ANTIBODY DRUG CONJUGATES COMPRISING ECTEINASCIDIN DERIVATIVES

Field of the Invention

The present invention relates to novel drug conjugates, drug linker compounds, to methods for their preparation, pharmaceutical compositions containing said drug conjugates and their use as antitumoral agents.

Background to the Invention

International patent application number PCT/EP2018/060868 is directed to novel ecteinascidin derivatives which demonstrate very promising anti-tumor activity. One of the compounds disclosed in such patent application is currently in Phase I clinical trials for the prevention and treatment of solid tumors.

The treatment of cancer has progressed significantly in recent years with the development of pharmaceutical entities that target and kill cancer cells more efficiently. Researchers have taken advantage of cell-surface receptors and antigens selectively expressed by target cells such as cancer cells to develop pharmaceutical entities based on antibodies that bind, in the example of tumors, the tumor-specific or tumor-associated antigens. In order to achieve this, cytotoxic molecules such as chemotherapeutic drugs, bacteria and plant toxins and radionuclides have been chemically linked to monoclonal antibodies that bind tumor-specific or tumor-associated cell surface antigens

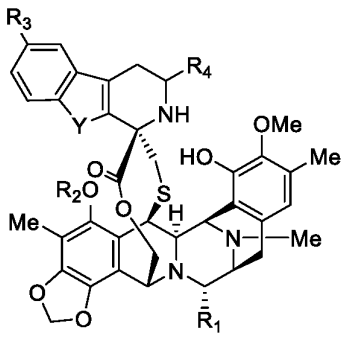
ADCs therefore represent a challenging area of development given the complex payload, linker and antibody structure but there remains a need for further ADCs to be developed.

Summary of the Invention

There is a need for novel active drug conjugates. The present invention addresses this need. It further provides novel drug linker compounds for use in the preparation of drug conjugates of the present invention, processes for the preparation of the novel drug conjugates of the present invention, pharmaceutical compositions containing said drug conjugates and their use as antitumoral agents, as well as a kit comprising the drug conjugate of the present invention for use in the treatment of cancer.

In a first aspect of the present invention there is provided a drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the drug conjugate having formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ wherein:

D is a drug moiety having the following formula (I) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof,



(I)

wherein:

D is covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_4 is selected from hydrogen, $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino,

with the optional proviso that when R_4 is hydrogen then Y is -O-;

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

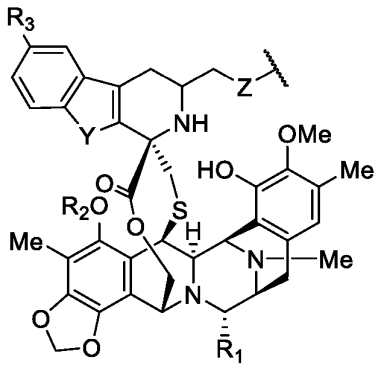
g is an integer of 0 or 1;

Ab is a moiety comprising at least one antigen binding site; and

n is the ratio of the group $[D-(X)_b-(AA)_w-(T)_g-(L)-]$ to the moiety comprising at least one antigen binding site and is in the range from 1 to 20.

In a further aspect of the present invention there is provided a drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the compound having formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab wherein:

D is a drug moiety having the following formula (IH) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof,



(IH)

wherein:

the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to (L);

each of Y and Z is independently selected from -NH- and -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl, wherein the optional substituents are one or more substituents R_x;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl, wherein the optional substituents are one or more substituents R_x;

substituents R_x are selected from the group consisting of C_1-C_{12} alkyl groups which may be optionally substituted with at least one group R_y, C_2-C_{12} alkenyl groups which may be optionally substituted with at least one group R_y, C_2-C_{12} alkynyl groups which may be optionally substituted with at least one group R_y, halogen atoms, oxo groups, thio groups, cyano groups, nitro groups, OR_y, $OCOR_y$, $OCOOR_y$, COR_y, $COOR_y$, $OCONR_yR_z$, $CONR_yR_z$, $S(O)R_y$, SO_2R_y, $P(O)(R_y)OR_z$, NR_yR_z, NR_yCOR_z, $NR_yC(=O)NR_yR_z$, $NR_yC(=NR_y)NR_yR_z$, aryl

groups having from 6 to 18 carbon atoms in one or more rings which may optionally be substituted with one or more substituents which may be the same or different selected from the group consisting of R_y, OR_y, $OCOR_y$, $OCOOR_y$, NR_yR_z, NR_yCOR_z, and $NR_yC(=NR_y)NR_yR_z$, aralkyl groups comprising an alkyl group having from 1 to 12 carbon atoms substituted with an optionally substituted aryl group as defined above, aralkyloxy groups comprising an alkoxy group having from 1 to 12 carbon atoms substituted with an optionally substituted aryl group as defined above, and a 5- to 14- membered saturated or unsaturated heterocyclic group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said heterocyclic group optionally being substituted with one or more substituents R_y, and where there is more than one optional substituents on any given group the optional substituents R_y may be the same or different;

each R_y and R_z is independently selected from the group consisting of hydrogen, C_1-C_{12} alkyl groups, C_1-C_{12} alkyl groups that are substituted with at least one halogen atom, aralkyl groups comprising a C_1-C_{12} alkyl group that is substituted with an aryl group having from 6 to 18 carbon atoms in one or more rings and heterocycloalkyl groups comprising a C_1-C_{12} alkyl group that is substituted with a 5- to 14- membered saturated or unsaturated heterocyclic group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s);

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

g is an integer of 0 or 1;

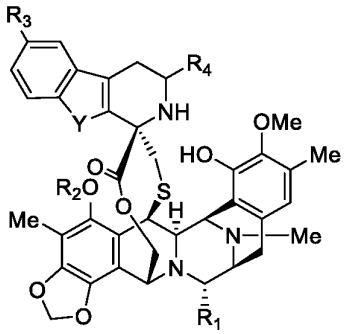
where b+g+w is optionally not 0;

Ab is a moiety comprising at least one antigen binding site; and

n is the ratio of the group $[D-(X)_b-(AA)_w-(T)_g-(L)-]$ to the moiety comprising at least one antigen binding site and is in the range from 1 to 20.

In a further aspect of the present invention there is provided a drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the drug conjugate having formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ wherein:

D is a drug moiety having the following formula (I) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof,



(I)

wherein:

D is covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_4 is selected from hydrogen, $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino,

with the optional proviso that when R_4 is hydrogen then Y is -O-;

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is an integer ranging from 0 to 12;

b is 1;

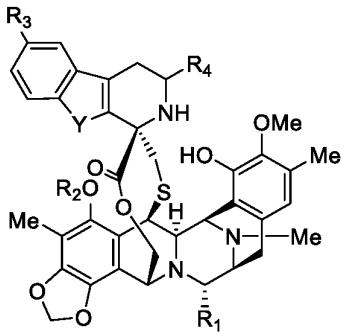
g is an integer of 0 or 1;

Ab is a moiety comprising at least one antigen binding site; and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] to the moiety comprising at least one antigen binding site and is in the range from 1 to 20.

In a further aspect of the present invention there is provided a drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the drug conjugate having formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab wherein:

D is a drug moiety having the following formula (I) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof,



(I)

wherein:

D is covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

R_4 is selected from hydrogen, -CH_2OH, -CH_2OC(=O)R_c, -CH_2NH_2, and -CH_2NHProt^{NH};

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

Prot^{NH} is a protecting group for amino,

with the optional proviso that when R_4 is hydrogen then Y is -O-;

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is 2;

b is 1;

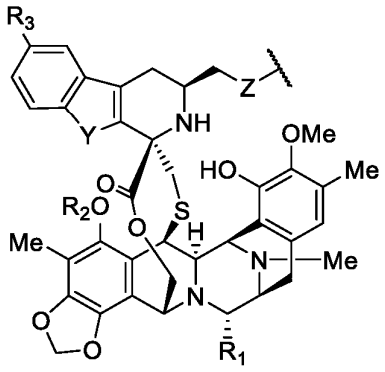
g is an integer of 0 or 1;

Ab is a moiety comprising at least one antigen binding site; and

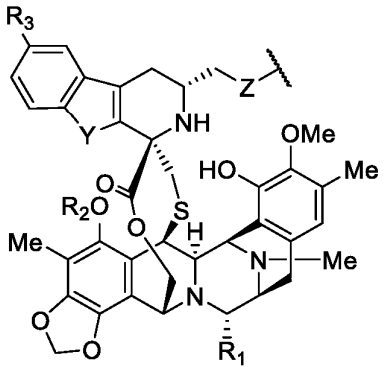
n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] to the moiety comprising at least one antigen binding site and is in the range from 1 to 20.

As we shall explain and exemplify in greater detail below, the drug conjugates of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab of the present invention represent a breakthrough in addressing the problems outlined above of requiring further drug conjugates in addition to those based on the three main families of cytotoxic drugs that have been used as payloads to date, that show excellent antitumor activity.

In preferred embodiments of the present invention, there is provided a drug conjugate as defined herein, or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein D is a drug moiety selected from formulas (IHa) and (IHb):



(IHa)

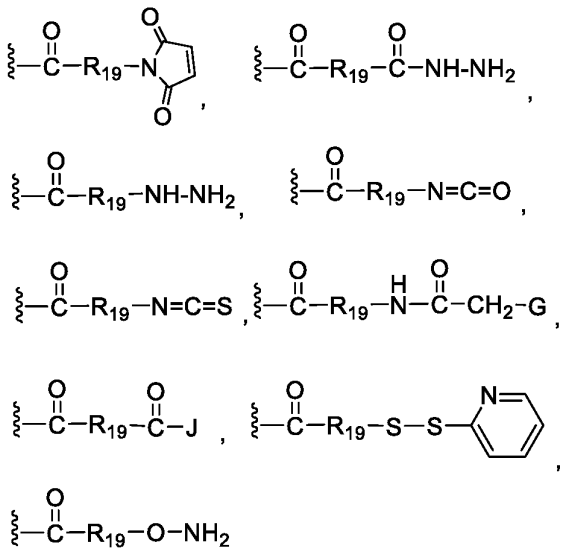


(IHb)

Where the wavy lines, R_1, R_2, R_3, Y, and Z are as defined for formula (IH).

In a further aspect of the present invention, there is provided a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 or of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H, wherein:

L_1 is a linker selected from the group of formulas consisting of:



each of the the wavy lines indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D;

G is selected from halo, -*O*-mesyl and -*O*-tosyl;

J is selected from halo, hydroxy, -*N*-succinimidoxy, -*O*-(4-nitrophenyl), -*O*-pentafluorophenyl, -*O*-tetrafluorophenyl and -O-C(O)-OR_{20};

R_{19} is selected from -C_1-C_{12} alkylene-, -C_3-C_8 carbocyclo, -O-(C_1-C_{12} alkylene), -C_6-C_{18} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{18} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{18} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_3-C_8 carbocyclo)-, -(C_3-C_8 carbocyclo)-C_1-C_{12} alkylene-, -C_5-C_{14} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{14} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{14} heterocyclo)-C_1-C_{12} alkylene-, wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$- and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{20} is a C_1-C_{12} alkyl or an aryl group having from 6 to 18 carbon atoms in one or more aromatic rings, said aryl groups optionally being substituted with one or more substituents R_x;

r is an integer ranging from 1-10;

g is an integer of 0 or 1;

b is an integer of 0 or 1;

w is an integer ranging from 0 to 12; and

each of D, R_x, X, T, and AA is as defined in the first aspect of the invention.

In preferred embodiments of the present invention, b+g+w is not 0. In further embodiments, b+w is not 0. In yet further embodiments, when w is not 0, then b is 1. In a further embodiment, when w is 0 then b is 1.

In a further aspect of the present invention, there is provided a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 or of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H, or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein each of D, X, AA, T, L_1, b, g and w are as defined herein; but further wherein if the compound is a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H then b+w+g≠0.

In a preferred emdboiment according to aspects of the present invention, n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] to the moiety comprising at least one antigen binding site and is in the range from 1 to 20. In further embodiments n is in the range from 1-12, 1-8, 3-8, 3-6, 3-5 or is 1, 2, 3, 4, 5 or 6 preferably, 3, 4 or 5 or 4.

In a further aspect of the present invention, there is provided a drug moiety D for use in an antibody drug conjugate. In a further aspect of the present invention, there is provided a drug moiety D for use as a payload in an antibody drug conjugate. In a further aspect of the present invention, there is provided the use of a drug moiety D as described herein, in the manufacture of an antibody drug conjugate.

In a further aspect of the present invention, there is provided a drug conjugate according to the present invention, for use as a medicament.

In a further aspect of the present invention, there is provided a drug conjugate according to the present invention for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred cancer is breast cancer.

In a further aspect of the present invention, there is provided a pharmaceutical composition comprising a drug conjugate according to the present invention and a pharmaceutically acceptable carrier.

In a further aspect of the present invention, there is provided a method for the prevention or treatment of cancer, comprising administering an effective amount of a drug conjugate according to the present invention to a patient in need thereof. Preferably, the cancer is selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred cancer is breast cancer.

In a further aspect of the present invention, there is provided the use of a drug conjugate according to the present invention in the preparation of a medicament for the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred cancer is breast cancer.

In a further aspect of of the present invention, there is provided a kit comprising a therapeutically effective amount of a drug conjugate according to the present invention and a pharmaceutically acceptable carrier. The kit is for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred cancer is breast cancer. A kit according to the present invention may comprise a therapeutically effective amount of a drug conjugate according to the present invention and, optionally, instructions for use of the drug conjugate in the treatment of cancer, particularly a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer; most preferably for use of the drug conjugate in the treatment of breast cancer.

In a further aspect of the present invention there is provided a process for the preparation of a drug conjugate according to the present invention comprising conjugating a moiety Ab comprising at least one antigen binding site and a drug D, Ab and D being as defined herein.

Detailed Description of Preferred Embodiments

The following apply to all aspects of the present invention:

In the compounds of the present invention, the alkyl groups may be branched or unbranched, and preferably have from 1 to about 12 carbon atoms. One more preferred class of alkyl groups has from 1 to about 6 carbon atoms. Even more preferred are alkyl groups having 1, 2, 3 or 4 carbon atoms. Methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-

butyl, isobutyl, *sec*-butyl and *tert*-butyl are particularly preferred alkyl groups in the compounds of the present invention.

In the compounds of the present invention, the alkenyl groups may be branched or unbranched, have one or more double bonds and from 2 to about 12 carbon atoms. One more preferred class of alkenyl groups has from 2 to about 6 carbon atoms. Even more preferred are alkenyl groups having 2, 3 or 4 carbon atoms. Ethenyl, 1-propenyl, 2-propenyl, 1-methylethenyl, 1-butenyl, 2-butenyl, and 3-butenyl are particularly preferred alkenyl groups in the compounds of the present invention.

In the compounds of the present invention, the alkynyl groups may be branched or unbranched, have one or more triple bonds and from 2 to about 12 carbon atoms. One more preferred class of alkynyl groups has from 2 to about 6 carbon atoms. Even more preferred are alkynyl groups having 2, 3 or 4 carbon atoms.

Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated and/or fused rings and from 6 to about 18 carbon ring atoms. Preferably aryl groups contain from 6 to about 10 carbon ring atoms. Specially preferred aryl groups included substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted phenanthryl and substituted or unsubstituted anthryl.

Suitable heterocyclic groups include heteroaromatic and heteroalicyclic groups containing from 1 to 3 separated and/or fused rings and from 5 to about 18 ring atoms. Preferably heteroaromatic and heteroalicyclic groups contain from 5 to about 10 ring atoms, most preferably 5, 6, or 7 ring atoms. Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-coumarinyl, quinolyl including 8-quinolyl, isoquinolyl, pyridyl, pyrazinyl, pyrazolyl, pyrimidinyl, furyl, pyrrolyl, thienyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoxazolyl, oxazolyl, imidazolyl, indolyl, isoindolyl, indazolyl, indolizinyl, phthalazinyl, pteridyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, pyridazinyl, triazinyl, cinnolinyl, benzimidazolyl, benzofuranyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl and furopyridyl. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S and include, e.g., pyrrolidinyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydrothiopyranyl, piperidyl, morpholinyl, thiomorpholinyl, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridyl, 2-pirrolinyl, 3-pyrrolinyl, indolinyl, 2*H*-pyranyl, 4*H*-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexyl, 3-azabicyclo[4.1.0]heptyl, 3*H*-indolyl, and quinolizinyl.

The groups above mentioned may be substituted at one or more available positions by one or more suitable groups such as OR', =O, SR', SOR', SO_2R', NO_2, NHR', NR'R', =N-R', NHCOR', $N(COR')_2$, $NHSO_2R'$, NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', protected OH, protected amino, protected SH, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, where each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, COH, COalkyl, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. In addition, where there are more than one R' groups on a substituent, each R' may be the same or different.

In the compounds for the present invention, the halogen substituents include F, Cl, Br, and I.

More particularly, in the compounds of the present invention, the alkyl groups in the definitions of R_{20}, R_a, R_b, R_c, R_x, R_y and R_z may be straight chain or branched alkyl chain groups having from 1 to 12 carbon atoms, and they are preferably an alkyl group having from 1 to 6 carbon atoms, more preferably a methyl group, an ethyl group or an *i*-propyl group, and most preferably a methyl group. In the definitions of M and Q, they may be straight chain or branched alkyl chain groups having from 1 to 6 carbon atoms. Methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, isobutyl, *sec*-butyl and *tert*-butyl are particularly preferred alkyl groups in the compounds of the present invention.

In the compounds of the present invention, the alkenyl groups in the definitions of R_a, R_b, R_c and R_x are branched or unbranched, and may have one or more double bonds and from 2 to 12 carbon atoms. Preferably, they have from 2 to 6 carbon atoms, and more preferably they are branched or unbranched alkenyl groups having 2, 3 or 4 carbon atoms. Ethenyl, 1-propenyl, 2-propenyl, 1-methylethenyl, 1-butenyl, 2-butenyl, and 3-butenyl are particularly preferred alkenyl groups in the compounds of the present invention.

In the compounds of the present invention, the alkynyl group in the definitions of R_a, R_b, R_c and R_x are branched or unbranched, and may have one or more triple bonds and from 2 to 12 carbon atoms. Preferably, they have from 2 to 6 carbon atoms, and more preferably they are branched or unbranched alkynyl groups having 2, 3 or 4 carbon atoms.

In the compounds of the present invention, the halogen substituents in the definitions of R_x, R_y and R_z include F, Cl, Br and I, preferably Cl.

In the compounds of the present invention, the 5- to 14-membered saturated or unsaturated heterocyclic group in the definitions of R_x is a heterocyclic group having one or more rings, comprising at least one oxygen, nitrogen or sulphur atom in said ring(s). The heterocyclic group is a group which may be a heteroaromatic group or a heteroalicyclic group, the latter of which may be partially unsaturated, both the aromatic and the alicyclic heterocyclic group containing from 1 to 3 separated or fused rings. Preferably the heteroaromatic and heteroalicyclic group contain from 5 to 10 ring atoms. Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O and S atoms and include, for example, quinolyl including 8-quinolyl, isoquinolyl, coumarinyl including 8-coumarinyl, pyridyl, pyrazinyl, pyrazolyl, pyrimidinyl, furyl, pyrrolyl, thienyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoxazolyl, oxazolyl, imidazolyl, indolyl, isoindolyl, indazolyl, indolizinyl, phthalazinyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, pyridazinyl, triazinyl, cinnolinyl, benzimidazolyl, benzofuranyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl and furopyridyl. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O and S atoms and include, for example, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydrothiopyranyl, piperidyl, morpholinyl, thiomorpholinyl, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexyl, 3-azabicyclo[4.1.0]heptyl, 3H-indolyl, and quinolizinyl.

In the compounds of the present invention, the aryl group in the definition of R_x and R_{20} is a single or multiple ring compound that contain separate and/or fused aryl groups and has from 6 to 18 ring atoms and is optionally substituted. Typical aryl groups contain from 1 to 3 separated or fused rings. Preferably aryl groups contain from 6 to 12 carbon ring atoms. Particularly preferred aryl groups include substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted phenanthryl and substituted or unsubstituted anthryl, and most preferred substituted or unsubstituted phenyl, wherein the substituents are as indicated above.

In the compounds of the present invention, the aralkyl groups in the definitions of R_x, R_y and R_z comprise an alkyl group as defined and exemplified above which is substituted with one or more aryl groups as defined and exemplified above. Preferred examples include optionally substituted benzyl, optionally substituted phenylethyl and optionally substituted naphthylmethyl.

In the compounds of the present invention, the aralkyloxy groups in the definitions of R_x comprise an alkoxy group having from 1 to 12 carbon atoms which is substituted with one

or more aryl groups as defined and exemplified above. Preferably, the alkoxy moiety has from 1 to 6 carbon atoms and the aryl group contains from 6 to about 12 carbon ring atoms, and most preferably the aralkyloxy group is optionally substituted benzyloxy, optionally substituted phenylethoxy and optionally substituted naphthylmethoxy.

In the compounds of the present invention, the heterocycloalkyl groups in the definitions of R_y and R_z comprise an alkyl group as defined and exemplified above which is substituted with one or more heterocyclyl groups as defined and exemplified above. Preferably, the heterocycloalkyl groups comprise an alkyl group having from 1 to 6 carbon atoms which is substituted with a heterocyclyl group having from 5 to 10 ring atoms in 1 or 2 ring atoms and can be aromatic, partially saturated or fully saturated. More preferably, the heterocycloalkyl groups comprise a methyl or ethyl group which is substituted with a heterocyclyl group selected from the group consisting of pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, tetrahydrofuranyl, oxanyl, thianyl, 8-quinolyl, isoquinolyl, pyridyl, pyrazinyl, pyrazolyl, pyrimidinyl, furyl, pyrrolyl, thienyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoxazolyl, oxazolyl and benzimidazole.

In the compounds of the present invention, the alkylene groups in the definition of R_{19} are straight or branched alkylene groups having from 1 to 12 carbon atoms and the alkylene groups in the definitions of M, X, T, and R_{30} are straight or branched alkylene groups having from 1 to 6 carbon atoms. Preferably, the alkylene groups in the definition of R_{19} are straight or branched alkylene groups having from 1 to 8 carbon atoms, more preferably straight or branched alkylene groups having from 1 to 6 carbon atoms. For M, preferred are straight or branched alkylene groups having from 1 to 3 carbon atoms. In the definition of X, the alkylene groups in the definition of X are preferably straight or branched alkylene groups having from 2 to 4 carbon atoms. For T, preferred are straight or branched alkylene groups having from 2 to 4 carbon atoms. In the definition of R_{30}, preferred are straight or branched alkylene groups having from 2 to 4 carbon atoms, being most preferred a straight alkylene group having 3 carbon atoms. For the avoidance of doubt, the term “alkylene” is used to refer to alkanediyl groups.

In the compounds of the present invention, the carbocyclo groups in the definitions of R_{19} and M are cycloalkyl groups having from 3 to 8 carbon atoms which have two covalent bonds at any position on the cycloalkyl ring connecting said cycloalkyl group to the remainder of the drug conjugate. Preferably, the carbocyclo groups in the definitions of R_{19} and M are cycloalkyl groups having from 3 to 7 carbon atoms, and more preferably carbocyclo groups having from 5 to 7 carbon atoms.

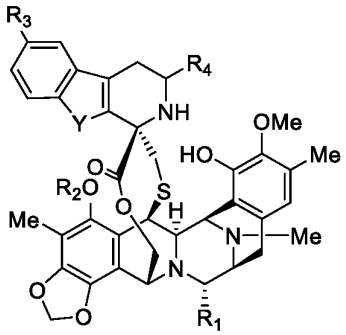
In the compounds of the present invention, the arylene groups in the definition of R_{19} are aryl groups having from 6 to 18 carbon atoms in one or more rings which have two covalent bonds at any position on the aromatic ring system connecting said arylene groups to the remainder of the drug conjugate. Preferably, the arylene groups in the definition of R_{19} are

aryl groups having from 6 to 12 carbon atoms in one or more rings which have two covalent bonds at any position on the aromatic ring system, and most preferably they are phenylene groups.

In the compounds of the present invention, the heterocyclo groups in the definition of R_{19} are heterocyclyl groups containing from 1 to 3 separated or fused rings having from 5 to 14 ring atoms and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), wherein there are two covalent bonds at any position on the ring system of said heterocyclic groups. The heterocyclic groups are groups which may be heteroaromatic groups or heteroalicyclic groups (the latter may be partially unsaturated). Preferably, the heterocyclo groups in the definition of R_{19} are heterocyclyl groups containing from 1 to 3 separated or fused rings having from 5 to 12 ring atoms and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), wherein there are two covalent bonds at any position on the ring system of said heterocyclic groups.

Where there are more than one optional substituents R_x, R_y or R_z on a substituent, each substituent R_x may be the same or different, each substituent R_y may be the same or different and each R_z may be the same or different.

In an embodiment, D may be a compound of formula **I** or a pharmaceutically acceptable salt or ester thereof:



I

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

R_4 is selected from hydrogen, -CH_2OH, -CH_2OC(=O)R_c, -CH_2NH_2, and -CH_2NHProt^{NH};

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

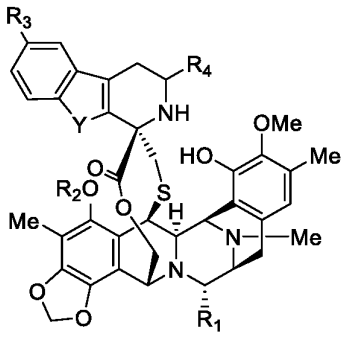
R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In an embodiment, the compound of formula I has the proviso that when R_4 is hydrogen then Y is -O-.

In a further embodiment, the compound of formula **I** may be a compound of formula **IC**, or a pharmaceutically acceptable salt or ester thereof:



IC

wherein:

Y is -NH-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_4 is selected from $-CH_2OH$, $-CH_2O-(C=O)R_c$, $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

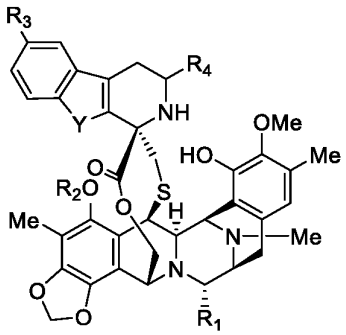
R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **ID**, or a pharmaceutically acceptable salt or ester thereof:



ID

wherein:

Y is -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

R_4 is selected from hydrogen, -CH_2OH, -CH_2O-(C=O)R_c, -CH_2NH_2 and -CH_2NH$Prot^{NH}$;

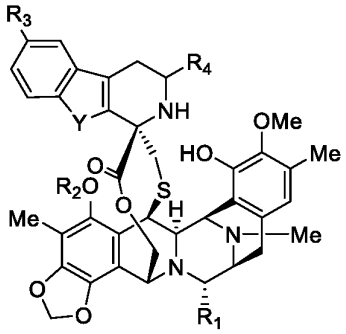
R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **IE**, or a pharmaceutically acceptable salt or ester thereof:



IE

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

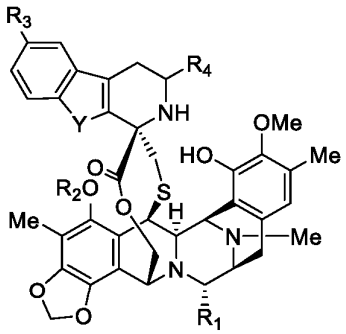
R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **IA** or a pharmaceutically acceptable salt or ester thereof:



IA

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen;

R_4 is selected from hydrogen, -CH_2OH, -CH_2O-(C=O)R_c, -CH_2NH_2 and -$CH_2NHProt^{NH}$;

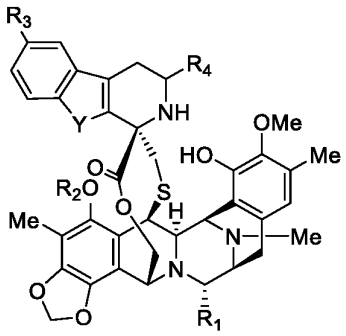
R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In an embodiment, the compound of formula **IA** has the proviso that when R_4 is hydrogen then Y is -O-.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **IB** or a pharmaceutically acceptable salt or ester thereof:



IB

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is a $-OR_b$ group;

R_4 is selected from hydrogen, $-CH_2OH$, $-CH_2O-(C=O)R_c$, $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

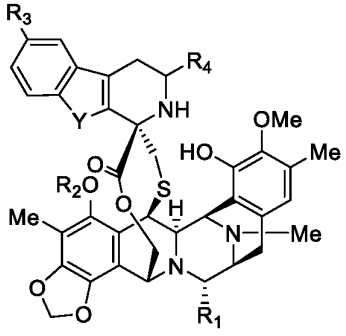
R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In an embodiment, the compound of formula **IB** has the proviso that when R_4 is hydrogen then Y is -O-.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **IF** or a pharmaceutically acceptable salt or ester thereof:



IF

wherein:

Y is -NH- or -O-;

R_1 is -OH;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

R_4 is selected from hydrogen, -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

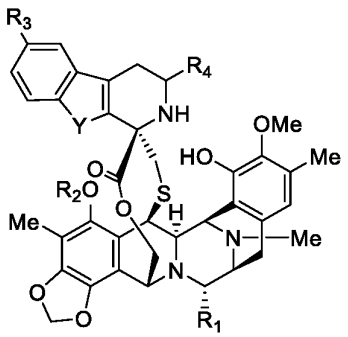
R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

In an embodiment, the compound of formula **IF** has the proviso that when R_4 is hydrogen then Y is -O-.

In a yet further embodiment, the compound of formula **I** may be a compound of formula **IG** or a pharmaceutically acceptable salt or ester thereof:



IG

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is acetyl;

R_3 is hydrogen or a -OR_b group;

R_4 is selected from hydrogen, -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$;

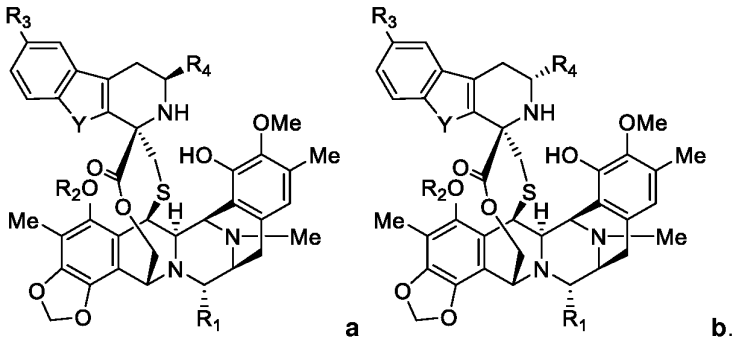
R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino

In an embodiment, the compound of formula IG has the proviso that when R_4 is hydrogen then Y is -O-.

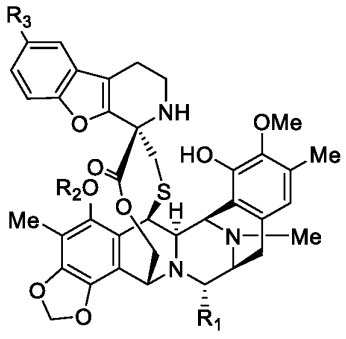
Preferred compounds of the compounds of formula **I**, **IA**, **IB**, **IC**, **ID**, **IE**, **IF**, or **IG**, are those having general formula **a** or **b**, or a pharmaceutically acceptable salt or ester thereof:



a **b**.

Note where the compounds have general formula a or b, R_4 may not be hydrogen.

Preferred compounds of the compounds of formula **I**, **IA**, **IB**, **ID, IF**, or **IG** may be those having formula **c** or a pharmaceutically acceptable salt or ester thereof:



c

wherein:

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group;

R_3 is hydrogen or a -OR_b group;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl.

For the avoidance of doubt, the compounds above may be the drug moiety D and are covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L). Thus, when conjugated, a covalent bond replaces a proton on a hydroxy or amine group on the compound.

Preferred compounds include compounds of general formula **I, IA, IB, IE, IF, IG, Ia, IAa, IBa, IEa, IFa, IGa, Ib, IAb, IBb, IEb, IFb,** and **IGb,** wherein:

Y is -NH-;

and R_1; R_2; R_3; R_4; R_a; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Preferred compounds include compounds of general formula **I, IA, IB, IE, IF, IG, Ia, IAa, IBa, IEa, IFa, IGa, Ib, IAb, IBb, IEb, IFb,** and **IGb,** wherein:

Y is -O-;

and R_1; R_2; R_3; R_4; R_a; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, ID, IE, IG, Ia, IAa, IBa, ICa, IDa, IEa, IGa, Ib, IAb, IBb, ICb, IDb, IEb,** and **IGb**, wherein:

R_1 is -OH;

and Y; R_2; R_3; R_4; R_a; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, ID, IE, IF, Ia, IAa, IBa, ICa, IDa, IEa, IFa, Ib, IAb, IBb, ICb, IDb, IEb, and IFb**, wherein:

R_2 is a -C(=O)R_a group where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and Y; R_1; R_3; R_4; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IB, IC, ID, IE, IF, IG, Ia, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IBb, ICb, IDb, IEb, IFb,** and **IGb**, wherein:

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, or IGb; and R_3 is a -OR_b group for compounds of formula

IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and Y; R_1; R_2; R_4; R_a; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb**, wherein:

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from $-CH_2OH$ and $-CH_2NH_2$. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and Y; R_1; R_2; R_3; R_a; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is –OH;

and R_2; R_3; R_4; R_a; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_2 is a $-C(=O)R_a$ for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or

unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_1; R_3; R_4; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_2; R_4; R_a; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, or substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1; R_2; R_3; R_a; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_3; R_4; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a -OR_b group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_2; R_4; R_a; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_4 is selected from -CH_2OH, -CH_2OC(=O)R_c, -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from

substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_2; R_3; R_a; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_4; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or

unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1; R_3; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1; R_2; and R_a; are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a -OR_b group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_4; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from-CH_2NH_2, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_3; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-

butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1 is as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb**, wherein:

Y is -NH-;

R_1 is -OH;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

and R_2; R_3; R_4; R_a; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_1; R_3; R_4; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_3 is hydrogen or a -OR_b group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a -OR_b group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 is hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_2; R_4; R_a; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from $-CH_2OH$ and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1; R_2; R_3; R_a; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is –OH;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_3; R_4; R_b; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl,

substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_2; R_4; R_a; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from $-CH_2OH$ and CH_2NH_2. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_2; R_3; R_a; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or

unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_4; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 is selected from -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from -CH_2OH and -CH_2NH_2. More preferably, R_4 may be -CH_2NH_2. Most preferred R_4 is -CH_2OH;

and R_1; R_3; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_3 is hydrogen or a -OR_b group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a -OR_b group for compounds of formula IB, IBa or IBb; where R_b is a substituted or

unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from $-CH_2OH$ and $-CH_2NH_2$. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1; R_2; and R_a; are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted

tert-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_4; R_c; and $Prot^{NH}$ are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 is selected from -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from -CH_2OH and -CH_2NH_2. More preferably, R_4 may be -CH_2NH_2. Most preferred R_4 is -CH_2OH;

and R_3; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-

butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from $-CH_2OH$, $-CH_2OC(=O)R_c$, $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from $-CH_2NH_2$, and $-CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from $-CH_2OH$ and $-CH_2NH_2$. More preferably, R_4 may be $-CH_2NH_2$. Most preferred R_4 is $-CH_2OH$;

and R_1 is as defined as above.

Further preferred compounds include compounds of general formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb**, wherein:

Y is -O-;

R_1 is -OH;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa, or IAb; and R_3

is a -OR_b group for compounds of formula IB, IBa or IBb; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 is selected from -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb, or IGb; and R_4 is selected from -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from -CH_2OH and -CH_2NH_2. More preferably, R_4 may be -CH_2NH_2. Most preferred R_4 is -CH_2OH.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc,** and **IGc** wherein:

R_1 is -OH;

and R_2; R_3; R_a and R_b are as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_2 is a -$C(=O)R_a$ group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_1; R_3; R_b are as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_3 is hydrogen or a -OR_b group for compounds of formula Ic, IDc, IFc, or IGc; R_3 is hydrogen for compounds of formula IAc; and R_3 is a -OR_b group for compounds of formula IBc; where

R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_2; and R_a are as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_3; and R_b are as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_1 is -OH;

R_3 is hydrogen or a -OR_b group for compounds of formula Ic, IDc, IFc, or IGc; R_3 is hydrogen for compounds of formula IAc; and R_3 is a -OR_b group for compounds of formula IBc; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_2; and R_a are as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_2 is a -C(=O)R_a group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly

preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR_b group for compounds of formula Ic, IDc, IFc, or IGc; R_3 is hydrogen for compounds of formula IAc; and R_3 is a -OR_b group for compounds of formula IBc; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1 is as defined as above.

Further preferred compounds include compounds of general formula **Ic, IAc, IBc, IDc, IFc,** and **IGc**, wherein:

R_1 is -OH;

R_2 is a -C(=O)R_a group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc; where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR_b group for compounds of formula Ic, IDc, IFc, or IGc; R_3 is hydrogen for compounds of formula IAc; and R_3 is a -OR_b group for compounds of formula IBc; where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group.

The following preferred substituents (where allowed by possible substituent groups) apply to compounds of formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, Ic, IAc, IBc, IDc, IFc,** and **IGc:**

In compounds of the present invention, particularly preferred R_1 is -OH.

In compounds of the present invention, particularly preferred R_2 is a -C(=O)R_a group where R_a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl.

In compounds of the present invention, particularly preferred R_3 is hydrogen or a -OR_b group where R_b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group.

In compounds of the present invention, particularly preferred R_4 is selected from H, -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$ where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from H, CH_2OH and CH_2NH_2. Most preferred R_4 is -CH_2OH.

In compounds of general formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** particularly preferred R_4 is selected from -CH_2OH, -$CH_2OC(=O)R_c$, -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb, or IGb; and R_4 is selected from -CH_2NH_2, and -$CH_2NHProt^{NH}$ for compounds of formula IE, IEa or IEb; where R_c is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R_c is a substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl, and substituted or unsubstituted *tert*-butyl. Most preferred R_c is methyl. More preferred R_4 is selected from CH_2OH and CH_2NH_2. Most preferred R_4 is -CH_2OH.

Being particularly preferred compounds of formula **Ia, IAa, IBa, ICa, IDa, IFa, IGa** when R_4 is -CH_2OH or -$CH_2OC(=O)R_c$ and compounds of formula **Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb** when R_4 is -CH_2NH_2 or -$CH_2NHProt^{NH}$.

In compounds of the present invention, particularly preferred Y is -NH-.

Alternatively, in compounds of the present invention, particularly preferred Y is -O-.

Preferred compounds according to the present invention include:

- Compounds of formula **I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb,** and **IGb** wherein:

  R_4 is selected from $-CH_2OH$ and $-CH_2OC(=O)R_c$;

  Being particularly preferred compounds of formula **Ia, IAa, IBa, ICa, IDa, IFa**, and **IGa** and/or compounds where R_4 is $-CH_2OH$.

- Compounds of formula **I, IA, IB, IC, ID, IE IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** wherein

  R_4 is selected from $-CH_2NH_2$ and $-CH_2NHProt^{NH}$; and

  $Prot^{NH}$ is a protecting group for amino.

  Being particularly preferred compounds of formula **Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **Ic, IAc, IBc, IDc, IFc, IGc** wherein

  R_2 is a $-C(=O)R_a$ group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc;

  R_3 is hydrogen or a $-OR_b$ group for compounds of formula Ic, IDc, IFc, IGc; R_3 is hydrogen for compounds of formula IAc; or R_3 is a $-OR_b$ group for compounds of formula IBc;

  R_a is selected from hydrogen, and substituted or unsubstituted C_1-C_6 alkyl; and

  R_b is substituted or unsubstituted C_1-C_6 alkyl.

Particularly preferred compounds according to the present invention include:

- Compounds of formula **I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb,** and **IGb** wherein

  Y is -NH-;

  R_4 is selected from $-CH_2OH$, and $-CH_2OC(=O)R_c$;

  and

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl.

Being more preferred compounds of formula **Ia, IAa, IBa, ICa, IFa, IGa** and/or compounds where R_4 is $-CH_2OH$.

- Compounds of formula **I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb,** and **IGb** wherein

Y is -O-;

R_4 is selected from $-CH_2OH$ and $-CH_2OC(=O)R_c$;

and

R_c is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl.

Being more preferred compounds of formula **Ia, IAa, IBa, IDa, IFa, IGa** and/or compounds where R_4 is $-CH_2OH$.

- Compounds of formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb** wherein

Y is -NH-;

R_4 is selected from $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

and

$Prot^{NH}$ is a protecting group for amino.

Being more preferred compounds of formula **Ib, IAb, IBb, ICb, IEb, IFb, IGb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb** wherein

Y is -O-;

R_4 is selected from $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

and

$Prot^{NH}$ is a protecting group for amino.

Being more preferred compounds of formula **Ib, IAb, IBb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb, IGb** wherein

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, ID, IF, Ia, IAa, IBa, ICa, IDa, IFa, Ib, IAb, IBb, ICb, IDb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, ID, IF, IG, Ia, ICa, IDa, IFa, IGa, Ib, ICb, IDb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb;

R_4 is selected from $-CH_2OH$, and $-CH_2OC(=O)R_c$;

R_a is selected from hydrogen, and substituted or unsubstituted C_1-C_6 alkyl;

R_b is substituted or unsubstituted C_1-C_6 alkyl; and

R_c is substituted or unsubstituted C_1-C_6 alkyl.

Being more preferred compounds of formula **Ia, IAa, IBa, ICa, IDa, IFa, IGa** and/or compounds where R_4 is $-CH_2OH$.

- Compounds of formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** wherein

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, ID, IF, Ia, IAa, IBa, ICa, IDa, IFa, Ib, IAb, IBb, ICb, IDb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb;

R_4 is selected from $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

R_a is selected from hydrogen, and substituted or unsubstituted C_1-C_6 alkyl;

R_b is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being more preferred compounds of formula **Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **Ic, IAc, IBc, IDc, IFc, IGc** wherein

R_2 is a $-C(=O)R_a$ group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula Ic, IDc, IFc, IGc; R_3 is hydrogen for compounds of formula IAc; or R_3 is a $-OR_b$ group for compounds of formula IBc;

R_a is substituted or unsubstituted C_1-C_6 alkyl; and

R_b is substituted or unsubstituted C_1-C_6 alkyl.

More preferred compounds according to the present invention include

- Compounds of formula **I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb,** and **IGb** wherein

Y is -NH-;

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, IF, Ia, IAa, IBa, ICa, IFa, Ib, IAb, IBb, ICb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a $-OR_b$ group for compounds of formula I, IC, IF, IG, Ia, ICa, IFa, IGa, Ib, ICb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a $-OR_b$ group for compounds of formula IB, IBa or IBb;

R_4 is $-CH_2OH$;

R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl; and

R_b is substituted or unsubstituted C_1-C_6 alkyl.

Being particularly more preferred compounds of formula **Ia, IAa, or IBa, ICa, IFa, IGa**.

- Compounds of formula **I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb,** and **IGb** wherein

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IF, Ia, IAa, IBa, IDa, IFa, Ib, IAb, IBb, IDb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a -OR_b group for compounds of formula I, ID, IF, IG, Ia, IDa, IFa, IGa, Ib, IDb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;

R_4 is -CH_2OH;

R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl; and

R_b is substituted or unsubstituted C_1-C_6 alkyl.

Being particularly more preferred compounds of formula **Ia, IAa, IBa, IDa, IFa**, or **IGa**.

- Compounds of formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb** wherein

Y is -NH-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl;

R_b is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being particularly more preferred compounds of formula **Ib, IAb, IBb, ICb, IEb, IFb, IGb** and/or compounds where R_4 is -CH_2NH_2.

- Compounds of formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb** wherein

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a -OR_b group for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl;

R_b is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being particularly more preferred compounds of formula **Ib, IAb, IBb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is CH_2NH_2.

- Compounds of formula **I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb,** and **IGb** wherein

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, ID, IF, Ia, IAa, IBa, ICa, IDa, IFa, Ib, IAb, IBb, ICb, IDb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, ID, IF, IG, Ia, ICa, IDa, IFa, IGa, Ib, ICb, IDb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;

R_4 is -CH_2OH;

R_a is substituted or unsubstituted C_1-C_6 alkyl; and

R_b is substituted or unsubstituted C_1-C_6 alkyl.

Being particularly more preferred compounds of formula **Ia, IAa, IBa, ICa, IDa, IFa**, or **IGa**.

- Compounds of formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** wherein

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, ID, IE, IF, Ia, IAa, IBa, ICa, IDa, IEa, IFa, Ib, IAb, IBb, ICb, IDb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is substituted or unsubstituted C_1-C_6 alkyl;

R_b is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being particularly more preferred compounds of formula **Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is -CH_2NH_2.

- Compounds of formula **I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb,** and **IGb** wherein

Y is -NH-;
R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IF, Ia, IAa, IBa, ICa, IFa, Ib, IAb, IBb, ICb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;
R_3 is hydrogen or a -OR_b group for compounds of formula I, IC, IF, IG, Ia, ICa, IFa, IGa, Ib, ICb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is a -OR_b group for compounds of formula IB, IBa or IBb;
R_4 is -$CH_2OC(=O)R_c$;
R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl;
R_b is substituted or unsubstituted C_1-C_6 alkyl; and
R_c is a substituted or unsubstituted C_1-C_6 alkyl.

Being more preferred compounds of formula **Ia, IAa, IBa, ICa, IFa**, or **IGa**.

- Compounds of formula **Ic, IAc, IBc, IDc, IFc**, and **IGc** wherein

R_2 is a -C(=O)R_a group for compounds of formula Ic, IAc, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc;

R_3 is hydrogen or methoxy for compounds of formula Ic, IDc, IFc, or IGc; R_3 is hydrogen for compounds of formula IAc; or R_3 is methoxy for compounds of formula IBc; and

R_a is substituted or unsubstituted C_1-C_6 alkyl.

Particularly more preferred compounds according to the present invention include:

- Compounds of formula **I, IA, IB, IC, IF, IG, Ia, IAa, IBa, ICa, IFa, IGa, Ib, IAb, IBb, ICb, IFb,** and **IGb** wherein

  Y is -NH-;

  R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IF, Ia, IAa, IBa, ICa, IFa, Ib, IAb, IBb, ICb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

  R_3 is hydrogen or methoxy for compounds of formula I, IC, IF, IG, Ia, ICa, IFa, IGa, Ib, ICb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; and R_3 is methoxy for compounds of formula IB, IBa or IBb;

  R_4 is -CH_2OH; and

  R_a is substituted or unsubstituted C_1-C_6 alkyl.

  Being even more preferred compounds of formula **Ia, IAa, IBa, ICa, IFa, IGa**.

- Compounds of formula **I, IA, IB, ID, IF, IG, Ia, IAa, IBa, IDa, IFa, IGa, Ib, IAb, IBb, IDb, IFb,** and **IGb** wherein

  Y is -O-;

  R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IF, Ia, IAa, IBa, IDa, IFa, Ib, IAb, IBb, IDb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;;

  R_3 is hydrogen or methoxy for compounds of formula I, ID, IF, IG, Ia, IDa, IFa, IGa, Ib, IDb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is methoxy for compounds of formula IB, IBa or IBb;

  R_4 is -CH_2OH; and

  R_a is substituted or unsubstituted C_1-C_6 alkyl.

  Being even more preferred compounds of formula **Ia, IAa, IBa, IDa, IEa, IFa, IGa**.

- Compounds of formula **I, IA, IB, IC, IE, IF, IG, Ia, IAa, IBa, ICa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IEb, IFb,** and **IGb** wherein

  Y is -NH-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, IE, IF, Ia, IAa, IBa, ICa, IEa, IFa, Ib, IAb, IBb, ICb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or methoxy for compounds of formula I, IC, IE, IF, IG, Ia, ICa, IEa, IFa, IGa, Ib, ICb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is methoxy for compounds of formula IB, IBa or IBb;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being even more preferred compounds of formula **Ib, IAb, IBb, ICb, IEb, IFb, IGb** and/or compounds where R_4 is -CH_2NH_2.

- Compounds of formula **I, IA, IB, ID, IE, IF, IG, Ia, IAa, IBa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, IDb, IEb, IFb,** and **IGb** wherein

Y is -O-;

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, ID, IE, IF, Ia, IAa, IBa, IDa, IEa, IFa, Ib, IAb, IBb, IDb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or methoxy for compounds of formula I, ID, IE, IF, IG, Ia, IDa, IEa, IFa, IGa, Ib, IDb, IEb, IFb, or IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is methoxy for compounds of formula IB, IBa or IBb;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R_a is substituted or unsubstituted C_1-C_6 alkyl; and

$Prot^{NH}$ is a protecting group for amino.

Being even more preferred compounds of formula **Ib, IAb, IBb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is -CH_2NH_2.

- Compounds of formula **I, IA, IB, IC, ID, IF, IG, Ia, IAa, IBa, ICa, IDa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IFb,** and **IGb** wherein

R_2 is a -C(=O)R_a group for compounds of formula I, IA, IB, IC, ID, IF, Ia, IAa, IBa, ICa, IDa, IFa, Ib, IAb, IBb, ICb, IDb, or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or methoxy for compounds of formula I, IC, ID, IF, IG, Ia, ICa, IDa, IFa, IGa, Ib, ICb, IDb, IFb, and IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is methoxy for compounds of formula IB, IBa or IBb;

R_4 is $-CH_2OH$; and

R_a is selected from methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl.

Being even more preferred compounds of formula **Ia, IAa, IBa, ICa, IDa, IEa, IFa,** or **IGa**.

- Compounds of formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb,** and **IGb** wherein

R_2 is a $-C(=O)R_a$ group for compounds of formula I, IA, IB, IC, ID, IE, IF, Ia, IAa, IBa, ICa, IDa, IEa, IFa, Ib, IAb, IBb, ICb, IDb, IEb or IFb; and R_2 is acetyl for compounds of formula IG, IGa or IGb;

R_3 is hydrogen or a methoxy for compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, and IGb; R_3 is hydrogen for compounds of formula IA, IAa or IAb; or R_3 is methoxy for compounds of formula IB, IBa or IBb;

R_4 is selected from $-CH_2NH_2$ and $-CH_2NHProt^{NH}$;

R_a is selected from methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl; and

$Prot^{NH}$ is a protecting group for amino.

Being even more preferred compounds of formula **Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **Ic** or **IAc, IDc, IFc,** and **IGc** wherein

R_2 is a $-C(=O)R_a$ group for compounds of formula Ic, IAc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc;

R_3 is hydrogen; and

R_a is selected from methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl.

- Compounds of formula **Ic, IBc, IDc, IFc,** and **IGc** wherein

R_2 is a -C(=O)R_a group for compounds of formula Ic, IBc, IDc, or IFc; and R_2 is acetyl for compounds of formula IGc;

R_3 is methoxy; and

R_a is selected from methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl.

Even more preferred compounds according to the present invention include:

- Compounds of formula **I, IA, IC, IF, IG, Ia, IAa, ICa, IFa, IGa, Ib, IAb, ICb, IFb,** and **IGb** wherein

  Y is -NH-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is -CH_2OH.

  Being most preferred compounds of formula **Ia, IAa, ICa, IFa,** or **IGa**.

- Compounds of formula **I, IA, ID, IF, IG, Ia, IAa, IDa, IFa, IGa, Ib, IAb, IDb, IFb,** and **IGb** wherein

  Y is -O-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is -CH_2OH.

  Being most preferred compounds of formula **Ia, IAa, IDa, IFa, or IGa**

- Compounds of formula **I, IA, IC, IE, IF, IG, Ia, IAa, ICa, IEa, IFa, IGa, Ib, IAb, ICb, IEb, IFb,** and **IGb** wherein

  Y is -NH-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is -CH_2NH_2.

Being most preferred compounds of formula **Ib, IAb, ICb, IEb, IFb,** or **IGb**.

- Compounds of formula **I, IA, ID, IE, IF, IG, Ia, IAa, IDa, IEa, IFa, IGa, Ib, IAb, IDb, IEb, IFb,** and **IGb** wherein

  Y is -O-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2NH_2$.

  Being most preferred compounds of formula **Ib, IAb, IDb, IEb, IFb,** or **IGb**.

- Compounds of formula **I, IA, IC, ID, IF, IG, Ia, IAa, ICa, IDa, IFa, IGa, Ib, IAb, ICb, IDb, IFb,** and **IGb** wherein

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2OH$.

  Being most preferred compounds of formula **Ia, IAa, ICa, IDa, IFa** or **IGa**.

- Compounds of formula **I, IA, IC, ID, IF, IG, Ia, IAa, ICa, IDa, IFa, IGa, Ib, IAb, ICb, IDb, IFb,** and **IGb** wherein

  R_1 is -OH;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2OH$.

  Being most preferred compounds of formula **Ia, IAa, ICa, IDa, IFa** or **IGa**.

- Compounds of formula **I, IA, IC, ID, IE, IF, IG, Ia, IAa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, ICb, IDb, IEb, IFb,** and **IGb** wherein

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2NH_2$.

Being most preferred compounds of formula **Ib, IAb, ICb, IDb, IEb, IFb,** or **IGb**.

- Compounds of formula **Ic** or **IAc, IDc, IFc, IGc** wherein

  R_2 is acetyl; and

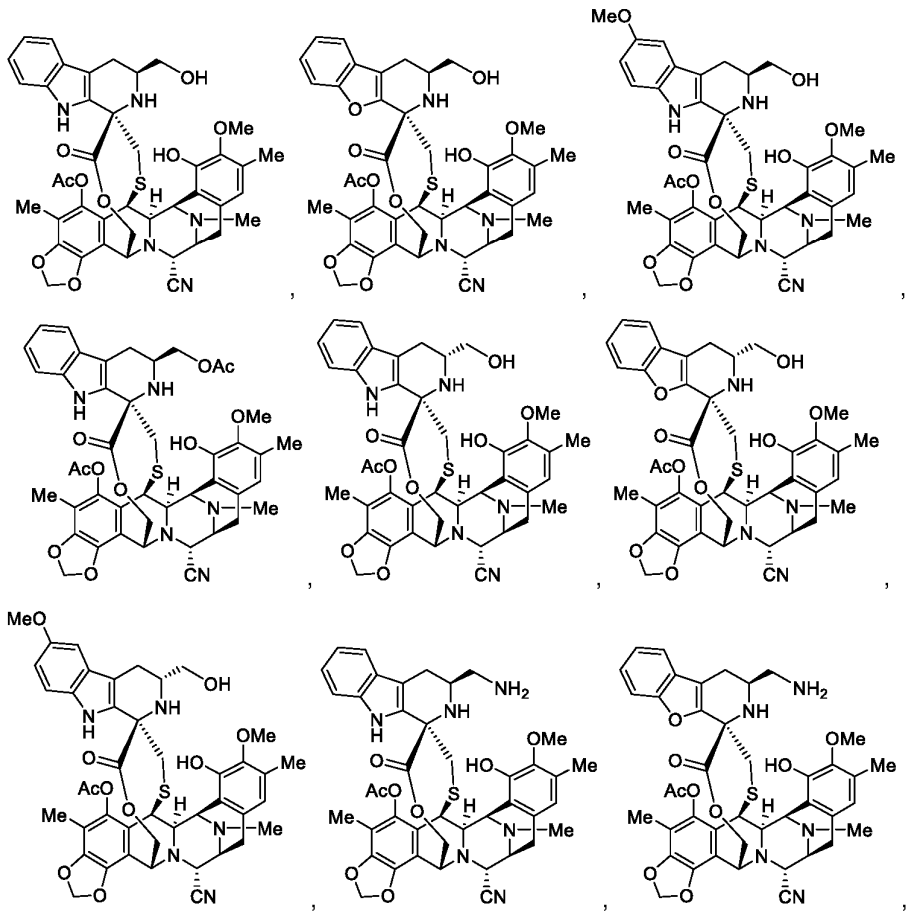
  R_3 is hydrogen.

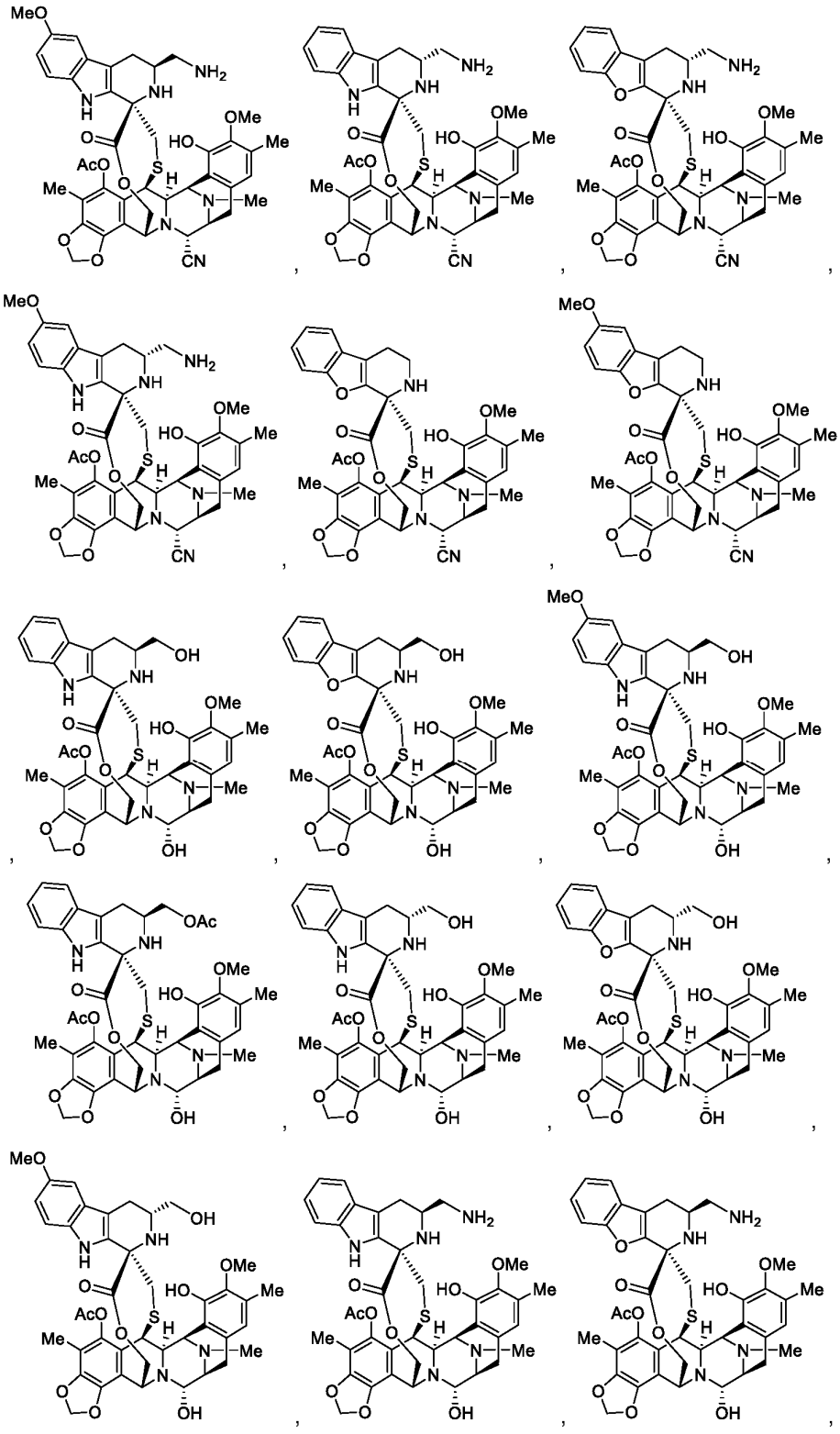
- Compounds of formula **Ic** or **IBc, IDc, IFc, IGc** wherein

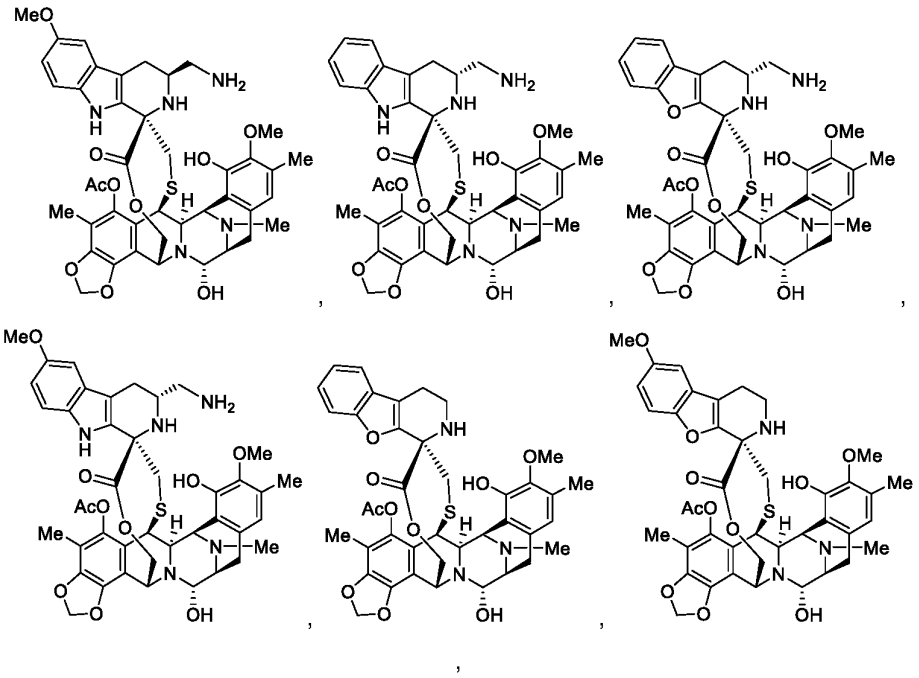
  R_2 is acetyl; and

  R_3 is methoxy.

- A compound according to the present invention of formula:

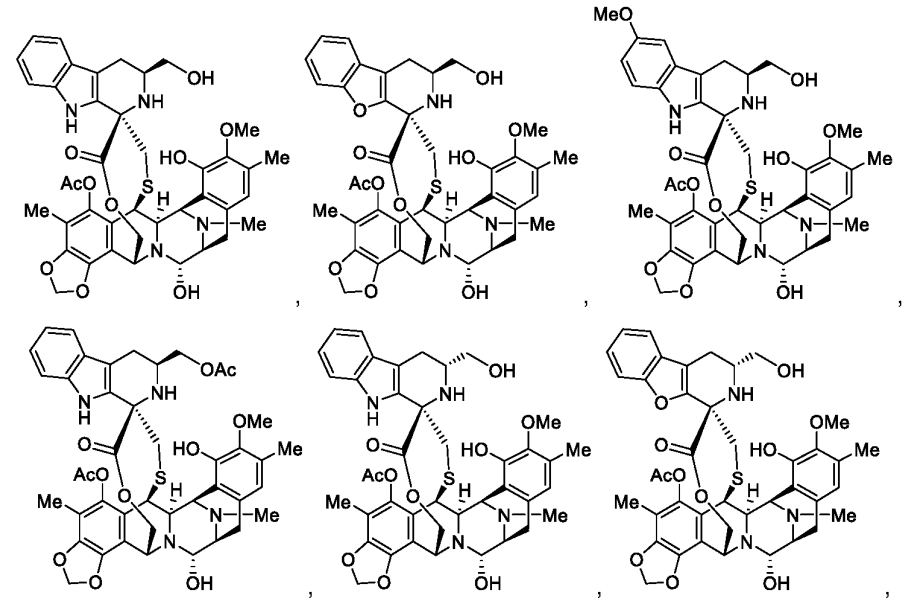


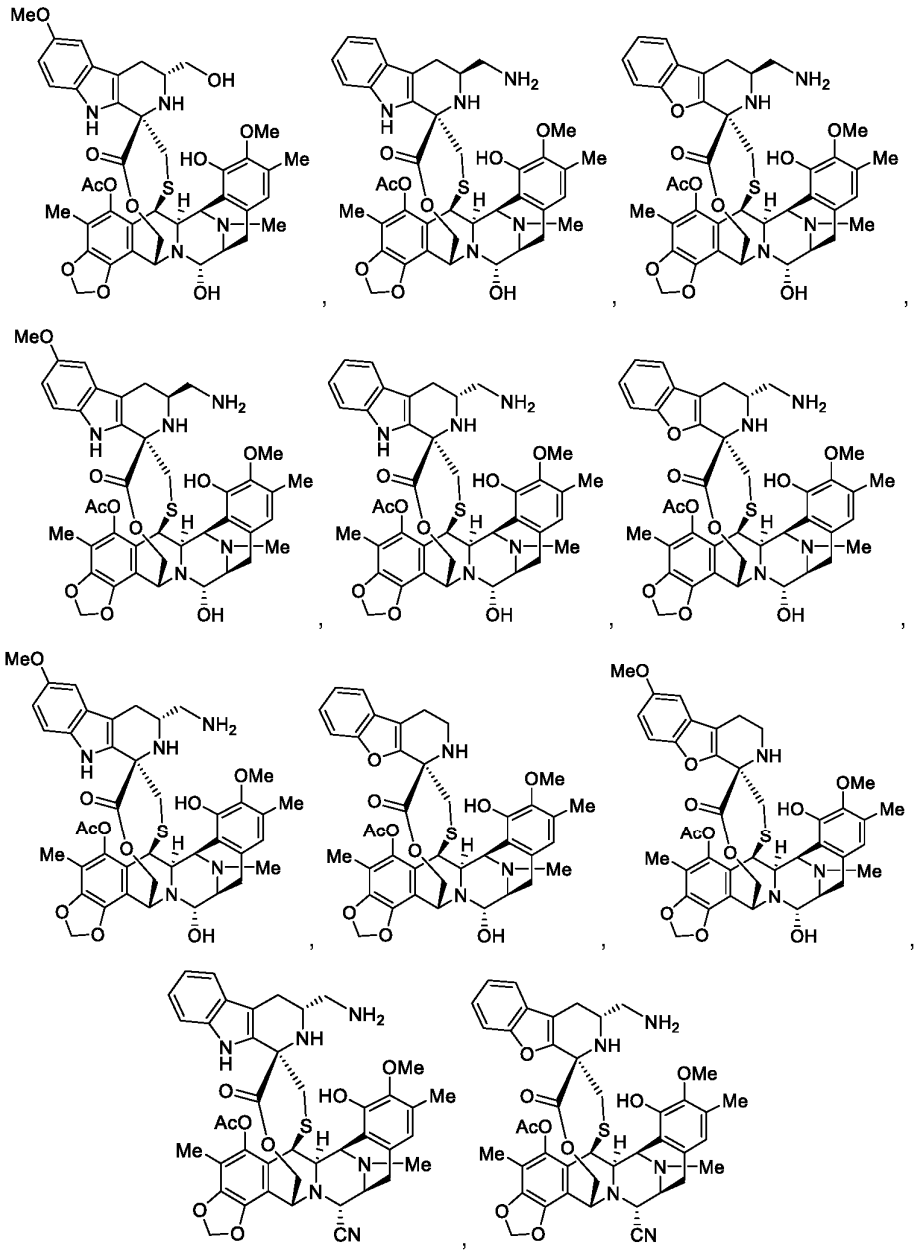




or a pharmaceutically acceptable salt or ester thereof.

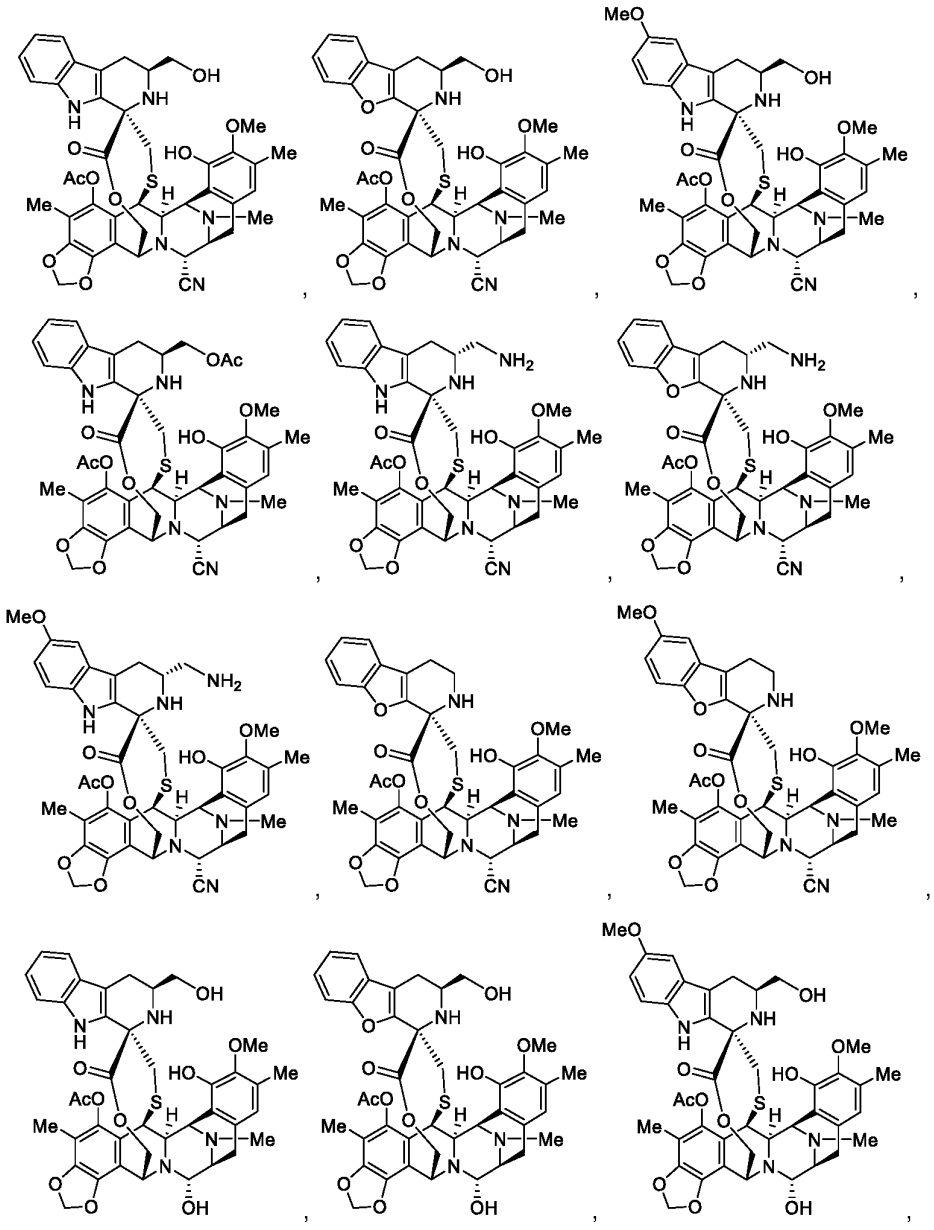
Being particularly preferred a compound of formula:

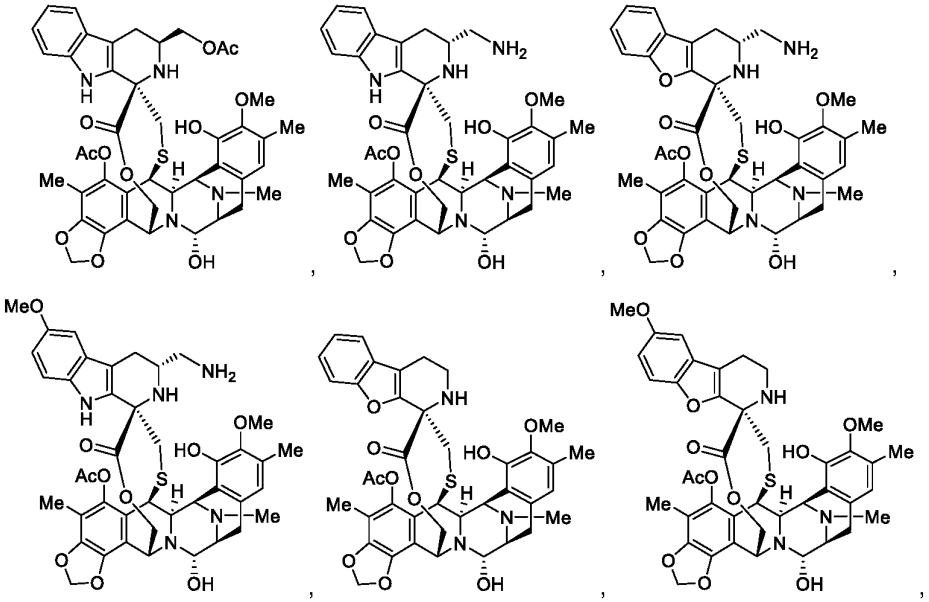




or a pharmaceutically acceptable salt or ester thereof.

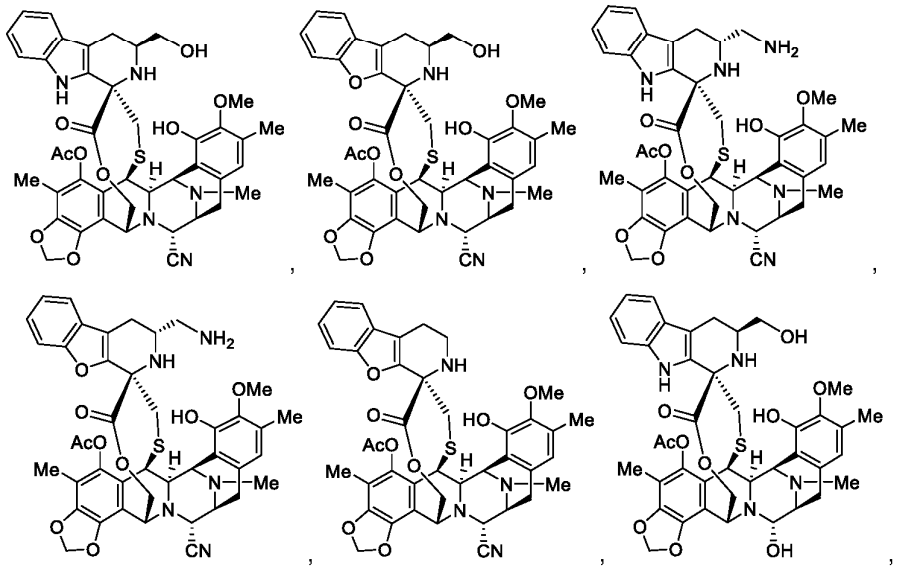
- A compound according to the present invention of formula:

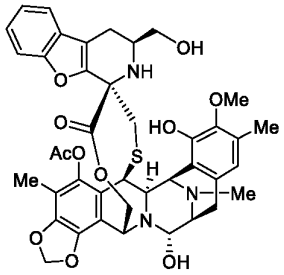
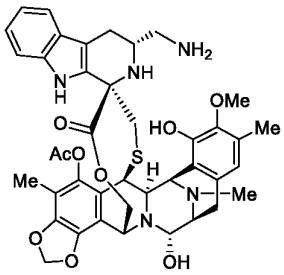
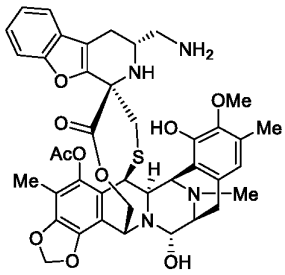
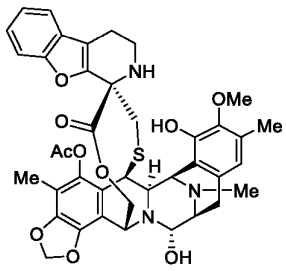




or a pharmaceutically acceptable salt or ester thereof.

