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(54) **Title:** LINKERS AND APPLICATION TOWARDS ADC THEREOF

(57) **Abstract:** Disclosed are linkers, containing amide surrogates with a regular or novel lysosomal enzymatic cleavable dipeptidic unit, to connect cytotoxic drugs to antibodies. Also disclosed are ADCs (antibody-drug conjugates) derived from these amide surrogate linkers for the treatment of cancer.



LINKERS AND APPLICATION TOWARDS ADC THEREOF

FIELD OF THE INVENTION

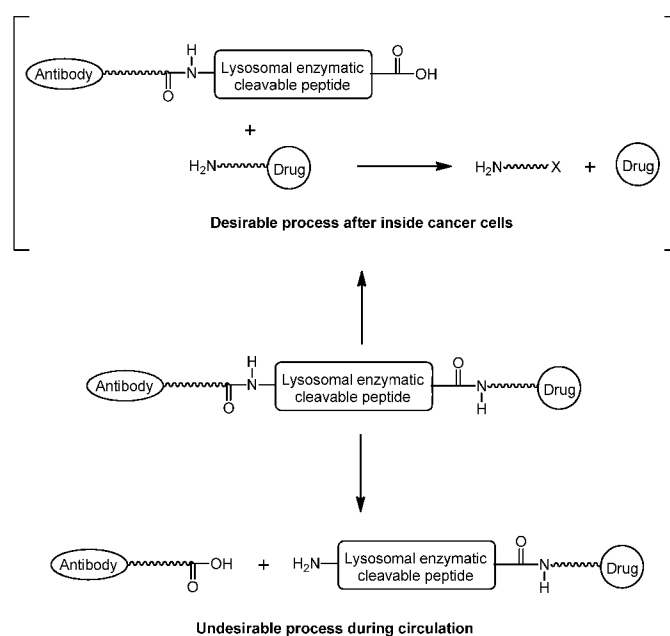
The present invention relates to linkers, containing amide surrogates with a regular or a novel lysosomal enzymatic cleavable dipeptidic unit, to connect cytotoxic drugs to antibodies. The present invention also relates to ADCs (antibody-drug conjugates) derived from these amide surrogate linkers for the treatment of cancers.

BACKGROUND OF THE INVENTION

Antibody drug conjugates (ADCs) hold the promise as a target specific and effective treatment for a number of diseases e.g. bacterial infection and cancers, but at the same time with an improved systemic side effect profile.

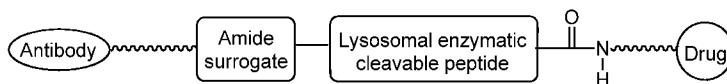
The concept to combine a target specific antibody with a cytotoxic compound is very simple and elegant. An antibody is well known for its ability to recognize an antigen in specific manner and the often insufficient cell killing activity following an immune response, limited its therapeutic potential. On the other hand, cytotoxic compounds are widely used in the treatment of cancers and their effectiveness are hindered by non-specific systemic adverse events associated with higher dose usage. The combination of an antibody with a cytotoxic compound can therefore lead to a therapeutic agent with enhancing efficacy to the antibody and reduced systemic side effects to the cytotoxic drug, attributing to the target specific delivery by the antibody and minimize of normal tissues from drug exposures.

One of the keys to the success of ADCs relies on the identification of suitable linkers that are stable during circulation in the blood stream, but rapidly cleave to release the cytotoxic compound at the target for cell killing activity. A demonstrated successful example is the linker, such as the one in Adcertris, which incorporates a lysosomal enzymatic cleavable peptide component. The C-terminal amide bond in this part of the linker that can be easily cleaved at target cells by lysosomal enzymes is essential to the drug releasing mechanism. Other amide bonds on the linker if undergo prematurely cleavage may lead to undesirable side effects (Formular B-1).



(B-1)

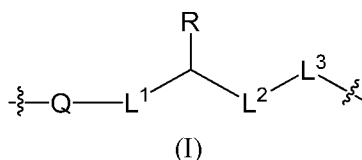
This application describes the replacement of the N-terminal amide bond of the lysosomal enzymatic cleavable peptide with an amide surrogate (Formullar B-2). This modification can further improved stability of the resulting ADCs in circulation.



(B-2)

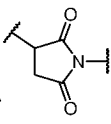
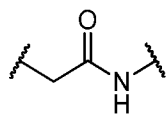
DESCRIPTION OF THE INVENTION

The present invention provides a linker of formula (I) to connect drugs to antibodies,



wherein,

R is selected from the group consisting of CF₂H, CF₃, CF₂CF₃, and PhSO₂Me;

Q is selected from the group consisting of  and  ;
 L¹ is selected from the group consisting of -(CR¹R²)_m-(CR³R⁴)-(CR¹R²)_n-,
 -(CR¹R²)_m-(CR³R⁴)-O-(CR¹R²)_n-, and -[(CR¹R²)(CR¹R²)X]_p-(CR¹R²)_q-;

m is 1, 2, 3, or 4;

n is 1, 2, 3, or 4;

p is 1, 2, 3, 4, 5, or 6;

q is 1 or 2;

R¹ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂.

R² is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R³ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R⁴ is selected from the group consisting of H, C₁₋₃ alkyl, OH, NR⁵R⁶, CO₂H, P(O)(OH)₂, and SO₃H, said C₁₋₃ alkyl is optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

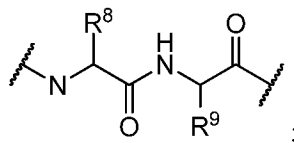
R⁵ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R⁶ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

X is selected from the group consisting of NR^7 , O, S(O)_r ;

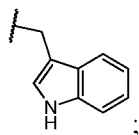
r is 0, 1, or 2;

R^7 is selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , NHMe , and NMe_2 ;

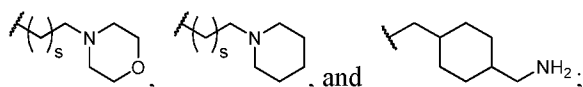


L^2 is a di-peptide unit selected from

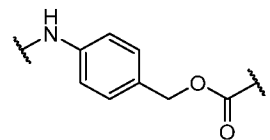
R^8 is selected from the group consisting of methyl, propyl, isopropyl, sec-butyl, benzyl, and



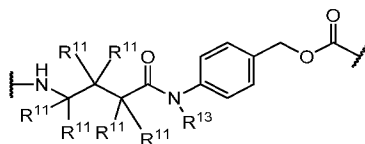
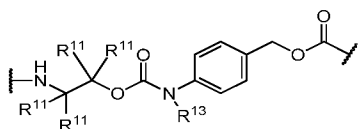
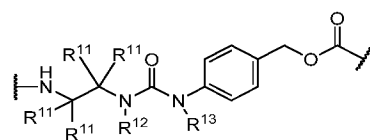
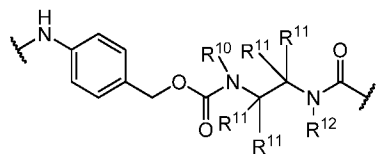
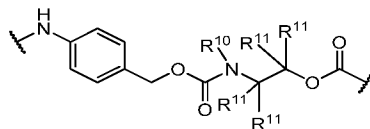
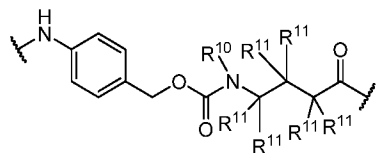
R^9 is selected from the group consisting of $(\text{CH}_2)_4\text{NH}_2$, $(\text{CH}_2)_3\text{NHCONH}_2$, $(\text{CH}_2)_3\text{NHC(=NH)NH}_2$,



s is 0, 1, 2, 3, or 4;



L^3 is a self-immolative unit selected from the group consisting of



R^{10} is selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , NHMe , and NMe_2 ;

each R^{11} is independently selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , NHMe , and NMe_2 ;

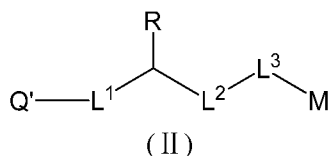
two geminal R^{11} may be optionally joined together to form a 3-6 membered ring with a carbon atom to which they are attached;

two adjacent R¹¹ may be optionally joined together to form a 5-7 membered ring with a carbon atom to which they are attached;

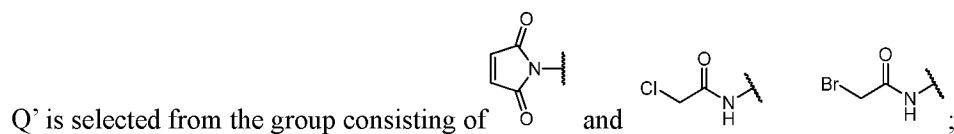
R¹² is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂; and

R¹³ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂.

The present invention also provides a drug-linker conjugate of formula (II),



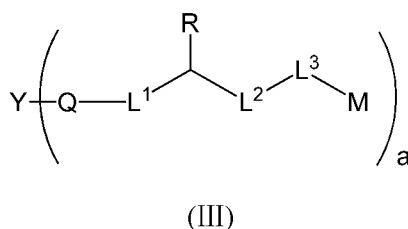
wherein,



M is a drug; and

other variables are as defined above.

The present invention also provides an ADC of formula (III) containing the linker of claim 1,



wherein,

Y is an antibody;

a is 1, 2, 3, 4, 5, 6, 7, or 8;

M is a drug; and

other variables are as defined above.

In certain embodiment of this invention, the aforesaid antibody is selected from the group consisting of a resurfaced monoclonal antibody, a resurfaced single chain monoclonal antibody, or a resurfaced monoclonal antibody fragment that preferentially binds to a target cell.

In certain embodiment of this invention, the aforesaid is selected from the group consisting of a humanized monoclonal antibody, a humanized single chain monoclonal antibody, or a humanized monoclonal antibody fragment.

In certain embodiment of this invention, the aforesaid antibody is selected from the group consisting of a chimeric antibody, a chimeric antibody fragment, a domain antibody, or a domain antibody fragment thereof.

In certain embodiment of this invention, the aforesaid antibody is selected from the group consisting of MY9, anti-B4, EpCAM, CD2, CD3, CD4, CD5, CD6, CD11, CD19, CD20, CD22, CD26, CD30, CD33, CD37, CD38, CD40, CD44, CD56, CD79, CD105, CD138, EphA receptors,

EphB receptors, EGFR, EGFRvIII, HER2, HER3, mesothelin, cripto, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ integrin or C242.

In certain embodiment of this invention, the aforesaid antibody is selected from the group consisting of My9-6, B4, C242, N901, DS6, EphA2 receptor, CD38, IGF-IR, CNTO 95, B-B4, Trastuzumab, Tertuzumab, Bevatuzumab, Sibrotuzumab, Rituximab, and Adalimumab.

In certain embodiment of this invention, the aforesaid antibody binds to target cells selected from tumor cells; virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melanocytes; cells expressing one or more of IGF-IR, CanAg, EGFR, MUC1, MUC1 6, VEGF, TF, MY9, anti-B4, EpCAM, CD2, CD3, CD4, CD5, CD6, CD11, CD11a, CD18, CD19, CD20, CD22, CD26, CD30, CD33, CD37, CD38, CD40, CD44, CD56, CD70, CD79, CD105, CD138, EphA receptors, EphB receptors, EGFRvIII, HER2/neu, HER3, mesothelin, cripto, $\alpha_v\beta_3$ integrin, $\alpha_v\beta_5$ integrin, $\alpha_v\beta_6$ integrin, Apo2, and C242 antigens; or cells expressing insulin growth factor receptor, epidermal growth factor receptor, and folate receptor.

In certain embodiment of this invention, the aforesaid tumor cells are selected from breast cancer cells, prostate cancer cells, ovarian cancer cells, colorectal cancer cells, gastric cancer cells, squamous cancer cells, small-cell lung cancer cells, and testicular cancer cells.

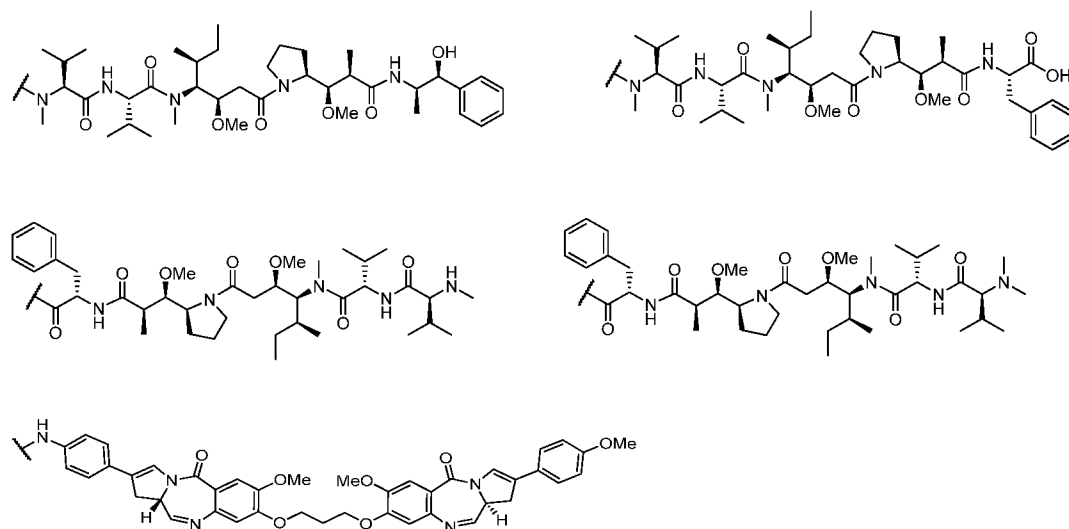
Drug herein can include various kind such radio-labeled compounds as well.

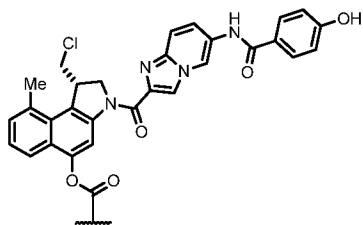
In certain embodiment of this invention, the aforesaid drug is a cytotoxic drug.

In certain embodiment of this invention, the aforesaid drug is selected from the group consisting of maytansinoid, DNA-binding drug and its analog, calicheamicin, doxorubicin and its analog, vinca alkaloid, cryptophycin, dolastatin, auristatin and analog thereof, tubulysin, epothilone, taxoid and siRNA.

In certain embodiment of this invention, the aforesaid DNA-binding drug is CC-1065.

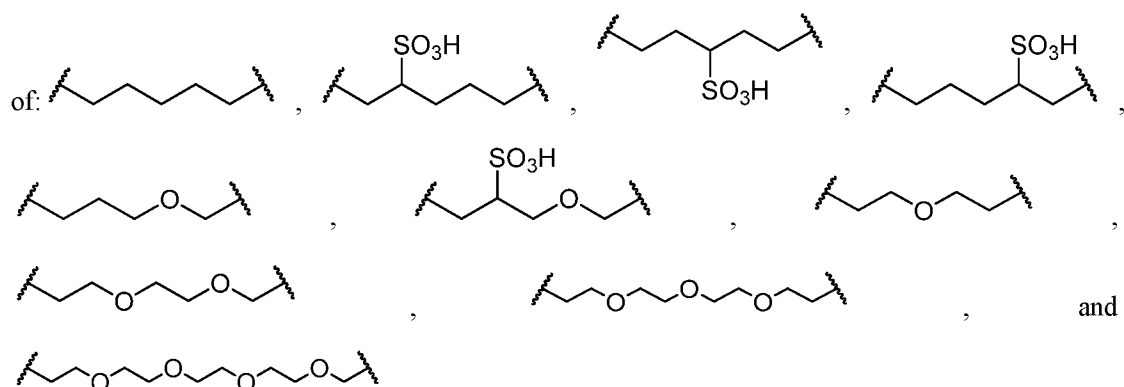
In certain embodiment of this invention, the aforesaid M is selected from the group consisting of:



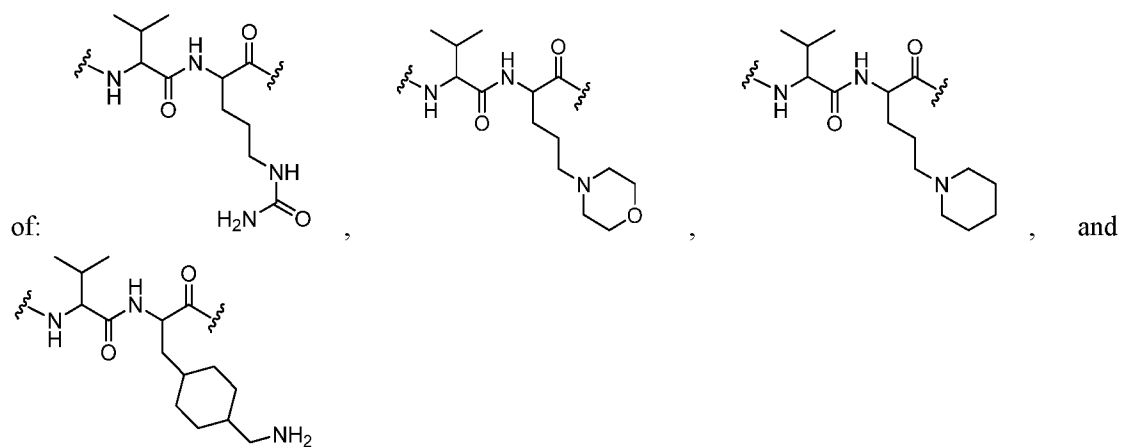


In certain embodiment of this invention, the aforesaid drug is a diagnostic or detection reagent, concretely the drug is a radio-labeled compound, more concretely the drug is labeled with ^3H , ^{18}F , ^{11}C , ^{13}N , ^{15}O , ^{201}Tl , ^{32}P , ^{51}Cr , ^{67}Ga , ^{123}I , ^{125}I , ^{131}I , ^{132}I , ^{131}Cs , ^{113}Xe , ^{133}Xe , ^{169}Yb , ^{198}Au , ^{203}Hg , $^{99\text{m}}\text{Tc}$, $^{113\text{m}}\text{In}$, $^{133\text{m}}\text{In}$, ^{75}Se , ^{186}Re , ^{153}Sm , and ^{89}Sr .

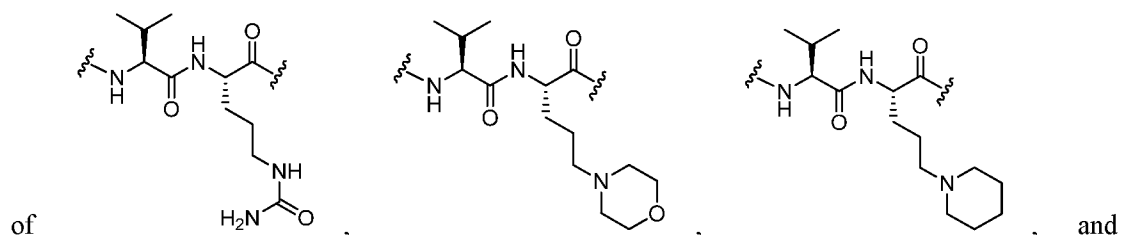
In certain embodiment of this invention, the aforesaid L^1 is selected from the group consisting

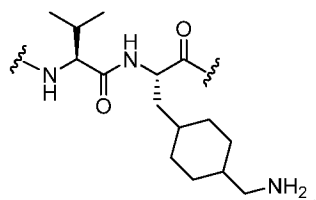


In certain embodiment of this invention, the aforesaid L^2 is selected from the group consisting



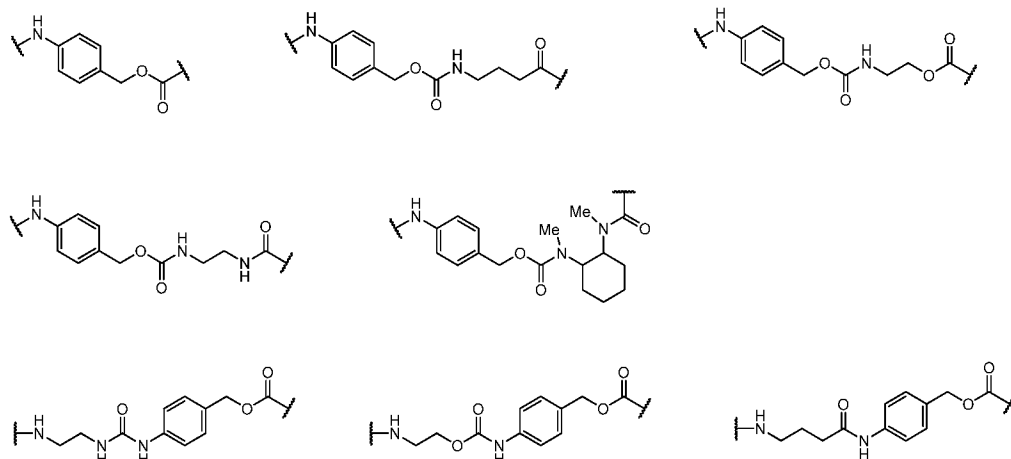
In certain embodiment of this invention, the aforesaid L^2 is selected from the group consisting



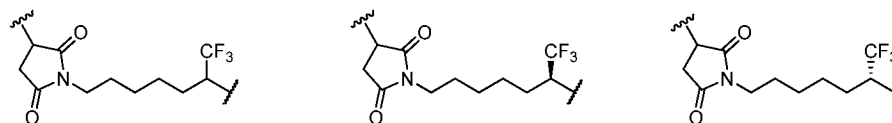


In certain embodiment of this invention, the aforesaid ring formed by two geminal R¹¹ or two adjacent R¹¹ is cyclohexyl.

