphs [00152], [00156], [00159], [00161] - [00163], [00173] paragraphs [00222] - [00224] paragraphs [00308], [00309], [00370], [00373] paragraphs [00403], [00420] ----- - / - -	12, 28
☒ Further documents are listed in the continuation of Box C.		
☒ See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance		
"E" earlier application or patent but published on or after the international filing date		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone		
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art		
"&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
12 June 2024		19/08/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Fernandez Rodriguez

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/016775

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/187740 A1 (SHASQI INC [US]) 11 October 2018 (2018-10-11)	1-9,11, 13, 17-20, 26,27, 29-45 12,28
Y	claims 1, 14, 16, 21-31, 38, 40-44 examples 1-4, 11-13, 15, 16, 18, 19, 22 paragraphs [00153], [00160] - [00162], [00170] paragraphs [00257], [00258], [00282] -----	
X	Kui Wu: "Click activated protodrugs against cancer increase the therapeutic potential of chemotherapy through local capture and activation", , 5 January 2021 (2021-01-05), XP093169359, DOI: 10.1039/d0sc06099b Retrieved from the Internet: URL:https://pubs.rsc.org/en/content/articlepdf/2021/sc/d0sc06099b [retrieved on 2024-06-07]	1-11,13, 14,19, 20,26, 27, 29-34,36
Y	figure 7 schemes 1, 5 section: "Results - Antitumor efficacy in a mouse model" -----	12,28
Y	AGNESE MAGGI ET AL: "Development of a novel antibody-tetrazine conjugate for bioorthogonal pretargeting", ORGANIC & BIOMOLECULAR CHEMISTRY, vol. 14, no. 31, 12 July 2016 (2016-07-12) , pages 7544-7551, XP055385205, ISSN: 1477-0520, DOI: 10.1039/C6OB01411A abstract figure 4 -----	28
Y	YAMAZAKI CHISATO M. ET AL: "Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance", NATURE COMMUNICATIONS, 10 June 2021 (2021-06-10), XP055940303, DOI: 10.1038/s41467-021-23793-7 Retrieved from the Internet: URL:https://www.nature.com/articles/s41467-021-23793-7.pdf [retrieved on 2024-06-07] abstract figure 1 ----- -/-	28

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/016775

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2023/077126 A1 (TAMBO INC [US]) 4 May 2023 (2023-05-04) claims 36-51 table 1; compounds 1, 6-8, 13-16, 20, 21 -----	1-3, 6-12, 15-19, 26,27, 29-38, 40-45
X,P	WO 2023/077129 A1 (TAMBO INC [US]) 4 May 2023 (2023-05-04) claims 1, 54, 58-69 examples 9-12 pages 105, 106 page 109 - page 113 -----	1-3, 6-12, 15-19, 26-38, 40-45
X,P	MCFARLAND JESSE M ET AL: "Click Chemistry Selectively Activates an Auristatin Protodrug with either Intratumoral or Systemic Tumor-Targeting Agents", ACS CENTRAL SCIENCE, 22 June 2023 (2023-06-22), pages 1400-1408, XP093171563, ISSN: 2374-7943, DOI: 10.1021/acscentsci.3c00365 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/a csentsci.3c00365 [retrieved on 2024-06-07] cited in the application figures 1-4 section: "Results and discussion - In Vivo Antitumor Efficacy of SQP22 in Various Murine Tumor Models" section: "conclusion" -----	1-3, 6-11, 17-19, 26-36, 41,42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/016775

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-20, 24 (completely); 25-45 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20, 24(completely); 25-45(partially)

A trans-cyclooctene-drug conjugate of Formula A-I or Formula A-II, its use in a method of treating cancer, and a pharmaceutical composition and a kit comprising said trans-cyclooctene-drug conjugate.

2. claims: 21-23(completely); 25-45(partially)

A trans-cyclooctene-drug conjugate of Formula B-I, its use in a method of treating cancer, and a pharmaceutical composition and a kit comprising said trans-cyclooctene-drug conjugate.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2024/016775

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
WO 2022032191	A1	10-02-2022	CN	116056765	A	02-05-2023	
			EP	4192506	A1	14-06-2023	
			EP	4295917	A2	27-12-2023	
			JP	2023537066	A	30-08-2023	
			US	2023256109	A1	17-08-2023	
			WO	2022032191	A1	10-02-2022	

WO 2018187740	A1	11-10-2018	AU	2018250312	A1	31-10-2019	
			CN	111093708	A	01-05-2020	
			EP	3606559	A1	12-02-2020	
			JP	7405616	B2	26-12-2023	
			JP	2020516621	A	11-06-2020	
			US	2021128733	A1	06-05-2021	
			US	2022168426	A1	02-06-2022	
			WO	2018187740	A1	11-10-2018	

WO 2023077126	A1	04-05-2023	CN	118414169	A	30-07-2024	
			WO	2023077126	A1	04-05-2023	

WO 2023077129	A1	04-05-2023	NONE				