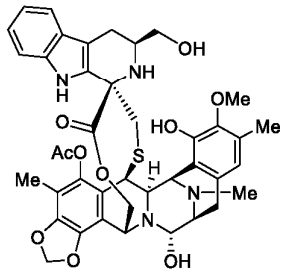
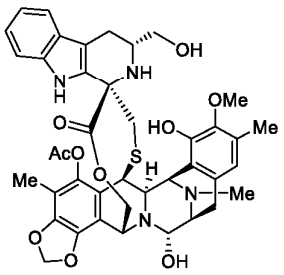
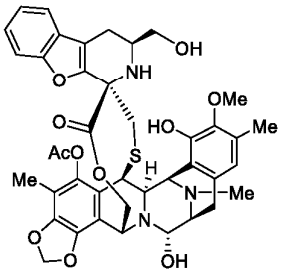
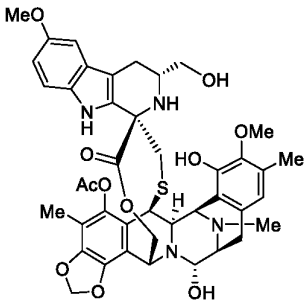
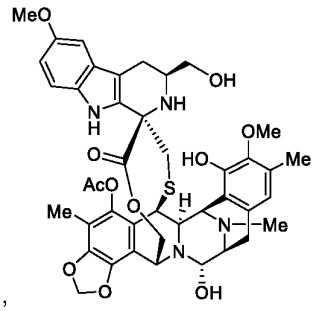
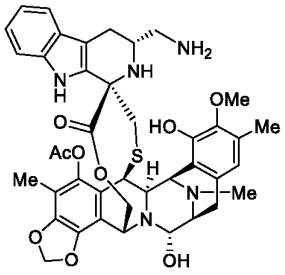
Being particularly preferred a compound of formula:

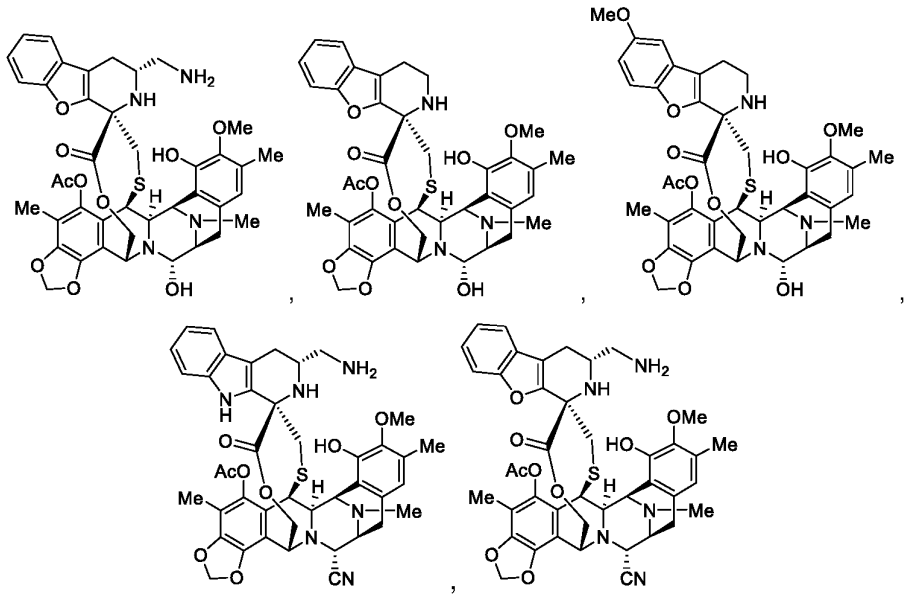



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or a pharmaceutically acceptable salt or ester thereof.

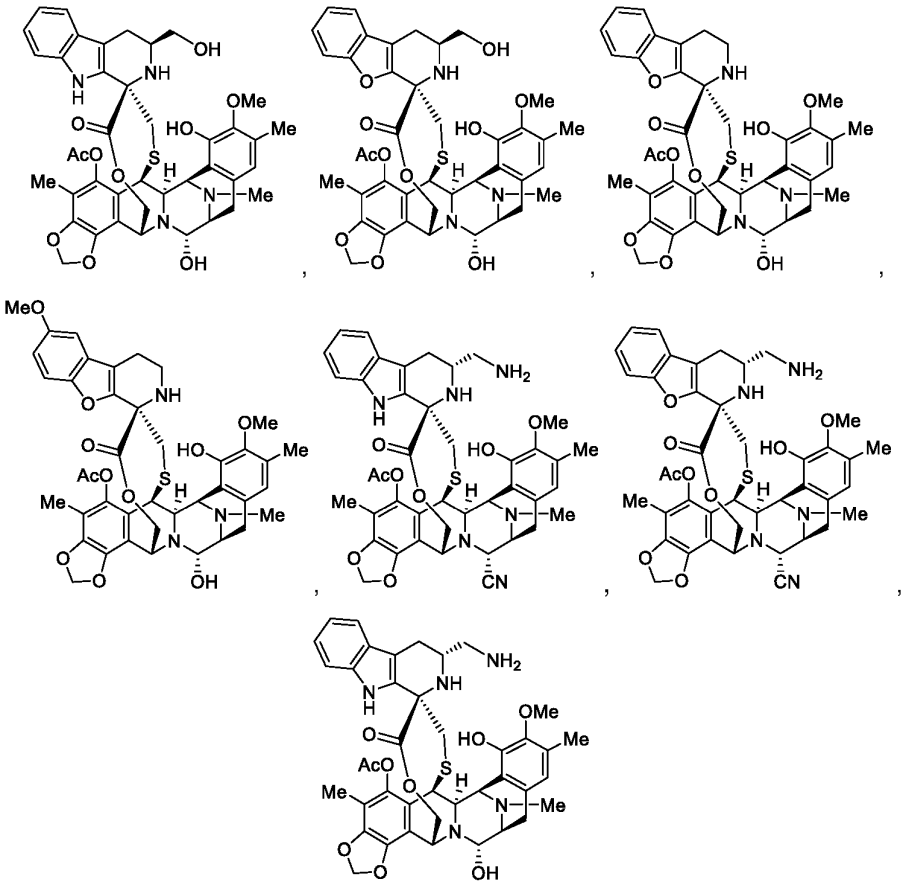
Being more preferred a compound of formula:


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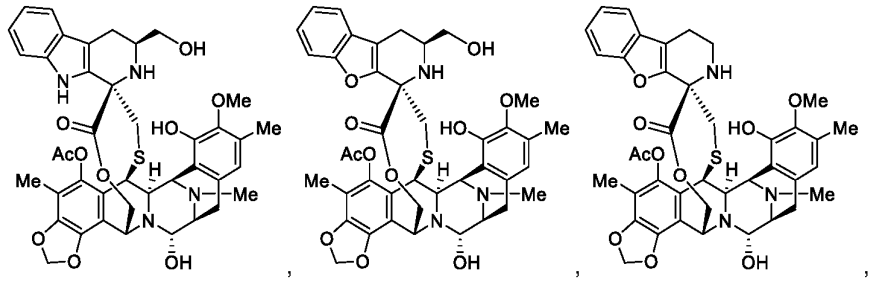
or a pharmaceutically acceptable salt or ester thereof.

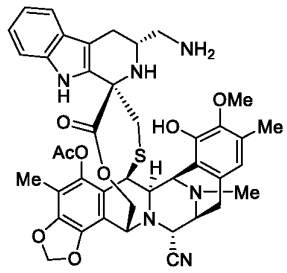
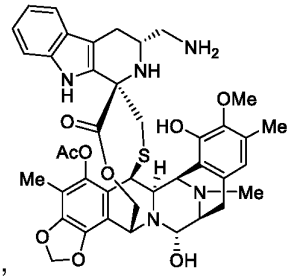
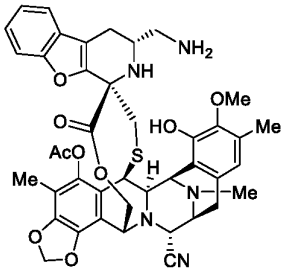
Being even more preferred compounds according to the present invention are compounds of formula:



or a pharmaceutically acceptable salt or ester thereof.

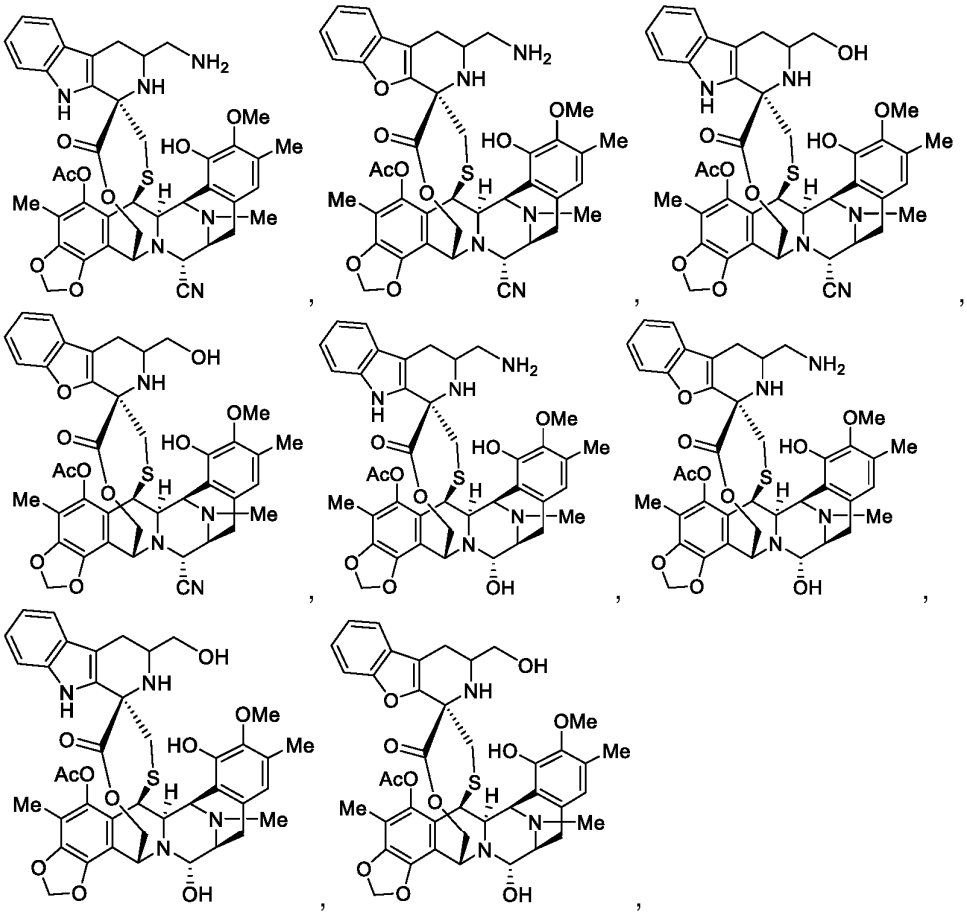
Further preferred compounds according to the present invention are compounds of formula:



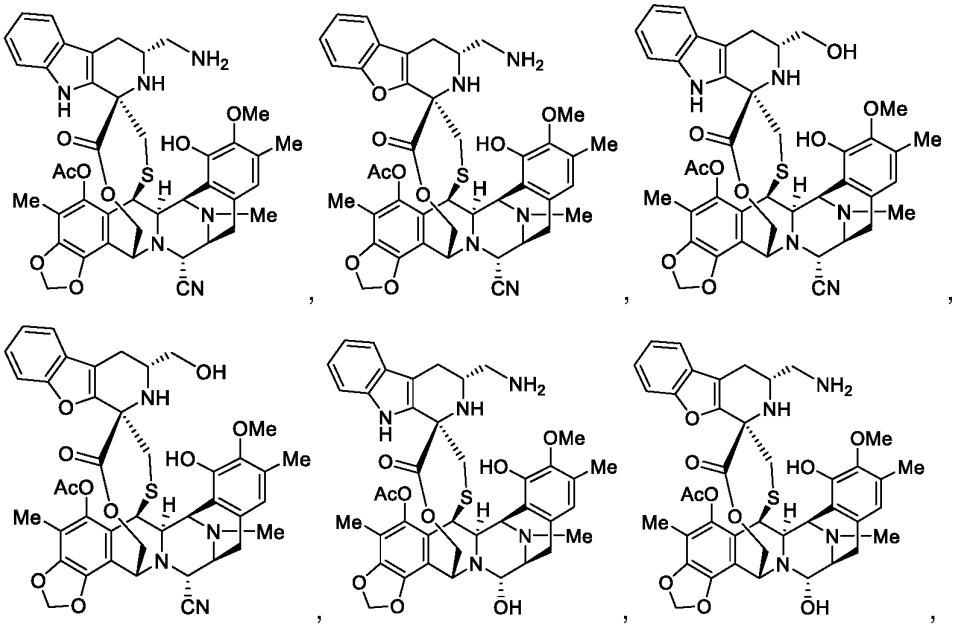
or a pharmaceutically acceptable salt or ester thereof.

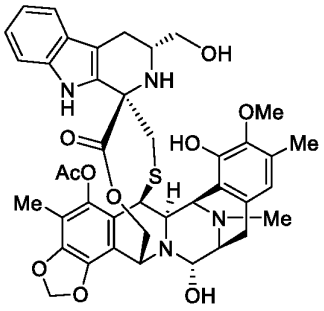
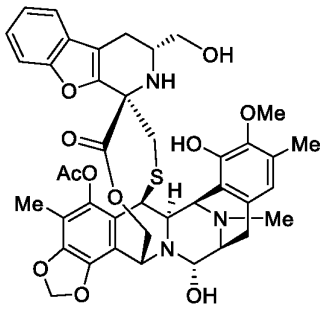
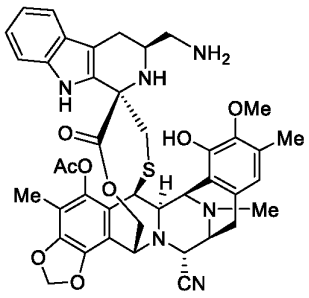
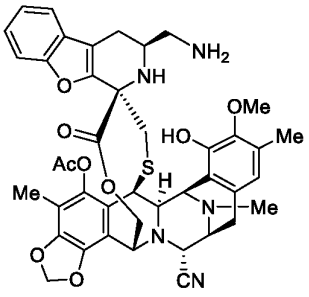
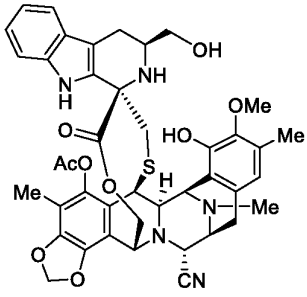
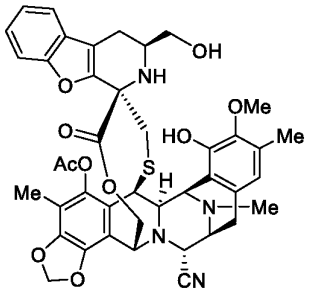
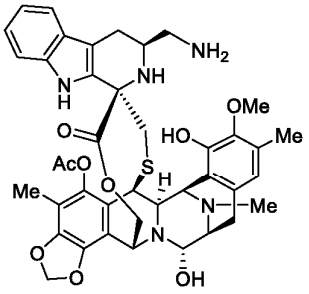
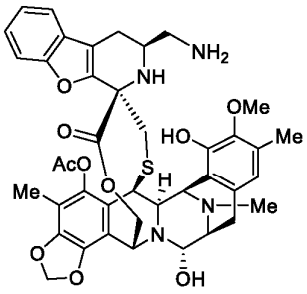
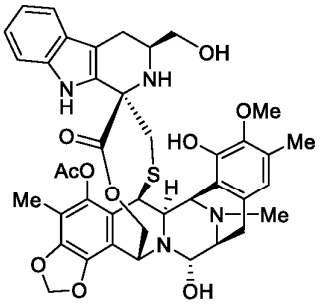
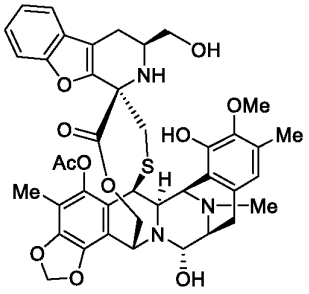
In a further embodiment, according to the present invention preferred compounds are compounds of formula:



or a pharmaceutically acceptable salt or ester thereof.

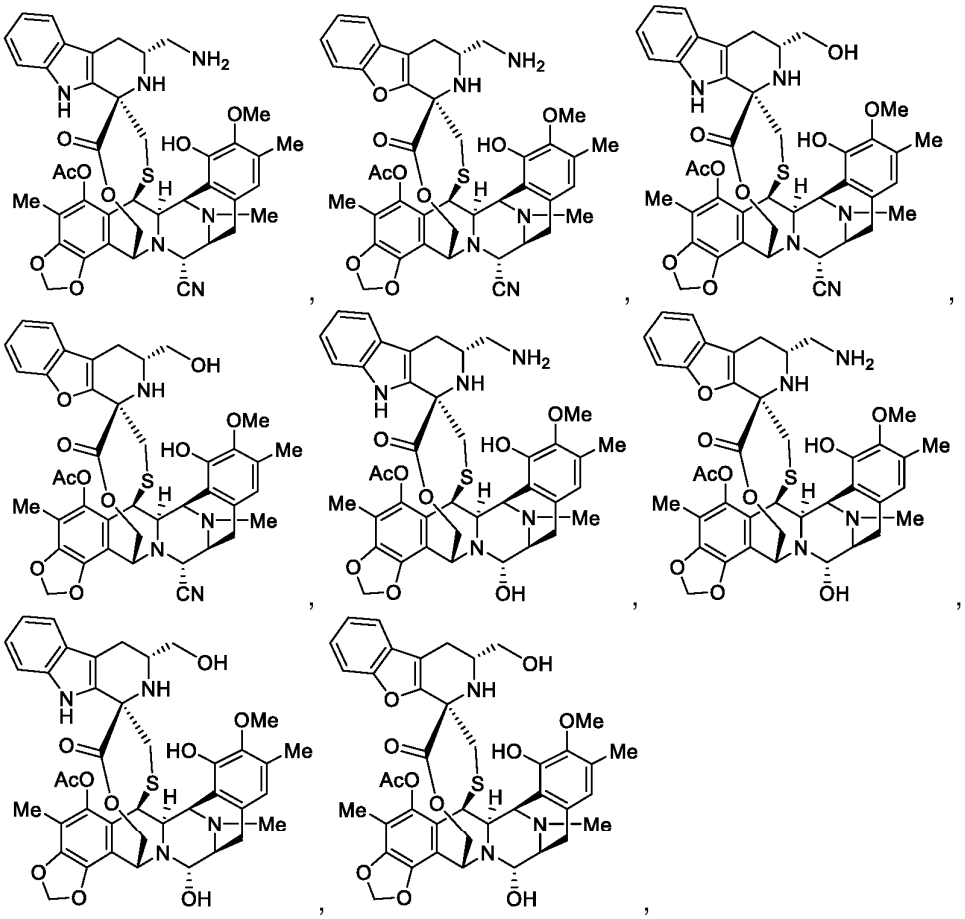
Further preferred compounds include a compound of formula:




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or a pharmaceutically acceptable salt or ester thereof.

Further preferred compounds include a compound of formula:



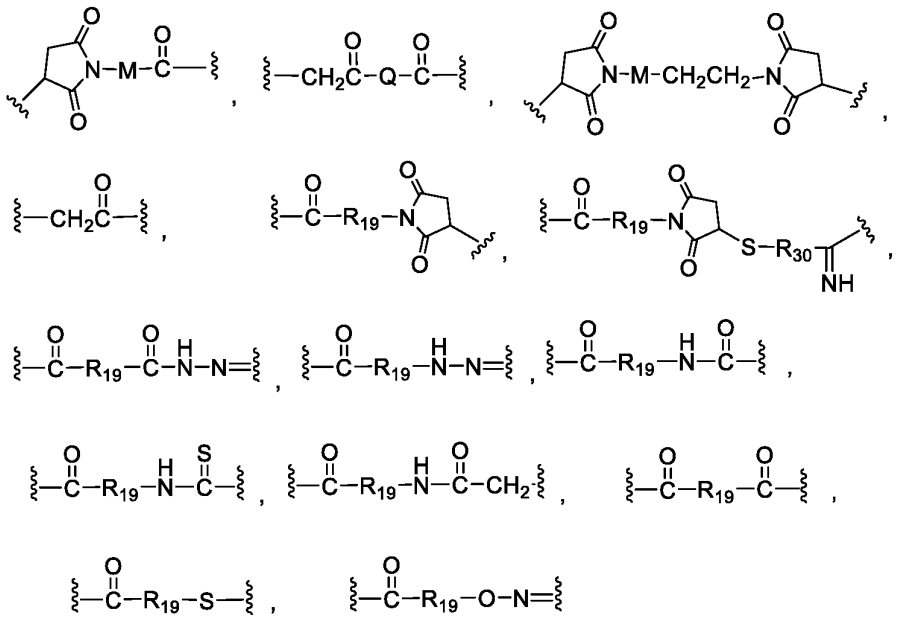
or a pharmaceutically acceptable salt or ester thereof.

In additional preferred embodiments, the preferences described above for the different substituents are combined. The present invention is also directed to such combinations of preferred substitutions (where allowed by possible substituent groups) in compounds of formula **I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, Ic, IAc, IBc, IDc, IFc or IGc** according to the present invention.

For the avoidance of doubt, the compounds above may be the drug moiety D and are covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L). Thus, when conjugated, a covalent bond replaces a proton on a hydroxy or amine group on the compound.

Preferred drug conjugates according to the the present invention are given below. The preferred definitions of $(X)_b$, $(AA)_w$, $(T)_g$, and (L) as set out below are applicable to all of the drug moiety D compounds described above. Preferred drug conjugates according to the the present invention include:

- a drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention wherein L is a linker group selected from the group consisting of:



wherein

the wavy lines indicate the point of covalent attachments to an Ab (the wavy line to the right) and to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D (the wavy line to the left);

R_{19} is selected from -C_1-C_{12} alkylene-, -C_3-C_8 carbocyclo, -O-(C_1-C_{12} alkylene), -C_6-C_{18} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{18} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{18} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_3-C_8 carbocyclo)-, -(C_3-C_8 carbocyclo)-C_1-C_{12} alkylene-, -C_5-C_{14} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{14} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{14} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$-, and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene

substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a -C_1-C_6 alkylene- group;

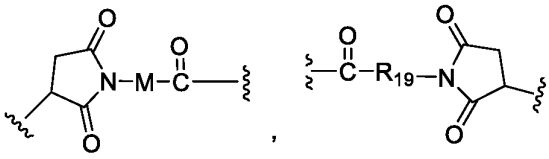
M is selected from the group consisting of -C_1-C_6 alkylene-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)-, -$(CH_2CH_2O)_s$-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)-CON(H or C_1-C_6 alkyl)-C_1-C_6 alkylene-, phenylene which may optionally be substituted with one or more substituents R_x, phenylene-C_1-C_6 alkylene- wherein the phenylene moiety may optionally be substituted with one or more substituents R_x and -C_1-C_6 alkylene-CON(H or C_1-C_6 alkyl)C_1-C_6 alkylene-;

Q is selected from the group consisting of -N(H or C_1-C_6 alkyl)phenylene- and -N(H or C_1-C_6 alkyl)-$(CH_2)_s$;

r is an integer ranging from 1 to 10; and

s is an integer ranging from 1 to 10.

- a drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention wherein L is selected from the group consisting of:



wherein:

the wavy lines indicate the point of covalent attachments to an Ab (the wavy line to the right) and to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D (the wavy line to the left);

R_{19} is selected from -C_1-C_{12} alkylene-, -O-(C_1-C_{12} alkylene), -C_6-C_{12} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{12} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_5-C_{12} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{12} heterocyclo)- wherein said

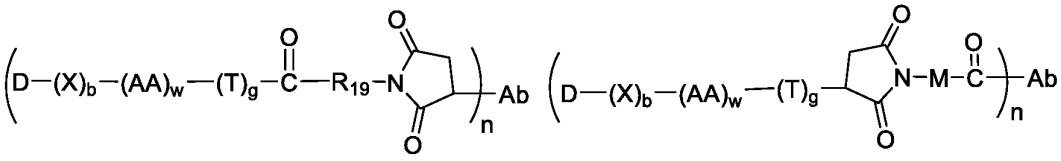
heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{12} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$-, and -CH_2-$(OCH_2CH_2)_r$- wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a -C_1-C_6 alkylene- group;

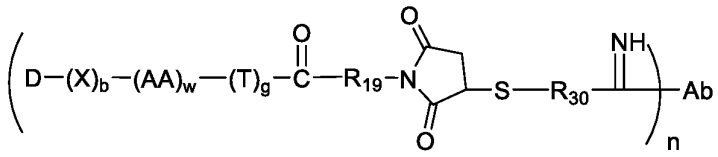
M is selected from the group consisting of -C_1-C_6 alkylene-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)- and phenylene which may optionally be substituted with one or more substituents R_x; and

r is an integer ranging from 1-6.

- a drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention selected from formulas (IV), (V) and (VI):



(IV) (V)



(VI)

wherein:

X and T are extending groups as defined herein;

each AA is independently an amino acid unit as defined herein;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

g is an integer of 0 or 1;

where b+g+w is optionally not 0;

D is a drug moiety;

Ab is a moiety comprising at least one antigen binding site;

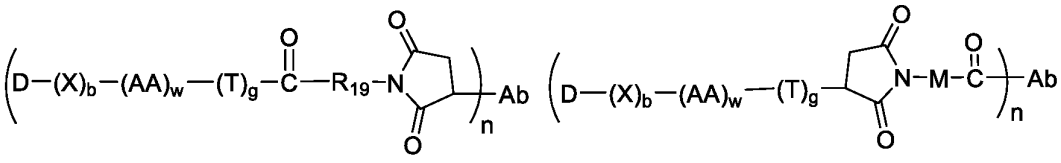
n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formula (IV), (V) or (VI) to the moiety comprising at least one antigen binding site and is in the range from 1 to 20;

R_{19} is selected from -C_1-C_8 alkylene-, -O-(C_1-C_8 alkylene), -C_1-C_8 alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, and -C_6-C_{12} arylene-C_1-C_8 alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

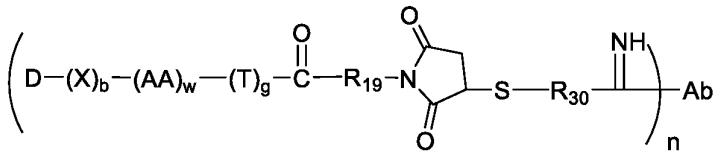
R_{30} is a –C_2-C_4 alkylene- group; and

M is selected from the group consisting of -C_1-C_3 alkylene- and -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-.

- a drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention, selected from formulas (IV), (V) and (VI):



(IV) (V)



(VI)

wherein:

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

g is an integer of 0 or 1;

where b+g+w is optionally not 0;

D is a drug moiety;

Ab is a moiety comprising at least one antigen binding site;

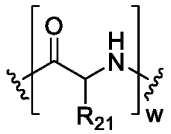
n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formulas (IV), (V) or (VI) to the moiety comprising at least one antigen binding site and is in the range from 1 to 20;

R_{19} is selected from -C_1-C_6 alkylene-, phenylene-C_1-C_6 alkylene- wherein the phenylene group may optionally be substituted with one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, wherein each of the above alkylene substituents whether alone or attached to another moiety in the carbon chain may optionally be substituted by one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, aryl groups having from 6 to 12 carbon atoms, halogen atoms, nitro groups and cyano groups, and preferably R_{19} is a -C_1-C_6 alkylene group;

R_{30} is a –C_2-C_4 alkylene- group; and

M is -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-.

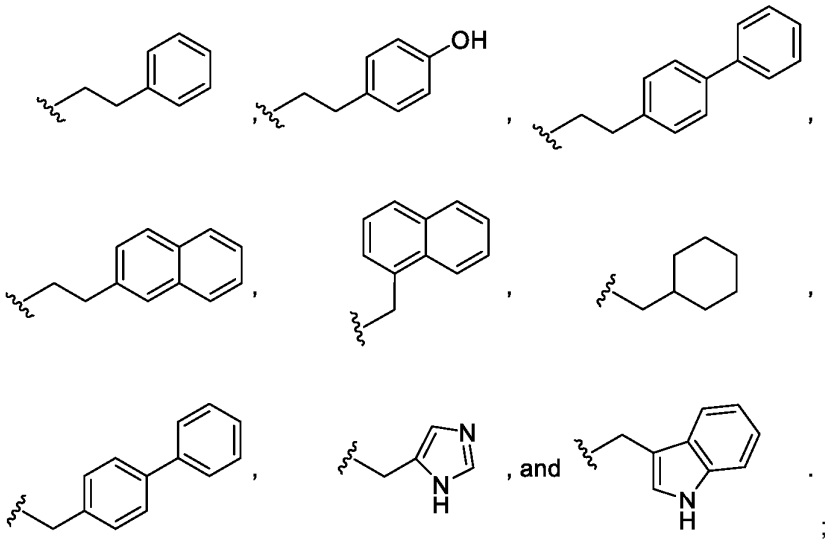
- It is preferred that in the definition of the drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab, L is as defined in the preferred definitions for said group above and $(AA)_w$ is of formula (II):



(II)

wherein the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right); and

R_{21} is, at each occurrence, selected from the group consisting of hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, $-CH_2OH$, $-CH(OH)CH_3$, $-CH_2CH_2SCH_3$, $-CH_2CONH_2$, $-CH_2COOH$, $-CH_2CH_2CONH_2$, $-CH_2CH_2COOH$, $-(CH_2)_3NHC(=NH)NH_2$, $-(CH_2)_3NH_2$, $-(CH_2)_3NHCOCH_3$, $-(CH_2)_3NHCHO$, $-(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_4NHCOCH_3$, $-(CH_2)_4NHCHO$, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NHCONH_2$, $-CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,

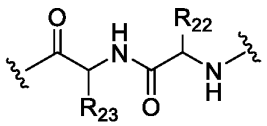


and w is an integer ranging from 0 to 12.

- a drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the first aspect of the present invention, wherein L is as defined in the preferred definitions for said group above and $(AA)_w$ is of formula (II) wherein:

  R_{21} is selected, at each occurrence, from the group consisting of hydrogen, methyl, isopropyl, sec-butyl, benzyl, indolylmethyl, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_3NHC(=NH)NH_2$ and $-(CH_2)_4NHC(=NH)NH_2$; and
  w is an integer ranging from 0 to 6.

- a drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the first aspect of the present invention, wherein L is as defined in the preferred definitions for said group above, wherein w is 0 or 2, and when w is 2, then $(AA)_w$ is of formula (III) wherein:



(III)

the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

R_{22} is selected from methyl, benzyl, isopropyl, *sec*-butyl and indolylmethyl; and

R_{23} is selected from methyl, $-(CH_2)_4NH_2$, $-(CH_2)_3NHCONH_2$ and $-(CH_2)_3NHC(=NH)NH_2$.

- In embodiments of the present invention b+g+w is not 0. In further embodiments, b+w is not 0. In yet further embodiments, when w is not 0, then b is 1. Further, it is preferred that in the definition of the drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab, L and $(AA)_w$ are as defined in the preferred definitions for said groups above and X is an extending group selected from:

  where D is conjugated via an amine group (for example where Z is -NH-):

  -COO-(C_1-C_6 alkylene)NH-;

  -COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

  -COO-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

  -$COCH_2NH$-$COCH_2$-NH-;

  -$COCH_2NH$-;

  -COO-(C_1-C_6 alkylene)S-;

  -COO-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-; or

  where D is conjugated via an hydroxy group (for example where Z is -O-):

  -CONH-(C_1-C_6 alkylene)NH-;

  -COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

  -CONH-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

  -$COCH_2NH$-$COCH_2$-NH-;

-$COCH_2NH$-;

-CONH-(C_1-C_6 alkylene)S-;

-CONH-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-; and

b is 0 or 1, preferably 1.

- a drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab according to the present invention, wherein L and $(AA)_w$ are as defined in the preferred definitions for said groups above and X is an extending group selected from the group consisting of:

where D is conjugated via an amine group (for example where Z is -NH-):

-COO-(C_2-C_4 alkylene)NH-;

-COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups;

-COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-;

-$COCH_2NH$-$COCH_2$-NH-;

-COO-(C_2-C_4 alkylene)S-;

-COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-; or

where D is conjugated via an hydroxy group (for example where Z is -O-):

-CONH-(C_2-C_4 alkylene)NH-;

-COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups;

-CONH-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-;

-$COCH_2$NH-$COCH_2$-NH-;

-CONH-(C_2-C_4 alkylene)S-;

-CONH-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-; and

b is 0 or 1, preferably 1.

- a drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention, wherein L and $(AA)_w$ are as defined in the preferred definitions for said groups above and X is an extending group selected from the group consisting of:

where D is conjugated via an amine group (for example where Z is -NH-):

-COO-CH_2-phenylene-NH-

-$COO(CH_2)_3NHCOOCH_2$-phenylene-NH-;

-$COO(CH_2)_3$NH-;

-$COO(CH_2)_3$-S-;

-$COO(CH_2)_3NHCO(CH_2)_2$S-; or

where D is conjugated via an hydroxy group (for example where Z is -O-):

-COO-CH_2-phenylene-NH-

-$CONH(CH_2)_3NHCOOCH_2$-phenylene-NH-;

-$CONH(CH_2)_3$NH-;

-$CONH(CH_2)_3$-S-;

-$CONH(CH_2)_3NHCO(CH_2)_2$S-; and

b is 0 or 1, preferably 1.

- a drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the present invention, wherein L, $(AA)_w$, and $(X)_b$ are as defined in the preferred definitions for said groups above and T is an extending group selected from the group consisting of:

  -CO-(C_1-C_6 alkylene)-NH-;

  -CO-(C_1-C_6 alkylene)-[O-(C_2-C_6 alkylene)]$_j$-NH-;

  -COO-(C_1-C_6 alkylene)-[O-(C_2-C_6 alkylene)]$_j$-NH-;

  where j is an integer from 1 to 25, and

  g is 0 or 1.

- A drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the present invention, wherein L, $(AA)_w$, and $(X)_b$ are as defined in the preferred definitions for said groups above and T is an extending group selected from the group consisting of:

  -CO-(C_1-C_4 alkylene)NH-

  -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-;

  -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-;

  where j is an integer from 1 to 10; and

  g is 0 or 1.

- A drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the present invention, wherein L, $(AA)_w$, and $(X)_b$ are as defined in the preferred definitions for said groups above and T is an extending group selected from the group consisting of:

  -CO-(C_1-C_4 alkylene)NH-

  -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-;

  -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-;

  where j is an integer from 1 to 5; and

  g is 0 or 1.

- A preferred drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, (IH), (IHa) or (IHb), or a

pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein R_1 is CN or OH in compounds of formula I, IA, IB, IC, ID, IE, IG, Ia, IAa, IBa, ICa, IDa, IEa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IGb, (IH), (IHa) or (IHb); R_1 is OH in compounds of formula IF, IFa and IFb, and more preferably R_1 is CN.

- Another preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula I, IA, IB, IC, ID, IE, IF, Ia, IAa, IBa, ICa, IDa, IEa, IFa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, (IH), (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein R_2 is $C(=O)R_a$, wherein R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl, wherein the optional substituents are one or more substituents R_x, and more preferably R_2 is acetyl.

- Another preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, (IH), (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein R_3 is hydrogen or a $-OR_b$ group in compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, IGb, (IH), (IHa) or (IHb); R_3 is hydrogen in compounds of formula IA, IAa, or IAb; R_3 is a $-OR_b$ group in compounds of formula IB, IBa, or IBb, wherein R_b is a substituted or unsubstituted C_1-C_6 alkyl group, wherein the optional substituents are one or more substituents R_x, and more preferably R_3 is hydrogen or methoxy. Most preferably R_3 is hydrogen.

- Another preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula (IH), (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein Y is -NH- or -O-.

- Another preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula (IH), (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein Z is -NH- or -O-, and more preferably Z is -NH-.

- A further preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein:

R_1 is -CN or -OH;

R_2 is -C(=O)R_a, wherein R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl, wherein the optional substituents are one or more substituents R_x;

R_3 is hydrogen or a -OR_b group wherein R_b is a substituted or unsubstituted C_1-C_6 alkyl group, wherein the optional substituents are one or more substituents R_x,

Y is -NH- or -O-; and

Z is -NH- or -O-.

- A further preferred drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein:

R_1 is -CN or -OH;

R_2 is acetyl;

R_3 is hydrogen or methoxy, more preferably hydrogen;

Y is -NH- or -O-; and

Z is -NH- or -O-.

- A further preferred drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is a compound of formula (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein:

R_1 is -CN;

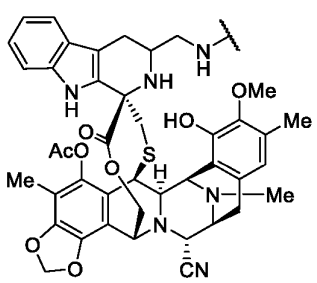
R_2 is acetyl:

R_3 is hydrogen;

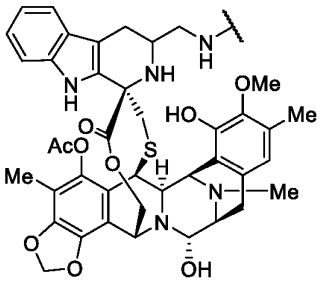
Y is -NH- or -O-; and

Z is -NH-.

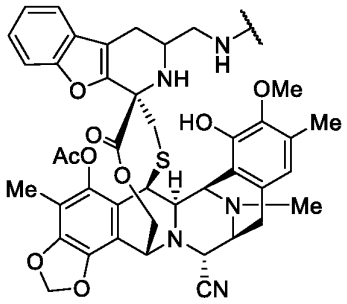
- A further preferred drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is selected from:



,

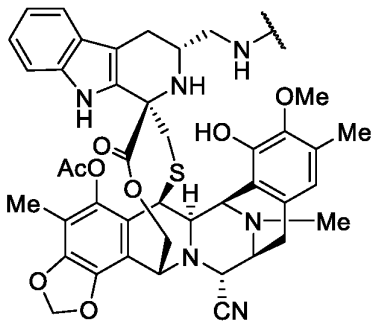
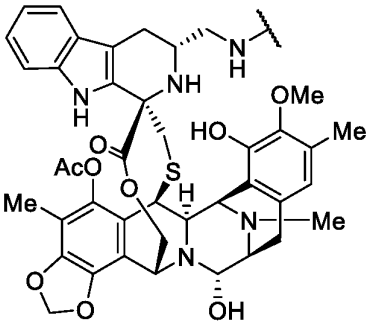
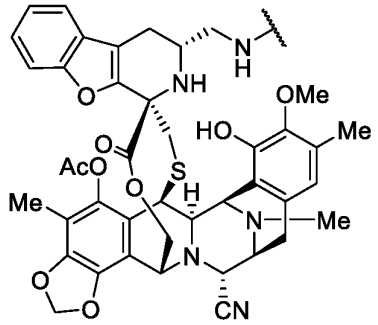


and



or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy lines indicate the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any or to (L).

- A further preferred drug conjugate of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, and $(T)_g$ are as defined above and wherein D is selected from:



and


or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy lines indicate the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any or to (L).

- A further preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and wherein the moiety Ab comprising at least one antigen binding site is an antigen-binding peptide.

- A further preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and the moiety Ab comprising at least one antigen binding site is an antibody, a single domain antibody or an antigen-binding fragment thereof.

- A further preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and the moiety Ab comprising at least one antigen binding site is a monoclonal, polyclonal antibody or bispecific antibody and wherein the antibody or antigen-binding fragment thereof is derived from any species, preferably a human, mouse or rabbit.

- A further preferred drug conjugate of formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and the moiety Ab comprising at least one antigen binding site is an antibody or antigen-binding fragment thereof which is selected from the group consisting of a human antibody, an antigen-binding fragment of a human antibody, a humanized

antibody, an antigen-binding fragment of a humanized antibody, a chimeric antibody, an antigen-binding fragment of a chimeric antibody, a glycosylated antibody and a glycosylated antigen binding fragment.

- A further preferred drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and the moiety Ab comprising at least one antigen binding site is an antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is an antigen-binding fragment selected from the group consisting of an Fab fragment, an Fab' fragment, an F(ab')2 fragment and an Fv fragment.

- A further preferred drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab according to the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined above and the moiety Ab comprising at least one antigen binding site is an antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody which immunospecifically binds to cancer cell antigens, viral antigens, antigens of cells that produce autoimmune antibodies associated with autoimmune disease, microbial antigens, and preferably a monoclonal antibody which immunospecifically binds to cancer cell antigens.

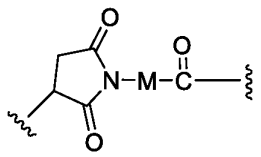
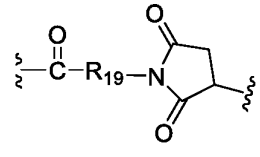
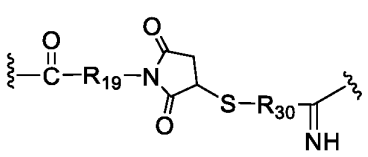
- A further preferred drug conjugate of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab according to the the present invention is one wherein L, $(AA)_w$, $(X)_b$, $(T)_g$ and D are as defined herein and the moiety Ab comprising at least one antigen binding site is an antibody selected from the group consisting of Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Coltuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab, Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Lifastuzumab, Lorvotuzumab, Milatuzumab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Pinatuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab, Sofituzumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, wherein preferably the antibody is selected from Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab,

Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, and yet more preferably Abciximab, Alemtuzumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denosumab, Dinutuximab, Durvalumab, Elotuzumab, Gemtuzumab, Ibritumomab, Inotuzumab, Ipilimumab, Labetuzumab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, Rovalpituzumab, Siltuximab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof. Of these, particularly preferred are Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof; or the antibody is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologicallly active portion thereof.

- Particularly preferred drug conjugates of formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n-Ab$ according to the present invention include the following:

(a) a drug conjugate according to the present invention wherein:

L is selected from the group consisting of:


, 
and 

wherein:

the wavy lines indicate the point of covalent attachments to an Ab (the wavy line to the right) and to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to (D) (the wavy line to the left);

R_{19} is selected from $-C_1-C_{12}$ alkylene-, $-O-(C_1-C_{12}$ alkylene), $-C_6-C_{12}$ arylene in one or more rings which may optionally be substituted with one or more substituents R_x, $-C_1-C_{12}$ alkylene-

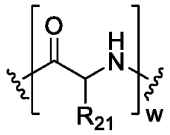
C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{12} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_5-C_{12} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{12} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{12} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$- and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a -C_1-C_6 alkylene- group;

M is selected from the group consisting of -C_1-C_6 alkylene-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)- and phenylene which may optionally be substituted with one or more substituents R_x;

r is an integer ranging from 1-6;

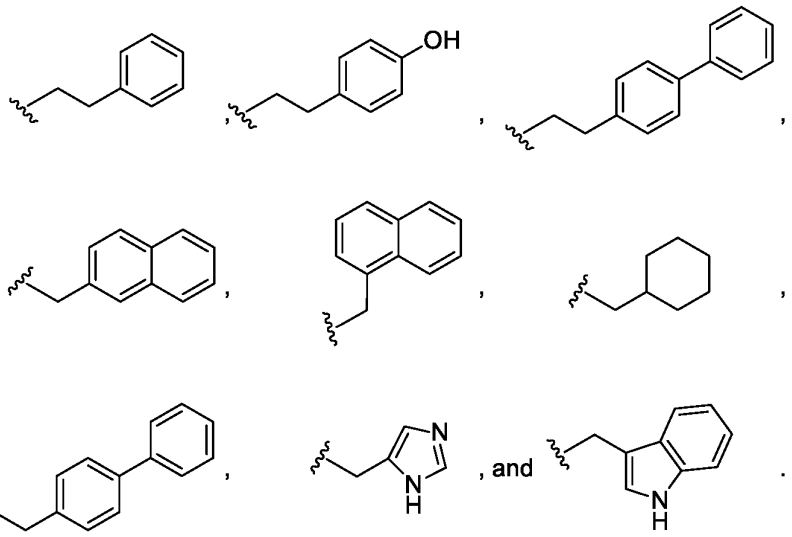
$(AA)_w$ is of formula (II):



(II)

wherein the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

R_{21} is, at each occurrence, selected from the group consisting of hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, -CH_2OH, -$CH(OH)CH_3$, -$CH_2CH_2SCH_3$, -CH_2CONH_2, -CH_2COOH, -$CH_2CH_2CONH_2$, -CH_2CH_2COOH, -$(CH_2)_3NHC(=NH)NH_2$, -$(CH_2)_3NH_2$, -$(CH_2)_3NHCOCH_3$, -$(CH_2)_3NHCHO$, -$(CH_2)_4NHC(=NH)NH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_4NHCOCH_3$, -$(CH_2)_4NHCHO$, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NHCONH_2$, -$CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



w is an integer ranging from 0 to 12;

wherein X is an extending group selected from

where D is conjugated via an amine group (for example where Z is -NH-): -COO-(C_1-C_6 alkylene)NH-, -COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-, -COO-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-, -$COCH_2$NH-$COCH_2$-NH-, -$COCH_2$-NH-, -COO-(C_1-C_6 alkylene)S-, -COO-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-; or

where D is conjugated via an hydroxy group (for example where Z is -O-): -CONH-(C_1-C_6 alkylene)NH-, -COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-, -CONH-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-, -$COCH_2$NH-$COCH_2$-NH-, -$COCH_2$NH-, -CONH-(C_1-C_6 alkylene)S-, and -CONH-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-;

b is 0 or 1, preferably 1;

wherein T is an extending group selected from -CO-(C_1-C_6 alkylene)-NH-, -CO-(C_1-C_6 alkylene)-[O-(C_2-C_6 alkylene)]$_j$-NH-, and -COO-(C_1-C_6 alkylene)-[O-(C_2-C_6 alkylene)]$_j$-NH-, where j is an integer from 1 to 25;

g is 0 or 1;

D is a drug moiety of formula I, IA, IB, IC, ID, IE, IF, IG, IH, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof wherein:

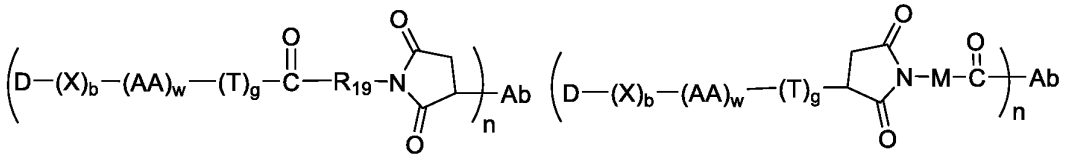
R_2 is $C(=O)R_a$, in compounds of formuma I, IA, IB, IC, ID, IE, IF, Ia, IAa, IBa, ICa, IDa, IEa, IFa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, (IH), (IHa) or (IHb); R_2 is acetyl in compounds of formula IG, IGa or IGb, wherein R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl, wherein the optional substituents are one or more substituents R_x;

R_3 is hydrogen or a $-OR_b$ group in compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, IGb, (IH), (IHa) or (IHb); R_3 is hydrogen in compounds of formula IA, IAa, or IAb; R_3 is a $-OR_b$ group in compounds of formula IB, IBa, or IBb, wherein R_b is a substituted or unsubstituted C_1-C_6 alkyl group, wherein the optional substituents are one or more substituents R_x;

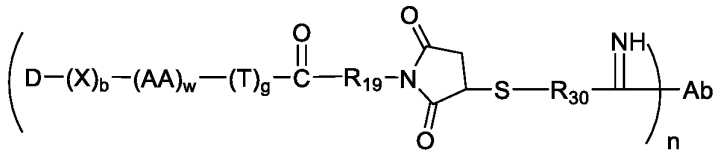
the moiety Ab comprising at least one antigen binding site is an antibody or an antigen-binding fragment thereof and it is selected from the group consisting of a human antibody, an antigen-binding fragment of a human antibody, a humanized antibody, an antigen-binding fragment of a humanized antibody, a chimeric antibody, an antigen-binding fragment of a chimeric antibody, a glycosylated antibody and a glycosylated antigen binding fragment; and

n is the ratio of the group $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]$ to the moiety Ab comprising at least one antigen binding site and is in the range from 1 to 12.

(b) a drug conjugate according to the present invention selected from the formulas (IV), (V) and (VI):



(IV) (V)



(VI)

wherein:

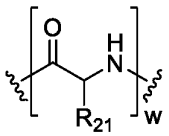
R_{19} is selected from $-C_1$-C_8 alkylene-, $-O-(C_1$-C_8 alkylene), $-C_1$-C_8 alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x and $-C_6$-C_{12} arylene-C_1-C_8 alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x,

wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a $–C_2$-C_4 alkylene- group;

M is selected from the group consisting of -C_1-C_3 alkylene- and -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-;

$(AA)_w$ is of formula (II)



(II) ,

wherein:

the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

R_{21} is, at each occurrence, selected from the group consisting of hydrogen, methyl, isopropyl, sec-butyl, benzyl, indolylmethyl, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_3NHC(=NH)NH_2$ and -$(CH_2)_4NHC(=NH)NH_2$;

w is an integer from 0 to 6;

X is an extending group selected from the group consisting of

where D is conjugated via an amine group (for example where Z is -NH-): -COO-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2NH$-$COCH_2$-NH-, -COO-(C_2-C_4 alkylene)S-, and -COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-; or

where D is conjugated via an hydroxy group (for example where Z is -O-): -CONH-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms,

halogen atoms, nitro groups and cyano groups, -CONH-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2NH$-$COCH_2$-NH-, -CONH-(C_2-C_4 alkylene)S-, and -CONH-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-;

b is 0 or 1, preferably 1;

wherein T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 10;

g is 0 or 1;

D is a drug moiety of formula I, IA, IB, IC, ID, IE, IF, IG, IH, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, IGb, (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof wherein:

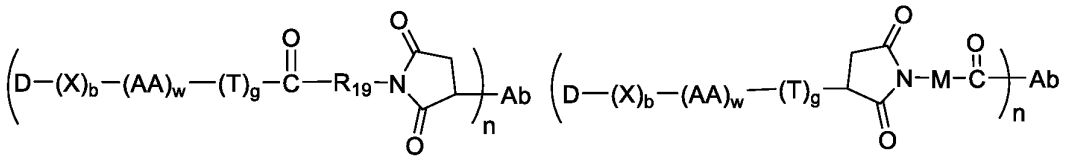
R_2 is acetyl;

R_3 is hydrogen or methoxy in compounds of formula I, IC, ID, IE, IF, IG, Ia, ICa, IDa, IEa, IFa, IGa, Ib, ICb, IDb, IEb, IFb, IGb, (IH), (IHa) or (IHb); R_3 is hydrogen in compounds of formula IA, IAa, or IAb; R_3 is a methoxy group in compounds of formula IB, IBa, or IBb, preferably R_3 is hydrogen;

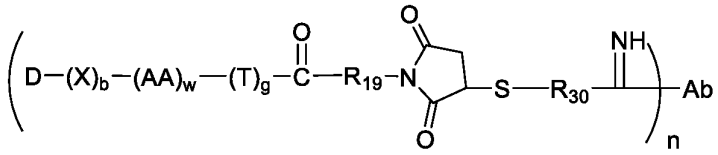
the moiety Ab comprising at least one antigen binding site is an antibody or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment is a monoclonal antibody which immunospecifically binds to cancer cell antigens, viral antigens, antigens of cells that produce autoimmune antibodies associated with autoimmune disease, microbial antigens, and preferably a monoclonal antibody which immunospecifically binds to cancer cell antigens; and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formulas (IV), (V) or (VI) to the moiety Ab comprising at least one antigen binding site and is in the range from 3 to 8.

(c) a drug conjugate according to the present invention selected from the formulas (IV), (V) and (VI):



(IV) (V)



(VI)

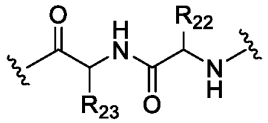
wherein:

R_{19} is selected from -C_1-C_6 alkylene-, -phenylene-C_1-C_6 alkylene- wherein the phenylene group may optionally be substituted with one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, wherein each of the above alkylene substituents whether alone or attached to another moiety in the carbon chain may optionally be substituted by one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, aryl groups having from 6 to 12 carbon atoms, halogen atoms, nitro groups and cyano groups, and preferably R_{19} is a C_1-C_6 alkylene group;

R_{30} is a –C_2-C_4 alkylene- group;

M is -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

wherein the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

R_{22} is selected from methyl, benzyl, isopropyl, *sec*-butyl and indolylmethyl;

R_{23} is selected from methyl, -$(CH_2)_4NH_2$, -$(CH_2)_3NHCONH_2$ and -$(CH_2)_3NHC(=NH)NH_2$;

X is an extending group selected from the group consisting of -COO-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups or cyano groups)-NH-, -$COCH_2$NH-$COCH_2$-NH-,-COO-(C_2-C_4 alkylene)S-, and -COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-;

b is 0 or 1, preferably 1;

wherein T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 5;

g is 0 or 1;

D is a drug moiety of formula I, IA, IC, ID, IE, IG, IH, Ia, IAa, ICa, IDa, IEa, IGa, Ib, IAb, ICb, IDb, IEb, IGb, (IHa) or (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof wherein:

R_1 is CN;

R_2 is acetyl:

R_3 is hydrogen;

Y is -NH- or -O-;

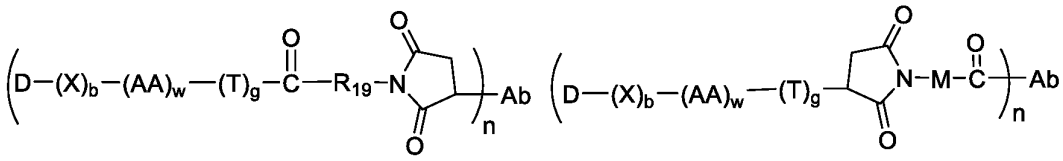
Z is -NH-;

- the moiety Ab comprising at least one antigen binding site is a monoclonal antibody selected from the group consisting of Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Coltuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab, Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Lifastuzumab, Lorvotuzumab, Milatuzumab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Pinatuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab,

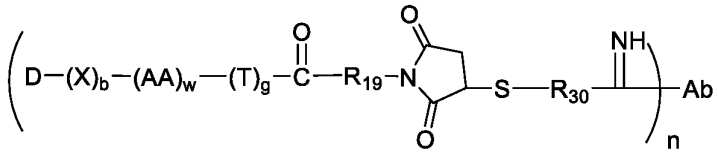
Sofituzumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, wherein preferably the antibody is selected from Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab, Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, and yet more preferably Abciximab, Alemtuzumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denosumab, Dinutuximab, Durvalumab, Elotuzumab, Gemtuzumab, Ibritumomab, Inotuzumab, Ipilimumab, Labetuzumab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, Rovalpituzumab, Siltuximab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof. Of these, particularly preferred are Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof; or the antibody is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologicallly active portion thereof;and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formulas (IV), (V) or (VI) to the moiety Ab comprising at least one antigen binding site and is in the range from 3 to 5.

(d) A drug conjugate according to the present invention selected from the formulas (IV), (V) and (VI):



(IV) (V)



(VI)

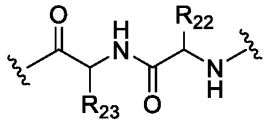
wherein:

R_{19} is -C_2-C_6 alkylene-;

R_{30} is a -C_2-C_4 alkylene- ;

M is -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

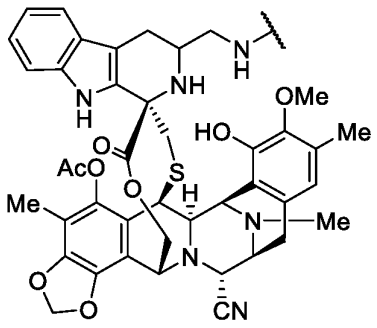
wherein R_{22} is isopropyl, R_{23} is selected from methyl and -$(CH_2)_3NHCONH_2$, wherein the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

X is an extending group selected from the group consisting of -COO-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2$NH-$COCH_2$-NH-, -COO-(C_2-C_4 alkylene)S-, and -COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-;

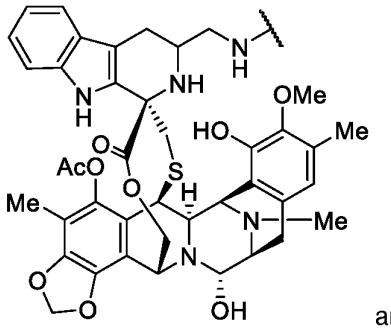
b is 0 or 1, preferably 1; wherein T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 5;

g is 0 or 1;

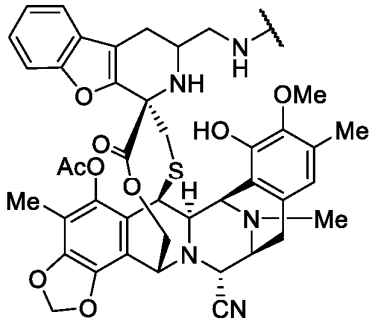
D is a drug moiety selected from:



,



and

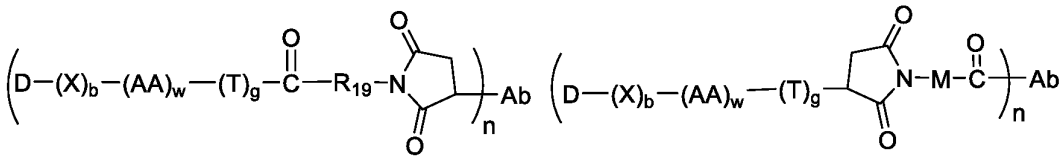


or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to (L);

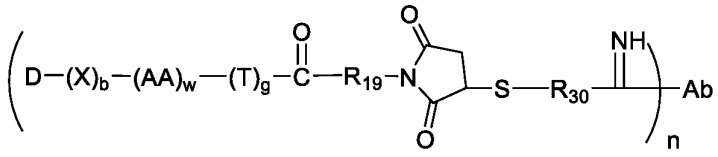
the moiety Ab comprising at least one antigen binding site is selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologicallly active portion thereof; and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formulas (IV), (V) or (VI) to the moiety Ab comprising at least one antigen binding site and is in the range from 3 to 5.

(e) A drug conjugate according to the present invention selected from the formulas (IV), (V), and (VI):



(IV) (V)



(VI)

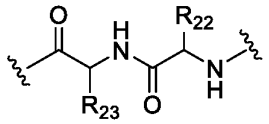
wherein:

R_{19} is -C_2-C_6 alkylene-;

R_{30} is -C_2-C_4 alkylene-;

M is -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

wherein R_{22} is isopropyl, R_{23} is selected from methyl and -$(CH_2)_3NHCONH_2$, and the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

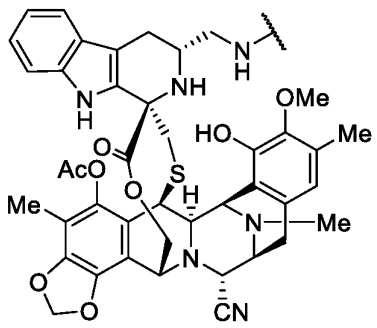
X is an extending group selected from the group consisting of -COO-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2NH$-$COCH_2$-NH-, -COO-(C_2-C_4 alkylene)S-, and -COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-;

b is 0 or 1, preferably 1;

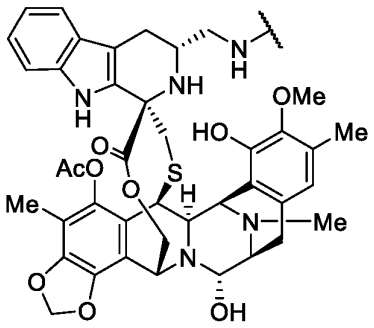
wherein T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 5;

g is 0 or 1;

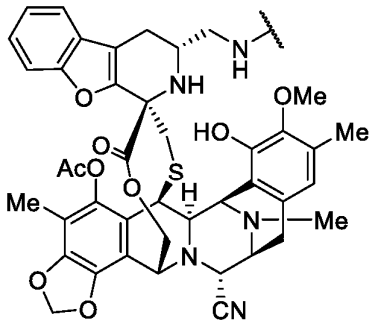
D is a drug moiety selected from:



,



and

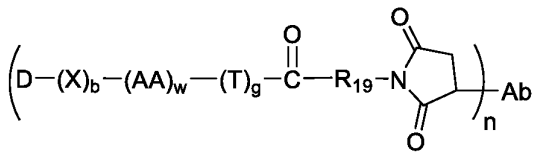


or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof ; wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to (L);

the moiety Ab comprising at least one antigen binding site is selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologicallly active portion thereof; and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formulas (IV), (V) or (VI) to the moiety comprising at least one antigen binding site and is in the range from 3 to 5.

(f) A drug conjugate according to the present invention of formula (IV):

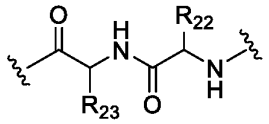


(IV)

wherein:

R_{19} is C_2-C_5 alkylene-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

wherein R_{22} is isopropyl, R_{23} is selected from methyl and $-(CH_2)_3NHCONH_2$, and the wavy lines indicate the point of covalent attachments to $(X)_b$ (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right); and

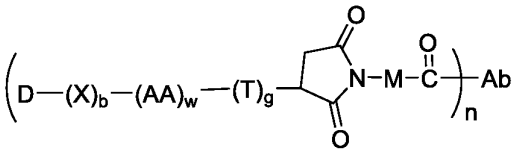
X is a $-COOCH_2$-phenylene-NH group;

b is 1;

T is an extending group of formula -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_4$-NH-;

g is 0 or 1;

or of formula (V)



(V)

wherein M is -methyl-cyclohexylene-;

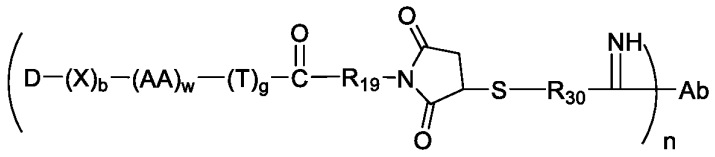
b is 1;

w is 0;

X is an extending group selected from $-(CH_2)_3S$- and $-(CH_2)_3NHCO(CH_2)_2S$-

g is 0;

or of formula (VI)

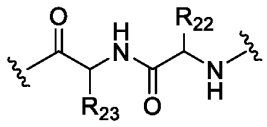


(VI)

wherein R_{19} is -C_2-C_5 alkylene-;

R_{30} is -C_3 alkylene-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

wherein R_{22} is isopropyl, R_{23} is selected from methyl and -$(CH_2)_3NHCONH_2$, and the wavy lines indicate the point of covalent attachments to $(X)_b$ (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right); and

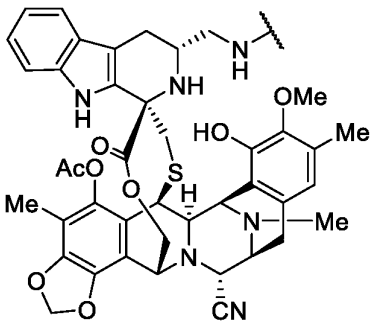
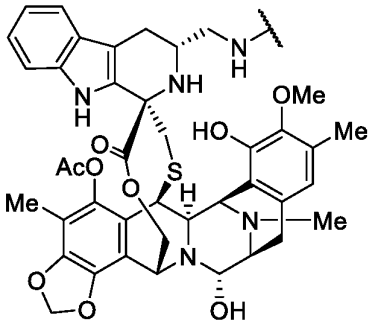
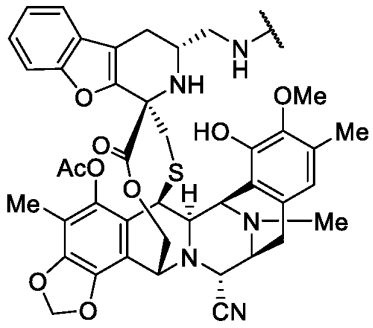
X is a -$COOCH_2$-phenylene-NH group;

b is 1;

T is an extending group of formula -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_4$-NH-;

g is 0 or 1;

D is a drug moiety selected from:

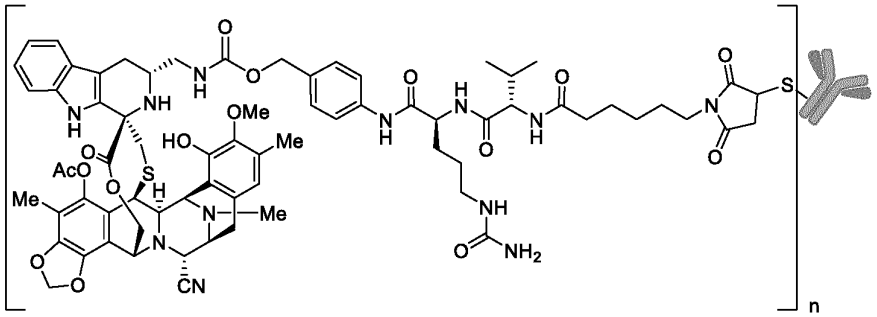


and


or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy line indicates the point of covalent attachment to $(X)_b$;

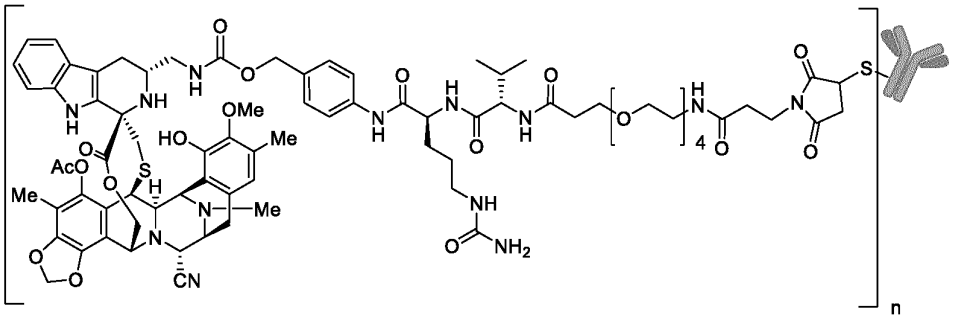
the moiety Ab comprising at least one antigen binding site is Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologicallly active portion thereof; and

n is the ratio of the group $[D-(X)_b-(AA)_w-(T)_g-(L)-]$ wherein L is as defined in formula (IV) to the moiety Ab comprising at least one antigen binding site and is in the range from 3 to 5, and preferably 4.

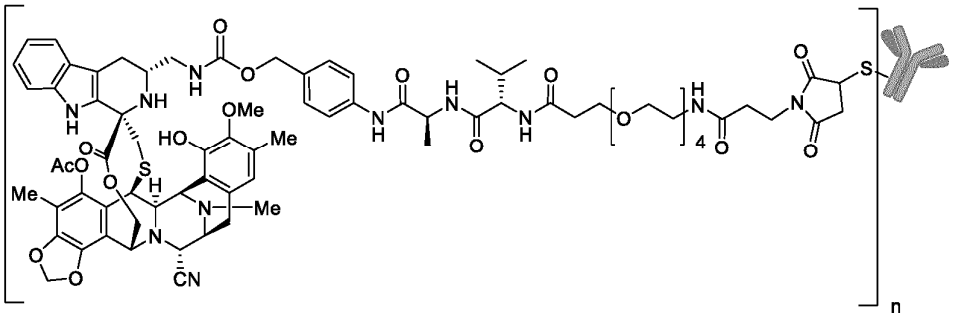
g) an antibody drug conjugate according according to the present invention, selected from the group consisting of:



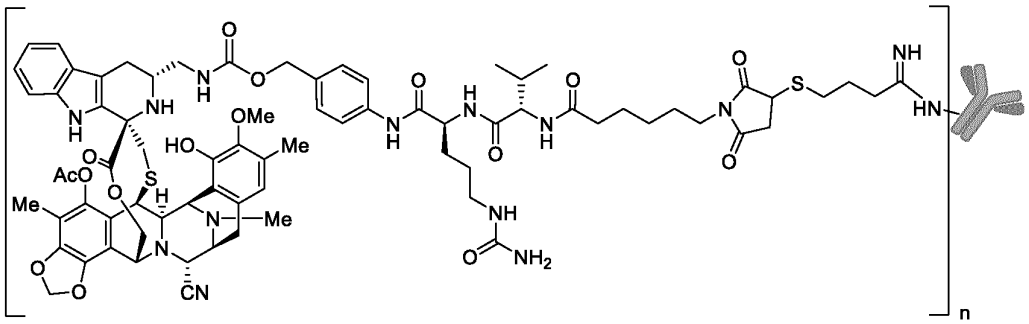
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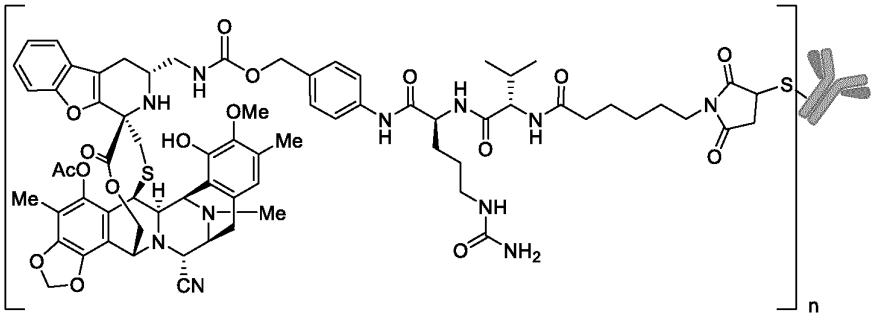
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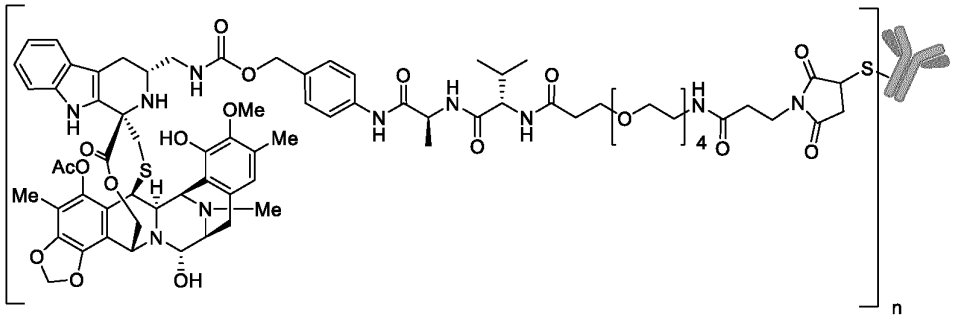
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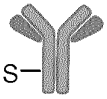


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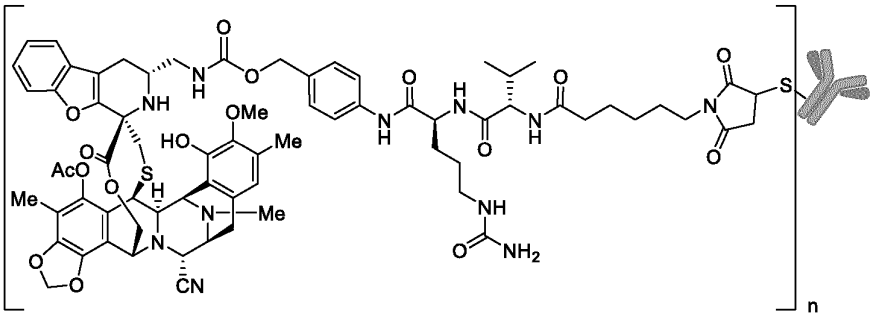


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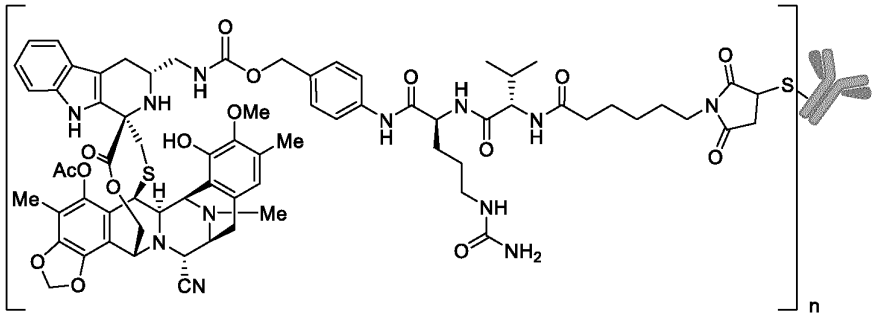
wherein n is from 2 to 6, more preferably 3, 4, or 5 and each  and  is independently selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof.

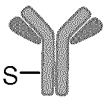
In an embodiment, antibody drug conjugates according to the present invention excludes:

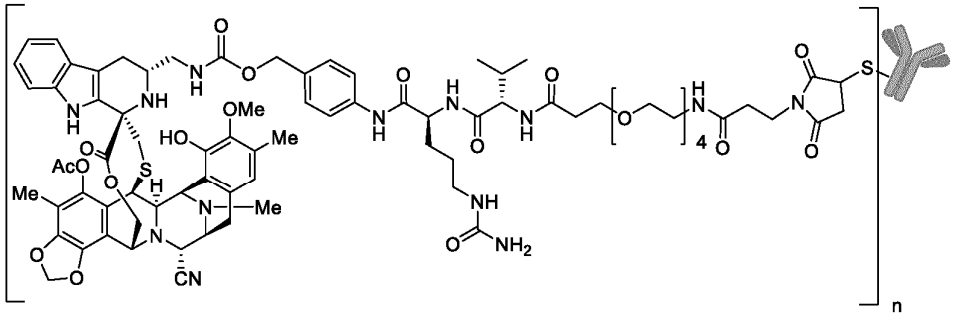


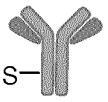
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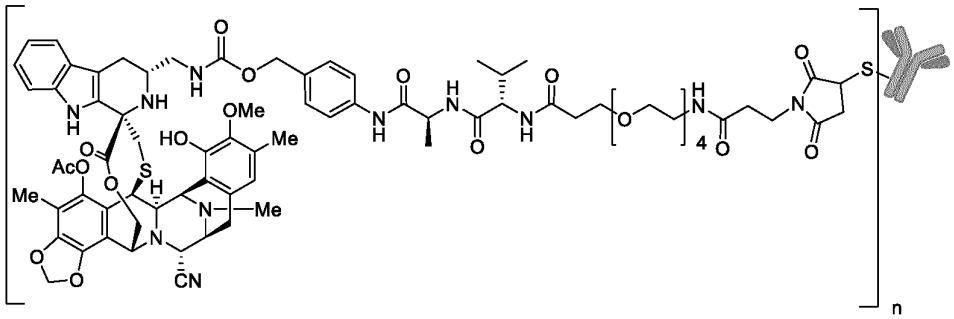
More preferably the antibody drug conjugate is selected from the group consisting of:

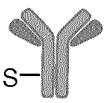


wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is selected from Trastuzumab and an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, more preferably is Trastuzumab or an antigen binding fragment or an immunologically active portion thereof,

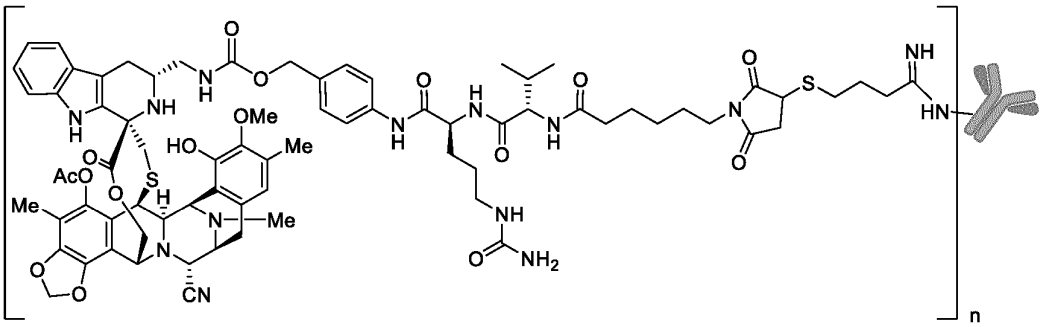


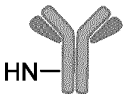
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

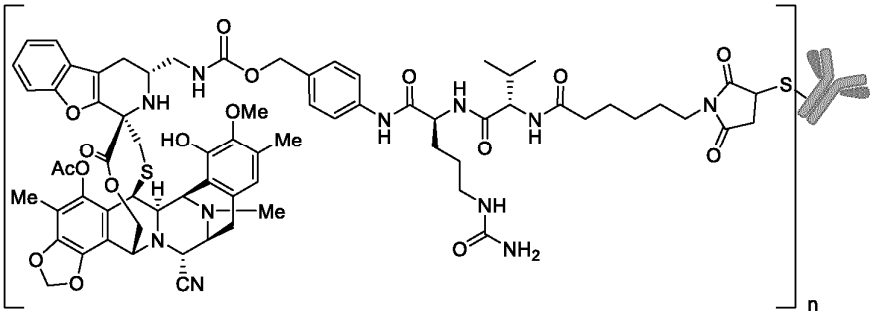


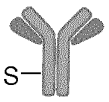
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is selected from Trastuzumab and an anti-CD13 antibody or an antigen-binding fragment or an

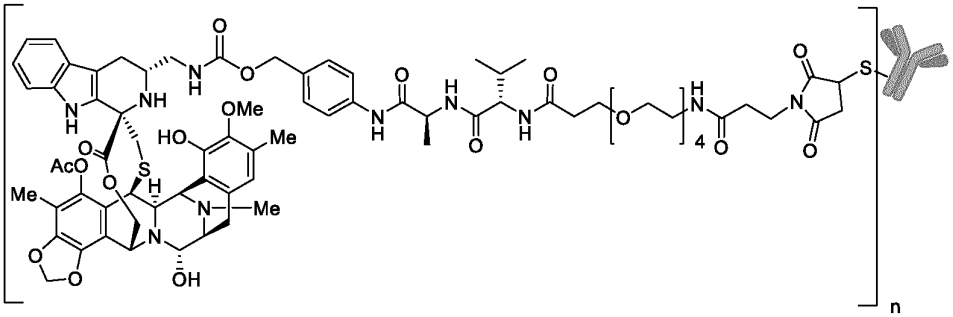
immunologically active portion thereof, more preferably is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

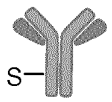


wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

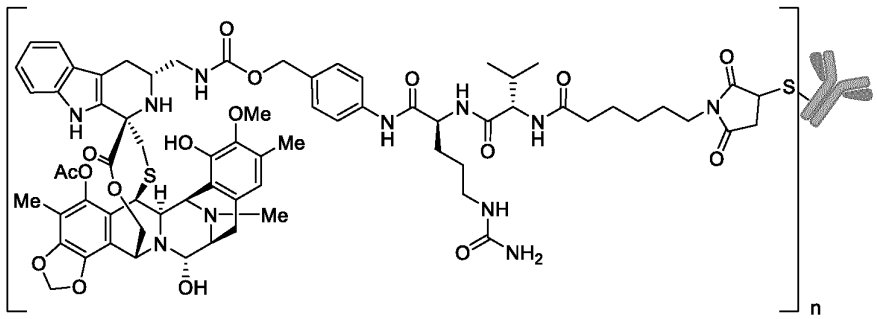


wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof, and

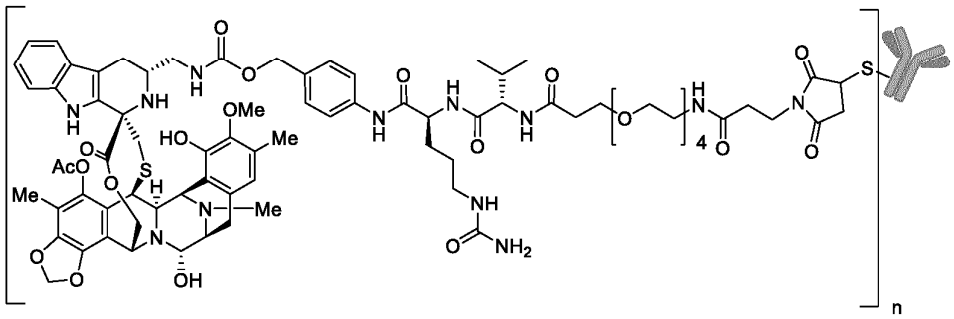


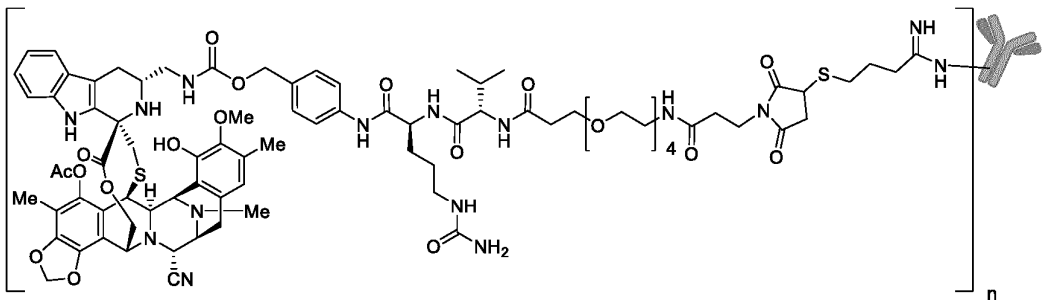
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is selected from Trastuzumab and an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, more preferably is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof.