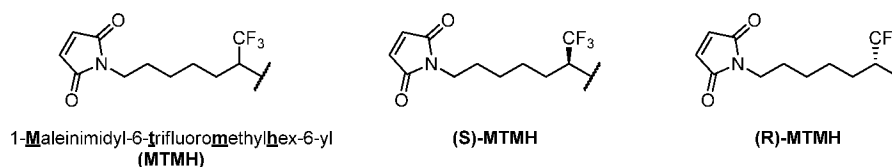
In certain embodiment of this invention, the aforesaid L³ is selected from the group consisting of:



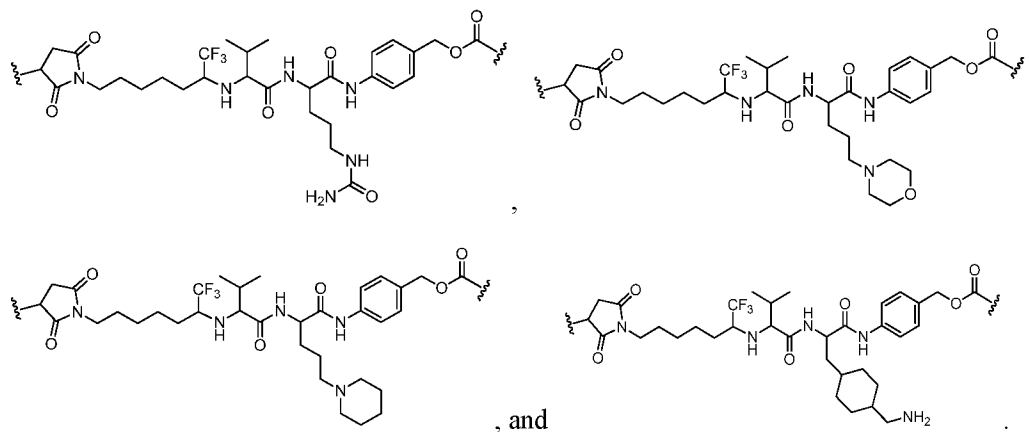
In certain embodiment of this invention, the aforesaid moiety of $\text{---Q---L}^1\text{---}$ is selected from the group consisting of:



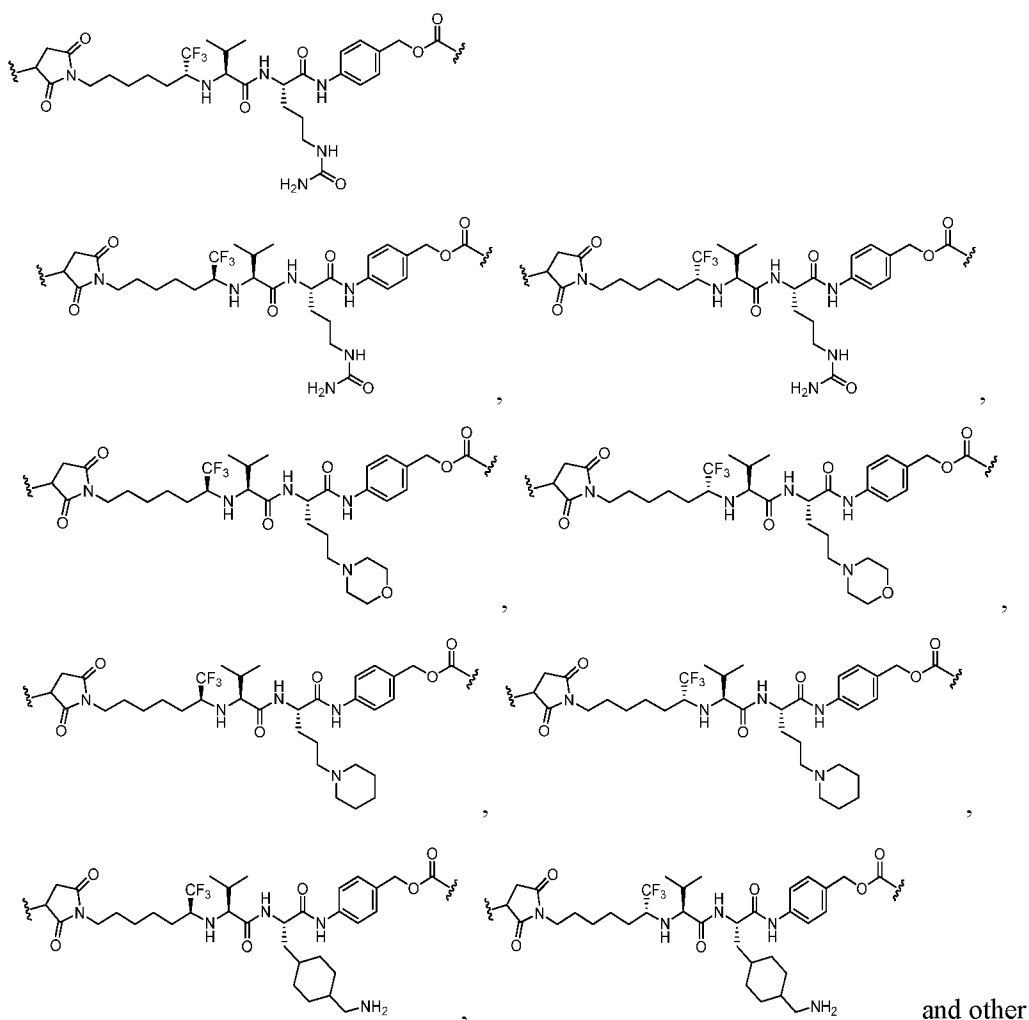
In certain embodiment of this invention, the aforesaid moiety of $\text{Q'---L}^1\text{---}$ is selected from the group consisting of:



In certain embodiment of this invention, the aforesaid linker is selected from the group consisting of:

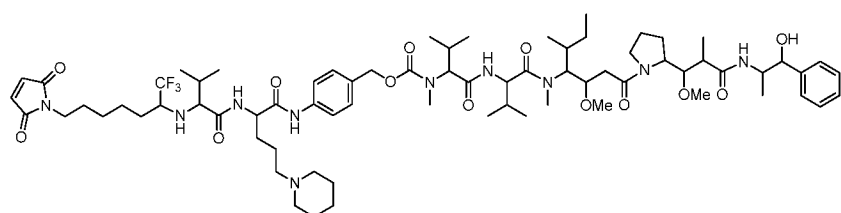
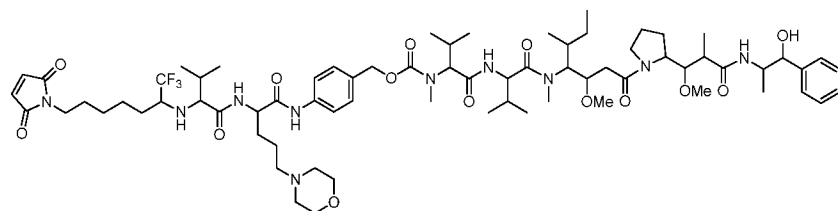
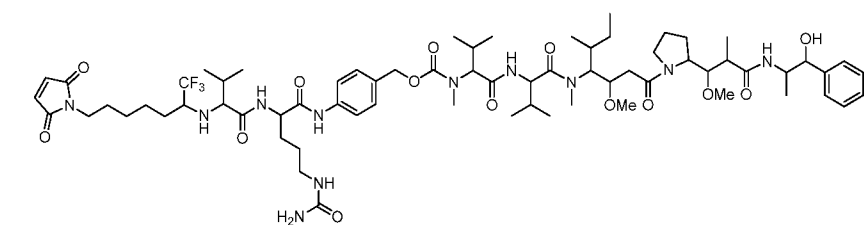


In certain embodiment of this invention, the aforesaid linker is selected from the group consisting of:

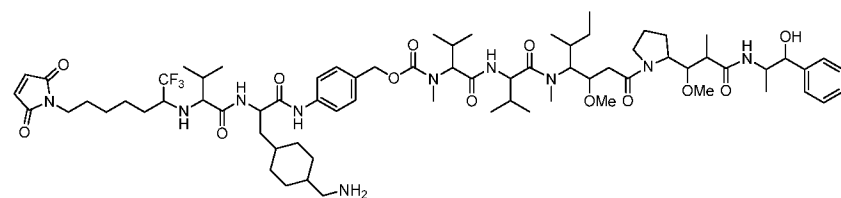


diastereomers.

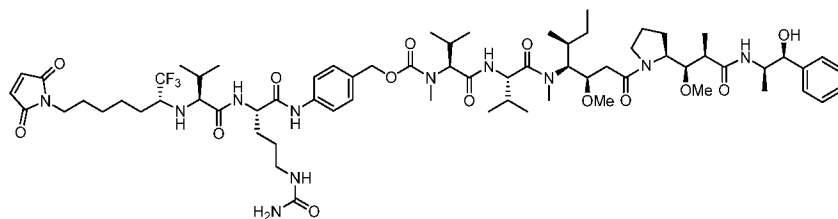
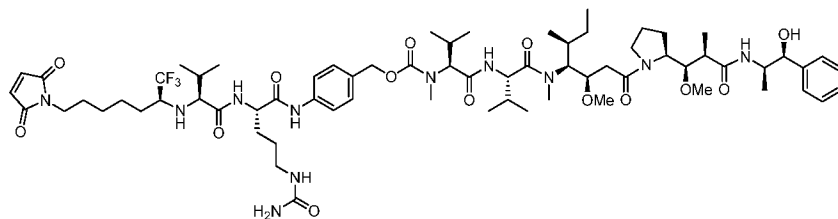
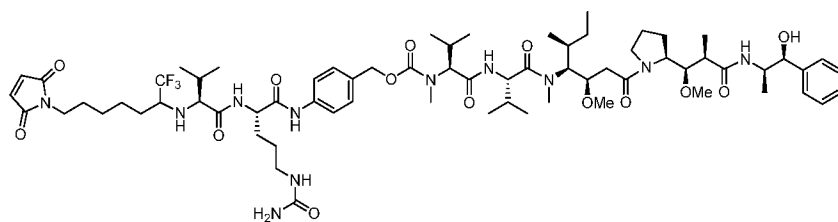
In certain embodiment of this invention, the aforesaid conjugate is selected from the group consisting of:

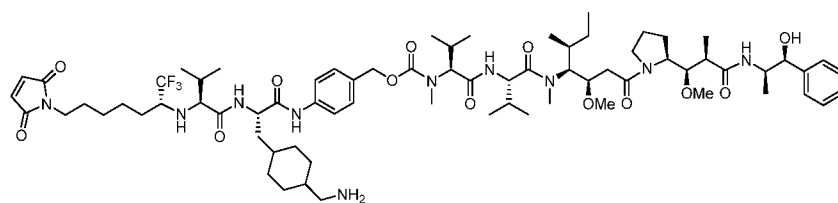
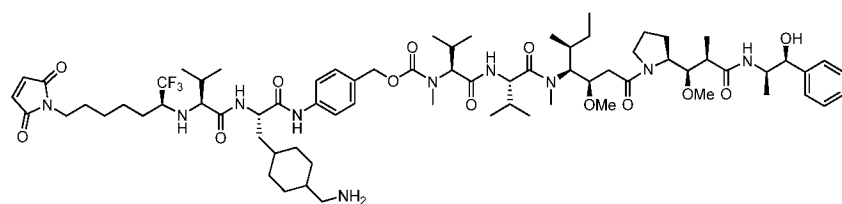
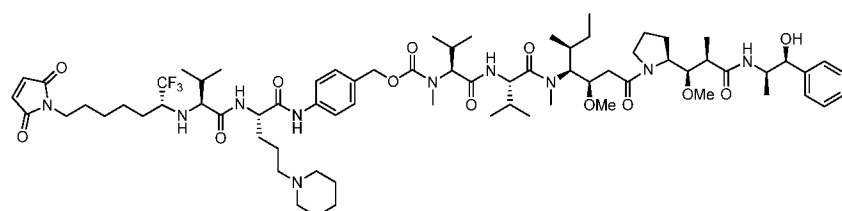
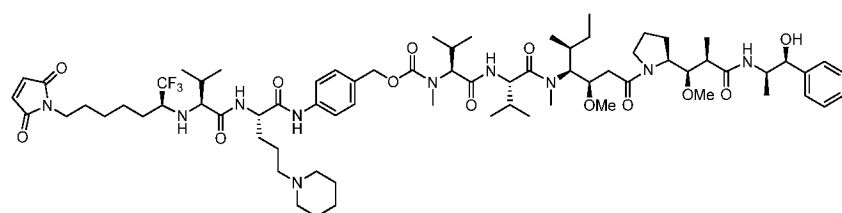
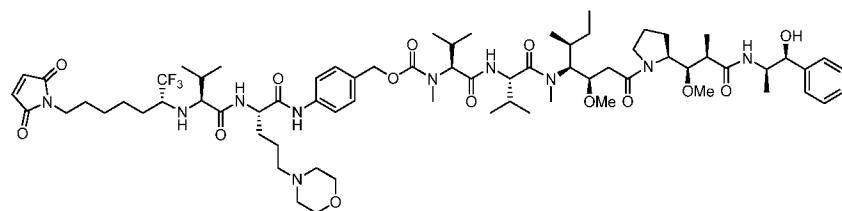
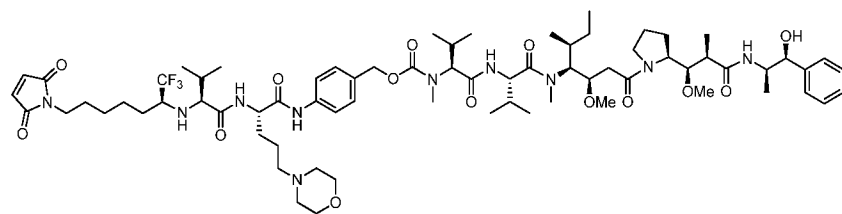


, and



In certain embodiment, the aforesaid conjugate is selected from the group consisting of:

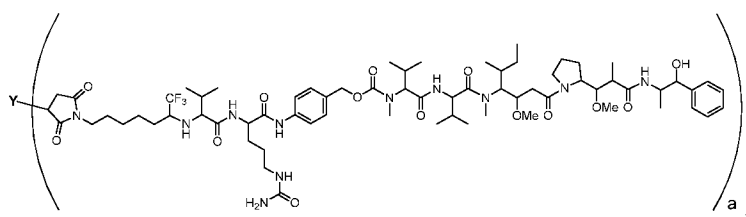


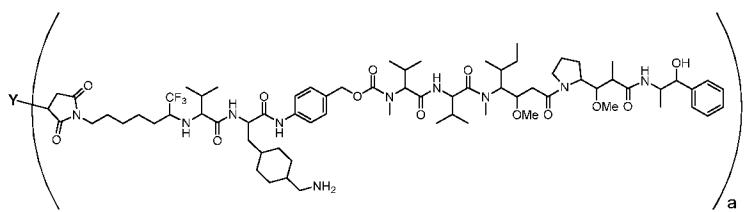
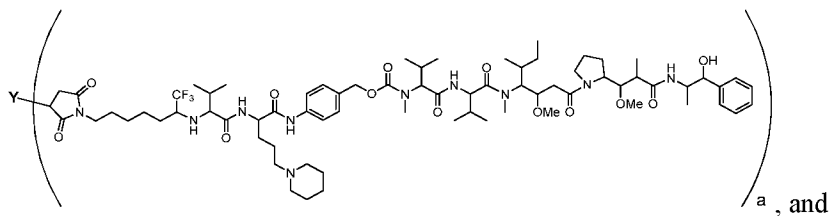
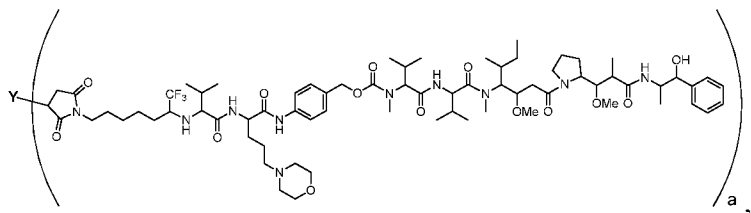


and other

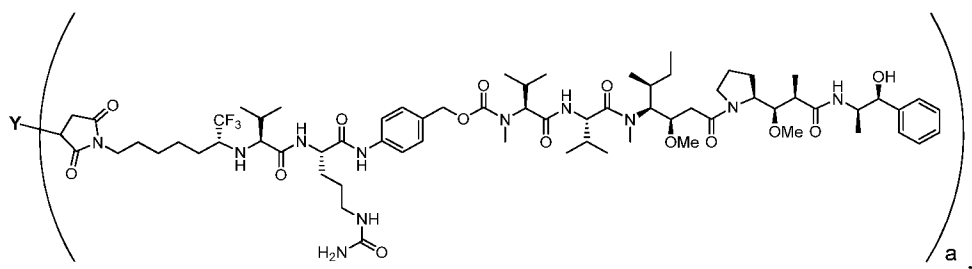
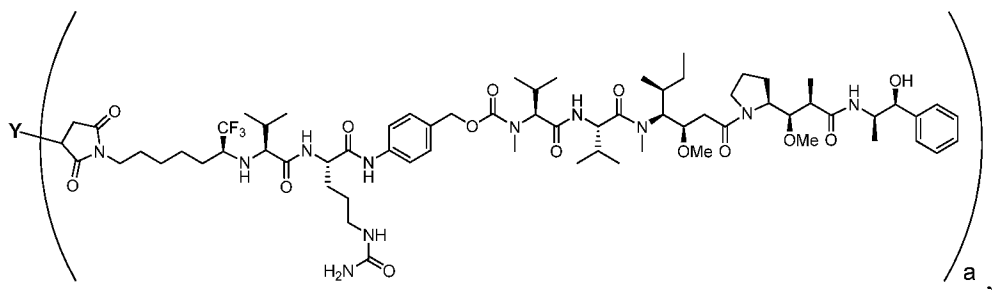
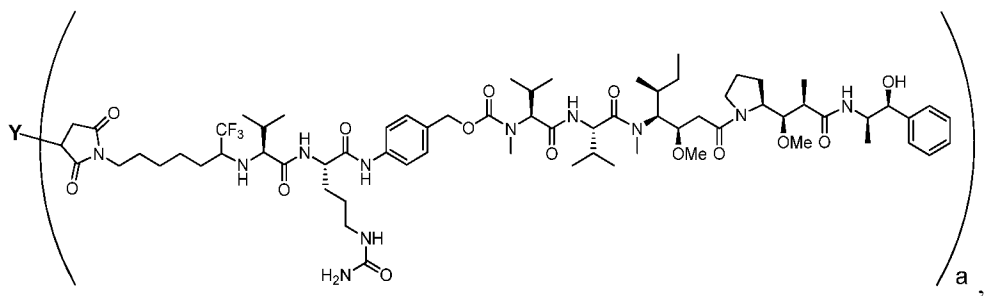
diastereomers.

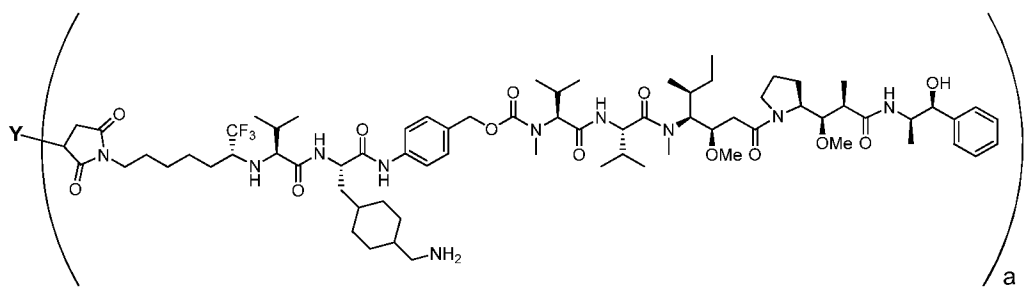
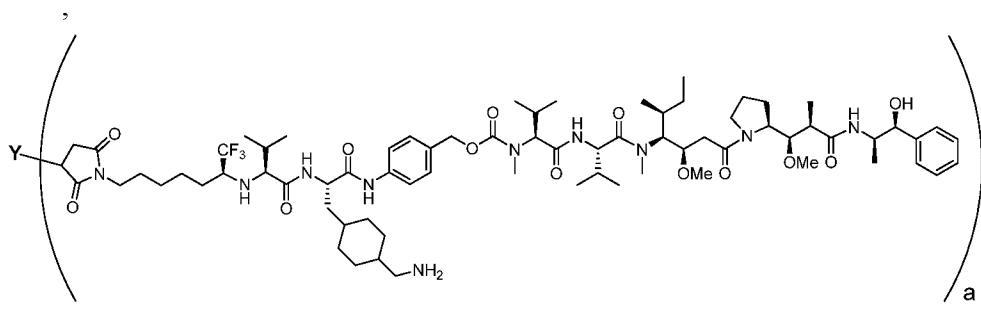
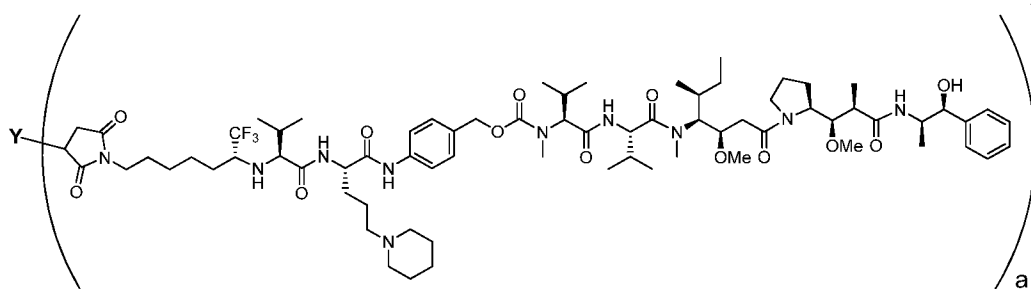
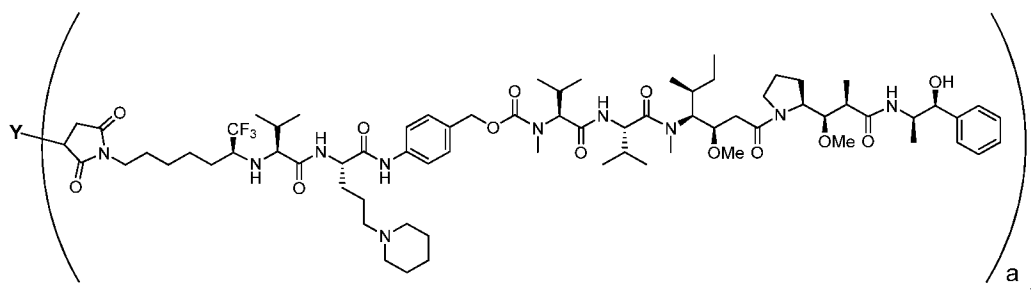
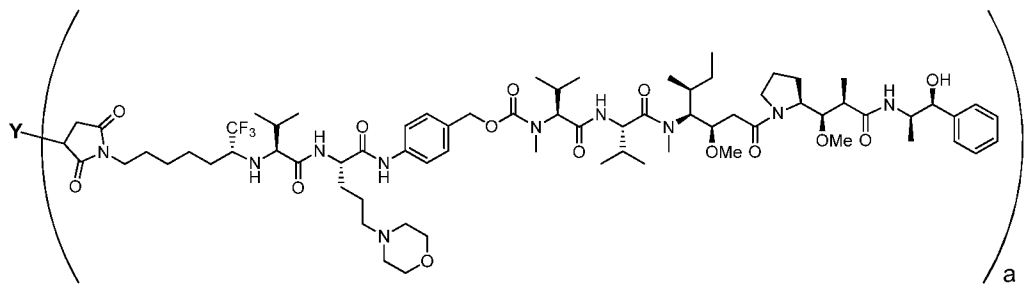
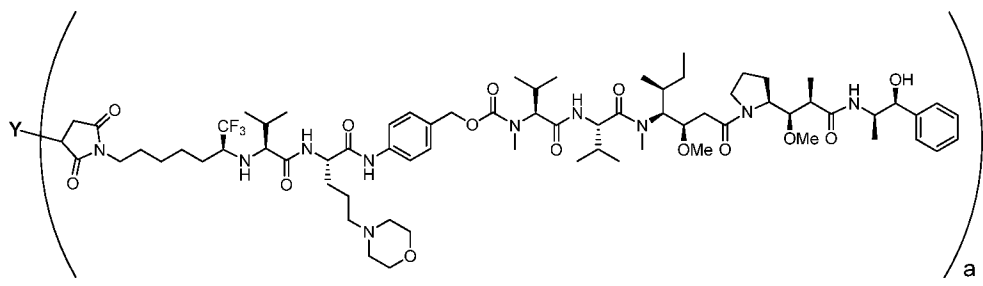
In certain embodiment of this invention, the aforesaid ADC is selected from the group consisting of:

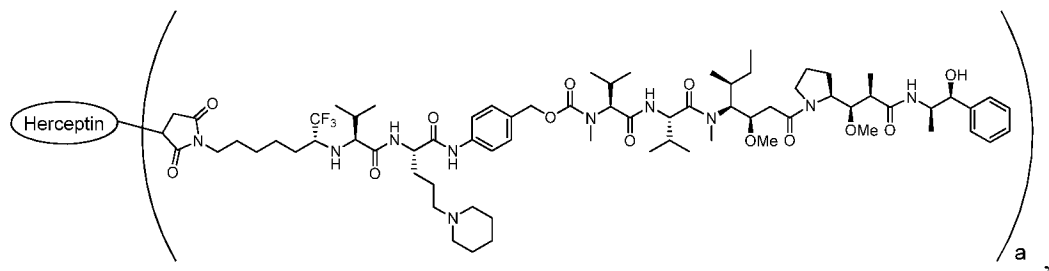
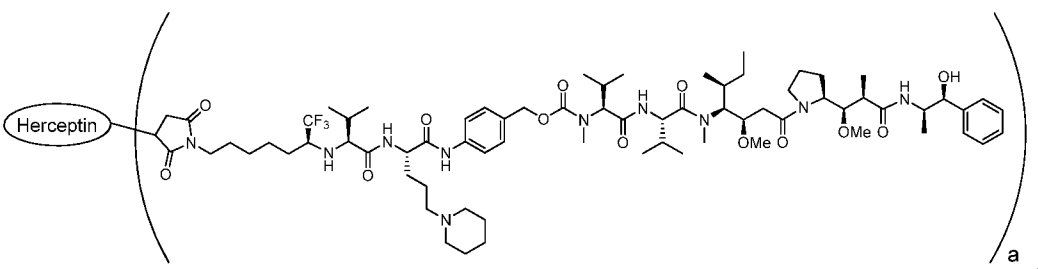
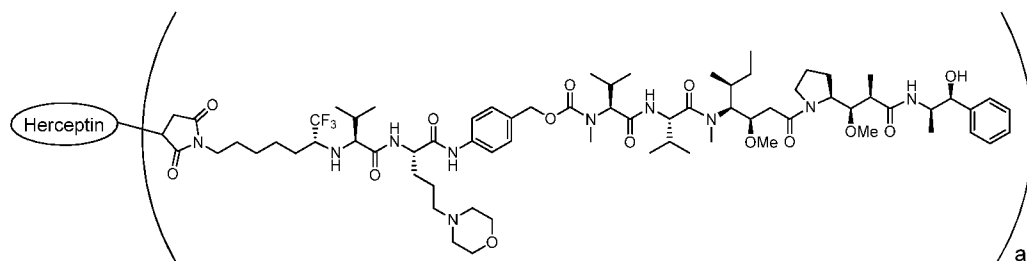
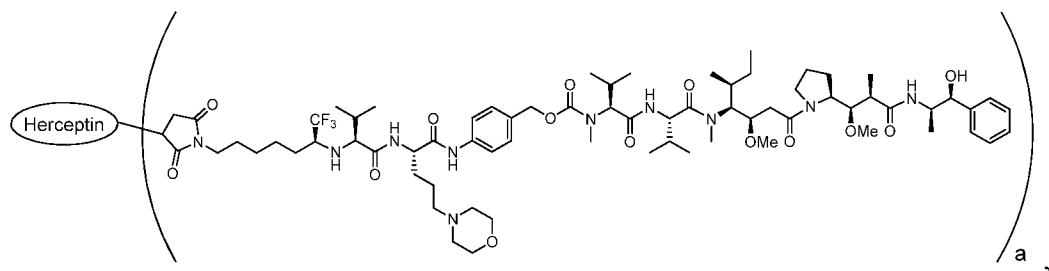
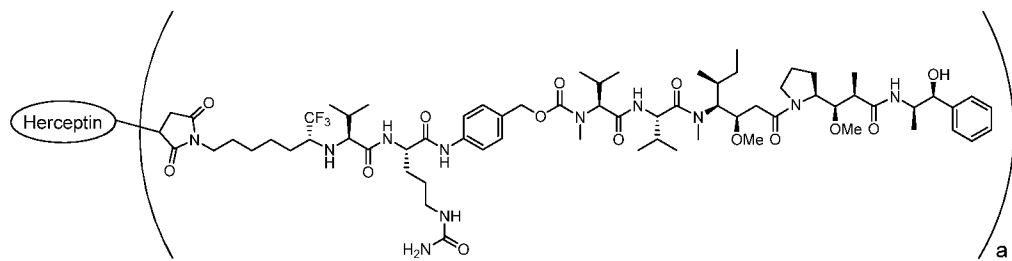
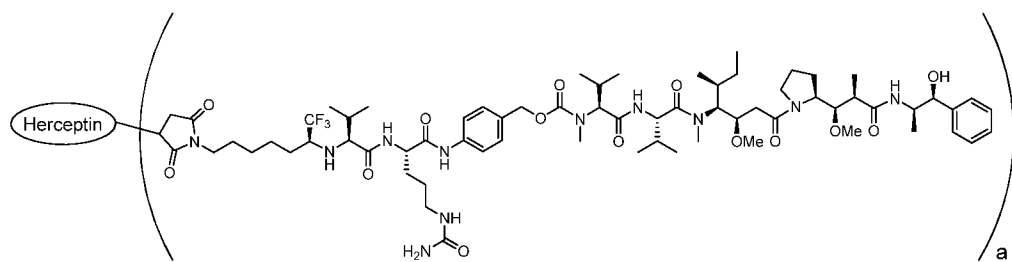


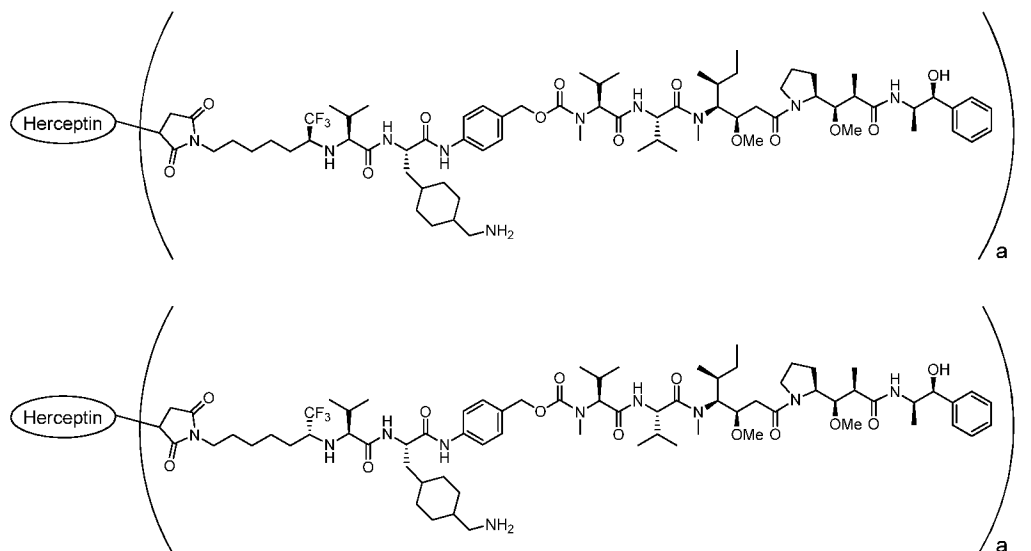


In certain embodiment, the aforesaid ADC is selected from the group consisting of:









and other diastereomers,

a is 2, 3, 4, 5, or 6.

The present invention also provides a pharmaceutical composition comprising an effective amount of the aforesaid ADC and a pharmaceutically acceptable carrier, diluent or excipient.

The present invention also provides a use of the aforesaid linker, conjugate, or ADC or the aforesaid composition in the preparation of a medicament for the treatment of cancers.

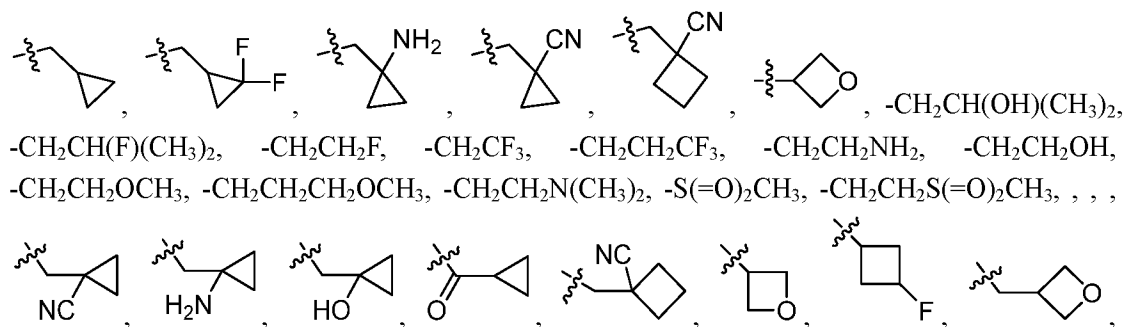
The present invention also provides a method for treating or diagnosing cancers, comprising administering the subject in need thereof an effective amount of the aforesaid ADC or the aforesaid pharmaceutical composition.

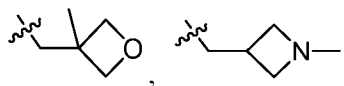
Definitions and Explanations

C₁₋₆ is selected from the group consisting of C₁, C₂, C₃, C₄, C₅, and C₆, the number denotes numbers of carbon atoms; C₃₋₆ is selected from the group consisting of C₃, C₄, C₅, and C₆.

C₁₋₆ alkyl, C₁₋₆ heteroalkyl, C₃₋₆ cyclohydrocarbyl, C₃₋₆ heterocyclohydrocarbyl, C₁₋₆ alkyl substituted by C₃₋₆ cyclohydrocarbyl or C₃₋₆ heterocyclohydrocarbyl, and C₁₋₆ heteroalkyl substituted by C₃₋₆ cyclohydrocarbyl or C₃₋₆ heterocyclohydrocarbyl include, but are not limited to: methyl, ethyl, n-propyl, isopropyl, -CH₂C(CH₃)(CH₃)(OH), cyclopropyl, cyclobutyl, propylmethylene, cyclopropylacetyl, benzyloxy, cyclopropylalkylene, trifluoromethyl, aminomethyl, hydroxymethyl, methyloxy, methylacetyl, methyloxycarbonyl, methylsulfonyl, methylsulfinyl, ethyloxy, ethylacetyl, ethylsulfonyl, ethyloxycarbonyl, dimethylamino, diethylamino, dimethylaminocarbonyl, and diethylaminocarbonyl;

N(CH₃)₂, NH(CH₃), -CH₂CF₃, -CH₂CH₂CF₃, -CH₂CH₂F, -CH₂CH₂S(=O)₂CH₃, -CH₂CH₂CN,





The term "substituted", as used herein, means that any one or more hydrogens on the designated atom is replaced with a selection from the indicated group, including deuterium "D" atom, a variant of hydrogen, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens on the atom are replaced. Keto substituents are not present on aromatic moieties. The term "optionally substituted", as used herein, means that the designated atom can be substituted or unsubstituted by the substituents, and unless otherwise stated, the species and number the substituents are not defined provided that they can be achieved in Chemistry.

When any variable (e.g., R) occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R, then said group may optionally be substituted with up to two R groups and R at each occurrence is selected independently from the definition of R. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

When a bond to a substituent is shown to cross a bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring. When a substituent is listed without indicating the atom via which such substituent is bonded to the rest of the compound of a given formula, then such substituent may be bonded via any atom in such substituent. Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generically referred to as "alkyl group substituents," and they can be one or more of a variety of groups selected from, but not limited to: -R', -OR', =O, =NR', =N-OR', -NR'R'',

-SR', -halogen, -SiR'R''R''', OC(O)R', -C(O)R', -CO₂R', -CONR'R'',

-OC(O)NR'R'', -NR''C(O)R', NR' C(O)NR''R''', -NR''C(O)₂R',

-NR''''C(NR'R'R''')=NR''', NR''' C(NR'R'')=NR''', -S(O)R', -S(O)₂R',

-S(O)₂NR'R'', NR''SO₂R', -CN, -NO₂, -N₃, -CH(Ph)₂, fluoro(C1-C4)alkoxy, and fluoro(C1-C4)alkyl, in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''', R'''' and R''''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, e.g., aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', R'''' and R''''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are generically referred to as "aryl group substituents." The substituents are selected from, for example: $-R'$, $-OR'$, $-NR'R''$, $-SR'$, -halogen, $-SiR'R''R'''$, $OC(O)R'$, $-C(O)R'$, $-CO_2R'$, $-CONR'R''$, $-OC(O)NR'R''$, $-NR''C(O)R'$, $NR' C(O)NR''R'''$, $-NR''C(O)_2R'$, $-NR''''-C(NR'R''R''')=NR''''$, $NR'''' C(NR'R'')=NR''''$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R''$, $NR''SO_2R'$, $-CN$, $-NO_2$, $-N_3$, $-CH(Ph)_2$, fluoro(C1-C4)alkoxy, and fluoro(C1-C4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R' , R'' , R''' , R'''' and R''''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R' , R'' , R''' , R'''' and R''''' groups when more than one of these groups is present.

Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-T-C(O)-(CRR')_q-U-$, wherein T and U are independently $-NR-$, $-O-$, $-CRR'-$ or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A(CH_2)_rB-$, wherein A and B are independently $-CRR'-$, $-O-$, $-NR-$, $-S-$, $-S(O)-$, $S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CRR')_s-X-(CR''R''')_d-$, where s and d are independently integers of from 0 to 3, and X is $-O-$, $-NR'-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituents R, R' , R'' and R''' are preferably independently selected from hydrogen or substituted or unsubstituted (C1-C6)alkyl.

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C1-C4)alkyl" is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

Examples of haloalkyl include, but are not limited to, trifluoromethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl. "Alkoxy" represents an alkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge. C1-6 alkoxy, is intended to include C1, C2, C3, C4, C5, and C6 alkoxy groups. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxo, and s-pentoxo. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, or cyclopentyl. 3-7 cycloalkyl is intended to include C3, C4, C5, C6, and C7 cycloalkyl groups. Alkenyl" is intended to include hydrocarbon chains of either straight or branched configuration and one or more unsaturated carbon-carbon bonds that may occur in any stable point along the chain, such as ethenyl and propenyl.

"Halo" or "halogen" as used herein refers to fluoro, chloro, bromo, and iodo.

As used herein, the term "hetero", mean, unless otherwise stated, "heteroatom" or "heteroradical" (namely radical containing heteroatom), including atoms other than carbon (C) and hydrogen (H), also including the radicals containing these aforesaid heteroatoms. Examples include oxygen (O), nitrogen (N) sulfur (S), silicon (Si), germanium (Ge), aluminum (Al) and

boron (B), also include optionally substituted -C(=O)N(H)-, -N(H)-, -C(=NH)-, -S(=O)₂N(H)-, or -S(=O)N(H)-.

“Ring” as used herein, means a substituted or unsubstituted cycloalkyl, heterocycloalkyl, cycloalkenyl, heterocycloalkenyl, cycloalkynyl, heterocycloalkynyl, aryl, or heteroaryl. A ring includes mono, bi, spiro, fused, and bridged ring moieties. The number of atoms in a ring is typically defined by the number of members in the ring. For example, a “5- to 7-membered ring” means there are 5 to 7 atoms in the encircling arrangement. Unless otherwise specified, the ring optionally includes one to three heteroatoms. Thus, the term “5- to 7-membered ring” includes, for example phenyl, pyridinyl and piperidinyl. The term “5- to 7-membered heterocycloalkyl ring”, on the other hand, would include pyridinyl and piperidinyl, but not phenyl. The term “ring” further includes a ring system comprising more than one “ring”, wherein each “ring” is independently defined as above.

As used herein, the term “heterocycle” or “heterocyclic group” is intended to mean a stable monocyclic, bicyclic, or tricyclic ring containing heteroatom or heteroradical, which is saturated, partially unsaturated or unsaturated (aromatic), and which consists of carbon atoms and 1, 2, 3, or 4 ring heteroatoms independently selected from the group consisting of N, O and S and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The nitrogen and sulfur heteroatoms may optionally be oxidized (i. e., NO and S (O) p). The nitrogen atom may be substituted or unsubstituted (i.e., N or NR wherein R is H or another substituent, if defined). The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. A nitrogen in the heterocycle may optionally be quaternized. It is preferred that when the total number of S and O atoms in the heterocycle exceeds 1, then these heteroatoms are not adjacent to one another. It is preferred that the total number of S and O atoms in the heterocycle is not more than 1. As used herein, the term “aromatic heterocyclic group” or “heteroaryl” is intended to mean a stable 5, 6, or 7-membered monocyclic or bicyclic or 7, 8, 9, or 10-membered bicyclic heterocyclic aromatic ring which consists of carbon atoms and 1, 2, 3, or 4 heterotams independently selected from the group consisting of N, O and S. The nitrogen atom may be substituted or unsubstituted (i.e., N or NR wherein R is H or another substituent, if defined). The nitrogen and sulfur heteroatoms may optionally be oxidized (i.e., NO and S (O) p). It is to be noted that total number of S and O atoms in the aromatic heterocycle is not more than 1. Bridged rings are also included in the definition of heterocycle. A bridged ring occurs when one or more atoms (i.e., C, O, N, or S) link two non-adjacent carbon or nitrogen atoms. Preferred bridges include, but are not limited to, one carbon atom, two carbon atoms, one nitrogen atom, two nitrogen atoms, and a carbon-nitrogen group. It is noted that a bridge always converts a monocyclic ring into a tricyclic ring. When a ring is bridged, the substituents recited for the ring may also be present on the bridge.

Examples of heterocycles include, but are not limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazoliny, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, 4aH-carbazolyl, carboliny, chromanyl, chromenyl, cinnoliny, decahydroquinoliny, 2H, 6H-1, 5,2-dithiazinyl, dihydrofuro [2, 3-b] tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazoliny, imidazolyl, 1H-indazolyl, indolenyl, indoliny, indoliziny, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindoliny, isoindolyl, isoquinoliny,

isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2, 5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinoliziny, quinoxaliny, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2, 4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2, 3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

The term “hydrocarbyl” or its lower concept (such as alkyl, alkenyl, alkynyl and phenyl etc.) by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (i.e. C₁-C₁₀ means one to ten carbons). “Hydrocarbyl” include, but are not limited to, aliphatic hydrocarbyl and aromatic hydrocarbyl, and the aliphatic hydrocarbyl include linear and cyclic ones, specifically including but not limited to, alkyl, alkenyl, and alkynyl, and the aromatic hydrocarbyl include, but are not limited to, 6-12 membered aromatic hydrocarbyl, for example, benzene, and naphthalene. In some embodiments, the term “alkyl” means a straight or branched chain, or combinations thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers.

The term “heterohydrocarbyl” or its lower concept (such as heteroalkyl, heteroalkenyl, heteroalkynyl and heteroaryl etc.) by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom. In some embodiments, the term “heteroalkyl,” by itself or in combination with another term, means a stable straight or branched chain, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom. In an exemplary embodiment, the heteroatoms can be selected from the group consisting of B, O, N and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) B, O, N and S may be placed at any interior position of the heterohydrocarbyl group (including the position at which the hydrocarbyl group is attached to the remainder of the molecule). Examples include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -CH₂-CH=N-OCH₃, and -CH=CH-N(CH₃)-CH₃. Up to two heteroatoms

may be consecutive, such as, for example, $-\text{CH}_2\text{-NH-OCH}_3$.

The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

The terms "cyclohydrocarbyl", "heterocyclohydrocarbyl", or their lower concepts (such as aryl, heteroaryl, cycloalkyl, heterocycloalkyl, cycloalkenyl, heterocycloalkenyl, cycloalkynyl, and heterocycloalkynyl etc.) by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "hydrocarbyl" or "heterohydrocarbyl", respectively. Additionally, for heterohydrocarbyl or heterocyclohydrocarbyl (such as heteroalkyl and heterocycloalkyl), a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Non-limiting examples of heterocycloalkyl moieties include 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl.

The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, substituent that can be a single ring or multiple rings (preferably from 1 to 3 rings), which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms. In an exemplary embodiment, the heteroatom is selected from B, N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalyl, 5-quinoxalyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

For brevity, the term "aryl" when used in combination with other terms (e.g., aryloxy, arylthio, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

The term "leaving group" means a functional group or atom which can be displaced by another functional group or atom in a substitution reaction, such as a nucleophilic substitution reaction. By way of example, representative leaving groups include triflate, chloro, bromo and iodo groups; sulfonic ester groups, such as mesylate, tosylate, brosylate, nosylate and the like; and acyloxy groups, such as acetoxymethyl, trifluoroacetoxymethyl and the like.

The term "protecting group" includes but not limited to "amino-protecting group", "hydroxy-protecting group" and "thiol-protecting group". The term "amino-protecting group" means a protecting group suitable for preventing undesired reactions at an amino nitrogen. Representative amino-protecting groups include, but are not limited to, formyl; acyl groups, for

example alkanoyl groups, such as acetyl, trichloroacetyl or trifluoroacetyl; alkoxy carbonyl groups, such as tert-butoxycarbonyl (Boc); arylmethoxycarbonyl groups, such as benzyloxycarbonyl (Cbz) and 9-fluorenylmethoxycarbonyl (Fmoc); arylmethyl groups, such as benzyl (Bn), trityl (Tr), and 1,1-di-(4'-methoxyphenyl)methyl; silyl groups, such as trimethylsilyl (TMS) and tert-butyldimethylsilyl (TBS); and the like. The term "hydroxy-protecting group" means a protecting group suitable for preventing undesired reactions at a hydroxy group. Representative hydroxy-protecting groups include, but are not limited to, alkyl groups, such as methyl, ethyl, and tert-butyl; acyl groups, for example alkanoyl groups, such as acetyl; arylmethyl groups, such as benzyl (Bn), p-methoxybenzyl (PMB), 9-fluorenylmethyl (Fm), and diphenylmethyl (benzhydryl, DPM); silyl groups, such as trimethylsilyl (TMS) and tert-butyldimethylsilyl (TBS); and the like.

Where the "antibody" is a cell-binding agent, it binds to an antigen that is a polypeptide and may be a transmembrane molecule (e.g. receptor) or a ligand such as a growth factor.

Exemplary antigens include molecules such as renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor vmc, factor IX, tissue factor (TF), and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin, such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta- lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT4, NT-5, or NT-6), or a nerve growth factor such as NGF- β ; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF- β , TGF- β 2, TGF-beta[3], TGF- β 4, or TGF-beta5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins, EpCAM, GD3, FLT3, PSMA, PSCA, MUC1, MUC16, STEAP, CEA, TENB2, EphA receptors, EphB receptors, folate receptor, FOLR1, mesothelin, cripto, alphavbetae, integrins, VEGF, VEGFR, transferrin receptor, IRTA1, IRTA2, IRTA3, IRTA4, IRTA5; CD proteins such as CD2, CD3, CD4, CD5, CD6, CD8, CD11, CD14, CD19, CD20, CD21, CD22, CD23, CD25, CD26, CD28, CD30, CD33, CD36, CD37, CD38, CD40, CD44, CD52, CD55, CD56, CD59, CD70, CD79, CD80, CD81, CD103, CD105, CD134, CD137, CD138, CD152; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon, such as interferon- alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs), e.g., IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the HIV envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins, such as CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4 and VCAM; a tumor associated antigen

such as HER2, HER3 or HER4 receptor; and fragments of any of the above-listed polypeptides, antibody mimics Adnectins (US appl 20070082365), or an antibody which binds to one or more tumor-associated antigens or cell-surface receptors disclosed in US Publication No. 20080171040 or US Publication No. 20080305044 and are incorporated in their entirety by reference.