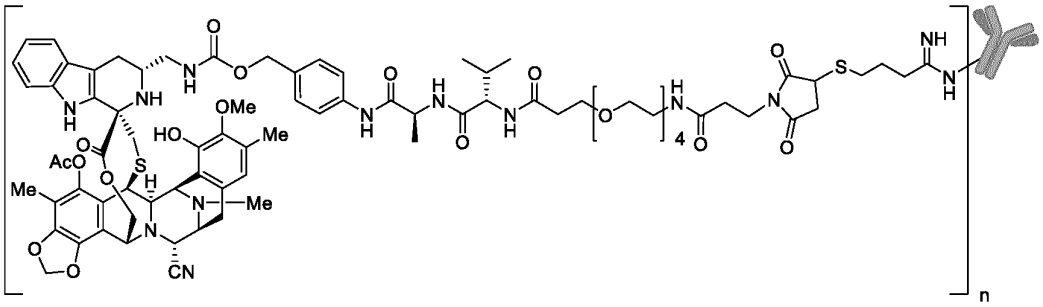
h) an antibody drug conjugate according to the present invention, selected from the group consisting of:

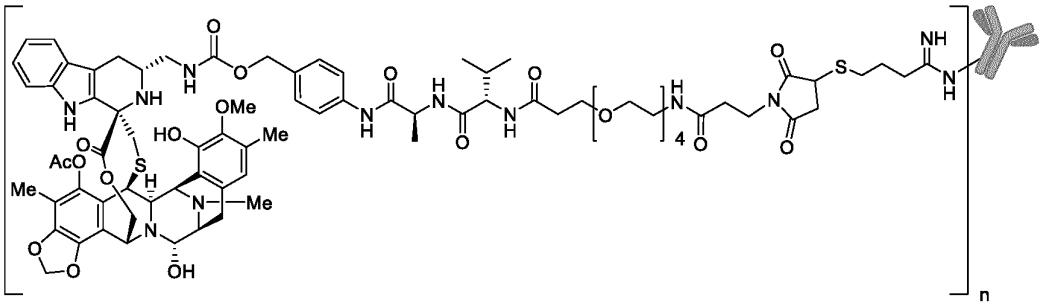


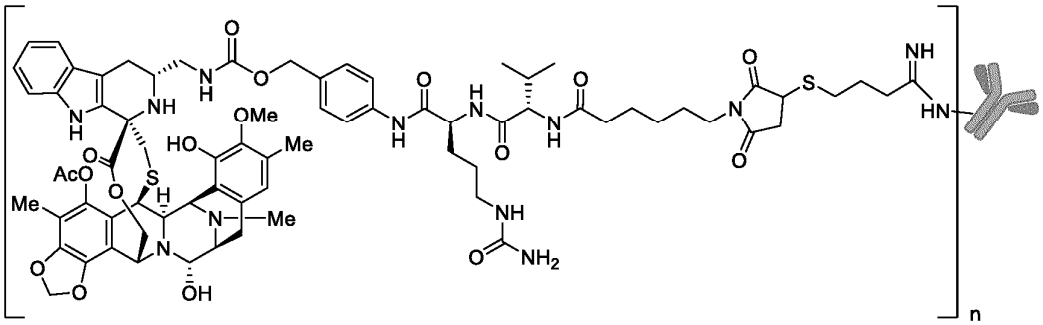
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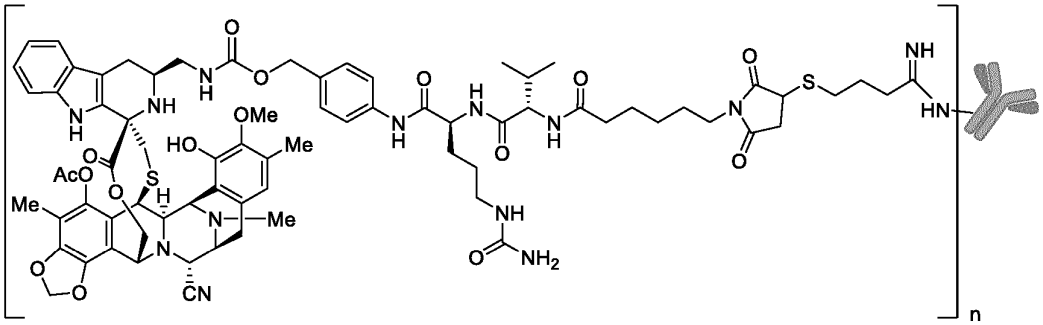


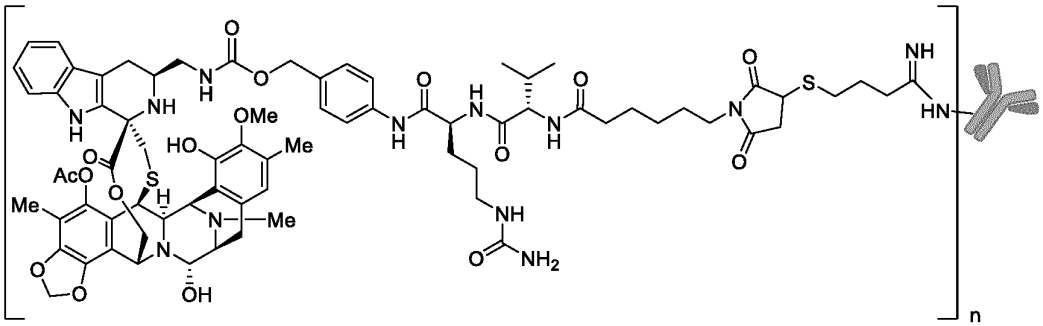


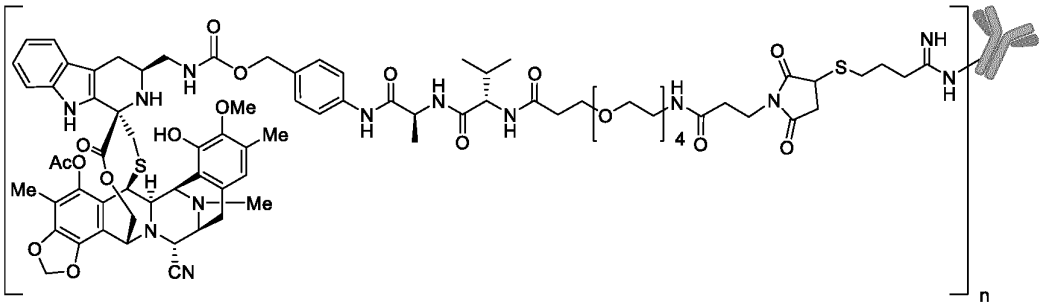


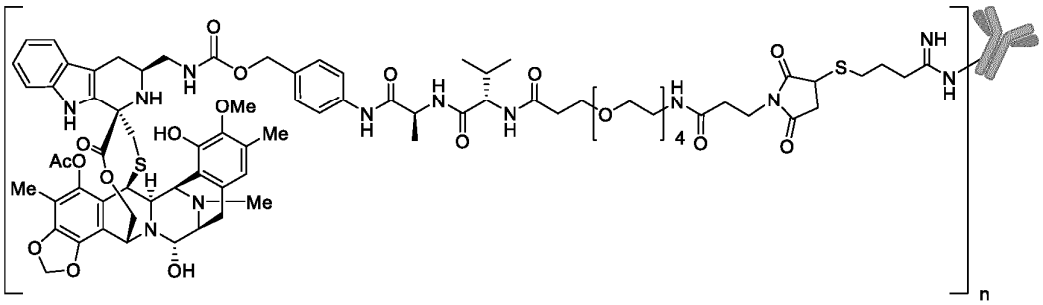


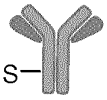
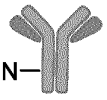


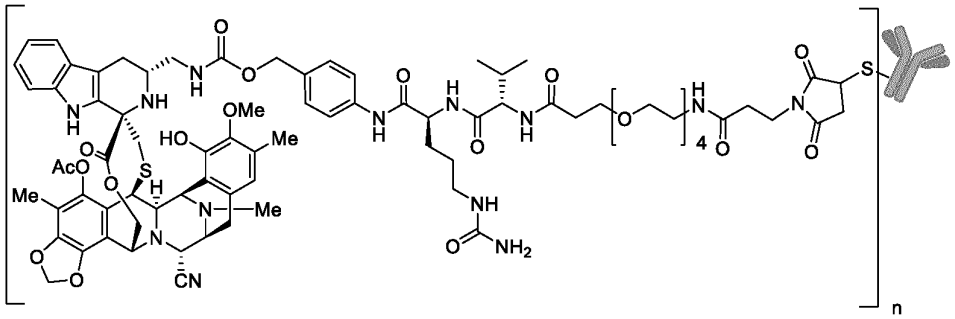


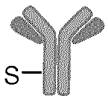




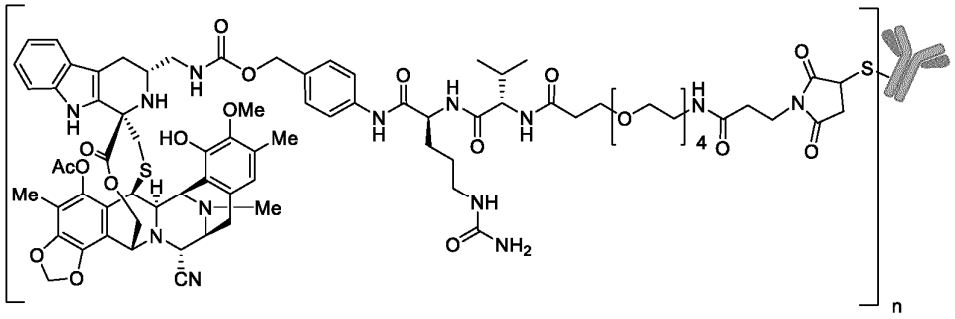


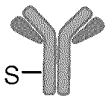
wherein n is from 2 to 6, more preferably 3, 4, or 5 and each  and  is independently selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof; or

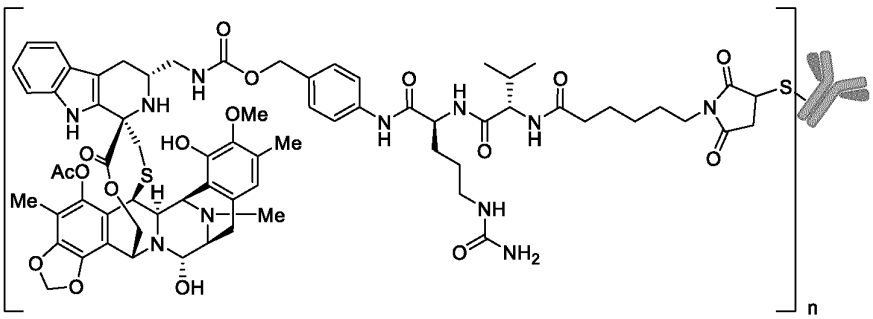


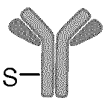
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof.

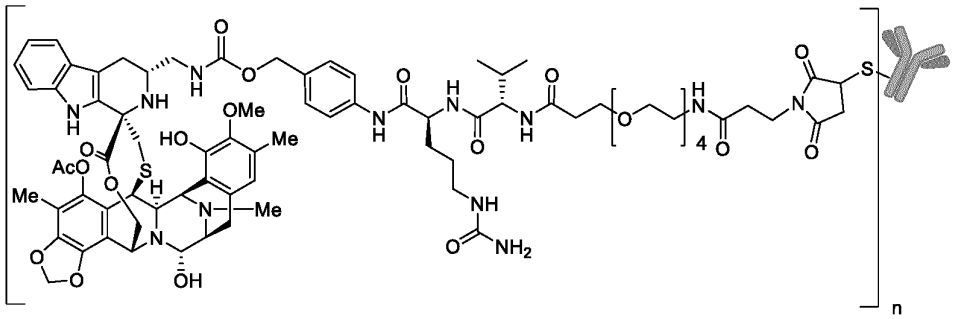
More preferably the antibody drug conjugate is selected from the group consisting of:

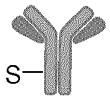


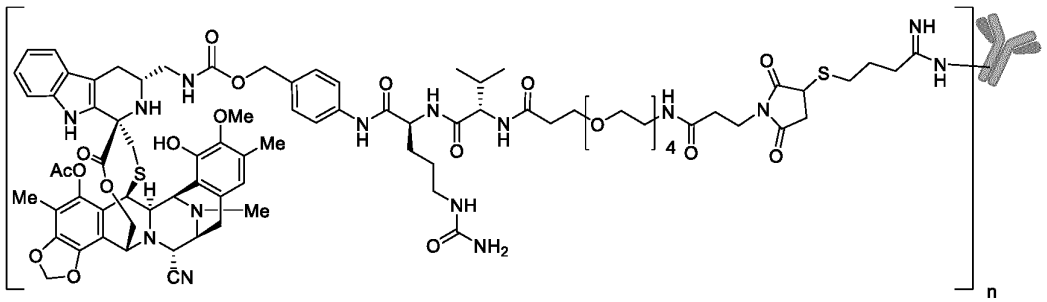
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof.

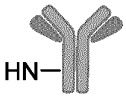


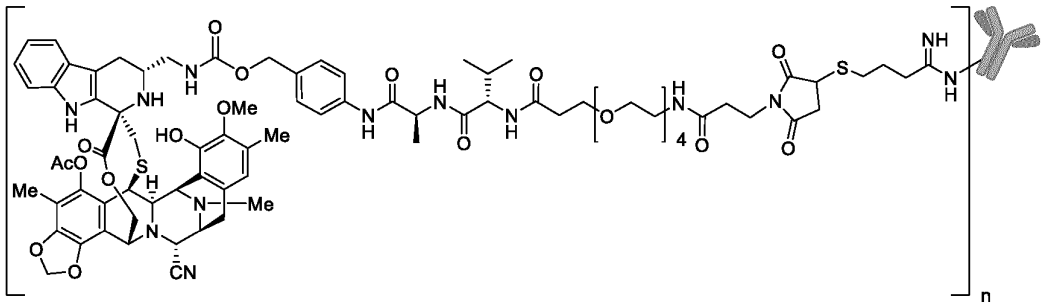
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

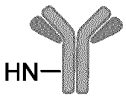


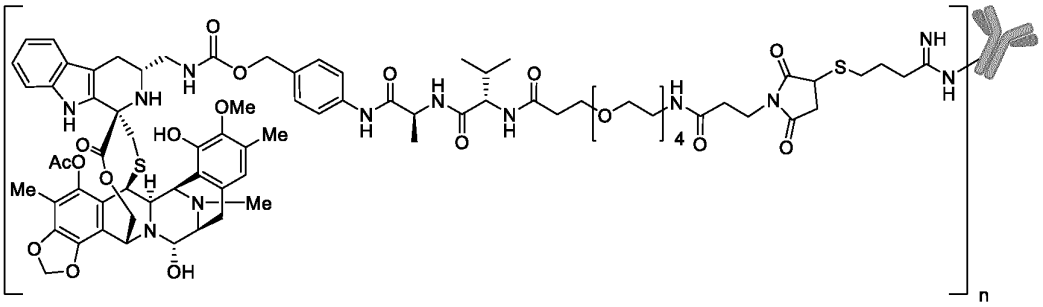
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

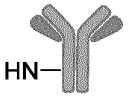


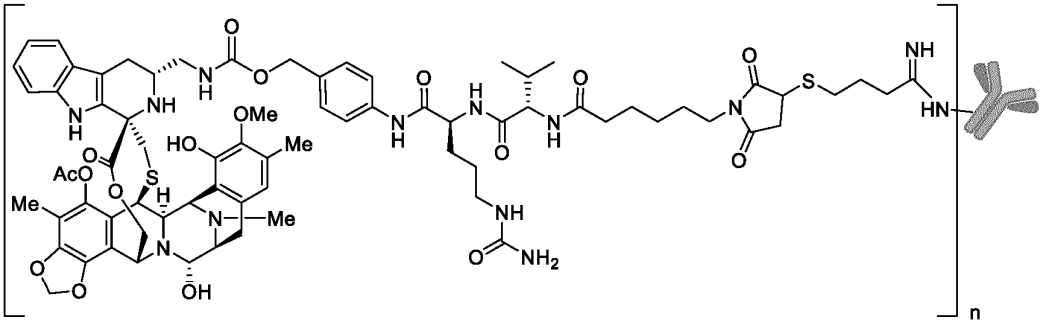
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

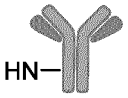


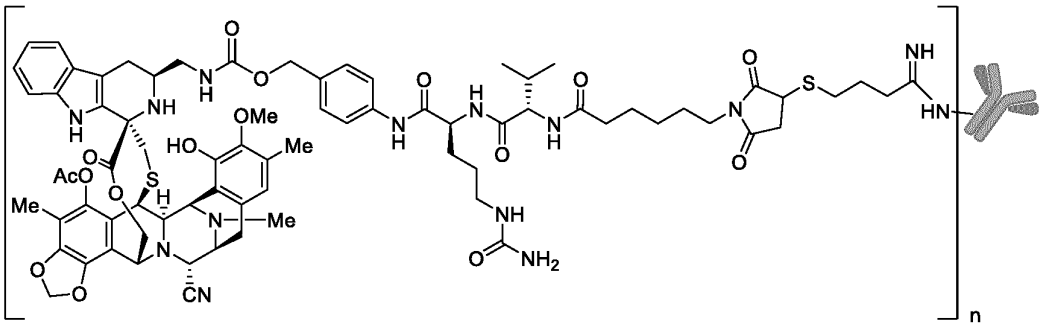
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,



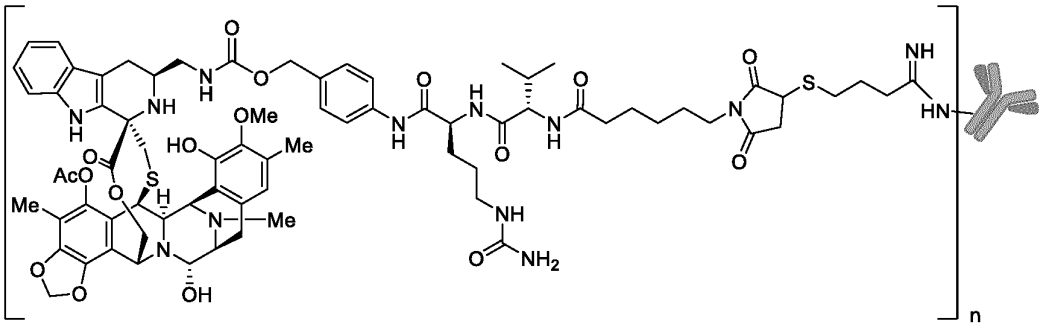
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,



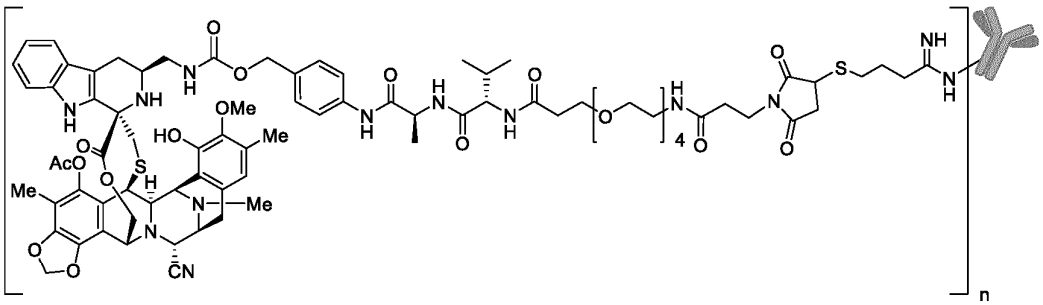
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

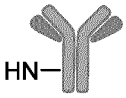


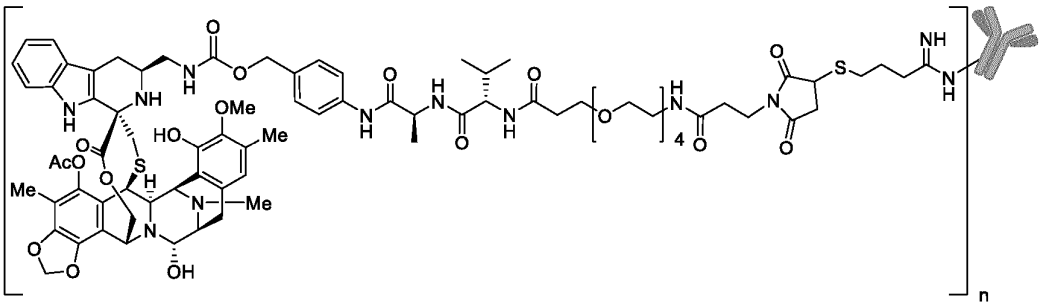
wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

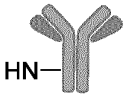


wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,



wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,



wherein n is form 2 to 6, more preferably 3, 4, or 5 and and  is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof,

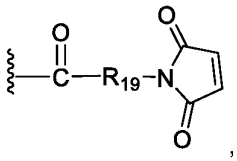
Particularly preferably, the antibody drug conjugates according to the present invention should be in isolated or purified form.

Preferred compounds of formula $D-(X)_b-(AA)_w-(T)_g-L_1$ or of formula $D-(X)_b-(AA)_w-(T)_g-H$ according to the present invention include:

- a compound of formula $D-(X)_b-(AA)_w-(T)_g-L_1$ or of formula $D-(X)_b-(AA)_w-(T)_g-H$ wherein each of D, X, AA, T, L_1, b, g and w are as defined herein in the present invention; but further wherein if the compound is a compound of formula $D-(X)_b-(AA)_w-(T)_g-H$ then $b+w+g\neq 0$.

- a compound of formula $D-(X)_b-(AA)_w-(T)_g-L_1$ or of formula $D-(X)_b-(AA)_w-(T)_g-H$ according to the present invention wherein:

L_1 is a linker of formula:



,

wherein:

the wavy line indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D;

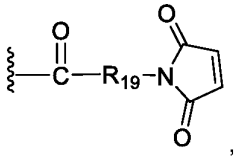
R_{19} is selected from $-C_1-C_{12}$ alkylene-, $-O-(C_1-C_{12}$ alkylene), $-C_6-C_{12}$ arylene in one or more rings which may optionally be substituted with one or more substituents R_x, $-C_1-C_{12}$ alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, $-C_6-C_{12}$ arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, $-C_5-C_{12}$ heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, $-C_1-C_{12}$ alkylene-(C_5-C_{12} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{12} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, $-(OCH_2CH_2)_r-$ and $-CH_2-(OCH_2CH_2)_r-$, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

r is an integer ranging from 1-6; and

each of D, R_x, X, AA, T, b, g and w is as defined in the present invention; but wherein if the compound is a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H then b+w+g≠0.

- a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 or of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H according to the present invention wherein:

L_1 is linker of formula:



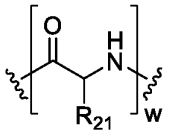
,

wherein:

the wavy line indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D;

R_{19} is selected from -C_1-C_8 alkylene-, -O-(C_1-C_8 alkylene), -C_1-C_8 alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, and -C_6-C_{12} arylene-C_1-C_8 alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

$(AA)_w$ is of formula (II):



(II)

,

wherein the wavy lines indicate the point of covalent attachments to $(X)_b$, if any, or to D (the wavy line to the left) and to $(T)_g$ if any, or L_1 or to a hydrogen atom (the wavy line to the right);

wherein R_{21} is selected, at each occurrence, from the group consisting of hydrogen, methyl, isopropyl, *sec*-butyl, benzyl, indolylmethyl, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_3NHC(=NH)NH_2$ and -$(CH_2)_4NHC$-(=NH)NH_2, and w is an integer from 0 to 6;

X is an extending group selected from the group consisting of

where D is conjugated via an amine group (for example where Z is -NH-): -COO-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH, wherein said phenylene group may optionally be

substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2NH$-$COCH_2$-NH-, -COO-(C_2-C_4 alkylene)S-, and -COO-(C_2-C_4 alkylene)-NHCO(C_1-C_3 alkylene)S- or

where D is conjugated via an hydroxy group (for example where Z is -O-): -CONH-(C_2-C_4 alkylene)NH-, -COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, -CONH-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-, -$COCH_2NH$-$COCH_2$-NH-, -CONH-(C_2-C_4 alkylene)S-, and -CONH-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-;

T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-; -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH- and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 10;

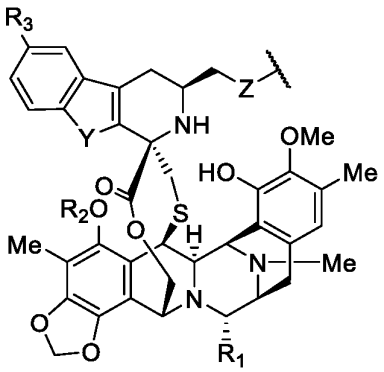
b is 0 or 1;

g is 0 or 1;

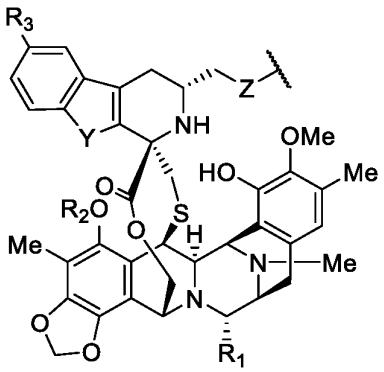
wherein if the compound is a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H then b+w+g≠0; and

D is a drug moiety of formula I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, and IGb; and is covalently attached via a hydroxy or amine group; or

is a drug moiety of formula (IHa) or a formula (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof wherein:



(IHa)



(IHb)

wherein the wavy lines of (IHa) and (IHb) indicate the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to L_1;

R_1 is -OH or -CN;

R_2 is a -C(=O)R_a group, wherein R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl, wherein the optional substituents are one or more substituents R_x;

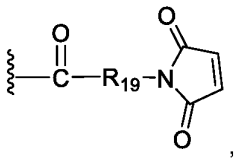
R_3 is hydrogen or a -OR_b group wherein R_b is a substituted or unsubstituted C_1-C_6 alkyl group, wherein the optional substituents are one or more substituents R_x.

Y is –NH- or -O-; and

Z is –NH- or -O-.

- a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 or of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H according to the present invention wherein:

L_1 is a group of formula:



,

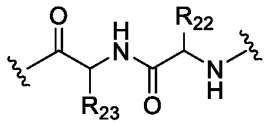
wherein:

the wavy line indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D;

R_{19} is selected from -C_1-C_6 alkylene-, phenylene-C_1-C_6 alkylene- wherein the phenylene group may optionally be substituted with one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, wherein each of the above

alkylene substituents whether alone or attached to another moiety in the carbon chain may optionally be substituted by one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, aryl groups having from 6 to 12 carbon atoms, halogen atoms, nitro groups and cyano groups, and preferably R_{19} is a C_1-C_6 alkylene group;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

wherein the wavy lines indicate the point of covalent attachments to X (the wavy line to the left) and to $(T)_g$ if any, or L_1 or to a hydrogen atom (the wavy line to the right);

R_{22} is selected from methyl, benzyl, isopropyl, *sec*-butyl and indolylmethyl;

R_{23} is selected from methyl, $-(CH_2)_4NH_2$, $-(CH_2)_3NHCONH_2$ and $-(CH_2)_3NHC(=NH)NH_2$;

X is an extending group selected from

where D is conjugated via an amine group (for example where Z is -NH-): $-COO-CH_2$-phenylene-NH-, $-COO(CH_2)_3NHCOO-CH_2$-phenylene-NH, $-COO-(CH_2)_3)NH-$, $-COO(CH_2)_3-S-$, and $-COO-(CH_2)_3NHCO-(CH_2)_2S-$, or

where D is conjugated via an hydroxy group (for example where Z is -O-): $-COO-CH_2$-phenylene-NH-, $-CONH(CH_2)_3NHCOOCH_2$-phenylene-NH-, $-CONH(CH_2)_3NH-$, $-CONH(CH_2)_3-S-$, and $-CONH(CH_2)_3NHCO(CH_2)_2S-$.

wherein T is an extending group selected from -CO-(C_1-C_4 alkylene)-NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, and -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, where j is an integer from 1 to 5;

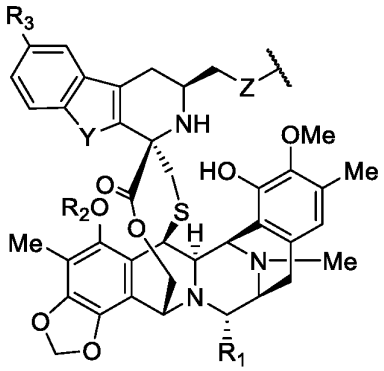
b is 0 or 1;

g is 0 or 1;

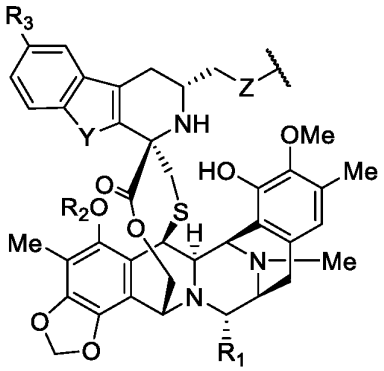
wherein if the compound is a compound of formula $D-(X)_b-(AA)_w-(T)_g-H$ then $b+w+g\neq0$; and

D is a drug moiety of formula I, IA, IB, IC, ID, IE, IF, IG, Ia, IAa, IBa, ICa, IDa, IEa, IFa, IGa, Ib, IAb, IBb, ICb, IDb, IEb, IFb, and IGb; and is covalently attached via a hydroxy or amine group; or

is a drug moiety of formula (IHa) or a formula (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof:



(IHa)



(IHb)

wherein the wavy lines of (IHa) and (IHb) indicate the point of covalent attachment;

R_1 is -CN or -OH;

R_2 is acetyl;

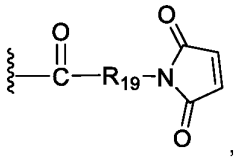
R_3 is hydrogen or methoxy, preferably hydrogen;

Y is -NH- or -O-. and

Z is -NH- or -O-.

- a compound of formula $D-(X)_b-(AA)_w-(T)_g-L_1$ or of formula $D-(X)_b-(AA)_w-(T)_g-H$ according to the present invention wherein:

L_1 is a linker of formula:



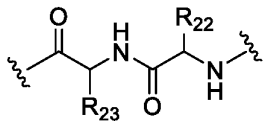
,

wherein:

the wavy line indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any, or to D;

R_{19} is $-C_2-C_6$ alkylene-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

R_{22} is isopropyl, R_{23} is selected from methyl and $-(CH_2)_3NHCONH_2$, wherein the wavy lines indicate the point of covalent attachments to X (the wavy line to the left) and to $(T)_g$ if any, or L_1 or to a hydrogen atom (the wavy line to the right);

X is an extending group selected from $-COO-CH_2$-phenylene-NH-, $-COO(CH_2)_3NHCOO-CH_2$-phenylene-NH, $-COO-(CH_2)_3)NH-$, $-COO(CH_2)_3-S-$, and $-COO-(CH_2)_3NHCO-(CH_2)_2S-$;

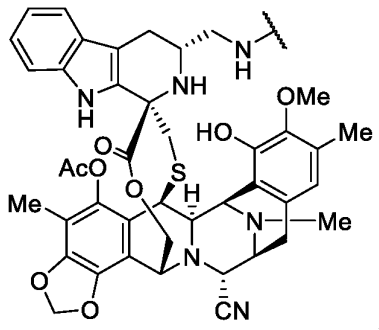
wherein T is an extending group selected from $-CO-(C_1-C_4$ alkylene)-NH-, $-CO-(C_1-C_4$ alkylene)-$[O-(C_2-C_4$ alkylene)$]_j$-NH-, and $-COO-(C_1-C_4$ alkylene)-$[O-(C_2-C_4$ alkylene)$]_j$-NH-, where j is an integer from 1 to 5;

b is 0 or 1;

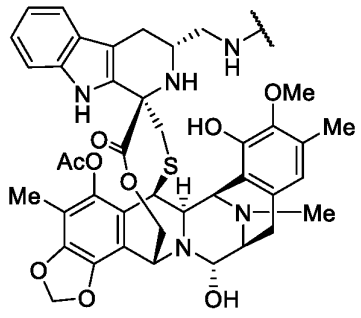
g is 0 or 1;

wherein if the compound is a compound of formula $D-(X)_b-(AA)_w-(T)_g-H$ then $b+w+g\neq0$; and

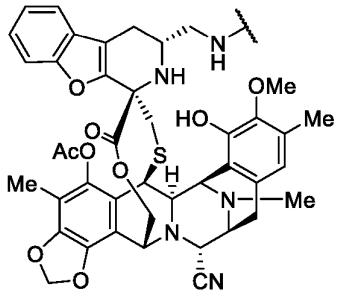
D is a drug moiety selected from:



,



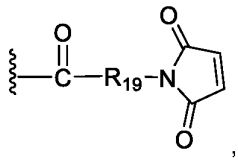
and



or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy line indicates the point of covalent attachment.

- a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 or of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H according to the present invention wherein:

L_1 is a group of formula:



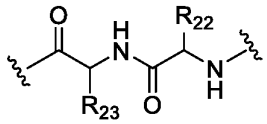
,

wherein:

the wavy line indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$, if any or to D;

R_{19} is a -C_2-C_5 alkylene-;

w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

,

wherein R_{22} is isopropyl, R_{23} is selected from methyl and -$(CH_2)_3NHCONH_2$, wherein the wavy lines indicate the point of covalent attachments to X (the wavy line to the left) and to $(T)_g$ if any, or L_1 or to a hydrogen atom (the wavy line to the right);

X is a -COO-CH_2-phenylene-NH- group;

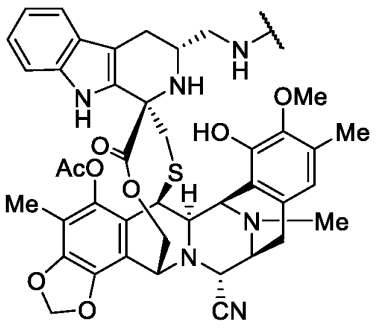
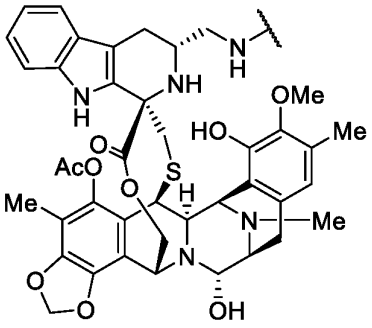
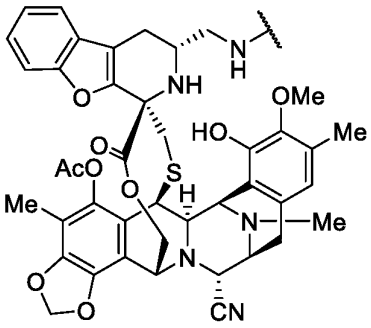
T is a -CO-$(CH_2)_2$-[O-$(CH_2)_2$]$_4$-NH- group;

b is 0 or 1;

g is 0 or 1;

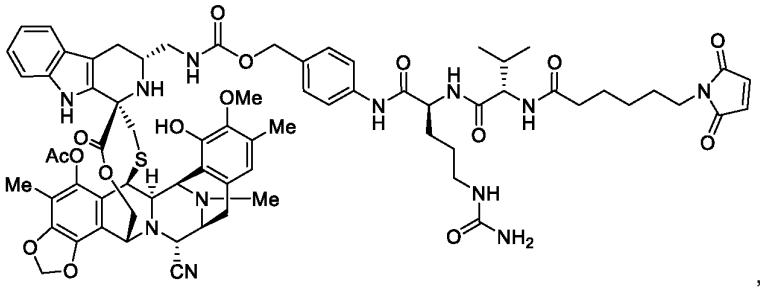
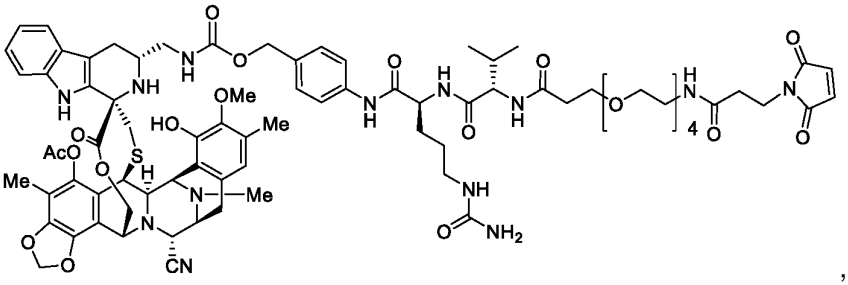
wherein if the compound is a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H then b+w+g≠0; and

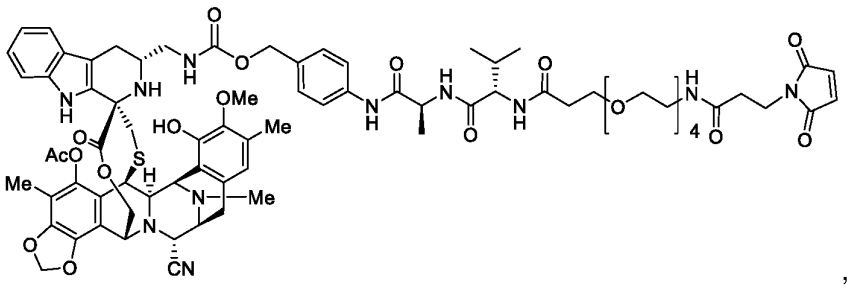
D is a drug moiety selected from:


,

and


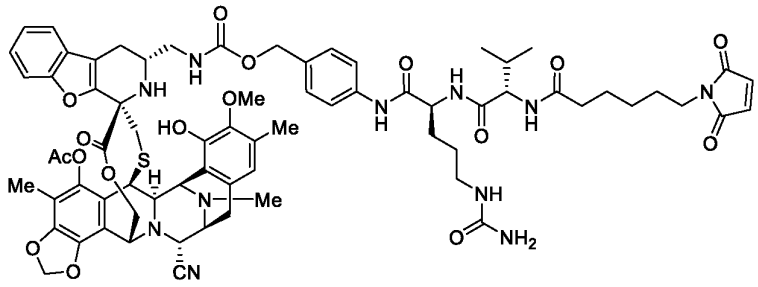
or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof; wherein the wavy line indicates the point of covalent attachment.

- a compound of formula D-X-$(AA)_w$-$(T)_g$-L_1 selected from:

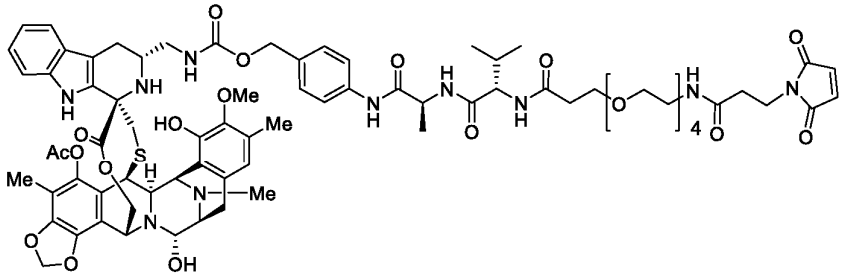

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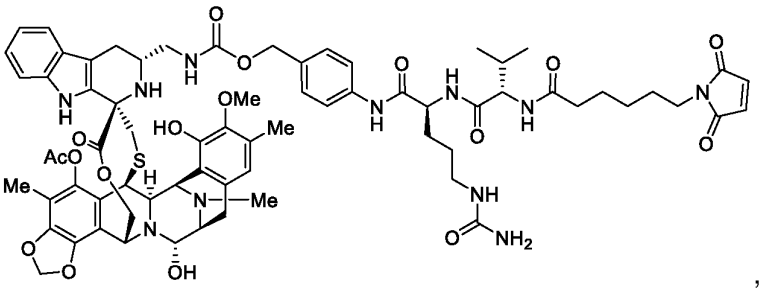


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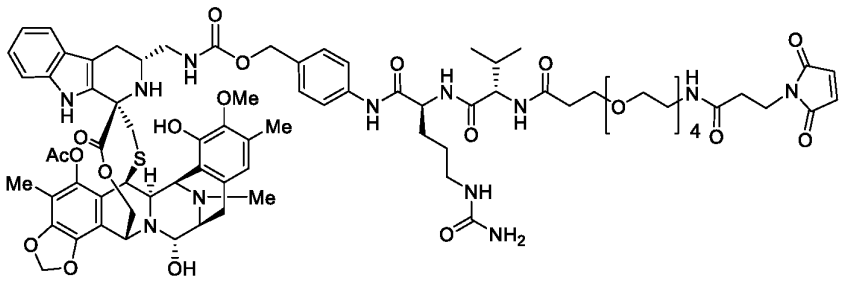


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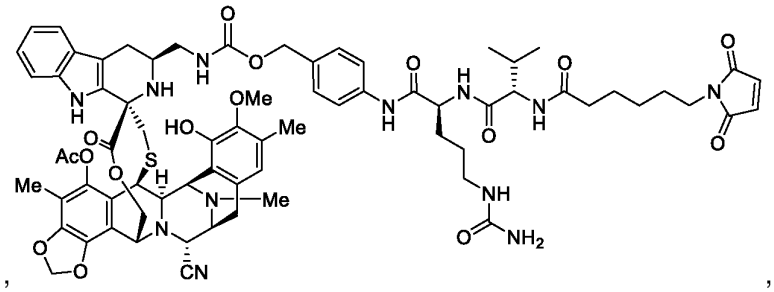
- a compound of formula D-X-$(AA)_w$-$(T)_g$-L_1 selected from:



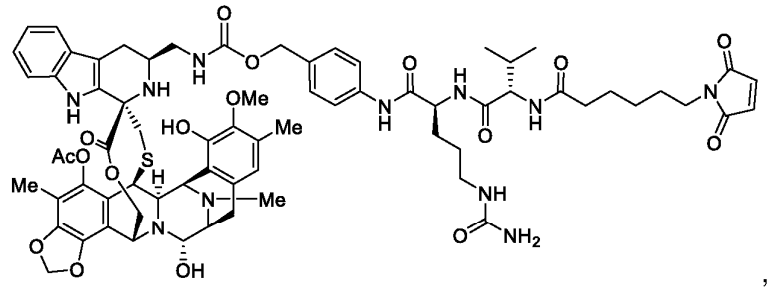
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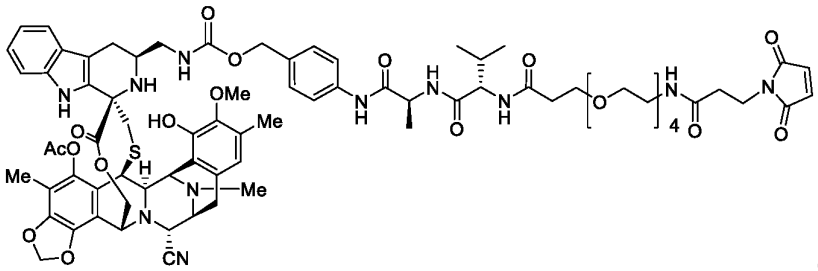
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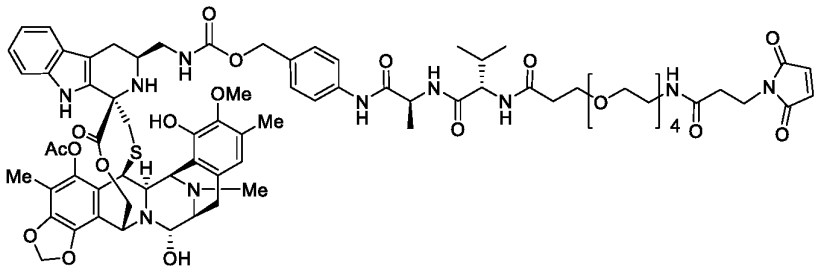
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and



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The term “pharmaceutically acceptable salts, esters, solvates, tautomers or stereoisomers” in the drug conjugates of the present invention refers to any pharmaceutically acceptable salt, ester, solvate, hydrate or stereosiomeric form or any other compound which, upon administration to the patient is capable of providing a compound as described herein,

whether directly or indirectly. However, it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention since those may be useful in the preparation of pharmaceutically acceptable salts. The preparation of salts, prodrugs and derivatives can be carried out by methods known in the art.

For instance, pharmaceutically acceptable salts of compounds provided herein are synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent or in a mixture of the two. Generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol or acetonitrile are preferred. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, trifluoroacetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulphonate and p-toluenesulphonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, *N*,*N*-dialkylenethanolamine, triethanolamine and basic aminoacids salts.

The drug conjugates of the present invention may be in crystalline form either as free compounds or as solvates (e.g. hydrates) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

Any compound that is a prodrug of the drug conjugate of the present invention is within the scope and spirit of the invention. The term “prodrug” is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester derivative. Many suitable prodrugs are well-known to the person in the art and can be found, for example, in Burger “Medicinal Chemistry and Drug Discovery 6th ed. (Donald J. Abraham ed., 2001, Wiley) and “Design and Applications of Prodrugs” (H. Bundgaard ed., 1985, Harwood Academic Publishers), the contents of which are incorporated herein by reference.

In relations to the compounds of the present invention, the pharmacologically acceptable esters are not particularly restricted, and can be selected by a person with an ordinary skill in the art. In the case of said esters, it is preferable that such esters can be cleaved by a biological process such as hydrolysis *in vivo*. The group constituting the said esters (the group shown as R when the esters thereof are expressed as -COOR) can be, for example, a C_1-C_4 alkoxy C_1-C_4 alkyl group such as methoxyethyl, 1-ethoxyethyl, 1-methyl-1-methoxyethyl, 1-(isopropoxy)ethyl, 2-methoxyethyl, 2-ethoxyethyl, 1,1-dimethyl-1-methoxymethyl, ethoxymethyl, propoxymethyl, isopropoxymethyl, butoxymethyl or t-

butoxymethyl; a C_1-C_4 alkoxylated C_1-C_4 alkoxy C_1-C_4 alkyl group such as 2-methoxyethoxymethyl; a C_6-C_{10} aryloxy C_1-C_4 alkyl group such as phenoxymethyl; a halogenated C_1-C_4 alkoxy C_1-C_4 alkyl group such as 2,2,2-trichloroethoxymethyl or bis(2-chloroethoxy)methyl; a C_1-C_4 alkoxycarbonyl C_1-C_4 alkyl group such as methoxycarbonylmethyl; a cyano C_1-C_4 alkyl group such as cyanomethyl or 2-cyanoethyl; a C_1-C_4 alkylthiomethyl group such as methylthiomethyl or ethylthiomethyl; a C_6-C_{10} arylthiomethyl group such as phenylthiomethyl or naphthylthiomethyl; a C_1-C_4 alkylsulfonyl C_1-C_4 lower alkyl group, which may be optionally substituted with a halogen atom(s) such as 2-methanesulfonylethyl or 2-trifluoromethanesulfonylethyl; a C_6-C_{10} arylsulfonyl C_1-C_4 alkyl group such as 2-benzenesulfonylethyl or 2-toluenesulfonylethyl; a C_1-C_7 aliphatic acyloxy C_1-C_4 alkyl group such as formyloxymethyl, acetoxymethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl, valeryloxymethyl, isovaleryloxymethyl, hexanoyloxymethyl, 1-formyloxyethyl, 1-acetoxyethyl, 1-propionyloxyethyl, 1-butyryloxyethyl, 1-pivaloyloxyethyl, 1-valeryloxyethyl, 1-isovaleryloxyethyl, 1-hexanoyloxyethyl, 2-formyloxyethyl, 2-acetoxyethyl, 2-propionyloxyethyl, 2-butyryloxyethyl, 2-pivaloyloxyethyl, 2-valeryloxyethyl, 2-isovaleryloxyethyl, 2-hexanoyloxyethyl, 1-formyloxypropyl, 1-acetoxypropyl, 1-propionyloxypropyl, 1-butyryloxypropyl, 1-pivaloyloxypropyl, 1-valeryloxypropyl, 1-isovaleryloxypropyl, 1-hexanoyloxypropyl, 1-acetoxybutyl, 1-propionyloxybutyl, 1-butyryloxybutyl, 1-pivaloyloxybutyl, 1-acetoxypentyl, 1-propionyloxypentyl, 1-butyryloxypentyl, 1-pivaloyloxypentyl or 1-pivaloyloxyhexyl; a C_5-C_6 cycloalkylcarbonyloxy C_1-C_4 alkyl group such as cyclopentylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl, 1-cyclopentylcarbonyloxyethyl, 1-cyclohexylcarbonyloxyethyl, 1-cyclopentylcarbonyloxypropyl, 1-cyclohexylcarbonyloxypropyl, 1-cyclopentylcarbonyloxybutyl or 1-cyclohexylcarbonyloxybutyl; a C_6-C_{10} arylcarbonyloxy C_1-C_4 alkyl group such as benzoyloxymethyl; a C_1-C_6 alkoxycarbonyloxy C_1-C_4 alkyl group such as methoxycarbonyloxymethyl, 1-(methoxycarbonyloxy)ethyl, 1-(methoxycarbonyloxy)propyl, 1-(methoxycarbonyloxy)butyl, 1-(methoxycarbonyloxy)pentyl, 1-(methoxycarbonyloxy)hexyl, ethoxycarbonyloxymethyl, 1-(ethoxycarbonyloxy)ethyl, 1-(ethoxycarbonyloxy)propyl, 1-(ethoxycarbonyloxy)butyl, 1-(ethoxycarbonyloxy)pentyl, 1-(ethoxycarbonyloxy)hexyl, propoxycarbonyloxymethyl, 1-(propoxycarbonyloxy)ethyl, 1-(propoxycarbonyloxy)propyl, 1-(propoxycarbonyloxy)butyl, isopropoxycarbonyloxymethyl, 1-(isopropoxycarbonyloxy)ethyl, 1-(isopropoxycarbonyloxy)butyl, butoxycarbonyloxymethyl, 1-(butoxycarbonyloxy)ethyl, 1-(butoxycarbonyloxy)propyl, 1-(butoxycarbonyloxy)butyl, isobutoxycarbonyloxymethyl, 1-(isobutoxycarbonyloxy)ethyl, 1-(isobutoxycarbonyloxy)propyl, 1-(isobutoxycarbonyloxy)butyl, t-butoxycarbonyloxymethyl, 1-(t-butoxycarbonyloxy)ethyl, pentyloxycarbonyloxymethyl, 1-(pentyloxycarbonyloxy)ethyl, 1-(pentyloxycarbonyloxy)propyl, hexyloxycarbonyloxymethyl, 1-(hexyloxycarbonyloxy)ethyl or 1-(hexyloxycarbonyloxy)propyl; a C_5-C_6 cycloalkyloxycarbonyloxy C_1-C_4 alkyl group such as cyclopentyloxycarbonyloxymethyl, 1-(cyclopentyloxycarbonyloxy)ethyl, 1-(cyclopentyloxycarbonyloxy)propyl, 1-(cyclopentyloxycarbonyloxy)butyl, cyclohexyloxycarbonyloxymethyl, 1-

(cyclohexyloxycarbonyloxy)ethyl, 1-(cyclohexyloxycarbonyloxy)propyl or 1-(cyclohexyloxycarbonyloxy)butyl; a [5-(C_1-C_4 alkyl)-2-oxo-1,3-dioxolen-4-yl]methyl group such as (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-ethyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-propyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-isopropyl-2-oxo-1,3-dioxolen-4-yl)methyl or (5-butyl-2-oxo-1,3-dioxolen-4-yl)methy; a [5-(phenyl, which may be optionally substituted with a C_1-C_4 alkyl, C_1-C_4 alkoxy or halogen atom(s))-2-oxo-1,3-dioxolen-4-yl]methyl group such as (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl, [5-(4-methylphenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, [5-(4-methoxyphenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, [5-(4-fluorophenyl)-2-oxo-1,3-dioxolen-4-yl]methyl or [5-(4-chlorophenyl)-2-oxo-1,3-dioxolen-4-yl]methyl; or a phthalidyl group, which may be optionally substituted with a C_1-C_4 alkyl or C_1-C_4 alkoxy group(s), such as phthalidyl, dimethylphthalidyl or dimethoxyphthalidyl, and is preferably a pivaloyloxymethyl group, phthalidyl group or (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl group, and more preferably a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl group.

Any compound referred to herein is intended to represent such specific compound as well as certain variations or forms. In particular, compounds referred to herein may have asymmetric centres and therefore exist in different enantiomeric forms. All optical isomers and stereoisomers of the compounds referred to herein, and mixtures thereof, are considered within the scope of the present invention. Thus any given compound referred to herein is intended to represent any one of a racemate, one or more enantiomeric forms, one or more diastereomeric forms, one or more atropisomeric forms, and mixtures thereof. Particularly, the drug conjugates of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)]$_n$-Ab and compounds of formula D-X-$(AA)_w$-$(T)_g$-L_1 or D-X-$(AA)_w$-$(T)_g$-H may include enantiomers depending on their asymmetry or diastereoisomers. Stereoisomerism about the double bond is also possible, therefore in some cases the molecule could exist as (E)-isomer or (Z)-isomer. If the molecule contains several double bonds, each double bond will have its own stereoisomerism, that could be the same or different than the stereoisomerism of the other double bonds of the molecule. The single isomers and mixtures of isomers fall within the scope of the present invention.

Furthermore, compounds referred to herein may exist as geometric isomers (i.e., *cis* and *trans* isomers), as tautomers, or as atropisomers. Specifically, the term tautomer refers to one of two or more structural isomers of a compound that exist in equilibrium and are readily converted from one isomeric form to another. Common tautomeric pairs are amine-imine, amide-imide, keto-enol, lactam-lactim, etc. Additionally, any compound referred to herein is intended to represent hydrates, solvates, and polymorphs, and mixtures thereof when such forms exist in the medium. In addition, compounds referred to herein may exist in isotopically-labelled forms. All geometric isomers, tautomers, atropisomers, hydrates, solvates, polymorphs, and isotopically labelled forms of the compounds referred to herein, and mixtures thereof, are considered within the scope of the present invention.

Protected forms of the compounds disclosed herein are considered within the scope of the present invention. Suitable protecting groups are well known for the skilled person in the art. A general review of protecting groups in organic chemistry is provided by Wuts, PGM and Greene TW in Protecting Groups in Organic Synthesis, 4th Ed. Wiley-Interscience, and by Kocienski PJ in Protecting Groups, 3rd Ed. Georg Thieme Verlag. These references provide sections on protecting groups for OH, amino and SH groups. All these references are incorporated by reference in their entirety.

Within the scope of the present invention an OH protecting group is defined to be the *O*-bonded moiety resulting from the protection of the OH through the formation of a suitable protected OH group. Examples of such protected OH groups include ethers, silyl ethers, esters, sulfonates, sulfenates and sulfinates, carbonates, and carbamates. In the case of ethers the protecting group for the OH can be selected from methyl, methoxymethyl, methylthiomethyl, (phenyldimethylsilyl)methoxymethyl, benzyloxymethyl, *p*-methoxybenzyloxymethyl, [(3,4-dimethoxybenzyl)oxy]methyl, *p*-nitrobenzyloxymethyl, *o*-nitrobenzyloxymethyl, [(*R*)-1-(2-nitrophenyl)ethoxy]methyl, (4-methoxyphenoxy)methyl, guaiacolmethyl, [(*p*-phenylphenyl)oxy]methyl, *t*-butoxymethyl, 4-pentenyloxymethyl, siloxymethyl, 2-methoxyethoxymethyl, 2-cyanoethoxymethyl, bis(2-chloroethoxy)methyl, 2,2,2-trichloroethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, menthoxymethyl, *O*-bis(2-acetoxy-ethoxy)methyl, tetrahydropyranyl, fluorous tetrahydropyranyl, 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl, 4-methoxy-tetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl *S,S*-dioxide, 1-[(2-chloro-4-methyl)-phenyl]-4-methoxypiperidin-4-yl, 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl, 1-(4-chlorophenyl)-4-methoxypiperidin-4-yl, 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3*a*,4,5,6,7,7*a*-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-hydroxyethyl, 2-bromoethyl, 1-[2-(trimethylsilyl)ethoxy]ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 1-methyl-1-phenoxyethyl, 2,2,2-trichloroethyl, 1,1-dianisyl-2,2,2-trichloroethyl, 1,1,1,3,3,3-hexafluoro-2-phenylisopropyl, 1-(2-cyanoethoxy)ethyl, 2-trimethylsilylethyl, 2-(benzylthio)ethyl, 2-(phenylselenyl)ethyl, *t*-butyl, cyclohexyl, 1-methyl-1'-cyclopropylmethyl, allyl, prenyl, cinnamyl, 2-phenallyl, propargyl, *p*-chlorophenyl, *p*-methoxyphenyl, *p*-nitrophenyl, 2,4-dinitrophenyl, 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, 2,6-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, pentadienylnitrobenzyl, pentadienylnitropiperonyl, halobenzyl, 2,6-dichlorobenzyl, 2,4-dichlorobenzyl, 2,6-difluorobenzyl, *p*-cyanobenzyl, fluorous benzyl, 4-fluorousalkoxybenzyl, trimethylsilylxylyl, *p*-phenylbenzyl, 2-phenyl-2-propyl, *p*-acylaminobenzyl, *p*-azidobenzyl, 4-azido-3-chlorobenzyl, 2-trifluoromethylbenzyl, 4-trifluoromethylbenzyl, *p*-(methylsulfinyl)benzyl, *p*-siletanylbenzyl, 4-acetoxybenzyl, 4-(2-trimethylsilyl)ethoxymethoxybenzyl, 2-naphthylmethyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxide, 2-quinolinylmethyl, 6-methoxy-2-(4-methylphenyl)-4-quinolinemethyl, 1-pyrenylmethyl, diphenylmethyl, 4-methoxydiphenylmethyl, 4-phenyldiphenylmethyl, *p,p*'-

dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, tris(4-*t*-butylphenyl)methyl, α-naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenyl-methyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxy)phenyldiphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 4,4'-dimethoxy-3''-[*N*-(imidazolylmethyl)]trityl, 4,4'-dimethoxy-3''-[*N*-(imidazolylethyl)carbamoyl]trityl, bis(4-methoxyphenyl)-1'-pyrenylmethyl, 4-(17-tetrabenzo[*a*,*c*,*g*,*i*]fluorenylmethyl)-4,4''-dimethoxytrityl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-phenylthioxanthyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, 4,5-bis(ethoxycarbonyl)-[1,3]-dioxolan-2-yl, benzisothiazolyl *S*,*S*-dioxide. In the case of silyl ethers the protecting group for the OH can be selected from trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylhexylsilyl, 2-norbornyldimethylsilyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl, tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-*t*-butylmethylsilyl, bis(*t*-butyl)-1-pyrenylmethoxysilyl, tris(trimethylsilyl)silyl, (2-hydroxystyryl)dimethylsilyl, (2-hydroxystyryl)diisopropylsilyl, *t*-butylmethoxyphenylsilyl, *t*-butoxydiphenylsilyl, 1,1,3,3-tetraisopropyl-3-[2-(triphenylmethoxy) ethoxy]disiloxane-1-yl, and fluorous silyl. In the case of esters the protecting group for the OH together with the oxygen atom of the unprotected OH to which it is attached form an ester that can be selected from formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trichloroacetamidate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, phenylacetate, diphenylacetate, 3-phenylpropionate, bisfluorous chain type propanoyl, 4-pentenoate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, 5[3-bis(4-methoxyphenyl)hydroxymethylphenoxy]levulinate, pivaloate, 1-adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate, 4-bromobenzoate, 2,5-difluorobenzoate, *p*-nitrobenzoate, picolinate, nicotinate, 2-(azidomethyl)benzoate, 4-azidobutyrate, (2-azidomethyl)phenylacetate, 2-{[(tritylthio)oxy]methyl}benzoate, 2-{[(4-methoxytritylthio)oxy]methyl}benzoate, 2-{[methyl(tritylthio)amino]methyl}benzoate, 2-{{[(4-methoxytrityl)thio]methylamino}methyl}benzoate, 2-(allyloxy)phenylacetate, 2-(prenyloxymethyl)benzoate, 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoate, 6-(levulinyloxymethyl)-3-methoxy-4-nitrobenzoate, 4-benzyloxybutyrate, 4-trialkylsilyloxybutyrate, 4-acetoxy-2,2-dimethylbutyrate, 2,2-dimethyl-4-pentenoate, 2-iodobenzoate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2-(chloroacetoxymethyl)benzoate, 2-[(2-chloroacetoxy)ethyl]benzoate, 2-[2-(benzyloxy)ethyl]benzoate, 2-[2-(4-methoxybenzyloxy)ethyl]benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate, *o*-(methoxycarbonyl)benzoate, α-naphthoate, nitrate, alkyl *N*,*N*,*N*',*N*'-tetramethylphosphorodiamidate, and 2-chlorobenzoate. In the case of sulfonates, sulfenates and sulfinates the protecting group for the OH together with the oxygen atom of the

unprotected OH to which it is attached form a sulfonate, sulfenate or sulfinates that can be selected from sulfate, allylsulfonate, methanesulfonate, benzylsulfonate, tosylate, 2-[(4-nitrophenyl)ethyl]sulfonate, 2-trifluoromethylbenzenesulfonate, 4-monomethoxytritylsulfenate, alkyl 2,4-dinitrophenylsulfenate, 2,2,5,5-tetramethylpyrrolidin-3-one-1-sulfinate, and dimethylphosphinothioyl. In the case of carbonates the protecting group for the OH together with the oxygen atom of the unprotected OH to which it is attached form a carbonate that can be selected from methyl carbonate, methoxymethyl carbonate, 9-fluorenylmethyl carbonate, ethyl carbonate, bromoethyl carbonate, 2-(methylthiomethoxy)ethyl carbonate, 2,2,2-trichloroethyl carbonate, 1,1-dimethyl-2,2,2-trichloroethyl carbonate, 2-(trimethylsilyl)ethyl carbonate, 2-[dimethyl(2-naphthylmethyl)silyl]ethyl carbonate, 2-(phenylsulfonyl)ethyl carbonate, 2-(triphenylphosphonio)ethyl carbonate, *cis*-[4-[[(methoxytrityl)sulfenyl]oxy]tetrahydrofuran-3-yl]oxy carbonate, isobutyl carbonate, *t*-butyl carbonate, vinyl carbonate, allyl carbonate, cinnamyl carbonate, propargyl carbonate, *p*-chlorophenyl carbonate, *p*-nitrophenyl carbonate, 4-ethoxy-1-naphthyl carbonate, 6-bromo-7-hydroxycoumarin-4-ylmethyl carbonate, benzyl carbonate, *o*-nitrobenzyl carbonate, *p*-nitrobenzyl carbonate, *p*-methoxybenzyl carbonate, 3,4-dimethoxybenzyl carbonate, anthraquinon-2-ylmethyl carbonate, 2-dansylethyl carbonate, 2-(4-nitrophenyl)ethyl carbonate, 2-(2,4-dinitrophenyl)ethyl carbonate, 2-(2-nitrophenyl)propyl carbonate, 2-(3,4-methylenedioxy-6-nitrophenyl)propyl carbonate, 2-cyano-1-phenylethyl carbonate, 2-(2-pyridyl)amino-1-phenylethyl carbonate, 2-[*N*-methyl-*N*-(2-pyridyl)]amino-1-phenylethyl carbonate, phenacyl carbonate, 3',5'-dimethoxybenzoin carbonate, methyl dithiocarbonate, and *S*-benzyl thiocarbonate. And in the case of carbamates the protecting group for OH together with the oxygen atom of the unprotected OH to which it is attached forms a carbamate that can be selected from dimethyl thiocarbamate, *N*-phenyl carbamate, and *N*-methyl-*N*-(*o*-nitrophenyl) carbamate.

Within the scope of the present invention an amino protecting group is defined to be the *N*-bonded moiety resulting from the protection of the amino group through the formation of a suitable protected amino group. Examples of protected amino groups include carbamates, ureas, amides, heterocyclic systems, *N*-alkyl amines, *N*-alkenyl amines, *N*-alkynyl amines, *N*-aryl amines, imines, enamines, N-metal derivatives, N-N derivatives, N-P derivatives, N-Si derivatives, and N-S derivatives. In the case of carbamates the protecting group for the amino group together with the amino group to which it is attached form a carbamate that can be selected from methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate, 2,6-di-*t*-butyl-9-fluorenylmethyl carbamate, 2,7-bis(trimethylsilyl)fluorenylmethyl carbamate, 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 17-tetrabenzo[*a,c,g,i*]fluorenylmethyl carbamate, 2-chloro-3-indenylmethyl carbamate, benz[*f*]inden-3-ylmethyl carbamate, 1,1-dioxobenzo[*b*]-thiophene-2-ylmethyl carbamate, 2-methylsulfonyl-3-phenyl-1-prop-2-enyl carbamate, 2,7-di-*t*-butyl-[9,(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate, 2,2,2-trichloroethyl carbamate, 2-trimethylsilylethyl carbamate, (2-phenyl-2-trimethylsilyl)ethyl carbamate, 2-phenylethyl carbamate, 2-chloroethyl

carbamate, 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate, 1,1-dimethyl-2,2,2-trichloroethyl carbamate, 2-(2'-pyridyl)ethyl carbamate, 2-(4'-pyridyl)ethyl carbamate, 2,2-bis(4'-nitrophenyl)ethyl carbamate, 2-[(2-nitrophenyl)dithio]-1-phenylethyl carbamate, 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate, fluorous BOC carbamate, 1-adamantyl carbamate, 2-adamantyl carbamate, 1-(1-adamantyl)-1-methylethyl carbamate, 1-methyl-1-(4-byphenylyl)ethyl carbamate, 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate, triisopropylsilyloxy carbamate, vinyl carbamate, allyl carbamate, prenyl carbamate, 1-isopropylallyl carbamate, cinnamyl carbamate, 4-nitrocinnamyl carbamate, 3-(3'-pyridyl)prop-2-enyl carbamate, hexadienyl carbamate, propargyl carbamate, 1,4-but-2-ynyl biscarbamate, 8-quinolyl carbamate, *N*-hydroxypiperidinyl carbamate, alkyl dithiocarbamate, benzyl carbamate, 3,5-di-*t*-butylbenzyl carbamate, *p*-methoxybenzyl carbamate, *p*-nitrobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate, 4-trifluoromethylbenzyl carbamate, fluorous benzyl carbamate, 2-naphthylmethyl carbamate, 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 4-phenylacetoxybenzyl carbamate, 4-azidobenzyl carbamate, 4-azido-methoxybenzyl carbamate, *m*-chloro-*p*-acyloxybenzyl carbamate, *p*-(dihydroxyboryl)-benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-toluenesulfonyl)ethyl carbamate, 2-(4-nitrophenylsulfonyl)ethyl carbamate, 2-(2,4-dinitrophenylsulfonyl)ethyl carbamate, 2-(4-trifluoromethylphenylsulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate, 2-phosphonioethyl carbamate, 2-[phenyl(methyl)sulfonio]ethyl carbamate, 1-methyl-1-(triphenylphosphonio)ethyl carbamate, 1,1-dimethyl-2-cyanoethyl carbamate, 2-dansylethyl carbamate, 2-(4-nitrophenyl)ethyl carbamate, 4-methylthiophenyl carbamate, 2,4-dimethylthiophenyl carbamate, *m*-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, *α*-methylnitropiperonyl carbamate, *o*-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(*o*-nitrophenyl)methyl carbamate, 2-nitrophenylethyl carbamate, 6-nitroveratryl carbamate, 4-methoxyphenacyl carbamate, 3',5'-dimethoxybenzoin carbamate, 9-xanthenylmethyl carbamate, *N*-methyl-*N*-(*o*-nitrophenyl) carbamate, *t*-amyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, cyclobutyl carbamate, cyclopentyl carbamate, cyclohexyl carbamate, isobutyl carbamate, isobornyl carbamate, cyclopropylmethyl carbamate, *p*-decyloxybenzyl carbamate, diisopropylmethyl carbamate, 2,2-dimethoxy-carbonylvinyl carbamate, *o*-(*N,N*-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(*N,N*-dimethyl-carboxamido)propyl carbamate, butynyl carbamate, 1,1-dimethylpropynyl carbamate, 2-iodoethyl carbamate, 1-methyl-1-(4'-pyridyl)ethyl carbamate, 1-methyl-1-(*p*-phenylazophenyl)ethyl carbamate, *p*-(*p'*-methoxyphenylazo)benzyl carbamate, *p*-(phenylazo)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, isonicotinyl carbamate, 4-(trimethyl-ammonium)benzyl carbamate, *p*-cyanobenzyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, phenyl carbamate, 2,4,6-tri-*t*-butylphenyl carbamate, 1-methyl-1-phenylethyl carbamate, and *S*-benzyl

thiocarbamate. In the case of ureas the protecting groups for the amino group can be selected from phenothiazinyl-(10)-carbonyl, *N'*-*p*-toluenesulfonylaminocarbonyl, *N'*-phenylaminothiocarbonyl, 4-hydroxyphenylaminocarbonyl, 3-hydroxytryptaminocarbonyl, and *N'*-phenylaminothiocarbonyl. In the case of amides the protecting group for the amino together with the amino group to which it is attached form an amide that can be selected from formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, pent-4-enamide, picolinamide, 3-pyridylcarboxamide, *N*-benzoylphenylalanyl amide, benzamide, *p*-phenylbenzamide, *o*-nitrophenylacetamide, 2,2-dimethyl-2-(*o*-nitrophenyl)acetamide, *o*-nitrophenoxyacetamide, 3-(*o*-nitrophenyl)propanamide, 2-methyl-2-(*o*-nitrophenoxy)propanamide, 3-methyl-3-nitrobutanamide, *o*-nitrocinnamide, *o*-nitrobenzamide, 3-(4-*t*-butyl-2,6-dinitrophenyl)-2,2-dimethylpropanamide, *o*-(benzoyloxyme-thyl)benzamide, 2-(acetoxymethyl)benzamide, 2-[(*t*-butyldiphenylsiloxy)methyl]benzamide, 3-(3',6'-dioxo-2',4',5'-trimethylcyclohexa-1',4'-diene)-3,3-dimethylpropionamide, *o*-hydroxy-*trans*-cinnamide, 2-methyl-2-(*o*-phenylazophenoxy)propanamide, 4-chlorobutanamide, aceto-acetamide, 3-(*p*-hydroxyphenyl)propanamide, (*N'*-dithiobenzyloxycarbonylamino)acetamide, and *N*-acetylmethionine amide. In the case of heterocyclic systems the protecting group for the amino group together with the amino group to which it is attached form a heterocyclic system that can be selected from 4,5-diphenyl-3-oxazolin-2-one, *N*-phthalimide, *N*-dichlorophthalimide, *N*-tetrachlorophthalimide, *N*-4-nitrophthalimide, *N*-thiodiglycoloyl, *N*-dithiasuccinimide, *N*-2,3-diphenylmaleimide, *N*-2,3-dimethylmaleimide, *N*-2,5-dimethylpyrrole, *N*-2,5-bis(triisopropylsiloxy)pyrrole, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct, *N*-1,1,3,3-tetramethyl-1,3-disilaisoindoline, *N*-diphenylsilyldiethylene, *N*-5-substituted-1,3-dimethyl-1,3,5-triazacyclohexan-2-one, *N*-5-substituted-1,3-benzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, and 1,3,5-dioxazine. In the case of *N*-alkyl, *N*-alkenyl, *N*-alkynyl or *N*-aryl amines the protecting group for the amino group can be selected from *N*-methyl, *N*-*t*-butyl, *N*-allyl, *N*-prenyl, *N*-cinnamyl, *N*-phenylallyl, *N*-propargyl, *N*-methoxymethyl, *N*-[2-(trimethylsilyl)ethoxy]methyl, *N*-3-acetoxypropyl, *N*-cyanomethyl, *N*-2-azanorbornenes, *N*-benzyl, *N*-4-methoxybenzyl, *N*-2,4-dimethoxybenzyl, *N*-2-hydroxybenzyl, *N*-ferrocenylmethyl, *N*-2,4-dinitrophenyl, *o*-methoxyphenyl, *p*-methoxyphenyl, *N*-9-phenylfluorenyl, *N*-fluorenyl, *N*-2-picolylamine N'-oxide, *N*-7-methoxycoumar-4-ylmethyl, *N*-diphenylmethyl, *N*-bis(4-methoxyphenyl)methyl, *N*-5-dibenzosuberyl, *N*-triphenylmethyl, *N*-(4-methylphenyl)diphenylmethyl, and *N*-(4-methoxyphenyl)diphenylmethyl. In the case of imines the protecting group for the amino group can be selected from *N*-1,1-dimethylthiomethylene, *N*-benzylidene, *N*-*p*-methoxybenzylidene, *N*-diphenylmethylene, *N*-[2-pyridyl)mesityl]methylene, *N*-(*N',N'*-dimethylaminomethylene), *N*-(*N',N'*-dibenzylaminomethylene), *N*-(*N'*-*t*-butylaminome-thylene), *N,N'*-isopropylidene, *N*-*p*-nitrobenzylidene, *N*-salicylidene, *N*-5-chlorosalicylidene, *N*-(5-chloro-2-hydroxyphenyl)phenylmethylene, *N*-cyclohexylidene, and *N*-*t*-butylidene. In the case of enamines the protecting group for the amino group can be selected from *N*-(5,5-dimethyl-3-

oxo-1-cyclohexenyl), *N*-2,7-dichloro-9-fluorenylmethylene, *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, *N*-(1,3-dimethyl-2,4,6-(1*H*,3*H*,5*H*)-trioxopyrimidine-5-ylidene)-methyl, *N*-4,4,4-trifluoro-3-oxo-1-butenyl, and *N*-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl). In the case of N-metal derivatives the protecting group for the amino group can be selected from N-borane, *N*-diphenylborinic ester, *N*-diethylborinic ester, *N*-9-borabicyclononane, *N*-difluoroborinic ester, and 3,5-bis(trifluoromethyl)phenylboronic acid; and also including *N*-phenyl(pentacarbonylchromium)carbenyl, *N*-phenyl(pentacarbonyl-tungsten)carbenyl, *N*-methyl(pentacarbonylchromium)carbenyl, *N*-methyl(pentacarbonyltungsten)carbenyl, *N*-copper chelate, *N*-zinc chelate, and a 18-crown-6-derivative. In the case of N-N derivatives the protecting group for the amino group together with the amino group to which it is attached form a N-N derivative that can be selected from *N*-nitroamino, *N*-nitrosoamino, amine *N*-oxide, azide, triazene derivative, and *N*-trimethylsilylmethyl-*N*-benzylhydrazine. In the case of N-P derivatives the protected group for the amino group together with the amino group to which it is attached form a N-P derivative that can be selected from diphenylphosphinamide, dimethylthiophosphinamide, diphenylthiophosphinamide, dialkyl phosphoramidate, dibenzyl phosphoramidate, diphenyl phosphoramidate, and iminotriphenylphosphorane. In the case of N-Si derivatives the protecting group for the NH_2 can be selected from *t*-butyldiphenylsilyl and triphenylsilyl. In the case of N-S derivatives the protected amino group can be selected from N-sulfenyl or N-sulfonyl derivatives. The N-sulfenyl derivatives can be selected from benzenesulfenamide, 2-nitrobenzenesulfenamide, 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfe-namide, 1-(2,2,2-trifluoro-1,1-diphenyl)ethylsulfenamide, and *N*-3-nitro-2-pyridinesulfenamide. The N-sulfonyl derivatives can be selected from methanesulfonamide, trifluoromethanesulfonamide, *t*-butylsulfonamide, benzylsulfonamide, 2-(trimethylsilyl) ethanesulfonamide, *p*-toluenesulfonamide, benzenesulfonamide, *o*-anisylsulfonamide, 2-nitrobenzenesulfonamide, 4-nitrobenzenesulfonamide, 2,4-dinitrobenzenesulfonamide, 2-naphthalenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide, 2-(4-methylphenyl)-6-methoxy-4-methylsulfonamide, 9-anthracenesulfonamide, pyridine-2-sulfonamide, benzothiazole-2-sulfonamide, phenacylsulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide, 2,4,6-trimethoxybenzenesulfonamide, 2,6-dimethyl-4-methoxy-benzenesulfonamide, pentamethylbenzenesulfonamide, 2,3,5,6-tetramethyl-4-methoxyben-zenesulfonamide, 4-methoxybenzenesulfonamide, 2,4,6-trimethylbenzenesulfonamide, 2,6-dimethoxy-4-methylbenzenesulfonamide, 3-methoxy-4-*t*-butylbenzenesulfonamide, and 2,2,5,7,8-pentamethylchroman-6-sulfonamide.