Concrete antigens for antibodies encompassed by the present invention include CD proteins such as CD2, CD3, CD4, CD5, CD6, CD8, CD11, CD14, CD18, CD19, CD20, CD21, CD22, CD25, CD26, CD27, CD28, CD30, CD33, CD36, CD37, CD38, CD40, CD44, CD52, CD55, CD56, CD70, CD79, CD80, CD81, CD103, CD105, CD134, CD137, CD138, and CD152; members of the ErbB receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-I, MacI, p150.95, VLA-4, ICAM-I, VCAM, EpCAM, alpha4/beta7 integrin, and alpha5/beta3 integrin including either alpha or beta subunits thereof (e.g. anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF; tissue factor (TF); TGF-beta; alpha interferon (alpha-IFN); an interleukin, such as IL-8; IgE; blood group antigens Apo2, death receptor; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C etc.

The most concrete targets herein are IGF-IR, CanAg, EphA2, MUC1, MUC16, VEGF, TF, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD56, CD138, CA6, Her2/neu, EpCAM, CRIPTO (a protein produced at elevated levels in a majority of human breast cancer cells), darpins, alpha_v/beta₃ integrin, alpha_v/beta₅ integrin, alpha_v/beta₆ integrin, TGF-beta, CD11a, CD18, Apo2 and C242 or an antibody which binds to one or more tumor-associated antigens or cell-surface receptors disclosed in US Publication No. 20080171040 or US Publication No. 20080305044 and are incorporated in their entirety by reference.

Concrete antigens for antibodies encompassed by the present invention also include CD proteins such as CD3, CD4, CD8, CD19, CD20, CD27, CD34, CD37, CD38, CD46, CD56, CD70 and CD138; members of the ErbB receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-I, MacI, p150.95, VLA-4, ICAM-I, VCAM, EpCAM, alpha4/beta7 integrin, and alpha v/beta3 integrin including either alpha or beta subunits thereof (e.g. anti-CD11a, anti-CD 18 or anti-CD11b antibodies); growth factors such as VEGF; tissue factor (TF); TGF-β; alpha interferon (alpha-IFN); an interleukin, such as IL-8; IgE; blood group antigens Apo2, death receptor; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C, etc. The most concrete targets herein are IGF-IR, CanAg, EGF-R, EGF-RvIII, EphA2, MUC1, MUC16, VEGF, TF, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD56, CD70, CD 138, CA6, Her2/neu, CRIPTO (a protein produced at elevated levels in a majority of human breast cancer cells), alpha v/beta3 integrin, alpha v/beta5 integrin, TGF-β, CD11a, CD18, Apo2, EpCAM and C242.

Monoclonal antibody techniques allow for the production of specific cell-binding agents in the form of monoclonal antibodies. Particularly well known in the art are techniques for creating monoclonal antibodies produced by immunizing mice, rats, hamsters or any other mammal with the antigen of interest such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus, and viral proteins such as viral coat proteins. Sensitized human cells can also be used. Another method of creating monoclonal antibodies is the use of phage libraries of sFv (single chain variable region), specifically human sFv (see, e.g., Griffiths et al, U.S. Patent No. 5,885,793; McCafferty et al, WO 92/01047; Liming et al, WO 99/06587.)

Selection of the antibody is a matter of choice that depends upon the particular cell population that is to be targeted, but in general monoclonal antibodies and epitope binding

fragments thereof are preferred, if an appropriate one is available.

For example, the monoclonal antibody My9 is a murine IgG2a antibody that is specific for the CD33 antigen found on Acute Myeloid Leukemia (AML) cells (Roy et al. Blood 77:2404-2412 (1991)) and can be used to treat AML patients. Similarly, the monoclonal antibody anti-B4 is a murine IgG1 that binds to the CD 19 antigen on B cells (Nadler et al, J Immunol. 131 :244-250 (1983)) and can be used if the target cells are B cells or diseased cells that express this antigen such as in non-Hodgkin's lymphoma or chronic lymphoblastic leukemia. The antibody N901 is a murine monoclonal IgG1 antibody that binds to CD56 found on small cell lung carcinoma cells and on cells of other tumors of neuroendocrine origin (Roy et al. J Nat. Cancer Inst. 88:1136-1145 (1996)); huC242 is an antibody that binds to the CanAg antigen; Trastuzumab is an antibody that binds to HER2/neu; and anti-EGF receptor antibody binds to EGF receptor.

The drug unit used in this invention can be any cytotoxic, cytostatic or immunosuppressive drug also referred to herein as a cytotoxic, cytostatic or immunosuppressive agent. Examples of drugs include radioactive isotopes, chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogues and derivatives thereof. The Drug unit has an atom that can form a bond with the Linker Unit, in some embodiments, the Drug unit has a nitrogen atom that can form a bond with the Linker unit. In other embodiments, the Drug unit has a carboxylic acid that can form a bond with the Linker unit. In other embodiments, the Drug unit has a sulfhydryl group that can form a bond with the Linker unit. In other embodiments, the Drug unit has a hydroxyl group or ketone that can form a bond with the Linker unit.

Useful classes of cytotoxic or immunosuppressive agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cis-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinoids, pre-formirsg compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. Particularly examples of useful classes of cytotoxic agents include, for example, DNA minor groove binders, DNA alkylating agents, and tubulin inhibitors. Exemplary cytotoxic agents include, for example, auristatins, camptothecins, duocarmycins, etoposides, maytansines and maytansinoids (e.g., DM1 and DM4), taxanes, benzodiazepines (e.g., pyrrolo [1,4] benzodiazepines (PBDs), indolinobenzodiazepines, and oxazolidinobenzodiazepines) and vinca alkaloids. Select benzodiazepine containing drugs are described in WO 2010/091 150, WO 2012/1 12708, WO 2007/085930, and WO 201 1/023883.

Individual cytotoxic or immunosuppressive agents include, for example, an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, calicheamicin, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, etoposide, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gemcitabine, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, iomustine (CCNU), maytansine, mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C,

mitoxantrone, nitroimidazole, paclitaxei, paly toxin, plicamycin, procarbazine, rhizoxin, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP- 16 and VM-26.

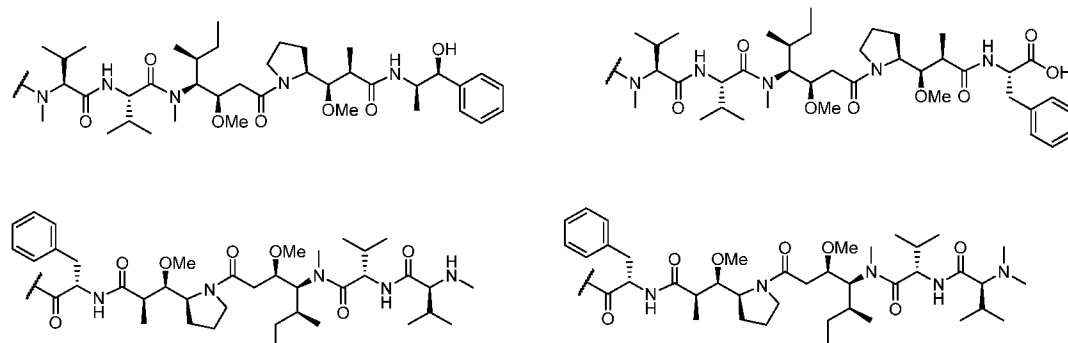
In certain typical embodiments, suitable cytotoxic agents include, for example, DNA minor groove binders (e.g., enediynes and exitropsins, a CBI compound; see also U.S. Patent No. 6,130,237), duocarmycins (see U.S. Publication No. 20060024317), taxanes (e.g., paclitaxei and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino- doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemarotin, maytansinoids, discodermoidin, eleutherobin, and mitoxantrone.

In certain embodiments, the Drug unit is an anti-tubulin agent. Examples of anti-tubulin agents include, but are not limited to, taxanes (e.g., Taxol® (paclitaxei), Taxotere® (docetaxel)), T67 (Tularik) and vinca alkaloids (e.g., vincristine, vinblastine, vindesine, and vinorelbine). Other antitubulin agents include, for example, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine and colcemid, estramustine, cryptophysins, cemarotin, maytansinoids, combretastatins, discodermoidin, and eleutherobin.

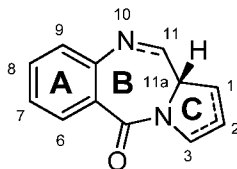
In certain embodiments, the cytotoxic agent is maytansine or a maytansinoid, another group of anti-tubulin agents. (ImmunoGen, Inc.: see also Chari et al, 1992, Cancer Res. 52:127- 131 and U.S. Patent No. 8,163,888).

In certain embodiments, the Drug unit is an auristatin. Auristatins include, but are not limited to, AE, AFP, AEB, AEVB, MMAF, and MMAE. The synthesis and structure of auristatins are described in U.S. Patent Application Publication Nos. 2003-0083263, 2005- 0238649 2005-0009751 , 2009-01 1 1756, and 201 1 -0020343; International Patent Publication No. WO 04/010957, international Patent Publication No. WO 02/088172, and U.S. Patent Nos. 7,659,241 and 8,343,928; each of which is incorporated by reference in its entirety and for all purposes. Exemplary auristatins of the present invention bind tubulin and exert a cytotoxic or cytostatic effect on the desired cell line.

Exemplary auristatin Drug units have the following formula or a pharmaceutically acceptable salt thereof wherein the wavy line indicates site of attachment to the linker unit:



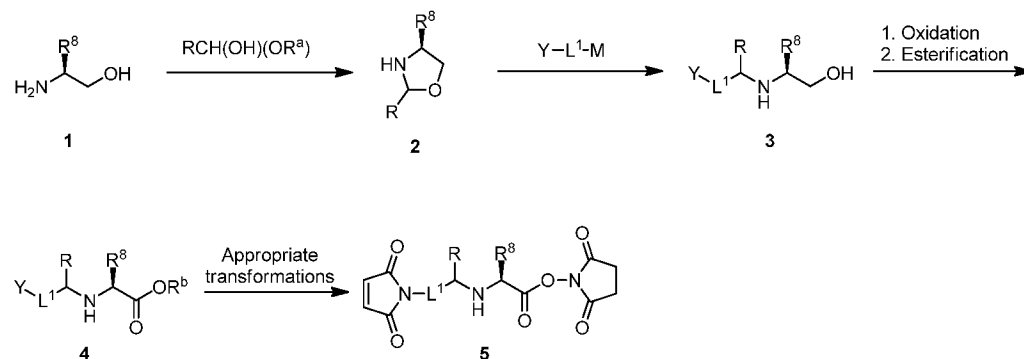
In certain embodiments, the Drug is a benzodiazepine (including benzodiazepine containing drugs e.g., pyrrolo[1,4]benzodiazepines (PBDs), indolinobenzodiazepines, and oxazolidinobenzodiazepines). PBDs are of the general structure:



but can differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine ($\text{N}=\text{C}$), a carbinolamine ($\text{NH}-\text{CH}(\text{OH})\text{R}$) or a carbinolamine methyl ether ($\text{NH}-\text{CH}(\text{OMe})\text{R}$) at the N10-C11 position, which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have a (S)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site. The ability of PBDs to form an adduct in the minor groove enables them to interfere with DNA processing, hence their use as antitumour agents. The biological activity of these molecules can be potentiated by, for example, joining two PBD units together through their C8/C8'-hydroxyl functionalities via a flexible alkylene linker. The PBD dimers are thought to form sequence-selective DNA lesions such as the palindromic 5'-Pu-GATC-Py-3' interstrand cross-link which is thought to be mainly responsible for their biological activity.

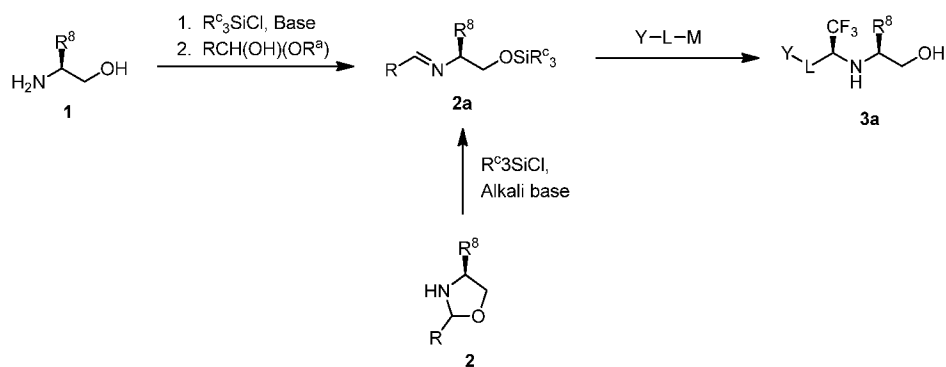
General Method to Prepare Linkers, Drug-Linker conjugates and ADCs:

Method 1



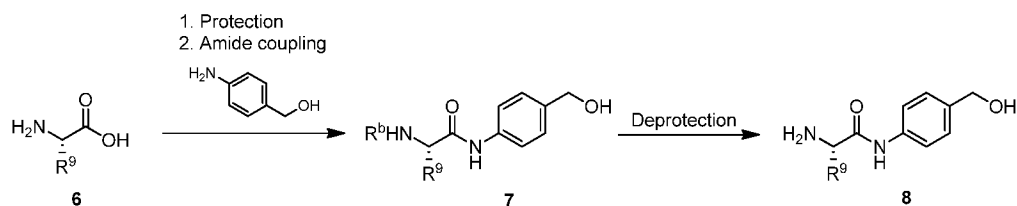
An amino alcohol **1** is reacted with a hemiacetal such as trifluoroacetaldehyde methyl hemiacetal in a solvent such as toluene at refluxing temperature with concomitant removal of water to give an oxazolidine intermediate **2**. Oxazolidine **2** is then reacted with a Grignard or alkali reagent derived from an appropriate precursor to afford an amino alcohol intermediate **3**. Oxidation under an appropriate condition such as Jones reagent or periodic acid with catalytic amount of chromium trioxide and followed by esterification furnishes the ester intermediate **4**. Appropriate manipulation of functional groups on intermediate **4** affords intermediate **5** with suitable handles for further transformations.

Method 2



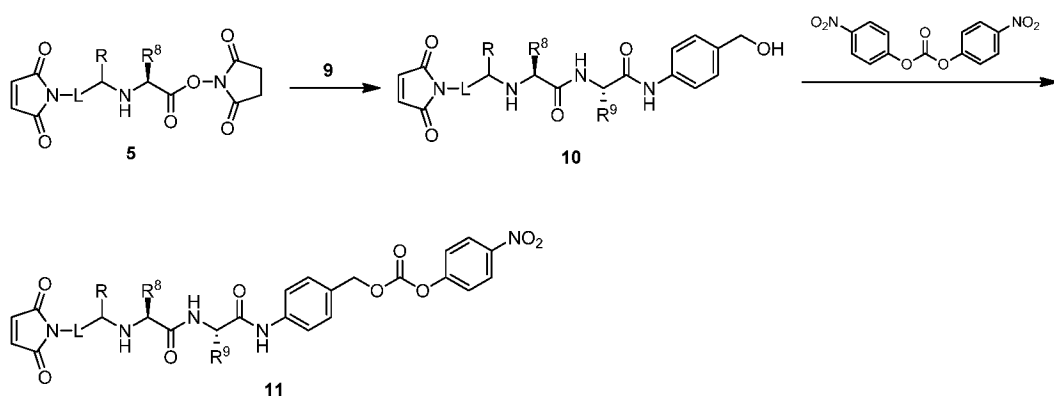
The amino alcohol **3** in Method 1 can also be prepared in a diastereo-selective manner as described in Gosselin, F., et. al., *Org. Lett.* **2004**, 6, 641 and Roy, A., et. al., *J. Org. Chem.* **2006**, 71, 4320 to give **3a** with high distereoselectivity.

Method 3



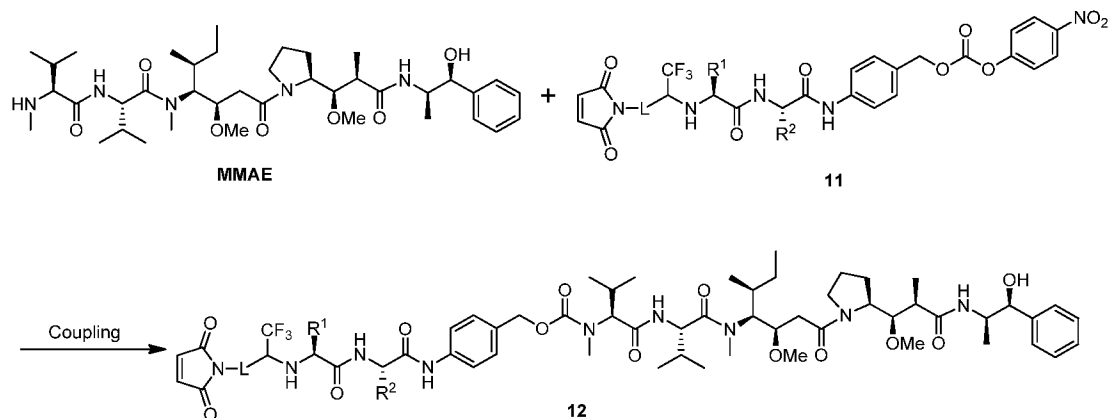
Amino acid **6** is protected with an appropriate protecting group on the amino moiety and then coupling with 4-amino benzyl alcohol to give intermediate **7** in a standard manner. The protecting group on the amine is then removed to give intermediate **8**.

Method 4



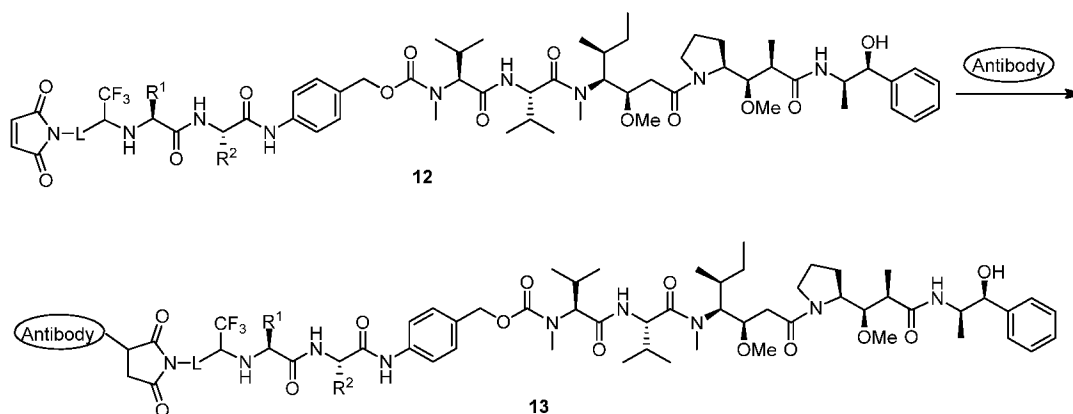
Intermediate **5** from Method 1 and intermediate **8** from Method 3 is reacted under an appropriate condition to give intermediate **10**. Intermediate **10** is then converted to the 4-nitrophenyl carbonate intermediate **11**.

Method 5



Intermediate **11** from Method 4 is reacted with **MMAE** under an appropriate condition to give linker-drug intermediate **12**.

Method 6



Intermediate **12** is coupled with the desired antibody under an appropriate condition as described in the corresponding experimental section.

General Procedure for Conjugation of Drug-Linker Intermediate to Herceptin:

Step 1: Reduction of disulfide bond of antibody and purification

To produce 240 mg ADC, 8.955 mL Herceptin (26.8mg/mL, around 30 mg/mL) was mixed with 8.95 μ L 0.5 M TCEP (final concentration of TCEP in reaction mixture was 0.5 mM), and the reaction was performed at room temperature for 2 hour. Then the reaction solution was loaded on an 1 mL Protein A affinity column to get rid of the remained TCEP and other non-mAb impurities. After the purification, pH of the eluent was adjusted to around 6.0 with 1 M Tris, and the concentration of mAb was measured by nanodrop. The final volume of the solution was about 20mL, and mAb concentration was 11.6 mg/mL.

Step 2: Conjugation of drug-linker and antibody

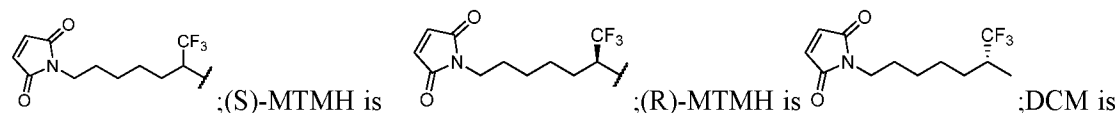
The solution was divided equally into two tubes (10 mL each), and 1 mL DMSO solution of drug-linker intermediate (25 mg/mL) was added to each tube. The ratio of drug-linker intermediate to mAb is 5:1. The reaction was carried out at room temperature for ca 12 hour. When finished, solutions from the two tubes were pooled, centrifuged at 5000rpm for 5min and loaded onto a Protein A affinity column to remove the un-reacted drug-linker intermediate. The

pH of the eluent from the column was adjusted to 6.0 with 1 M Tris buffer.

The invention is now further described by examples. The examples given below are for illustration purpose and not meant to limit this invention. The compounds of the present invention can be prepared in a number of ways known to one skilled in the art of organic synthesis. The examples of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or by variations thereon as appreciated by those skilled in the art. Concrete methods include, but are not limited to, those described below.

All solvents used are commercially available and are used without further purification. Reactions are typically run using anhydrous solvents under an inert atmosphere of nitrogen. Proton NMR are recorded on Bruker Avance III 400 (400 MHz) spectrometer and chemical shifts are reported as (ppm) down field from tetramethylsilane. Mass spectra are determined on Agilent 1200 series plus 6110 (& 1956A). LC/MS, or Shimadzu MS consisting of a DAD: SPD-M20A (LC) and Shimadzu Micromass 2020 detector. The mass spectrometer is equipped with an electrospray ion source (ESI) operated in a positive or negative mode.