Within the scope of the present invention a protecting group for SH is defined to be the *S*-bonded moiety resulting from the protection of the SH group through the formation of a suitable a protected SH group. Examples of such protected SH groups include thioethers, disulfides, silyl thioethers, thioesters, thiocarbonates, and thiocarbamates. In the case of thioethers the protecting group for the SH can be selected from *S*-alkyl, *S*-benzyl, *S*-*p*-methoxybenzyl, *S*-*o*-hydroxybenzyl, *S*-*p*-hydroxybenzyl, *S*-*o*-acetoxybenzyl, S-*p*-

acetoxybenzyl, *S*-*p*-nitrobenzyl, *S*-*o*-nitrobenzyl, *S*-2,4,6-trimethylbenzyl, *S*-2,4,6-trimethoxybenzyl, *S*-4-picolyl, *S*-2-picolyl-*N*-oxide, *S*-2-quinolinylmethyl, *S*-9-anthrylmethyl, *S*-9-fluorenylmethyl, *S*-xanthenyl, *S*-ferrocenylmethyl, *S*-diphenylmethyl, *S*-bis(4-methoxyphenyl)methyl, *S*-5-dibenzosuberyl, *S*-triphenylmethyl, 4-methoxytrityl, *S*-diphenyl-4-pyridylmethyl, *S*-phenyl, *S*-2,4-dinitrophenyl, *S*-2-quinolyl, *S*-*t*-butyl, *S*-1-adamantyl, *S*-methoxymethyl, *S*-isobutoxymethyl, *S*-benzyloxymethyl, *S*-1-ethoxyethyl, *S*-2-tetrahydropyranyl, *S*-benzylthiomethyl, S-phenylthiomethyl, *S*-acetamidomethyl (Acm), *S*-trimethylacetamidomethyl, *S*-benzamidomethyl, *S*-allyloxycarbonylaminomethyl, *S*-*N*-[2,3,5,6-tetrafluoro-4-(*N*'-piperidino)-phenyl-*N*-allyloxycarbonylaminomethyl, *S*-phthalimidomethyl, *S*-phenylacetamidomethyl, *S*-acetylmethyl, *S*-carboxymethyl, *S*-cyanomethyl, *S*-(2-nitro-1-phenyl)ethyl, *S*-2-(2,4-dinitrophenyl)ethyl, *S*-2-(4'-pyridyl)ethyl, *S*-2-cyanoethyl, *S*-2-(trimethylsilyl)ethyl, *S*-2,2-bis(carboethoxy)ethyl, *S*-(1-*m*-nitrophenyl-2-benzoyl)ethyl, *S*-2-phenylsulfonylethyl, *S*-1-(4-methylphenylsulfonyl)-2-methylprop-2-yl, and *S*-*p*-hydroxyphenacyl. In the case of disulfides the protected SH group can be selected from *S*-ethyl disulfide, *S*-*t*-butyl disulfide, *S*-2-nitrophenyl disulfide, *S*-2,4-dinitrophenyl disulfide, *S*-2-phenylazophenyl disulfide, *S*-2-carboxyphenyl disulfide, and *S*-3-nitro-2-pyridyl disulfide. In the case of silyl thioethers the protecting group for the SH can be selected from the list of groups that was listed above for the protection of OH with silyl ethers. In the case of thioesters the protecting group for the SH can be selected from *S*-acetyl, *S*-benzoyl, *S*-2-methoxyisobutyryl, *S*-trifluoroacetyl, *S*-*N*-[[*p*-biphenylyl)-isopropyloxy]carbonyl]-*N*-methyl-γ-aminothiobutyrate, and *S*-*N*-(*t*-butoxycarbonyl)-*N*-methyl-γ-aminothiobutyrate. In the case of thiocarbonate protecting group for the SH can be selected from *S*-2,2,2-trichloroethoxycarbonyl, *S*-*t*-butoxycarbonyl, *S*-benzyloxycarbonyl, *S*-*p*-methoxybenzyloxycarbonyl, and *S*-fluorenylmethylcarbonyl. In the case of thiocarbamate the protected SH group can be selected from *S*-(*N*-ethylcarbamate) and *S*-(*N*-methoxymethylcarbamate).

The mention of these groups should not be interpreted as a limitation of the scope of the invention, since they have been mentioned as a mere illustration of protecting groups for OH, amino and SH groups, but further groups having said function may be known by the skilled person in the art, and they are to be understood to be also encompassed by the present invention.

To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term “about”. It is understood that, whether the term “about” is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

"Antibody-drug-conjugates (ADCs)" represent a targeted strategy to deliver a cytotoxic molecule to a cancer cell (see, for example, International Patent Applications WO-A-2004/010957, WO-A-2006/060533 and WO-A-2007/024536). Such compounds are typically referred to as drug, toxin and radionuclide "conjugates". Tumor cell killing occurs upon binding of the drug conjugate to a tumor cell and release and/or activation of the cytotoxic activity of the drug moiety. The selectivity afforded by drug conjugates minimizes toxicity to normal cells, thereby enhancing tolerability of the drug in the patient. Three examples of drug antibody conjugates of this type that have received marketing approval are: Gemtuzumab ozogamicin for acute myelogenous leukemia, Brentuximab vedotin for relapsed and refractory Hodgkin lymphoma and anaplastic large cell lymphoma, and ado-Trastuzumab emtansine for breast cancer, especially HER2+.

The effectiveness of drugs for cancer chemotherapy generally relies on differences in growth rates, biochemical pathways, and physiological characteristics between cancer and normal tissues. Consequently, most standard chemotherapeutics are relatively nonspecific and exhibit dose-limiting toxicities that contribute to suboptimal therapeutic effects. One approach to selectively target malignant cells and not healthy tissues is to use specific monoclonal antibodies (mAbs) that recognize tumor-associated antigens expressed on the surface of tumor cells [Meyer, D.L. & Senter, P.D. (2003) Recent advances in antibody drug conjugates for cancer therapy. Annu. Rep. Med. Chem., 38, 229-237; Chari, R.V. (2008) Targeted cancer therapy: conferring specificity to cytotoxic drugs. Acc. Chem. Res. 41, 98−107]. More than 30 G-type immunoglobulins (IgG) and related agents have been approved over the past 25 years mainly for cancers and inflammatory diseases.

An alternative strategy is to look to chemically conjugate small anti-neoplastic molecules to mAbs, used both as carriers (increased half-life) and as targeting agents (selectivity). Considerable effort has been directed toward the use of monoclonal antibodies (mAbs) for targeted drug delivery due to their high selectivities for tumor-associated antigens, favorable pharmacokinetics, and relatively low intrinsic toxicities. The mAb-drug conjugates (ADCs) are formed by covalently linking anticancer drugs to mAbs, usually through a conditionally stable linker system. Upon binding to cell surface antigens, mAbs used for most ADCs are actively transported to lysosomes or other intracellular compartments, where enzymes, low pH, or reducing agents facilitate drug release. There are, however, currently limited ADCs in development.

Antigens must have high tumor cell selectivity to limit toxicity and off-target effects. A plethora of tumor-associated antigens have been investigated in pre-clinical models and in clinical trials including antigens over-expressed in B-cells (e.g., CD20, CD22, CD40, CD79), T-cells (CD25, CD30), carcinoma cells (HER2, EGFR, EpCAM, EphB2, PSMA), endothelial (endoglin), or stroma cells (fibroblast activated protein), to name a few [Teicher BA. Antibody-drug conjugate targets. Curr Cancer Drug Targets 9(8):982-1004, 2009]. An important

property for ADC targets is their ability to be internalized; this can be an intrinsic feature of the antigen by itself, or it can be induced by the binding of the antibody to its antigen. Indeed, ADC internalization is crucial to reduce toxicity associated with an extracellular delivery of the drug payload.

Regarding the conjugated small molecules and in contrast to the vast variety of putative antigen targets, a limited number of families of cytotoxic drugs used as payloads in ADCs are currently actively investigated in clinical trials: calicheamycin (Pfizer), duocarmycins (Synthon), pyrrolobenzodiazepines (Spirogen), irinotecan (Immunomedics), maytansinoids (DM1 and DM4; ImmunoGen + Genentech/Roche, Sanofi-Aventis, Biogen Idec, Centocor/Johnson & Johnson, Millennium/Takeda), and auristatins (MMAE and MMAF; Seattle Genetics + Genentech/Roche, MedImmune/AstraZeneca, Bayer-Schering, Celldex, Progenics, Genmab). Calicheamycin, duocarmycins and pyrrolobenzodiazepines are DNA minor groove binders, irinotecan is a topoisomerase I inhibitor, whereas maytansinoids and auristatins are tubulin depolymerization agents.

Interestingly, a representative of three of these cytotoxic-derived ADCs has reached late stage clinical trials. Trastuzumab emtansine (T-DM1), trastuzumab linked to a maytansinoid hemi-synthetic drug by a stable linker (FDA approval on February 22, 2013 for advanced HER2 positive breast cancer); Inotuzumab ozogamicin (CMC-544), a humanized anti-CD22 mAb (G5/44, IgG4) conjugated to calicheamycin with an acid labile linker (acetylphenoxy-butanoic) (B-cell non-Hodgkin's lymphoma); Brentuximab vedotin, a humanized anti-CD30 mAb linked to monomethyl auristatin E (MMAE), via a maleimidecaproyl-valyl-citrullinyl-*p*-aminobenzylcarbamate linker (FDA approval on August 19, 2011 for anaplastic large cell lymphoma and Hodking´s lymphoma).

Linkers represent the key component of ADC structures. Several classes of second generation linkers have been investigated, including acid-labile hydrazone linkers (lysosomes) (e.g. gemtuzumab and inotuzumab ozogamicin); disulfide-based linkers (reductive intracellular environment); non-cleavable thioether linkers (catabolic degradation in lysosomes) (e.g., trastuzumab emtansine); peptide linkers (e.g. citruline-valine) (lysosomal proteases like cathepsin-B) (e.g. brentuximab vedotin): see, for example, WO-A-2004/010957, WO-A-2006/060533 and WO-A-2007/024536. Purification of antibody-drug conjugates by size exclusion chromatography (SEC) has also been described [see, e.g., Liu et al., Proc. Natl. Acad. Sci. USA, 93: 8618-8623 (1996), and Chari et al., Cancer Research, 52: 127-131 (1992)].

Trastuzumab (Herceptin) is a monoclonal antibody that interferes with the HER2/neu receptor. Its main use is to treat certain breast cancers. The HER receptors are proteins that are embedded in the cell membrane and communicate molecular signals from outside the cell (molecules called EGFs) to inside the cell, and turn genes on and off. The HER proteins

stimulate cell proliferation. In some cancers, notably certain types of breast cancer, HER2 is over-expressed, and causes cancer cells to reproduce uncontrollably.

The HER2 gene is amplified in 20-30% of early-stage breast cancers, which makes it overexpress epidermal growth factor (EGF) receptors in the cell membrane. In some types of cancer, HER2 may send signals without growth factors arriving and binding to the receptor, making its effect in the cell constitutive; however, trastuzumab is not effective in this case.

The HER2 pathway promotes cell growth and division when it is functioning normally; however when it is overexpressed, cell growth accelerates beyond its normal limits. In some types of cancer the pathway is exploited to promote rapid cell growth and proliferation and hence tumor formation. In cancer cells the HER2 protein can be expressed up to 100 times more than in normal cells (2 million versus 20,000 per cell). This overexpression leads to strong and constant proliferative signaling and hence tumor formation. Overexpression of HER2 also causes deactivation of checkpoints, allowing for even greater increases in proliferation.

In the compounds of the present invention, Ab is a moiety comprising at least one antigen binding site. In an alternative embodiment, Ab can be any suitable agent that is capable of binding to a target cell, preferably an animal cell and more preferably, a human cell. Examples of such agents include lymphokines, hormones, growth factors and nutrient-transport molecules (e.g. transferrin). In another example, Ab may be an aptamer, and may include a nucleic acid or a peptide aptamer.

Where Ab is a moiety comprising at least one antigen binding site, the moiety is preferably an antigen-binding peptide or polypeptide. In a preferred embodiment, the moiety is an antibody or an antigen-binding fragment thereof.

The term ‘antibody’ in the drug conjugates of the present invention refers to any immunolglobulin, preferably a full-length immunoglobulin. Preferably, the term covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies, such as bispecific antibodies, and antibody fragments thereof, so long as they exhibit the desired biological activity. Antibodies may be derived from any species, but preferably are of rodent, for examples rat or mouse, human or rabbit origin. Alternatively, the antibodies, preferably monoclonal antibodies, may be humanised, chimeric or antibody fragments thereof. The term ‘chimeric antibodies’ may also include "primatised" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc) and human constant region sequences. The immunoglobulins can also be of any type (e.g. IgG, IgE, IgM, IgD, and IgA), class (e.g., IgGI, IgG2, IgG3, IgG4, IgAI and IgA2) or subclass of immunoglobulin molecule.

The term 'monoclonal antibody' refers to a substantially homogenous population of antibody molecules (i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts), produced by a single clone of B lineage cells, often a hybridoma. Importantly, each monoclonal has the same antigenic specificity - i.e. it is directed against a single determinant on the antigen.

The production of monoclonal antibodies can be carried out by methods known in the art. However, as an example, the monoclonal antibodies can be made by the hybridoma method (Kohler et al (1975) Nature 256:495), the human B cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4: 72), or the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Alternatively, the monoclonal antibody can be produced using recombinant DNA methods (see, US 4816567) or isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597.

Polyclonal antibodies are antibodies directed against different determinants (epitopes). This heterogenous population of antibody can be derived from the sera of immunised animals using various procedures well known in the art.

The term 'bispecific antibody' refers to an artificial antibody composed of two different monoclonal antibodies. They can be designed to bind either to two adjacent epitopes on a single antigen, thereby increasing both avidity and specificity, or bind two different antigens for numerous applications, but particularly for recruitment of cytotoxic T- and natural killer (NK) cells or retargeting of toxins, radionuclides or cytotoxic drugs for cancer treatment (Holliger & Hudson, Nature Biotechnology, 2005, 23(9), 1126-1136). The bispecific antibody may have a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation (WO 94/04690; Suresh et al., Methods in Enzymology, 1986, 121:210; Rodrigues et al., 1993, J. of Immunology 151:6954-6961; Carter et al., 1992, Bio/Technology 10:163-167; Carter et al., 1995, J. of Hematotherapy 4:463-470; Merchant et al., 1998, Nature Biotechnology 16:677-681.

Methods to prepare hybrid or bispecific antibodies are known in the art. In one method, bispecific antibodies can be produced by fusion of two hybridomas into a single 'quadroma' by chemical cross-linking or genetic fusion of two different Fab or scFv modules (Holliger & Hudson, Nature Biotechnology, 2005, 23(9), 1126-1136).

The term 'chimeric' antibody refers to an antibody in which different portions are derived from different animal species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. In contrast, a 'humanised antibody' comes predominantly from a human, even though it contains non-human portions. Specifically, humaised antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from hypervariable regions of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanised antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanised antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

Recombinant antibodies such as chimeric and humanised monoclonal antibodies can be produced by recombinant DNA techniques known in the art. Completely human antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harboured by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, for example, US Patent Nos. 5625126; 5633425; 5569825; 5661016; 5545806; each of which is incorporated herein by reference in its entirety. Other human antibodies can be obtained commercially from, for example, Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA).

The term 'antigen-binding fragment' in the drug conjugates of the present invention refers to a portion of a full length antibody where such antigen-binding fragments of antibodies retain the antigen-binding function of a corresponding full-length antibody. The antigen-binding fragment may comprise a portion of a variable region of an antibody, said portion comprising at least one, two, preferably three CDRs selected from CDR1, CDR2 and CDR3. The antigen-binding fragment may also comprise a portion of an immunoglobulin light

and heavy chain. Examples of antibody fragments include Fab, Fab', F(ab')2, scFv, di-scFv, sdAb, and BiTE (Bi-specific T-cell engagers), Fv fragments including nanobodies, diabodies, diabody-Fc fusions, triabodies and, tetrabodies; minibodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above that immunospecifically bind to a target antigen such as a cancer cell antigens, viral antigens or microbial antigens, single-chain or single-domain antibody molecules including heavy chain only antibodies, for example, camelid VHH domains and shark V-NAR; and multispecific antibodies formed from antibody fragments. For comparison, a full length antibody, termed 'antibody' is one comprising a VL and VH domains, as well as complete light and heavy chain constant domains.

The antibody may also have one or more effector functions, which refer to the biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region engineered according to methods in the art to alter receptor binding) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

The antibody can also be a functionally active fragment (also referred to herein as an immunologically active portion), derivative or analog of an antibody that immunospecifically binds to a target antigen such as a cancer cell antigen, viral antigen, or microbial antigen or other antibodies bound to tumour cells. In this regard, functionally active means that the fragment, derivative or analog is able to elicit anti-idiotype antibodies that recognise the same antigen that the antibody from which the fragment, derivative or analog is derived recognised. Specifically, in an exemplary embodiment the antigenicity of the idiotype of the immunoglobulin molecule can be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art (e.g., the BIA core assay), see, for example, Kabat et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md; Kabat E et al., 1980, J. of Immunology 125(3):961-969).

The term 'antibody' may also include a fusion protein of an antibody, or a functionally active fragment thereof, for example in which the antibody is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, such as at least 10, 20 or 50 amino acid portion of the protein) that is not the antibody. The antibody or fragment thereof may be covalently linked to the other protein at the N-terminus of the constant domain.

Furthermore, the antibody or antigen-binding fragments of the present invention may include analogs and derivatives of antibodies or antigen-binding fragments thereof that are either modified, such as by the covalent attachment of any type of molecule as long as such covalent attachment permits the antibody to retain its antigen binding immunospecificity. Examples of modifications include glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular antibody unit or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, etc. Additionally, the analog or derivative can contain one or more unnatural amino acids.

The antibodies or antigen-binding fragments of the present invention may also have modifications (e.g., substitutions, deletions or additions) in the Fc domain of the antibody. Specifically, the modifications may be in the Fc-hinge region and result in an increased binding for the FcRn receptor (WO 97/34631).

In one embodiment, the antibody in the drug conjugate of the present invention may be any antibody or antigen-binding fragment thereof, preferably a monoclonal antibody that is useful in the treatment of a disease, preferably cancer. The cancer may be breast cancer, colorectal cancer, endometrial cancer, kidney cancer melanoma, leukaemias, lung cancer, multiple myeloma, lymphomas (e.g. Hodgkin's disease and non-Hodgkin's Lymphoma), solid tumors such as sarcoma and carcinomas, melanoma, mesothelioma, osteosarcoma, ovarian cancer and renal cancer. In a preferred embodiment the cancer is lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. In a more preferred embodiment the cancer is colorectal cancer, breast cancer, leukaemia, lymphoma, and ovarian cancer

Antibodies that may be useful in the treatment of cancer include, but are not limited to, antibodies against the following antigens: CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas) for example EGF receptor 2 protein (breast cancer), MAGE-I (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE-4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MUCI-KLH (breast cancer), CEA (colorectal), gplOO (melanoma), MARTI (melanoma), PSA (prostate), IL-2 receptor (T-cell leukemia and lymphomas), CD20 (non-Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia),CD22 (lymphoma), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD40 (lymphoma), mucin (carcinomas), P21 (carcinomas), MPG (melanoma), and Neu oncogene product (carcinomas). Some specific, useful antibodies include, but are not limited to, BR96 mAb (Trail, P. A., et al Science (1993) 261, 212-215), BR64 (Trail, PA, et al

Cancer Research (1997) 57, 100-105, mAbs against the CD40 antigen, such as S2C6 mAb (Francisco, J. A., et al Cancer Res. (2000) 60:3225-3231), mAbs against the CD70 antigen, such as 1F6 mAb, and mAbs against the CD30 antigen, such as AClO (Bowen, M. A., et al (1993) J. Immunol., 151:5896- 5906; Wahl et al., 2002 Cancer Res. 62(13):3736-3742). Many other internalizing antibodies that bind to tumor associated antigens can be used and have been reviewed (Franke, A. E., et al Cancer Biother Radiopharm. (2000) 15:459-476; Murray, J. L., (2000) Semin Oncol, 27:64-70; Breitling, F., and Dubel, S., Recombinant Antibodies, John Wiley, and Sons, New York, 1998).

The present invention encompasses treating cancers associated with these antibodies.

Other tumour-associated antigens include, but are not limited to, BMPR1B, E16, STEAP1, STEAP2, 0772P. MPF, Napi3b, Sema5b, PSCA hlg, ETBR, MSG783, TrpM4, CRIPTO, CD21, CD79b, FcRH2, HER2, NCA, MDP, IL20Rα, Brevican, EphB2R, ASLG659, PSCA, GEDA, BAFF-R, CD79A, CXCR5, HLA-DOB, P2X5, CD72, LY64, FCRH1, IRTA2 and TENB2.

In an alternative embodiment, the antibody in the drug conjugate of the present invention may be an antibody or antigen-binding fragment thereof, preferably a monoclonal antibody, that immunospecifically binds to a viral antigen, microbial antigen or an antigen of a cell that produces autoimmune antibodies associated with autoimmune disease.

The viral antigen may include, but is not limited to, any viral peptide, polypeptide or protein such as HIV gpl20, HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g., Gb, Gc, Gd, and Ge) and hepatitis B surface antigen that is capable of eliciting an immune response.

The microbial antigen may include, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacterial, fungi, pathogenic protozoa, or yeast polypeptide including, e.g., LPS and capsular polysaccharide) that is capable of eliciting an immune response.

In a further embodiment, the antibody or antigen-binding fragment binds to an epitope that is present on a cell, such as a tumour cell. Preferably, where the cell is a tumour cell, the tumour cell epitope is not present on non-tumour cells, or is present at a lower concentration or in a different steric configuration than in tumour cells.

In one embodiment, the antibody or antigen-binding fragment binds to an epitope present in the context of one of the following antigens: CA125, CA15-3, CA19-9 L6, Lewis Y, Lewis X, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific antigen, prostatic acid phosphatase, epidermal growth factor for example EGF receptor 2 protein,

MAGE-I, MAGE-2, MAGE-3, MAGE-4, anti-transferrin receptor, p97, MUCI-KLH, CEA, gplOO, MARTI, PSA, IL-2 receptor, CD20, CD52, CD33 ,CD22, human chorionic gonadotropin, CD38, CD40, mucin, P21, MPG, Neu oncogene product, BMPR1B, E16, STEAP1, STEAP2, 0772P. MPF, Napi3b, Sema5b, PSCA hlg, ETBR, MSG783, TrpM4, CRIPTO, CD21, CD79b, FcRH2, HER2, NCA, MDP, IL20Rα, Brevican, EphB2R, ASLG659, PSCA, GEDA, BAFF-R, CD79A, CXCR5, HLA-DOB, P2X5, CD72, LY64, FCRH1, IRTA2, TENB2, a viral antigen (such as any viral peptide, polypeptide or protein such as HIV gpl20, HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g., Gb, Gc, Gd, and Ge) and hepatitis B surface antigen) that is capable of eliciting an immune response), microbial antigen (any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacterial, fungi, pathogenic protozoa, or yeast polypeptide including, e.g., LPS and capsular polysaccharide) that is capable of eliciting an immune response) or an antigen of a cell that produces autoimmune antibodies associated with autoimmune disease.

In one embodiment, where the antigen is ErBB2 (also known as ERBB2, CD340 or HER2; such terms may be used interchangeably), the antibody or antigen-binding fragment may bind to one or more of the following epitopes: ARHC L (SEQ ID NO: 1), QNGS (SEQ ID NO: 2) and PPFCVARC PSG (SEQ ID NO: 3). These epitopes correspond to positions 557-561, 570-573 and 593-603 respectively of the human HER2 polypetide sequence (Accession: NM_004448, Version: NM_004448.3).

In another embodiment, the antibody may be any antibody known for the treatment or prevention of viral or microbial infection - i.e. an infectious disease. Examples of such antibodies include, but are not limited to, PRO542 (Progenies) which is a CD4 fusion antibody useful for the treatment of HIV infection; OsTAVIR (Protein Design Labs, Inc., CA) which is a human antibody useful for the treatment of hepatitis B virus; PROTOVIR. (Protein Design Labs, Inc., CA) which is a humanised IgG1 antibody useful for the treatment of cytomegalovirus (CMV); and anti-LPS antibodies.

Other antibodies useful in the treatment of infectious diseases include, but are not limited to, antibodies against the antigens from pathogenic strains of bacteria (Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria gonorrheae, Neisseria meningitidis, Corynebacterium diphtheriae, Clostridium botulinum, Clostridium perfringens, Clostridium tetani, Hemophilus influenzae, Klebsiella pneumoniae, Klebsiella ozaenas, Klebsiella rhinoscleromotis, Staphylococcus aureus, Vibrio colerae, Escherichia coli, Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Bacillus cereus, Edwardsiella tarda, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pallidum, Treponema pertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospira icterohernorrhagiae, Mycobacterium tuberculosis, Pneumocystis

carinii, Francisella tularensis, Brucella abortus, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsia prowazeki, Rickettsia tsutsugumushi, Chlamydia spp.); pathogenic fungi (Coccidioides immitis, Aspergillus fumigatus, Candida albicans, Blastomyces dermatitidis, Cryptococcus neoformans, Histoplasma capsulatum); protozoa (Entomoeba histolytica, Toxoplasma gondii, Trichomonas tenas, Trichomonas hominis, Trichomonas vaginalis, Tryoanosoma gambiense, Trypanosoma rhodesiense, Trypanosoma cruzi, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria); or Helminiths (Enterobius vermicularis, Trichuris trichiura, Ascaris lumbricoides, Trichinella spiralis, Strongyloides stercoralis, Schistosoma japonicum, Schistosoma mansoni, Schistosoma haematobium, and hookworms).

Other antibodies useful for the treatment of viral disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxviridae, Herpesviridae, Herpes Simplex virus 1, Herpes Simplex virus 2, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis E virus, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, and Human Immunodeficiency Virus.

In an alternative embodiment, the antibody of the drug conjugate of the present invention may also be any antibody known for the treatment of prevention of autoimmune disorders, such as, but not limited to, Th2-lymphocyte related disorders (e.g. atopic dermatitis, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, and graft versus host disease); Th1 lymphocyte-related disorders (e.g. rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, and tuberculosis); activated B lymphocyte-related disorders (e.g. systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes); and Active Chronic Hepatitis, Addison's Disease, Allergic Alveolitis, Allergic Reaction, Allergic Rhinitis, Alport's Syndrome, Anaphlaxis, Ankylosing Spondylitis, Anti-phosholipid Syndrome, Arthritis, Ascariasis, Aspergillosis, Atopic Allergy, Atropic Dermatitis, Atropic Rhinitis, Behcet's Disease, Bird-Fancier's Lung, Bronchial Asthma, Caplan's Syndrome, Cardiomyopathy, Celiac Disease, Chagas' Disease, Chronic Glomerulonephritis, Cogan's Syndrome, Cold Agglutinin Disease, Congenital Rubella Infection, CREST Syndrome, Crohn's Disease, Cryoglobulinemia, Cushing's Syndrome, Dermatomyositis, Discoid Lupus, Dresser's Syndrome, Eaton-Lambert Syndrome, Echovirus Infection, Encephalomyelitis, Endocrine opthalmopathy, Epstein-Barr Virus Infection, Equine Heaves, Erythematosis, Evan's Syndrome, Felty's Syndrome, Fibromyalgia, Fuch's Cyclitis, Gastric Atrophy, Gastrointestinal Allergy, Giant Cell Arteritis, Glomerulonephritis, Goodpasture's Syndrome, Graft v. Host Disease, Graves' Disease, Guillain-Barre Disease,

Hashimoto's Thyroiditis, Hemolytic Anemia, Henoch-Schonlein Purpura, Idiopathic Adrenal Atrophy, Idiopathic Pulmonary Fibritis, IgA Nephropathy, Inflammatory Bowel Diseases, Insulin-dependent Diabetes Mellitus, Juvenile Arthritis, Juvenile Diabetes Mellitus (Type I), Lambert-Eaton Syndrome, Laminitis, Lichen Planus, Lupoid Hepatitis, Lupus Lymphopenia, Meniere's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Polyglandular Syndromes, Presenile Dementia, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Psoriatic Arthritis, Raynauds Phenomenon, Recurrent Abortion, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sampter's Syndrome, Schistosomiasis, Schmidt's Syndrome, Scleroderma, Shulman's Syndrome, Sjorgen's Syndrome, Stiff-Man Syndrome, Sympathetic Ophthahnia, Systemic Lupus Erythematosis, Takayasu's Arteritis, Temporal Arteritis, Thyroiditis, Thrombocytopenia, Thyrotoxicosis, Toxic Epidermal Necrolysis, Type B Insulin Resistance, Type I Diabetes Mellitus, Ulcerative Colitis, Uveitis, Vitiligo, Waldenstrom's Macroglobulemia and Wegener's Granulomatosis.

Antibodies immunospecific for an antigen of a cell that is responsible for producing autoimmune antibodies can be obtained by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. Examples of autoimmune antibodies include, but are not limited to, Anti-Nuclear Antibody; Anti ds DNA; Anti ss DNA, Anti Cardiolipin Antibody IgM, IgG; Anti Phospholipid Antibody IgM, IgG; Anti SM Antibody; Anti Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody; Thyroglobulin Antibody; Anti SCL-70; Anti- Jo; Anti-U1RNP; Anti- La/SSB; Anti SSA; Anti SSB; Anti Perital Cells Antibody; Anti Histones; Anti-RNP; C-ANCA; P-ANCA; Anti centromere; Anti-Fibrillarin, and Anti-GBM Antibody.

In another embodiment, the antibody of the drug conjugate of the present invention can be one that binds to both a receptor or a receptor complex expressed on an activated lymphocyte, such as one associated with an autoimmune disease. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member, a TNF receptor superfamily member, an integrin, an interleukin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin, or a complement control protein. Non-limiting examples of suitable immunoglobulin superfamily members are CD2, CD3, CD4, CD5, CD8, CD13, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-I, and ICOS. Non-limiting examples of suitable TNF receptor superfamily members are CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, TNF-RI, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAEL-RI, TRAIL-R2, TRAIL-R3, TRABL-R4, and APO-3. Non-limiting examples of suitable integrins are CDI Ia, CDIIb, CDIIc, CD18, CD29, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD103, and CD104. Non-limiting examples of suitable lectins are C-type, S- type, and I-type lectin.

An antibody that binds a molecular target or an antigen of interest, e.g., ErbB2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. Where the antibody is one which binds ErbB2, it will usually preferentially bind ErbB2 as opposed to other ErbB receptors, and may be one which does not significantly cross-react with other proteins such as EGFR, ErbB 3 or ErbB4. In such embodiments, the extent of binding of the antibody to these non- ErbB2 proteins (e.g., cell surface binding to endogenous receptor) will be less than 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). Sometimes, the anti- ErbB2 antibody will not significantly cross-react with the rat neu protein, e.g., as described in Schecter et al., Nature 312:513-516 (1984) and Drebin et al., Nature 312:545-548 (1984).

In another embodiment, the antibody of the drug conjugate or target of the present invention may be selected from an antibody or target in the below table. Such antibodies are immunospecific for a target antigen and can be obtained commercially or produced by any method known in the art such as, e.g., recombinant expression techniques.

Table 1: Therapeutic monoclonal antibodies

Name	**Trade name**	**Target**
3F8		GD2 ganglioside
8H9		B7-H3
Abagovomab		CA-125 (imitation)
Abciximab	ReoPro	CD41 7E3
Abituzumab		CD51
Abrilumab		Integrin α4β7
Actoxumab		*Clostridium difficile*
Adalimumab	Humira	TNF-α
Adecatumumab		EpCAM
Atidortoxumab		*Staphylococcus aureus* alpha toxin
Aducanumab		Beta-amyloid
Afasevikumab		IL17A and IL17F
Afutuzumab		CD20
Alemtuzumab	Campath, Lemtrada	CD52
Alirocumab	Praluent	PCSK9
Altumomab	Hybri-ceaker	CEA
Amatuximab		Mesothelin
Andecaliximab		gelatinase B
Anetumab		MSLN
Anifrolumab		interferon α/β receptor

Name	Trade name	Target
Anrukinzumab		IL-13
Apolizumab		HLA-DR β-chain
Aprutumab		FGFR2
Ascrinvacumab		Activin receptor-like kinase 1
Aselizumab		L-selectin (CD62L)
Atezolizumab	Tecentriq	PD-L1
Atidortoxumab		Staphylococcus aureus alpha toxin
Atinumab		RTN4
Atorolimumab		Rhesus factor
Avelumab	Bavencio	PD-L1
Azintuxizumab		CD319
Bapineuzumab		beta amyloid
Basiliximab	Simulect	CD25 (α chain of IL-2 receptor)
Bavituximab		phosphatidylserine
BCD-100		PD-1
Bectumomab	LymphoScan	CD22
Begelomab		DPP4
Belantamab		BCMA
Belimumab	Benlysta	BAFF
Bemarituzumab		FGFR2
Benralizumab	Fasenra	CD125
Berlimatoxumab		*Staphylococcus aureus* bi-component leukocidin
Bersanlimab		ICAM-1
Bertilimumab		CCL11 (eotaxin-1)
Besilesomab	Scintimun	CEA-related antigen
Bevacizumab	Avastin	VEGF-A
Bezlotoxumab	Zinplava	*Clostridium difficile*
Blinatomumab	Blincyto	CD19, CD3
Bimagrumab		ACVR2B
Bimekizumab		IL 17A and IL17F
Birtamimab		Serum amyloid A protein
Bivatuzumab		CD44 v6
BIVV009		C1s
Bleselumab		CD40
Blontuvetmab	Blontress	CD20
Blosozumab		SOST
Bococizumab		Neural apoptosis-regulated proteinase 1

Name	Trade name	Target
Brazikumab		IL23
Brentuximab	Adcentris	CD30 (TNFRSF8)
Briakinumab		IL-12, IL-23
Brodalumab	Siliz	IL-17
Brontictuzumab		Notch 1
Burosumab	Crysvita	FGF 23
Cabiralizumab		CSF1R
Camidanlumab		CD25
Camrelizumab		Programmed cell death 1
Canakinumab	Ilaris	IL-1
Cantuzumab		MUC-1
Capromab	Prostascint	prostatic carcinoma cells
Carlumab		MCP-1
Carotuximab		endoglin
Catumaxomab	Removab	EpCAM, CD3
CC49		TAG-72
cBR96		Lewis-Y antigen
Cedelizumab		CD4
Cemiplimab		PCDC1
Cergutuzumab		IL2
Cetrelimab		Programmed cell death 1
Cetuximab	Erbitux	EGFR
Cibisatamab		CEACAM5
Cixutumumab		IGF-1 receptor (CD221)
Clazakizumab		IL6
Clenoliximab		CD4
Clivatuzumab	hPAM4-Cide	MUC1
Codrituzumab		glypican 3
Cofetuzumab		PTK7
Coltuximab		CD19
Conatumumab		TRAIL-R2
Concizumab		TFPI
Cosfroviximab	ZMapp	Ebolavirus glycoprotein
CR6261		Influenza A hemagglutinin
Crenezumab		1-40-β-amyloid
Crizanlizumab		Selectin P
Crotedumab		GCGR
Cusatuzumab		CD70

Name	Trade name	Target
Dacetuzumab		CD40
Daclizumab	Zenapax	CD25 (α chain of IL-2 receptor)
Dalotuzumab		IGF-1 receptor (CD221)
Dapirolizumab pegol		CD154 (CD40L)
Daratumumab	Darzalex	CD38
Dectrekumab		IL-13
Demcizumab		DLL4
Denintuzumab		CD19
Denosumab	Prolia	RANKL
Depatuxizumab		EGFR
Derlotuximab		Histone complex
Detumomab		B-lymphoma cell
Dezamizumab		Serum amyloid P component
Dinutuximab	Unituxin	GD2 ganglioside
Diridavumab		hemagglutinin
Domagrozumab		GDF-8
Drozitumab		DR5
Duligotuzumab		ERBB3 (HER3)
Dupilumab	Dupixent	IL4
Durvalumab	Imfinzi	PD-L1
Dusigitumab		ILGF2
Ecromeximab		GD3 ganglioside
Eculizumab	Soliris	C5
Edobacomab		endotoxin
Edrecolomab	Panorex	EpCAM
Efalizumab	Raptiva	LFA-1 (CD11a)
Eldelumab		interferon gamma-induced protein
Elezanumab		RGMA
Elgemtumab		ERBB3 (HER3)
Elotuzumab	Empliciti	SLAMF7
Elsilimomab		IL-6
Emactuzumab		CSF1R
Emapalumab	Gamifant	Interferon gamma
Emibetuzumab		HHGFR
Emicizumab	Hemlibra	Activated F9, F10
Enapotamab		AXL
Enavatuzumab		TWEAK receptor

Name	Trade name	Target
Enfortumab		nectin-4
Enlimomab pegol		ICAM-1 (CD54)
Enoblituzumab		CD276
Enokizumab		IL9
Enoticumab		DLL4
Ensituximab		5AC
Epitumomab		episialin
Epratuzumab		CD22
Eptinezumab		Calcitonin gene-related peptide
Erenumab	Aimovig	CGRP
Ertumaxomab	Rexomun	HER2/neu, CD3
Etaracizumab	Abegrin	integrin $\alpha_v\beta_3$
Etigilimab		TIGIT
Etrolizumab		integrin β_7
Evinacumab		Angiopoietin 3
Evolocumab	Repatha	PCSK9
Exbivirumab		hepatitis B surface antigen
Fanolesomab	NeutroSpec	CD15
Faralimomab		interferon receptor
Faricimab		VEGF-A and Ang-2
Farletuzumab		folate receptor 1
Fasinumab		HNGF
FBTA05	Lymphomun	CD20
Felvizumab		respiratory syncytial virus
Fezakinumab		IL-22
Fibatuzumab		Ephrin receptor A3
Ficlatuzumab		HGF
Figitumumab		IGF-1 receptor (CD221)
Firivumab		Influenza A virus hemagglutinin
Flanvotumab		TYRP1 (glycoprotein 75)
Fletikumab		IL-20
Fontolizumab	HuZAF	IFN-γ
Foralumab		CD3 epsilon
Foravirumab		rabies virus glycoprotein
Fremanezumab		Calcitonin gene-related peptide alpha
Fresolimumab		TGF-β
Frunevetmab		NGF
Fulranumab		NGF

Name	Trade name	Target
Futuximab		EGFR
Galcanezumab		calcitonin
Galiximab		CD80
Ganitumab		1 receptor (CD221)
Gantenerumab		beta amyloid
Gatipotuzumab		MUC1
Gavilimomab		CD147 (basigin)
Gedivumab		Hemagglutinin HA
Gemtuzumab	Mylotarg	CD33
Gevokizumab		IL-1β
Gilvetmab		PCDC1
Gimsilumab		CSF2
Girentuximab	Rencarex	carbonic anhydrase 9 (CA-IX)
Glembatumumab		GPNMB
Golimumab	Simponi	TNF-α
Gomiliximab		CD23 (IgE receptor)
Gosuranemab		tau protein
Guselkumab	Tremfya	IL23
Ianalumab		BAFF-R
Ibalizumab	Trogarzo	CD4
Ibritumomab	Zevalin	CD20
Icrucumab		VEGFR-1
Idarucizumab	Praxbind	dabigatran
Ifabotuzumab		EPHA3
Iladatuzumab		CD97B
IMAB362		CLDN18.2
Imalumab		MIF
Imaprelimab		MCAM
Imciromab	Myoscint	cardiac myosin
Imgatuzumab		EGFR
Inclacumab		selectin P
Indatuximab		SDC1
indusatumab		GUCY2C
inebilizumab		CD19
Infliximab	Remicade	TNF-α
Inolimomab		CD25 (α chain of IL-2 receptor)
Inotuzumab	Besponsa	CD22
Intetumumab		CD51

Name	Trade name	Target
Ipilimumab	Yervoy	CD152
Iomab-B		CD45
Iratumumab		CD30 (TNFRSF8)
Isatuximab		CD38
Iscalimab		CD40
Istiratumab		IGF1R, CD221
Itolizumab	Alzumab	CD6
Ixekizumab	Taltz	IL-17A
Keliximab		CD4
Labetuzumab	CEA-Cide	CEA
Lacnotuzumab		CSF1, MCSF
Ladiratuzumab		LIV-1
Lanadelumab		kallikrein
Landogrozumab		GDF-8
Laprituximab		EGFR
Larcaviximab		Ebolavirus glycoprotein
Lebrikizumab		IL-13
Lemalesomab		NCA-90 (granulocyte antigen)
Lendalizumab		C5
Lenvervimab		Hepatitis B surface antigen
Lenzilumab		CSF2
Lerdelimumab		TGF beta 2
Leronlimab		CCR5
Lesofavumab		Hemagglutinin HA
Lexatumumab		TRAIL-R2
Libivirumab		hepatitis B surface antigen
Lifastuzumab		Phosphate-sodium co-transporter
Ligelizumab		IGHE
Lilotomab		CD37
Lintuzumab		CD33
Lirilumab		KIR2D
Lodelcizumab		PCSK9
Lokivetmab	Cytopoint	*Canis lupus familiaris* IL31
Loncastuximab		CD19
Losatuxizumab		EGFR, ERBB1 HER1
Lorvotuzumab		CD56
Lucatumumab		CD40
Lulizumab pegol		CD28

Name	Trade name	Target
Lumiliximab		CD23 (IgE receptor)
Lumretuzumab		ERBB3 (HER3)
Lupartumab		LYPD3
Lutikizumab		Interleukin 1 alpha
MABp1	Xilonix	IL1A
Mapatumumab		TRAIL-R1
Margetuximab		HER2
Marstacimab		TFPI
Maslimomab		T-cell receptor
Mavrilimumab		GMCSF receptor α-chain
Matuzumab		EGFR
Mepolizumab	Bosatria	IL-5
Metelimumab		TGF beta 1
Milatuzumab		CD74
Minretumomab		TAG-72
Mirikizumab		IL23A
Mirvetuximab		Folate receptor alpha
Mitumomab		GD3 ganglioside
Modotuximab		EGFR extracellular domain III
Mogamulizumab	Poteligeo	CCR4
Monalizumab		NKG2A
Morolimumab		Rhesus factor
Mosunetuzumab		CD3E, MS4A1, CD20
Motavizumab	Numax	respiratory syncytial virus
Moxetumomab		CD22
Muromonab-CD3	Orthoclone OKT3	CD3
Namilumab		CSF2
Naratuximab		CD37
Narnatumab		RON
Natalizumab	Tysabri	integrin α_4
Navicixizumab		DLL4
Navivumab		Influenza A virus hemagglutinin HA
Naxitamab		C-Met
Nebacumab		endotoxin
Necitumumab	Portrazza	EGFR
Nemolizumab		IL31RA
Nerelimomab		TNF-α
Nesvacumab		angiopoietin 2

Name	Trade name	Target
Netakimab		Interleukin 17A
Nimotuzumab	Theracim, Theraloc	EGFR
Nirsevimab		RSVFR
Nivolumab	Opdivo	PD-1
Obiltoxaximab	Anthim	*Bacillus anthracis* anthrax
Obinutuzumab	Gazyva	CD20
Ocaratuzumab		CD20
Ocrelizumab	Ocrevus	CD20
Odulimomab		LFA-1 (CD11a)
Ofatumumab	Arzerra	CD20
Olaratumab	Lartruvo	PDGF-R α
Oleclumab		5'-nucleotidase
Olendalizumab		Complement C5a
Olokizumab		IL6
Omalizumab	Xolair	IgE Fc region
OMS721		MASP-2
Onartuzumab		human scatter factor receptor kinase
Ontuxizumab		TEM1
Onvatilimab		VSIR
Opicinumab		LINGO-1
Oregovomab	OvaRex	CA-125
Orticumab		oxLDL
Otelixizumab		CD3
Otilimab		GMCSF
Otlertuzumab		CD37
Oxelumab		OX-40
Ozanezumab		NOGO-A
Ozoralizumab		TNF-α
Pagibaximab		lipoteichoic acid
Palivizumab	Synagis, Abbosynagis	F protein of respiratory syncytial virus
Pamrevlumab		CTGF
Panitumumab	Vectibix	EGFR
Pankomab		Tumor specific glycosylation of MUC1
Panobacumab		*Pseudomonas aeruginosa*
Parsatuzumab		EGFL7
Pascolizumab		IL-4
Pasotuxizumab		Folate hydrolase

Name	Trade name	Target
Pateclizumab		LTA
Patritumab		ERBB3 (HER3)
Pembrolizumab	Keytruda	PD1
Pemtumomab	Theragyn	MUC1
Perakizumab		IL17A
Pertuzumab	Omnitarg	HER2/neu
Pidilizumab		PD-1
Pinatuzumab		CD22
Pintumomab		adenocarcinoma antigen
Placulumab		human TNF
Plozalizumab		CCR2
Pogalizumab		TNFR superfamily member 4
Polatuzumab		CD79B
Ponezumab		human beta-amyloid
Porgaviximab		Zaire evolavirus glycoprotein
Prasinezumab		NACP
Prezalizumab		ICOSL
Priliximab		CD4
Pritoxaximab		*E. coli* shiga toxin type-1
Pritumumab		vimentin
PRO 140		CCR5
Quilizumab		IGHE
Racotumomab	Vaxira	NGNA ganglioside
Radretumab		fibronectin extra domain-B
Rafivirumab		rabies virus glycoprotein
Ralpancizumab		Neural apoptosis-regulated proteinase 1
Ramucirumab	Cyramza	VEGFR2
Ranevetmab		NGF
Ravagalimab		CD40
Ravulizumab		C5
Raxibacumab		anthrax toxin, protective antigen
Refanezumab		Myelin-associated glycoprotein
Regavirumab		cytomegalovirus glycoprotein B
Relatlimab		LAG3
Remtolumab		Interleukin 17 alpha, TNF
Reslizumab	Cinqair	IL-5
Rilotumumab		HGF
Rinucumab		Platelet-derived growth factor receptor beta.

Name	Trade name	Target
Risankizumab		IL23A
Rituximab	MabThera, Rituxan	CD20
Rivabazumab pegol		*Pseudomonas aeruginosa* type III secretion system
Robatumumab		IGF-1 receptor (CD221)
Rmab	RabiShield	Rabies virus G glycoprotein
Roledumab		RHD
Romilkimab		Interleukin 13
Romosozumab	Evenity	sclerostin
Rontalizumab		IFN-α
Rosmantuzumab		Root plate-specific spondin 3
Rovalpituzumab		DLL3
Rovelizumab	LeukArrest	CD11, CD18
Rozanolixizumab		FCGRT
Ruplizumab	Antova	CD154 (CD40L)
SA237		IL-6R
Sacituzumab		TROP-2
Samalizumab		CD200
Samrotamab		LRRC15
Sapelizumab		IL6R
Sarilumab	Kevzara	IL6
Satralizumab		IL6 receptor
Satumomab		TAG-72
Secukinumab	Cosentyx	IL-17A
Selicrelumab		CD40
Seribantumab		ERBB3 (HER3)
Setoxaximab		*E. coli* shiga toxin type-2
Setrusumab		SOST
Sevirumab		cytomegalovirus
Sibrotuzumab		FAP
SGN-CD19A		CD19
SHP647		Mucosal addressin cell adhesion molecule
Sifalimumab		IFN-α
Siltuximab	Sylvant	IL-6
Simtuzumab		LOXL2
Sintilimab		PD-1
Siplizumab		CD2
Sirtratumab		SLITRK6

Name	Trade name	Target
Sirukumab		IL-6
Sofituzumab		CA-125
Solanezumab		beta amyloid
Sonepcizumab		sphingosine-1-phosphate
Sontuzumab		episialin
Spartalizumab		PDCD1, CD279
Stamulumab		myostatin
Suptavumab		RSVFR
Sutimlimab		C1S
Suvizumab		HIV-1
Suvratoxumab		*Staphylococcus aureus* alpha toxin
Tabalumab		BAFF
Tacatuzumab	AFP-Cide	alpha-fetoprotein
Talacotuzumab		CD123
Talizumab		IgE
Tamtuvetmab	Tactress	CD52
Tanezumab		NGF
Taplitumomab		CD19
Tarextumab		Notch receptor
Tavolimab		CD134
Tefibazumab	Aurexis	clumping factor A
Telisotuzumab		HGFR
Tenatumomab		tenascin C
Teneliximab		CD40
Teplizumab		CD3
Tepoditamab		Dendritic cell-associated lectin 2
Teprotumumab		IGF-1 receptor (CD221)
Tesidolumab		C5
Tetulomab		CD37
Tezepelumab		TSLP
Tibulizumab		BAFF
Tildrakizumab	Ilumya	IL23
Tigatuzumab		TRAIL-R2
Timigutuzumab		HER2
Timolumab		AOC3
Tiragotumab		TIGIT
Tislelizumab		PCDC1, CD279
Tisotumab		Coagulation factor III

Name	Trade name	Target
Tocilizumab	Actemra, RoActemra	IL-6 receptor
Tomuzotuximab		EGFR, HER1
Toralizumab		CD154 (CD40L)
Tosatoxumab		*Staphylococcus aureus*
Tositumomab	Bexxar	CD20
Tovetumab		CD140a
Tralokinumab		IL-13
Trastuzumab	Herceptin	HER2/neu
TRBS07	Ektomab	GD2 ganglioside
Tregalizumab		CD4
Tremelimumab		CTLA-4
Trevogrumab		Growth differentiation factor 8
Tucotuzumab		EpCAM
Tuvirumab		hepatitis B virus
Ublituximab		MS4A1
Ulocuplumab		CXCR4 (CD184)
Urelumab		4-1BB (CD137)
Urtoxazumab		*Escherichia coli*
Ustekinumab	Stelara	IL-12, IL-23
Utomilumab		4-1BB (CD137)
Vadastuximab		CD33
Vanalimab		CD40
Vandortuzumab		STEAP1
Vantictumab		Frizzled receptor
Vanucizumab		angiopoietin 2
Vapaliximab		AOC3 (VAP-1)
Varisacumab		VEGF-A
Varlilumab		CD27
Vatelizumab		ITGA2 (CD49b)
Vedolizumab	Entyvio	integrin $\alpha_4\beta_7$
Veltuzumab		CD20
Vepalimomab		AOC3 (VAP-1)
Vesencumab		NRP1
Visilizumab	Nuvion	CD3
Volociximab		integrin $\alpha_5\beta_1$
Vonlerolizumab		CD134
Vopratelimab		ICOS
Vorsetuzumab		CD70

Name	Trade name	Target
Votumumab	HumaSPECT	tumor antigen CTAA16.88
Vunakizumab		Interleukin 17 alpha
Xentuzumab		IGF1, IGF2
XMAB-5574		CD19
Zalutumumab	HuMax-EGFr	EGFR
Zanolimumab	HuMax-CD4	CD4
Zatuximab		HER1
Zenocutuzumab		ERBB3, HER3
Ziralimumab		CD147 (basigin)
Zolbetuximab		CLDN18
Zolimomab		CD5

In addition to the above, the antibody of the drug antibody conjugate of the present invention may be Vitaxin which is a humanised antibody for the treatment of sarcoma; Smart IDIO which is a humanised anti-HLA-DR antibody for the treatment of non-Hodgkin's lymphoma; Oncolym which is a radiolabeled murine anti-HLA-DrIO antibody for the treatment of non-Hodgkin's lymphoma; and Allomune which is a humanised anti-CD2 mAb for the treatment of Hodgkin's Disease or non-Hodgkin's lymphoma.

The antibody of the drug conjugate of the present invention may also be any antibody-fragment known for the treatment of any disease, preferably cancer. Again, such antibody fragments are immunospecific for a target antigen and can be obtained commercially or produced by any method known in the art such as, e.g., recombinant expression techniques. Examples of such antibodies available include any from the below table.

Table 2: Therapeutic monoclonal antibody fragments

Fragment type/format	Name	Trade name	Target
Fab/chimeric	abciximab	ReoPro	CD41 (integrin alpha-IIb)
Fab/humanised	abrezekimab		Interleukin 13
F(ab')$_2$/mouse	Afelimomab		TNF-α
F(ab')$_2$/humanised	Alacizumab pegol		VEGFR2
Fab/mouse	Anatumomab		TAG-72
Fab/ovine		CroFab	Snake venom
Fab/ovine		DigiFab	Digoxin
Fab/ovine		Digibind	Digoxin

Fragment type/format	Name	Trade name	Target
Fab'/mouse	arcitumomab	CEA-scan	CEA
Fab'/mouse	bectumomab	LymphoScan	CD22
Fab'/mouse	biciromab	FibriScint	fibrin II, beta chain
BiTE/mouse	Blinatumomab	Blincyto	CD19
scFv/humanised	brolucizumab		VEGFA
sdAb/humanised	caplacizumab	Cablivi	VWF
Fab'/PEGylated humanised	certolizumab pegol	Cimzia	TNF-α
Fab/humanised	citatuzumab		EpCAM
$F(ab')_2$/mouse	dorlimomab		unknown
scFv/chimeric humanised	duvortuxizumab		CD19, CD3E
scFv/human	efungumab	Mycograb	Hsp90
$F(ab')_2$/humanised	erlizumab		ITGB2 (CD18)
Di-scFy	flotetuzumab		IL-3 receptor
scFv/human	gancotamab		unknown
$F(ab')_2$/mouse	igovomab	Indimacis-125	CA-125
Fab/humanised	lampalizumab		CFD
scFv/humanised	letolizumab		TRAP
Fab/mouse	nacolomab		C242 antigen
Fab/mouse	naptumomab		5T4
Fab/mouse	nofetumomab		unknown
scFv/humanised	oportuzumab	Vicinium	EpCAM
Fab/humanised	ranibizumab	Lucentis	VEGF-A
BiTE/mouse	Solitomab		EpCAM
Fab'/mouse	sulesomab	LeukoScan	NCA-90 (granulocyte antigen)
Fab	Tadocizumab		integrin $\alpha_{IIb}\beta_3$
Fab/mouse	Telimomab		unknown
scFv/humanised	Vobarilizumab		IL6R
Fab/humanised		Thromboview	D-dimer
Fab/PEGylated humanised	CDP791		VEGF
Fab/bispecific humanised	MDX-H210		Her2/Neu & CD64 (γFcR1)
scFv/humanised	Pexelizumab		Complement C5

Fragment type/format	Name	Trade name	Target
$(ScFv)_4$ fused to streptavidin mouse	CC49		TAG-72 Pancarcinoma antigen
ScFv fused to β-lactamase human	SGN-17		P97 antigen
ScFv fused to PEG human	F5 scFv-PEG Immunoliposome		Her2
Diabody $(V_H\text{-}V_L)_2$ human	C6.5K-A		Her2/Neu
Diabody $(V_H\text{-}V_L)_2$ human	L19 L19-γIFN		EDB domain of fibronectin
Diabody $(V_L\text{-}V_H)_2$ human	T84.66		CEA
Minibody $(scF_v\text{-}C_H3)_2$ murine-human chimera (minibody)	T84.66		CEA
Minibody murine-human chimera (minibody)	10H8		Her2
S_cF_v dimer Fc $(S_cF_v)_2$-Fc murine-human chimera (minibody)	T84.66		CEA
Bispecific scFv $(V_L\text{-}V_H\text{-}V_H\text{-}V_L)$ mouse	r28M		CD28 and MAP
Bispecific scFv $(V_L\text{-}V_H\text{-}V_H\text{-}V_L)$ origin unknown	BiTE MT103		CD19 and CD3
Bispecific scFv $(V_L\text{-}V_H\text{-}V_H\text{-}V_L)$ origin unknown	BiTE		Ep-CAM and CD3
Bispecific tandem diabody	Tandab		CD19 & CD3

Fragment type/format	Name	Trade name	Target
(VH-VL- VH -VL) (mouse)			
VhH-β-lactamase fusion camelid	Nanobody		CEA
Dab/human	Anti-TNFα dAb		TNFα
VhH/camelid	Nanobody		TNFα
VhH/camelid	Nanobody		Von Willebrand factor

Fab fragment, antigen-binding (one arm)

$F(ab')_2$ fragment, antigen-binding, including hinge region (both arms)

Fab'fragment, antigen-binding, including hinge region (one arm)

scFv single-chain variable fragment

di-scFv dimeric, single-chain variable fragment

(Holliger & Hudson, Nature Biotechnology, 2005, 23(9), 1126-1136).

In a preferred embodiment, the antibody in the drug conjugates of the present invention targets a cell surface antigen.

In preferred embodiments, the antibody in the drug conjugates of the present invention may bind to a receptor encoded by the ErbB gene. The antibody may bind specifically to an ErbB receptor selected from EGFR, HER2, HER3 and HER4. Preferably, the antibody in the drug conjugate may specifically bind to the extracellular domain of the HER2 receptor and inhibit the growth of tumour cells which overexpress the HER2 receptor. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody. Preferably, the humanised antibody may be huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 or huMAb4D5-8 (Trastuzumab), particularly preferably Trastuzumab. The antibody may also be an antibody fragment, e.g. a Fab fragment.

Other preferred antibodies include:

(i) anti-CD4 antibodies. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody;

(ii) anti-CD5 antibodies. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody;

(iii) anti-CD13 antibodies. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody;

(iv) anti-CD20 antibodies. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody. Preferably, the humanised antibody is Rituximab or an antibody fragment thereof, e.g. a Fab fragment; and

(v) anti-CD30 antibodies. The antibody of the drug conjugate may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanised antibody. Preferably the humanised antibody is Brentuximab vedotin or an antibody fragment thereof.

In one embodiment of the invention, the drug antibody conjugate may demonstrate one or more of the following: (i) increased cytotoxicity (or a decrease in cell survival), (ii) increased cytostatic activity (cytostasis), (iii) increased binding affinity to the target antigen or epitope, (iv) increased internalisation of the conjugate, (v) reduction of patient side effects, and/or (vi) improved toxicity profile. Such increase may be relative to a known drug antibody conjugate in the art that binds the same or a different epitope or antigen.

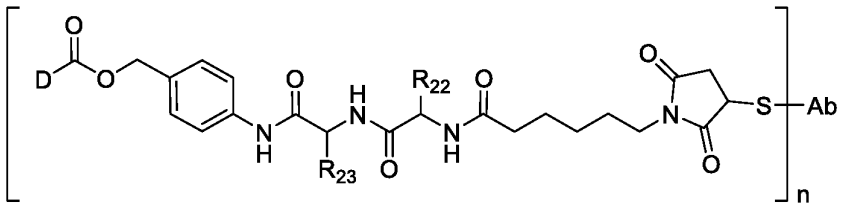
Processes For The Preparation Of The Drug Antibody Conjugates

The drug antibody conjugates of the present invention can be prepared according to techniques that are well known in the art. Processes for conjugating moieties comprising at least one antigen binding site antibodies such as antibodies to a number of different drugs using different processes have been described and exemplified previously in, for example, WO-A-2004/010957, WO-A-2006/060533 and WO-A-2007/024536, the contents of which are incorporated herein by reference thereto. These involve use of a linker group that derivatises the drug, toxin or radionuclide in such a way that it can then be attached to the moiety such as an antibody. Attachment to the moiety such as an antibody is typically by one of three routes: via free thiol groups in cysteines after partial reduction of disulfide groups in the antibody; via free amino groups in lysines in the antibody; and via free hydroxyl groups in serines and/or threonines in the antibody. The attachment method varies depending upon the site of attachment on the moiety such as an antibody. Purification of antibody-drug conjugates by size exclusion chromatography (SEC) has also been described [see, e.g., Liu et al., Proc. Natl. Acad. Set (USA), 93: 8618-8623 (1996), and Chari et al., Cancer Research, 52: 127-131 (1992)].

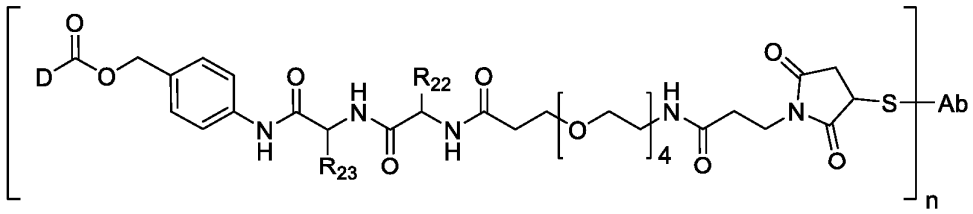
As previously noted, the drug payloads of the drug conjugates of the present invention are ecteinascidin derivatives disclosed in, or fall within the scope of, International patent application no. PCT/EP2018/060868, the contents of which are incorporated herein by reference thereto. These compounds are synthesised according to the processes described in the present application.

As noted earlier, there is provided a process for the preparation of a drug conjugate according to the present invention comprising conjugating a moiety Ab comprising at least one antigen binding site and a drug D of formula (IH), (IHa) or (IHb), Ab and D being as defined herein.

One example of a process for the preparation of a drug conjugate of the present invention involves the preparation of drug antibody conjugates of formula (G) or (G') of the present invention as follows:



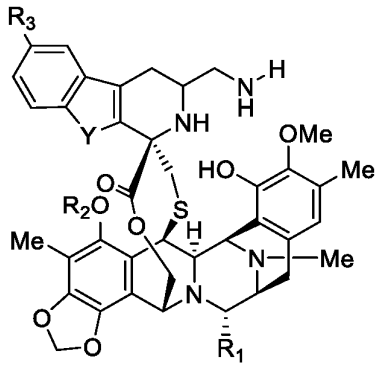
(G)



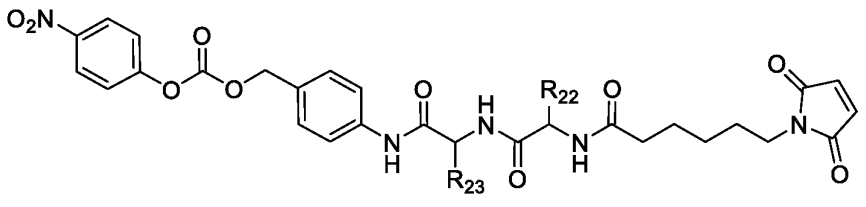
(G')

said process comprising the following steps:

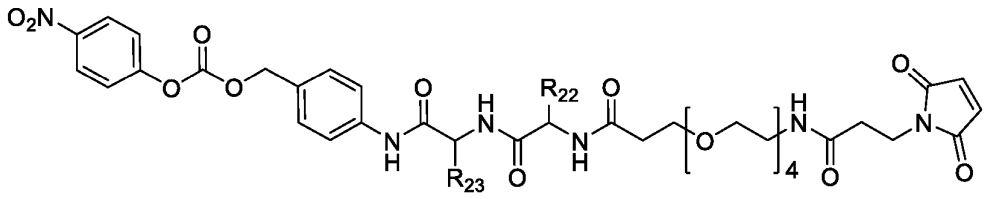
(i) reacting a drug (D-H) of formula (IH)-H:



wherein the substituents in the definitions of (IH)-H are as defined above for formula (IH), with a compound of formula (D') or (E):

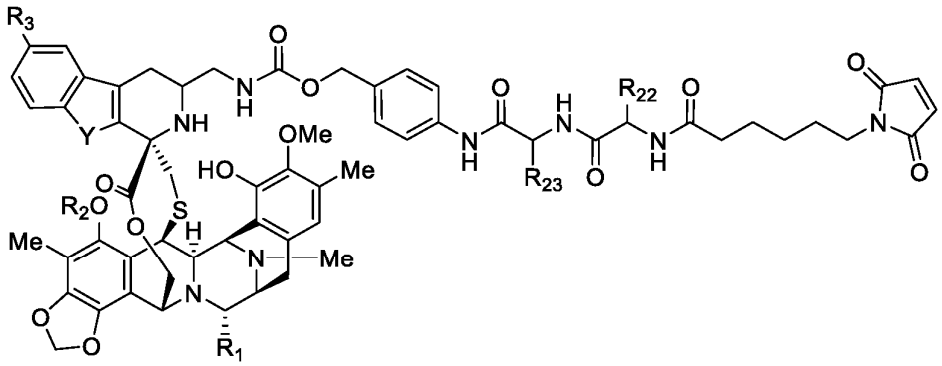


(D')

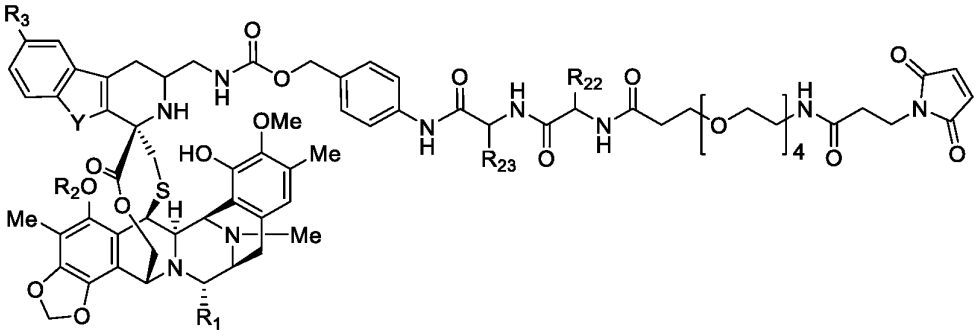


(E)

to give a compound of formula (F) or (F'), respectively:

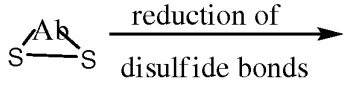


(F)



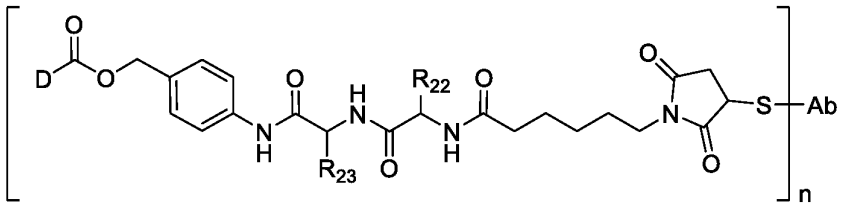
(F')

(ii) partial reduction of one or more disulfide bonds in the antibody to be conjugated to give a reduced antibody Ab-SH having free thiol groups:

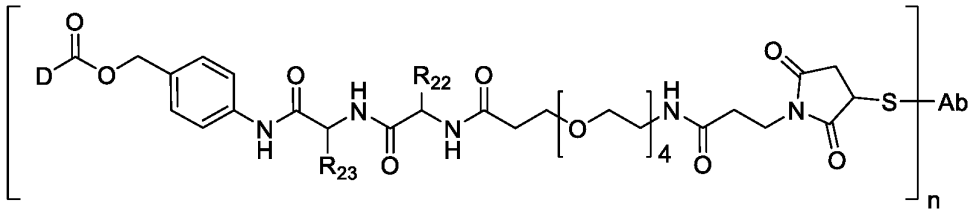


; and

(iii) reaction of the partially reduced antibody Ab-SH having free thiol groups with the compound of formula (F) or (F') produced in step (i) to give the desired drug antibody conjugate of formula (G) or (G') respectively:



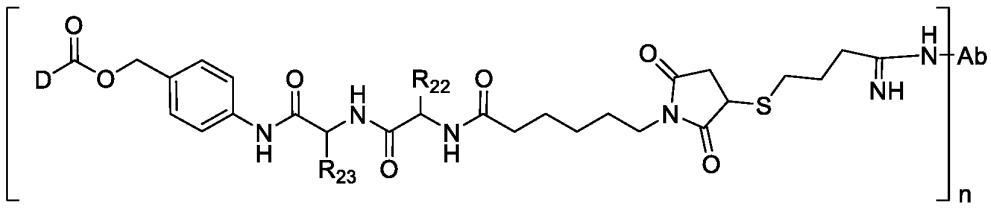
(G)



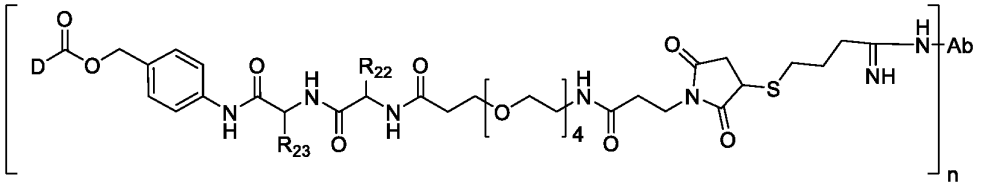
(G')

In another preferred embodiment of this process, the antibody is selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, or it is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, and most preferably it is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof. Furthermore, the partial reduction of this monoclonal antibodody is performed using tris[2-carboxyethyl]phosphine hydrochloride (TCEP).

Another example of a process for the preparation of a drug conjugate of the present invention involves the preparation of drug antibody conjugates of formula (W) or (W') of the present invention as follows:



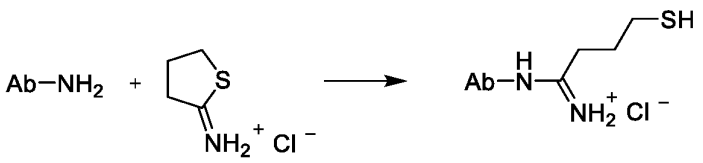
(W)



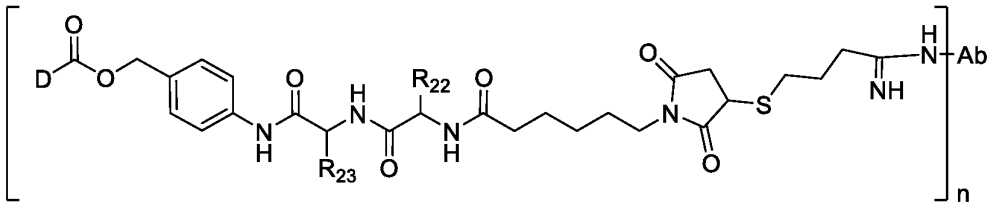
(W')

said process comprising the following steps:

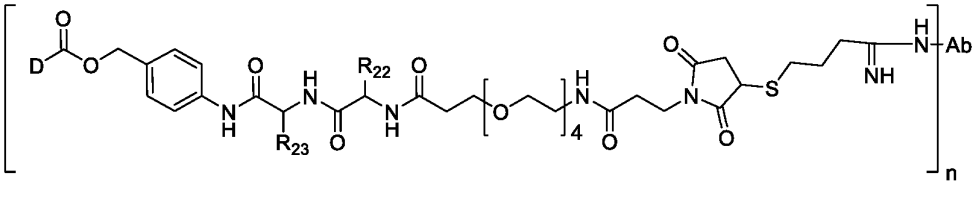
(i) reacting the antibody with 2-iminothiolane hydrochloride (Traut´s reagent) to give a thiol-activated antibody:



(ii) reacting the thiol-activated antibody with the compound of formula (F) or (F'), to give the desired drug antibody conjugate of formula (W) or (W'), respectively.



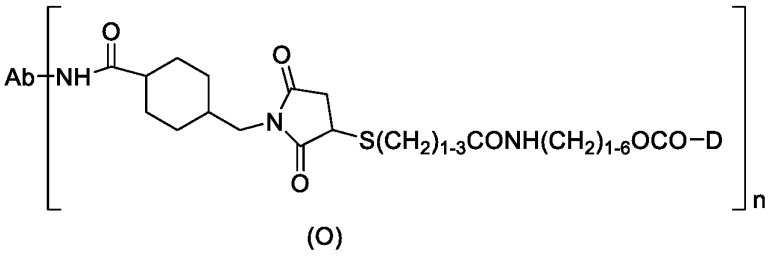
(W)



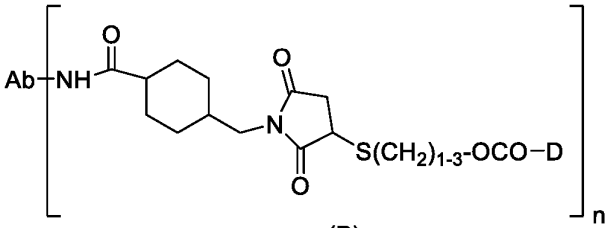
(W').

In another preferred embodiment of this process, the antibody is selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, or it is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, and most preferably it is Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof.

Another example of a process for the preparation of a drug antibody conjugate of the present invention, involves the preparation of drug antibody conjugates of formula (O) or (P) as follows:



(O)



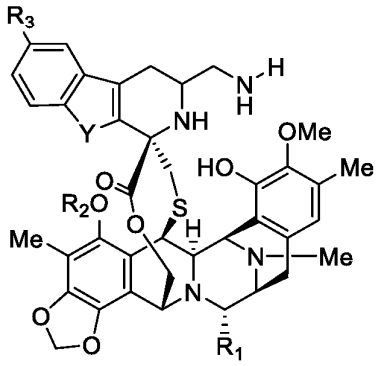
(P)

:

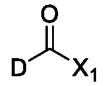
said process comprising the following steps:

(i) either:

(a) reacting a drug (D-H) of formula (IH)-H:



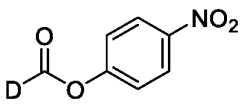
wherein the substituents in the definitions of (IH)-H are as defined above, with a compound of formula X_2-C(O)-X_1 wherein X_1 and X_2 are leaving groups to give a compound of formula (B):



(B)

and the point of attachment of the -(C=O)X_1 moiety is the free -NH_2 group of the compound of formula D-H, or

(b) reacting said drug (D-H) of formula (IH)-H as defined above with 4-nitro-phenylchloroformate to give a compound of formula (J):

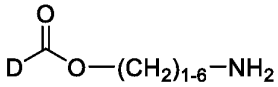


(J)

and the point of attachment of the (4-nitrophenyl)-O-CO- group is the same as that for the $X_1(CO)$ moiety in (a) above;

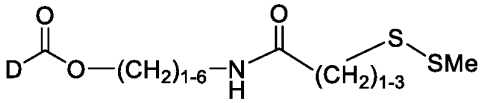
(ii)either:

(c) reacting the compound of formula (B) produced in step (i) with a hydroxy compound of formula HO-$(CH_2)_{1-6}$NHProt^{NH} and removing the Prot^{NH} group from the coupled compound to give a compound of formula (C):



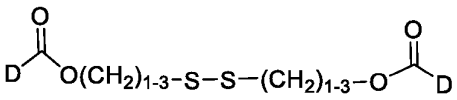
(C)

and then reacting the resulting compound of formula (C) with a compound of formula Me-S-S-$(CH_2)_{1-3}$-CO_2H to give a compound of formula (K):



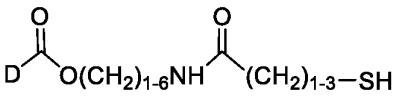
(K) , or

(d) reacting the compound (J) produced in step (i) with a compound of formula HO-$(CH_2)_{1-3}$SProt^{SH} and removing the Prot^{SH} group from the coupled compound to give a compound of formula (L):

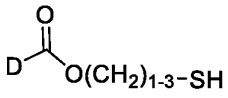


(L)

(iii) reacting (K) or (L) produced in step (ii) with dithiothreitol under disulfide reducing conditions to give compounds of formula (M) and (N) respectively:

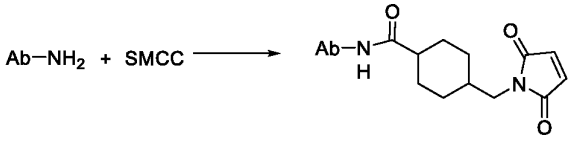


(M)

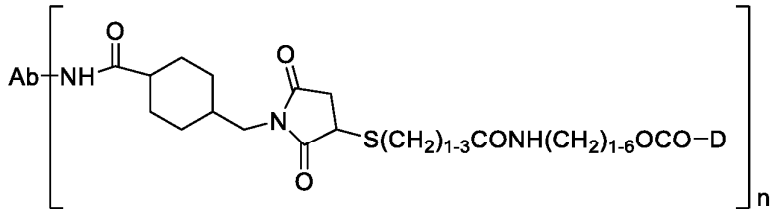


(N)

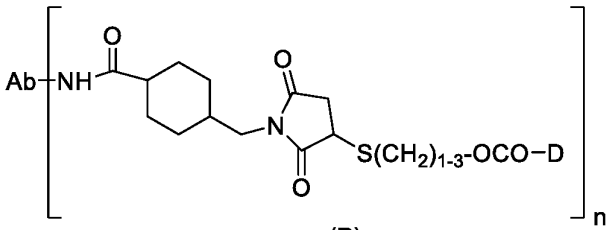
(iv) reacting the antibody to be conjugated with succininimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate to derivatise said antibody at one or more lysine groups with a succininimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carbonyl group:



(v) reacting the derivatised antibody produced in step (iv) with either (M) or (N) produced in step (iii) to give the desired drug antibody conjugate of formula (O) or (P):



(O)



(P)

The compound of formula X_2-C(O)-X_1 is preferably 1,1'-carbonyldiimidazole. Similarly, the hydroxy compound reacted with the compound of formula (B) is preferably HO-$(CH_2)_{2-4}$-NHProtNH, and more preferably HO-$(CH_2)_3$-NHProt^{NH}.