The following abbreviations are used: ADC is antibody-drug conjugate; aq is aqueous; mAb is monoclonal antibody; MTMH is 1-Maleinimidyl-6-trifluoromethylhex-6-yl with the structure of



dichloromethane; PE is petroleum ether; DMF is *N,N*-dimethylformamide; DMSO is dimethylsulfoxide; EtOAc is ethyl acetate; EtOH is ethanol; MeOH is methanol; Cbz is benzyloxycarbonyl, an amine protecting group; BOC is *tert*-butylcarbonyl, amine protecting group; HOAc is acetic acid; NaBH(OAc)₃ is sodium triacetoxyborohydride; r.t. is room temperature; THF is tetrahydrofuran; Boc₂O is di-*tert*-butyl dicarbonate; TFA is trifluoroacetic acid; DIPEA is diisopropylethylamine;

Pd(dppf)Cl₂ is

[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II); POCl₃ is phosphorus oxychloride; NaH is sodium hydride; LAH is Lithium Aluminium Hydride; Pd(OAc)₂ is Palladium(II) acetate; Pd₂(dba)₃ is tris(dibenzylideneacetone)dipalladium; Pd(PPh₃)₄ is tetrakis(triphenylphosphine)palladium; Et₃SiH is triethylsilane; PPh₃ is triphenyl phosphine; Xantphos is 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene; MeSO₃H is methanesulfonic acid; Xphos is 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl; Lawesson's reagent is 2,4-Bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide; NBS is N-bromosuccinimide; t-BuOK is potassium *tert*-butoxide;

Compounds are named either manually or by using ChemDraw®, or using vendors catalogue name if commercially available.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 showed the antiproliferative activity of Herceptin and different ADCs and the result suggested that the study ADC001 and the control ADC-HA showed comparable activity in the inhibition on the growth of breast cancer cell line HCC1954 *in vitro*. Herceptin alone is ineffective in this assay.

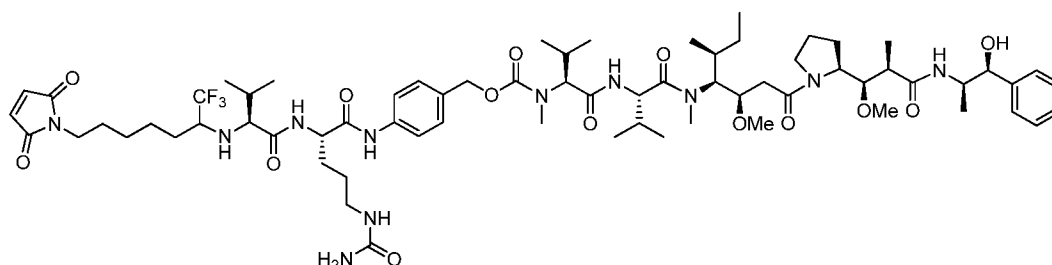
Figure 2 showed the tumor growth curves from each group. The result suggested that the

study ADC001 and the control ADC-HA displayed comparable activity to inhibit tumor growth in the HCC1954 xenograft mice model.

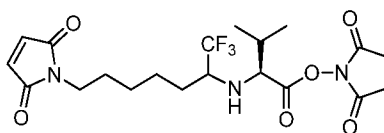
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

To describe the present invention in more detail, the following examples are provided. However, the scope of the present invention is not limited to these.

EXAMPLE 1

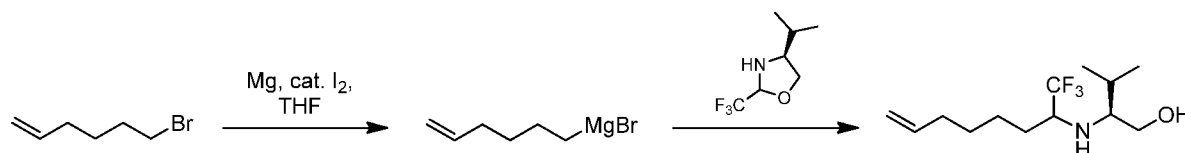


Example 1-1



(2S)-2,5-Dioxopyrrolidin-1-yl-2-[(1,1,1-trifluoroheptan-7-en-2-yl)amino]-3-methylbutanoate

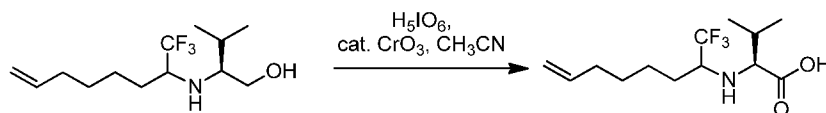
Step 1: (2S)-3-Methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butan-1-ol



To a stirred suspension of Mg (10.2 g, 420 mmol) with a few small crystals of iodine in 80 mL of THF was added a small portion of a solution of 6-bromohex-1-ene (22.8 g, 140 mmol) in anhydrous THF (40 mL). After the reaction initiated, the remaining 6-bromohex-1-ene THF solution was added dropwise at a rate to maintain a gentle refluxing. After complete addition, the resulting mixture was heated at 70°C for 1 h. The Grignard reagent (~120 mL in THF) obtained was then used directly for the next step.

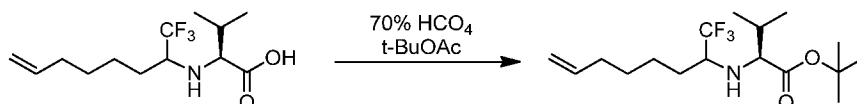
The Grignard reagent from above was cooled to -78 °C and a solution of (4S)-4-isopropyl-2-(trifluoromethyl)oxazolidine (17 g, 93.4 mmol) in THF (40 mL) was added dropwise. The resulting mixture was warmed up to 0 °C and stirred for 1.5 h. The reaction was quenched with sat. NH₄Cl (50 mL), extracted with EtOAc (4 x 100 mL). The organic phase was dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography elution with Pet. ether/EtOAc (0 - 4 %) to give the title compound (11.5 g, 46%) as a yellow oil.

Step 2: (2S)-3-Methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butanoic acid



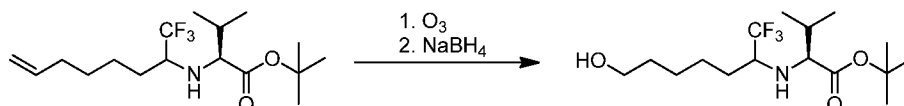
To a solution of H_5IO_6 (33.2 g, 145.8 mmol) in anhydrous CH_3CN (100.0 mL) was added CrO_3 (486 mg, 4.86 mmol) and stirred at room temperature for 1 h. The mixture was then cooled to 0°C and a solution of (2S)-3-methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butan-1-ol (6.5 g, 24.3 mmol, in 20 mL CH_3CN) was added dropwise. The resulting mixture was stirred at 0°C for 1.5 h, quenched with K_2HPO_4 (16.3 g in 50 mL of H_2O) and extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash column chromatography elution with Pet. ether/EtOAc (0 - 4% - 10% EtOAc) to give the title compound (1.04 g, 12.7%) as a yellow oil.

Step 3: (2S)-Tert-butyl 3-methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butanoate



To a solution of (2S)-3-methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butanoic acid (650 mg, 2.3 mmol) in t-BuOAc (10.0 mL) was added dropwise 70% HClO_4 (398 mg, 2.8 mmol) at 0°C and stirred for 1 h at this temperature. After warming to room temperature and further stirred for 16 h, the reaction mixture was poured into EtOAc (50 mL), washed successively with H_2O (30 mL), sat. aq. NaHCO_3 (30 mL), and brine, dried over Na_2SO_4 , filtered, and concentrated. The residue was purified by flash column chromatography elution with Pet. ether/EtOAc (0~2%~5%~10% EtOAc) to give the title compound (0.56 g, 72%) as a colorless oil.

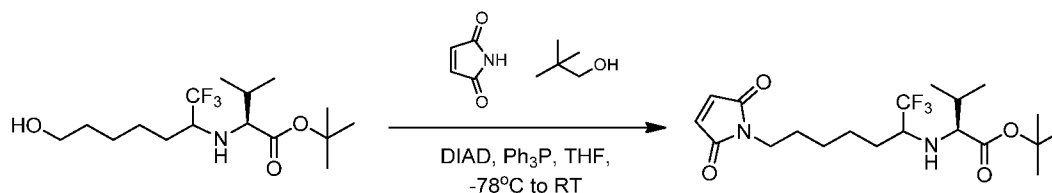
Step 4: (2S)-Tert-butyl 3-methyl-2-[(1,1,1-trifluoro-7-hydroxyheptan-2-yl)amino]butanoate



To a solution of (2S)-tert-butyl 3-methyl-2-[(1,1,1-trifluorooct-7-en-2-yl)amino]butanoate (1.4 g, 4.2 mmol) in anhydrous DCM (6 mL) and MeOH (6 mL) was cooled to -78°C and charged with O_3 for 2 min. The mixture was then warmed to 0°C and NaBH_4 (481 mg, 12.7 mmol) was added. The resulting mixture was stirred at the same temperature for 2 h, quenched with sat. aq. NH_4Cl (4 mL), dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash column chromatography elution with Pet. ether/EtOAc (4%~10% EA) to give the title compound 7 (1.05 g, 72%) as a colorless oil.

^1H NMR (CDCl_3) δ 3.63 (t, $J=6.5$ Hz, 2H), 3.06 (d, $J=6.0$ Hz, 1H), 3.01 (d, $J=4.6$ Hz, 1H), 2.90 - 2.79 (m, 1H), 1.92 - 1.80 (m, 1H), 1.67 - 1.55 (m, 5H), 1.44 (s, 8H), 1.42 - 1.31 (m, 3H), 0.95 - 0.83 (m, 6H).

Step 5: (2S)-Tert-butyl 2-((7-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,1,1-trifluoroheptan-2-yl)amino)-3-methylbutanoate

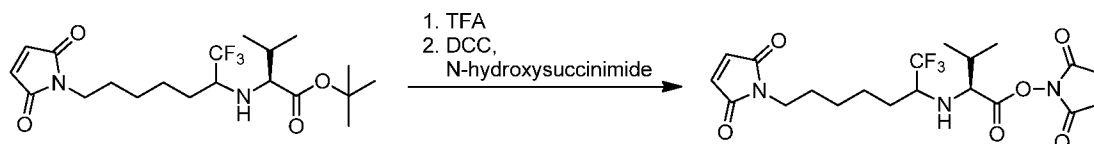


To a solution of triphenyl phosphine (845 mg, 3.2 mmol) in THF (35 mL) at -78°C under N_2

was added DIAD (651 mg, 3.2 mmol) dropwise. The yellow reaction mixture was stirred at -78°C for 5 min and (2S)-tert-butyl 3-methyl-2-[(1,1,1-trifluoro-7-hydroxyheptan-2-yl)amino]butanoate (1.1 g, 3.22 mmol) in THF (5 mL) was added. After further stirring for 5 min, neopentyl alcohol (142 mg, 1.61 mmol) and 1H-pyrrole-2,5-dione (313 mg, 3.22 mmol) were added to the reaction mixture as solids. The reaction was then stirred overnight at ambient temperature and concentrated. The residue was purified by flash column chromatography elution with Pet. ether/EtOAc (0~15 % EtOAc) to give the title compound (550 mg, 40.7 %) as a colorless oil.

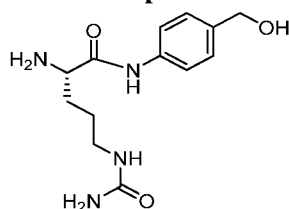
^1H NMR (CDCl_3) δ 6.69 (s, 2 H), 3.52 (t, $J = 7.2$ Hz, 2 H), 3.06-3.01 (m, 1 H), 2.84-2.82 (m, 1 H), 1.89-1.87 (m, 1 H), 1.66-1.52 (m, 8 H), 1.50-1.25 (m, 9 H), 0.95-0.86 (m, 6 H).

Step 6: (2S)-2,5-Dioxopyrrolidin-1-yl-2-{[7-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,1,1-trifluoro-heptan-2-yl]amino}-3-methylbutanoate



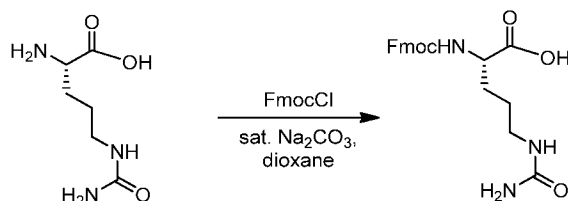
To a solution of (2S)-tert-butyl 2-((7-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,1,1-trifluoroheptan-2-yl)amino)-3-methylbutanoate (350 mg, 0.84 mmol) in anhydrous DCM (6 mL) under N_2 at 0°C was added TFA (6 mL) dropwise. The resulting mixture was stirred at room temperature for 2 h, concentrated and co-evaporated with toluene (2×10 mL) to remove TFA. The residue was then re-dissolved in anhydrous THF (5 mL) under N_2 at 0°C , and triethylamine (251 mg, 2.492 mmol) was added followed by DCC (171 mg, 0.83 mmol). After stirring at room temperature for 5 min, N-hydroxysuccinimide (96 mg, 0.83 mmol) was added. The mixture was stirred at room temperature for 16 h, filtered and concentrated to give the crude title compound (260 mg), which was used for next reaction step without further purification.

Example 1-2



Cit-PAB-OH

Step 1: Fmoc-Cit

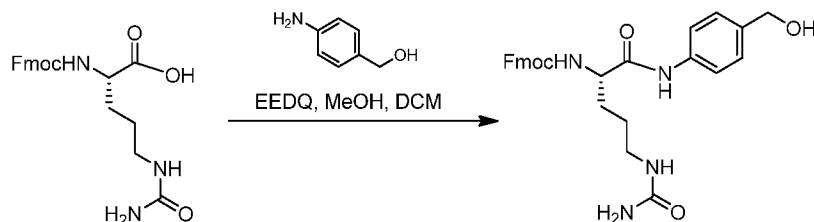


To a solution of L-citrulline (2.0 g, 11.42 mmol) in 10 % aq. Na_2CO_3 (50 mL) at 0°C was added Fmoc-Cl (3.54 g, 13.70 mmol) in dioxane (10 mL) dropwise. The mixture was stirred at 0°C for 5 min and then at room temperature for 2 h. After dilution with H_2O (50 mL), the mixture was washed with EtOAc (50 mL). The aqueous phase was separated, adjusted to pH ~2-3 with 2N aqueous HCl and extracted with EtOAc (100 mL $\times$ 2). The combined organic phases were dried

over Na₂SO₄, filtered and concentrated to give the crude title compound (5.0 g) as white foam.

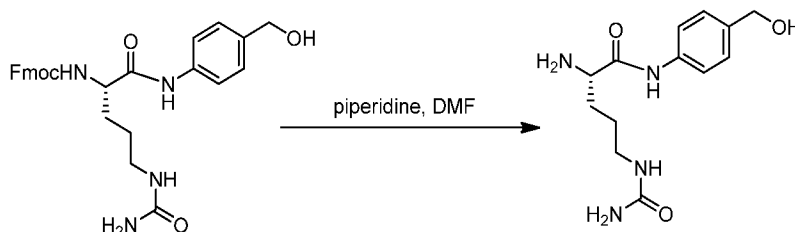
¹H NMR (DMSO-d₆) δ 7.89 (d, *J* = 7.6 Hz, 1 H), 7.74-7.67 (m, 3 H), 7.42 (d, *J* = 7.2 Hz, 2 H), 7.33 (d, *J* = 7.2 Hz, 2 H), 5.95 (d, *J* = 5.6 Hz, 1 H), 5.39 (s, 2 H), 4.28-4.22 (s, 3 H), 3.96-3.91 (m, 1 H), 2.98-2.93 (m, 2 H), 1.72-1.38 (m, 4 H).

Step 2: Fmoc-Cit-PAB-OH



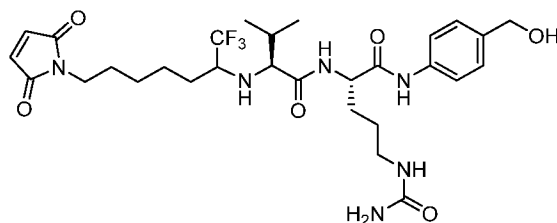
To a solution of crude Fmoc-cit (5.0 g, 11.4 mmol) and 4-aminobenzyl alcohol (1.8 g, 14.9 mmol) in 2:1 DCM/MeOH (150 mL) was added EEDQ (5.65 g, 22.84 mmol). The mixture was stirred in the dark at room temperature for 16 h and then concentrated. The residue was triturated with tert-butyl methyl ether (100 mL). The resulting suspension was sonicated and then left to stand for about 30 min. The white solid was collected by filtration and washed repeatedly with tert-butyl methyl ether to give the crude title compound (5.5 g).

Step 3: Cit-PAB-OH



To a solution of Fmoc-cit-PAB-OH (284 mg, 0.56 mmol) in DMF (3 mL) at room temperature was added piperidine (0.6 mL). The mixture was stirred at room temperature for 2 h. Solvent was removed *in vacuo* and the residue was co-evaporated with DMF (10 mL x 2) to remove piperidine. The thick oily residue was washed with Pet. ether (10 mL x 2) and dried to give the title compound as brown oil, which was used for next step without further purification.

Example 1-3

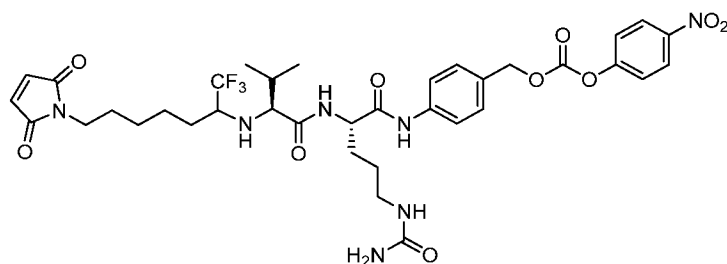


MTMH-Val-Cit-PAB-OH

To a solution of crude (2S)-2,5-dioxopyrrolidin-1-yl-2-{[7-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,1,1-trifluoro-heptan-2-yl]amino}-3-methylbutanoate (0.56 mmol) from step 6 of Example 1-1 in DMF (5 mL) was added crude Cit-PAB-OH (0.56 mmol) from step 2 of Example 1-2 under N₂. The resulting mixture was stirred at room temperature for 16 h. Solvent was removed *in vacuo*. The thick oily residue was washed with tert-butyl methyl ether (10 mL x 2) to give the crude title

compound, which was used for next step without further purification. LCMS(ESI): 627.3 (MH⁺)

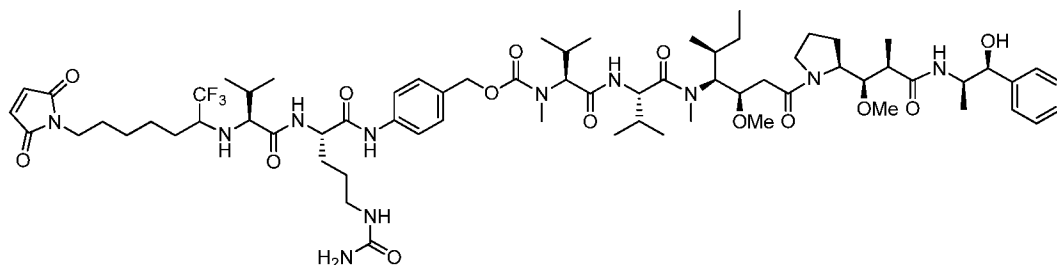
Example 1-4



MTMH-Val-Cit-PAB-(4-nitrophenyl) carbonate

To a solution of MTMH-Val-Cit-PAB-OH (260 mg crude, 0.56 mmol) in DMF (3 mL) was added bis(4-nitrophenyl) carbonate (340 mg 1.12 mmol) and DIPEA (108 mg, 0.84 mmol). The mixture was stirred at room temperature for about 1 h and concentrated. The residue was washed with tert-butyl methyl ether (10 mL x 2) to give the title compound, which was used directly for next step without further purification. LCMS(ESI): 792.3 (MH⁺)

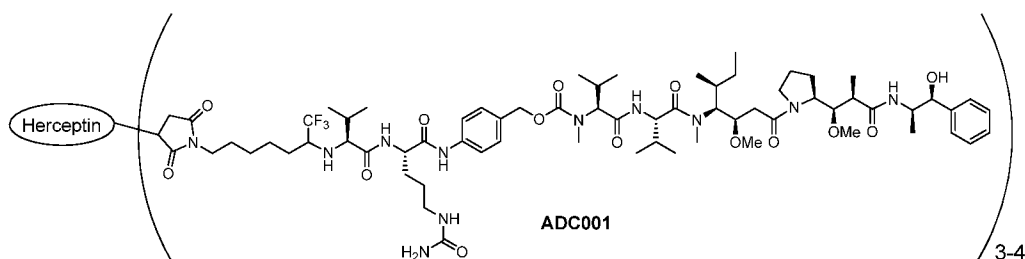
Example 1-5



MTMH-Val-Cit-PAB-MMAE

To a solution of MMAE (0.28 mmol) and MTMH-Val-Cit-PAB-(4-nitrophenyl) carbonate (0.56 mmol) in anhydrous DMF (2.5 mL) was added HOBt (23 mg, 0.17 mmol). After stirring at room temperature for 2 min, pyridine (0.5 mL) was added. The mixture was stirred at room temperature for 16 h and then concentrated. The residue was purified by Prep-HPLC to give the title compound (40 mg, 10.4 %) as a white solid, LCMS(ESI): = 685.9 [(M/2)H⁺]

Example 2



Herceptin (240 mg) and drug-linker intermediate 1 (11.3 mg) was coupled according to the general procedure for conjugation of drug-linker intermediate to herceptin to give the title ADC

(168 mg, 70%). The drug antibody ratio (DAR) of 3 to 4 was estimated by intact and de-glycosylated mass spectroscopy.

Screening of suitable dipeptide units for drug-linker intermediates

A convenient method was developed to identify suitable dipeptide units for the use in drug-linker intermediates. Cbz-protected dipeptides incorporated a fluorescent tag (7-amino-4-methylcoumarin, 7-AMC) via a para-aminobenzyl carbonyl fragment were prepared by standard peptide chemistry as shown in Scheme 1. These dipeptide substrates were then incubated with bovine cathepsin B under a similar condition as described by Dubowchik (Biorg. Med. Chem. Lett. **1998**, 8, 3341-3346). Rate of hydrolysis by bovine cathepsin B was measured by the release of 7-AMC (Table 1). The reference substrates **A** and **B** with the known Val-Cit and Phe-Lys respectively showed the hydrolysis rates as in the expected order. Novel dipeptides with hydrolysis rates slower than the substrate **A** were rejected. The three dipeptide units in **FS-9**, **FS-10** and **FS-11** were selected as additional dipeptide units for drug-linker intermediate preparation.

Scheme 1. Synthesis of cbz-protected dipeptide fluorescent substrates

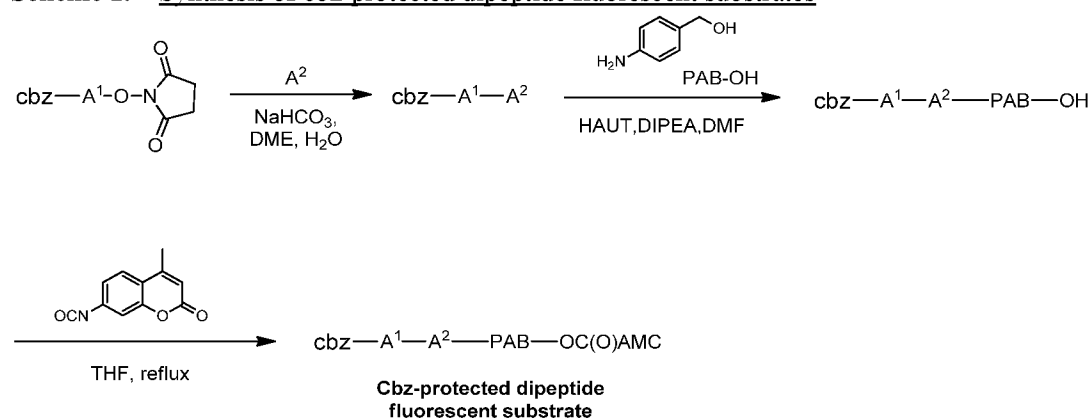
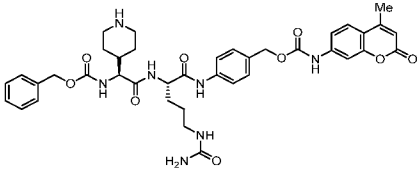
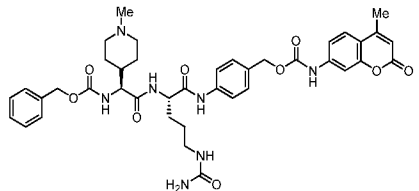
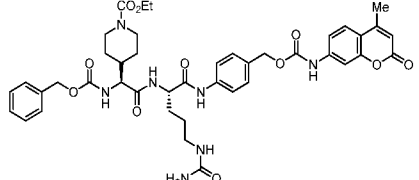
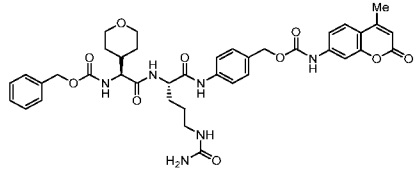
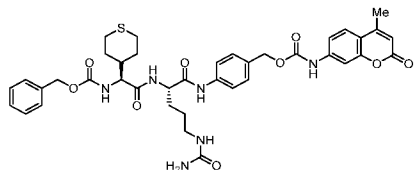
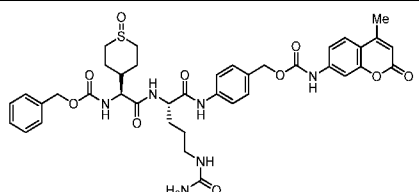
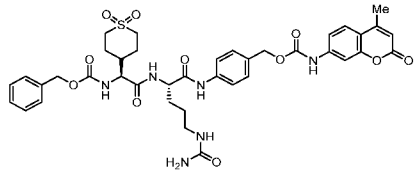
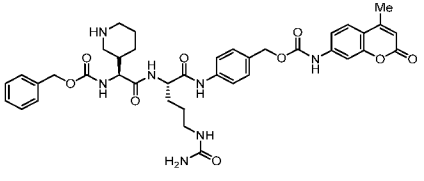
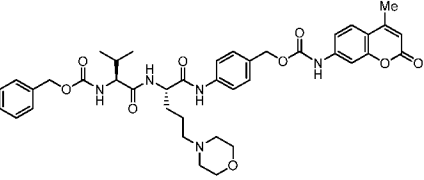
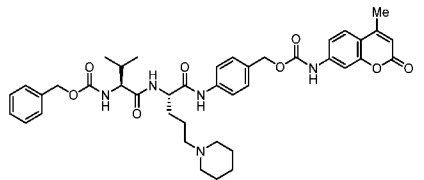
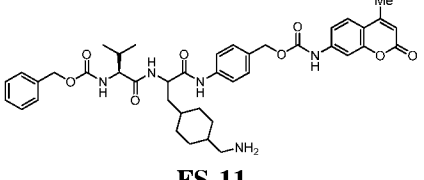


Table 1. Rate of hydrolysis under the incubation with bovine cathepsin B

Fluorescent Substrate	Rate of Hydrolysis (nmol/min/mg)
A	2.67
B	8.46

 FS-1	0.84
 FS-2	0.59
 FS-3	0.63
 FS-4	0.84
 FS-5	0
 FS-6	0.55
 FS-7	0

 FS-8	0
 FS-9	27.2
 FS-10	25.1
 FS-11	20.9

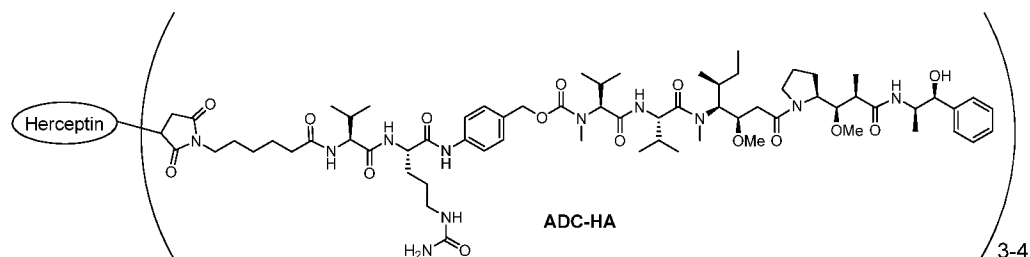
In Vitro Cytotoxicity Study

Breast cancer cell line HCC1954 was grown in appropriate medium supplemented with 10% fetal bovine serum and maintained in an atmosphere of 5% CO₂ in a humidified 37 °C incubator.

The day before treatment, cells were collected and seeded into 96-well plates (2,000 cells per well). On the second day, cells were treated with a dilute concentration of Herceptin and ADCs of this invention (10, 3.33, 1.11, 0.37, 0.123, 0.041, 0.014, 0.0048, 0.0015 and 0.0005 µg/ml). Each treatment was performed in triplicate.

After 72 hours treatment, cell viability was assessed by Cell Titer-Glo kit (Promega) according to the manufacturer's instruction.

The study was compared to a control ADC (ADC-HA) with the normal amide bond at the carbon centre bearing the trifluoromethyl group.

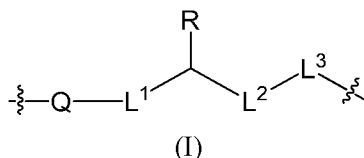


In Vivo Efficacy Study

7-8 Week old female NOD-SCID mice were injected with HCC1954 cells subcutaneously (Estrogen tablets were implanted subcutaneously three days before cell injection.). Treatment was started 9 days after cell injection once the mean estimated tumor mass reached 170 mm³. Mice were grouped randomly according to the tumor volume and body weight (8 animals per group). Animals were treated with ADCs of this invention (15 mpk), Herceptin (15 mpk) as well as vehicle control on day 0 and day 15. Animals were euthanized if the tumor volume of vehicle control group reached a mass of 1500 mm³.

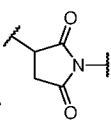
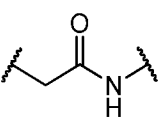
What is claimed is:

1. A linker of formula (I) to connect drugs to antibodies,



wherein,

R is selected from the group consisting of CF₂H, CF₃, CF₂CF₃, and PhSO₂Me;

Q is selected from the group consisting of  and  ;
 L¹ is selected from the group consisting of -(CR¹R²)_m-(CR³R⁴)-(CR¹R²)_n-,
 -(CR¹R²)_m-(CR³R⁴)-O-(CR¹R²)_n-, and -[(CR¹R²)(CR¹R²)X]_p-(CR¹R²)_q-;

m is 1, 2, 3, or 4;

n is 1, 2, 3, or 4;

p is 1, 2, 3, 4, 5, or 6;

q is 1 or 2;

R¹ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂.

R² is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R³ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R⁴ is selected from the group consisting of H, C₁₋₃ alkyl, OH, NR⁵R⁶, CO₂H, P(O)(OH)₂, and SO₃H, said C₁₋₃ alkyl is optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

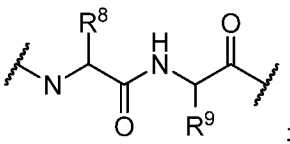
R⁵ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

R⁶ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

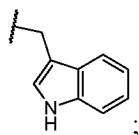
X is selected from the group consisting of NR⁷, O, S(O)_r;

r is 0, 1, or 2;

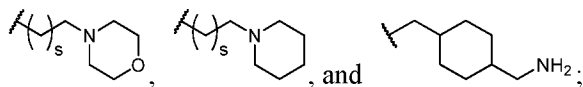
R⁷ is selected from the group consisting of H and C₁₋₃ alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH₂, NHMe, and NMe₂;

L² is a di-peptide unit selected from  ;

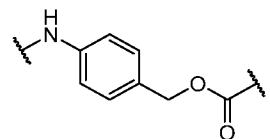
R^8 is selected from the group consisting of methyl, propyl, isopropyl, sec-butyl, benzyl, and



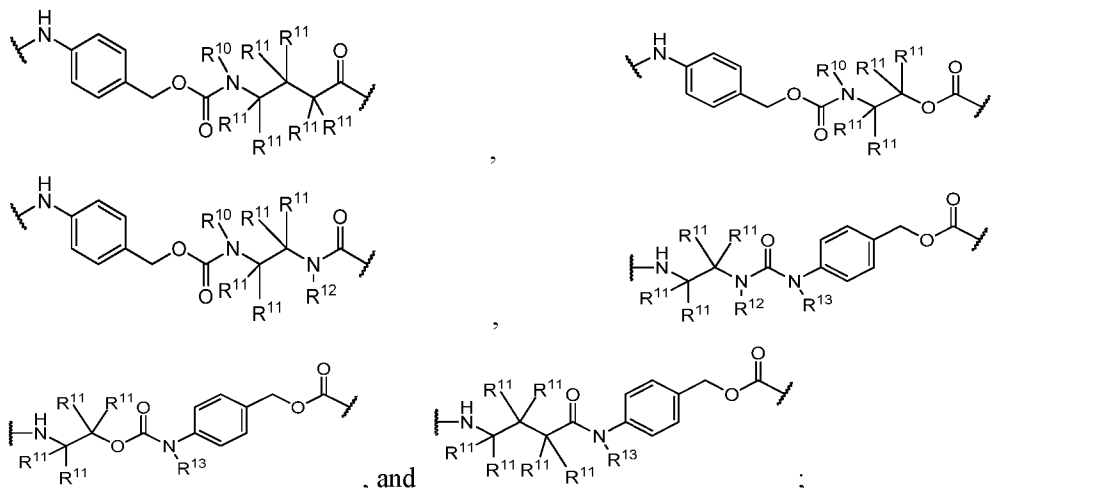
R^9 is selected from the group consisting of $(CH_2)_4NH_2$, $(CH_2)_3NHCONH_2$, $(CH_2)_3NHC(=NH)NH_2$,



s is 0, 1, 2, 3, or 4;



L^3 is a self-immolative unit selected from the group consisting of



R^{10} is selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , $NHMe$, and NMe_2 ;

each R^{11} is independently selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , $NHMe$, and NMe_2 ;

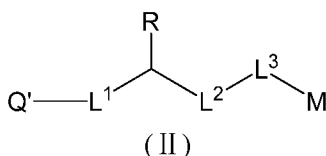
two geminal R^{11} may be optionally joined together to form a 3-6 membered ring with a carbon atom to which they are attached;

two adjacent R^{11} may be optionally joined together to form a 5-7 membered ring with a carbon atom to which they are attached;

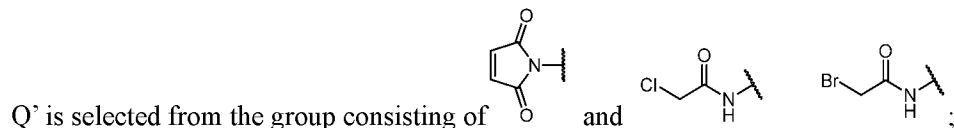
R^{12} is selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , $NHMe$, and NMe_2 ; and

R^{13} is selected from the group consisting of H and C_{1-3} alkyl optionally substituted with 1, 2 or 3 of substituents independently selected from the group consisting of halogen, OH, NH_2 , $NHMe$, and NMe_2 .

2. A drug-linker conjugate of formula (II),



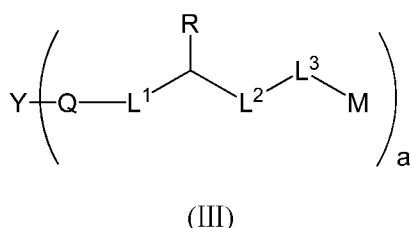
wherein,



M is a drug; and

other variables are as defined in claim 1.

3. An ADC of formula (III) containing the linker of claim 1,



wherein,

Y is an antibody;

a is 1, 2, 3, 4, 5, 6, 7, or 8;

M is a drug; and

other variables are as defined in claim 1.

4. The linker of claim 1 or the ADC of claim 3, wherein the antibody is selected from the group consisting of a resurfaced monoclonal antibody, a resurfaced single chain monoclonal antibody, or a resurfaced monoclonal antibody fragment that preferentially binds to a target cell;

or the antibody is selected from the group consisting of a humanized monoclonal antibody, a humanized single chain monoclonal antibody, or a humanized monoclonal antibody fragment;

or the antibody is selected from the group consisting of a chimeric antibody, a chimeric antibody fragment, a domain antibody, or a domain antibody fragment thereof;

or the antibody is selected from the group consisting of MY9, anti-B4, EpCAM, CD2, CD3, CD4, CD5, CD6, CD11, CD19, CD20, CD22, CD26, CD30, CD33, CD37, CD38, CD40, CD44, CD56, CD79, CD105, CD138, EphA receptors, EphB receptors, EGFR, EGFRvIII, HER2, HER3, mesothelin, cripto, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ integrin or C242;

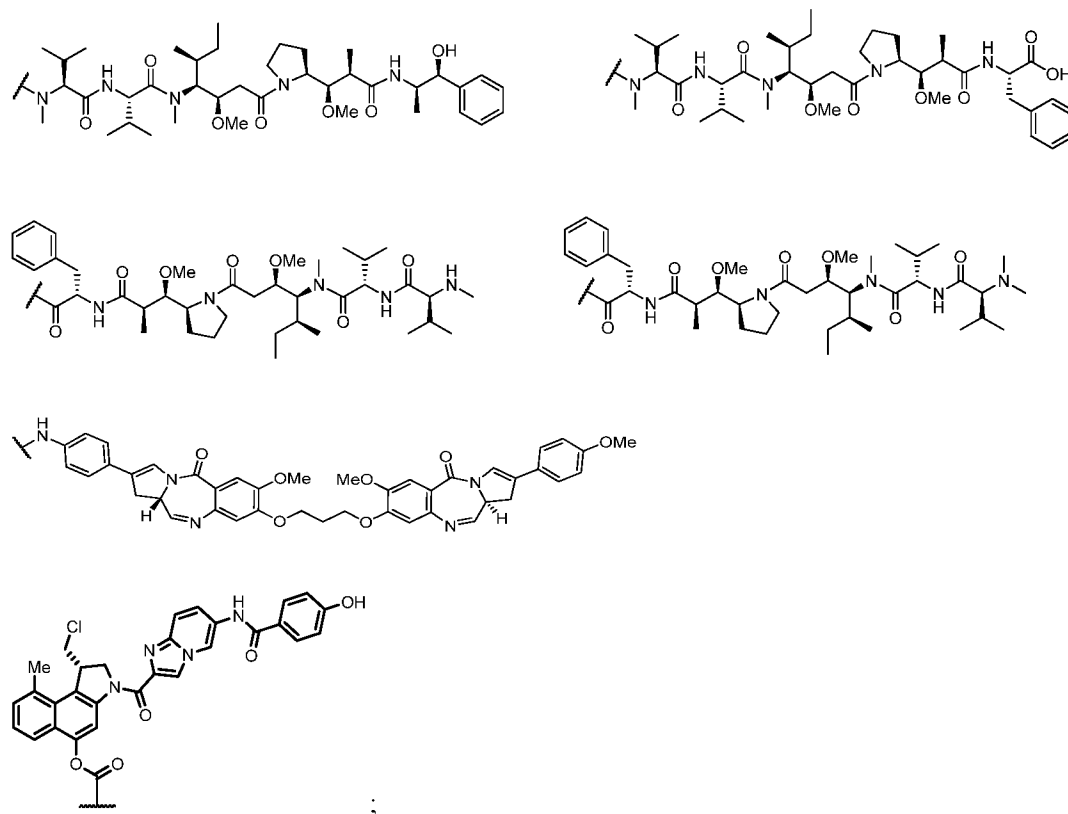
or the antibody is selected from the group consisting of My9-6, B4, C242, N901, DS6, EphA2 receptor, CD38, IGF-IR, CNTO 95, B-B4, Trastuzumab, Tertuzumab, Bevatuzumab, Sibrotuzumab, Rituximab, and Adalimumab.

5. The linker of claim 1 or the ADC of claim 3, wherein the antibody binds to target cells selected from tumor cells; virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melanocytes; cells expressing one or more of IGF-IR, CanAg, EGFR, MUC1, MUC1 6, VEGF, TF, MY9, anti-B4, EpCAM, CD2, CD3, CD4, CD5, CD6, CD11, CD11a, CD18, CD19, CD20, CD22, CD26, CD30, CD33, CD37, CD38, CD40, CD44, CD56, CD70, CD79, CD105, CD138, EphA receptors, EphB receptors, EGFRvIII, HER2/neu, HER3, mesothelin, cripto, $\alpha_v\beta_3$ integrin, $\alpha_v\beta_5$

integrin, alpha₆ integrin, Apo2, and C242 antigens; or cells expressing insulin growth factor receptor, epidermal growth factor receptor, and folate receptor, concretely the tumor cells are selected from breast cancer cells, prostate cancer cells, ovarian cancer cells, colorectal cancer cells, gastric cancer cells, squamous cancer cells, small-cell lung cancer cells, and testicular cancer cells.

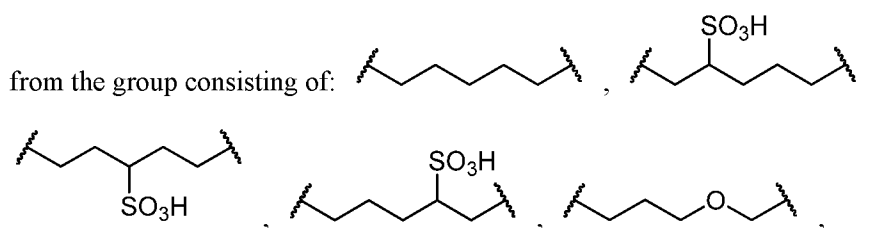
6. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein the drug is a cytotoxic drug.

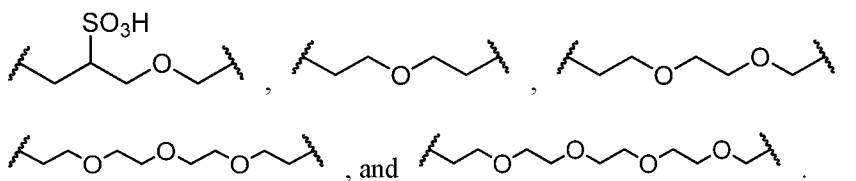
concretely the drug is selected from the group consisting of maytansinoid, DNA-binding drug and its analog, calicheamicin, doxorubicin and its analog, vinca alkaloid, cryptophycin, dolastatin, auristatin and analog thereof, tubulysin, epothilone, taxoid and siRNA, more concretely the DNA-binding drug is CC-1065, , more concretely M is selected from the group consisting of:



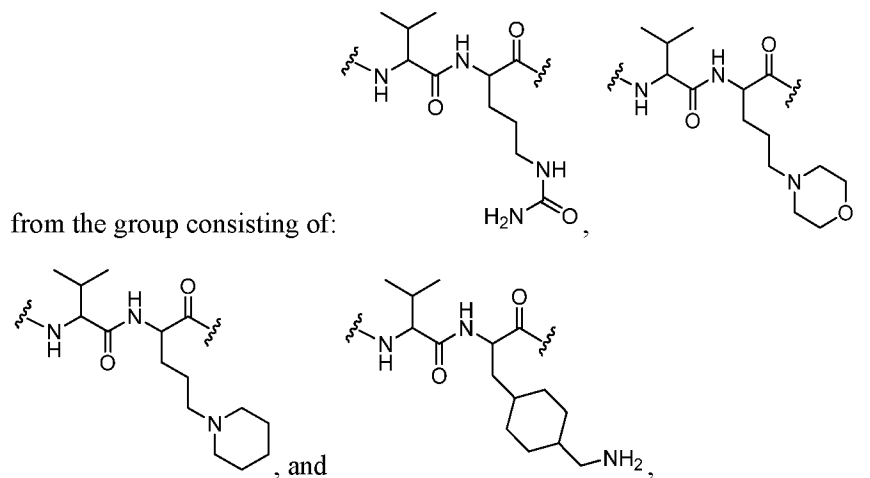
or the drug is a diagnostic or detection reagent, concretely the drug is a radio-labeled compound, concretely the drug is labeled with ^3H , ^{18}F , ^{11}C , ^{13}N , ^{15}O , ^{201}Tl , ^{32}P , ^{51}Cr , ^{67}Ga , ^{123}I , ^{125}I , ^{131}I , ^{132}I , ^{131}Cs , ^{113}Xe , ^{133}Xe , ^{169}Yb , ^{198}Au , ^{203}Hg , $^{99\text{m}}\text{Tc}$, $^{113\text{m}}\text{In}$, $^{133\text{m}}\text{In}$, ^{75}Se , ^{186}Re , ^{153}Sm , and ^{89}Sr .

7. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein L¹ is selected

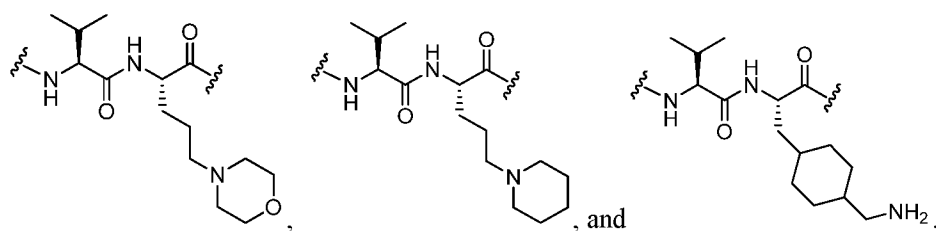




8. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein L^2 is selected

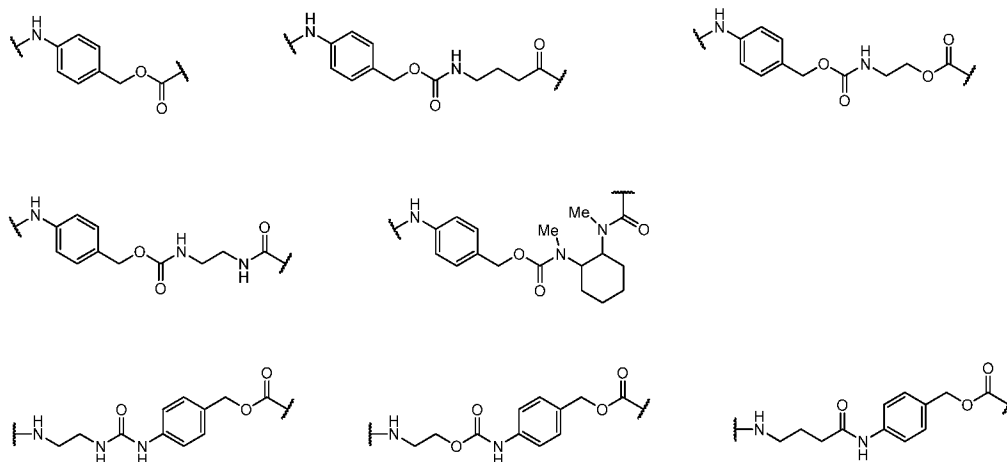


concretely L^2 is selected from the group consisting of:

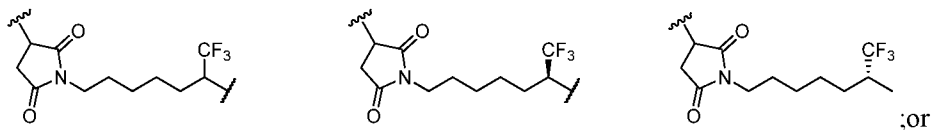
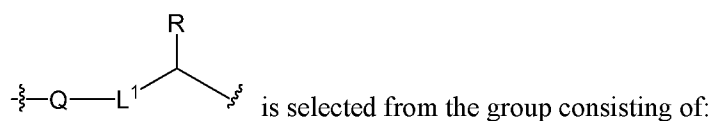


9. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein the ring formed by two geminal R^{11} or two adjacent R^{11} is cyclohexyl.