In one preferred embodiment of this invention, the compound reacted with the compound of formula (C) to give the compound of formula (K) is 3-(methyldisulfanyl)propanoic acid.

In another preferred embodiment, the compound HO-$(CH_2)_{1-3}$SProt^{SH} that is reacted with a compound of formula (J) to give a compound of formula (L) is HO-$(CH_2)_3$SProt^{SH}.

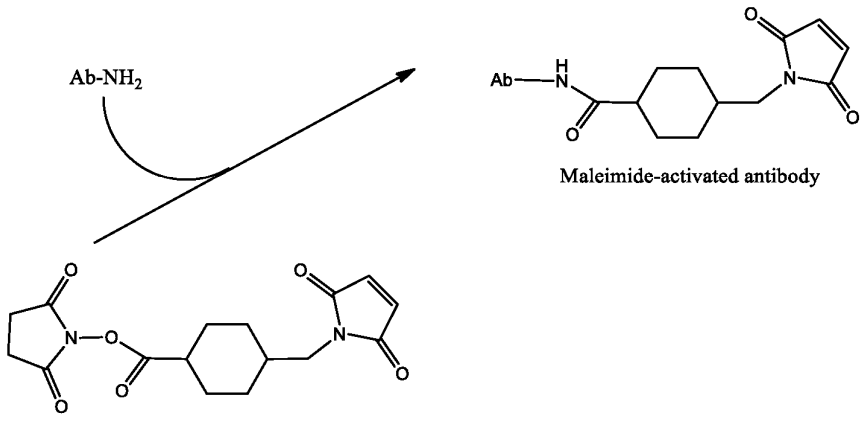
Where attachment to the drug linker moiety is via free thiol groups in cysteines after partial reduction of disulfide groups in the moiety comprising at least one antigen binding site

such as a monoclonal antibody, the partial reduction is typically conducted by first diluting to a suitable concentration and buffering the solution before partial reduction of the disulfide bonds by means of the addition of a suitable reducing agent such as tris[2-carboxyethyl]phosphine hydrochloride (TCEP) or dithiothreitol (DTT). By choosing appropriate ratios of the moiety to be reduced such as a monoclonal antibody and the reducing agent, the reaction conditions and the time of the reduction it is possible to obtain a desired free thiol to moiety ratio, e.g. four free thiol groups per monoclonal antibody.

The partially reduced moiety such as the partially reduced monoclonal antibody having the free thiol groups, prepared as described above, is then reacted with drug-linker compounds of the invention of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1 (wherein the group L_1 in such compound is a maleimide group which is free to react with the thiol groups). The resulting drug antibody conjugates are purified by any suitable means known in the art, e.g. by size exclusion chromatography (SEC) [see, e.g., Liu et al., Proc. Natl. Acad. Sci. USA, 93: 8618-8623 (1996), and Chari et al., Cancer Research, 52: 127-131 (1992)].

In one preferred embodiment of this invention, the partially reduced monoclonal antibody is Trastuzumab or an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, preferably Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof; or preferably an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof.

In an alternative embodiment of the invention, lysines in the moiety comprising at least one antigen binding site such as a monoclonal antibody can first be reacted with succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate. A free amine group on an antibody can react with the N-hydroxysuccinimide ester to give a maleimide-activated antibody:

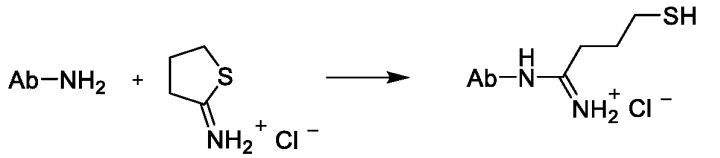


Maleimide-activated antibody

SMCC

The maleimide-activated antibody can then be reacted with a compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-H having a reactive thiol moiety.

In an alternative embodiment of the invention, lysines in the moiety comprising at least one antigen binding site such as a monoclonal antibody can first be reacted with 2-iminothiolane hydrochloride (Traut´s reagent). A free amine group on an antibody can react with the imidic thiolactone to give a thiol-activated antibody.



thiol-activated antibody

One specific example of processes for the preparation of drug antibody conjugates of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab of the present invention by conjugation via free thiol groups in cysteines after partial reduction of disulfide groups in the antibody is shown in Figure 1.

Another specific example of processes for the preparation of drug antibody conjugates of formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab of the present invention by conjugation with free amino groups in lysines after reaction of the antibody with Traut's reagent is shown in Figure 2.

Compositions Comprising the Drug Antibody Conjugate of the Invention and Uses Thereof

There is also provided a pharmaceutical composition comprising a drug conjugate according to the present invention and a pharmaceutically acceptable carrier. Examples of the administration form of a drug conjugate having the general formula [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-]$_n$-Ab of the present invention include without limitation oral, topical, parenteral, sublingual, rectal, vaginal, ocular, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Preferably, the compositions are administered parenterally. Pharmaceutical compositions of the invention can be formulated so as to allow a drug conjugate of the present invention to be bioavailable upon administration of the composition to an animal, preferably human. Compositions can take the form of one or more dosage units, where for example, a tablet can be a single dosage unit, and a container of a drug antibody conjugate of the present invention in aerosol form can hold a plurality of dosage units.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s)

can be gaseous, so as to provide an aerosol composition useful in, for example, inhalatory administration. The term "carrier" refers to a diluent, adjuvant or excipient, with which a drug antibody conjugate of the present invention is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to an animal, the drug antibody conjugates of the present invention or compositions and pharmaceutically acceptable carriers are sterile. Water is a preferred carrier when the drug antibody conjugates of the present invention are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, corn starch and the like; lubricants such as magnesium stearate; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent.

When the composition is in the form of a capsule (e.g. a gelatin capsule), it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The composition can be in the form of a liquid, e.g. an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The preferred route of administration is parenteral administration including, but not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, intranasal, intracerebral, intraventricular, intrathecal, intravaginal or transdermal. The preferred mode of administration is left to the discretion of the practitioner, and will depend in part upon the site of the medical condition (such as the site of cancer). In a more preferred embodiment, the present drug antibody conjugates of the present invention are administered intravenously.

The liquid compositions of the invention, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or digylcerides, polyethylene glycols, glycerin, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in an ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is a preferred adjuvant.

The amount of the drug conjugate of the present invention that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The compositions comprise an effective amount of a drug conjugate of the present invention such that a suitable dosage will be obtained. The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and its particular site, host and the diease being treated, e.g. cancer and, if so, what type of tumor. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The drug conjugate of the present invention or compositions can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings.

In specific embodiments, it can be desirable to administer one or more drug conjugates of the present invention or compositions locally to the area in need of treatment. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue. In another embodiment, administration

can be by direct injection at the site (or former site) of a manifestation of an autoimmune disease.

Pulmonary administration can also be employed, e.g. by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the drug antibody conjugate of the present invention or compositions can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained- release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The pharmaceutical compositions can be prepared using methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining a drug conjugate of the present invention with water so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension.

We have found that the drug conjugates and compositions of the present invention are particularly effective in the treatment of cancer.

Thus, as described earlier, the present invention provides a method of treating a patient in need thereof, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a drug conjugate or a composition of the present invention. The present invention provides a drug conjugate according to the present invention for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred cancer is breast cancer.

The drug conjugates and compositions of the present invention are useful for inhibiting the multiplication of a tumor cell or cancer cell, or for treating cancer in an animal. The drug conjugates and compositions of the present invention can be used accordingly in a variety of settings for the treatment of animal cancers. The conjugates of the invention comprising Drug -Linker-Moiety comprising at least one antigen binding site can be used to deliver a Drug or Drug unit to a tumor cell or cancer cell. Without being bound by theory, in one embodiment, the Moiety comprising at least one antigen binding site of a drug conjugate of the present invention binds to or associates with a cancer-cell or a tumor-cell-associated antigen, and the drug conjugate of the present invention can be taken up inside a tumor cell

or cancer cell through receptor-mediated endocytosis. The antigen can be attached to a tumor cell or cancer cell or can be an extracellular matrix protein associated with the tumor cell or cancer cell. Once inside the cell, one or more specific sequences within the Linker unit are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases or hydrolases, resulting in release of a Drug or a Drug-Linker Compound. The released Drug or Drug-Linker Compound is then free to migrate in the cell and induce cytotoxic activities. In an alternative embodiment, the Drug or Drug unit is cleaved from the drug conjugate of the present invention outside the tumor cell or cancer cell, and the Drug or Drug-Linker Compound subsequently penetrates the cell.

In one embodiment, the Moiety comprising at least one antigen binding site binds to the tumor cell or cancer cell. In another embodiment, the Moiety comprising at least one antigen binding site binds to a tumor cell or cancer cell antigen which is on the surface of the tumor cell or cancer cell. In yet another embodiment, the Moiety comprising at least one antigen binding site binds to a tumor cell or cancer cell antigen which is an extracellular matrix protein associated with the tumor cell or cancer cell.

The specificity of the Moiety comprising at least one antigen binding site for a particular tumor cell or cancer cell can be important for determining those tumors or cancers that are most effectively treated. For example, drug conjugates of the present invention having a Trastuzumab unit can be useful for treating antigen positive carcinomas including leukaemias, lung cancer, colon cancer, lymphomas (e.g. Hodgkin's disease, non-Hodgkin's Lymphoma), solid tumors such as, sarcoma and carcinomas, Multiple myeloma, kidney cancer and melanoma. The cancer may preferably be lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma or ovarian cancer. For example, drug conjugates of the present invention having a Rituximab unit can be useful for treating CD-20 expressing tumors such as haematological cancers including leukemias and lymphomas. For example, drug conjugates of the present invention having an anti-CD4 antibody unit can be useful for treating CD-4 expressing tumors such as haematological cancers including lymphomas. For example, drug conjugates of the present invention having an anti-CD5 antibody unit can be useful for treating CD-5 expressing tumors such as haematological cancers including leukemias and lymphomas. For example, drug conjugates of the present invention having an anti-CD13 antibody unit can be useful for treating CD-13 expressing tumors such as haematological cancers including leukemias and lymphomas.

Other particular types of cancers that can be treated with drug conjugates of the present invention include, but are not limited to: blood-borne cancers including all forms of leukemia; lymphomas, such as Hodgkin's disease, non-Hodgkin's Lymphoma and Multiple myeloma.

In particular, the drug conjugates and compositions of the present invention show excellent activity in the treatment of breast cancer.

Drug conjugates and compositions of the present invention provide conjugation specific tumor or cancer targeting, thus reducing general toxicity of these conjugates. The Linker units stabilize the drug antibody conjugates in blood, yet are cleavable by tumor-specific proteases and hydrolases within the cell, liberating a Drug.

The drug conjugates and compositions of the present invention can be administered to an animal that has also undergone surgery as treatment for the cancer. In one embodiment of the present invention, the additional method of treatment is radiation therapy.

In a specific embodiment of the present invention, the drug conjugate or composition of the present invention may be administered with radiotherapy. Radiotherapy may be administered at the same time, prior to or after treatment with the drug conjugate or composition of the present invention. In an embodiment, the drug conjugate or composition of the present invention is administered concurrently with radiation therapy. In another specific embodiment, the radiation therapy is administered prior or subsequent to administration of a drug conjugate or composition of the present invention, preferably at least an hour, five hours, 12 hours, a day, a week, a month, more preferably several months (e.g. up to three months), prior or subsequent to administration of a drug antibody conjugate or composition of the present invention.

With respect to radiation, any radiation therapy protocol can be used depending upon the type of cancer to be treated. For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater that 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

In the present invention, there is provided a kit comprising a therapeutically effective amount of a drug conjugate according to the present invention and a pharmaceutically acceptable carrier. In an embodiment, there is provided a kit comprising a composition according to the present invention and, optionally, instructions for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer.

In one embodiment, the kit according to this aspect is for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer. Most preferred kit is for use in the treatment of breast cancer.

Brief Description of the Drawings

The invention is diagrammatically illustrated, by way of example, in the accompanying drawings in which:

Figure 1 is a schematic illustration of one process according to the present invention wherein conjugation to the antibody is via free thiol groups;

Figure 2 is a schematic illustration of one process according to the present invention wherein conjugation to the antibody is via free amino groups.

Figure 3. Dose-response curves corresponding to one representative experiment conducted to evaluate the antiproliferative potential of the **ADC 1** in HER2-positive (black symbols) or HER2-negative (hollow symbols) cell lines. Dots correspond to the average of triplicates with error bars denoting SD, drawing lines correspond to the best fitting by nonlinear regression of the experimental points to a four-parameters logistic curve used to obtain the IC_{50} values reported in Table 13.

Figure 4. Histograms showing the effect on cell survival of either Trastuzumab at 20 µg/mL or the **ADC 1** at 16 or 2.5 µg/mL in several cell lines, either HER2-positive (SK-BR-3 and HCC-1954) or HER2-negative (MDA-MB-231 and MCF-7). Bars correspond to the average of three determinations with error bars denoting SD. Statistical significance was measured using an unpaired two-tailed t-test, p values are summarized as follows: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$

Figure 5. Dose-response curves showing the antiproliferative potential of the **ADC 2** in HER2-positive (black symbols) or HER2-negative (hollow symbols) cell lines. Dots correspond to the average of triplicates with error bars denoting SD, drawing lines correspond to the best fitting by nonlinear regression of the experimental points to a four-parameters logistic curve used to obtain the IC_{50} values reported in Table 15.

Figure 6. Histograms showing the effect on cell survival of either Trastuzumab at 20 µg/mL or the **ADC 2** at 16 or 2.5 µg/mL in several cell lines, either HER2-positive (SK-BR-3 and HCC-1954) or HER2-negative (MDA-MB-231 and MCF-7). Bars correspond to the average of three determinations with error bars denoting SD. Statistical significance was measured using an unpaired two-tailed t-test, p values are summarized as follows: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$.

Figure 7. Dose-response curves showing the antiproliferative potential of the **ADC 3** in HER2-positive (black symbols) or HER2-negative (hollow symbols) cell lines. Dots correspond to the average of triplicates with error bars denoting SD, drawing lines correspond to the best fitting by nonlinear regression of the experimental points to a four-parameters logistic curve used to obtain the IC_{50} values reported in Table 17.

Figure 8. Histograms showing the effect on cell survival of either Trastuzumab at 20 µg/mL or the **ADC 3** at 20 or 3 µg/mL in several cell lines, either HER2-positive (SK-BR-3 and HCC-1954) or HER2-negative (MDA-MB-231 and MCF-7). Bars correspond to the average of three determinations with error bars denoting SD. Statistical significance was measured using an unpaired two-tailed t-test, p values are summarized as follows: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$.

Figure 9. Dose-response curves showing the antiproliferative potential of the **ADC 4** in HER2-positive (black symbols) or HER2-negative (hollow symbols) cell lines. Dots correspond to the average of triplicates with error bars denoting SD, drawing lines correspond to the best fitting by nonlinear regression of the experimental points to a four-parameters logistic curve used to obtain the IC_{50} values reported in Table 18.

Figure 10. Histograms showing the effect on cell survival of either Trastuzumab at 3 µg/mL or the **ADC 4** at 3 or 0.2 µg/mL in several cell lines, either HER2-positive (SK-BR-3 and HCC-1954) or HER2-negative (MDA-MB-231 and MCF-7). Bars correspond to the average of three determinations with error bars denoting SD. Statistical significance was measured using an unpaired two-tailed t-test, p values are summarized as follows: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$.

Figure 11. Dose-response curves showing the antiproliferative potential of the **ADC 6** in HER2-positive (black symbols) or HER2 negative (hollow symbols) cell lines. Dots correspond to the average of triplicates with error bars denoting SD, drawing lines correspond to the best fitting by nonlinear regression of the experimental points to a four-parameters logistic curve used to obtain the IC_{50} values reported in Table 21.

Figure 12. Tumor volume evaluation of BT-474 tumors in mice treated with placebo, **11-R** (at 5 mg/kg), **ADC 1 (2.2 TCEP)** (at 1.6 and 6.5 mg/kg) and **ADC 1** (at 2.24 mg/kg).

Figure 13. Tumor volume evaluation of JIMT-1 tumors in mice treated with placebo, **11-R** (at 5.0 mg/kg) and **ADC 1** (at 5, 10 and 30 mg/kg).

Figure 14. Tumor volume evaluation of SKOV3 tumors in mice treated with placebo, **11-R** (at 5.0 mg/kg) and **ADC 1** (at 5, 10 and 30 mg/kg).

Figure 15. Tumor volume evaluation of N87 tumors in mice treated with placebo, **11-R** (at 5 mg/kg) and **ADC 1 (2.2 TCEP)** (at 5, 10 and 30 mg/kg)-

Figure 16 Tumor volume evaluation of Gastric-008 (PDX) tumors in mice treated with placebo, **11-R** (at 5 mg/kg) and **ADC-1** (at 5, 10 and 30 mg/kg).

Examples

The present invention is further illustrated by way of the following, non-limiting examples. In the examples, the following abbreviations are used:

CDI, 1,1'-Carbonyldiimidazole
DIPEA, *N*,*N*-Diisopropylethylamine
Hex, Hexane
EtOAc, Ethyl acetate
DCM, Dichloromethane
NMP, *N*-Methyl-2-pyrrolidone
DMF, Dimethylformamide
EDC, *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride
EDTA, Ethylenediaminetetraacetic acid
MeOH, Methanol
DTT, Dithiothreitol
Py, Pyridine
THF, Tetrahydrofuran
TCEP, Tris[2-carboxyethyl]phosphine hydrochloride
MC, 6-Maleimidocaproyl
Fmoc, 9-Fluorenylmethoxycarbonyl
Cit, Citrulline
Val, Valine
DMSO, Dimethylsulfoxide
Trt, Triphenylmethyl
HOBt, 1-Hydroxybenzotriazole
DIPCDI, *N*,*N'*-Diisopropylcarbodiimide
TFA, Trifluoroacetic acid
PABOH, 4-Aminobenzyl alcohol
bis-PNP, bis(4-Nitrophenyl) carbonate
NAC, *N*-Acetylcysteine
SEC, Size-Exclusion Chromatography
HPLC, High Performance Liquid Chromatography
ADC, Antibody Drug Conjugate
ATCC, American Type Culture Collection
DMEM, Dulbecco's Modified Eagle's Medium
RPMI, Rosmell Park Memorial Institute Medium
ITS, Insulin-transferrin-sodium selenite media supplement
FCS, Fetal Calf Serum
SRB, Sulforhodamine B
PBS, Phosphate Buffered Saline
DR, Dose-Response
UV, Ultraviolet

SMCC, Succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate

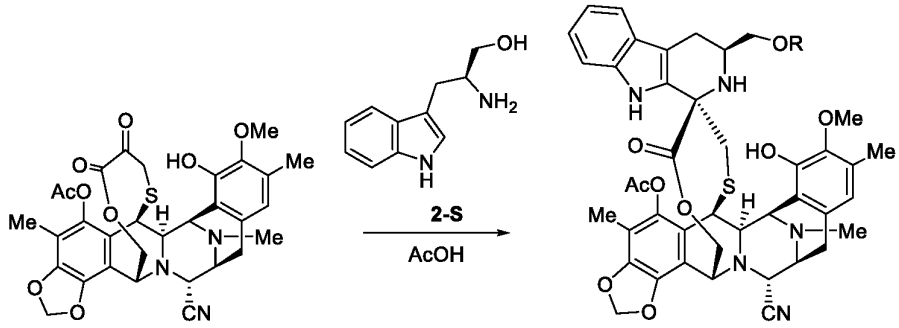
LAR, Linker to Antibody Ratio

Synthesis of compounds

Compound **1** was prepared as described in Example 20 of **WO 01/87895**.

Example 0-1.

A)



1 **3-S** R = H + **3a-S** R = Ac

To a solution of **1** (0.5 g, 0.80 mmol) in acetic acid (20 mL, 0.04 M) was added *L*-tryptophanol (**2-S**) (533 mg, 3.0 mmol, Sigma-Aldrich). The reaction mixture was stirred at 23 ºC for 16 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compounds **3-S** (616 mg, 97%) and **3a-S** (12 mg, 2%).

3-S

R_f= 0.50 (Hexane:EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.36 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.27 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.13 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.26 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.3 Hz, 1H), 5.75 (s, 1H), 5.14 (dd, *J* = 11.7, 1.2 Hz, 1H), 4.60 (s, 1H), 4.41 (s, 1H), 4.36-4.24 (m, 2H), 4.21 (d, *J* = 2.7 Hz, 1H), 3.82 (s, 3H), 3.52 (s, 1H), 3.50-3.47 (m, 1H), 3.45 (dq, *J* = 8.4, 2.2 Hz, 1H), 3.35 (t, *J* = 10.1 Hz, 1H), 3.01-2.78 (m, 5H), 2.62 (dd, *J* = 15.3, 4.7 Hz, 1H), 2.41 (s, 1H), 2.38 (s, 3H), 2.37-2.31 (m, 1H), 2.28 (s, 3H), 2.17 (s, 3H), 2.06 (s, 3H).

ESI-MS *m/z*: 794.2 $(M+H)^+$.

3a-S

R_f= 0.70 (Hexane:EtOAc, 1:1).

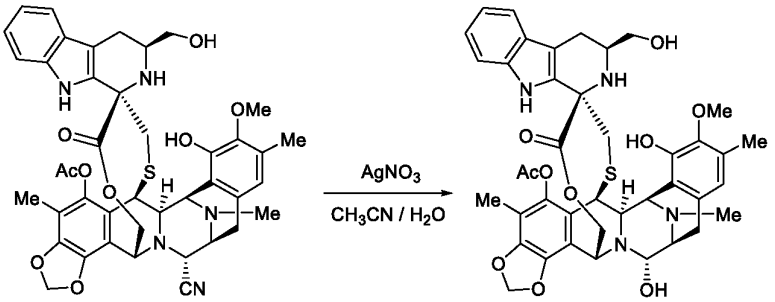
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (s, 1H), 7.38 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.25 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.11 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.02 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.24 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.3 Hz, 1H), 5.79 (s, 1H), 5.13 (d, *J* = 11.7 Hz, 1H), 4.60

(s, 1H), 4.39 (s, 1H), 4.36-4.22 (m, 3H), 4.17-4.09 (m, 1H), 3.91 (dd, *J* = 10.5, 8.6 Hz, 1H), 3.83 (s, 3H), 3.51-3.41 (m, 2H), 3.04-2.92 (m, 3H), 2.72 (dd, *J* = 15.1, 4.0 Hz, 1H), 2.54-2.41 (m, 2H), 2.38 (s, 3H), 2.35-2.30 (m, 1H), 2.29 (s, 3H), 2.21-2.16 (m, 1H), 2.18 (s, 3H), 2.12 (s, 3H); 2.05 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 170.7, 168.6, 147.5, 145.8, 143.0, 141.1, 140.4, 135.6, 130.1, 129.5, 126.7, 122.2, 121.2, 120.9, 119.4, 118.4, 118.2, 118.2, 113.6, 113.5, 110.9, 110.0, 109.1, 102.1, 91.4, 67.2, 63.4, 61.3, 60.4, 59.7, 59.1, 54.8, 54.6, 47.7, 42.0, 41.6, 31.6, 24.0, 22.6, 21.0, 15.9, 14.2, 9.7.

ESI-MS *m/z*: 836.2 $(M+H)^+$.

B)



3-S **4-S**

To a solution of **3-S** (616 mg, 0.77 mmol) in CH_3CN:H_2O (1.39:1, 51 mL, 0.015 M) was added $AgNO_3$ (3.40 g, 23.3 mmol). After 3 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **4-S** (471 mg, 78%).

R_f= 0.50 (CH_2Cl_2:CH_3OH, 9:1).

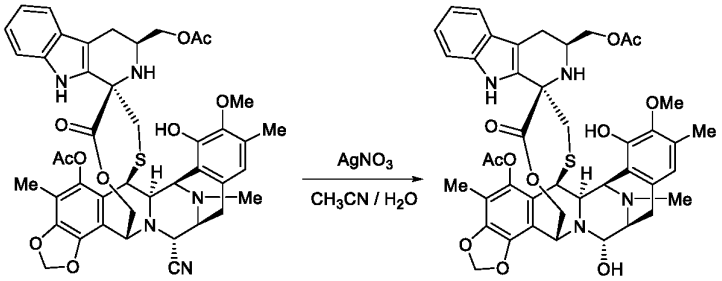
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.36 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.26 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 6.64 (s, 1H), 6.23 (d, *J* = 1.3 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 5.75 (s, 1H), 5.25 (d, *J* = 11.4 Hz, 1H), 4.92 (s, 1H), 4.52 (br s, 3H), 4.22 (dd, *J* = 11.4, 2.2 Hz, 1H), 4.19 (s, 1H), 3.83 (s, 3H), 3.54 (br s, 2H), 3.35 (t, *J* = 10.2 Hz, 1H), 3.26 (s, 1H), 3.01-2.93 (m, 3H), 2.88 (br s, 3H), 2.63 (dd, *J* = 15.2, 4.8 Hz, 1H), 2.38 (s, 3H), 2.36-2.31 (m, 2H), 2.28 (s, 3H), 2.05 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.9, 168.6, 147.5, 145.4, 142.9, 141.2, 140.7, 135.5, 130.4, 126.8, 122.3, 122.0, 121.3, 119.4, 118.4, 115.2, 112.8, 111.0, 110.0, 109.6, 101.8, 81.9, 76.8, 65.2, 62.8, 62.5, 60.4, 58.1, 57.9, 55.9, 55.1, 53.4, 51.6, 41.8, 41.3, 39.6, 24.1, 23.8, 20.5, 15.8, 9.7.

ESI-MS *m/z*: 767.3 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z* 767.2788 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$: 767.2745).

B')



3a-S 4a-S

To a solution of **3a-S** (30 mg, 0.035 mmol) in CH_3CN:H_2O (1.39:1, 2.4 mL, 0.015 M) was added $AgNO_3$ (180 mg, 1.07 mmol). After 3 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **4a-S** (24 mg, 83%).

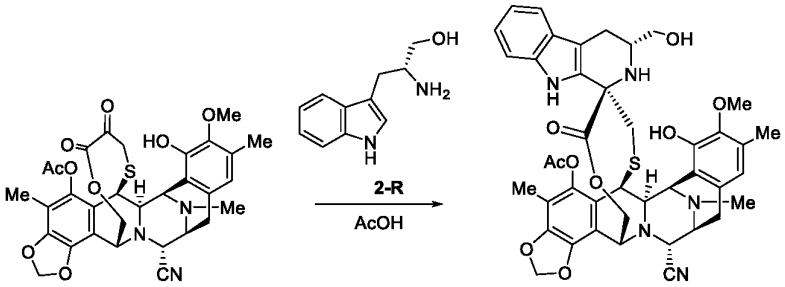
R_f= 0.60 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.30-7.21 (m, 1H), 7.06 (dddt, *J* = 34.7, 8.0, 7.1, 1.1 Hz, 2H), 6.63 (s, 1H), 6.22 (d, *J* = 1.3 Hz, 1H), 6.02 (dd, *J* = 12.9, 1.4 Hz, 1H), 5.74 (s, 1H), 5.25-5.21 (m, 1H), 4.89 (d, *J* = 8.7 Hz, 1H), 4.55-4.45 (m, 2H), 4.30-4.18 (m, 1H), 4.14 (dd, *J* = 10.5, 4.2 Hz, 1H), 4.00-3.88 (m, 2H), 3.82 (s, 3H), 3.56-3.44 (m, 2H), 3.23 (d, *J* = 9.0 Hz, 1H), 2.95 (d, *J* = 15.7 Hz, 2H), 2.87-2.78 (m, 2H), 2.71 (dd, *J* = 15.0, 3.9 Hz, 1H), 2.48 (dd, *J* = 15.1, 9.6 Hz, 1H), 2.37 (s, 3H), 2.35-2.29 (m, 1H), 2.28 (s, 3H), 2.22-2.16 (m, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H).

ESI-MS *m/z*: 809.2 $(M-H_2O+H)^+$.

Example 0-2

A)



1 3-R

To a solution of **1** (0.5 g, 0.80 mmol) in acetic acid (20 mL, 0.04 M) was added D-tryptophanol (**2-R**) (533 mg, 3.0 mmol, Sigma-Aldrich). The reaction mixture was stirred at 23 ºC for 16 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added

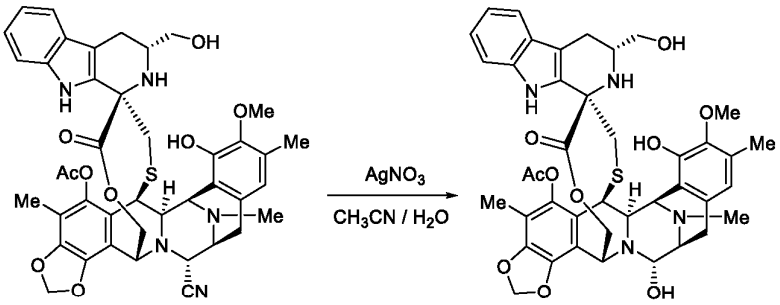
and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **3-R** (479 mg, 75%).

R_f= 0.44 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 9.6 Hz, 1H), 7.12 (t, *J* = 7.3 Hz, 1H), 7.03 (t, *J* = 7.3 Hz, 1H), 6.60 (s, 1H), 6.25 (s, 1H), 6.03 (s, 1H), 5.75 (s, 1H), 5.04 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.37 (s, 1H), 4.32-4.25 (m, 1H), 4.22 (d, *J* = 2.7 Hz, 1H), 4.19-4.09 (m, 1H), 3.82 (s, 3H), 3.77 (s, 1H), 3.64 (d, *J* = 9.0 Hz, 1H), 3.49-3.41 (m, 2H), 3.02-2.90 (m, 2H), 2.60-2.52 (m, 2H), 2.45 (d, *J* = 14.7 Hz, 2H), 2.40 (s, 3H), 2.28 (s, 3H), 2.22-2.14 (m, 2H), 2.18 (s, 3H), 2.10 (m, 3H).

ESI-MS *m/z*: 794.3 $(M+H)^+$.

B)



3-R **4-R**

To a solution of **3-R** (479 mg, 0.60 mmol) in CH_3CN:H_2O (1.39:1, 40 mL, 0.015 M) was added $AgNO_3$ (3.03 g, 18.1 mmol). After 3 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **4-R** (428 mg, 91%).

R_f= 0.45 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.11 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.02 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1H), 6.61 (s, 1H), 6.22 (d, *J* = 1.3 Hz, 1H), 5.99 (d, *J* = 1.3 Hz, 1H), 5.73 (s, 1H), 5.17 (dd, *J* = 11.5, 1.2 Hz, 1H), 4.86 (s, 1H), 4.56-4.47 (m, 2H), 4.17 (dd, *J* = 5.1, 1.6 Hz, 1H), 4.08 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.81 (s, 3H), 3.78 (d, J = 3.8 Hz, 1H), 3.64 (dd, *J* = 10.8, 3.8 Hz, 2H), 3.51 (d, *J* = 5.1 Hz, 1H), 3.48-3.43 (m, 2H), 3.24 (d, *J* = 8.6 Hz, 1H), 3.00-2.80 (m, 2H), 2.57 (s, 1H), 2.55-2.43 (m, 1H), 2.40 (s, 3H), 2.27 (s, 3H), 2.19-2.12 (m, 1H), 2.16 (s, 3H), 2.08 (s, 3H).

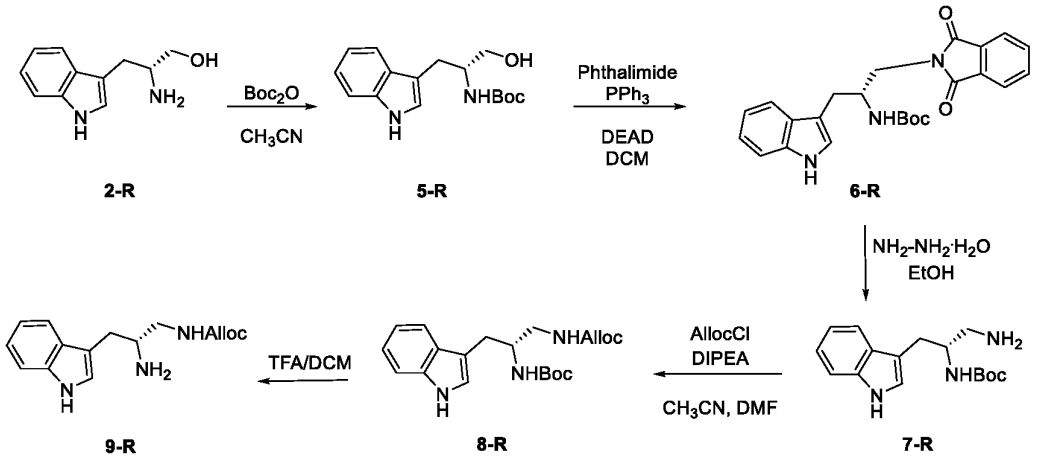
^{13}C NMR (101 MHz, $CDCl_3$): δ 171.8, 168.6, 147.6, 145.4, 143.0, 141.3, 140.7, 136.0, 131.1, 130.0, 129.6, 126.6, 122.1, 121.6, 121.2, 119.4, 118.4, 115.6, 112.9, 111.1, 110.6, 101.8,

81.7, 65.8, 62.7, 61.8, 60.4, 60.3, 57.9, 57.8, 56.1, 55.0, 52.1, 42.2, 41.3, 41.1, 23.8, 23.4, 20.5, 15.7, 9.8.

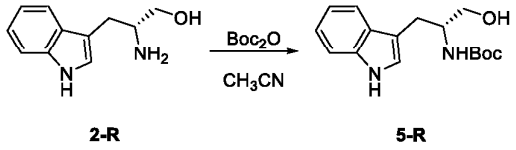
ESI-MS *m/z*: 767.6 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 767.2799 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$: 767.2745).

Example 0-3. Synthesis of allyl *N*-[(*R*)-(2-amino-3-(*1H*-indol-3-yl)propyl)]carbamate (**9-R**)



A)

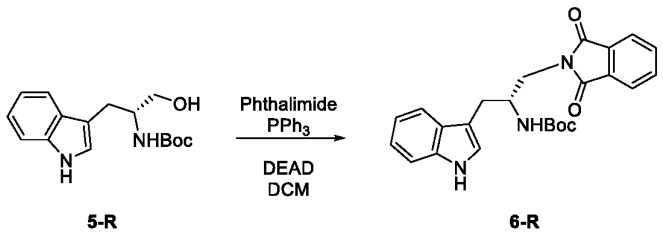


To a solution of D-tryptophanol (**2-R**) (2.0 g, 10.4 mmol) in CH_3CN (42 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (4.6 g, 20.8 mmol). The reaction mixture was stirred at 23 ºC for 3 h and concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH from 99:1 to 85:15) to afford **5-R** (2.2 g, 73%).

R_f= 0.5 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.67 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.38 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.29-7.10 (m, 2H), 7.06 (s, 1H), 4.82 (s, 1H), 4.00 (s, 1H), 3.71 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.62 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.01 (d, *J* = 6.7 Hz, 2H), 2.14 (s, 1H), 1.44 (s, 9H).

B)



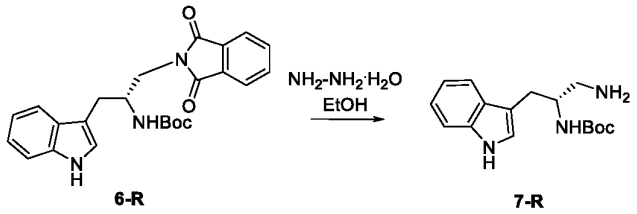
To a solution of **5-R** (2.4 g, 8.2 mmol) in CH_2Cl_2 (50 mL, 6 mL/mmol) was added phthalimide (2.7 g, 18.2 mmol), triphenylphosphine (4.8 g, 18.2 mmol) and the mixture was cooled at 0 ºC.

A solution of diethyl azodicarboxylate (DEAD) solution in CH_2Cl_2 (25 mL, 3 mL/mmol) was added for 15 min. The reaction was stirred at 23 ºC for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **6-R** (3.3 g, 96%).

R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.81 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.66 (dd, *J* = 5.6, 3.2 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.19-7.04 (m, 3H)., 4.81 (s, 1H), 4.40 (s, 1H), 3.83 (dd, *J* = 13.9, 3.7 Hz, 1H), 3.72 (dd, *J* = 13.9, 9.9 Hz, 1H), 3.08-3.01 (m, 2H), 1.23 (s, 9H).

C)



To a solution of **6-R** (3.25 g, 7.74 mmol) in ethanol (231 mL, 30 mL/mmol) was added hydrazine monohydrate (37 mL, 774 mmol). The reaction mixture was stirred at 80 ºC in sealed tube for 2.25 h, concentrated under vacuum. Flash chromatography (EtOAc:CH_3OH, from 100:1 to 50:50) afforded **7-R** (2.15 g, 96%).

R_f= 0.2 (EtOAc:CH_3OH, 6:4).

^{1}H NMR (400 MHz, CD_3OD): δ 7.60 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.13-7.04 (m, 2H), 7.05-6.96 (m, 1H), 4.02-3.94 (m, 1H), 2.99-2.87 (m, 3H), 2.78 (dd, *J* = 13.1, 9.7 Hz, 1H), 1.39 (s, 9H).

ESI-MS *m/z*: 290.2 $(M+H)^+$.

D)



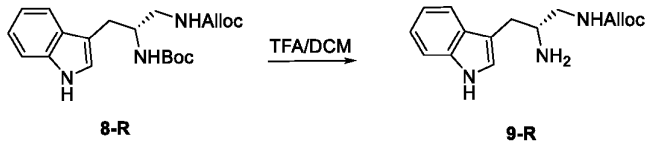
To a solution of **7-R** (2.15 g, 7.4 mmol) in CH_3CN (74 mL, 10 mL/mmol) and DMF (7.4 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (1.06 mL, 5.9 mmol) and allyl chloroformate (7.9 mL, 74 mmol). The reaction was stirred at 23 ºC for 16 h. The mixture was diluted with EtOAc, NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **8-R** (1.69 g, 61%).

R_f= 0.4 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.35 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.16 (dddd, *J* = 27.8, 8.0, 7.0, 1.1 Hz, 2H), 7.04 (d, *J* = 2.4 Hz, 1H), 5.90 (ddt, *J* = 17.3, 10.7, 5.6 Hz, 1H), 5.34-5.22 (m, 1H), 5.20 (dt, *J* = 10.5, 1.4 Hz, 1H), 5.12 (s, 1H), 4.82 (s, 1H), 4.55 (dq, *J* = 5.4, 1.7 Hz, 2H), 4.02 (s, 1H), 3.35 (dt, *J* = 10.0, 4.7 Hz, 1H), 3.21 (s, 1H), 2.95 (ddd, *J* = 21.6, 15.4, 9.1 Hz, 2H), 1.42 (s, 9H).

ESI-MS *m/z*: 274.3 $(M\text{-}Boc+H)^+$.

E)



8-R **9-R**

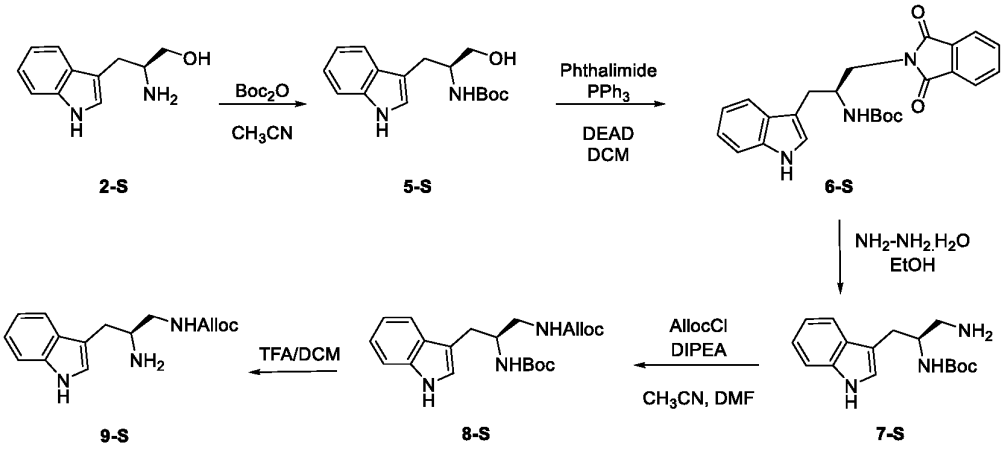
To a solution of **8-R** (1.30 g, 3.50 mmol) in CH_2Cl_2 (58 mL, 16.6 mL/mmol) was added trifluoroacetic acid (30 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 1.5 h, concentrated under vacuum to give crude **9-R** which was used in the next steps without further purification.

R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 5.87 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.34-5.13 (m, 2H), 4.50 (d, *J* = 5.5 Hz, 2H), 3.62 (bs, 1H), 3.42 (dd, *J* = 14.9, 3.9 Hz, 1H), 3.36-3.20 (m, 1H), 3.11-3.00 (m, 2H).

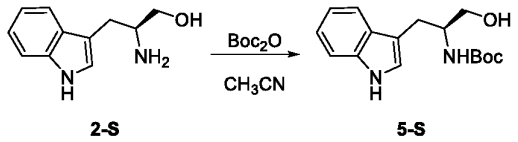
ESI-MS *m/z*: 274.3 $(M+H)^+$.

Example 0-4. Synthesis of allyl *N*-[(*S*)-(2-amino-3-(*1H*-indol-3-yl)propyl)]carbamate (**9-S**)



2-S **5-S** **6-S** **7-S** **8-S** **9-S**

A)



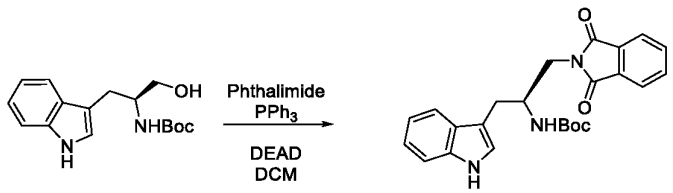
2-S 5-S

To a solution of L-tryptophanol (**2-S**) (2.0 g, 10.4 mmol) in CH_3CN (42 mL, 4 mL/mmol) was added Di-*tert*-butyl dicarbonate (4.6 g, 20.8 mmol). The reaction mixture was stirred at 23 ºC for 3 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **5-S** (2.24 g, 73%).

R_f= 0.5 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.65 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.37 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.23-7.11 (m, 2H), 7.06 (s, 1H), 4.81 (s, 1H), 3.99 (s, 1H), 3.70 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.61 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.00 (d, *J* = 6.7 Hz, 2H), 2.01 (s, 1H), 1.42 (s, 9H).

B)



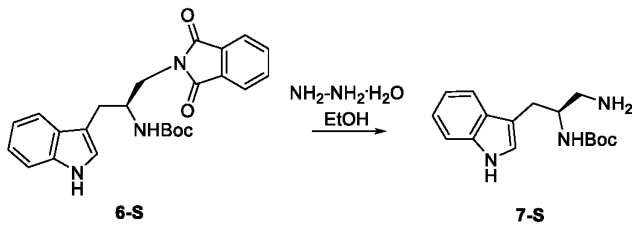
5-S 6-S

To a solution of **5-S** (1.2 g, 4.13 mmol) in CH_2Cl_2 (24.8 mL, 6 mL/mmol) was added phthalimide (1.33 g, 9.1 mmol), triphenylphosphine (2.4 g, 9.1 mmol) and the mixture was cooled at 0 ºC. A solution of diethyl azodicarboxylate (DEAD) solution (3 mL, 10.32 mmol) in CH_2Cl_2 (12.4 mL, 3 mL/mmol) was added for 15 min. The reaction was stirred at 23 ºC for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **6-S** (2.8 g, >100%).

R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H), 7.80 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.66 (dd, *J* = 5.6, 3.2 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.21-7.04 (m, 3H)., 4.74 (s, 1H), 4.42 (s, 1H), 3.83 (dd, *J* = 13.9, 3.7 Hz, 1H), 3.72 (dd, *J* = 13.9, 9.9 Hz, 1H), 3.10-3.01 (m, 2H), 1.23 (s, 9H).

C)



6-S 7-S

To a solution of **6-S** (0.86 g, 2.07 mmol) in ethanol (72 mL, 36 mL/mmol) was added hydrazine monohydrate (10 mL, 207 mmol). The reaction mixture was stirred at 80 ºC in

sealed tube for 2.25 h, concentrated under vacuum. Flash chromatography (EtOAc:CH_3OH, from 100:1 to 50:50) to afford **7-S** (1.0 g, 84%).

R_f= 0.2 (EtOAc:CH_3OH, 6:4).

^1H NMR (400 MHz, CD_3OD): δ 7.61 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.13-6.97 (m, 2H), 7.09 (s, 1H), 4.06-3.96 (m, 1H), 3.01-2.76 (m, 4H), 1.38 (s, 9H).

ESI-MS *m/z*: 290.3 $(M+H)^+$.

D)



7-S **8-S**

To a solution of **7-S** (0.95 g, 3.3 mmol) in CH_3CN (33 mL, 10 mL/mmol) and DMF (3.3 mL, 1 mL/mmol) was added *N*,*N*-diisopropylethylamine (0.5 mL, 2.6 mmol) and allyl chloroformate (3.5 mL, 33 mmol). The reaction was stirred at 23 ºC for 20 h. The mixture was diluted with EtOAc, NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **8-S** (0.88 g, 73%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^1H NMR (400 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.20 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.13 (dddd, *J* = 27.8, 8.0, 7.0, 1.1 Hz, 2H), 7.06 (d, *J* = 2.4 Hz, 1H), 5.90 (ddt, *J* = 17.3, 10.7, 5.6 Hz, 1H), 5.31-5.18 (m, 2H), 5.09 (s, 1H), 4.80 (s, 1H), 4.59-4.52 (m, 2H), 4.03 (s, 1H), 3.37 (dt, *J* = 10.0, 4.7 Hz, 1H), 3.21 (s, 1H), 3.05-2.87 (m, 2H), 1.42 (s, 9H).

ESI-MS *m/z*: 274.3 $(M-Boc+H)^+$.

E)



8-S **9-S**

To a solution of **8-S** (0.875 g, 2.3 mmol) in CH_2Cl_2 (38 mL, 16.6 mL/mmol) was added trifluoroacetic acid (19 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 2 h, concentrated under vacuum to give crude **9-S** which was used in the next steps without further purification.

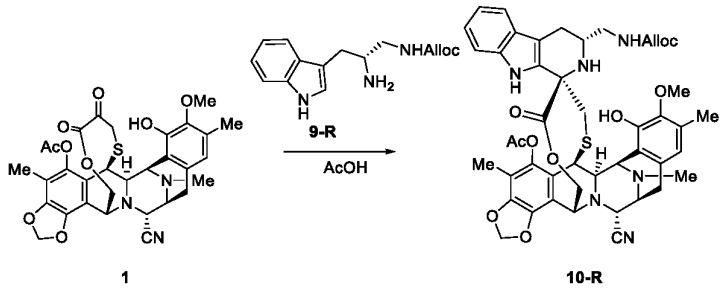
R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, CD_3OD): δ 7.56 (d, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.21 (s, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 5.94 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.34-5.16 (m, 2H), 4.56 (d, *J* = 5.5 Hz, 2H), 3.60 (bs, 1H), 3.43 (dd, *J* = 14.9, 3.9 Hz, 1H), 3.37-3.31 (m, 1H), 3.14-2.99 (m, 2H).

ESI-MS *m/z*: 274.3 $(M+H)^+$.

Example 0-5

A)



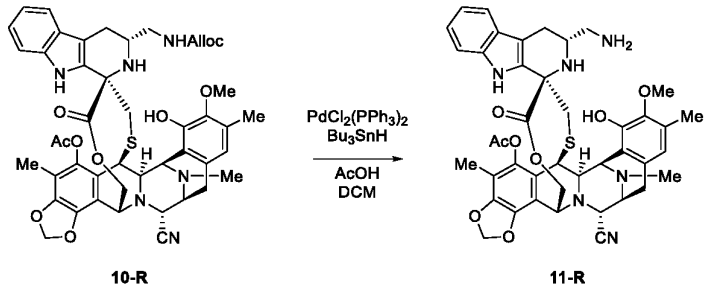
To a solution of **1** (1.45 g, 2.33 mmol) in acetic acid (58 mL, 0.08 M) was added **9-R** (0.95 g, 3.50 mmol). The reaction mixture was stirred a 50 ºC for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **10-R** (1.3 g, 64%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.10 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.01 (td, *J* = 7.5, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.23 (d, *J* = 1.4 Hz, 1H), 6.01 (d, J = 1.4 Hz, 1H), 5.99-5.89 (m, 1H), 5.79 (s, 1H), 5.44-5.21 (m, 2H), 5.14-4.99 (m, 2H), 4.63 (ddd, *J* = 7.3, 4.4, 1.5 Hz, 2H), 4.36 (s, 1H), 4.33-4.24 (m, 1H), 4.29-4.26 (m, 1H), 4.21 (d, J = 2.7 Hz, 1H), 4.19-4.13 (m, 3H), 3.80 (s, 3H), 3.56 (s, 1H), 3.48-3.43 (m, 3H), 3.27 (dt, *J* = 13.2, 4.0 Hz, 1H), 3.04-2.88 (m, 2H), 2.56 (dd, *J* = 15.2, 3.8 Hz, 1H), 2.49-2.35 (m, 2H), 2.31 (s, 3H), 2.28 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H).

ESI-MS *m/z*: 877.3 $(M+H)^+$.

B)



To a solution of **10-R** (600 mg, 0.68 mmol) in CH_2Cl_2 (12 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (77 mg, 0.1 mmol) and acetic acid (0.4 mL, 6.8 mmol). Tributyltin hydride (1.1 mL, 4.08 mmol) was added at 0 ºC, the reaction mixture was stirred at 0 ºC for 0.5 h and concentrated under vacuum. The crude obtained was diluted with EtOAc, saturated aqueous solution of NH_4Cl was added, and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and

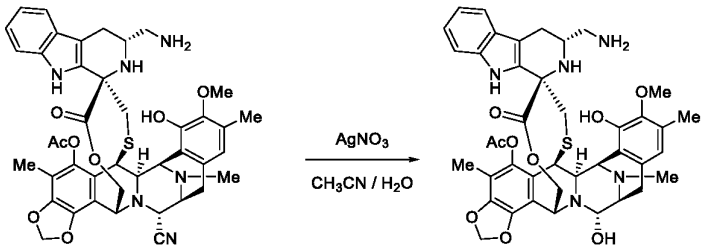
concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **11-R** (440 mg, 82%).

R_f= 0.5 (CH_2Cl_2:CH_3OH, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.11 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.03 (ddt, J = 8.3, 7.0, 1.4 Hz, 1H), 6.58 (s, 1H), 6.24 (d, *J* = 1.5 Hz, 1H), 6.02 (d, *J* = 1.5 Hz, 1H), 5.02 (d, *J* = 11.8 Hz, 1H), 4.63 (s, 1H), 4.36 (s, 1H), 4.28 (d, *J* = 5.1 Hz, 1H), 4.21 (d, *J* = 2.2 Hz, 1H), 4.16 (s, 1H), 3.80 (s, 3H), 3.51-3.39 (m, 4H), 3.32-3.13 (m, 3H), 2.95 (d, *J* = 8.9 Hz, 2H), 2.89-2.76 (m, 2H), 2.73-2.57 (m, 1H), 2.42 (d, *J* = 14.8 Hz, 1H), 2.36 (s, 3H), 2.25 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H).

ESI-MS *m/z*: 793.2 $(M+H)^+$.

C)



11-R **12-R**

To a solution of **11-R** (850 mg, 1.07 mmol) in CH_3CN:H_2O (1.39:1, 70 mL, 0.015 M) was added $AgNO_3$ (3.64 g, 21.4 mmol). After 17 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **12-R** (553 mg, 66%).

R_f= 0.3 (CH_2Cl_2:CH_3OH, 9:1).

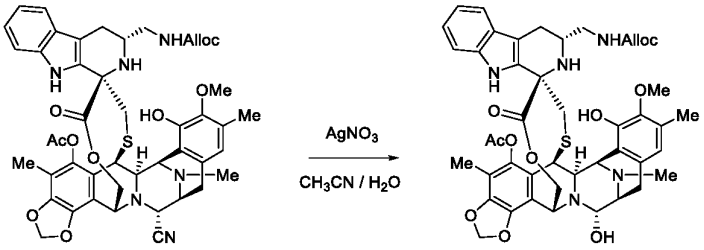
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 1H), 7.11 (ddt, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.02 (ddt, *J* = 8.3, 7.1, 1.2 Hz, 1H), 6.58 (s, 1H), 6.22 (s, 1H), 6.00 (s, 1H), 5.16 (d, *J* = 11.5 Hz, 1H), 4.87 (s, 1H), 4.54 (s, 1H), 4.51 (d, *J* = 3.3 Hz, 1H), 4.17 (d, *J* = 5.4 Hz, 1H), 4.07 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.81 (s, 3H), 3.52 (d, *J* = 5.1 Hz, 1H), 3.24 (d, *J* = 8.8 Hz, 2H), 2.99-2.78 (m, 4H), 2.66 (dd, *J* = 14.9, 3.5 Hz, 1H), 2.49-2.39 (m, 2H), 2.38 (s, 3H), 2.28 (m, 2H), 2.25 (s, 3H), 2.21-2.16 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD): δ 171.7, 169.4, 148.7, 145.9, 143.7, 141.4, 140.9, 136.9, 130.8, 130.0, 129.7, 126.0, 121.4, 121.0, 119.7, 119.1, 118.4, 117.5, 114.9, 110.8, 107.5, 106.4, 102.1, 91.3, 63.2, 60.0, 59.0, 58.6, 55.3, 54.6, 52.7, 52.4, 48.4, 45.8, 42.5, 40.2, 24.5, 23.2, 19.2, 15.0, 8.2.

ESI-MS *m/z*: 766.2 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 766.2972 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{44}N_5O_8S^+$: 766.2905).

C')



10-R 13-R

To a solution of **10-R** (700 mg, 0.8 mmol) in CH_3CN:H_2O (1.39:1, 52.5 mL, 0.015 M) was added $AgNO_3$ (2.66 g, 16 mmol). After 20 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2: CH_3OH, from 99:1 to 85:15) to give **13-R** (438 mg, 63%).

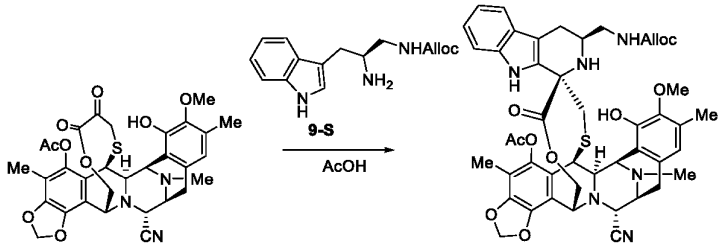
Rf= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.32-7.20 (m, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.62 (s, 1H), 6.21 (s, 1H), 6.05-5.90 (m, 1H), 5.99 (s, 1H), 5.75 (d, *J* = 6.0 Hz, 1H), 5.40-5.07 (m, 4H), 4.88 (d, *J* = 14.7 Hz, 1H), 4.68-4.50 (m, 3H), 4.28-4.13 (m, 1H), 4.08 (dt, *J* = 11.4, 2.4 Hz, 1H), 3.83 (s, 3H), 3.68-3.40 (m, 4H), 3.37-3.19 (m, 2H), 2.98-2.79 (m, 2H), 2.59-2.36 (m, 3H), 2.29 (s, 3H), 2.27 (s, 3H), 2.14 (s, 3H), 2.10-2.16 (m, 1H), 2.08 (s, 3H).

ESI-MS *m/z*: 850.3 $(M-H_2O+H)^+$.

Example 0-6

A)



1 10-S

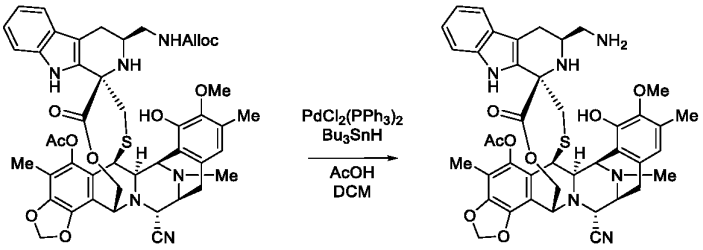
To a solution of **1** (955 mg, 1.5 mmol) in acetic acid (37.5 mL, 0.08 M) was added **9-S** (627 mg, 2.29 mmol). The reaction mixture was stirred a 50 ºC for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **10-S** (756 mg, 58%).

R_f= 0.4 (Hexane: EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 7.9 Hz, 1H), 7.10 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.01 (td, *J* = 7.5, 7.0, 1.0 Hz, 1H), 6.68 (s, 1H), 6.23 (d, *J* = 1.4 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 6.07-5.93 (m, 1H), 5.82 (s, 1H), 5.41-5.19 (m, 2H), 5.1 (d, *J* = 11.7 Hz, 1H), 4.66 (dt, *J* = 5.9, 1.3 Hz, 1H), 4.57 (s, 1H), 4.37 (s, 1H), 4.33-4.20 (m, 3H), 3.81 (s, 3H), 3.46 (d, *J* = 4.2 Hz, 2H), 3.22-3.13 (m, 1H), 3.11-2.88 (m, 4H), 2.66 (dd, *J* = 15.2, 4.2 Hz, 1H), 2.51 (dd, *J* = 15.3, 6.0 Hz, 1H), 2.43-2.32 (m, 2H), 2.31 (s, 3H), 2.26 (s, 3H), 2.19 (s, 3H), 2.04 (s, 3H).

ESI-MS *m/z*: 877.3 $(M+H)^+$.

B)



10-S **11-S**

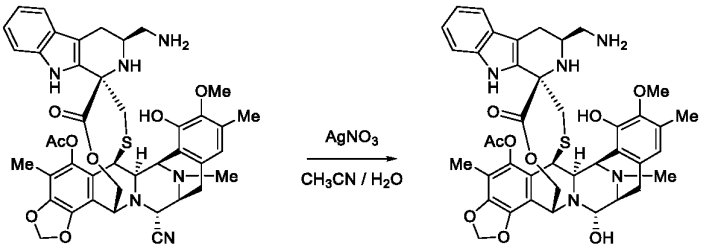
To a solution of **10-S** (650 mg, 0.72 mmol) in CH_2Cl_2 (13.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (83 mg, 0.11 mmol) and acetic acid (0.42 mL, 7.4 mmol). Tributyltin hydride (1.2 mL, 4.4 mmol) was added at 0 ºC, the reaction mixture was stirred at 23 ºC for 0.5 h, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **11-S** (445 mg, 78%).

R_f= 0.5 (CH_2Cl_2:CH_3OH, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.12 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.02 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 6.62 (s, 1H), 6.26 (d, *J* = 1.5 Hz, 1H), 6.04 (d, *J* = 1.5 Hz, 1H), 5.12 (d, *J* = 11.8 Hz, 1H), 4.59 (s, 1H), 4.42 (s, 1H), 4.36-4.17 (m, 3H), 3.81 (s, 3H), 3.51-3.39 (m, 3H), 2.98-2.75 (m, 4H), 2.69-2.60 (m, 2H), 2.47 (d, *J* = 16.1 Hz, 1H), 2.38 (s, 3H), 2.35-2.17 (m, 2H), 2.28 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H).

ESI-MS *m/z*: 793.3 $(M+H)^+$.

C)



11-S **12-S**

To a solution of **11-S** (435 mg, 0.55 mmol) in CH_3CN:H_2O (1.39:1, 38.5 mL, 0.015 M) was added $AgNO_3$ (1.84 g, 11 mmol). After 24 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **12-S** (152 mg, 35%).

R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

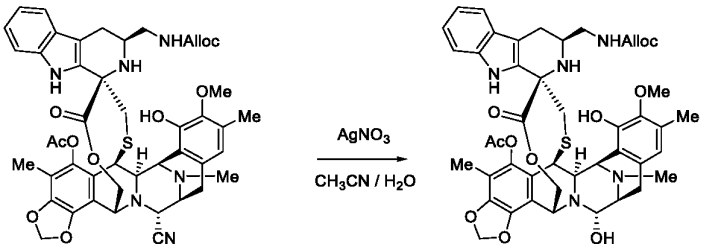
^{1}H NMR (500 MHz, CD_3OD): δ 7.34 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.28 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.04 (ddt, *J* = 8.2, 7.0, 1.1 Hz, 1H), 6.95 (ddt, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.55 (s, 1H), 6.31-6.25 (m, 1H), 6.15-6.05 (m, 1H), 5.31 (d, *J* = 11.4 Hz, 1H), 4.91 (s, 1H), 4.64 (s, 1H), 4.40-4.19 (m, 3H), 3.76 (s, 3H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.44 (d, *J* = 9.0 Hz, 1H), 3.03-2.85 (m, 4H), 2.85-2.65 (m, 2H), 2.59 (d, *J* = 15.6 Hz, 1H), 2.52-2.39 (m, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H).

^{13}C NMR (126 MHz, CD_3OD): δ 171.4, 169.3, 148.6, 145.8, 143.5, 141.2, 140.8, 136.5, 131.2, 130.3, 129.5, 126.3, 121.6, 121.2, 119.8, 119.4, 118.6, 117.5, 114.9, 111.0, 107.5, 107.4, 102.2, 91.1, 63.5, 60.5, 59.2, 58.5, 55.3, 54.7, 53.4, 52.7, 48.6, 44.7, 42.7, 39.9, 24.3, 23.4, 19.2, 15.1, 8.2.

ESI-MS *m/z*: 766.2 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 766.2958 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{44}N_5O_8S$: 766.2905).

C')



10-S **13-S**

To a solution of **10-S** (5 mg, 0.006 mmol) in CH_3CN:H_2O (1.39:1, 0.5 mL, 0.015 M) was added $AgNO_3$ (29 mg, 0.17 mmol). After 20 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **13-S** (5 mg, 100%).

R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

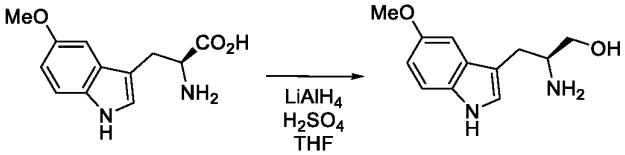
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.32-7.20 (m, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.84 (s, 1H), 6.24 (s, 1H), 6.08-5.97 (m, 1H), 6.01 (s,

1H), 5.87 (s, 1H), 5.42-5.19 (m, 4H), 4.88 (s, 1H), 4.69-4.65 (m, 2H), 4.58 (s, 1H), 4.28-4.13 (m, 2H), 3.84 (s, 3H), 3.68-3.40 (m, 2H), 3.24-3.15 (m, 2H), 3.08-2.90 (m, 2H), 2.73-2.57 (m, 2H), 2.53-2.37 (m, 3H), 2.34 (s, 3H), 2.25 (s, 3H), 2.14 (s, 3H), 2.10-2.16 (m, 1H), 2.03 (s, 3H).

ESI-MS *m/z*: 850.3 $(M-H_2O+H)^+$.

Example 0-7.

A) Synthesis of (*S*)-5-methoxy-tryptophanol (**17-S**)



16-S **17-S**

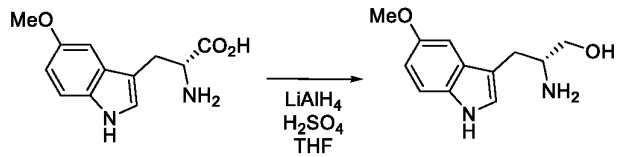
To a solution of $LiAlH_4$ (23.4 mL, 1.0 M in THF, 23.4 mmol) at -40 ºC was added carefully H_2SO_4 (0.31 mL, 5.57 mmol) and a suspension of 5-methoxy-L-tryptophan (**16-S**) (1.0 g, 4.26 mmol, Chem-Impex) in THF (13.4 mL, 0.3 M). The reaction mixture was left evolution at 23 ºC, heated for 3 h at 80 ºC and 18 h at 23 ºC. Cool at -21 ºC the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum to give **17-S** as a crude which was used in the next step without further purification.

R_f= 0.2 (CH_2Cl_2:CH_3OH, 4:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.19 (dt, *J* = 8.8, 0.7 Hz, 1H), 7.06-7.00 (m, 2H), 6.72 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.77 (s, 3H), 3.63-3.48 (m, 1H), 3.42-3.33 (m, 1H), 3.17-3.06 (m, 1H), 2.86 (ddt, *J* = 14.3, 6.1, 0.8 Hz, 1H), 2.66 (dd, *J* = 14.3, 7.5 Hz, 1H).

ESI-MS *m/z*: 221.4 $(M+H)^+$.

B) Synthesis of (*R*)-5-methoxy-tryptophanol (**17-R**)



16-R **17-R**

To a solution of $LiAlH_4$ (11.7 mL, 1.0 M in THF, 11.7 mmol) at -40 ºC was added carefully H_2SO_4 (0.31 mL, 5.75 mmol) and a suspension of 5-methoxy-D-tryptophan (**16-R**) (0.5 g, 2.13 mmol, Aldrich) in THF (6.7 mL, 0.3 M). The reaction mixture was left evolution at 23 ºC, heated for 3.5 h at 80 ºC and 18 h at 23 ºC. Cool at -21 ºC the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through

Celite® and washed with CH_3OH. The crude was concentrated under vacuum to give **17-R** as a crude which was used in the next step without further purification.

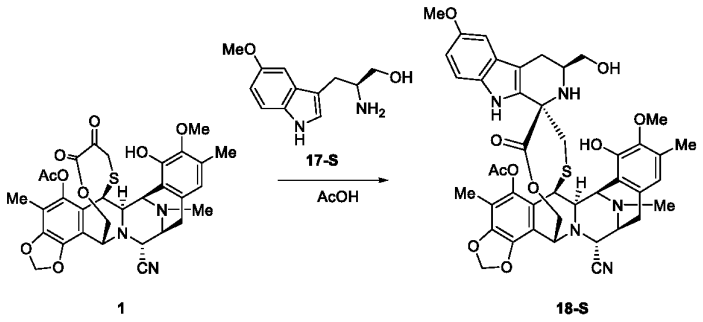
R_f= 0.2 (CH_2Cl_2:CH_3OH, 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.20 (d, *J* = 8.9 Hz, 1H), 7.06-6.96 (m, 2H), 6.71 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.75 (s, 3H), 3.62-3.52 (m, 1H), 3.37 (dd, *J* = 10.8, 7.0 Hz, 1H), 3.09 (br s, 1H), 2.82 (dd, *J* = 14.3, 5.9 Hz, 1H), 2.62 (dd, *J* = 14.4, 7.6 Hz, 1H).

ESI-MS *m/z*: 221.6 $(M+H)^+$.

Example 0-8

A)



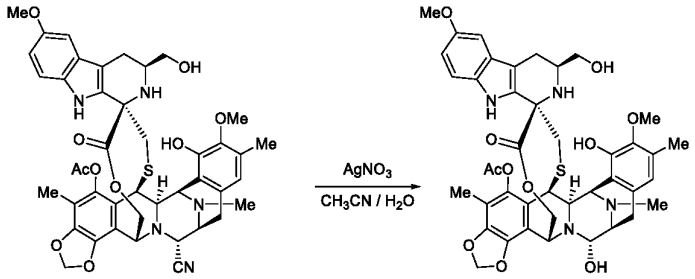
To a solution of **1** (530 mg, 0.85 mmol) in acetic acid (10.6 mL, 0.08 M) was added **17-S** (469 mg, 2.13 mmol). The reaction mixture was stirred at 50 ºC for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **18-S** (420 mg, 60%).

R_f= 0.3 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.13 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.51 (s, 1H), 6.27 (s, 1H), 6.11 (s, 1H), 5.21 (d, *J* = 11.7 Hz, 1H), 4.67 (s, 1H), 4.49-4.29 (m, 4H), 3.75 (s, 3H), 3.73 (s, 3H), 3.47 (t, *J* = 5.8 Hz, 3H), 3.37 (d, *J* = 5.1 Hz, 1H), 3.01-2.81 (m, 2H), 2.75 (d, *J* = 7.4 Hz, 1H), 2.66 (dd, *J* = 15.1, 4.1 Hz, 1H), 2.55-2.35 (m, 4H), 2.34 (s, 3H), 2.28 (s, 3H), 2.11 (s, 3H), 1.99 (s, 3H).

ESI-MS *m/z*: 824.3 $(M+H)^+$.

B)



18-S 19-S

To a solution of 18-S (420 mg, 0.519 mmol) in CH_3CN:H_2O (1.39:1, 36 mL, 0.015 M) was added $AgNO_3$ (2.60 g, 15.3 mmol). After 3 h at 23 ºC, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain 19-S (250 mg, 60%).

R_f= 0.45 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (500 MHz, CD_3OD): δ 7.15 (dd, *J* = 8.9, 0.6 Hz, 1H), 6.82 (dd, *J* = 2.4, 0.6 Hz, 1H), 6.68 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.54 (s, 1H), 6.27 (d, *J* = 1.3 Hz, 1H), 6.08 (d, *J* = 1.3 Hz, 1H), 5.30 (d, *J* = 11.5 Hz, 1H), 4.62 (s, 1H), 4.34 (dd, *J* = 11.4, 2.0 Hz, 1H), 4.31-4.27 (m, 2H), 3.76 (s, 3H), 3.75 (s, 3H), 3.66-3.58 (m, 1H), 3.55-3.45 (m, 2H), 3.42 (d, *J* = 7.8 Hz, 1H), 2.93-2.73 (m, 3H), 2.68 (dd, *J* = 15.1, 4.2 Hz, 1H), 2.54 (d, *J* = 15.4 Hz, 1H), 2.42 (dd, *J* = 15.1, 10.1 Hz, 2H), 2.35 (s, 3H), 2.29 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H).

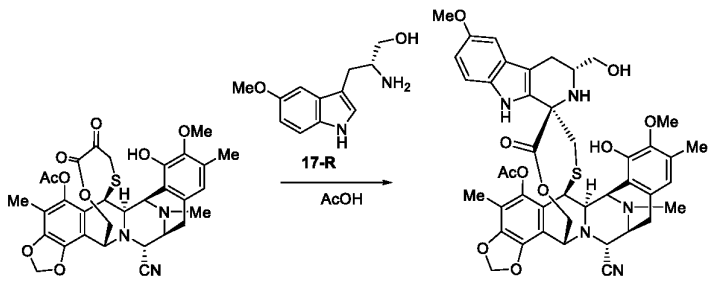
^{13}C NMR (126 MHz, CD_3OD): δ 172.7, 170.8, 155.1, 149.9, 147.2, 145.0, 142.6, 142.2, 133.1, 132.4, 132.1, 131.3, 128.1, 122.5, 121.6, 120.3, 116.4, 113.0, 112.9, 111.4, 109.0, 103.6, 100.8, 92.5, 66.6, 65.0, 61.7, 60.4, 59.9, 56.7, 56.1, 54.8, 54.1, 51.7, 44.1, 41.3, 30.7, 25.4, 24.7, 20.6, 16.3, 9.5.

ESI-MS *m/z*: 798.1 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 797.2899 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{42}H_{45}N_4O_{10}S$ 797.2851).

Example 0-9

A)



1 18-R

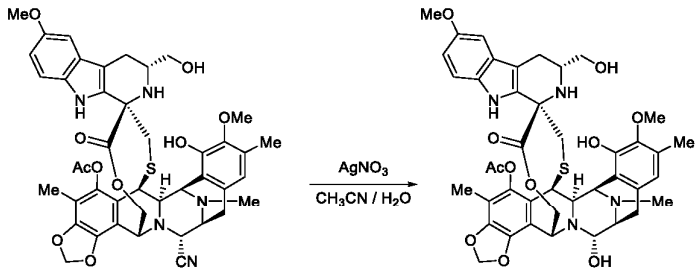
To a solution of **1** (311 mg, 0.50 mmol) in acetic acid (6.25 mL, 0.08 M) was added **17-R** (220 mg, 1.0 mmol). The reaction mixture was stirred at 50 ºC for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **18-R** (280 mg, 68%).

R_f= 0.3 (Hexane: EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.60 (s, 1H), 6.23 (s, 1H), 6.02 (s, 1H), 5.76 (s, 1H), 5.04 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.36 (s, 1H), 4.28 (d, *J* = 5.0 Hz, 1H), 4.24-4.09 (m, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.64 (s, 1H), 3.47-3.40 (m, 3H), 3.01-2.90 (m, 2H), 2.53 (d, *J* = 6.9 Hz, 2H), 2.45-2.41 (m, 1H), 2.40 (s, 3H), 2.27 (s, 3H), 2.22-2.14 (m, 1H), 2.18 (s, 3H), 2.06 (s, 3H).

ESI-MS *m/z*: 824.3 $(M+H)^+$.

B)



18-R **19-R**

To a solution of 18-R (330 mg, 0.40 mmol) in CH_3CN:H_2O (1.39:1, 28 mL, 0.015 M) was added $AgNO_3$ (2.04 g, 12.0 mmol). After 3 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain 19-R (224 mg, 69%).

R_f= 0.44 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (500 MHz, CD_3OD): δ 7.14 (dd, *J* = 8.8, 0.5 Hz, 1H), 6.83 (d, *J* = 2.5 Hz, 1H), 6.68 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.59 (s, 1H), 6.26 (d, *J* = 1.4 Hz, 1H), 6.07 (d, *J* = 1.4 Hz, 1H), 5.21 (d, *J* = 11.5 Hz, 1H), 4.68-4.55 (m, 1H), 4.32-4.25 (m, 2H), 4.12 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.60 (d, *J* = 5.2 Hz, 1H), 3.57-3.45 (m, 3H), 3.41 (d, *J* = 8.8 Hz, 1H), 2.97-2.83 (m, 3H), 2.73 (dd, *J* = 15.0, 3.4 Hz, 1H), 2.69 (d, *J* = 14.9 Hz, 1H), 2.34 (s, 3H), 2.30 (s, 3H), 2.20 (dd, *J* = 15.1, 10.4 Hz, 1H), 2.12 (s, 3H), 2.11-2.08 (m, 1H), 2.05 (s, 3H).

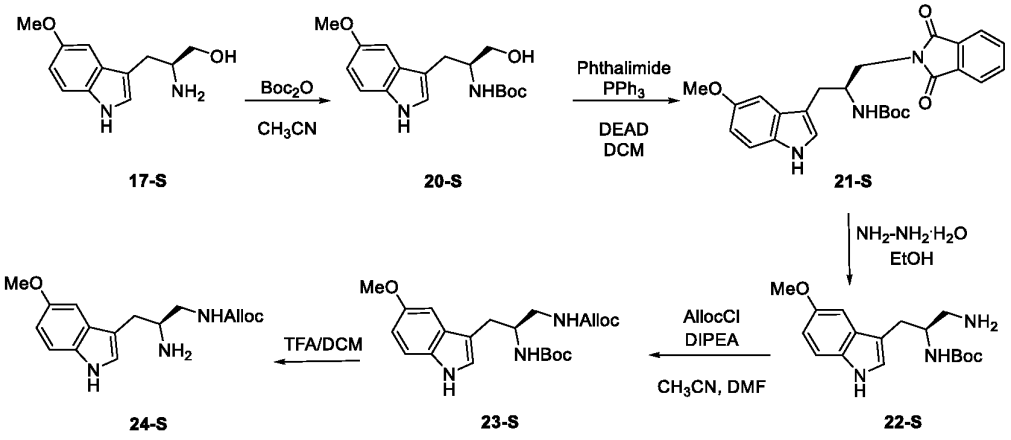
^{13}C NMR (126 MHz, CD_3OD): δ 173.0, 170.8, 155.0, 149.8, 147.3, 145.0, 142.8, 142.3, 133.5, 133.1, 132.2, 132.1, 131.1, 130.5, 127.8, 122.5, 121.7, 120.0, 116.4, 113.5, 112.9, 111.4,

110.2, 103.5, 100.9, 92.6, 66.8, 64.5, 61.3, 60.4, 60.0, 56.8, 56.1, 55.9, 54.1, 44.1, 41.3, 25.6, 24.5, 20.6, 16.2, 9.6.

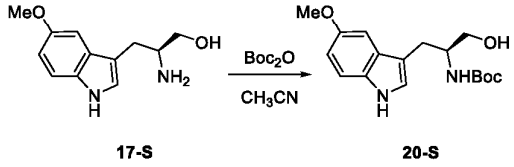
ESI-MS *m/z*: 797.4 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 797.2896 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{42}H_{45}N_4O_{10}S$ 797.2851).

Example 0-10. Synthesis of allyl *N*-[(*S*)-2-amino-3-(5-methoxy-*1H*-indol-3-yl)propyl)]carbamate (**24-S**)



A)

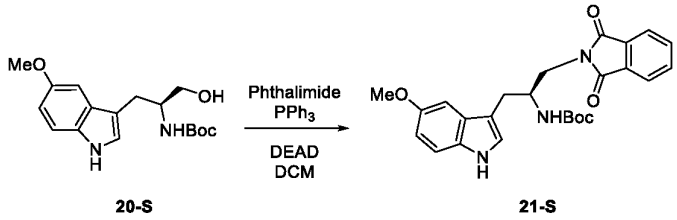


To a solution of **17-S** (6.9 g, 31.4 mmol) in CH_3CN (126 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (13.7 g, 62.8 mmol). The reaction mixture was stirred at 23 ºC for 5.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) gives **20-S** (4.5 g, 45%).

R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 7.03 (s, 1H), 6.87 (dd, *J* = 8.8, 2.5 Hz, 1H), 4.83 (s, 1H), 3.98 (s, 1H), 3.87 (s, 3H), 3.73-3.58 (m, 2H), 2.96 (d, *J* = 6.6 Hz, 2H), 1.42 (s, 9H).

B)

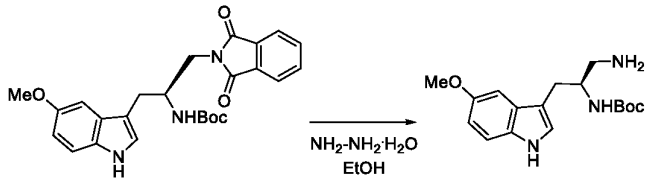


To a solution of **20-S** (4.5 g, 14 mmol) in CH_2Cl_2 (84 mL, 6 mL/mmol) was added phthalimide (4.5 g, 30.9 mmol), triphenylphosphine (8.1 g, 30.9 mmol) and the mixture was cooled at 0 ºC. A solution of 40% of diethyl azodicarboxylate (DEAD) in CH_2Cl_2 (10.4 mL, 35 mmol) was added for 15 min. The reaction was stirred at 23 ºC for 18 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 85:15) to yield **21-S** (5.8 g, 92%).

R_f= 0.55 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 7.78 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.69-7.61 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.06 (dd, *J* = 18.5, 2.4 Hz, 2H), 6.81 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.87 (s, 1H); 4.39 (s, 1H), 3.87 (s, 3H), 3.83-3.66 (m, 2H), 2.98 (d, *J* = 6.1 Hz, 2H), 1.20 (s, 9H).

C)



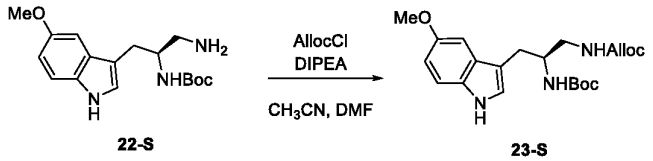
21-S **22-S**

To a solution of **21-S** (6.29 g, 14 mmol) in ethanol (420 mL, 30 mL/mmol) was added hydrazine monohydrate (61.1 mL, 1260 mmol). The reaction mixture was stirred at 80 ºC in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) affords **22-S** (4.2 g, 95%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 2.4 Hz, 1H), 7.06 (s, 1H), 6.76 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.06-3.97 (m, 1H), 3.82 (s, 3H), 3.06-2.82 (m, 4H), 1.37 (s, 9H).

D)



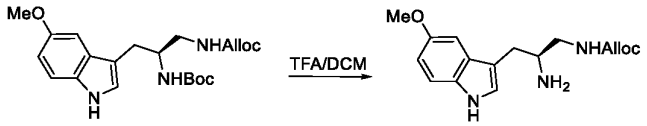
22-S **23-S**

To a solution of **22-S** (4.0 g, 12.52 mmol) in CH_3CN (125 mL, 10 mL/mmol) and DMF (12 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (1.8 mL, 10 mmol) and allyl chloroformate (13.3 mL, 125 mmol). The reaction was stirred at 23 ºC for 5 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to obtain **23-S** (2.65 g, 52%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.28-7.20 (m, 1H), 7.04 (d, *J* = 13.1 Hz, 2H), 6.85 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.97-5.82 (m, 1H), 5.33-5.24 (m, 1H), 5.19 (dt, *J* = 10.4, 1.3 Hz, 1H), 5.11 (s, 1H), 4.82 (s, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 4.01 (s, 1H), 3.86 (s, 3H), 3.37 (d, *J* = 13.7 Hz, 1H), 3.21 (s, 1H), 2.89 (dd, *J* = 14.5, 7.0 Hz, 1H), 1.41 (s, 9H).

E)



23-S **24-S**

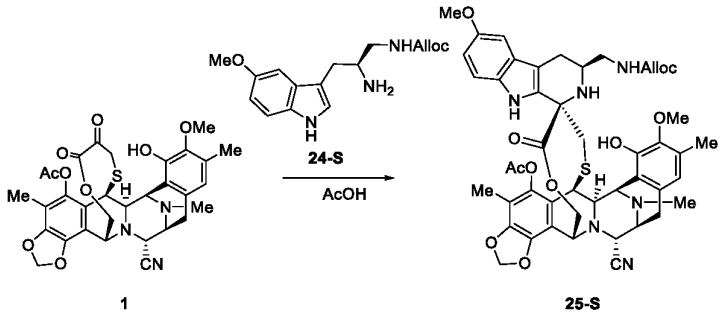
To a solution of **23-S** (2.60 g, 6.44 mmol) in CH_2Cl_2 (106 mL, 16.6 mL/mmol) was added trifluoroacetic acid (54 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 1.5 h, concentrated under vacuum to afford **24-S** (3.9 g, 100%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, CD_3OD): δ 8.27 (s, 1H), 7.25 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.10 (s, 1H), 6.96 (d, *J* = 2.3 Hz, 1H), 6.87 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.81 (ddt, *J* = 16.3, 10.9, 5.7 Hz, 1H), 5.23 (dd, *J* = 19.3, 13.6 Hz, 2H), 4.49 (d, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.81-3.55 (m, 1H), 3.62-3.39 (m, 2H), 3.08 (qd, *J* = 15.1, 7.3 Hz, 2H).

Example 0-11

A)



1 **25-S**

To a solution of **1** (120 mg, 0.19 mmol) in acetic acid (6 mL, 0.08 M) was added **24-S** (117 mg, 0.35 mmol). The reaction mixture was stirred at 23 ºC for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **25-S** (95 mg, 54%).

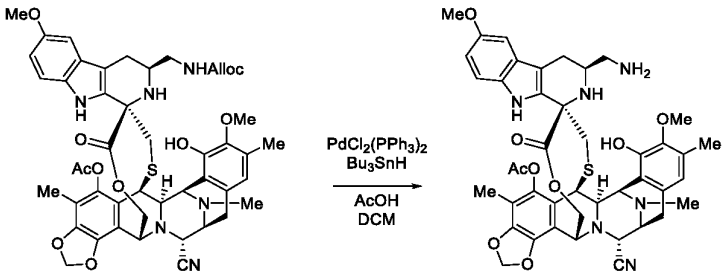
R_f= 0.4 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.80 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.68 (s, 1H), 6.24 (s, 1H), 6.03 (s, 1H), 6.02-5.93 (m, 1H), 5.76 (s, 1H), 5.38 (d, *J* = 10.5 Hz, 1H), 5.26 (d, *J* = 10.5 Hz, 1H), 5.11 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 2H),

4.57 (s, 1H), 4.37 (s, 1H), 4.33-4.19 (m, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.46 (s, 2H), 3.17 (s, 1H), 3.10-2.90 (m, 3H), 2.68-2.45 (m, 2H), 2.38-2.33 (m, 1H), 2.32 (s, 3H), 2.27 (s, 3H), 2.16 (s, 3H), 2.04 (s, 2H).

ESI-MS *m/z*: 907.1 $(M+H)^+$.

B)



25-S 26-S

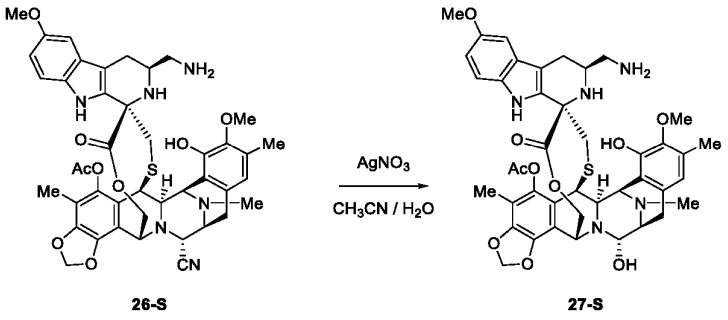
To a solution of **25-S** (90 mg, 0.1 mmol) in CH_2Cl_2 (2 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II)dichloride (12 mg, 0.1 mmol) and acetic acid (0.056 mL, 0.99 mmol). Tributyltin hydride (0.16 mL, 0.60 mmol) was added at 0 ºC, the reaction mixture was stirred at 0 ºC for 0.5 h, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **26-S** (75 mg, 92%).

R_f= 0.25 (CH_2Cl_2:CH_3OH, 1:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.15 (d, *J* = 9.3 Hz, 1H), 6.81-6.76 (m, 2H), 6.72 (s, 1H), 6.25 (d, *J* = 1.2 Hz, 1H), 6.03 (d, *J* = 1.2 Hz, 1H), 5.12 (d, *J* = 11.7 Hz, 1H), 4.57 (s, 1H), 4.41 (s, 1H), 4.36-4.24 (m, 2H), 4.20 (d, *J* = 11.7 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.44 (dd, *J* = 22.0, 7.1 Hz, 2H), 3.08-2.78 (m, 4H), 2.73-2.64 (m, 2H), 2.41-2.22 (m, 3H), 2.28 (s, 3H), 2.25-2.15 (m, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H).

ESI-MS *m/z*: 823.3 $(M+H)^+$.

C)



26-S 27-S

To a solution of **26-S** (70 mg, 0.085 mmol) in CH_3CN:H_2O (1.39:1, 6 mL, 0.015 M) was added $AgNO_3$ (335 mg, 1.7 mmol). After 18 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over

anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **27-S** (23 mg, 33%).

R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

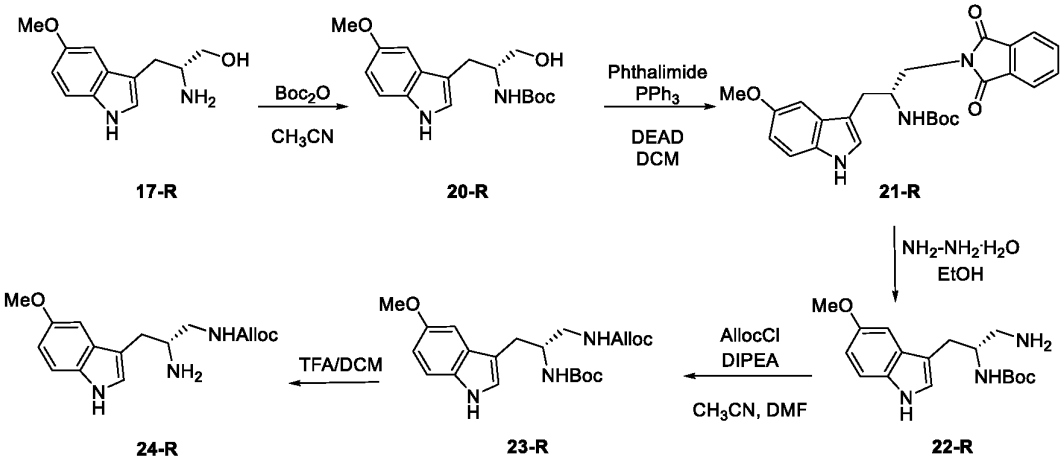
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.78 (s, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.78 (s, 1H), 5.22 (d, *J* = 11.5 Hz, 1H), 4.90 (s, 1H), 4.58-4.42 (m, 3H), 4.29-4.10 (m, 2H), 3.84-3.80 (m, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.53-3.48 (m, 2H), 3.22 (d, *J* = 8.7 Hz, 1H), 3.12 (s, 1H), 3.02 (d, *J* = 12.8 Hz, 1H), 2.89-2.64 (m, 3H), 2.46 (s, 3H), 2.42-2.34 (m, 2H), 2.27 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.1, 168.7, 154.0, 147.6, 145.6, 143.0, 141.2, 140.8, 131.6, 130.6, 129.6, 127.1, 121.8, 120.9, 118.4, 115.2, 112.5, 111.8, 101.8, 100.2, 81.5, 62.6, 60.6, 58.0, 57.8, 56.0, 55.8, 55.0, 42.3, 41.4, 31.9, 29.7, 27.8, 26.9, 25.6, 24.0, 22.7, 20.5, 16.0, 14.1, 13.6, 9.7.

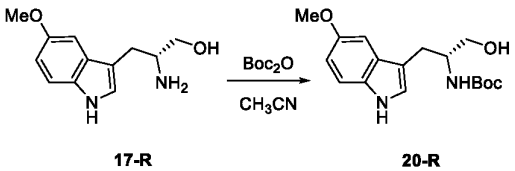
ESI-MS *m/z*: 796.3 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 796.3062 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{42}H_{46}N_5O_9S$ 796.3011).

Example 0-12. Synthesis of allyl *N*-[(*R*)-2-amino-3-(5-methoxy-*1H*-indol-3-yl)propyl)]carbamate (**24-R**)



A)



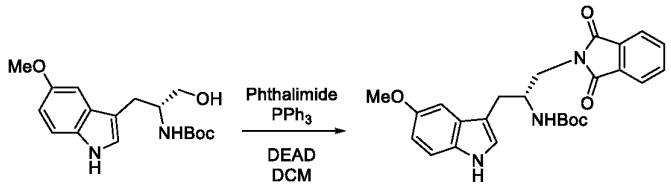
To a solution of **17-R** (2.35 g, 10.7 mmol) in CH_3CN (43 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (4.67 g, 21.4 mmol). The reaction mixture was stirred at 23 ºC for 2.5 h,

concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) afforded **20-R** (1.7 g, 50%).

R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.83 (s, 1H), 3.98 (s, 1H), 3.87 (s, 3H), 3.69 (td, *J* = 9.2, 7.5, 5.3 Hz, 1H), 3.61 (dd, *J* = 10.9, 5.6 Hz, 1H), 2.95 (d, *J* = 6.8 Hz, 2H), 1.42 (s, 9H).

B)



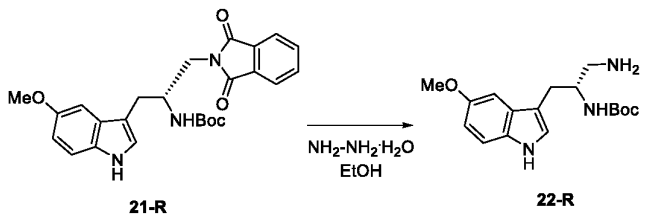
20-R **21-R**

To a solution of **20-R** (1.7 g, 5.3 mmol) in CH_2Cl_2 (32 mL, 6 mL/mmol) was added phthalimide (1.72 g, 11.7 mmol), triphenylphosphine (3.06 g, 11.7 mmol) and the mixture was cooled at 0 ºC. A solution of 40% of diethyl azodicarboxylate (DEAD) in CH_2Cl_2 (4.0 mL, 13.2 mmol) was added for 15 min. The reaction was stirred at 23 ºC for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 85:15) to afford **21-R** (2.0 g, 84%).

R_f= 0.45 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 7.80 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.67 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.30-7.12 (m, 2H), 7.08 (dd, *J* = 15.2, 2.4 Hz, 1H), 6.84 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.85 (d, *J* = 9.2 Hz, 1H), 4.43 (q, *J* = 5.3 Hz, 1H), 3.86 (s, 3H), 3.83-3.68 (m, 2H), 3.01 (d, *J* = 5.4 Hz, 2H), 1.22 (s, 9H).

C)



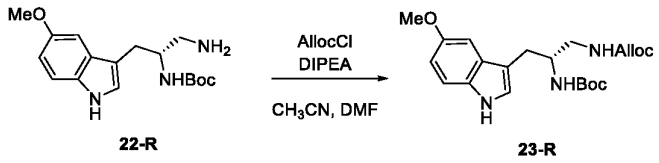
21-R **22-R**

To a solution of **21-R** (2.0 g, 4.45 mmol) in ethanol (133 mL, 30 mL/mmol) was added hydrazine monohydrate (21.6 mL, 445 mmol). The reaction mixture was stirred at 80 ºC in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford **22-R** (1.15 g, 81%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.8 Hz, 1H), 7.12 (s, 1H), 7.05 (s, 1H), 6.75 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.95 (ddd, *J* = 10.7, 8.7, 5.4 Hz, 1H), 3.82 (s, 3H), 2.98-2.79 (m, 3H), 2.75 (dd, *J* = 13.1, 9.4 Hz, 1H), 1.37 (s, 9H).

D)



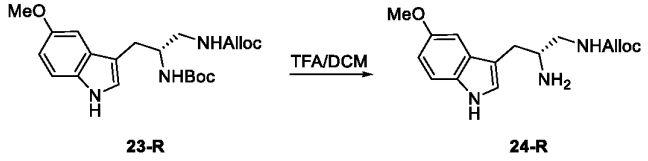
22-R **23-R**

To a solution of **22-R** (1.1 g, 3.4 mmol) in CH_3CN (34 mL, 10 mL/mmol) and DMF (3.4 mL, 1 mL/mmol) was added *N*,*N*-diisopropylethylamine (0.5 mL, 2.7 mmol) and allyl chloroformate (3.7 mL, 34 mmol). The reaction was stirred at 23 ºC for 19 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **23-R** (0.95 g, 69%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 7.05 (s, 1H), 6.98-6.87 (m, 1H), 6.82 (dt, *J* = 8.8, 1.8 Hz, 1H), 5.96-5.81 (m, 1H), 5.37-5.22 (m, 2H), 5.22-5.14 (m, 1H), 5.02-4.97 (m, 1H), 4.60-4.47 (m, 2H), 4.00 (s, 1H), 3.84 (s, 3H), 3.31 (s, 1H), 3.19 (s, 1H), 2.88 (td, *J* = 14.5, 13.3, 5.9 Hz, 2H), 1.40 (s, 9H).

E)



23-R **24-R**

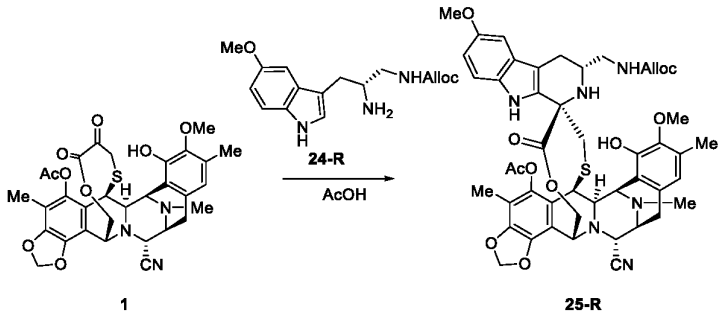
To a solution of **23-R** (0.94 g, 2.3 mmol) in CH_2Cl_2 (39 mL, 16.6 mL/mmol) was added trifluoroacetic acid (19 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 1.5 h, concentrated under vacuum to afford **24-R** (0.72 g, 100%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.27 (d, *J* = 8.8, 1H), 7.18 (s, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.80 (ddd, *J* = 8.8, 2.4, 0.9 Hz, 1H), 5.95 (ddt, *J* = 16.4, 10.8, 5.5 Hz, 1H), 5.32 (d, *J* = 17.1 Hz, 1H), 5.20 (d, *J* = 10.5 Hz, 1H), 4.60-4.53 (m, 2H), 3.83 (s, 3H), 3.59 (dt, *J* = 11.4, 5.5 Hz, 1H), 3.47-3.30 (m, 2H), 3.13-2.94 (m, 2H).

Example 0-13

A)



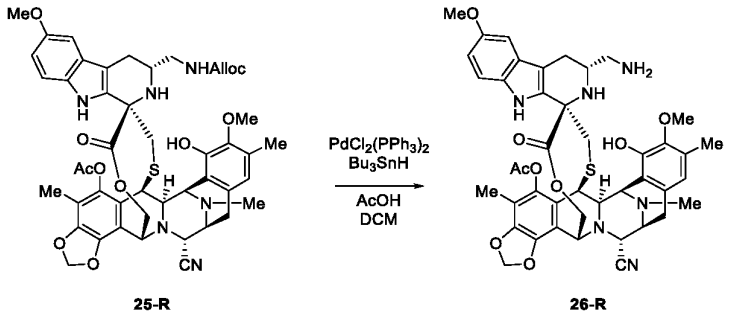
To a solution of **1** (0.71 g, 1.14 mmol) in acetic acid (45 mL, 0.08 M) was added **24-R** (0.54 mg, 1.8 mmol). The reaction mixture was stirred at 23 ºC for 7 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **25-R** (670 mg, 65%).

R_f= 0.4 (Hexane: EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.83-6.73 (m, 2H), 6.61 (s, 1H), 6.23 (d, *J* = 1.0 Hz, 1H), 6.02 (d, *J* = 1.0 Hz, 1H), 6.05-5.89 (m, 1H), 5.75 (s, 1H), 5.44-5.30 (m, 1H), 5.25 (d, *J* = 10.4 Hz, 1H), 5.13-4.99 (m, 2H), 4.71-4.59 (m, 2H), 4.36 (s, 1H), 4.30-4.07 (m, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.61-3.53 (m, 1H); 3.48-3.41 (m, 3H), 3.26 (dt, *J* = 13.3, 3.8 Hz, 1H), 3.04-2.88 (m, 2H), 2.52 (dd, *J* = 14.9, 3.7 Hz, 1H), 2.46-2.35 (m, 2H), 2.31 (s, 3H), 2.29 (s, 3H), 2.16 (s, 3H), 2.12-2.02 (m, 1H), 2.09 (s, 3H).

ESI-MS *m/z*: 907.3 $(M+H)^+$.

B)



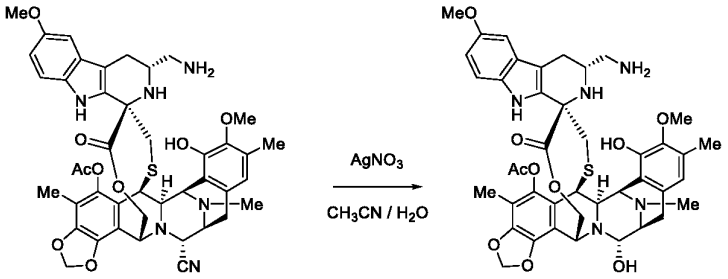
To a solution of **25-R** (745 mg, 0.82 mmol) in CH_2Cl_2 (15 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (92 mg, 0.1 mmol) and acetic acid (0.47 mL, 8.2 mmol). Tributyltin hydride (1.33 mL, 4.9 mmol) was added at 0 ºC, the reaction mixture was stirred at 0 ºC for 0.75 h and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **26-R** (680 mg, >100%).

R_f= 0.25 (CH_2Cl_2:CH_3OH, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 6.85-6.72 (m, 2H), 6.57 (s, 1H), 6.21 (d, *J* = 1.4 Hz, 1H), 6.00 (d, *J* = 1.3 Hz, 1H), 5.05-4.97 (m, 1H), 4.63 (s, 1H), 4.35 (s, 1H), 4.31-4.09 (m, 4H), 3.80 (s, 3H), 3.78 (s, 3H), 3.50-3.40 (m, 3H), 3.24 (dq, *J* = 9.9, 5.3 Hz, 1H), 2.95 (s, 1H), 2.91-2.75 (m, 2H), 2.62 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.43-2.28 (m, 2H), 2.36 (s, 3H), 2.25 (s, 3H), 2.22-2.14 (m, 1H), 2.15 (s, 3H), 2.08 (s, 3H).

ESI-MS *m/z*: 823.3 $(M+H)^+$.

C)



26-R **27-R**

To a solution of **26-R** (660 mg, 0.80 mmol) in CH_3CN:H_2O (1.39:1, 56 mL, 0.015 M) was added $AgNO_3$ (2.70 g, 16.0 mmol). After 16.5 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **27-R** (271 mg, 42%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (s, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 6.83 (s, 1H), 6.72 (d, *J* = 8.9 Hz, 1H), 6.58 (s, 1H), 6.20 (d, *J* = 1.8 Hz, 1H), 5.99 (d, *J* = 1.8 Hz, 1H), 5.76 (s, 1H), 5.15 (d, *J* = 11.4 Hz, 1H), 4.86 (s, 1H), 4.52 (m, 2H), 4.17 (d, *J* = 5.3 Hz, 1H), 4.07 (d, *J* = 11.4 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.55-3.43 (m, 2H), 3.32-3.20 (m, 2H), 3.01-2.82 (m, 4H), 2.68-2.59 (m, 1H), 2.44-2.31 (m, 1H), 2.38 (s, 3H), 2.30-2.19 (m, 1H), 2.26 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H).

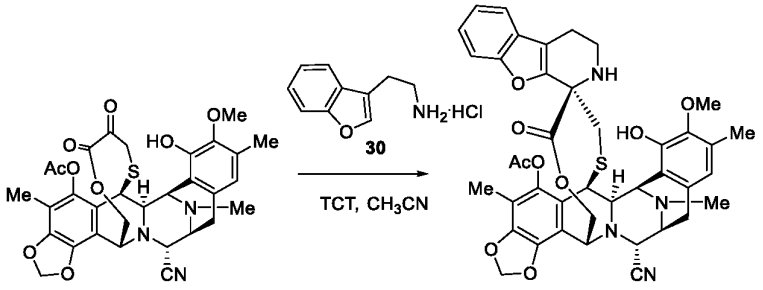
^{13}C NMR (101 MHz, CD_3OD): δ 171.7, 171.3, 153.8, 153.3, 148.0, 147.6, 145.4, 145.4, 143.1, 141.3, 140.7, 131.6, 131.4, 131.2, 129.3, 126.8, 121.6, 120.9, 118.3, 115.6, 112.2, 111.8, 101.8, 100.2, 81.7, 63.5, 63.1, 61.7, 58.0, 57.8, 56.1, 55.8, 55.0, 42.2, 42.1, 41.4, 41.0, 25.1, 23.8, 20.5, 16.0, 9.7.

ESI-MS *m/z*: 796.3 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 796.3045 $[M-H_2O+H]^+$ (Calcd. for $C_{42}H_{46}N_5O_9S$ 796.3011).

Example 0-14

A)



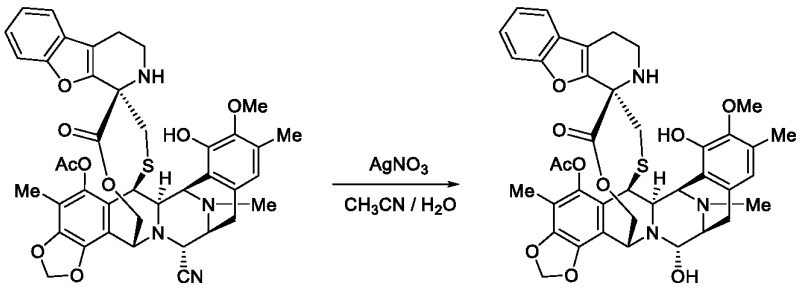
1 31

To a solution of compound **1** (2.0 g, 3.21 mmol) in acetonitrile (200 mL, 0.01 M) was added 2-benzofuran-3-yl-ethylamine hydrochloride (**30**) (1.90 g, 9.65 mmol, Sigma Aldrich) and cyanuric chloride (TCT) (200 mg, 10%). The reaction mixture was stirred at 85 ºC for 24 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **31** (1.95 g, 79%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.36 (m, 2H), 7.19-7.10 (m, 2H), 6.64 (s, 1H), 6.20 (d, *J* = 1.5 Hz, 1H), 6.05 (d, *J* = 1.5 Hz, 1H), 5.76 (s, 1H), 5.05 (d, *J* = 11.7 Hz, 1H), 4.54 (s, 1H), 4.33-4.24 (m, 2H), 4.23-4.16 (m, 2H), 3.81 (s, 3H), 3.49-3.38 (m, 2H), 3.28-3.21 (m, 1H), 3.06-2.78 (m, 5H), 2.57-2.50 (m, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 2.21 (m, 3H), 2.08 (s, 3H).
ESI-MS *m/z*: 765.3 $(M+H)^+$.

B)



31 32

To a solution of compound **31** (380 mg, 0.49 mmol) in CH_3CN:H_2O (1.39:1, 25 mL, 0.015 M) was added $AgNO_3$ (1.30 g, 7.45 mmol). After 5 h at 23 ºC, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **32** (175 mg, 47%).

R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (ddd, *J* = 10.7, 7.6, 1.1 Hz, 2H), 7.14 (dtd, *J* = 19.7, 7.3, 1.3 Hz, 2H), 6.65 (s, 1H), 6.16 (d, *J* = 1.5 Hz, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.75 (s, 1H), 5.15 (dd, *J* = 11.5, 1.2 Hz, 1H), 4.80 (s, 1H), 4.48 (d, *J* = 3.2 Hz, 1H), 4.44 (s, 1H), 4.20-4.06 (m, 2H), 3.81 (s, 1H), 3.50 (d, *J* = 18.8 Hz, 1H), 3.30 (ddd, *J* = 12.6, 7.9, 5.1 Hz, 1H), 3.22 (d, *J* = 9.1 Hz, 1H), 2.99 (d, *J* = 17.9 Hz, 1H), 2.84 (dd, *J* = 19.2, 12.0 Hz, 3H), 2.59-2.49 (m, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 2.21-2.14 (m, 1H), 2.18 (s, 3H), 2.06 (s, 3H).

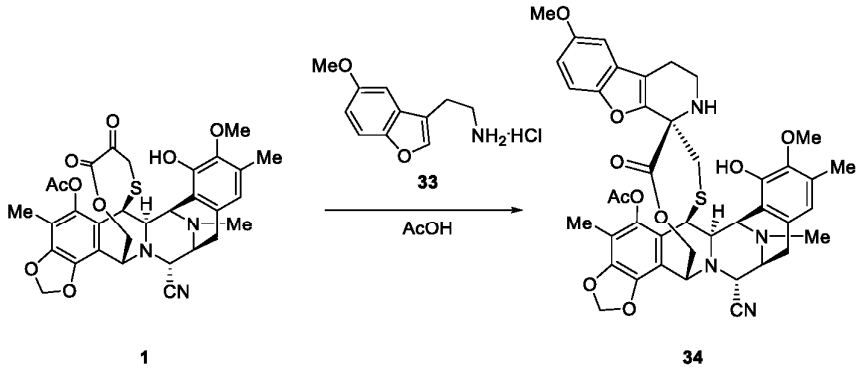
^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 168.7, 154.4, 150.0, 147.9, 145.5, 142.9, 140.9, 140.8, 131.3, 129.0, 127.7, 123.7, 122.2, 121.2, 120.8, 118.9, 118.3, 115.5, 113.5, 111.7, 101.7, 82.1, 62.7, 61.7, 60.3, 57.8, 57.4, 55.9, 55.0, 42.2, 41.3, 39.7, 38.2, 29.7, 23.7, 21.3, 20.6, 15.9, 9.7.

ESI-MS *m/z*: 738.6 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 756.2654 $[M+H]^+$ (Calcd. for $C_{40}H_{42}N_3O_{10}S$ 756.2585).

Example 0-15

A)



1 **34**

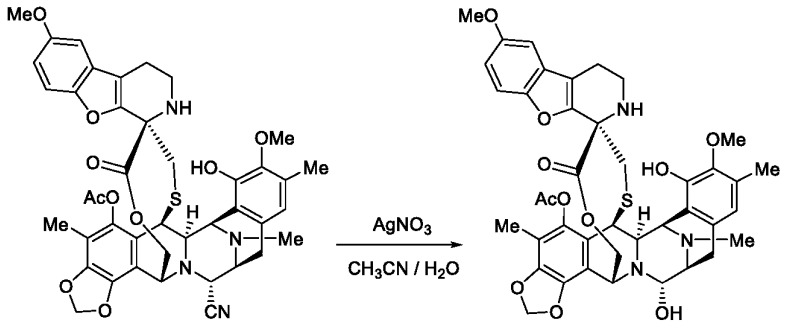
To a solution of **1** (500 mg, 0.80 mmol) in acetic acid (10 mL, 0.08 M) was added 2-(5-methoxybenzofuran-3-yl)-ethylamine hydrochloride (**33**) (Diverchim, ref: DW04590) (444 mg, 1.60 mmol). The reaction mixture was stirred at 50 ºC for 6 days and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) affords **34** (270 mg, 43%).

R_f= 0.3 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, *J* = 9.1 Hz, 1H), 6.80-6.73 (m, 2H), 6.63 (s, 1H), 6.18 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.4 Hz, 1H), 5.78 (s, 1H), 5.03 (dd, *J* = 11.5, 1.3 Hz, 1H), 4.52 (s, 1H), 4.29 (s, 1H), 4.26 (dd, *J* = 4.7, 1.5 Hz, 1H), 4.23-4.16 (m, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.46-3.43 (m, 1H), 3.43-3.37 (m, 1H), 3.24 (s, 1H), 3.03 (d, *J* = 18.0 Hz, 1H), 2.91 (dd, *J* = 17.9, 9.2 Hz, 1H), 2.87-2.72 (m, 2H), 2.53-2.47 (m, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.06 (s, 3H).

ESI-MS *m/z*: 795.8 $(M+H)^+$.

B)



34 35

To a solution of **34** (345 mg, 0.43 mmol) in CH_3CN:H_2O (1.39:1, 30 mL, 0.015 M) was added $AgNO_3$ (2.20 g, 13.0 mmol). After 3 h at 23 ºC, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain **35** (175 mg, 51%).

R_f= 0.35 (CH_2Cl_2:CH_3OH, 9:1).

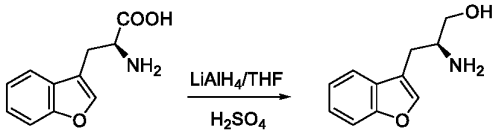
^{1}H NMR (500 MHz, CD_3OD): δ 7.27 (d, *J* = 9.0 Hz, 1H), 6.90 (d, *J* = 2.6 Hz, 1H), 6.80 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.57 (s, 1H), 6.23 (d, *J* = 1.2 Hz, 1H), 6.05 (d, *J* = 1.2 Hz, 1H), 5.23 (d, *J* = 11.5 Hz, 1H), 4.27-4.08 (m, 4H), 3.77 (s, 3H), 3.75 (s, 3H), 3.63 (d, *J* = 14.1 Hz, 2H), 3.40-3.34 (m, 2H), 2.93-2.87 (m, 5H), 2.80 (d, *J* = 15.5 Hz, 1H), 2.57-2.54 (m, 2H), 2.34 (s, 3H), 2.30 (s, 3H), 2.14 (s, 3H), 2.05 (s, 3H).

^{13}C NMR (126 MHz, CD_3OD): δ 171.9, 170.6, 157.5, 147.0, 145.0, 142.3, 141.0, 132.2, 131.1, 129.1, 122.2, 120.9, 120.2, 116.3, 115.1, 114.0, 112.7, 111.4, 103.5, 102.7, 92.9, 62.0, 60.3, 59.8, 59.4, 56.5, 56.2, 56.0, 54.0, 43.8, 41.2, 40.7, 30.8, 30.3, 28.7, 24.5, 21.6, 20.6, 16.2, 9.6.

ESI-MS *m/z*: 768.6 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 768.2630 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585).

Example 0-16



36-S 37-S

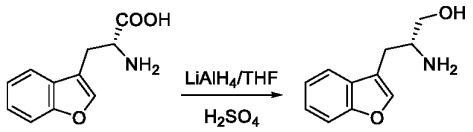
To a solution of $LiAlH_4$ (148 mL, 1.0 M in THF, 148 mmol) at -40 ºC was added carefully H_2SO_4 (7.14 mL, 72.9 mmol) and a suspension of (*S*)-2-amino-3-(benzofuran-3-yl)propanoic acid (**36-S**) (prepared as described in *Tetrahedron Asymmetry* **2008**, 19, 500-511) (5.54 g,

26.9 mmol) in THF (85 mL, 0.003 M). The reaction mixture was left evolution at 23 ºC, heated at 80 ºC for 3 h and 18 h at 23 ºC. Cool at -21 ºC the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum to afford compound **37-S** (3.93 g, >100%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.67-7.62 (m, 1H), 7.61 (s, 1H), 7.51-7.41 (m, 1H), 7.34-7.18 (m, 2H), 3.69-3.48 (m, 1H), 3.44 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.18 (dtd, *J* = 7.4, 6.4, 4.6 Hz, 1H), 2.88 (ddd, *J* = 14.4, 6.1, 1.0 Hz, 1H), 2.68 (ddd, *J* = 14.4, 7.5, 0.9 Hz, 1H).

Example 0-17



36-R **37-R**

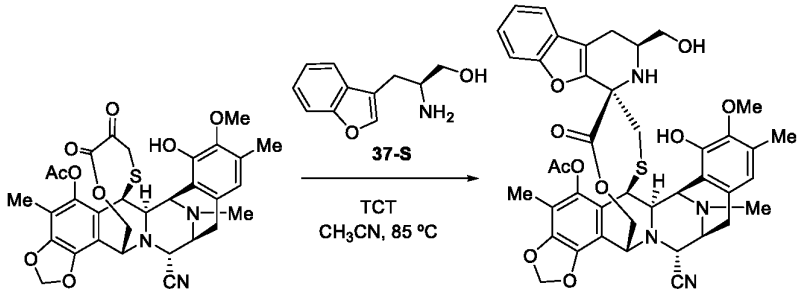
To a solution of $LiAlH_4$ (118 mL, 1.0 M in THF, 118 mmol) at -40 ºC was added carefully H_2SO_4 (3.1 mL, 57.8 mmol) and a suspension of (*R*)-2-amino-3-(benzofuran-3-yl)propanoic acid (**36-R**) (prepared as described in *Tetrahedron Asymmetry* **2008**, 19, 500-511) (4.4 g, 21.4 mmol) in THF (67.4 mL, 0.003 M). The reaction mixture was left evolution at 23 ºC, heated at 80 ºC for 3 h and 18 h at 23 ºC. Cool at -21 ºC the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15, Silice amine) to afford compound **37-R** (2.77 g, 68%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.63-7.52 (m, 1H), 7.56 (s, 1H), 7.46-7.33 (m, 1H), 7.21 (dtd, *J* = 19.9, 7.3, 1.3 Hz, 2H), 3.57 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.42 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.15 (dtd, *J* = 7.6, 6.3, 4.6 Hz, 1H), 2.84 (ddd, *J* = 14.4, 6.0, 1.0 Hz, 1H), 2.64 (ddd, *J* = 14.4, 7.5, 0.9 Hz, 1H).

Example 0-18

A)



1 38-S

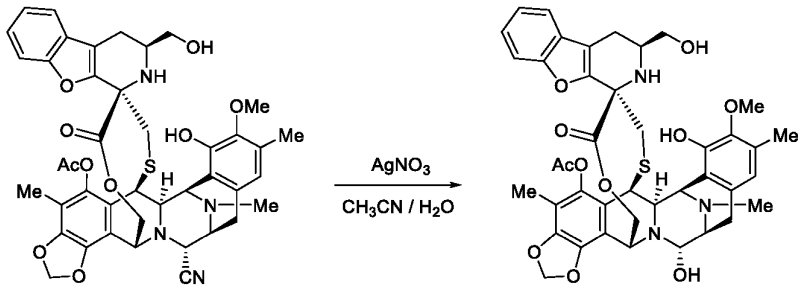
To a solution of compound **1** (850 mg, 1.36 mmol) in CH_3CN (136 mL, 0.01 M) was added (*S*)-2-amino-3-(benzofuran-3-yl)propan-1-ol (**37-S**) (1.30 g, 6.83 mmol and cyanuric chloride (TCT) (170 mg, 20%). The reaction mixture was stirred at 85 ºC for 24 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **38-S** (750 mg, 69%).

R_f= 0.25 (Hexane:EtOAc, 1:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.39-7.33 (m, 1H), 7.33-7.29 (m, 1H), 7.20 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.14 (td, *J* = 7.4, 1.0 Hz, 1H), 6.61 (s, 1H), 6.21 (d, *J* = 1.4 Hz, 1H), 6.06 (d, *J* = 1.4 Hz, 1H), 5.74 (s, 1H), 5.08 (d, *J* = 11.2 Hz, 1H), 4.58 (s, 1H), 4.37 (s, 1H), 4.32-4.23 (m, 2H), 4.19 (d, *J* = 2.7 Hz, 1H), 3.81 (s, 3H), 3.52-3.41 (m, 3H), 3.36-3.29 (m, 1H), 3.13 (d, *J* = 9.8 Hz, 1H), 3.00-2.81 (m, 3H), 2.57 (dd, *J* = 15.7, 4.9 Hz, 1H), 2.50 (d, *J* = 15.2 Hz, 1H), 2.37 (s, 3H), 2.31-2.25 (m, 1H), 2.29 (s, 3H), 2.16 (s, 3H), 2.10 (d, *J* = 7.2 Hz, 1H), 2.05 (s, 3H).

ESI-MS *m/z*: 795.2 $(M)^+$.

B)



38-S 39-S

To a solution of compound **38-S** (890 mg, 1.12 mmol) in CH_3CN:H_2O (1.39:1, 75 mL, 0.015 M) was added $AgNO_3$ (4.70 g, 28.0 mmol). After 18 h at 23 ºC, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was

purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **39-S** (500 mg, 57%).

R_f= 0.30 (CH_2Cl_2:CH_3OH, 9:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.33 (m, 1H), 7.33-7.28 (m, 1H), 7.23-7.16 (m, 1H), 7.16-7.09 (m, 1H), 6.62 (s, 1H), 6.18 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.4 Hz, 1H), 5.71 (s, 1H), 5.19 (d, *J* = 11.2 Hz, 1H), 4.85 (s, 1H), 4.49 (s, 2H), 4.24-4.10 (m, 3H), 3.81 (s, 3H), 3.54 (d, *J* = 4.9 Hz, 1H), 3.49 (d, *J* = 2.3 Hz, 3H), 3.33 (t, *J* = 10.1 Hz, 2H), 3.22 (s, 1H), 2.98 (s, 1H), 2.84 (d, *J* = 7.6 Hz, 2H), 2.62-2.53 (m, 2H), 2.37 (s, 3H), 2.30-2.24 (m, 1H), 2.28 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H).

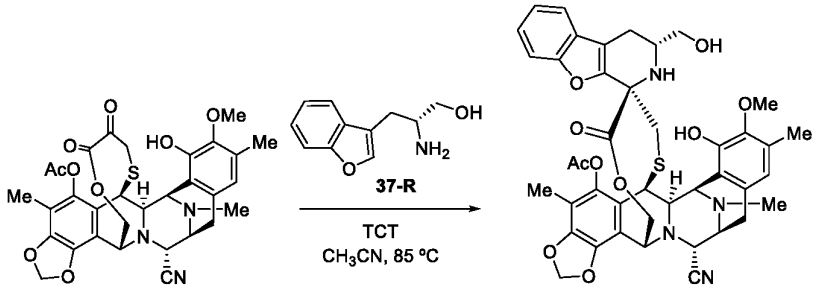
^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 170.7, 156.1, 150.6, 149.9, 147.1, 145.0, 142.4, 142.2, 132.0, 131.4, 128.7, 125.5, 123.8, 122.6, 121.6, 120.1, 116.5, 114.4, 112.3, 103.5, 92.6, 66.0, 65.1, 62.2, 60.4, 59.7, 56.6, 56.1, 54.8, 54.1, 51.6, 44.0, 41.3, 38.3, 30.8, 24.8, 20.6, 16.3, 9.6.

ESI-MS *m/z*: 768.2 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 768.2652 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585)

Example 0-19

A)



1 **38-R**

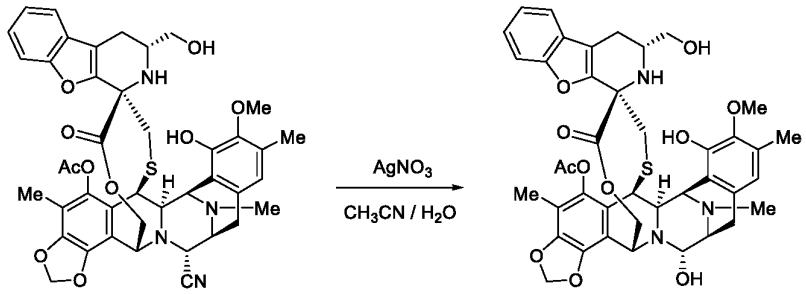
To a solution of compound **1** (100 mg, 0.16 mmol) in CH_3CN (16 mL, 0.01 M) was added (*R*)-2-amino-3-(benzofuran-3-yl)propan-1-ol (**37-R**) (307 mg, 1.6 mmol) and cyanuric chloride (TCT) (40 mg, 40%). The reaction mixture was stirred at 85 ºC for 44 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **38-R** (95 mg, 75%).

R_f= 0.3 (Hexane:EtOAc, 1:1).

^1H NMR (400 MHz, $CDCl_3$): δ 7.42-7.27 (m, 2H), 7.28 -7.09 (m, 2H), 6.58 (s, 1H), 6.20 (d, *J* = 1.4 Hz, 1H), 6.05 (d, *J* = 1.4 Hz, 1H), 5.79 (s, 1H), 5.00 (d , *J* = 11.4 Hz, 1H), 4.59 (s, 1H), 4.34 (s, 1H), 4.31-4.16 (m, 4H), 3.80 (s, 3H), 3.79-3.76 (m, 1H), 3.63 (s, 1H), 3.54-3.40 (m, 4H), 2.99-2.87 (m, 2H), 2.68 (d, *J* = 15.0 Hz, 1H), 2.56-2.47 (m, 1H), 2.38 (s, 3H), 2.27 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H).

ESI-MS *m/z*: 795.2 $(M+H)^+$.

B)



38-R **39-R**

To a solution of compound **38-R** (95 mg, 0.11 mmol) in CH_3CN:H_2O (1.39:1, 11 mL, 0.015 M) was added $AgNO_3$ (601 mg, 3.58 mmol). After 18 h at 23 ºC, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **39-R** (66 mg, 70%).

R_f= 0.3 (CH_2Cl_2:CH_3OH, 9:1).

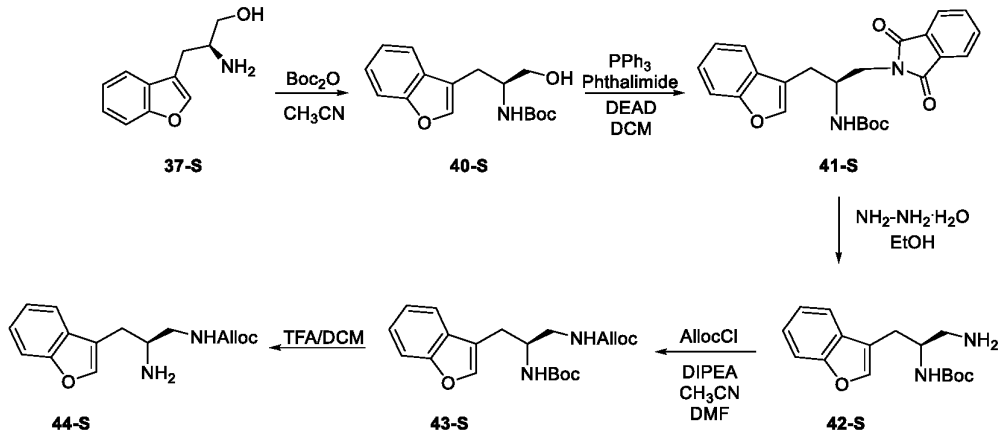
^1H NMR (400 MHz, $CDCl_3$): δ 7.39-7.31 (m, 2H), 7.23-7.07 (m, 2H), 6.59 (s, 1H), 6.17 (d, *J* = 1.4 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 5.75 (s, 1H), 5.12 (dd, *J* = 11.3, 1.2 Hz, 1H), 4.84 (s, 1H), 4.56-4.43 (m, 2H), 4.19-4.07 (m, 3H), 3.79 (s, 3H), 3.83-3.74 (m, 1H), 3.66-3.51 (m, 3H), 3.24 (s, 1H), 2.99-2.79 (m, 2H), 2.75-2.64 (m, 1H), 2.59-2.43 (m, 2H), 2.38 (s, 3H), 2.27 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD): δ 170.5, 169.1, 154.9, 148.9, 148.5, 145.7, 143.6, 141.1, 140.8, 130.6, 129.9, 127.1, 124.1, 122.4, 122.4, 121.2, 120.3, 118.7, 118.2, 115.1, 113.6, 110.9, 102.1, 91.1, 65.0, 63.3, 60.2, 59.0, 58.4, 55.4, 54.5, 52.7, 52.3, 42.5, 38.7, 29.4, 23.5, 23.2, 19.1, 14.8, 8.3.

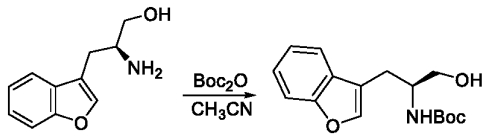
ESI-MS *m/z*: 768.2 $(M-H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 767.2628 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585).

Example 0-20. Synthesis of allyl-*N*-[(*S*)-2-amino-3-(benzofuran-3-yl)propyl]carbamate (**44-S**).



A)

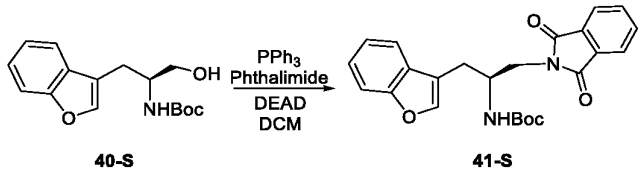


To a solution of compound **37-S** (1.0 g, 5.22 mmol) in CH_3CN (21 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (2.28 g, 10.4 mmol). The reaction mixture was stirred at 23 ºC for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **40-S** (0.5 g, 33%).

R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.49 (s, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.36-7.19 (m, 2H), 4.94 (s, 1H), 3.98 (s, 1H), 3.71-3.56 (m, 2H), 2.93 (d, *J* = 6.9 Hz, 2H), 1.41 (s, 9H).

B)

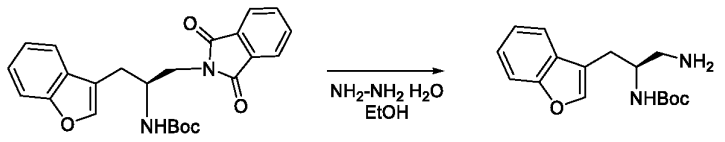


To a solution of compound **40-S** (0.5 g, 1.71 mmol) in CH_2Cl_2 (11 mL, 6 mL/mmol) was added phthalimide (0.55 g, 3.77 mmol), triphenylphosphine (0.99 g, 3.77 mmol) and the mixture was cooled at 0 ºC. A solution of 40% of diethyl azodicarboxylate (DEAD) in CH_2Cl_2 (1.26 mL, 4.29 mmol) was added for 15 min. The reaction was stirred at 23 ºC for 18 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 40:60) to afford compound **41-S** (0.68 g, 94%).

R_f= 0.8 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89-7.79 (m, 2H), 7.83-7.62 (m, 2H), 7.65-7.55 (m, 2H), 7.49-7.42 (m, 1H), 7.33-7.20 (m, 2H), 4.83 (d, *J* = 9.0 Hz, 1H), 4.39 (ddt, *J* = 12.1, 6.3, 2.9 Hz, 1H), 3.88-3.70 (m, 2H), 2.96 (d, *J* = 6.4 Hz, 2H), 1.24 (s, 9H).

C)



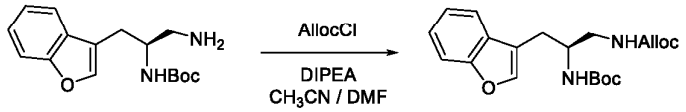
41-S **42-S**

To a solution of compound **41-S** (345 mg, 0.82 mmol) in ethanol (25 mL, 30 mL/mmol) was added hydrazine monohydrate (3.6 mL, 73.8 mmol). The reaction mixture was stirred at 80 ºC in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **42-S** (233 mg, 98%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 7.5 Hz, 1H), 7.49-7.42 (m, 2H), 7.33-7.18 (m, 2H), 4.85 (d, *J* = 8.8 Hz, 1H), 3.91 (s, 1H), 2.91-2.76 (m, 3H), 2.67 (dd, *J* = 13.1, 6.8 Hz, 1H), 1.25 (s, 9H).

D)



42-S **43-S**

To a solution of compound **42-S** (280 mg, 0.96 mmol) in CH_3CN (10 mL, 10 mL/mmol) and DMF (16 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (0.14 mL, 0.77 mmol) and allyl chloroformate (1.02 mL, 9.64 mmol). The reaction was stirred at 23 ºC for 2 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford compound **43-S** (445 mg, >100%).

R_f= 0.5 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.52-7.43 (m, 2H), 7.34-7.20 (m, 2H), 5.90 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.32-5.17 (m, 2H), 4.93-4.86 (m, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 4.08-3.98 (m, 1H), 3.40-3.21 (m, 2H), 2.88 (m, 2H), 1.25 (s, 9H).

E)



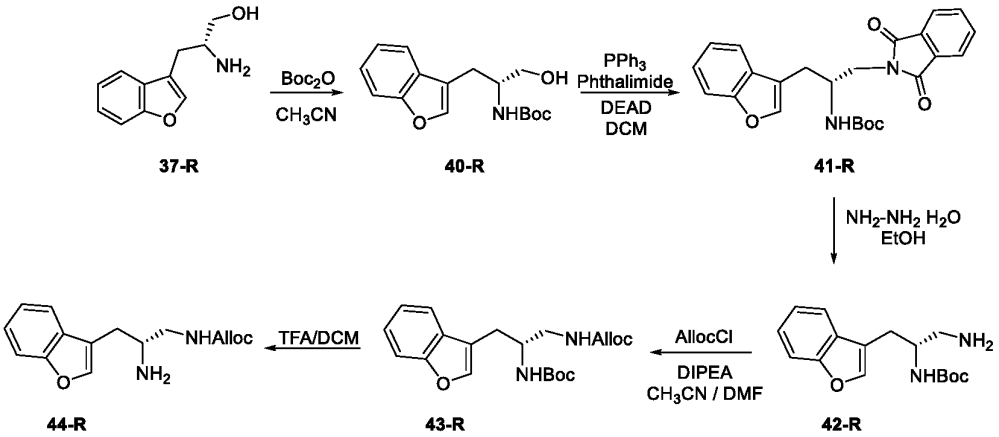
43-S **44-S**

To a solution of compound **43-S** (160 mg, 0.43 mmol) in CH_2Cl_2 (8 mL, 16.6 mL/mmol) was added trifluoroacetic acid (4 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 1.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **44-S** (175 mg, >100%).

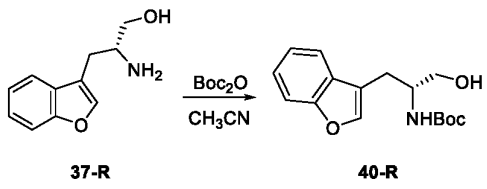
R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.72 (s, 1H), 7.64 (dt, *J* = 8.4, 0.9 Hz, 1H), 7.49 (dt, *J* = 8.4, 0.9 Hz, 1H), 7.37-7.22 (m, 2H), 5.94 (ddt, *J* = 16.3, 10.7, 5.5 Hz, 1H), 5.32 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.19 (dq, *J* = 10.6, 1.5 Hz, 1H), 4.56 (dt, *J* = 5.7, 1.5 Hz, 2H), 3.56 (qd, *J* = 7.0, 4.4 Hz, 1H), 3.46-3.32 (m, 1H), 3.32-3.24 (m, 1H), 3.03 (dd, *J* = 14.8, 6.9 Hz, 1H), 2.91 (ddd, *J* = 14.8, 7.1, 0.9 Hz, 1H).

Example 0-21. Synthesis of allyl-*N*-[(*R*)-2-amino-3-(benzofuran-3-yl)propyl]carbamate (**44-R**).



A)

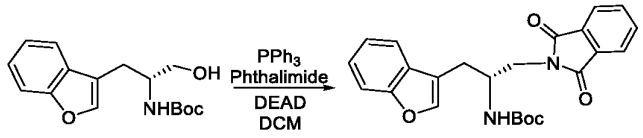


To a solution of compound **37-R** (2.75 g, 14.4 mmol) in CH_3CN (58 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (6.27 g, 28.76 mmol). The reaction mixture was stirred at 23 ºC for 2.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **40-R** (3.7 g, 88%).

R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.52-7.43 (m, 2H), 7.35-7.20 (m, 2H), 4.85 (d, *J* = 8.2 Hz, 1H), 4.00 (bs, 1H), 3.69 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.62 (dd, *J* = 10.9, 5.1 Hz, 1H), 2.94 (d, *J* = 6.9 Hz, 2H), 1.42 (s, 9H).

B)



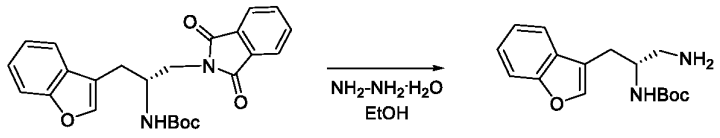
40-R **41-R**

To a solution of compound **40-R** (3.7 g, 12.7 mmol) in CH_2Cl_2 (76 mL, 6 mL/mmol) was added phthalimide (4.1 g, 28 mmol), triphenylphosphine (7.3 g, 28 mmol) and the mixture was cooled at 0 ºC. A solution of 40% of diethyl azodicarboxylate (DEAD) in CH_2Cl_2 (9.4 mL, 31.7 mmol) was added for 15 min. The reaction was stirred at 23 ºC for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **41-R** (4.05 g, 76%).

R_f= 0.8 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67-7.68 (m, 4H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.58 (s, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.27 (dtd, *J* = 17.2, 7.3, 1.4 Hz, 2H), 4.84 (d, *J* = 9.0 Hz, 1H), 4.46-4.30 (m, 1H), 3.89-3.66 (m, 2H), 2.97 (d, *J* = 6.4 Hz, 2H), 1.24 (s, 9H).

C)



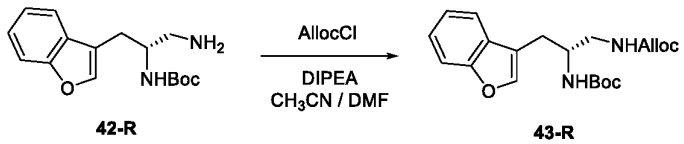
41-R **42-R**

To a solution of compound **41-R** (4.0 g, 9.5 mmol) in ethanol (285 mL, 30 mL/mmol) was added hydrazine monohydrate (41.5 mL, 856 mmol). The reaction mixture was stirred at 80 ºC in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **42-R** (2.2 g, 80%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.5 Hz, 1H), 7.45 (s, 1H), 7.44 (d, *J* = 7.1 Hz, 1H), 7.25 (dtd, *J* = 18.8, 7.3, 1.3 Hz, 2H), 4.94 (d, *J* = 8.8 Hz, 1H), 3.98-3.78 (m, 1H), 2.90-2.77 (m, 2H), 2.65 (dd, *J* = 13.1, 7.0 Hz, 1H), 1.40 (s, 9H).

D)



42-R **43-R**

To a solution of compound **42-R** (2.2 g, 7.6 mmol) in CH_3CN (76 mL, 10 mL/mmol) and DMF (7.6 mL, 1 mL/mmol) was added *N*,*N*-diisopropylethylamine (1.1 mL, 6.08 mmol) and allyl chloroformate (8.05 mL, 76 mmol). The reaction was stirred at 23 ºC for 7 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The

combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford compound **43-R** (2.3 g, 81%).

R_f= 0.7 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.5 Hz, 1H), 7.52-7.43 (m, 2H), 7.34-7.20 (m, 2H), 5.90 (ddt, *J* = 17.3, 10.8, 5.6 Hz, 1H), 5.29 (d, *J* = 17.2, 1H), 5.20 (d, *J* = 10.4, 1H), 5.10 (t, *J* = 6.2 Hz, 1H), 4.86 (d, *J* = 8.4 Hz, 1H), 4.56 (d, *J* = 5.4, 2H), 4.08-3.97 (m, 1H), 3.36 (dt, *J* = 10.7, 4.7 Hz, 1H), 3.30-3.23 (m, 1H), 2.87 (td, *J* = 14.8, 6.5 Hz, 2H), 1.41 (s, 9H).

E)



43-R **44-R**

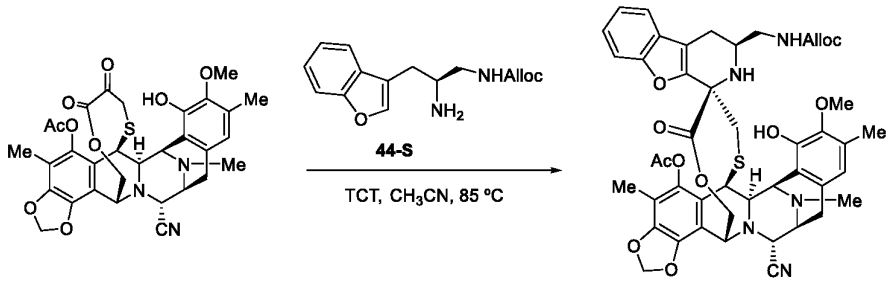
To a solution of compound **43-R** (1.32 g, 3.52 mmol) in CH_2Cl_2 (60 mL, 16.6 mL/mmol) was added Trifluoroacetic acid (30 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 ºC for 1.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **44-R** (0.90 g, 94%).

R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.69-7.61 (m, 1H), 7.54-7.46 (m, 1H), 7.39-7.24 (m, 2H), 5.95 (ddt, *J* = 16.3, 10.8, 5.5 Hz, 1H), 5.32 (dd, *J* = 17.3, 1.8 Hz, 1H), 5.24-5.16 (m, 1H), 4.57 (dt, *J* = 5.7, 1.5 Hz, 2H), 3.68 (qd, *J* = 7.1, 4.2 Hz, 1H), 3.48 (dd, *J* = 14.8, 4.2 Hz, 1H), 3.42-3.30 (m, 1H), 3.14-2.95 (m, 2H).

Example 0-22

A)



1 **45-S**

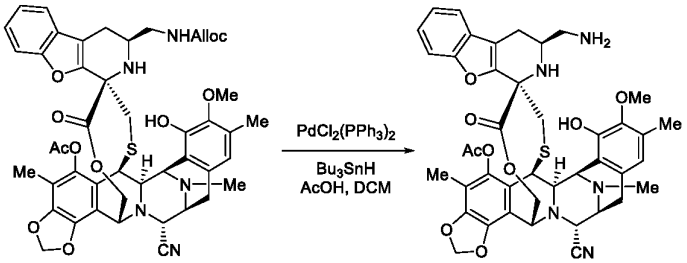
To a solution of compound **1** (750 mg, 1.2 mmol) in CH_3CN (120 mL, 0.01 M) was added compound **44-S** (1370 mg, 6 mmol) and cyanuric chloride (TCT) (184 mg, 20%). The reaction mixture was stirred at 85 ºC for 23 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **45-S** (755 mg, 72%).

R_f= 0.36 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.28 (m, 2H), 7.23-7.08 (m, 2H), 6.67 (s, 1H), 6.19 (d, *J* = 1.4 Hz, 1H), 6.09-5.95 (m, 1H), 6.04 (d, *J* = 1.4 Hz, 1H), 5.92 (s, 1H), 5.80 (s, 1H), 5.44-5.34 (m, 1H), 5.26 (dq, *J* = 10.4, 1.3 Hz, 1H), 5.08 (dd, *J* = 11.4, 1.1 Hz, 1H), 4.70-4.63 (m, 2H), 4.56 (s, 1H), 4.34 (s, 1H), 4.31-4.18 (m, 3H), 3.80 (s, 3H), 3.50-3.39 (m, 2H), 3.24-3.15 (m, 1H), 3.00 (dt, *J* = 12.2, 6.0 Hz, 2H), 2.95 (d, *J* = 5.2 Hz, 2H), 2.60 (dd, *J* = 15.4, 4.5 Hz, 2H), 2.44 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.25-2.20 (m, 1H), 2.18 (s, 3H), 2.12 (s, 1H), 2.04 (s, 3H).

ESI-MS *m/z*: 878.2 $(M+H)^+$.

B)



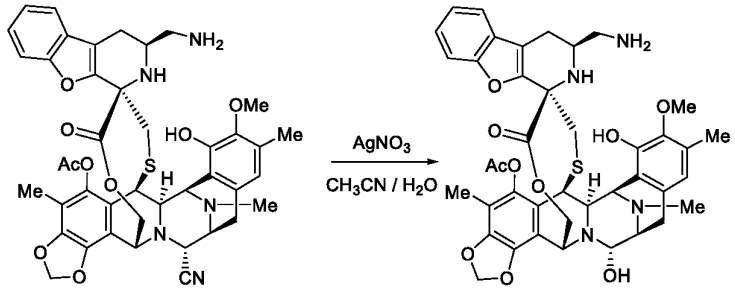
To a solution of compound **45-S** (750 mg, 0.85 mmol) in CH_2Cl_2 (15.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (96 mg, 0.14 mmol) and acetic acid (0.5 mL, 8.5 mmol). Tributyltin hydride (1.4 mL, 5.1 mmol) was added at 0 ºC, and the reaction mixture was stirred at 0 ºC for 30 minutes, and was concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and CH_2Cl_2:CH_3OH, from 100:1 to 1:100) to afford compound **46-S** (430 mg, 64%).

R_f= 0.3 (CH_2Cl_2:CH_3OH, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.29 (m, 2H), 7.22-7.11 (m, 2H), 6.57 (s, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 6.06 (d, *J* = 1.5 Hz, 1H), 5.07 (d, *J* = 11.5 Hz, 1H), 4.57 (s, 1H), 4.37 (s, 1H), 4.29-4.23 (m, 2H), 4.14 (s, 1H), 3.79 (s, 3H), 3.50-3.47 (m, 2H), 3.38 (d, *J* = 8.7 Hz, 1H), 2.95-2.71 (m, 4H), 2.68-2.52 (m, 2H), 2.51-2.38 (m, 1H), 2.35 (s, 3H), 2.33-2.26 (m, 1H), 2.29 (s, 3H), 2.17-2.08 (m, 1H), 2.10 (s, 3H), 2.04 (s, 3H).

ESI-MS *m/z*: 794.3 $(M+H)^+$.

C)



46-S 47-S

To a solution of compound **46-S** (550 mg, 0.7 mmol) in CH_3CN:H_2O (1.39:1, 49 mL, 0.015 M) was added $AgNO_3$ (2.4 g, 14 mmol). After 16 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give compound **47-S** (53 mg, 10%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (d, 7.9 Hz, 1H), 7.33 (d, 7.4 Hz, 1H), 7.23 (t, *J* = 7.4 Hz, 1H), 7.16 (t, *J* = 7.4 Hz, 1H), 6.77 (s, 1H), 6.20 (s, 1H), 6.04 (s, 1H), 5.92 (s, 1H), 5.20 (d, *J* = 11.1 Hz, 1H), 4.90 (s, 1H), 4.50 (s, 1H), 4.46-4.39 (m, 1H), 4.25 (d, *J* = 11.1 Hz, 1H), 4.20 (s, 1H), 3.84 (s, 3H), 3.81 (d, *J* = 4.2 Hz, 1H), 3.58 (s, 1H), 3.40-3.14 (m, 3H), 2.90 (t, *J* = 13.0 Hz, 1H), 2.76 (m, 3H), 2.50 (s, 3H), 2.46-2.37 (m, 1H), 2.32-2.26 (m, 2H), 2.30 (s, 3H), 2.15 (s, 3H), 2.04 (s, 3H).

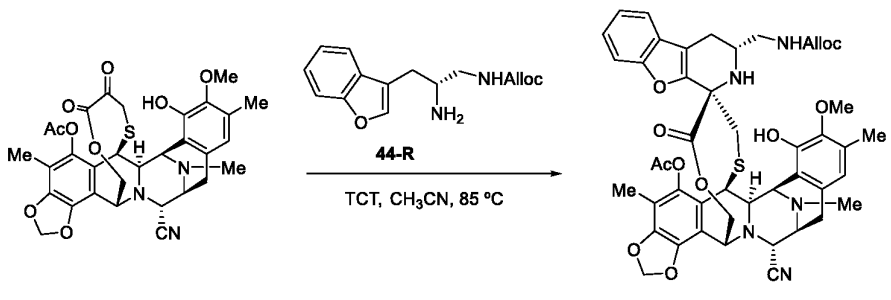
^{13}C NMR (126 MHz, CD_3OD): δ 170.5, 169.2, 154.6, 149.1, 148.7, 145.7, 143.5, 141.0, 140.9, 131.2, 129.6, 126.9, 124.4, 122.5, 121.4, 119.7, 118.7, 115.0, 112.7, 111.0, 110.7, 102.1, 91.2, 63.5, 61.2, 59.2, 58.5, 55.3, 54.7, 53.4, 52.7, 43.3, 42.5, 39.9, 36.9, 29.3, 24.1, 23.6, 19.1, 15.0, 8.2.

ESI-MS *m/z*: 767.2 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 767.2794 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$ 767.2745).

Example 0-23.

A)



1 45-R

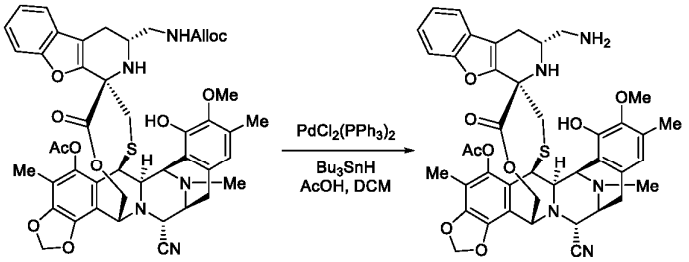
To a solution of compound **1** (621 mg, 1 mmol) in CH_3CN (100 mL, 0.01 M) was added compound **44-R** (825 mg, 3 mmol) and cyanuric chloride (TCT) (248 mg, 40%). The reaction mixture was stirred at 85 ºC for 66 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **45-R** (530 mg, 58%).

R_f= 0.4 (Hexane:EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.28 (m, 2H), 7.23-7.08 (m, 2H), 6.60 (s, 1H), 6.20 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.4 Hz, 1H), 6.01-5.92 (m, 1H), 5.77 (s, 1H), 5.44-5.20 (m, 2H), 5.09 (s, 1H), 5.04-4.96 (m, 1H), 4.71-4.55 (m, 2H), 4.34 (s, 1H), 4.30-4.18 (m, 3H), 3.79 (s, 3H), 3.53 (dd, *J* = 10.2, 4.4 Hz, 1H), 3.46 (m, 2H), 3.50-3.40 (m, 1H), 3.03-2.87 (m, 2H), 2.67 (d, *J* = 15.0 Hz, 1H), 2.47 (dd, *J* = 15.6, 3.7 Hz, 1H), 2.40-2.32 (m, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.19-2.12 (m, 2H), 2.16 (s, 3H), 2.09 (s, 3H).

ESI-MS *m/z*: 878.3 $(M+H)^+$.

B)



45-R 46-R

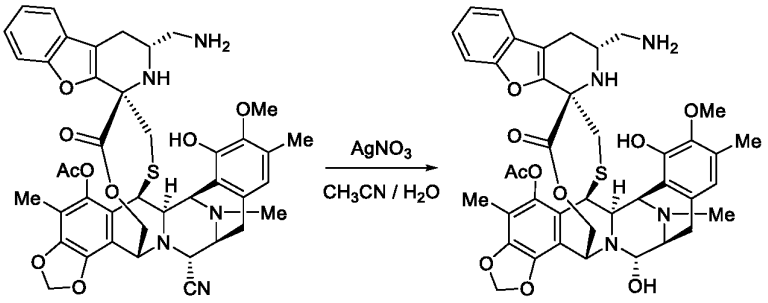
To a solution of compound **45-R** (552 mg, 0.63 mmol) in CH_2Cl_2 (11.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (70.7 mg, 0.1 mmol) and acetic acid (0.36 mL, 6.3 mmol). Tributyltin hydride (1.02 mL, 3.8 mmol) was added at 0 ºC and the reaction mixture was stirred at 0 ºC for 0.5 h, and concentrated under vacuum The crude obtained was diluted with EtOAc, saturated aqueous solution of NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford compound **46-R** (423 mg, 85%).

R_f= 0.3 (CH_2Cl_2:CH_3OH, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.28 (m, 2H), 7.23-7.08 (m, 2H), 6.56 (s, 1H), 6.19 (d, *J* = 1.4 Hz, 1H), 6.05 (d, *J* = 1.4 Hz, 1H), 4.98 (d, *J* = 11.5 Hz, 1H), 4.59 (s, 1H), 4.34 (s, 1H), 4.27 (dd, *J* = 5.1, 1.7 Hz, 1H), 4.22-4.16 (m, 2H), 3.80 (s, 3H), 3.49-3.39 (m, 2H), 3.31 (dq, *J* = 9.8, 5.5, 4.5 Hz, 2H), 2.95 (s, 1H), 2.83 (d, *J* = 5.6 Hz, 2H), 2.74-2.51 (m, 3H), 2.35 (s, 3H), 2.32-2.21 (m, 2H), 2.26 (s, 3H); 2.16 (s, 3H), 2.06 (s, 3H).

ESI-MS *m/z*: 794.3 $(M+H)^+$.

C)



46-R 47-R

To a solution of compound **46-R** (412 mg, 0.52 mmol) in CH_3CN:H_2O (1.39:1, 36 mL, 0.015 M) was added $AgNO_3$ (1.76 g, 10.4 mmol). After 22 h at 23 ºC, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give compound **47-R** (175 mg, 43%).

R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 (dd, *J* = 11.1, 7.9 Hz, 2H), 7.22-7.07 (m, 2H), 6.57 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 6.01 (d, *J* = 1.2 Hz, 1H), 5.11 (d, *J* = 11.2 Hz, 1H), 4.84 (s, 1H), 4.53-4.47 (m, 2H), 4.21-4.07 (m, 2H), 3.80 (s, 3H), 3.56 (d, *J* = 5.1 Hz, 1H), 3.43 (s, 1H), 3.24 (d, *J* = 9.1 Hz, 1H), 2.98-2.78 (m, 4H), 2.72-2.58 (m, 2H), 2.38 (s, 3H), 2.35-2.27 (m, 2H), 2.28 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD): δ 170.6, 169.1, 155.0, 148.8, 145.6, 143.7, 141.1, 140.8, 130.9, 129.7, 126.9, 124.2, 122.4, 121.1, 119.6, 118.9, 118.7, 115.0, 113.2, 112.5, 111.0, 102.1, 91.3, 63.3, 60.4, 59.0, 58.4, 55.3, 54.6, 52.6, 51.1, 44.9, 42.4, 39.8, 38.7, 29.4, 24.0, 23.2, 19.1, 15.0, 8.3.

ESI-MS *m/z*: 767.2 $(M\text{-}H_2O+H)^+$.

(+)-HR-ESI-TOF-MS *m/z*: 767.2806 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$ 767.2745).