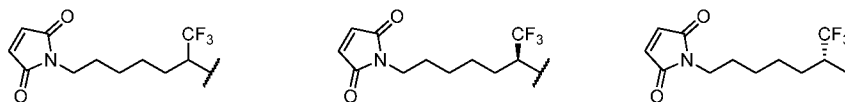
10. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein L^3 is selected from the group consisting of:



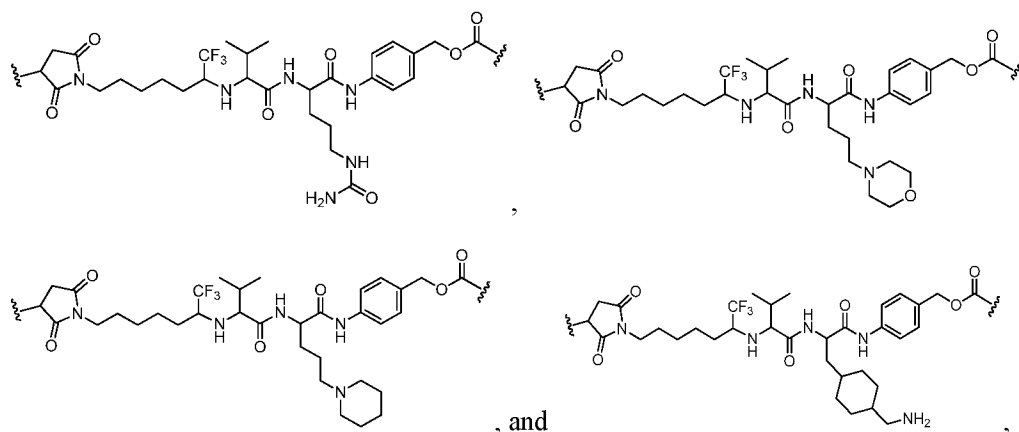
11. The linker of claim 1, the conjugate of claim 2, or the ADC of claim 3, wherein the moiety of



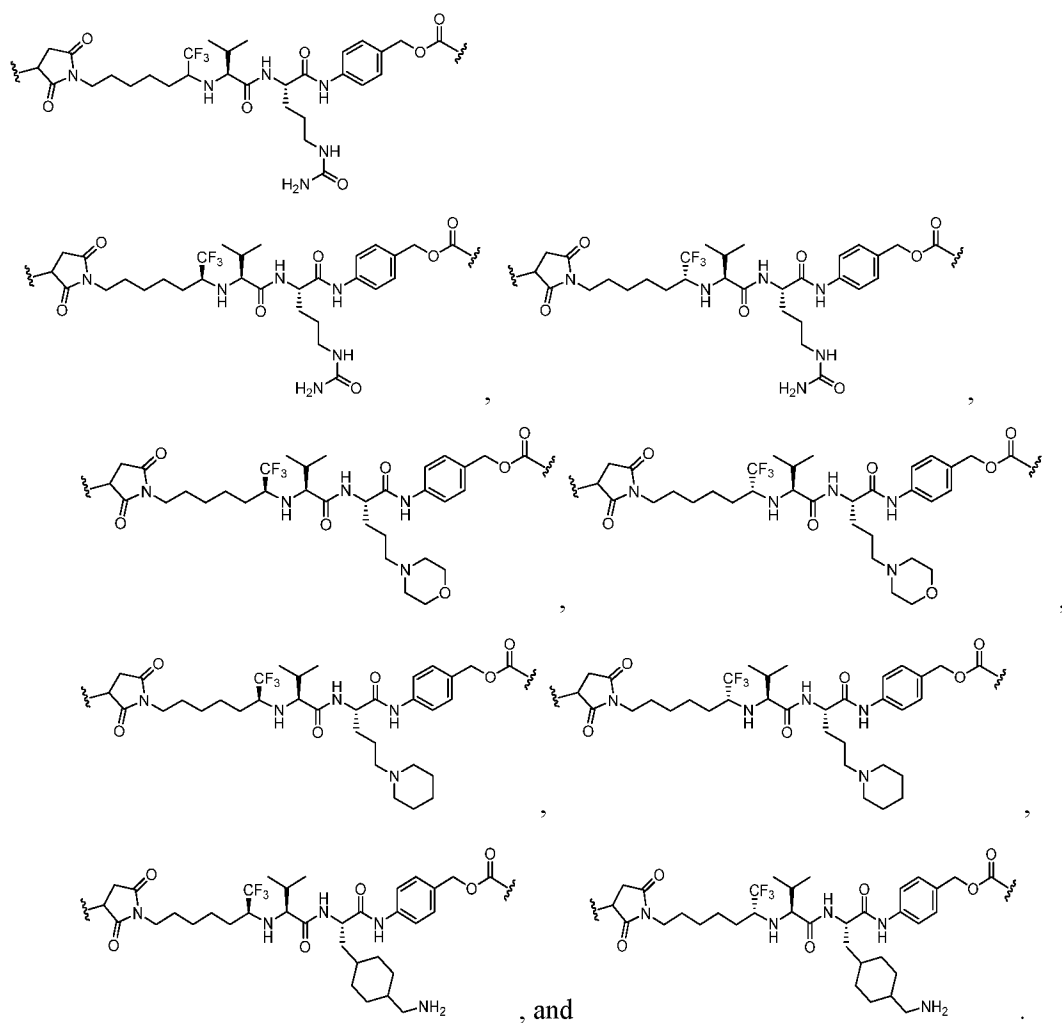
the moiety of $Q'\text{---}L^1\text{---}\overset{\text{R}}{\underset{\text{---}}{\text{C}}}\text{---}$ is selected from the group consisting of:



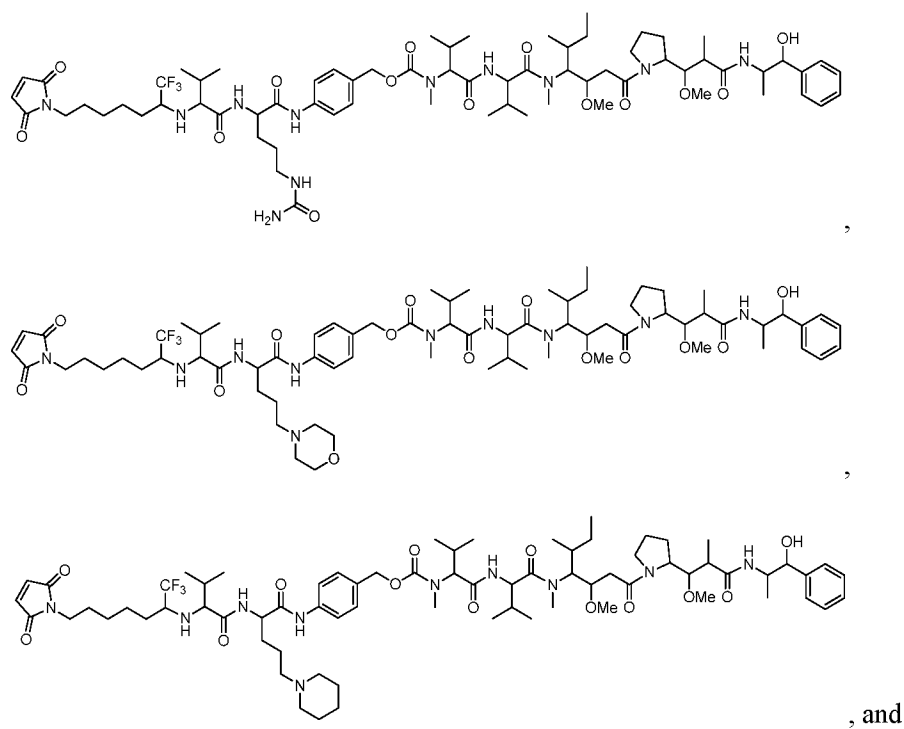
12. The linker of claim 1 or the ADC of claim 3, wherein the linker is selected from the group consisting of:

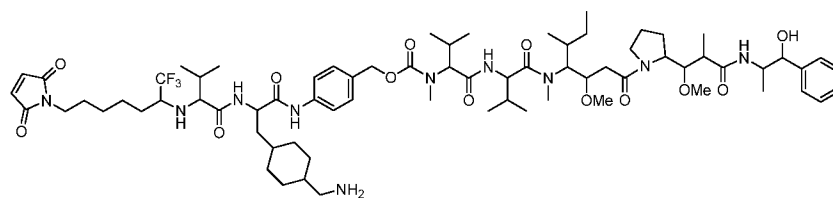


concretely the linker is selected from the group consisting of:

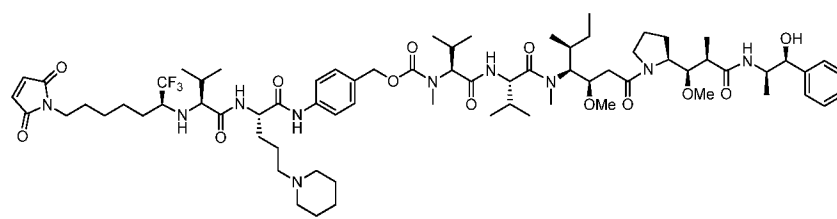
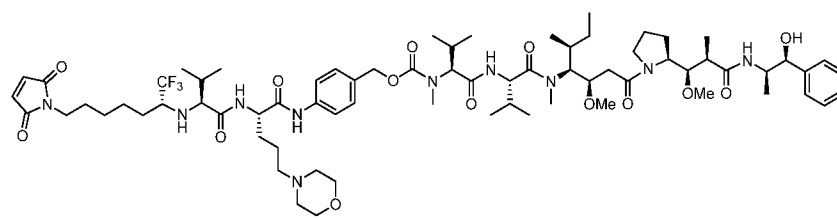
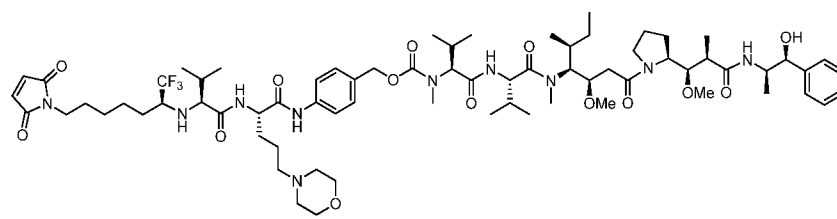
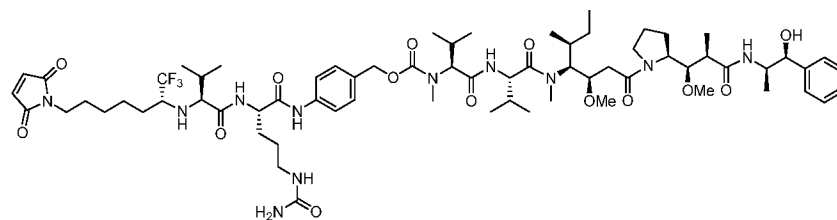
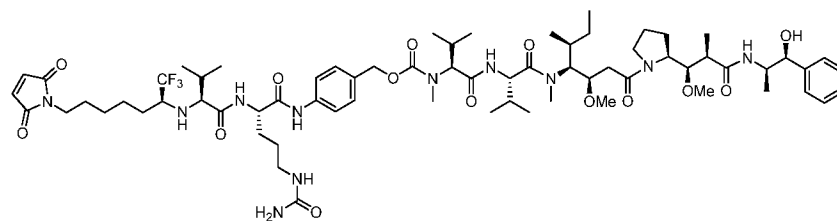
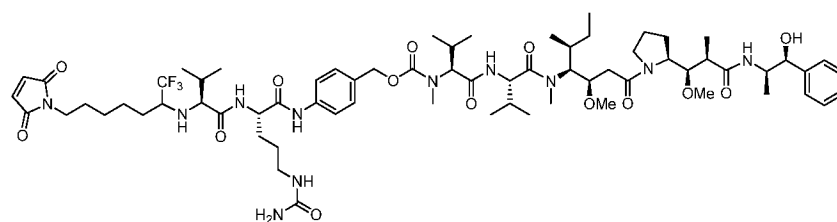


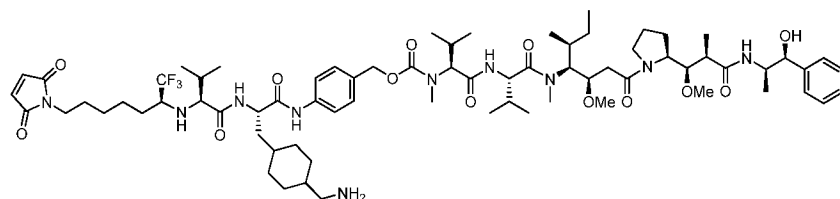
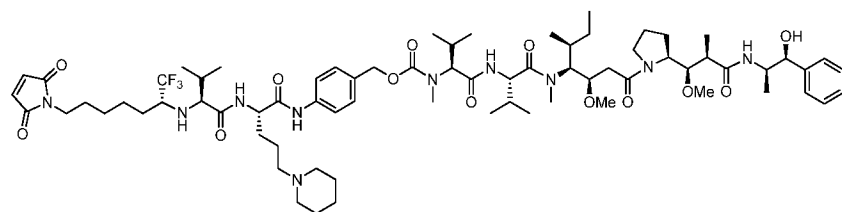
13. The conjugate of claim 2, it is selected from the group consisting of:



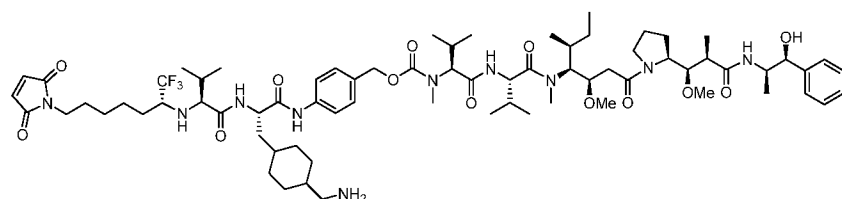


concretely it is selected from the group consisting of:

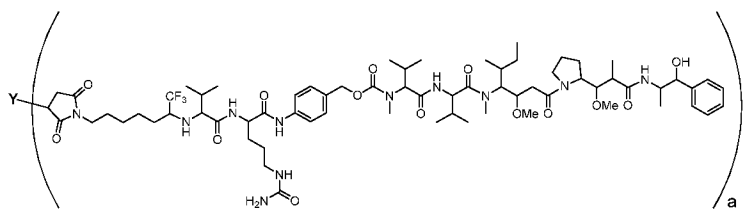




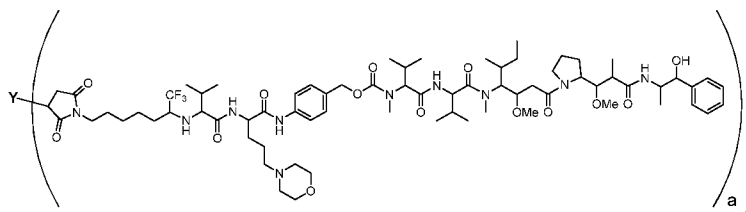
, and



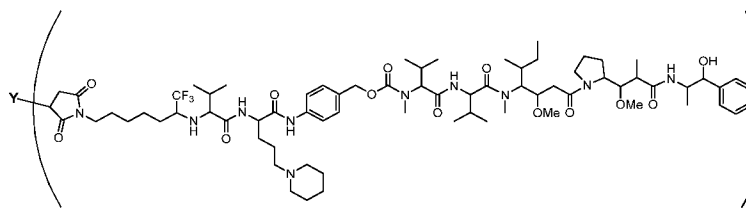
14. The ADC of claim 3, it is selected from the group consisting of:



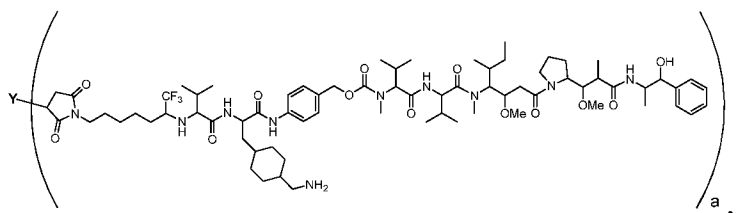
a ,



a ,



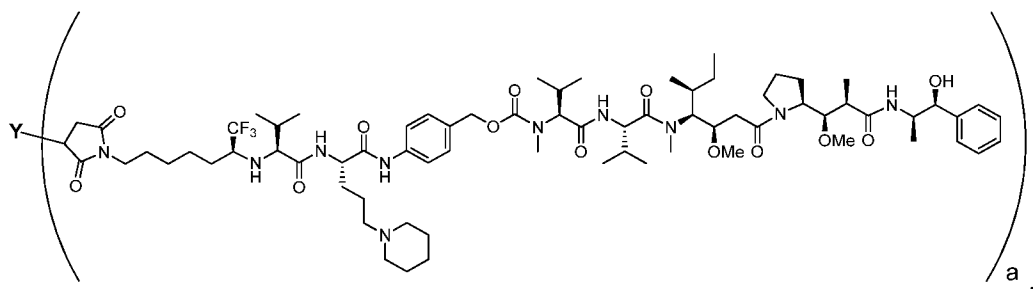
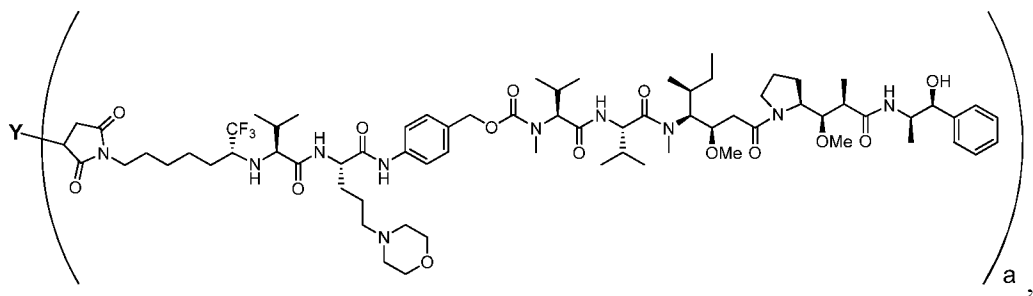
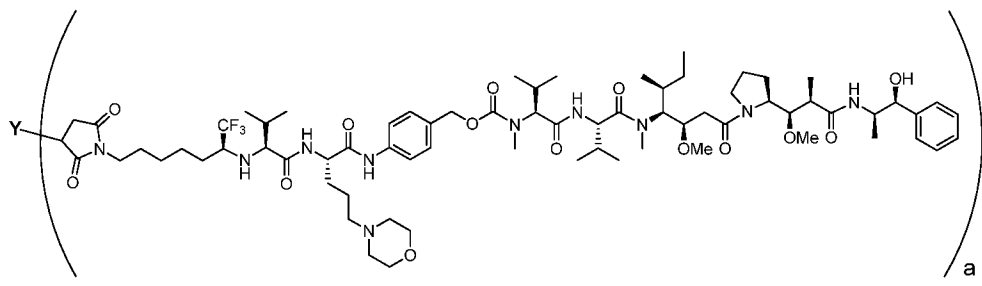
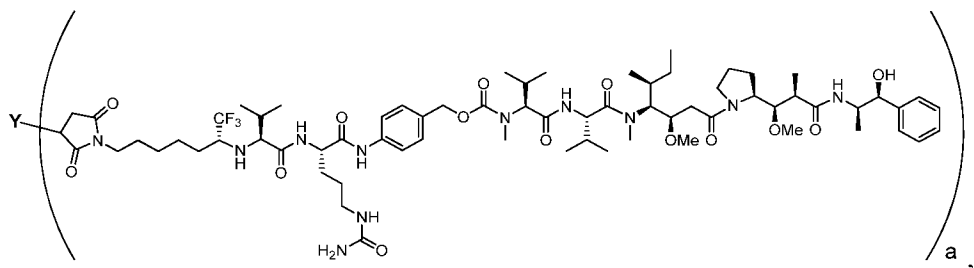
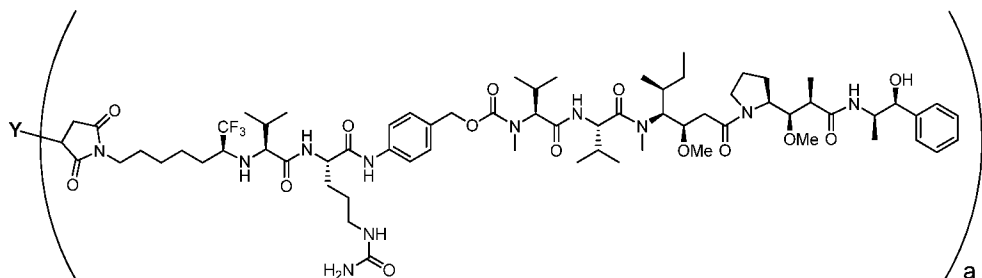
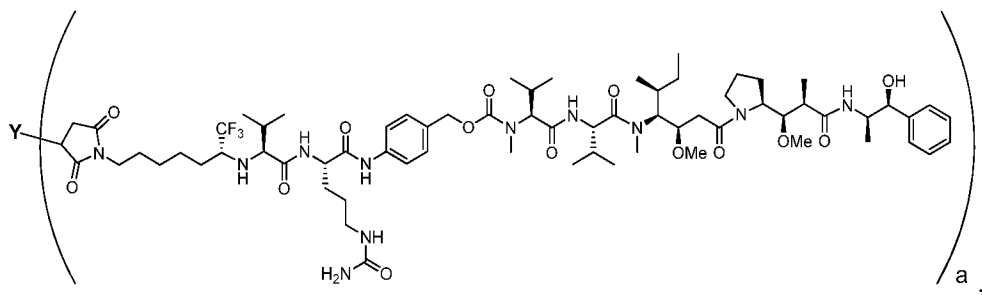
a , and