Bioactivity example of the payloads

The aim of this assay is to evaluate the *in vitro* cytostatic (ability to delay or arrest tumor cell growth) or cytotoxic (ability to kill tumor cells) activity of the samples being tested.

CELL LINES

Name	Nº ATCC	Species	Tissue	Characteristics
A549	CCL-185	human	Lung	lung carcinoma (NSCLC)
HT29	HTB-38	human	Colon	colorectal adenocarcinoma
MDA-MB-231	HTB-26	human	Breast	breast adenocarcinoma
PSN1	CRM-CRL-3211	human	Pancreas	pancreas adenocarcinoma
PC-3	CRL-1435	human	Prostate	prostate adenocarcinoma
22Rv1	CRL-2505	human	Prostate	prostate carcinoma

EVALUATION OF CYTOTOXIC ACTIVITY USING THE SRB AND THE MTT COLORIMETRIC ASSAYS

A colorimetric assay, using Sulforhodamine B (SRB) reaction has been adapted to provide a quantitative measurement of cell growth and viability (following the technique described by Skehan et al. *J. Natl. Cancer Inst.* 1990, 82, 1107-1112). Another colorimetric assay based on 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to a purple formazan has been also used to assess the antiproliferative activity (following the technique described by Mosmann et al. *J. Immunol. Meth.* 1983, 65, 55-63).

These forms of assays employ 96-well cell culture microplates following the standards of the American National Standards Institute and the Society for Laboratory Automation and Screening (ANSI SLAS 1-2004 (R2012) 10/12/2011. All the cell lines used in this study were obtained from the American Type Culture Collection (ATCC) and derive from different types of human cancer.

A549, HT29, MDA-MB-231 and PSN1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) while PC-3 and 22Rv1 cells were maintained in Roswell Park Memorial Institute Medium (RPMI). All cell lines were supplemented with 10% Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 ºC, 5% CO_2 and 98% humidity. For the experiments, cells were harvested from subconfluent cultures using trypsinization and resuspended in fresh medium before counting and plating.

A549, HT29, MDA-MB-231 and PSN1 cells were seeded in 96 well microtiter plates, at 5000 cells per well in aliquots of 150 μL, and allowed to attach to the plate surface for 18 hours (overnight) in drug free medium. After that, one control (untreated) plate of each cell line was fixed (as described below) and used for time zero reference value. Culture plates were then treated with test compounds (50 μL aliquots of 4X stock solutions in complete culture medium plus 4% DMSO) using ten 2/5 serial dilutions (concentrations ranging from 10 to 0.003 μg/mL) and triplicate cultures (1% final concentration in DMSO). After 72 hours treatment, the antitumor effect was measured by using the SRB methodology: Briefly, cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution at room

temperature, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells were then rinsed several times with 1% acetic acid solution and air-dried at room temperature. SRB was then extracted in 10 mM trizma base solution and the absorbance measured in an automated spectrophotometric plate reader at 490 nm.

An appropriate number of PC-3 and 22Rv1 cells, to reach a final cell density in the assay ranging from 5,000 to 15,000 cells per well depending on the cell line, were seeded in 96-well plates and allowed to stand in culture medium for 24 h at 37ºC under 5% CO2 and 98% humidity. Then, compounds or DMSO in culture medium were added to reach a final volume of 200 µL and the intended compound concentration in a range covering ten serial 2/5 dilutions starting from 0.1 µg/mL in 1% (v/v) DMSO. At this point a set of "time zero control plates" treated with 1% (v/v) DMSO were processed with MTT as described below. The rest of the plates were incubated during 72 h under the aforementioned environmental conditions. Afterwards 50 µL of a 1 mg/mL MTT solution in culture medium were added to the wells and incubated for 6-8 hours at 37ºC to allow formazan crystals generation. Culture medium was then removed and 100 µL of neat DMSO added to each well to dissolve the formazan product into a coloured solution whose absorbance at 540 nm was finally measured in a PolarStar Omega microplate multilabel reader (BMG Labtech, Ortenberg, Germany).

Effects on cell growth and survival were estimated by applying the NCI algorithm (Boyd MR and Paull KD. Drug Dev. Res. 1995, 34, 91-104).The values obtained in triplicate cultures were fitted by nonlinear regression to a four-parameters logistic curve by nonlinear regression analysis. Three reference parameters were calculated (according to the aforementioned NCI algorithm) by automatic interpolation of the curves obtained by such fitting: GI_{50} = compound concentration that produces 50% cell growth inhibition, as compared to control cultures; TGI = total cell growth inhibition (cytostatic effect), as compared to control cultures, and LC_{50} = compound concentration that produces 50% net cell killing cytotoxic effect).

Tables 3-9 illustrate data on the biological activity of compounds of the present invention.

Table 3. Biological activity (Molar)

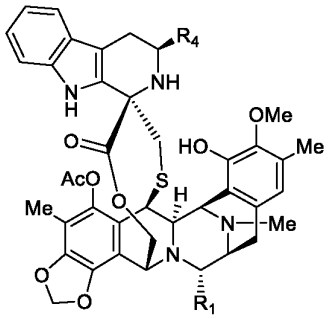
	Compound						
					**3-S** R_1 = CN, R_4 = -CH_2OH **3a-S** R_1 = CN, R_4 = -CH_2OAc **10-S** R_1 = CN, R_4 = -$CH_2NHAlloc$ **11-S** R_1 = CN, R_4 = -CH_2NH_2 **4-S** R_1 = OH, R_4 = -CH_2OH **4a-S** R_1 = OH, R_4 = -CH_2OAc **12-S** R_1 = OH, R_4 = -CH_2NH_2 **13-S** R_1 = OH, R_4 = -$CH_2NHAlloc$		
		A549	**HT29**	**MDA-MB-231**	**PSN1**	**PC-3**	**22Rv1**
GI_{50}	**3-S**	4.03E-10	2.77E-10	4.91E-10	9.95E-10		
TGI		6.17E-10	>1.26E-07	5.29E-10	1.64E-09		
LC_{50}		>1.26E-07	>1.26E-07	6.17E-10	>1.26E-07		
GI_{50}	**3a-S**	3.11E-09	2.99E-09	2.87E-09	2.15E-09		
TGI		3.23E-09	3.23E-09	3.59E-09	3.59E-09		
LC_{50}		>1.20E-07	>1.20E-07	4.90E-09	1.20E-08		
GI_{50}	**10-S**	2.05E-08	1.14E-08	4.79E-09	7.64E-09		
TGI		3.08E-08	1.25E-08	8.44E-09	1.25·E-08		
LC_{50}		7.53E-08	>1.14E-06	1.60E-08	2.39E-08		
GI_{50}	**11-S**	8.45E-09	3.41E-09	2.27E-09	3.28E-09		
TGI		2.65E-08	>1.26E-07	3.41E-09	4.54E-09		
LC_{50}		>1.26E-07	>1.26E-07	6.43E-09	8.07E-09		
GI_{50}	**4-S**	1.27E-09	1.27E-09	1.22E-09	1.78E-09	8.08E-10	3.58E-10
TGI		1.40E-09	1.40E-09	2.55E-09	2.29E-09		
LC_{50}		>1.27E-07	>1.27E-07	6.50E-09	3.44E-09		
GI_{50}	**4a-S**	3.99E-09	3.14E-09	3.39E-09	3.02E-09		
TGI		6.17E-09	3.39E-09	5.44E-09	3.27E-09		
LC_{50}		>1.21E-07	>1.21E-07	1.00E-08	3.51E-09		
GI_{50}	**12-S**	2.04E-08	4.85E-09	5.23E-09	3.44E-09		
TGI		5.61E-08	8.42E-09	8.42E-09	5.49E-09		
LC_{50}		>1.28E-07	>1.28E-07	1.53E-08	1.21E-08		
GI_{50}	**13-S**	1.15E-08	1.15E-08	1.15E-08	1.96E-08		
TGI		1.61E-08	1.27E-08	1.27E-08	2.88E-08		
LC_{50}		2.42E-08	>1.15E-06	1.38E-08	4.61E-08		

Table 4. Biological activity (Molar)

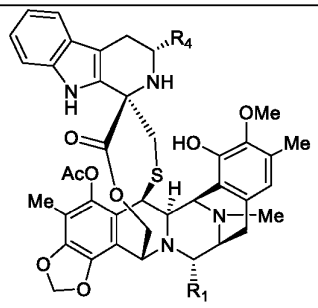
	Compound				
			**3-R** R_1 = CN, R_4 = -CH_2OH **10-R** R_1 = CN, R_4 = -$CH_2NHAlloc$ **11-R** R_1 = CN, R_4 = -CH_2NH_2 **4-R** R_1 = OH, R_4 = -CH_2OH **12-R** R_1 = OH, R_4 = -CH_2NH_2 **13-R** R_1 = OH, R_4 = -$CH_2NHAlloc$		
		A549	**HT29**	**MDA-MB-231**	**PSN1**
GI_{50}	**3-R**	4.03E-10	2.77E-10	2.77E-10	3.90E-10
TGI		5.79E-10	>1.26E-07	5.04E-10	6.05E-10
LC_{50}		>1.26E-07	>1.26E-07	1.25E-09	>1.26E-07
GI_{50}	**10-R**	3.76E-09	3.08E-09	2.85E-09	2.62E-09
TGI		5.93E-09	>1.14E-07	4.33E-09	3.88E-09
LC_{50}		>1.14E-07	>1.14E-07	7.18E-09	6.61E-09
GI_{50}	**11-R**	1.77E-09	1.39E-09	1.01E-09	1.39E-09
TGI		4.54E-09	>1.26E-07	1.51E-09	1.89E-09
LC_{50}		>1.26E-07	>1.26E-07	2.65E-09	>1.26E-07
GI_{50}	**4-R**	1.27E-09	1.26E-09	1.27E-09	4.59E-10
TGI		1.40E-09	1.40E-09	1.40E-09	8.54E-10
LC_{50}		> 1.27E-07	>1.27E-07	1.53E-09	2.55E-09
GI_{50}	**12-R**	1.40E-09	5.74E-10	3.19E-10	4.98E-10
TGI		2.93E-09	1.10E-09	6.76E-10	1.22E-09
LC_{50}		1.22E-08	2.93E-09	1.40E-09	>1.28E-07
GI_{50}	**13-R**	7.26E-09	6.91E-09	4.95E-09	2.88E-09
TGI		7.72E-09	7.60E-09	7.95E-09	3.11E-09
LC_{50}		>1.15E-07	>1.15E-07	1.38E-08	3.46E-09

Table 5. Biological activity (Molar)

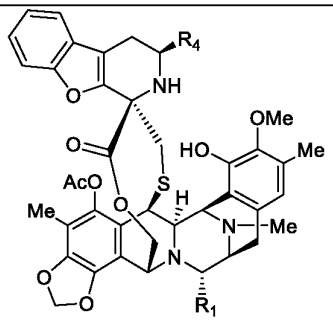
	Compound						
	 **38-S** R_1 = CN, R_4 = -CH_2OH **45-S** R_1 = CN, R_4 = -$CH_2NHAlloc$ **46-S** R_1 = CN, R_4 = -CH_2NH_2 **39-S** R_1 = OH, R_4 = -CH_2OH **47-S** R_1 = OH, R_4 = -CH_2NH_2						
		A549	**HT29**	**MDA-MB-231**	**PSN1**	**PC-3**	**22Rv1**
GI_{50}	**38-S**	8.05E-09	4.53E-09	2.52E-09	5.03E-09		
TGI		8.55E-09	7.05E-09	4.28E-09	8.18E-09		
LC_{50}		9.44E-09	>1.26E-07	7.80E-09	1.51E-08		
GI_{50}	**45-S**	1.82E-08	1.82E-08	1.71E-08	1.94E-08		
TGI		1.94E-08	1.94E-08	2.16E-08	2.62E-08		
LC_{50}		2.16E-08	>1.14E-07	2.96E-08	3.64E-08		
GI_{50}	**46-S**	8.19E-09	2.77E-09	3.65E-09	3.15E-09		
TGI		2.14E-08	6.17E-09	6.80E-09	4.79E-09		
LC_{50}		>1.26E-07	>1.26E-07	1.26E-08	9.20E-09		
GI_{50}	**39-S**	4.84E-09	3.94E-09	3.44E-09	8.02E-09	2.78E-09	4.81E-10
TGI		8.27E-09	6.74E-09	7.13E-09	1.02E-08		
LC_{50}		1.65E-08	>1.27E-07	1.78E-08	1.27E-08		
GI_{50}	**47-S**	1.40E-08	4.33E-09	6.24E-09	5.99E-09		
TGI		2.80E-08	6.75E-09	9.68E-09	8.54E-09		
LC_{50}		>1.27E-07	>1.27E-07	1.66E-08	1.27E-08		

Table 6. Biological activity (Molar)

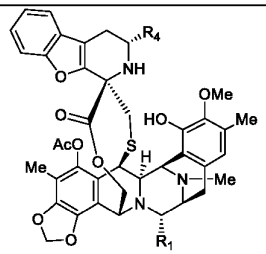
		Compound			
					
		38-R R_1 = CN, R_4 = -CH_2OH			
		45-R R_1 = CN, R_4 = -$CH_2NHAlloc$			
		46-R R_1 = CN, R_4 = -CH_2NH_2			
		39-R R_1 = OH, R_4 = -CH_2OH			
		47-R R_1 = OH, R_4 = -CH_2NH_2			
		A549	**HT29**	**MDA-MB-231**	**PSN1**
GI_{50}	**38-R**	6.54E-10	5.41E-10	4.53E-10	6.54E-10
TGI		1.04E-09	5.91E-10	8.43E-10	9.94E-10
LC_{50}		>1.26E-07	>1.26E-07	2.01E-09	1.76E-09
GI_{50}	**45-R**	1.82E-08	1.25E-08	9.57E-09	1.06E-08
TGI		1.94E-08	2.28E-08	1.94E-08	1.94E-08
LC_{50}		2.39E-08	>1.14E-07	4.33E-08	3.76E-08
GI_{50}	**46-R**	1.51E-09	1.21E-09	1.23E-09	9.95E-10
TGI		2.77E-09	1.39E-09	1.39E-09	1.51E-09
LC_{50}		>1.26E-07	>1.26E-07	1.51E-09	2.65E-09
GI_{50}	**39-R**	2.67E-10	2.93E-10	2.04E-10	3.65E-10
TGI		4.33E-10	6.24E-10	5.98E-10	5.73E-10
LC_{50}		>1.27E-07	>1.27E-07	2.80E-09	1.06E-09
GI_{50}	**47-R**	2.04E-09	8.03E-10	5.99E-10	1.40E-09
TGI		3.82E-09	1.40E-09	1.17E-09	2.04E-09
LC_{50}		1.40E-08	>1.27E-07	2.55E-09	3.31E-09

Table 7. Biological activity (Molar)

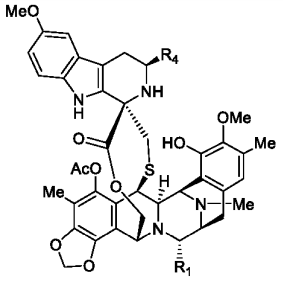
		Compound			
					
		18-S R_1 = CN, R_4 = -CH_2OH			
		25-S R_1 = CN, R_4 = -$CH_2NHAlloc$			
		26-S R_1 = CN, R_4 = -CH_2NH_2			
		19-S R_1 = OH, R_4 = -CH_2OH			
		27-S R_1 = OH, R_4 = -CH_2NH_2			
		A549	**HT29**	**MDA-MB-231**	**PSN1**
GI_{50}	**18-S**	1.70E-09	1.21E-09	1.21E-09	9.59E-10
TGI		3.03E-09	1.34E-09	1.34E-09	1.34E-09
LC_{50}		>1.21E-07	>1.21E-07	1.58E-09	>1.21E-07
GI_{50}	**25-S**	7.17E-09	7.17E-09	5.84E-09	6.84E-09
TGI		7.61E-09	7.72E-09	9.04E-09	9.26E-09
LC_{50}		>1.10E-07	>1.10E-07	1.54E-08	1.43E-08
GI_{50}	**26-S**	1.12E-08	2.79E-09	1.34E-09	3.04E-09
TGI		2.19E-08	3.16E-09	1.94E-09	3.28E-09
LC_{50}		>1.22E-07	>1.22E-07	3.89E-09	3.52E-09
GI_{50}	**19-S**	3.07E-09	1.35E-09	1.96E-09	2.95E-09
TGI		3.31E-09	1.60E-09	3.31E-09	3.19E-09
LC_{50}		>1.23E-07	>1.23E-07	1.10E-08	>1.23E-07
GI_{50}	**27-S**	6.02E-09	1.23E-09	1.19E-09	1.97E-09
TGI		1.12E-08	1.35E-09	1.23E-09	2.83E-09
LC_{50}		>1.23E-07	>1.23E-07	1.35E-09	4.55E-09

Table 8. Biological activity (Molar)

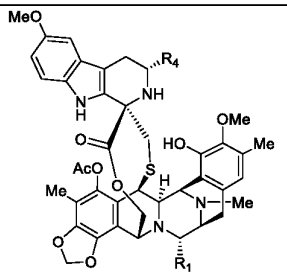
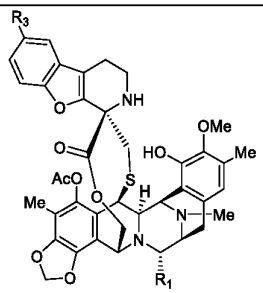
		Compound			
					
		18-R R_1 = CN, R_4 = -CH_2OH **25-R** R_1 = CN, R_4 = -$CH_2NHAlloc$ **26-R** R_1 = CN, R_4 = -CH_2NH_2 **19-R** R_1 = OH, R_4 = -CH_2OH **27-R** R_1 = OH, R_4 = -CH_2NH_2			
		A549	**HT29**	**MDA-MB-231**	**PSN1**
GI_{50}	**18-R**	1.21E-09	1.21E-09	1.21E-09	5.70E-10
TGI		1.34E-09	1.34E-09	1.34E-09	1.06E-09
LC_{50}		>1.21E-07	>1.21E-07	1.46E-09	>1.21E-07
GI_{50}	**25-R**	1.32E-09	1.54E-09	1.21E-09	1.21E-09
TGI		2.43E-09	2.76E-09	2.54E-09	2.32E-09
LC_{50}		9.92E-09	>1.10E-07	8.38E-09	6.73E-09
GI_{50}	**26-R**	1.94E-09	7.29E-10	1.17E-09	9.72E-10
TGI		3.40E-09	1.58E-09	1.22E-09	1.70E-09
LC_{50}		>1.22E-07	>1.22E-07	1.46E-09	3.52E-09
GI_{50}	**19-R**	1.47E-09	1.72E-09	1.23E-09	1.23E-09
TGI		3.56E-09	1.72E-09	1.35E-09	1.35E-09
LC_{50}		>1.23E-07	>1.23E-07	>1.23E-07	1.47E-09
GI_{50}	**27-R**	2.09E-09	5.04E-10	3.07E-10	6.39E-10
TGI		3.93E-09	5.53E-10	5.41E-10	1.17E-09
LC_{50}		1.01E-08	>1.23E-07	8.60E-10	2.46E-09

Table 9. Biological activity (Molar)

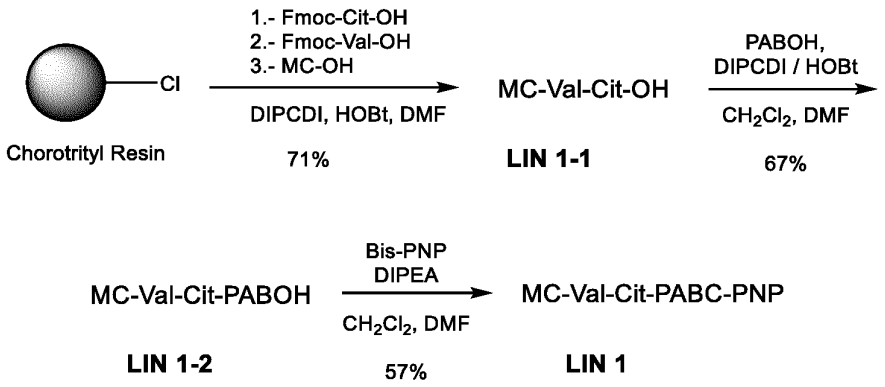
	Compound				
	 **31** R_1 = CN, R_3 = H **32** R_1 = OH, R_3 = H **34** R_1 = CN, R_3 = OMe **35** R_1 = OH, R_3 = OMe				
		A549	**HT29**	**MDA-MB-231**	**PSN1**
GI_{50}	**31**	1.96E-08	1.05E-08	8.89E-09	6.80E-09
TGI		2.09E-08	1.57E-08	1.70E-08	1.57E-08
LC_{50}		2.35E-08	>1.31E-07	3.53E-08	4.31E-08
GI_{50}	**32**	6.88E-09	6.88E-09	4.76E-09	6.09E-09
TGI		>1.32E-08	>1.32E-08	1.05E-08	8.34E-09
LC_{50}		>1.32E-08	>1.32E-08	>1.32E-08	1.20E-08
GI_{50}	**34**	5.91E-08	5.41E-08	4.53E-08	5.41E-08
TGI		8.05E-08	8.55E-08	7.67E-08	5.91E-08
LC_{50}		>1.26E-07	1.25E-07	1.12E-07	>1.26E-07
GI_{50}	**35**	8.14E-09	7.89E-09	4.58E-09	6.24E-09
TGI		8.78E-09	8.65E-09	8.27E-09	9.03E-09
LC_{50}		>1.27E-07	>1.27E-07	1.65E-08	1.40E-08

This data demonstrates that the payloads employed in the present invention have high potency *in vitro*.

Synthesis of linkers

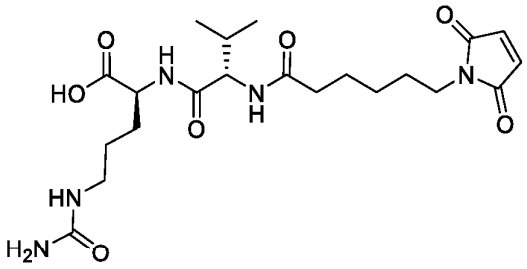
Preparation of LIN 1: MC-Val-Cit-PABC-PNP

Reaction Scheme



(a) **Preparation of LIN 1-1: MC-Val-Cit-OH**

LIN 1-1



Cl-TrtCl-resin (20 g, 1.49 mmol/g) (Iris Biotech, Ref.: BR-1065, 2-Chlorotrityl chloride resin (200-400 mesh, 1% DVB, 1.0-1.6 mmol/g), CAS 42074-68-0) was placed in a filter plate. 100 mL of DCM was added to the resin and the mixture was stirred for 1 h. The solvent was eliminated by filtration under vacuum. A solution of Fmoc-Cit-OH (11.83 g, 29.78 mmol) and DIPEA (17.15 mL, 98.45 mmol) in DCM (80 mL) was added and the mixture was stirred for 10 min. After that DIPEA (34.82 mmol, 199.98 mmol) was added and the mixture was stirred for 1 h. The reaction was terminated by addition of MeOH (30 mL) after stirring for 15 minutes. The Fmoc-Cit-O-TrtCl-resin produced as a result was subjected to the following washing/treatments: DCM (5 x 50 mL x 0.5 min), DMF (5 x 50 mL x 0.5 min), piperidine:DMF (1:4, 1 x 1 min, 2 x 10 min), DMF (5 x 50 mL x 0.5 min), DCM (5 x 50 mL x 0.5 min). The final piperidine wash gave NH_2-Cit-O-TrtCl-resin. The loading was calculated: 1.15 mmol/g.

The NH_2-Cit-O-TrtCl-resin produced above was washed with DMF (5 x 50 mL x 0.5 min) and a solution of Fmoc-Val-OH (31.22 g, 91.98 mmol), HOBt (11.23 g, 91.98 mmol) in DMF (100 mL) was added to the NH_2-Cit-O-TrtCl-resin, stirred and DIPCDI (14.24 mL, 91.98 mmol) was added and the mixture was stirred for 1.5 h. The reaction was terminated by washing with DMF (5 x 50 mL x 0.5 min). The Fmoc-Val-Cit-O-TrtCl-resin thus produced was treated with piperidine:DMF (1:4, 1 x 1 min, 2 x 10 min) and washed with DMF (5 x 50 mL x 0.5 min). The final piperidine wash gave NH_2-Val-Cit-O-TrtCl-resin.

A solution of 6-maleimidocaproic acid (MC-OH) (9.7 g, 45.92 mmol), HOBt (6.21 g, 45.92 mmol) in DMF (100 mL) was added to the NH_2-Val-Cit-O-TrtCl-resin produced above, stirred and DIPCDI (7.12 mL, 45.92 mmol) was added and the mixture was stirred for 1.5 h. The reaction was terminated by washing with DMF (5 x 50 mL x 0.5 min) and DCM (5 x 50 mL x 0.5 min).

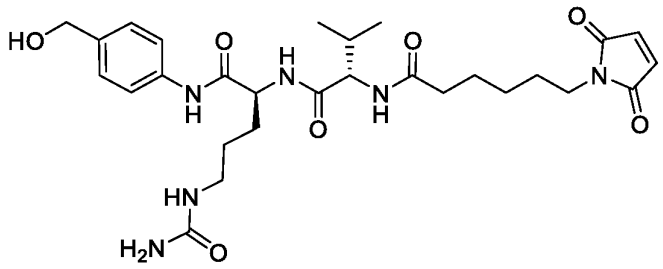
The peptide was cleaved from the resin by treatments with TFA:DCM (1:99, 5 x 100 mL). The resin was washed with DCM (7 x 50 mL x 0.5 min). The combined filtrates were evaporated to dryness under reduced pressure and the solid obtained was triturated with Et_2O and filtrated to obtain **LIN 1-1** (7.60 g, 71%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 12.47 (s, 1H), 8.13 (d, J = 7.3 Hz, 1H), 7.74 (d, J = 9.0 Hz, 1H), 6.99 (s, 2H), 5.93 (s, 1H), 5.35 (s, 2H), 4.20 (dd, J = 9.0, 6.8 Hz, 1H), 4.15-4.07 (m, 1H), 3.36 (t, J = 7.0 Hz, 2H), 3.00-2.88 (m, 2H), 2.21-2.12 (m, 1H), 2.11-2.03 (m, 1H), 1.98-1.86 (m, 1H), 1.74-1.62 (m, 1H), 1.61-1.50 (m, 1H), 1.50-1.31 (m, 6H), 1.21-1.11 (m, 2H), 0.84 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.8 Hz, 3H).

ESI-MS *m/z*: Calcd. for $C_{21}H_{33}N_5O_7$: 467.2. Found: 468.3 $(M+H)^+$.

(b) **Preparation of LIN 1-2: MC-Val-Cit-PABOH**

LIN 1-2



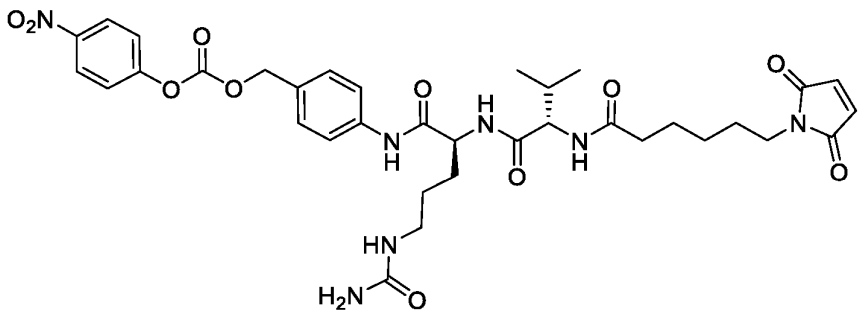
To a solution of **LIN 1-1** (1.6 g, 3.42 mmol) and 4-aminobenzyl alcohol (PABOH) (0.84 g, 6.84 mmol) in DCM (60 mL) was added a solution of HOBt (0.92 g, 6.84 mmol) in DMF (5 mL). DIPCDI (1.05 mL, 6.84 mmol) was added, the reaction mixture was stirred for 2 h at 23 ºC, Et_2O (150 mL) was added, and the solid obtained was filtrated in a filter plate under vacuum to obtain **LIN 1-2** (1.31 g, 67%).

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.88 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.77 (dd, J = 12.2, 8.5 Hz, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.2 Hz, 2H), 6.99 (s, 3H), 6.01-5.92 (m, 1H), 5.39 (s, 2H), 5.07 (s, 1H), 4.41 (s, 2H), 4.39-4.31 (m, 1H), 4.23-4.12 (m, 1H), 3.36 (t, J = 7.0 Hz, 2H), 3.06-2.97 (m, 1H), 2.96-2.90 (m, 1H), 2.22-2.03 (m, 2H), 2.01-1.88 (m, 1H), 1.76-1.62 (m, 1H), 1.63-1.28 (m, 6H), 1.25-1.11 (m, 2H), 0.84 (d, J = 6.9 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H).

ESI-MS *m/z*: Calcd. for $C_{28}H_{40}N_6O_7$: 572.3. Found: 573.3 $(M+H)^+$.

(c) **Preparation of LIN 1: MC-Val-Cit-PAB-PNP**

LIN 1



To a solution of **LIN 1-2** (500 mg, 0.87 mmol) and bis(4-nitrophenyl) carbonate (bis-PNP) (2.64 g, 8.72 mmol) in DCM:DMF (8:2, 25 mL) was added DIPEA (0.45 mL, 2.61 mmol). The reaction mixture was stirred for 20 h at 23 ºC and poured onto a silica gel column ($DCM:CH_3OH$, from 50:1 to 10:1) to afford pure target **LIN 1** (364 mg, 57%).

R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

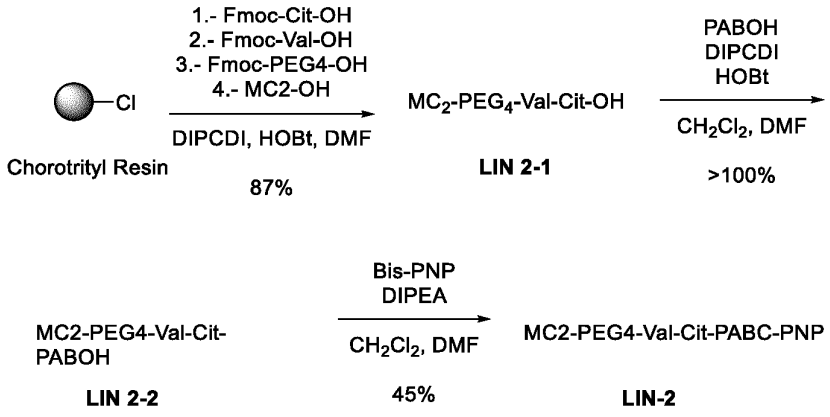
^1H NMR (400 MHz, $CDCl_3/CD_3OD$): δ 9.45 (s, 1H), 8.23 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.5 Hz, 2H), 6.65 (s, 2H), 5.20 (s, 2H), 4.56 (dt, *J* = 10.5, 5.4 Hz, 1H), 4.15 (d, *J* = 7.2 Hz, 1H), 3.46 (dd, *J* = 8.0, 6.4 Hz, 2H), 3.16-2.89 (m, 2H), 2.21 (dd, *J* = 8.3, 6.6 Hz, 2H), 2.06-1.97 (m, 1H), 1.90-1.83 (m, 1H), 1.73-1.46 (m, 7H), 1.34-1.20 (m, 2H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$) δ 174.4, 172.4, 171.1, 170.6, 160.5, 155.5, 152.5, 145.3, 138.7, 134.1, 129.9, 129.5, 125.2, 121.8, 120.0, 70.6, 59.0, 53.2, 37.5, 35.8, 30.6, 29.6, 29.3, 28.1, 26.2, 26.2, 25.1, 19.1, 18.1.

ESI-MS *m/z*: Calcd. for $C_{35}H_{43}N_7O_{11}$: 737.3. Found: 738.3 $(M+H)^+$.

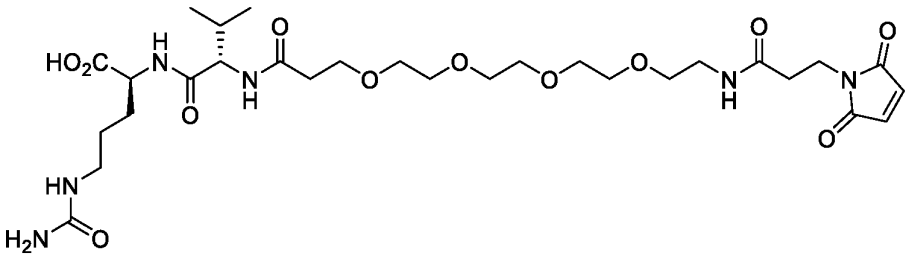
Preparation of LIN-2: MC2-PEG4-Val-Cit-PABC-PNP

Rection Scheme



a) **Preparation of LIN 2-1: MC2-PEG4-Val-Cit-OH**

LIN 2-1



Cl-TrtCl-resin (5 g, 1.49 mmol/g) was placed in a filter plate. To the resin was added CH_2Cl_2 (25 mL) and the mixture was stirred for 1 h at 23 ºC. The solvent was eliminated by filtration over vacuum. A solution of Fmoc-Cit-OH (2.95 g, 7.44 mmol) and DIPEA (4.29 mL, 24.61 mmol) in CH_2Cl_2 (20 mL) was added and the mixture was stirred for 10 min at 23 ºC. DIPEA (8.70 mL, 49.99 mmol) was additionally added and the mixture was stirred for 1 h at 23 ºC. The reaction was stopped by addition of MeOH (10 mL) and stirred 15 min at 23 ºC. The Fmoc-Cit-O-TrtCl-resin was subjected to the following washing/treatments: CH_2Cl_2 (5 x 15 mL x 0.5 min), DMF (5 x 15 mL x 0.5 min), piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min), DMF (5 x 15 mL x 0.5 min), CH_2Cl_2 (5 x 15 mL x 0.5 min). The loading was calculated: 1.17 mmol/g.

The NH_2-Cit-O-TrtCl-resin was washed with DMF (5 x 15 mL x 0.5 min) and a solution of Fmoc-Val-OH (7.80 g, 22.99 mmol) and HOBt (2.80 g, 24.5 mmol) in DMF (25 mL) was added to the NH_2-Cit-O-TrtCl-resin followed by addition of DIPCDI (3.56 mL, 24.5 mmol) at 23 ºC. The reaction mixture was stirred for 1.5 h at 23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min). The Fmoc-Val-Cit-O-TrtCl-resin was treated with piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min) and washed with DMF (5 x 15 mL x 0.5 min).

A solution of 15-(9-Fluorenylmethyloxycarbonyl)amino-4,7,10,13-tetraoxa-pentadecanoic acid (Fmoc-NH-PEG4-OH) (4.27 g, 8.75 mmol) and HOBt (1.18 g, 8.72 mmol) in DMF (30 mL) was added to the NH_2-Val-Cit-O-TrtCl-resin followed by addition of DIPCDI (1.35 mL, 8.72 mmol) at 23 ºC. The reaction mixture was stirred for 24 h at 23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min). The Fmoc-NH-PEG4-Val-Cit-O-TrtCl-resin was treated with piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min) and washed with DMF (5 x 15 mL x 0.5 min).

A solution of 3-(Maleimido)propionic acid (MC2-OH) (3.95 g, 23.35 mmol) and HOBt (3.16 g, 23.37 mmol) in DMF (30 mL) was added to the NH_2-PEG4-Val-Cit-O-TrtCl-resin followed by addition of DIPCDI (3.62 mL, 23.37 mmol) at 23 ºC. The reaction mixture was stirred for 2 h at

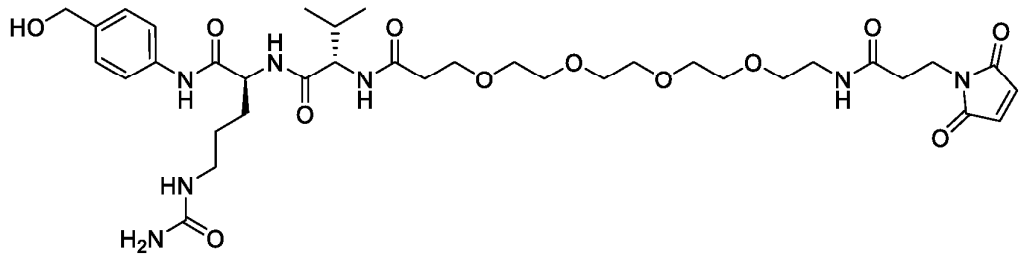
23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min) and CH_2Cl_2 (5 x 15 mL x 0.5 min).

The peptide was cleaved from the resin by treatments with TFA:CH_2Cl_2 (1:99, 5 x 50 mL). The resin was washed with CH_2Cl_2 (7 x 50 mL x 0.5 min). The combined filtrates were evaporated to dryness under reduced pressure, the solid obtained was triturated with Et_2O and filtrated to obtain **LIN 2-1** (4.59 g, 87% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67-7.57 (m, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.11 (t, *J* = 5.4 Hz, 1H), 6.73 (s, 2H), 4.49 (d, *J* = 7.2 Hz, 1H), 4.35 (t, *J* = 7.7 Hz, 1H), 3.82 (t, *J* = 7.0 Hz, 2H), 3.74 (t, *J* = 6.2 Hz, 2H), 3.68-3.56 (m, 13H), 3.56-3.45 (m, 2H), 3.39 (q, *J* = 5.4 Hz, 2H), 3.17 (s, 2H), 2.55 (q, *J* = 7.0, 6.0 Hz, 4H), 2.16-1.99 (m, 1H), 1.91 (s, 1H), 1.75 (s, 1H), 1.43 (s, 2H), 0.94 (d, = 9.7 Hz, 3H), 0.93 (d, = 9.7 Hz, 3H).
ESI-MS *m/z*: 673.3 $(M+H)^+$.

(b) **Preparation of LIN 2-2: MC2-PEG4-Val-Cit-PABOH**

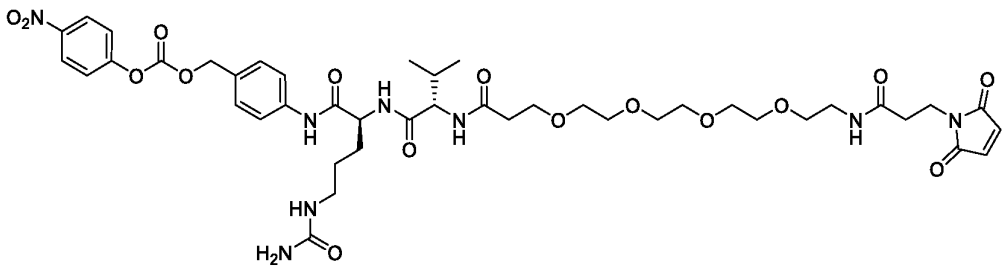
LIN 2-2



To a solution of **LIN 2-1** (1.5 g, 2.22 mmol) and 4-aminobenzyl alcohol (PABOH) (0.55 g, 4.45 mmol) in CH_2Cl_2 (60 mL) was added a solution of HOBt (0.60 g, 4.45 mmol) in DMF (5 mL) followed by addition of DIPCDI (0.69 mL, 4.45 mmol) at 23 ºC. The reaction mixture was stirred for 5 h at 23 ºC, Et_2O (150 mL) was added, and the solid obtained was filtrated under vacuum to obtain crude **LIN 2-2** (2.37 g, >100% yield) which was used in the next step without further purification.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.57 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 6.81 (s, 2H), 4.58 (s, 1H), 4.56 (s, 2H), 4.50 (dd, *J* = 9.1, 5.1 Hz, 1H), 4.21 (d, *J* = 7.0 Hz, 1H), 3.80-3.68 (m, 4H), 3.65-3.59 (m, 12H), 3.55-3.47 (m, 1H), 3.20 (dd, *J* = 13.6, 6.9 Hz, 1H), 3.12 (dt, *J* = 13.5, 6.7 Hz, 1H), 2.55 (td, *J* = 6.1, 2.1 Hz, 2H), 2.46 (t, *J* = 6.9 Hz, 2H), 2.15-2.07 (m, 1H), 1.95-1.88 (m, 1H), 1.79-1.70 (m, 1H), 1.67-1.50 (m, 2H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H).
ESI-MS *m/z*: 778.4 $(M+H)^+$.

(c) **Preparation of LIN 2: MC2-PEG4-Val-Cit-PABC-PNP**



To a solution of **LIN 2-2** (1.73 g, 2.22 mmol) and bis(4-nitrophenyl) carbonate (bis-PNP) (3.38 g, 11.12 mmol) in DCM:DMF (8:2, 75 mL) was added DIPEA (1.16 mL, 6.07 mmol) at 23 ºC. The reaction mixture was stirred for 19 h at 23 ºC and poured onto silica gel column (CH_2Cl_2:CH_3OH, from 50:1 to 10:1) to afford pure **LIN 2** (945 mg, 45% yield).

^{1}H NMR (500 MHz, CD_3OD): δ 8.22 (d, *J* = 9.2 Hz, 2H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 9.2 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.67 (s, 2H), 4.57-4.47 (m, 1H), 4.23-4.12 (m, 1H), 3.78-3.76 (m, 12H), 3.63-3.50 (m, 16H), 3.49-3.41 (m, 2H), 3.34-3.25 (m, 2H), 3.18-3.03 (m, 2H), 2.51 (t, *J* = 5.9 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 2H), 2.13-1.99 (m, 1H), 1.92-1.84 (m, 1H), 1.73-1.62 (m, 1H), 1.55-1.45 (m, 2H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H).

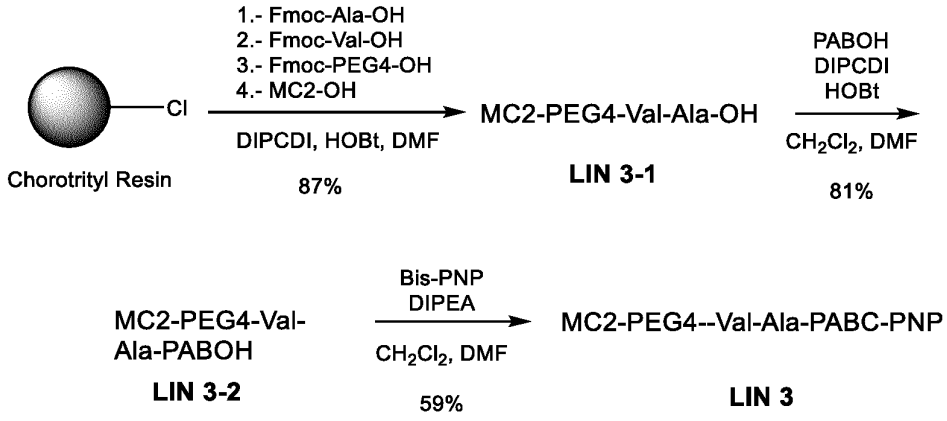
^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): δ 174.4, 172.9, 172.4, 172.4, 171.6, 170.9, 170.8, 170.7, 163.7, 155.8, 155.7, 152.5, 145.4, 138.8, 134.1, 131.3, 130.4, 129.2, 128.7, 125.7, 124.9, 121.8, 119.8 (x2), 115.1, 70.2 (x2), 70.1 (x2), 70.0, 69.9, 69.8, 69.0, 66.9, 59.2, 53.5, 39.0, 36.0, 34.4, 34.1, 30.4, 29.0, 18.5, 17.5.

ESI-MS *m/z*: 943.4 $(M+H)^+$.

R_f= 0.20 (CH_2Cl_2:CH_3OH, 9:1).

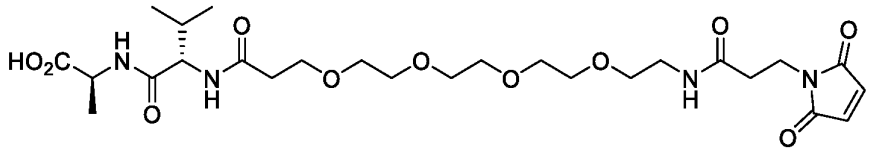
Preparation of LIN 3: MC2-PEG4-Val-Ala-PABC-PNP

Reaction Scheme



(a) **Preparation of LIN 3-1: MC2-PEG4-Val-Ala-OH**

LIN 3-1



Cl-TrtCl-resin (5 g, 1.49 mmol/g) was placed in a filter plate. To the resin was added CH_2Cl_2 (25 mL) and the mixture was stirred for 1 h at 23 ºC. The solvent was eliminated by filtration over vacuum. A solution of Fmoc-Ala-OH (2.31 g, 7.41 mmol) and DIPEA (4.28 mL, 24.61 mmol) in CH_2Cl_2 (20 mL) was added and the mixture was stirred for 10 min at 23 ºC. DIPEA (8.60 mL, 49.37 mmol) was additionally added and the reaction mixture was stirred for 1 h at 23 ºC. The reaction was stopped by addition of MeOH (10 mL) and stirred 15 min at 23 ºC. The Fmoc-Ala-O-TrtCl-resin was subjected to the following washing/treatments: CH_2Cl_2 (5 x 15 mL x 0.5 min), DMF (5 x 15 mL x 0.5 min), piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min), DMF (5 x 15 mL x 0.5 min), CH_2Cl_2 (5 x 15 mL x 0.5 min). The loading was calculated: 1.34 mmol/g.

The NH_2-Ala-O-TrtCl-resin was washed with DMF (5 x 15 mL x 0.5 min) and a solution of Fmoc-Val-OH (9.09 g, 26.79 mmol) and HOBt (3.62 g, 26.79 mmol) in DMF (25 mL) was added to the NH_2-Ala-O-TrtCl-resin followed by addition DIPCDI (4.14 mL, 26.79 mmol) at 23 ºC. The mixture was stirred for 1.5 h at 23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min). The Fmoc-Val-Ala-O-TrtCl-resin was treated with piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min) and washed with DMF (5 x 15 mL x 0.5 min).

A solution of 15-(9-Fluorenylmethyloxycarbonyl)amino-4,7,10,13-tetraoxa-pentadecanoic acid (Fmoc-NH-PEG4-OH) (4.90 g, 8.75 mmol) and HOBt (1.35 g, 9.98 mmol) in DMF (30 mL) was added to the NH_2-Val-Ala-O-TrtCl-resin followed by addition DIPCDI (1.55 mL, 10.0 mmol) at 23 ºC. The reaction mixture was stirred for 22 h at 23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min). The Fmoc-NH-PEG4-Val-Ala-O-TrtCl-resin was treated with piperidine:DMF (1:4, 15 mL, 1 x 1 min, 2 x 10 min) and washed with DMF (5 x 15 mL x 0.5 min).

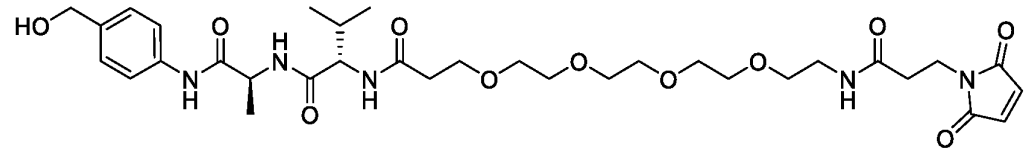
A solution of 3-(Maleimido)propionic acid (MC2-OH) (4.53 g, 26.78 mmol) and HOBt (3.62 g, 26.77 mmol) in DMF (30 mL) was added to the NH_2-PEG4-Val-Ala-O-TrtCl-resin followed by addition of DIPCDI (4.15 mL, 26.80 mmol) at 23 ºC. The reaction mixture was stirred for 2 h at 23 ºC. The reaction was stopped by washing with DMF (5 x 15 mL x 0.5 min) and CH_2Cl_2 (5 x 15 mL x 0.5 min).

The peptide was cleaved from the resin by treatments with TFA:CH_2Cl_2 (1:99, 5 x 50 mL). The resin was washed with CH_2Cl_2 (7 x 50 mL x 0.5 min). The combined filtrates were evaporated to dryness under reduced pressure, the solid obtained was triturated with Et_2O and filtrated to obtain **L 3-1** (4.73 g, 87% yield) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (bs, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.17 (d, *J* = 7.0 Hz, 1H), 6.85 (t, *J* = 5.6 Hz, 1H), 6.72 (s, 2H), 4.51 (q, *J* = 7.1 Hz, 1H), 4.38 (dd, *J* = 8.9, 6.9 Hz, 1H), 3.84 (t, *J* = 7.1 Hz, 2H), 3.75 (t, *J* = 5.9 Hz, 2H), 3.69-3.59 (m, 12H), 3.55 (t, *J* = 5.1 Hz, 2H), 3.41 (qd, *J* = 5.0, 1.7 Hz, 2H), 2.62-2.49 (m, 4H), 2.19-2.01 (m, 1H), 1.44 (d, *J* = 7.2 Hz, 3H), 0.95 (d, *J* = 11.9 Hz, 1H), 0.94 (d, *J* = 11.9 Hz, 1H).

(b) **Preparation of LIN 3-2: MC2-PEG4-Val-Ala-PABOH**

LIN 3-2

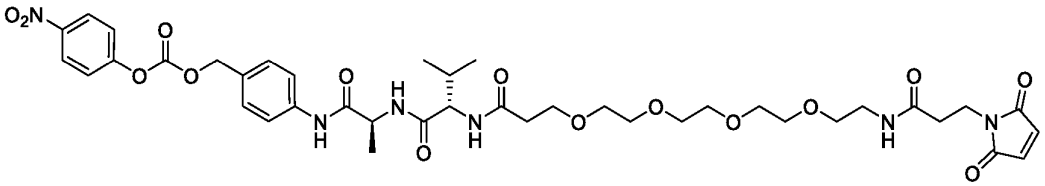


To a solution of **LIN 3-1** (1.84 g, 3.13 mmol) and 4-aminobenzyl alcohol (PABOH) (0.77 g, 6.27 mmol) in CH_2Cl_2 (70 mL) was added a solution of HOBt (0.84 g, 6.27 mmol) in DMF (5 mL) followed by addition of DIPCDI (0.97 mL, 6.27 mmol) at 23 ºC. The reaction mixture was stirred for 5 h at 23 ºC, Et_2O (150 mL) was added, and the solid obtained was filtrated under vacuum to obtain crude **LIN 3-2** (1.74 g, 81% yield) which was used in the next step without further purification.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.58 (d, *J* = 8.5 Hz, 2H), 7.30 (d, *J* = 8.5 Hz, 2H), 6.81 (s, 2H), 4.56 (s, 2H), 4.52-4.41 (m, 1H), 4.21 (d, *J* = 6.7 Hz, 1H). 3.91 (p, *J* = 6.5 Hz, 1H), 3.81-3.67 (m, 4H), 3.65-3.54 (m, 12H), 3.49 (t, *J* = 5.5 Hz, 2H), 2.56 (dd, *J* = 6.6, 5.5 Hz, 2H), 2.46 (t, *J* = 6.9 Hz, 2H), 2.12 (h, *J* = 6.8 Hz, 1H), 1.45 (d, *J* = 7.2 Hz, 3H), 1.00 (d, *J* = 12.1 Hz, 3H), 0.98 (d, *J* = 12.1 Hz, 3H).

(c) **Preparation of LIN 3: MC2-PEG4-Val-Ala-PABC-PNP**

LIN 3



To a solution of **LIN 3-2** (1.74 g, 2.51 mmol) and bis(4-nitrophenyl) carbonate (bis-PNP) (3.82 g, 12.57 mmol) in CH_2Cl_2:DMF (8:1, 70 mL) was added DIPEA (1.31 mL, 7.54 mmol) at 23 ºC. The reaction mixture was stirred for 20 h at 23 ºC and poured onto silica gel column (CH_2Cl_2:CH_3OH, from 50:1 to 10:1) to afford pure **LIN 3** (1.26 g, 59% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (s, 1H), 8.27 (d, *J* = 9.2 Hz, 2H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.38 (d, *J* = 9.1 Hz, 4H), 7.15 (dd, *J* = 21.8, 7.2 Hz, 2H), 6.69 (s, 2H), 6.62 (t, *J* = 5.7 Hz, 1H), 5.24 (s, 2H), 4.67 (p, *J* = 7.2 Hz, 1H), 4.24 (dd, *J* = 6.8, 5.7 Hz, 1H), 3.91-3.76 (m, 2H), 3.71 (ddd, *J* = 10.1, 6.1, 4.3 Hz, 1H), 3.66-3.54 (m, 14H), 3.53 (t, *J* = 5.1 Hz, 1H), 3.46-3.33 (m, 2H), 2.76-2.57 (m, 1H), 2.57-2.42 (m, 2H), 2.33-2.19 (m, 1H), 1.46 (d, *J* = 7.1 Hz, 3H), 1.01 (d, *J* = 12.1 Hz, 3H), 1.00 (d, *J* = 12.1 Hz, 3H).

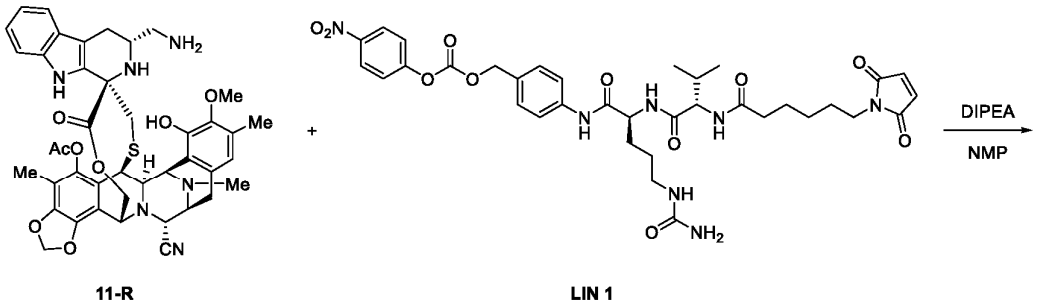
^{13}C NMR (75 MHz, CD_3OD): δ 173.0, 172.1, 171.6 (x2), 170.7, 163.8, 155.7, 152.5, 145.4, 140.3, 138.9, 134.1, 130.4, 129.1, 125.6, 124.8, 121.9, 119.7, 115.1, 70.2, 70.1 (x3), 70.0, 69.9, 69.8, 69.0, 66.9, 59.1, 53.4, 49.7, 39.0, 36.0, 34.3, 34.1, 30.4, 18.3, 17.3, 16.6.

ESI-MS *m/z*: 857.3 $(M+H)^+$.

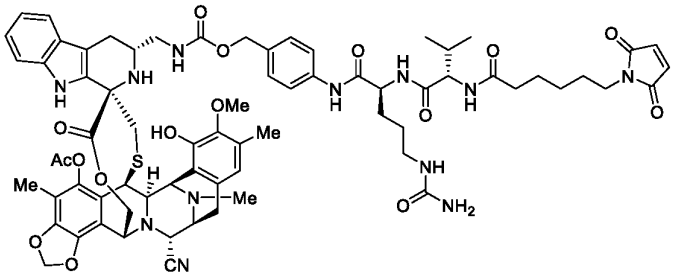
R_f= 0.45 (CH_2Cl_2:CH_3OH, 9:1).

Example 1: Synthesis of a compounds of formula D-X-$(AA)_w$-$(T)_g$-L_1

Preparation of Compound DL-1



11-R **LIN 1**



DL 1

To a solution of **11-R** (100 mg, 0.12 mmol) and **LIN 1** (465 mg, 0.63 mmol) in *N*-Methyl-2-pyrrolidone (NMP) (15 mL) was added *N,N*-diisopropylethylamine (DIPEA) (111 μL, 0.63 mmol) at 23 ºC. The reaction mixture was stirred for 3 days at 23 ºC, diluted with EtOAc (50 mL) and washed with H_2O (4 x 30 mL) and a saturated aqueous solution of NaCl (30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH, from 99:1 to 90:10) to obtain **DL 1** which was purified by HPLC preparative to yield pure **DL 1** (69 mg, 40% yield).

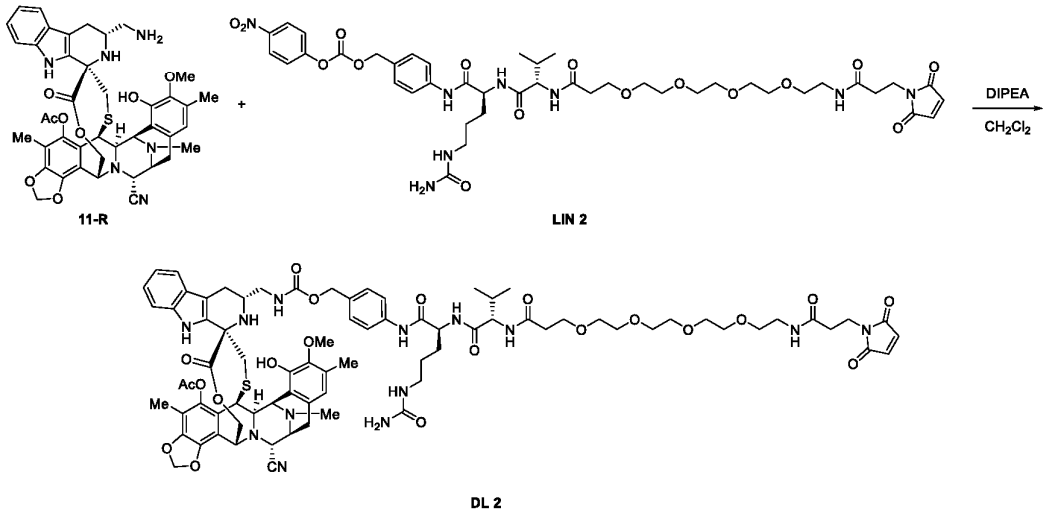
^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$): δ 7.85 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.91 (t, *J* = 7.5 Hz, 1H), 6.72 (s, 2H), 6.58 (s, 1H), 6.26 (s, 1H), 6.07 (s, 1H), 5.25-5.14 (m, 2H), 5.14-5.01 (m, 2H), 4.67 (bs, 1H), 4.51 (d, *J* = 6.7 Hz, 2H), 4.28 (dd, *J* = 16.2, 7.1 Hz, 4H), 4.21-4.05 (m, 3H), 3.71 (s, 3H), 3.51-3.40 (m, 2H), 3.36-3.32 (m, 2H), 3.23-2.99 (m, 2H), 2.99-2.72 (m, 2H), 2.65 (d, *J* = 14.9 Hz, 2H), 2.28 (s,·3H), 2.25 (s,·3H), 2.10 (s,·3H), 2.04 (s,·3H), 1.96-1.83 (m, 1H), 1.80-1.68 (m, 2H), 1.65-1.50 (m, 10H), 1.35-1.23 (m, 2H), 0.95 (d, *J* = 6.8Hz, 3H), 0.94 (d, *J* = 6.8Hz, 3H).

^{13}C NMR (75 MHz, $CD_3OH/CDCl_3$): δ 174.9, 172.5, 171.5, 171.1, 170.7, 169.5, 160.8, 157.4, 148.6, 146.1, 143.6, 141.1, 140.9, 138.0, 136.8, 133.9, 132.6, 130.5, 129.8, 129.6, 128.6, 126.0, 121.6, 120.4, 119.8, 119.0, 118.6, 118.0, 117.8, 116.7, 113.5, 112.8, 110.9, 109.1, 102.3, 66.0, 63.1, 62.9, 61.6, 60.2, 59.9, 59.2, 59.1, 58.9, 54.6, 54.6, 53.5, 50.7, 45.3, 42.1, 40.5, 37.1, 35.3, 30.3, 29.1, 27.9, 26.3, 26.0, 25.0, 24.5, 23.6, 19.4, 18.6, 17.7, 15.1, 8.6.

ESI-MS *m/z*: 1391.4 $(M+H)^+$.

R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

Preparation of compound DL 2



11-R LIN 2

DL 2

To a solution of **11-R** (50 mg, 0.063 mmol) and **LIN 2** (118 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) was added *N*,*N*-diisopropylethylamine (DIPEA) (22 μL, 0.12 mmol) at 23 ºC. The reaction mixture was stirred for 18 h at 23 ºC and poured onto silica gel column (CH_2Cl_2:CH_3OH, from 99:1 to 90:10) to yield **DL 2** which was purified by HPLC preparative to afford pure **DL 2** (30 mg, 30% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.76 (s, 2H), 6.58 (s, 1H), 6.28 (s, 1H), 6.09 (s, 1H), 5.25-5.14 (m, 2H), 5.14-5.01(m, 2H), 4.67 (bs, 1H), 4.51 (d, *J* = 6.7 Hz, 2H), 4.36-4.24 (m, 4H), 4.23-4.15 (m, 3H),

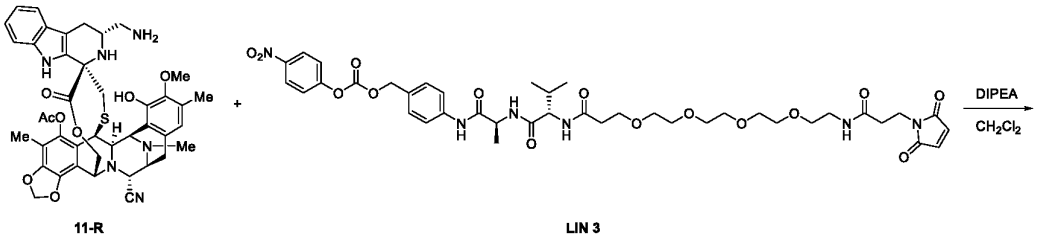
3.73 (s, 3H), 3.75-3.69 (m 3H), 3.58-3.50 (m, 14H), 3.51-3.40 (m, 2H), 3.36-3.32 (m, 2H), 3.23-3.05 (m, 2H), 2.99-2.88 (m, 2H), 2.68 (d, J = 14.9 Hz, 2H), 2.56-2.41 (m, 2H), 2.29 (s,·3H), 2.27 (s,·3H), 2.10 (s,·3H), 2.05 (s,·3H), 1.96-1.83 (m, 1H), 1.80-1.68 (m, 2H), 1.65-1.50 (m, 10H), 1.35-1.23 (m, 2H), 0.96 (d, J = 6.8Hz, 3H), 0.95 (d, J = 6.8Hz, 3H).

^{13}C NMR (75 MHz, CD_3OD): δ 173.0, 172.34, 171.6, 171.5 (x2), 170.8 (x2), 170.7, 169.4, 160.8, 157.4, 155.8, 148.7, 148.5, 146.2, 146.1, 143.7, 141.2, 141.1, 140.9, 138.4, 136.9, 134.0, 131.3, 129.8, 128.7, 128.5, 126.0, 121.5, 120.5, 119.9, 119.7, 118.5, 117.7, 112.7, 102.4, 70.1 (x5), 70.0 (x2), 69.9, 69.8, 69.7, 69.0, 68.9, 66.8, 61.6, 59.9, 59.2, 54.6, 54.0, 53.5, 40.4, 40.0, 39.0, 36.0, 35.1, 34.4, 34.1, 30.4, 29.0, 26.4, 24.6, 23.6, 19.3, 18.5, 17.4, 15.1, 8.4.

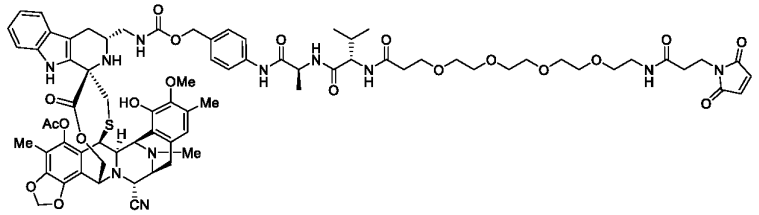
ESI-MS m/z: 1596.6 $(M+H)^+$.

R_f= 0.48 (CH_2Cl_2:CH_3OH, 9:1).

Preparation of compound DL 3



11-R **LIN 3**



DL 3

To a solution of **11-R** (50 mg, 0.063 mmol) and **LIN 3** (108 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) was added *N*,*N*-diisopropylethylamine (DIPEA) (22 μL, 0.12 mmol) at 23 ºC. The reaction mixture was stirred for 18 h at 23 ºC and poured onto silica gel column (CH_2Cl_2:CH_3OH, from 99:1 to 90:10) to yield **DL 3** which was purified by HPLC preparative to afford pure **DL 3** (25 mg, 26% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.64 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.30 (dt, J = 7.8, 1.0 Hz, 1H), 7.23 (dt, J = 8.4, 0.9 Hz, 1H), 7.01 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 6.91 (ddd, J = 7.9, 7.0, 1.0 Hz, 1H), 6.76 (s, 2H), 6.61 (s, 1H), 6.28 (s, 1H), 6.10 (s, 1H), 5.26-5.15 (m, 1H), 5.07 (dd, J = 12.1, 4.7 Hz, 2H), 4.66 (s, 1H), 4.57 (s, 1H), 4.47 (t, J = 7.1 Hz, 1H), 4.36 (s, 1H), 4.32-4.22 (m, 2H), 4.18 (d, J = 6.5 Hz, 2H), 3.75-3.68 (m, 6H), 3.72 (s, 3H), 3.59-3.46 (m, 8H), 3.44 (t, J = 5.3 Hz, 2H), 3.33-3.25 (m, 10H), 3.15 (dd, J = 9.7, 5.0 Hz, 1H), 2.95 (d, J = 17.9 Hz, 1H), 2.81 (dd, J = 18.0, 9.9 Hz, 2H), 2.73-2.59 (m, 2H), 2.52 (t, J = 6.1 Hz, 2H),

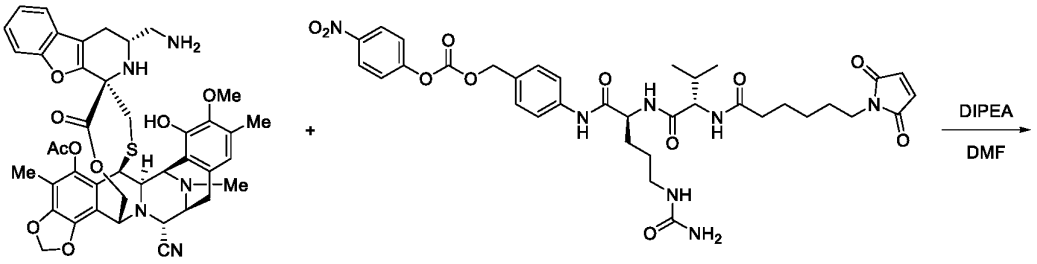
2.43 (t, *J* = 6.9 Hz, 2H), 2.29 (s, 3H), 2.27 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.45 (t, *J* = 8.7 Hz, 2H), 0.99 (d, *J* = 9.9 Hz, 3H), 0.97 (d, *J* = 9.9 Hz, 3H).

^{13}C NMR (75 MHz, CD_3OD): δ 173.1, 172.1, 172.1, 171.6, 171.5, 170.7, 169.4, 148.7, 146.1, 143.6, 140.9, 138.1, 136.9, 135.7, 134.0, 134.0, 132.7, 127.1, 126.0, 121.5, 120.5, 119.8, 119.7, 119.1, 118.5, 117.6, 113.4, 110.8, 105.8, 102.4, 99.9, 86.9, 70.1, 70.1, 70.1, 70.0, 70.0, 69.9, 69.8, 69.0, 67.7, 66.8, 65.9, 63.0, 62.0, 61.6, 60.1, 59.9, 59.2, 59.1, 54.7, 54.6, 50.8, 49.6, 42.1, 40.3, 40.0, 39.0, 36.0, 34.3, 34.1, 30.4, 28.8, 23.6, 19.2, 18.4, 17.3, 16.6, 14.9, 8.4.

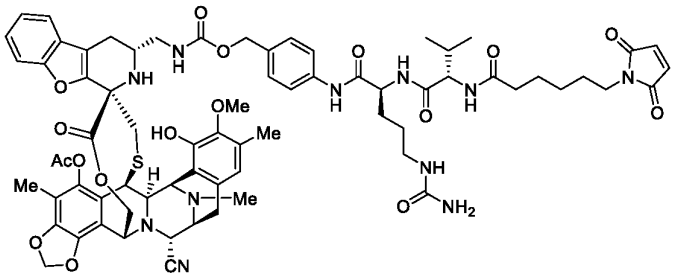
ESI-MS *m/z*: 1511.2 $(M+H)^+$.

R_f= 0.50 (CH_2Cl_2:CH_3OH, 9:1).

Preparation of compound DL 4



46-R **LIN 1**



DL 4

To a solution of **46-R** (26 mg, 0.032 mmol) and **LIN 1** (48 mg, 0.64 mmol) in DMF (2 mL) was added *N,N*-diisopropylethylamine (DIPEA) (12 μL, 0.64 mmol) at 23 ºC. The reaction mixture was stirred for 18 hours at 23 ºC, diluted with EtOAc (50 mL) and washed with H_2O (4 x 30 mL) and a saturated aqueous solution of NaCl (30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by HPLC preparative to yield pure **DL 4** (14 mg, 31% yield).

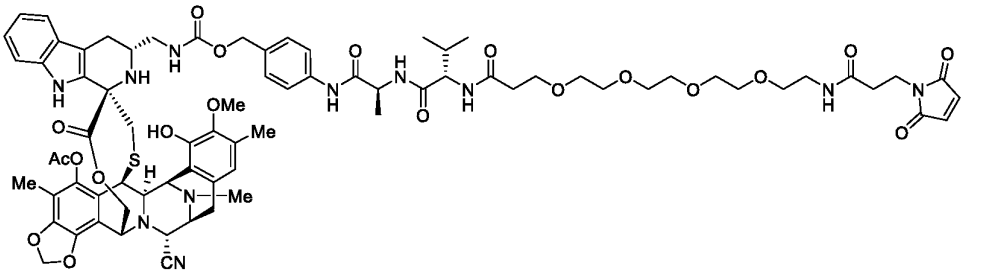
^{1}H NMR (400 MHz, CD_3OD): δ 7.88 (d, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.39-7.36 (m, 3H), 7.20-7.11 (m, 3H), 6.74 (s, 2H), 6.57 (s, 1H), 6.24 (s, 1H), 6.08 (s, 1H), 5.30-5.05 (m, 2H), 4.64 (s, 1H), 4.50 (d, *J* = 6.1 Hz, 2H), 4.34-4.27 (m, 4H), 4.21-4.13 (m, 3H), 3.70 (s, 3H), 3.45 (t, *J* = 7.2 Hz, 2H), 3.19-3.06 (m, 4H), 2.93-2.76 (m, 2H), 2.70-2.56 (m, 2H), 2.29-2.19

(m, 4H), 2.25 (s, 3H), 2.29-2.11 (m, 2H), 2.11 (s, 3H), 2.06 (s, 3H), 1.93-1.72 (m, 2H), 1.64-1.52 (m, 9H), 1.32-1.26 (m, 3H), 0.96-0.93 (m, 8H).

^{13}C NMR (100 MHz, CD_3OD): δ 173.4, 171.1, 169.7, 169.3, 169.1, 167.7, 159.3, 155.9, 153.5, 147.2, 147.1, 144.3, 142.1, 139.6, 139.2, 136.6, 132.5, 131.1, 129.1, 128.3, 127.2, 125.6, 122.7, 121.0, 119.2 (x2), 118.3, 117.5, 117.4, 112.2, 112.1, 111.7, 109.7, 100.8, 97.0, 64.6, 61.7, 60.0, 58.9, 58.4, 57.7 (x2), 57.4, 53.2, 53.1, 52.0, 48.9, 43.6, 40.5, 39.0, 35.6, 33.8, 28.8, 28.1, 27.6, 26.5, 24.6, 23.6, 22.6, 22.2, 21.5, 17.9, 17.1, 16.2, 13.6, 7.1.

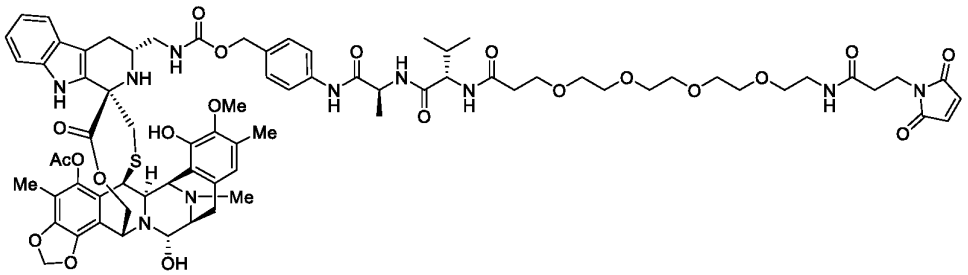
ESI-MS *m/z*: 1392.4 $(M+H)^+$.

Preparation of compound DL 5



DL 3

$AgNO_3$
$CH_3CN:H_2O$



DL 5

To a solution of **DL 3** (30 mg, 0.026 mmol) in $CH_3CN:H_2O$ (1.39:1, 6 mL, 0.015 M) was added $AgNO_3$ (132 mg, 0.79 mmol). After 18 h at 23 ºC, the reaction mixture was quenched with an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2 (x3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified in an automatic system for flash chromatography (SiO_2, $CH_2Cl_2:CH_3OH$, from 95:5 to 50:50) to obtain pure **DL 5** (34 mg, 87%).

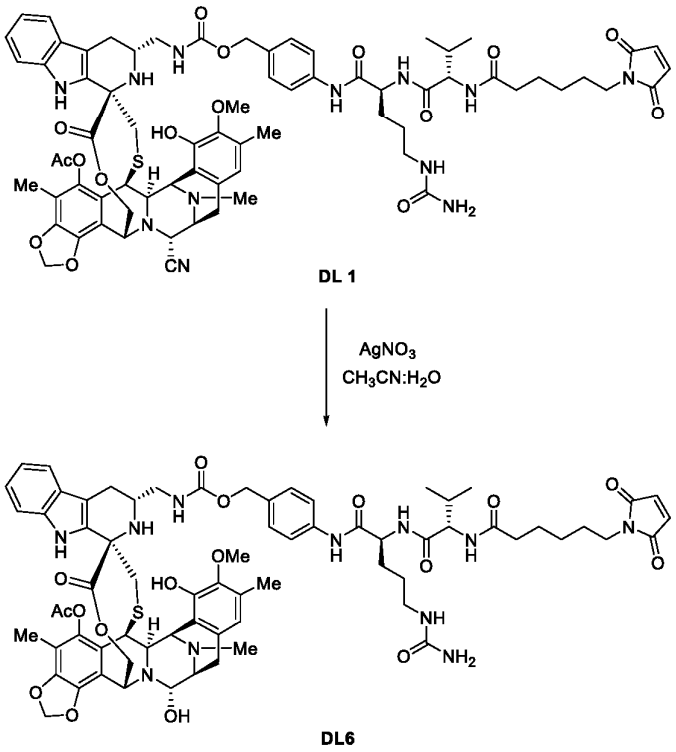
^{1}H NMR (400 MHz, CD_3OD): 7.64 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.00 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.91 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.75 (s, 2H), 6.64 (s, 1H), 6.25 (d, *J* = 1.4 Hz, 1H), 6.06 (d, *J* = 1.4 Hz, 1H),

5.23-5.06 (m, 3H), 4.60 (s, 1H), 4.47 (q, *J* = 7.1 Hz, 1H), 4.31 (dd, *J* = 16.3, 4.1 Hz, 2H), 4.18 (d, *J* = 6.6 Hz, 1H), 4.12 (d, *J* = 11.6 Hz, 1H), 3.76-3.66 (m, 2H), 3.61 (d, *J* = 5.3 Hz, 1H), 3.57-3.48 (m, 16H), 3.44 (t, *J* = 7.2 Hz, 4H), 3.27 (t, *J* = 5.3 Hz, 3H), 3.13 (dd, *J* = 13.4, 6.7 Hz, 1H), 2.96 (d, *J* = 18.0 Hz, 1H), 2.85 (dd, *J* = 18.1, 9.3 Hz, 1H), 2.75-2.61 (m, 2H), 2.56-2.48 (m, 2H), 2.43 (t, *J* = 6.9 Hz, 2H), 2.28 (s, 6H), 2.24 (s, 1H), 2.14 (s, 3H), 2.12-2.05 (m, 2H), 2.04 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, CD_3OD): δ 173.1, 172.1, 171.7, 171.6, 171.5, 170.7, 169.3, 162.5, 157.5, 148.9, 146.1, 144.1, 141.4, 140.9, 138.2, 136.9, 134.0, 132.7, 131.2, 129.7, 128.4, 126.0, 121.4, 120.1, 119.8, 119.7, 118.5, 117.6, 114.3, 112.5, 110.8, 102.3, 90.5, 70.1 (x2), 70.0 (x2), 69.9, 69.8, 69.0, 66.8, 65.9, 63.1, 60.2, 59.2, 59.1, 57.9, 55.6, 55.1, 53.6, 50.8, 49.6, 45.4, 42.1, 40.3, 39.5, 39.0, 35.9, 34.3, 34.0 (x2), 29.3, 24.7, 23.2, 19.1, 18.3, 17.3, 16.5, 15.0, 8.2.

ESI-MS *m/z*: 1483.4 $(M\text{-}H_2O+H)^+$.