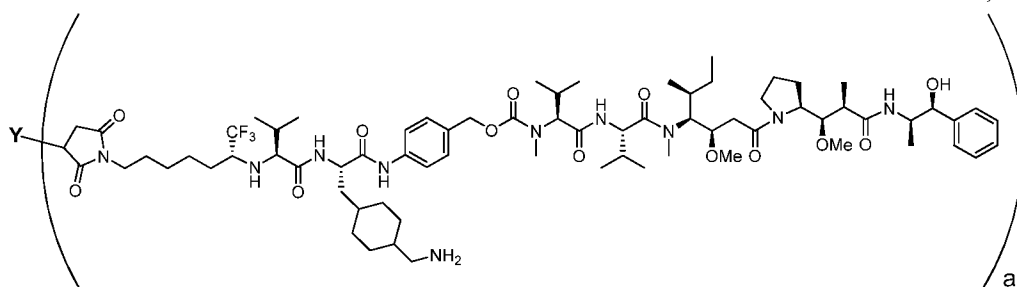
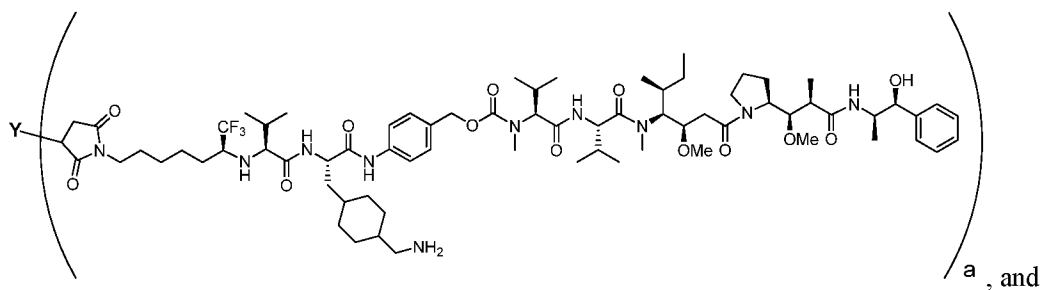
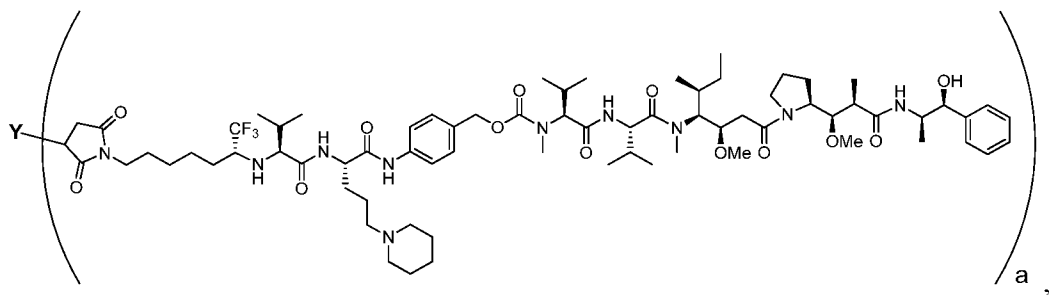


a ,

Y is an antibody, a is 1, 2, 3, 4, 5, 6, 7, or 8;

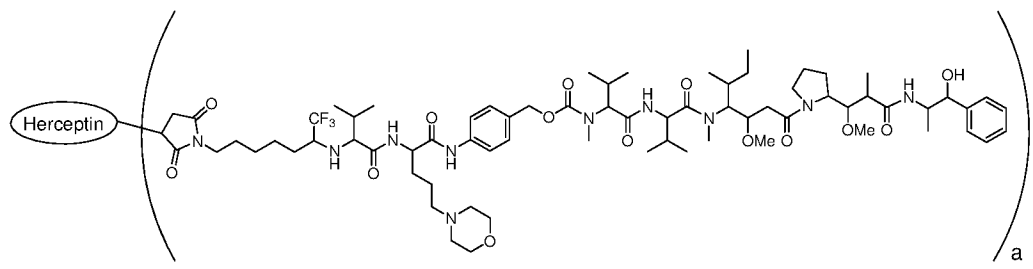
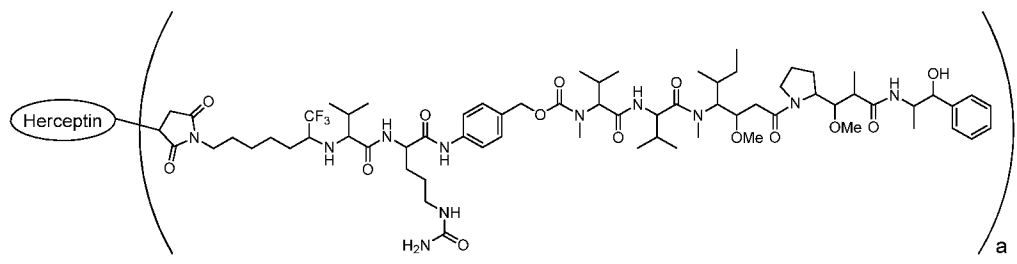
concretely the ADC is selected from the group consisting of:

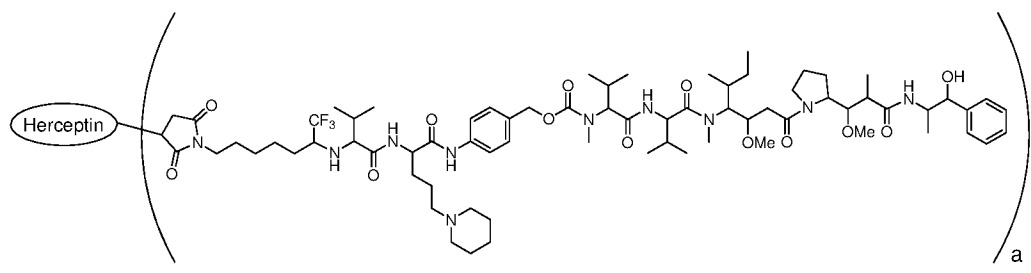




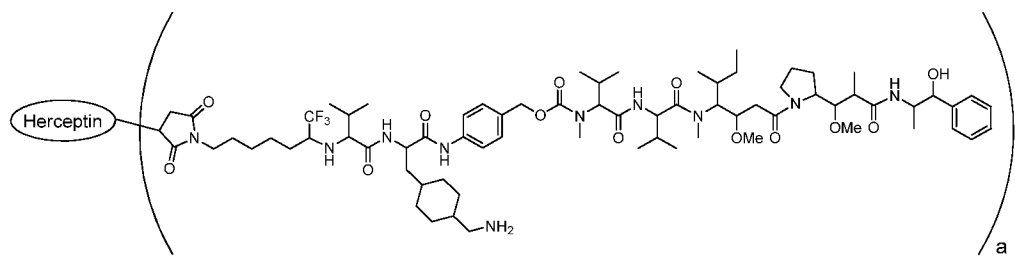
Y is an antibody, a is 1, 2, 3, 4, 5, 6, 7, or 8;

concretely the ADC is selected from the group consisting of:



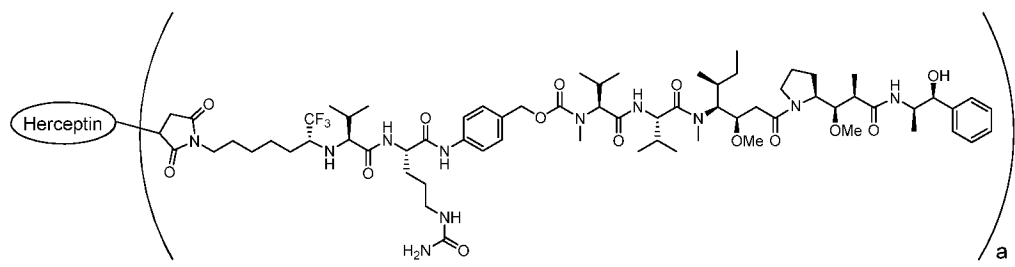
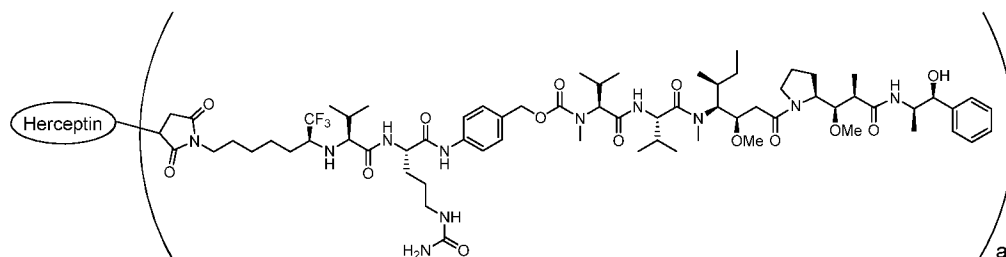
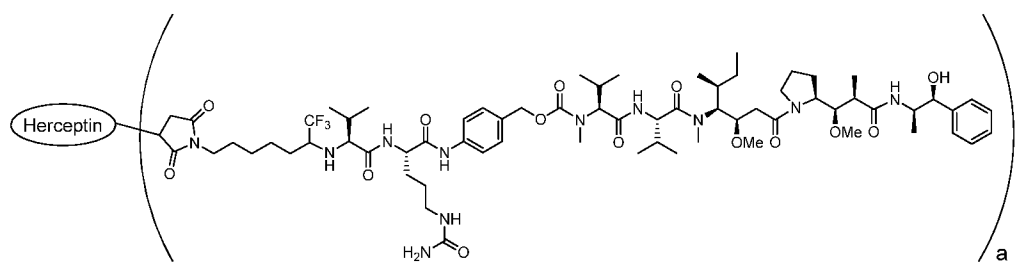


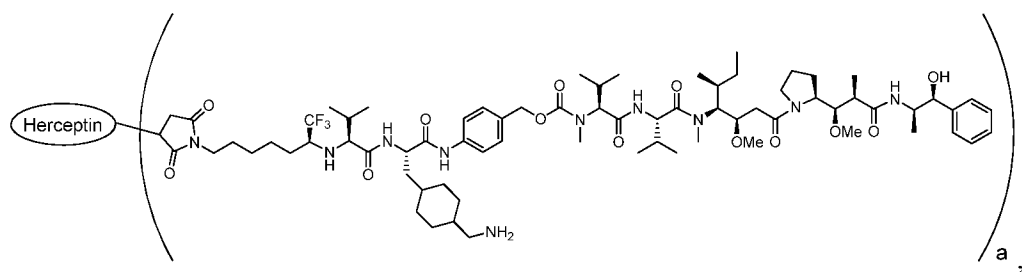
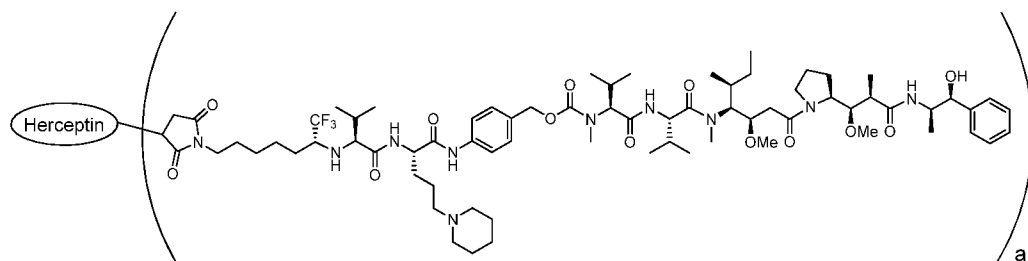
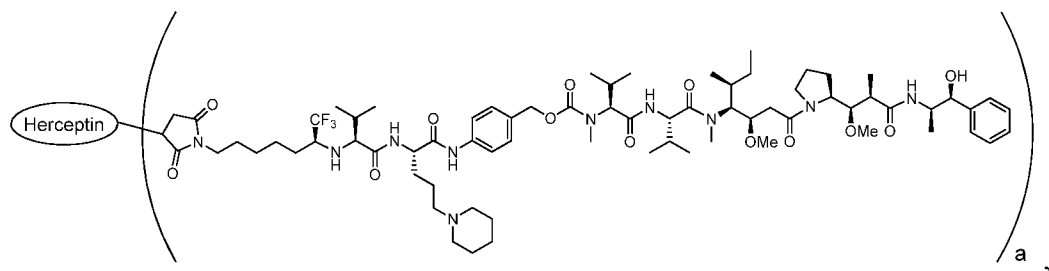
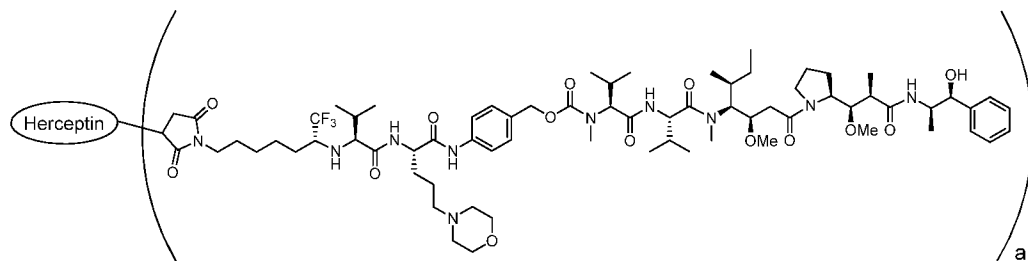
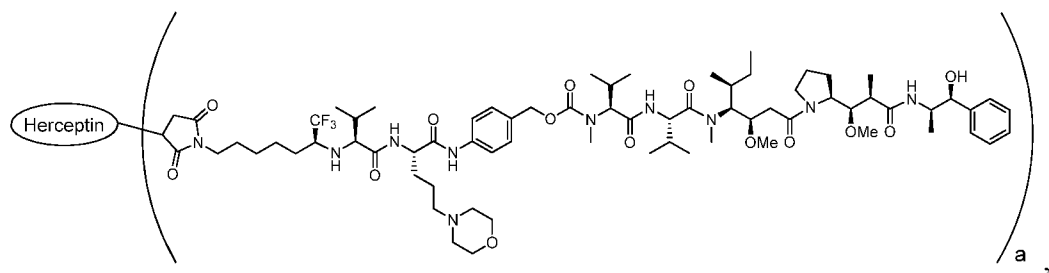
and



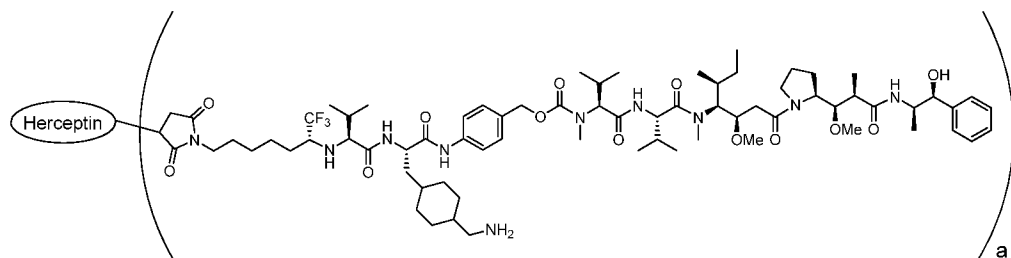
a is 2, 3, 4, 5, or 6; and

more concretely the ADC is selected from the group consisting of:





and



a is 2, 3, 4, 5, or 6.

15. A pharmaceutical composition comprising an effective amount of the ADC of any one of claim 2 to 14 and a pharmaceutically acceptable carrier, diluent or excipient.

16. A use of the linker, the conjugate, or the ADC in claims 1 to 14 or the composition of claim 15 in the preparation of a medicament for the treatment of cancers.

17. A method for treating or diagnosing cancers, comprising administering the subject in need thereof an effective amount of the ADC of any one of claims 2 to 14 or the pharmaceutical composition of claim 15.

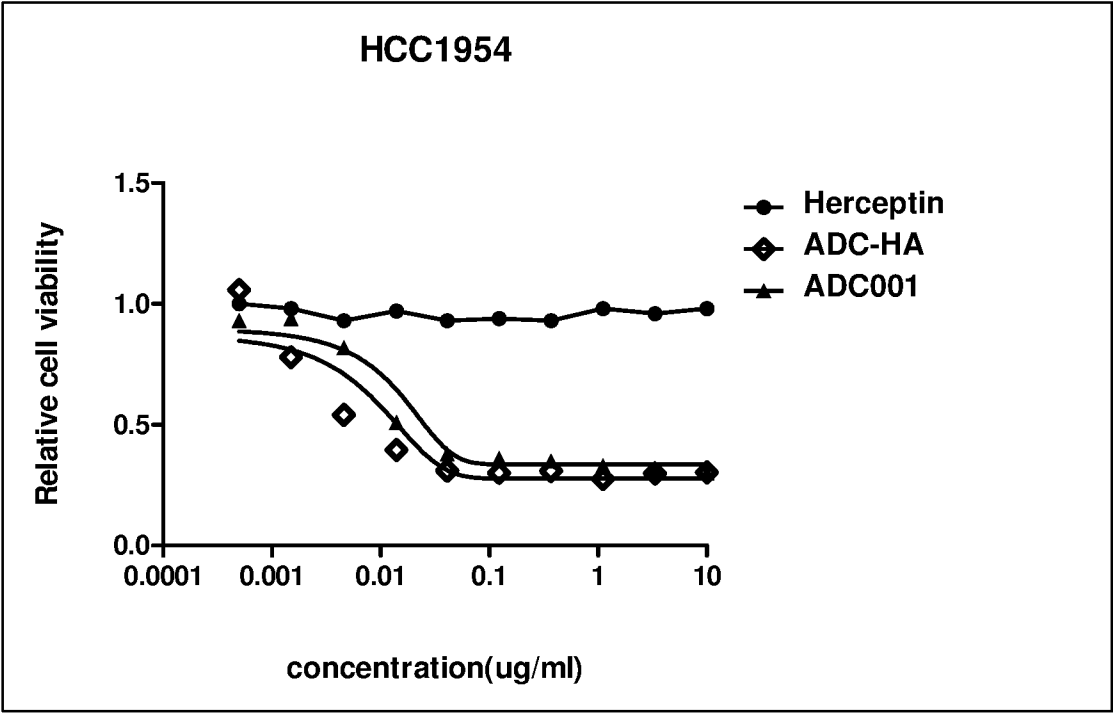


Figure 1

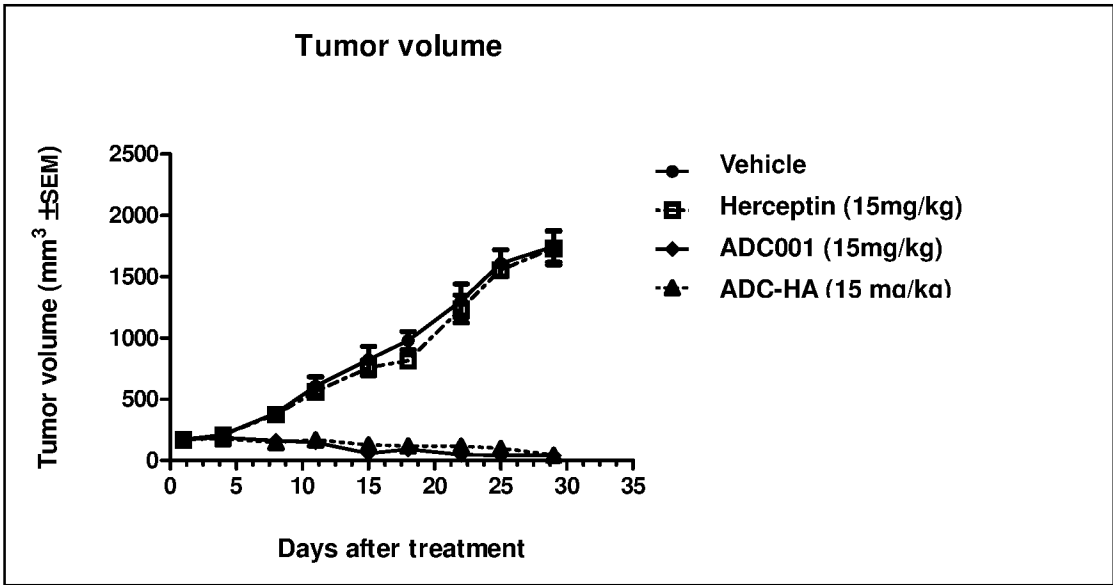


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/082292

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/48(2006.01)i; A61P 35/00(2006.01)i

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; 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)

EPODOC;WPI;CNTXT;USTXT;Web of science;Registry(STN);Caplus(STN);linker,antibody,drug,conjugate,cytotoxic,amide,lysosomal,enzymatic,surrogate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013163229 A1 (SEATTLE GENETICS, INC.ET AL.) 31 October 2013 (2013-10-31) paragraph [0019], claim 5	1-11(part), 12-14, 15-17(part)
A	WO 2004032828 A2 (SEATTLE GENETICS, INC.ET AL.) 22 April 2004 (2004-04-22) pages 57-58	1-11(part), 12-14, 15-17(part)
A	WO 2009099741 A1 (GENENTECH, INC.ET AL.) 13 August 2009 (2009-08-13) claim 9	1-11(part), 12-14, 15-17(part)
A	WO 2008141044 A2 (GENENTECH, INC.ET AL.) 31 December 2008 (2008-12-31) claims 22 and 25	1-11(part), 12-14, 15-17(part)

☐ 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

26 March 2015

Date of mailing of the international search report

27 April 2015

Name and mailing address of the ISA/CN

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Authorized officer

JIN,Ying

Telephone No. (86-10)82246758

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/082292

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.: **17**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Claim 17 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claim 17.
2. ☒ Claims Nos.: **1-11(part),15-17(part)**
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:
 - [1] Claims 1-11, 15-17, as written, encompasses an extremely wide range of linkers or conjugates that rendered a meaningful search impossible. The search was therefore limited to the linkers or the conjugates which defined in claims 12-14.
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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2014/082292

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2013163229	A1	31 October 2013	US	2013280282	A1	24 October 2013
				KR	20150003836	A	09 January 2015
				CA	2869846	A1	31 October 2013
				EP	2841103	A1	04 March 2015
				TW	201347775	A	01 December 2013
WO	2004032828	A2	22 April 2004	AU	2003294210	A1	04 May 2004
				WO	2004032828	A3	27 April 2006
				US	2005180972	A1	18 August 2005
				EP	1575514	A2	21 September 2005
WO	2009099741	A1	13 August 2009	US	2011076287	A1	31 March 2011
				US	2014227299	A1	14 August 2014
				EP	2240495	A1	20 October 2010
				US	8742076	B2	03 June 2014
WO	2008141044	A2	31 December 2008	AU	2008251608	A1	20 November 2008
				US	7723485	B2	25 May 2010
				CA	2683568	A1	20 November 2008
				EP	2144628	A2	20 January 2010
				CN	101687037	A	31 March 2010
				CN	101687037	B	10 July 2013
				AU	2008251608	B2	27 March 2014
				JP	2010526821	A	05 August 2010
				EP	2144628	B1	17 October 2012
				TW	200902554	A	16 January 2009
				WO	2008141044	A2	20 November 2008
				JP	5290276	B2	18 September 2013
				US	2008311134	A1	18 December 2008