Preparation of compound DL 6



To a solution of **DL 1** (50 mg, 0.035 mmol) in $CH_3CN:H_2O$ (1.39:1, 2.39 mL, 0.015 M) was added $AgNO_3$ (181 mg, 1.07 mmol). After 18 h at 23 ºC, the reaction mixture was quenched with an aqueous solution of $NaHCO_3$:NaCl (1:1) and extracted with CH_2Cl_2 (x3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under

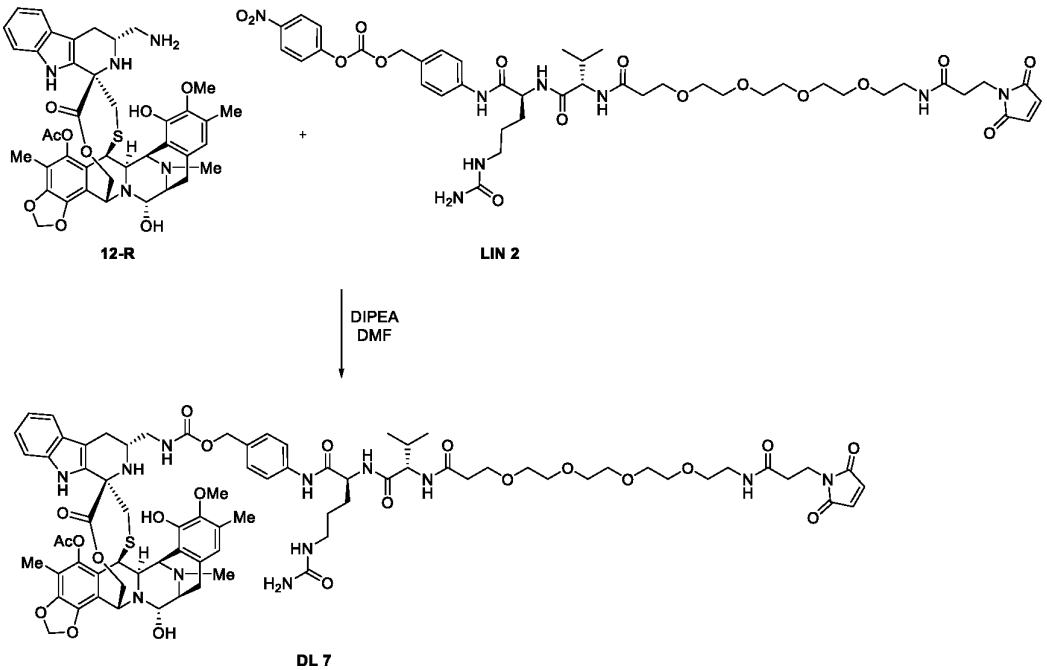
vacuum. The residue obtained was purified by HPLC preparative to obtain pure **DL 6** (23 mg, 47% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.12 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H), 7.01 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H), 6.82 (s, 1H), 6.75 (s, 2H), 6.32 (d, *J* = 1.3 Hz, 1H), 6.12 (d, *J* = 1.3 Hz, 1H), 5.32 (d, *J* = 11.8 Hz, 1H), 5.24-5.12 (m, 3H), 4.81 (m, 2H), 4.65 (s, 1H), 4.47 (s, 1H), 4.31 (d, *J* = 11.6 Hz, 1H), 4.18 (d, *J* = 9.0 Hz, 1H), 4.12 (d, *J* = 7.4 Hz, 1H), 3.93-3.83 (m, 2H), 3.76 (s, 3H), 3.65 (d, *J* = 12.3 Hz, 1H), 3.44 (t, *J* = 7.1 Hz, 2H), 3.23-2.99 (m, 2H), 2.92 (d, *J* = 15.9 Hz, 1H), 2.62 (s, 3H), 2.45 (d, *J* = 15.5 Hz, 1H), 2.35 (s, 3H), 2.30 (s, 3H), 2.28 (d, *J* = 13.3 Hz, 6H), 2.07 (s, 3H), 2.13-2.00 (m, 2H), 1.90 (m, 1H), 1.80-1.70 (m, 2H), 1.65-1.50 (m, 4H), 1.34-1.22 (m, 2H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, CD_3OD): δ 175.0, 172.6, 171.1, 170.9, 169.2, 169.1, 160.9, 157.7, 149.5, 147.0, 146.0, 144.8, 141.7, 141.2, 137.3, 136.8, 133.9, 132.5, 130.5, 129.8, 129.6, 128.5, 127.9, 122.6, 120.3, 119.8, 119.2, 118.4, 117.9, 116.7, 113.1, 112.8, 111.1, 108.4, 102.7, 101.5, 89.1, 66.2, 63.1, 62.9, 61.6, 59.3, 56.9, 56.1, 55.5, 54.7, 53.6, 50.7, 45.3, 42.2, 39.0, 38.9, 37.0, 35.1, 30.1, 29.3, 27.9, 26.5, 26.3, 26.0, 25.0, 23.1, 19.1, 18.4, 17.6, 15.2, 8.3.

ESI-MS *m/z*: 1364.4 $(M\text{-}H_2O+H)^+$.

Preparation of compound DL 7



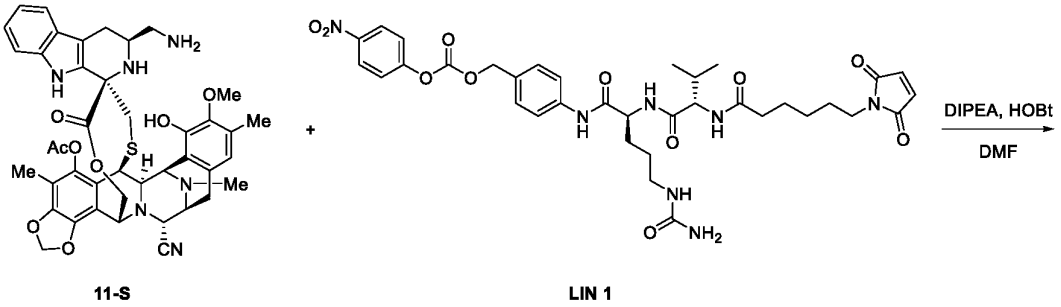
To a solution of **12-R** (100 mg, 0.12 mmol) and **LIN 2** (180 mg, 0.19 mmol) in Dimethylformamide (DMF) (2 mL, 0.06 M) was added *N,N*-Diisopropylethylamine (DIPEA) (90 μL, 0.51 mmol) at 23 ºC. After 18 hours the reaction mixture was purified by HPLC preparative to yield pure **DL 7** (125 mg, 62% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 7.01 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 6.82 (s, 1H), 6.76 (s, 2H), 6.32 (d, *J* = 1.3 Hz, 1H), 6.12 (d, *J* = 1.3 Hz, 1H), 5.32 (d, *J* = 11.8 Hz, 1H), 5.18 (d, *J* = 5.7 Hz, 2H), 4.82 (m, 2H), 4.64 (d, *J* = 2.8 Hz, 1H), 4.47 (s, 1H), 4.33 (dd, *J* = 12.0, 2.1 Hz, 1H), 4.22-4.12 (m, 2H), 3.96 (bs, 1H), 3.88 (d, *J* = 5.0 Hz, 1H), 3.78-3.67 (m, 8H), 3.60-3.52 (m, 14H), 3.56-3.41 (m, 3H), 3.27 (t, *J* = 5.4 Hz, 2H), 3.22-3.04 (m, 4H), 2.93 (d, *J* = 16.0 Hz, 1H), 2.70-2.58 (m, 3H), 2.55 (t, *J* = 6.9 Hz, 2H), 2.43 (t, *J* = 6.9 Hz, 2H), 2.36 (s, 3H), 2.30 (s, 3H), 2.15-2.07 (m, 1H), 2.06 (s, 3H), 2.02 (s, 2H), 1.95-1.87 (m, 1H), 1.80-1.70 (m, 1H), 1.65-1.50 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

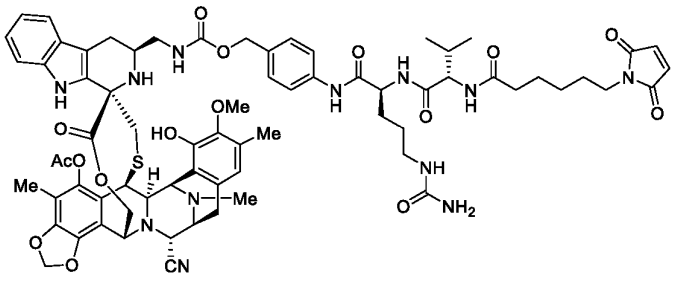
^{13}C NMR (125 MHz, CD_3OD): δ 173.1, 172.5, 171.6, 171.0, 170.7 (x2), 169.3, 168.5, 160.9, 160.0, 149.6, 147.1, 144.9, 141.8, 141.3, 138.2, 137.4, 134.1, 132.4, 128.6, 127.9, 125.2, 122.9, 120.2, 119.9, 119.4, 118.1 (x2), 117.1, 114.8, 113.2, 112.7, 111.3, 108.3, 102.7, 89.0, 70.2, 70.1 (x2), 70.0, 69.9, 69.8, 69.0, 66.9, 66.3, 65.5, 65.4, 61.9, 59.4, 56.9, 56.1, 55.4 (x2), 54.6, 53.7, 43.7, 42.4, 39.1, 39.0, 36.0, 34.4, 34.1, 31.4, 30.3, 28.9, 26.5, 23.1, 22.3, 19.2, 18.5, 17.5, 15.3, 13.2, 8.5.

ESI-MS *m/z*: 1570.4 $(M\text{-}H_2O+H)^+$.

Preparation of compound DL 8



11-S **LIN 1**



DL 8

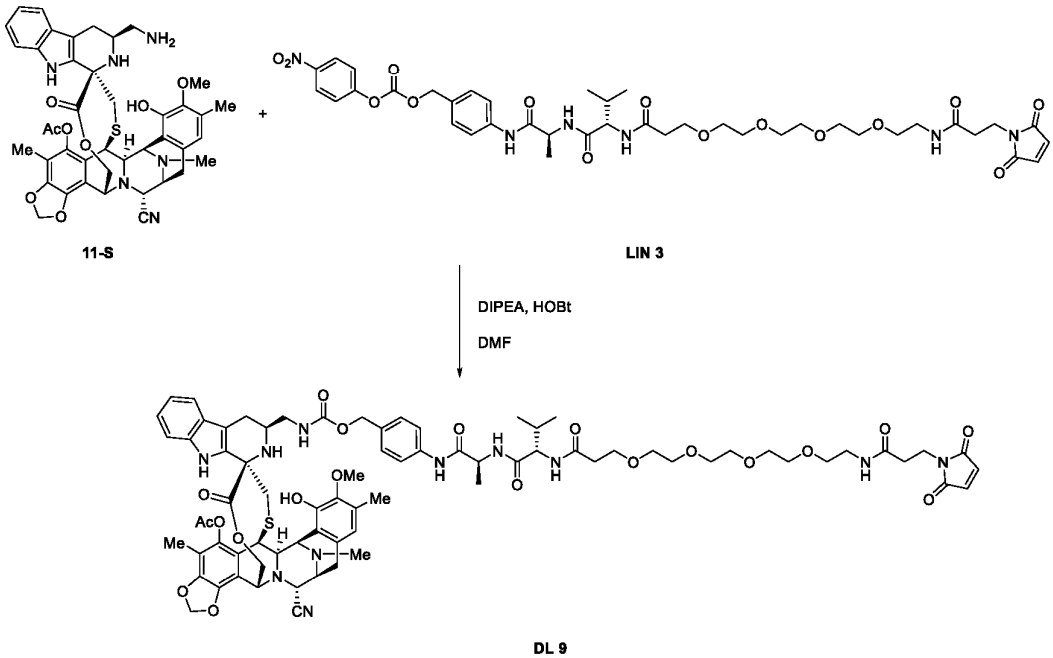
To a solution of **11-S** (30 mg, 0.037 mmol) and **LIN 1** (56 mg, 0.075 mmol) in Dimethylformamide (DMF) (2 mL, 0.018 M) was added *N,N*-Diisopropylethylamine (DIPEA) (26 μL, 0.15 mmol) and 1-Hydroxybenzotriazole (HOBt, 10 mg, 0.075 mmol) at 23 ºC. After 18 hours the reaction mixture was purified by HPLC preparative to yield pure **DL 8** (30 mg, 58% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.59 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.91 (t, *J* = 7.5 Hz, 1H), 6.74 (s, 2H), 6.50 (s, 1H), 6.27 (s, 1H), 6.09 (s, 1H), 5.20-5.03 (m, 2H), 4.65 (bs, 1H), 4.54-4.46 (m, 1H), 4.43-4.37 (m, 1H), 4.34-4.30 (m, 1H), 4.17-4.12 (m, 1H), 3.75 (s, 3H), 3.45 (t, *J* = 7.0 Hz, 4H), 3.32-3.23 (m, 2H), 3.38 (d, *J* = 7.6 Hz, 2H), 3.23-2.99 (m, 2H), 3.21-2.97 (m, 3H), 2.94-2.83 (m, 3H), 2.61-2.53 (m, 2H), 2.48-2.34 (m, 2H), 2.28 (s,·3H), 2.27-2.22 (m, 1H), 2.21 (s,·3H), 2.11 (s,·3H), 2.08-2.02 (m, 1H), 1.99 (s,·3H), 1.91-1.82 (m, 1H), 1.77-1.68 (m, 1H), 1.65-1.50 (m, 6H), 1.31-1.24 (m, 2H), 0.94 (d, *J* = 6.8Hz, 3H), 0.93 (d, *J* = 6.8Hz, 3H).

^{13}C NMR (100 MHz, CD_3OD): δ 174.9, 174.8, 172.5, 172.0, 171.2, 170.8, 169.4, 160.8, 157.5, 148.6, 146.0, 143.6, 140.9, 140.8, 138.0, 136.6, 136.5, 134.0, 132.7, 130.2, 129.9, 129.7, 128.7, 128.4, 126.3, 121.6, 120.5, 120.0, 119.8, 119.3, 118.7, 118.0, 117.7, 113.5, 112.9, 111.0, 107.8, 102.4, 65.9, 63.5, 61.4, 60.6, 59.7, 59.3, 59.2, 59.1, 58.8, 54.6, 54.6, 53.4, 44.8, 42.3, 40.6, 38.3, 37.1, 35.3 (x2), 30.3, 29.1, 28.0, 26.3, 26.0, 25.1, 24.0, 23.7, 19.5, 18.6, 17.7, 15.2, 8.6.

ESI-MS *m/z*: 1391.4 $(M+H)^+$.

Preparation of compound DL 9



To a solution of **11-S** (110 mg, 0.13 mmol) and **LIN-3** (119 mg, 0.13 mmol) in Dimethylformamide (DMF) (4 mL, 0.032 M) was added *N,N*-Diisopropylethylamine (DIPEA) (97 μL, 0.55 mmol) and 1-Hydroxybenzotriazole (HOBt, 38 mg, 0.27 mmol) at 23 ºC. After 18 hours the reaction mixture was purified by HPLC preparative to yield pure **DL 9** (120 mg, 57% yield).

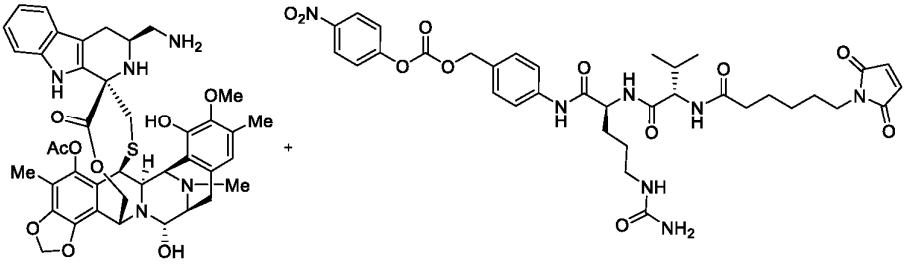
^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.00 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.91 (td, *J* = 7.5,

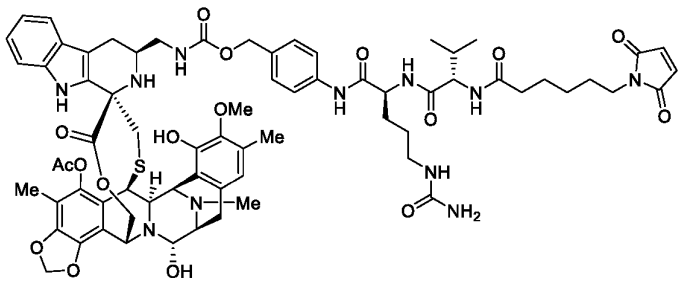
7.0, 1.1 Hz, 1H), 6.75 (s, 2H), 6.50 (s, 1H), 6.26 (d, *J* = 1.3 Hz, 1H), 6.08 (d, *J* = 1.4 Hz, 1H), 5.19 (d, *J* = 11.4 Hz, 1H), 5.09 (d, *J* = 10.4 Hz, 2H), 4.65 (s, 1H), 4.48 (p, *J* = 6.9 Hz, 1H), 4.42-4.38 (m, 2H), 4.34-4.30 (m, 2H), 4.22-4.14 (m, 1H), 3.78-3.64 (m, 5H), 3.61-3.50 (m, 8H), 3.45 (t, *J* = 5.4 Hz, 2H), 3.38 (d, *J* = 5.1 Hz, 1H), 3.33-3.23 (m, 3H), 3.02 (dd, *J* = 13.5, 5.5 Hz, 1H), 2.89 (d, *J* = 9.4 Hz, 2H), 2.62-2.33 (m, 7H), 2.27 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H), 2.15-2.05 (m, 1H), 1.99 (m, 3H), 1.42 (d, *J* = 7.1 Hz, 3H), 0.97 (dd, *J* = 6.8 Hz, 3H), 0.95 (dd, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, CD_3OD): δ 173.1 (x2), 172.1, 171.9, 171.6, 171.5, 171.5, 170.7, 169.3, 148.6, 146.0, 143.6, 140.9, 140.8, 138.1, 136.5, 134.1, 132.8, 130.3, 129.8, 128.4, 126.3, 121.6, 120.5, 120.0, 119.7, 119.3, 118.7, 118.0, 117.7, 113.5, 112.9, 111.0, 107.8, 102.4, 70.2, 70.1 (x2), 70.0 (x2), 69.8, 69.1, 66.9, 65.9, 63.5, 61.4, 60.6, 59.7, 59.3, 59.2, 59.1, 58.8, 54.7, 54.6, 49.6, 42.3, 40.5, 39.1, 39.0, 36.0, 34.4 (x2), 34.1, 30.4, 24.0, 23.7, 19.4, 18.5, 17.4, 16.8, 15.1, 8.5.

ESI-MS *m/z*: 1511.4 $(M+H)^+$.

Preparation of compound DL 10





To a solution of **12-S** (30 mg, 0.058 mmol) and **LIN 1** (98 mg, 0.13 mmol) in *N*-Methyl-2-pyrrolidone (NMP) (4 mL, 0.014 M) was added *N*,*N*-Diisopropylethylamine (DIPEA) (83 μL, 0.13 mmol) at 23 ºC. After 18 hours the reaction mixture was diluted with EtOAc (25 mL) and washed with H_2O (4 x 25 mL) and an aqueous saturated solution of NaCl. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum.

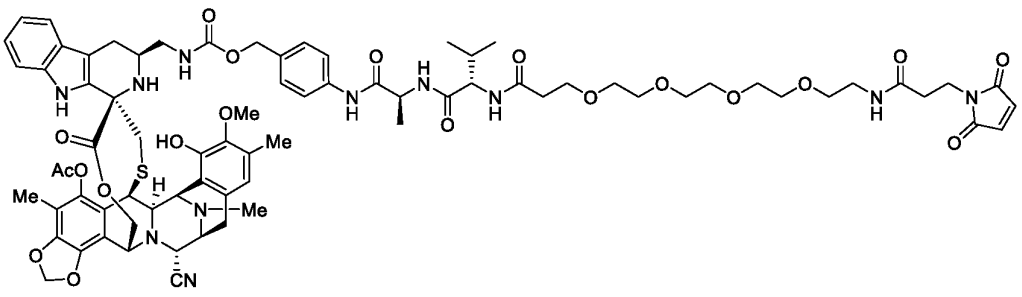
The residue was purified by flash chromatography on silica gel (CH_2Cl_2:CH_3OH, from 99:1 to 90:10) and the compound obtained purified by HPLC preparative to yield pure **DL 10** (11 mg, 21% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.61 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.08 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H), 6.98 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H), 6.76 (s, 2H), 6.66 (s, 1H), 6.29 (d, *J* = 1.3 Hz, 1H), 6.14 (d, *J* = 1.3 Hz, 1H), 5.13 (q, *J* = 12.3 Hz, 2H), 4.93 -.4.81 (m, 3H), 4.69 (s, 1H), 4.48 (s, 1H), 4.23 (d, *J* = 10.0 Hz, 1H), 4.13 (dd, *J* = 7.5, 4.0 Hz, 1H), 3.90 (d, *J* = 5.0, 1H), 3.76 (s, 3H), 3.49-3.41 (m, 2H), 3.40-3.27 (m, 1H), 3.28-3.24 (m, 4H), 3.22-3.05 (m, 4H), 2.80-2.65 (m, 3H), 2.63 (s, 3H), 2.29 (s, 3H), 2.28 (s, 3H), 2.13-2.00 (m, 2H), 2.03 (s, 3H), 1.93-1.83(m, 1H), 1.79-1.69 (m, 2H), 1.65-1.51 (m, 6H), 1.34-1.22 (m, 2H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, CD_3OD): δ 174.9, 172.7, 172.6, 171.1, 170.8 (x2), 169.1, 160.9, 149.4, 146.6, 144.7, 142.3, 141.5, 141.0, 138.1, 136.8, 133.9, 133.5, 132.6, 128.5, 127.5, 125.9, 122.2, 120.9, 119.8, 117.8, 113.2, 113.0, 111.2, 107.3, 102.6, 89.2, 66.2, 61.7, 61.3, 60.2, 59.4, 59.3, 57.4, 56.9, 55.8, 55.7, 55.2, 54.8, 53.5, 42.0, 39.1, 37.1, 35.2, 31.7, 30.2, 29.4, 29.1, 29.0 (x2), 27.9, 26.4, 26.0, 25.0, 23.3, 22.4, 19.2, 18.5, 17.7, 15.2, 13.2, 8.5.

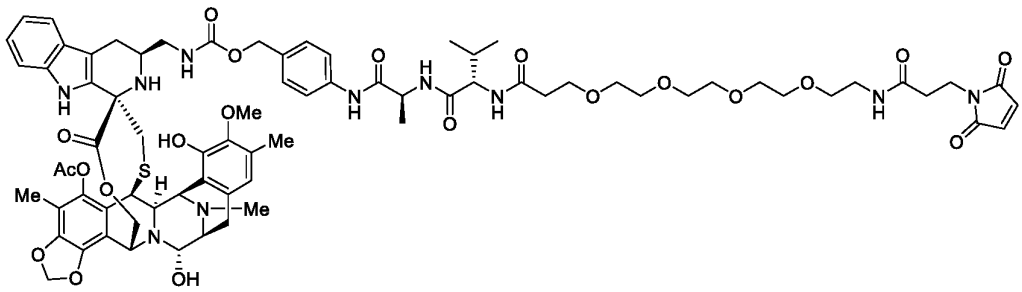
ESI-MS *m/z*: 1364.4 $(M\text{-}H_2O+H)^+$.

Preparation of compound DL 11



DL 9

$AgNO_3$
CH_3CN:H_2O



DL 11

To a solution of **DL 9** (90 mg, 0.059 mmol) in CH_3CN:H_2O (1.39:1, 4 mL, 0.015 M) was added $AgNO_3$ (298 mg, 1.78 mmol). After 21 h at 23 ºC, the reaction mixture was quenched with an aqueous solution of $NaHCO_3$:NaCl (1:1) and extracted with CH_2Cl_2 (x3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified in an automatic system for flash chromatography (SiO_2, CH_2Cl_2:CH_3OH, from 95:5 to 50:50) to obtain pure **DL 11** (65 mg, 73% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H),6.99 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.90 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.76 (s, 2H), 6.52 (s, 1H), 6.25 (d, *J* = 1.3 Hz, 1H), 6.06 (d, *J* = 1.3 Hz, 1H), 5.28 (d, *J* = 11.4 Hz, 1H), 5.09 (d, *J* = 10.4 Hz, 1H), 4.59 (s, 1H), 4.47 (q, *J* = 7.1 Hz, 1H), 4.33 (dd, *J* = 11.5, 1.9 Hz, 1H), 4.33-4.25 (m, 2H), 4.19 (d, *J* = 6.6 Hz, 1H), 3.75 (s, 3H), 3.75-3.67 m, 3H), 3.61 (d, *J* = 5.3 Hz, 1H), 3.58-3.51 (m, 12H), 3.45 (dd, *J* = 10.6, 5.2 Hz, 2H), 3.34 (s, 3H), 3.27 (t, *J* = 5.5 Hz, 3H), 3.04 (dd, *J* = 13.4, 6.7 Hz, 1H), 2.88-2.82 (m, 3H), 2.64-2.35 (m, 7H), 2.27 (s, 3H), 2.23 (s, 3H), 2.17-2.04 (m, 1H), 2.09 (s, 3H), 2.01 (d, *J* = 7.2 Hz, 1H), 1.98 (s, 3H), 1.42 (d, *J* = 7.2 Hz, 3H), 0.98 (dd, *J* = 6.8 Hz, 3H), 0.95 (dd, *J* = 6.8 Hz, 3H).
^{13}C NMR (100 MHz, CD_3OD): δ 173.1, 172.1, 172.0, 171.6, 171.5, 170.7, 169.4, 157.6, 148.6, 145.8, 143.7, 141.1, 140.8, 138.1, 136.5, 134.1, 132.8, 130.4, 130.0, 129.8, 128.4, 126.4, 126.2, 121.5, 120.9, 120.1, 119.8, 118.6, 117.7, 114.9, 112.4, 111.0, 107.7, 102.1, 91.0, 70.2, 70.1 (x2), 70.0 (x2), 69.8, 69.1, 66.9, 65.9, 63.5, 60.5, 59.3, 59.1, 58.2, 55.2, 54.7, 52.9, 49.6, 44.9, 42.6, 40.0, 39.0, 38.3, 36.0, 34.4, 34.1, 30.4, 29.4, 24.0, 23.3, 19.4, 18.5, 17.4, 16.8, 15.1, 8.4.
ESI-MS *m/z*: 1483.4 $(M\text{-}H_2O+H)^+$.

Example 2: Preparation of Antibody-Drug Conjugates (ADCs)

In this Example, syntheses of antibody-drug conjugates of the present invention are described. It should be noted that these syntheses are exemplary and that the processes described can be applied to all the compounds and antibodies described herein.

Example 2a Preparation of anti-CD13 monoclonal antibody

Anti-CD13 monoclonal antibodies were obtained following well known procedures commonly used in the art. Briefly BALB/c mice were immunized with human endothelial cells isolated from umbilical cord. To that end, 1.5E7 of the cells were injected to the mice intraperitoneally on days −45 and −30 and intravenously on day −3. On day 0 spleen from these animals were removed and spleen cells were fused with SP2 mouse myeloma cells at a ratio of 4:1 according to standard techniques to produce the hybridoma and distributed on 96-well tissue culture plates (Costar Corp., Cambridge, MA). After 2 weeks hybridoma culture supernatants were harvested and their reactivity against the cell line used in the immunization step was tested by flow cytometry. Positive supernatants were assayed by immunofluorescence staining the corresponding cells used as antigens. Hybridomas showing a specific staining,

immunoprecipitation pattern and cell distribution were selected and cloned and subcloned by limiting dilution.

Once the clones were selected, cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin at 37ºC during 3-4 days until the medium turned pale yellow. At that point, two thirds of the medium volume were removed, centrifuged at 1,000xg for 10 min to pellet the cells and the supernatant was either centrifuged again for further cleaning at 3,000xg for 10 min or filtered through 22 µm pore size membranes. The clarified supernatant was subjected to precipitation with 55% saturation ammonium sulphate and the resulting pellet was resuspended in 100 mM Tris-HCl pH 7.8 (1 mL per 100 mL of the original clarified supernatant) and dialyzed at 4 ºC for 16-24 h against 5 L of 100 mM Tris-HCl pH 7.8 with 150 mM NaCl, changing the dialyzing solution at least three times. The dialyzed material was finally loaded onto a Protein A-Sepharose column and the corresponding monoclonal antibody was eluted with 100 mM sodium citrate pH 3.0 or alternatively with 1M glycine pH 3.0. Those fractions containing the antibody were neutralized with 2M Tris-HCl pH 9.0 and finally dialyzed against PBS and stored at -80ºC until its use.

Preparation of Antibody-Drug Conjugate ADC1 with Trastuzumab and DL 1

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab.

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (13.9 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.33 mL, 4.6 mg, 30.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (17.5 µL, 87.6 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.0.

(b) Preparation of ADC 1.

To the solution of partially reduced Trastuzumab (140 µL, 1.4 mg, 9 nmol), *N,N*-Dimethylacetamide (DMA) was added (28.2 µL) followed by addition of a freshly prepared solution of **DL 1** (10 mM in DMA, 6.8 µL, 67.5 nmol, 7.5 eq.) Upon addition of **DL 1**, the solution turned turbid. The conjugation reaction was stirred for 30 min at 20 °C and the turbidity vanished during the conjugation reaction. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 6.8 µL, 67.5 nmol) followed by stirring the solution

for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 1** was concentrated to a final concentration of 6.05 mg/mL as determined by UV and 232 μL (1.4 mg, 9.3 nmol, 103%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (89%).

Preparation of Antibody-Drug Conjugate ADC 2 with Trastuzumab and DL 2.

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab.

Trastuzumab solution (0.33 mL, 4.6 mg, 30.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (17.5 μL, 87.6 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 3.9.

(b) Preparation of ADC 2.

To the solution of partially reduced Trastuzumab (140 μL, 1.4 mg, 9 nmol), *N,N*-Dimethylacetamide (DMA) was added (28.2 μL) followed by addition of a freshly prepared solution of **DL 2** (10 mM in DMA, 6.8 μL, 67.5 nmol, 7.5 eq.) Upon addition of **DL 2** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 6.8 μL, 67.5 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 2** was concentrated to a final concentration of 5.19 mg/mL as determined by UV and 270 μL (1.4 mg, 9.3 nmol, 103%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (65%).

Preparation of Antibody-Drug Conjugate ADC 3 with Trastuzumab and Compound DL 3.

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab.

Trastuzumab solution (0.33 mL, 4.6 mg, 30.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (17.5 μL, 87.6 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 3.9.

(b) Preparation of ADC 3.

To the solution of partially reduced Trastuzumab (140 μL, 1.4 mg, 9 nmol), *N,N*-Dimethylacetamide (DMA) was added (28.2 μL) followed by addition of a freshly prepared solution of **DL 3** (10 mM in DMA, 6.8 μL, 67.5 nmol, 7.5 eq.) Upon addition of **DL 3** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 6.8 μL, 67.5 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 3** was concentrated to a final concentration of 5.15 mg/mL as determined by UV and 280 μL (1.44 mg, 9.6 nmol, 107%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (93%).

Preparation of Antibody-Drug Conjugate ADC 4 with Traut's-modified Trastuzumab and Compound DL 1

(a) Reaction of Trastuzumab with 2-iminothiolane hydrochloride (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab solution (0.65 mL, 9 mg, 60 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8). Traut´s reagent was added (64.4 μL, 900 nmol, 15 eq.) and the reaction stirred for 2 h at 25 °C. The mixture was buffer exchanged using two Sephadex G25 NAP-5 columns into PBS buffer, and concentrated to a volume of 1.2 mL (7.5 mg/mL). Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 7.9.

(b) Preparation of ADC 4

To the solution of thiol-activated Trastuzumab (300 μL, 2.25 mg, 15 nmol), DMA was added (59.8 μL) followed by addition of a freshly prepared solution of **DL 1** (10 mM in DMA, 22.5 μL, 225 nmol, 15 eq.). Upon addition of **DL 1**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 4** was concentrated to a final concentration of 3.49 mg/mL as determined by UV and 252 μL (0.88 mg, 5.86 nmol, 39%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 5 with Trastuzumab and Compound DL 4.

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab.

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into

phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.5 mL, 8.55 mg, 57 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (24.5 μL, 122.4 nmol, 2.2 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 3.4.

(b) Preparation of ADC 5.

To the solution of partially reduced Trastuzumab (200 μL, 1.9 mg, 13.2 nmol), *N,N*-Dimethylacetamide (DMA) was added (42.1 μL) followed by addition of a freshly prepared solution of **DL 4** (10 mM in DMA, 7.9 μL, 79.2 nmol, 6 eq.) Upon addition of **DL 4**, the solution turned turbid. The conjugation reaction was stirred for 30 min at 20 °C and the turbidity vanished during the conjugation reaction. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 7.9 μL, 79.2 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 5** was concentrated to a final concentration of 5.30 mg/mL as determined by UV and 290 μL (1.54 mg, 1.0 nmol, 81%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (91%).

Preparation of Antibody-Drug Conjugate ADC 6 with Trastuzumab and Compound DL 5.

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab.

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.6 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.55 mL, 9.7 mg, 64.6 nmol) was diluted to a concentration of 12.8 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (29.2 μL, 146 nmol, 2.2 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 3.4.

(b) Preparation of ADC 6.

To the solution of partially reduced Trastuzumab (140 μL, 1.8 mg, 12 nmol), *N,N*-Dimethylacetamide (DMA) was added (28.2 μL) followed by addition of a freshly prepared solution of **DL 5** (10 mM in DMA, 6.8 μL, 67.5 nmol, 5.6 eq.) Upon addition of **DL 5** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 6.8 μL, 67.5 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 6** was concentrated to a final concentration of 4.29 mg/mL as determined by UV and 320 μL (1.37 mg, 9.1 nmol, 76%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (83%).

Preparation of Antibody-Drug Conjugate ADC 7 with Anti-CD13 and Compound DL 1.

(a) Partial reduction of Anti-CD13 to give Partially Reduced Anti-CD13.

Anti-CD13 solution (0.5 mL, 8.2 mg, 54.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (31.9 μL, 159 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.7.

(b) Preparation of ADC 7.

To the solution of partially reduced Anti-CD13 (200 μL, 2.0 mg, 13.3 nmol), *N,N*-Dimethylacetamide (DMA) was added (40 μL) followed by addition of a freshly prepared solution of **DL 1** (10 mM in DMA, 10 μL, 100 nmol, 7.5 eq.) Upon addition of **DL 1** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 10 μL, 100 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 7** was concentrated to a final concentration of 5.58 mg/mL as determined by UV and 350 μL (1.95 mg, 13 nmol, 98%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (90%).

Preparation of Antibody-Drug Conjugate ADC 8 with Anti-CD13 and Compound DL 3

(a) Partial reduction of Anti-CD13 to give Partially Reduced Anti-CD13.

Anti-CD13 solution (0.5 mL, 8.2 mg, 54.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (31.9 μL, 159 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at

20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.7.

(b) Preparation of ADC 8.

To the solution of partially reduced Anti-CD13 (200 μL, 2.0 mg, 13.3 nmol), *N,N*-Dimethylacetamide (DMA) was added (40 μL) followed by addition of a freshly prepared solution of **DL 3** (10 mM in DMA, 10 μL, 100 nmol, 7.5 eq.) Upon addition of **DL 3** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 10 μL, 100 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 8** was concentrated to a final concentration of 5.83 mg/mL as determined by UV and 380 μL (2.21 mg, 14.7 nmol, 111%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (94%).

Preparation of Antibody-Drug Conjugate ADC 9 with Anti-CD13 and Compound DL 5

(a) Partial reduction of Anti-CD13 to give Partially Reduced Anti-CD13.

Anti-CD13 solution (0.5 mL, 8.2 mg, 54.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (31.9 μL, 159 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.7.

(b) Preparation of ADC 9.

To the solution of partially reduced Anti-CD13 (200 μL, 2.0 mg, 13.3 nmol), *N,N*-Dimethylacetamide (DMA) was added (40 μL) followed by addition of a freshly prepared solution of **DL 5** (10 mM in DMA, 10 μL, 100 nmol, 7.5 eq.) Upon addition of **DL 5** the solution was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 10 μL, 100 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 9** was concentrated to a final concentration of 5.82 mg/mL as determined by UV and 380 μL (2.21 mg, 14.7 nmol, 111%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (89%).

Preparation of Antibody-Drug Conjugate ADC 10 with Anti-CD13 and Compound DL 2.

(a) Partial reduction of Anti-CD13 to give Partially Reduced Anti-CD13.

Anti-CD13 solution (0.5 mL, 8.2 mg, 54.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (31.9 μL, 159.6 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.7.

(b) Preparation of ADC 10.

To the solution of partially reduced Anti-CD13 (200 μL, 2 mg, 13.3 nmol), DMA was added (40 μL) followed by addition of a freshly prepared solution of **DL 2** (10 mM in DMA, 10 μL, 100 nmol, 7.5 eq.). Upon addition of **DL 2**, the solution turned turbid. The conjugation reaction was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 10 μL, 100 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 10** was concentrated to a final concentration of 6.61 mg/mL as determined by UV and 250 μL (1.65 mg, 11 nmol, 85%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (23%).

Preparation of Antibody-Drug Conjugate ADC 11 with Trastuzumab and Compound DL 6

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (13.9 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.33 mL, 4.6 mg, 30.6 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (17.5 μL, 87.6 nmol, 3 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.0.

(b) Preparation of ADC 11

To the solution of partially reduced Trastuzumab (140 μL, 1.4 mg, 9 nmol), DMA was added (28.2 μL) followed by addition of a freshly prepared solution of **DL 6** (10 mM in DMA, 6.8 μL,

67.5 nmol, 7.5 eq.). Upon addition of **DL 6**, the solution turned turbid. The conjugation reaction was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 6.8 μL, 67.5 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 11** was concentrated to a final concentration of 6.14 mg/mL as determined by UV and 218 μL (1.33 mg, 8.9 nmol, 99%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (38%).

Preparation of Antibody-Drug Conjugate ADC 12 with Trastuzumab and Compound DL 7

(a) Partial reduction of Trastuzumab to give Partially Reduced Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.5 mL, 8.5 mg, 57 nmol) was diluted to a concentration of 10 mg/mL with phosphate buffer (50 mM, pH 8). Partial reduction of the disulfide bonds in the antibody was performed by the addition of a 5.0 mM tris[2-carboxyethyl]phosphine hydrochloride (TCEP) solution (24.5 μL, 122.4 nmol, 2.2 eq.) The reduction reaction was left to stir for 90 min at 20 °C. Immediately after the reduction, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 3.4.

(b) Preparation of ADC 12

To the solution of partially reduced Trastuzumab (200 μL, 2 mg, 13.2 nmol), DMA was added (42.1 μL) followed by addition of a freshly prepared solution of **DL 7** (10 mM in DMA, 7.9 μL, 79 nmol, 6 eq.). Upon addition of **DL 7**, the solution turned turbid. The conjugation reaction was stirred for 30 min at 20 °C. The excess of drug was quenched by addition of *N*-acetylcysteine (NAC) (10 mM, 7.9 μL, 79 nmol) followed by stirring the solution for 20 min. The quenched conjugation reaction was purified by desalting using Sephadex G25 NAP-5 columns into PBS buffer. The final target product **ADC 12** was concentrated to a final concentration of 5.38 mg/mL as determined by UV and 270 μL (1.45 mg, 9.6 nmol, 72%) ADC solution was obtained. HIC HPLC runs were performed to determine the percentage of conjugation reaction (76%).

Preparation of Antibody-Drug Conjugate ADC 13 with Traut's modifiedTrastuzumab and compound DL 2

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (16.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.5 mL, 8.0 mg, 53.7 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8). Traut´s reagent was added (14 mM, 46.0 µL, 644 nmol, 12 eq.), and the reaction stirred for 2 h at 20 °C. The mixture was buffer exchanged using Sephadex G25 NAP-5 columns into PBS buffer, and concentrated to a volume of 0.8 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.4.

(b) Preparation of ADC 13

To the solution of thiol-activated Trastuzumab (200 µL, 2.0 mg, 13 nmol), DMA was added (37 µL) followed by addition of a freshly prepared solution of **DL 2** (10 mM in DMA, 13 µL, 130 nmol, 10 eq.). Upon addition of **DL 2**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 13** was concentrated to a final concentration of 2.83 mg/mL as determined by UV and 340 µL (0.96 mg, 6.4 nmol, 49%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 14 with Traut's modified Trastuzumab and Compound DL 3

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (16.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.5 mL, 8.0 mg, 53.7 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8). Traut´s reagent was added (14 mM, 46.0 µL, 644 nmol, 12 eq.), and the reaction stirred for 2 h at 20 °C. The mixture was buffer exchanged using Sephadex G25 NAP-5 columns into PBS buffer, and concentrated to

a volume of 0.8 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.4.

(b) Preparation of ADC 14

To the solution of thiol-activated Trastuzumab (200 μL, 2.0 mg, 13 nmol), DMA was added (37 μL) followed by addition of a freshly prepared solution of **DL 3** (10 mM in DMA, 13 μL, 130 nmol, 10 eq.). Upon addition of **DL 3**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 14** was concentrated to a final concentration of 0.75 mg/mL as determined by UV and 380 μL (0.28 mg, 1.9 nmol, 15%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 15 with Traut's modified Trastuzumab and Compound DL 5

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (16.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.5 mL, 8.0 mg, 53.7 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8). Traut´s reagent was added (14 mM, 46.0 μL, 644 nmol, 12 eq.), and the reaction stirred for 2 h at 20 °C. The mixture was buffer exchanged using Sephadex G25 NAP-5 columns into PBS buffer, and concentrated to a volume of 0.8 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.4.

(b) Preparation of ADC 15

To the solution of thiol-activated Trastuzumab (200 μL, 2.0 mg, 13 nmol), DMA was added (37 μL) followed by addition of a freshly prepared solution of **DL 5** (10 mM in DMA, 13 μL, 130 nmol, 10 eq.). Upon addition of **DL 5**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 15** was concentrated to a final concentration of 1.79 mg/mL as determined by UV and 440 μL (0.79 mg, 5.2 nmol, 40%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 16 with Traut's modified Trastuzumab and Compound DL 6

(a) Reaction of Trastuzumab with 2-iminothiolane hydrochloride (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.1 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (0.25 mL, 4.3 mg, 28.5 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8). Traut´s reagent was added (14 mM, 24.4 μL, 342 nmol, 12 eq.), and the reaction stirred for 2 h at 20 °C. The mixture was buffer exchanged using Sephadex G25 NAP-5 columns into PBS buffer, and concentrated to a volume of 0.43 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 4.6.

(b) Preparation of ADC 16

To the solution of thiol-activated Trastuzumab (200 μL, 2.0 mg, 13 nmol), DMA was added (37 μL) followed by addition of a freshly prepared solution of **DL 6** (10 mM in DMA, 13 μL, 130 nmol, 10 eq.). Upon addition of **DL 6**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 16** was concentrated to a final concentration of 5.63 mg/mL as determined by UV and 230 μL (1.29 mg, 8.6 nmol, 66%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 17 with Traut's modified Trastuzumab and Compound DL 8

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.7 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (1.5 mL, 26.5 mg, 177 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8) and fractionated in two

vials (1.3 mL each). Traut´s reagent was added (14 mM, 61.8 μL, 866 nmol, 10 eq.) in each vial, and the reactions stirred for 2 h at 20 °C. The reactions were mixed and buffer exchanged using Sephadex G25 NAP-10 columns into PBS buffer, and concentrated to a volume of 2.6 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 5.6.

(b) Preparation of ADC 17

To the solution of thiol-activated Trastuzumab (500 μL, 5.0 mg, 33 nmol), DMA was added (98.6 μL) followed by addition of a freshly prepared solution of **DL 8** (10 mM in DMA, 26.4 μL, 264 nmol, 8 eq.). Upon addition of **DL 8**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 17** was concentrated to a final concentration of 3.21 mg/mL as determined by UV and 390 μL (1.25 mg, 8.3 nmol, 25%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 18 with Traut's modified Trastuzumab and Compound DL 9

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.7 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (1.5 mL, 26.5 mg, 177 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8) and fractionated in two vials (1.3 mL each). Traut´s reagent was added (14 mM, 61.8 μL, 866 nmol, 10 eq.) in each vial, and the reactions stirred for 2 h at 20 °C. The reactions were mixed and buffer exchanged using Sephadex G25 NAP-10 columns into PBS buffer, and concentrated to a volume of 2.6 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 5.6.

(b) Preparation of ADC 18

To the solution of thiol-activated Trastuzumab (500 μL, 5.0 mg, 33 nmol), DMA was added (98.6 μL) followed by addition of a freshly prepared solution of **DL 9** (10 mM in DMA, 26.4 μL, 264 nmol, 8 eq.). Upon addition of **DL 9**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column

into PBS buffer. The final target product **ADC 18** was concentrated to a final concentration of 3.16 mg/mL as determined by UV and 390 μL (1.23 mg, 8.2 nmol, 25%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 19 with Traut's modified Trastuzumab and Compound DL 10

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.7 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (1.5 mL, 26.5 mg, 177 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8) and fractionated in two vials (1.3 mL each). Traut´s reagent was added (14 mM, 61.8 μL, 866 nmol, 10 eq.) in each vial, and the reactions stirred for 2 h at 20 °C. The reactions were mixed and buffer exchanged using Sephadex G25 NAP-10 columns into PBS buffer, and concentrated to a volume of 2.6 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 5.6.

(b) Preparation of ADC 19

To the solution of thiol-activated Trastuzumab (500 μL, 5.0 mg, 33 nmol), DMA was added (98.6 μL) followed by addition of a freshly prepared solution of **DL 10** (10 mM in DMA, 26.4 μL, 264 nmol, 8 eq.). Upon addition of **DL 10**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 19** was concentrated to a final concentration of 11.3 mg/mL as determined by UV and 290 μL (3.2 mg, 21.3 nmol, 64%) ADC solution was obtained.

Preparation of Antibody-Drug Conjugate ADC 20 with Traut's modified Trastuzumab and Compound DL 11

(a) Reaction of Trastuzumab with 2-iminothiolane (Traut´s reagent) to give thiol-activated Trastuzumab

Trastuzumab (Trastuzumab purchased from Roche as a white lyophilised powder for the preparation of a concentrated solution for infusion) was dissolved in 5 mL of phosphate buffer (50 mM, pH 8.0) and purified by desalting using Sephadex G25 PD-10 columns into

phosphate buffer (50 mM, pH 8.0). Concentration of Trastuzumab (17.7 mg/mL) was determined by measuring the absorbance at 280 nm.

Trastuzumab solution (1.5 mL, 26.5 mg, 177 nmol) was diluted to a concentration of 10 mg/mL using phosphate buffer (50 mM phosphate, 2 mM EDTA, pH 8) and fractionated in two vials (1.3 mL each). Traut´s reagent was added (14 mM, 61.8 μL, 866 nmol, 10 eq.) in each vial, and the reactions stirred for 2 h at 20 °C. The reactions were mixed and buffer exchanged using Sephadex G25 NAP-10 columns into PBS buffer, and concentrated to a volume of 2.6 mL. Immediately after, an Ellman assay was performed to give a Free Thiol to Antibody ratio (FTAR) of 5.6.

(b) Preparation of ADC 20

To the solution of thiol-activated Trastuzumab (500 μL, 5.0 mg, 33 nmol), DMA was added (98.6 μL) followed by addition of a freshly prepared solution of **DL 11** (10 mM in DMA, 26.4 μL, 264 nmol, 8 eq.). Upon addition of **DL 11**, the solution turned turbid. The conjugation reaction was stirred for 2 h at 25 °C and purified by desalting using a Sephadex G25 NAP-5 column into PBS buffer. The final target product **ADC 20** was concentrated to a final concentration of 3.73 mg/mL as determined by UV and 440 μL (1.6 mg, 10.6 nmol, 32%) ADC solution was obtained. **Example 3: Demonstrating the Cytotoxicity of the Antibody-Drug Conjugates of the Present Invention**

Bioassays for the detection of antitumor activity

The aim of the assay was to evaluate the *in vitro* cytostatic (ability to delay or arrest tumor cell growth) or cytotoxic (ability to kill tumor cells) activity of the samples being tested.

Cell lines and cell culture

The following human cell lines were obtained from the American Type Culture Collection (ATCC): SK-BR-3 (ATCC HB-30), HCC-1954 (ATCC CRL-2338) (Breast cancer, HER2+); MDA-MB-231 (ATCC HTB-26) and MCF-7 (ATCC HTB-22) (Breast cancer, HER2-), HT-1080 (ATCC CCL-121, fibrosarcoma, CD13+), Raji (ATCC CCL-86, Burkitt's lymphoma, CD13-) and RPMI 8226 (ATCC CRM-CCL-155, myeloma, CD13-). The human acute promyelocytic leukemia cell line NB 4 (ACC 207, CD13+) was obtained from the Leibniz-Institut DSMZ (Braunschweig, Germany). Cells were maintained at 37 ºC, 5% CO_2 and 95% humidity in Dulbecco's Modified Eagle's Medium (DMEM) (for SK-BR-3, MDA-MB-231 and MCF-7 cells), Eagle's Minimum Essential Medium (EMEM) (for HT-1080 cells) or RPMI-1640 (for the rest of the cell lines), all media supplemented with 10% Fetal Calf Serum (FCS), 2mM L-glutamine and 100 units/mL penicillin and streptomycin.

Cytotoxicity Assay

For SK-BR-3, HCC-1954, MDA-MB-231 and MCF-7 cells, a colorimetric assay using Sulforhodamine B (SRB) was adapted for quantitative measurement of cell growth and cytotoxicity, as described in V. Vichai and K. Kirtikara. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*, 2006, *1*, 1112-1116. Briefly, cells were seeded in 96-well microtiter plates and allowed to stand for 24 hours in drug-free medium before treatment with vehicle alone or with the tested substances for 72 hours. For quantification, cells were washed twice with phosphate buffered saline (PBS), fixed for 15 min in 1% glutaraldehyde solution, rinsed twice with PBS, stained in 0.4% (w/v) SRB with 1% (v/v) acetic acid solution for 30 min, rinsed several times with 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM Trizma base solution and the optical density measured at 490 nm in a microplate spectrophotometer.

For HT-1080, NB 4, Raji and RPMI-8226 cells, an alternative colorimetric assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used for quantitative measurement of cell viability as described by T. Mosmann in “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays”, *J Immunol Methods*, 1983, *65*: 55-63. Briefly, cells were seeded in 96-well trays and treated as above and after 72 hours of exposure to the tested substances cellular viability was estimated from conversion of MTT (Sigma, St Louis, MO, USA) to its coloured reaction product, MTT-formazan, which was dissolved in DMSO to measure its absorbance at 540 nm in a microplate spectrophotometer.

Cell survival was expressed as percentage of control, untreated cell survival. All evaluations were performed in triplicate and the resulting data were fitted by nonlinear regression to a four-parameters logistic curve from which the IC_{50} value (the concentration of compound causing 50% cell death as compared to the control cell survival) was calculated.

Bioactivity Example 1 - Cytotoxicity of the conjugate ADC 1 and related reagents against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the **ADC 1** along with the parent cytotoxic compounds **DL 1** and **11-R** and Trastuzumab were evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2-positive cells) as well as MDA-MB-231 and MCF-7 (HER2-negative cells). Standard dose-response (DR) curves for 72 hours incubation with the tested substances were performed.

Cytotoxicity of Trastuzumab

The *in vitro* cytotoxicty of Trastuzumab was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 50 to 0.01 µg/mL (3.33E-07-8.74E-11 M). Trastuzumab was completely inactive, not reaching the IC_{50} in any of

the cell lines tested, independently of their HER2 status as shown in Table 10 where results corresponding to the geometric mean of the IC_{50} values obtained in three independent experiments are presented.

Table 10. Summary of the *in vitro* cytotoxicity of Trastuzumab.

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	> 50	> 50	> 50	> 50
IC_{50}, M	> 3.4E-07	> 3.4E-07	> 3.4E-07	> 3.4E-07

Cytotoxicity of 11-R

The cytotoxicity of the intermediate compound **11-R** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 to 0.03 ng/mL (1.26E-07 – 3.3E-11 M).

As shown in Table 11, where results corresponding to the geometric mean of the IC_{50} values obtained in three independent experiments are presented, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the low nanomolar range, from 0.4 to 1.4 ng/mL (5.04E-10 to 1.70E-09 M). The geometric mean IC_{50} value across the whole cell panel was 0.79 ng/mL (9.94E-10 M), with the standard geometric deviation being 1.8 in agreement with the homogeneity of results across the four cell lines.

Table 11. Summary of the *in vitro* cytotoxicity of 11-R

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	5.76E-04	1.35E-03	3.99E-04	1.24E-03
IC_{50}, M	7.27E-10	1.70E-09	5.04E-10	1.57E-09

Cytotoxicity of DL 1

The cytotoxicity of the intermediate compound **DL 1** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 10 µg/mL to 2.6 ng/mL (7.58E-06 – 1.99E-09 M).

As shown in Table 12, where results corresponding to the geometric mean of the IC_{50} values

obtained in three independent experiments are presented, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the high nanomolar range, from 0.07 to 0.43 µg/mL (5.23E-08 to 3.11E-07 M). The geometric mean IC_{50} value across the whole cell panel was 0.16 µg/mL (1.15E-07 M), with the standard geometric deviation being 2.1 in agreement with the homogeneity of results across the four cell lines.

Table 12. Summary of the *in vitro* cytotoxicity of DL 1

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	1.16E-01	1.80E-01	7.28E-02	4.33E-01
IC_{50}, M	8.31E-08	1.29E-07	5.23E-08	3.11E-07

Cytotoxicity of ADC 1

The cytotoxicity of the **ADC 1** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). The evaluation was performed in three independent experiments, Figure 3 shows a representative DR curve corresponding to one of these experiments and Table 13 summarizes the results corresponding to the geometric mean of the IC_{50} values obtained in the three independent experiments.

As observed in Table 13, **ADC 1** showed a cytotoxicity which is similar to that shown by the parent drug **11-R** only in HER2-positive cells. However, in HER2-negative cells such toxicity is significantly lower: nearly 40-fold lower according to the selectivity ratio obtained by dividing the mean IC_{50} value in HER2-negative cells between that in HER2-positive cells. This selectivity leads us to conclude that the conjugate is acting through the interaction of the antibody with the membrane associated HER2 receptor on the tumor cells, followed by intracellular delivery of the cytotoxic drug.

Table 13. Summary of the *in vitro* cytotoxicity of ADC 1

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**			
IC_{50} (µg/mL)	3.20E-01	1.38E+00	1.36E+01	4.90E+01	6.64E-01	2.58E01	38.8
IC_{50} (M)	2.14E-09	9.20E-09	9.04E-08	3.27E-07	4.44E-09	1.72E-07	

To graphically compare the cytotoxicity of the monoclonal antibody Trastuzumab alone with that of the conjugate **ADC 1**, histograms showing the percentages of cell survival after treatment of the different cell lines with the monoclonal antibody alone (20 µg/mL) or **ADC 1** at 16 or 2.5 µg/mL, are shown in Figure 4. As observed, at similar concentrations (20 *versus* 16 µg/mL) Trastuzumab showed negligible cytotoxicity regardless the HER2 expression levels of the cell lines (cell survival between 68% and 100%), whilst the **ADC 1** showed a potent antiproliferative effect against the HER2-expressing cells HCC-1954 and SK-BR-3 (cell survival 23% and 8% respectively) and negligible against the HER2-negative cell line MCF-7 (100% cell survival). The effect of the ADC on MDA-MB-231 is noticeable at this concentration (22% cell survival) but it is very modest at lower concentrations (83% cell survival at 2.5 µg/mL) whereas it is still remarkable in the HER2-expressing cells (30% cell survival for both SK-BR-3 and HCC-1954).

These results clearly demonstrated the remarkable cytotoxicity and specificity of the **ADC 1** against HER2 expressing human tumor cells *in vitro*.

Bioactivity Example 2 - Cytotoxicity of the conjugate ADC 2 and related reagents against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the **ADC 2** along with the parent cytotoxic compound **DL 2** was evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2 positive cells) as well as MDA-MB-231 and MCF-7 (HER2 negative cells). Standard dose-response (DR) curves for 72 hours incubation with the tested substances were performed. The results are also compared with the parent cytotoxic compound **11-R** and the monoclonal antibody Trastuzumab described above.

Cytotoxicity of DL 2

The cytotoxicity of the intermediate compound **DL 2** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 10 µg/mL to 2.6 ng/mL (6.26E-06 – 1.64E-09 M).

As shown in Table 14, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the sub-micromolar range, from 0.2 to 0.47 µg/mL (1.25E-07 to 2.94E-07 M). The geometric mean IC_{50} value across the whole cell panel was 0.28 µg/mL (1.73E-07 M), with the standard geometric deviation being 1.5 in agreement with the homogeneity of results across the four cell lines.

Table 14. Summary of the *in vitro* cytotoxicity of DL 2

	HER2 positive		HER2 negative	
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7
IC_{50}, µg/mL	2.00E-01	3.10E-01	2.00E-01	4.69E-01
IC_{50}, M	1.25E-07	1.94E-07	1.25E-07	2.94E-07

Cytotoxicity of ADC 2

The cytotoxicity of the **ADC 2** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Figure 5 shows the DR curve corresponding to this experiment and Table 15 summarizes the IC_{50} values obtained.

As observed in Table 15, **ADC 2** showed higher cytotoxicity in HER2-positive cells than in HER2-negative cells: the ADC is nearly 10-fold more potent in the former according to the selectivity ratio obtained by dividing the mean IC_{50} value in HER2-negative cells between that in HER2-positive cells. Although this selectivity is rather modest, it still leads us to conclude that the conjugate is acting through the interaction of the antibody with the membrane associated HER2 receptor on the tumor cells, followed by intracellular delivery of the cytotoxic drug.

Table 15. Summary of the *in vitro* cytotoxicity of ADC 2

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	2.00E+00	5.60E+00	1.70E+01	4.01E+01	3.34E+00	2.61E+01	7.8
IC_{50} (M)	1.33E-08	3.73E-08	1.13E-07	2.67E-07	2.23E-08	1.74E-07	

To graphically compare the cytotoxicity of the monoclonal antibody Trastuzumab alone with that of the conjugate **ADC 2**, histograms showing the percentages of cell survival after treatment of the different cell lines with the monoclonal antibody alone (20 µg/mL) or **ADC 2** at 16 or 2.5 µg/mL, are shown in Figure 6. As observed, at similar concentrations (20 *versus* 16 µg/mL) Trastuzumab showed negligible cytotoxicity disregarding the HER2 expression levels of the cell lines (cell survival between 68% and 100%), whilst the **ADC 2** showed a significant antiproliferative effect against the HER2-expressing cells HCC-1954 and SK-BR-3 (cell survival 31% and 40% respectively), such effect being negligible against the HER2-negative cell line MCF-7 (94% cell survival). The effect of the ADC on the HER2-negative cell

line MDA-MB-231 is also significant at this concentration, (58% cell survival) but it is null at lower concentrations (100% cell survival at 2.5 µg/mL) whereas it is still remarkable in the HER2-expressing cells (63% cell survival for HCC-1954 and 51% for SK-BR-3).

Bioactivity Example 3 - Cytotoxicity of the conjugate ADC 3 and related reagents against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the **ADC 3** along with the parent cytotoxic compound **DL 3** was evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2 positive cells) as well as MDA-MB-231 and MCF-7 (HER2 negative cells). Standard dose-response (DR) curves for 72 hours incubation with the tested substances were performed. The results are also compared with the parent cytotoxic compound **11-R** and the monoclonal antibody Trastuzumab described above.

Cytotoxicity of DL 3

The cytotoxicity of the intermediate compound **DL 3** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 10 µg/mL to 2.6 ng/mL (6.62E-06 – 1.74E-09 M).

As shown in Table 16, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the sub-micromolar range, from 0.15 to 0.28 µg/mL (9.93E-08 to 1.85E-07 M). The geometric mean IC_{50} value across the whole cell panel was 0.2 µg/mL (1.33E-07 M), with the standard geometric deviation being 1.4 in agreement with the homogeneity of results across the four cell lines.

Table 16. Summary of the *in vitro* cytotoxicity of DL 3

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	1.60E-01	2.40E-01	1.50E-01	2.79E-01
IC_{50}, M	1.06E-07	1.59E-07	9.93E-08	1.85E-07

Cytotoxicity of ADC 3

The cytotoxicity of the **ADC 3** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 50 µg/mL to 13 ng/mL (3.33E-07 – 8.74E-11 M). Figure 7 shows the DR curve corresponding to this experiment and Table 17 summarizes the IC_{50} values obtained.

As observed in Table 17, **ADC 3** showed a cytotoxicity which only in HER2-positive cells is similar to that shown by the parent drug **11-R**. However, in HER2-negative cells such toxicity is significantly lower: 23-fold lower according to the selectivity ratio obtained by dividing the mean IC_{50} value in HER2-negative cells between that in HER2-positive cells. This selectivity leads us to conclude that the conjugate is acting through the interaction of the antibody with the membrane associated HER2 receptor on the tumor cells, followed by intracellular delivery of the cytotoxic drug as previously stated for **ADC 1** and **ADC 2**.

Table 17. Summary of the *in vitro* cytotoxicity of ADC 3

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	6.71E-01	8.10E-01	1.30E+01	2.21E+01	7.37E-01	1.70E+01	23.0
IC_{50} (M)	4.47E-09	5.40E-09	8.67E-08	1.47E-07	4.91E-09	1.13E-07	

To graphically compare the cytotoxicity of the monoclonal antibody Trastuzumab alone with that of the conjugate **ADC 3**, histograms showing the percentages of cell survival after treatment of the different cell lines with the monoclonal antibody alone (20 µg/mL) or **ADC 3** at 20 or 3 µg/mL, are shown in Figure 8. As observed, at 20 µg/mL Trastuzumab showed negligible cytotoxicity regardless the HER2 expression levels of the cell lines (cell survival between 68% and 100%), whilst the **ADC 3** showed a potent antiproliferative effect against the HER2-expressing cells HCC-1954 and SK-BR-3 (cell survival 23% and 32% respectively) and only marginal against the HER2-negative cell line MCF-7 (88% cell survival). The effect of the ADC on MDA-MB-231 is noticeable at this concentration (32% cell survival) but it is negligible at lower concentrations (91% cell survival at 3 µg/mL) whereas it is still remarkable in the HER2-expressing cells (34% cell survival for HCC-1954 and 45% for SK-BR-3).

Bioactivity Example 4 - Cytotoxicity of the conjugate ADC 4 against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the ADC **ADC 4** was evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2 positive cells) as well as MDA-MB-231 and MCF-7 (HER2 negative cells). Standard dose-response (DR) curves for 72 hours incubation with the ADC were performed. The results are also compared with the parent cytotoxic compounds **11-R** and **DL 1** as well as the monoclonal antibody Trastuzumab described above.

Cytotoxicity of ADC 4

The cytotoxicity of the **ADC 4** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 50 µg/mL to 13 ng/mL (3.33E-07 – 8.74E-11 M). Figure 9 shows the DR curve corresponding to this experiment and Table 18 summarizes the IC_{50} values obtained.

As observed in Table 18, **ADC 4** showed a cytotoxicity which only in HER2-positive cells is similar to that shown by the parent drug **11-R**. However, in HER2-negative cells such cytotoxicity is nearly 100-fold lower according to the selectivity ratio obtained by dividing the mean IC_{50} value in HER2-negative cells between that in HER2-positive cells. As already stated for **ADC 1**, such selectivity leads us to conclude that the conjugate is acting through the interaction of the antibody with the membrane associated HER2 receptor on the tumor cells, followed by intracellular delivery of the cytotoxic drug. Moreover, these results obtained with **ADC 4** demonstrate that the antitumoral potential endowed by the payload is similar regardless of the antibody residue involved in the covalent bond, either Cys or Lys.

Table 18. Summary of the *in vitro* cytotoxicity of ADC 4

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	3.20E-02	1.50E-01	3.50E+00	8.10E+00	6.92E-02	5.32E+00	76.86
IC_{50} (M)	2.13E-10	1.00E-09	2.33E-08	5.40E-08	4.62E-10	3.55E-08	

To graphically compare the cytotoxicity of the monoclonal antibody Trastuzumab alone with that of the conjugate **ADC 4**, histograms showing the percentages of cell survival after treatment of the different cell lines with the monoclonal antibody alone at 3 µg/mL or with the ADC at 3 or 0.2 µg/mL, are shown in Figure 10. As observed, at 3 µg/mL Trastuzumab showed scarce cytotoxicity irrespective of the HER2 expression levels of the cell lines (cell survival between 60% and 90%), whilst the **ADC 4** showed a potent antiproliferative effect against the HER2-expressing cells HCC-1954 and SK-BR-3 (cell survival 11% and 23% respectively) and nearly no effect on the HER2-negative cell line MCF-7. The effect of the ADC on the cell line MDA-MB-231 is noticeable at this concentration (55% cell survival) but it is negligible at lower concentrations (97-100% cell survival at 0.2 µg/mL) whereas it is clearly remarkable in the HER2-expressing cells (38% cell survival for HCC-1954 and 26% for SK-BR-3).

Bioactivity Example 5 - Cytotoxicity of the conjugate ADC 6 and related reagents against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the ADC 6 along with the parent cytotoxic compounds **DL 5** and **12**

R and were evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2-positive cells) as well as MDA-MB-231 and MCF-7 (HER2-negative cells). Standard dose-response (DR) curves for 72 hours incubation with the tested substances were performed.

Cytotoxicity of 12-R

The cytotoxicity of the intermediate compound **12-R** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 to 0.03 ng/mL (1.28E-07 – 3.83E-11 M).

As shown in Table 19, where results corresponding to the geometric mean of the IC_{50} values obtained in three independent experiments are presented, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the low nanomolar range, from 0.4 to 1.3 ng/mL (5.62E-10 to 1.62E-09 M). The geometric mean IC_{50} value across the whole cell panel was 0.71 ng/mL (9.06E-10 M), with the standard geometric deviation being 1.7 in agreement with the homogeneity of results across the four cell lines.

Table 19. Summary of the *in vitro* cytotoxicity of 12-R

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	4.41E-04	1.27E-03	4.93E-04	9.24E-04
IC_{50}, M	5.62E-10	1.62E-09	6.29E-10	1.18E-09

Cytotoxicity of DL 5

The cytotoxicity of the intermediate compound **DL 5** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 10 µg/mL to 2.6 ng/mL (6.66E-06 – 1.73E-09 M).

As shown in Table 20, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the high nanomolar range, from 0.10 to 0.19 µg/mL (6.53E-08 to 1.27E-07 M). The geometric mean IC_{50} value across the whole cell panel was 0.13 µg/mL (8.84E-08 M), with the standard geometric deviation being 1.4 in agreement with the homogeneity of results across the four cell lines.

Table 20. Summary of the *in vitro* cytotoxicity of DL 5

	HER2 positive		HER2 negative	
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7
IC_{50}, µg/mL	9.81E-02	1.91E-01	9.81E-02	1.70E-01
IC_{50}, M	6.53E-08	1.27E-07	6.53E-08	1.13E-07

Cytotoxicity of ADC 6

The cytotoxicity of the **ADC 6** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Figure 11 shows the DR curve obtained and Table 21 summarizes the deduced IC_{50} values.

As observed in Table 21, the cytotoxicity of **ADC 6** is comparable to that shown by the parent drug **12-R** only in HER2-positive cells. However, in HER2-negative cells such toxicity is lower: nearly 10-fold lower according to the selectivity ratio obtained by dividing the mean IC_{50} value in HER2-negative cells between that in HER2-positive cells. These data lead us to conclude that the conjugate is acting through the interaction of the antibody with the membrane associated HER2 receptor on the tumor cells, followed by intracellular delivery of the cytotoxic drug.

Table 21. Summary of the *in vitro* cytotoxicity of ADC 6

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	6.50E-01	4.10E+00	1.20E+01	1.70E+01	1.63E00	1.43E01	8.8
IC_{50} (M)	4.33E-09	2.73E-08	8.00E-08	1.13E-07	1.08E-08	9.51E-08	

Bioactivity Example 6 – Cytotoxicity of the conjugate ADC 11 and related reagents against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the **ADC 11** along with the parent cytotoxic compounds **DL 6** and **12 R** and were evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2-positive cells) as well as MDA-MB-231 and MCF-7 (HER2-negative cells). Standard dose-response (DR) curves for 72 hours incubation with the tested substances were performed.

Cytotoxicity of **DL 6**

The cytotoxicity of the intermediate compound DL 6 was evaluated against the different tumor cell lines by performing triplicated 10-points, 2.5-fold dilution DR curves ranging from 1 µg/mL to 0.26 ng/mL (7.23E-07 – 1.88E-10 M).

As shown in Table 22, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their HER2 expression, with IC_{50} values in the high nanomolar range, from 0.04 to 0.3 µg/mL (3.1E-08 to 1.97E-07 M). The geometric mean IC_{50} value across the whole cell panel was 0.11 µg/mL (7.89E-08 M), with the standard geometric deviation being 2.31 in agreement with the homogeneity of results across the four cell lines.

Table 22. Summary of the *in vitro* cytotoxicity of DL 6

	HER2 positive		HER2 negative	
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**
IC_{50}, µg/mL	7.09E-02	1.71E-01	4.29E-02	2.73E-01
IC_{50}, M	5.13E-08	1.24E-07	3.10E-08	1.97E-07

Cytotoxicity of **ADC 11**

The cytotoxicity of the **ADC 11** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 50 µg/mL to 13 ng/mL (3.33E-07 – 8.74E-11 M). Table 23 summarizes the deduced IC_{50} values.

Table 23. Summary of the *in vitro* cytotoxicity of ADC 11

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	**HCC-1954**	**MDA-MB-231**	**MCF-7**			
IC_{50} (µg/mL)	1.10E+01	3.60E+01	> 5.00E+01	> 5.00E+01	1.99E+01	> 5.00E+01	> 2.5
IC_{50} (M)	7.33E-08	2.40E-07	> 3.33E-07	> 3.33E-07	1.33E-07	> 3.33E-07	

Bioactivity Example 7 – Cytotoxicity of the conjugate ADC 12 against HER2 positive and negative breast cancer cells

The *in vitro* cytotoxicity of the **ADC 12** was evaluated against four different human breast cancer cell lines over-expressing or not the HER2 receptor, including SK-BR-3, HCC-1954 (HER2-positive cells) as well as MDA-MB-231 and MCF-7 (HER2-negative cells). Standard

dose-response (DR) curves for 72 hours incubation with the tested substances were performed.

The cytotoxicity of the **ADC 12** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 24 summarizes the deduced IC_{50} values. As observed, **ADC 12** is significantly more active in in HER2-positive cells, yielding a noticeable selectivity ratio higher than 20.

Table 24. Summary of the *in vitro* cytotoxicity of ADC 12

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	1.80E-01	2.90E-01	1.91E+00	1.30E+01	2.28E-01	4.98E+00	21.8
IC_{50} (M)	1.20E-09	1.93E-09	1.27E-08	8.67E-08	1.52E-09	3.32E-08	

Bioactivity Example 8 – Cytotoxicity of the conjugate ADC 13 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 13** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 25 summarizes the deduced IC_{50} values. As observed, **ADC 13** is significantly more active in HER2-positive cells, yielding an outstanding selectivity ratio close to 100.

Table 25. Summary of the *in vitro* cytotoxicity of ADC 13

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	1.50E-01	2.60E-01	1.10E+01	2.90E+01	1.97E-01	1.78E+01	90.43
IC_{50} (M)	1.00E-09	1.73E-09	7.33E-08	1.93E-07	1.32E-09	1.19E-07	

Bioactivity Example 9 – Cytotoxicity of the conjugate ADC 14 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 14** was evaluated against the different tumor cell lines by

performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 26 summarizes the deduced IC_{50} values. Remarkably, **ADC 14** has shown a cytotoxic activity in the low nM range comparable to that of the parental drug **11R** but only in HER2-expressing cells, whilst it has failed to show any activity in HER2-negative cells within the range of concentrations tested, thus confirming an outstanding selectivity.

Table 26. Summary of the *in vitro* cytotoxicity of ADC 14

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	1.10E-01	1.80E-01	> 2.51E+01	> 2.51E+01	1.41E-01	> 2.51E+01	>178
IC_{50} (M)	7.33E-10	1.20E-09	> 1.67E-07	> 1.67E-07	9.38E-10	> 1.67E-07	

Bioactivity Example 10 – Cytotoxicity of the conjugate ADC 15 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 15** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 50 µg/mL to 13 ng/mL (3.33E-07 – 8.74E-11 M). Table 27 summarizes the deduced IC_{50} values. As observed, **ADC 15** is significantly more active in in HER2-positive cells, yielding an outstanding selectivity ratio close to 200.

Table 27. Summary of the *in vitro* cytotoxicity of ADC 15

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	8.00E-02	1.80E-01	2.10E+01	2.21E+01	1.20E-01	2.15E+01	179.4
IC_{50} (M)	5.33E-10	1.20E-09	1.40E-07	1.47E-07	8.00E-10	1.43E-07	

Bioactivity Example 11 – Cytotoxicity of the conjugate ADC 16 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 16** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 28 summarizes the deduced IC_{50} values. As observed,

ADC 16 is significantly more active in in HER2-positive cells, yielding an outstanding selectivity ratio above 100.

Table 28. Summary of the *in vitro* cytotoxicity of ADC 16

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	4.01E-02	8.00E-01	1.30E+01	2.70E+01	1.79E-01	1.87E+01	104.7
IC_{50} (M)	2.67E-10	5.33E-09	8.67E-08	1.80E-07	1.19E-09	1.25E-07	

Bioactivity Example 12 – Cytotoxicity of the conjugate ADC 17 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 17** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 29 summarizes the deduced IC_{50} values.

Table 29. Summary of the *in vitro* cytotoxicity of ADC 17

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	7.40E+00	1.40E+01	4.40E+01	4.20E+01	1.02E+01	4.30E+01	4.22
IC_{50} (M)	4.93E-08	9.33E-08	2.93E-07	2.80E-07	6.78E-08	2.86E-07	

Bioactivity Example 13 – Cytotoxicity of the conjugate ADC 18 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 18** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 30 summarizes the deduced IC_{50} values.

Table 30. Summary of the *in vitro* cytotoxicity of ADC 18

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	4.40E+00	2.21E+01	4.31E+01	9.71E+01	9.84E+00	6.46E+01	6.57

IC_{50} (M)	2.93E-08	1.47E-07	2.87E-07	6.47E-07	6.56E-08	4.31E-07	

Bioactivity Example 14 – Cytotoxicity of the conjugate ADC 19 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 19** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 31 summarizes the deduced IC_{50} values.

Table 31. Summary of the *in vitro* cytotoxicity of ADC 19

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	8.30E+00	3.41E+01	> 1.00E+02	> 1.00E+02	1.68E+01	> 1.00E+02	>5.95
IC_{50} (M)	5.53E-08	2.27E-07	> 6.67E-07	> 6.67E-07	1.12E-07	> 6.67E-07	

Bioactivity Example 15 – Cytotoxicity of the conjugate ADC 20 against HER2 positive and negative breast cancer cells

The cytotoxicity of the **ADC 20** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 32 summarizes the deduced IC_{50} values.

Table 32. Summary of the *in vitro* cytotoxicity of ADC 20

	HER2 positive		HER2 negative		IC50 in HER2+ (geom. mean)	IC50 in HER2- (geom. mean	Selec. ratio
	SK-BR-3	HCC-1954	MDA-MB-231	MCF-7			
IC_{50} (µg/mL)	2.21E+00	1.61E+01	5.40E+01	4.01E+01	5.95E+00	4.65E+01	7.82
IC_{50} (M)	1.47E-08	1.07E-07	3.60E-07	2.67E-07	3.97E-08	3.10E-07	

Bioactivity Example 16 – Cytotoxicity of the conjugate ADC 7 and related reagents against CD13 positive and negative cancer cells

Cytotoxicity of **11-R**

The cytotoxicity of the intermediate compound 11-R was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 0.03 ng/mL (1.26E-07 – 3.3E-11 M).

As shown in Table 33, where results corresponding to the geometric mean of the IC_{50} values obtained in three different experiments are presented, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their CD13 expression, with IC_{50} values in the low nanomolar range, from 0.5 to 1.2 ng/mL (5.8E-10 to 1.51E-09 M). The geometric mean IC_{50} value across the whole cell panel was 0.84 ng/mL (1.06E-09 M) with the standard geometric deviation being 1.5 in agreement with the homogeneity of results across the four cell lines.

Table 33. Summary of the in vitro cytotoxicity of 11-R

	CD13 positive		CD13 negative	
	HT-1080	NB-4	Raji	RPMI8226
IC_{50}, µg/mL	1.20E-03	4.60E-04	9.67E-04	9.28E-04
IC_{50}, M	1.51E-09	5.80E-10	1.22E-09	1.17E-09

Cytotoxicity of **ADC 7**

The cytotoxicity of the **ADC 7** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 34 summarizes the deduced IC_{50} values. Remarkably, **ADC 7** has shown a cytotoxic activity in the low nM range comparable to that of the parental drug **11-R** but only in CD13-expressing cells, whereas its activity in CD13-negative cells is rather modest. Consequently, an outstanding selectivity fair above 100 can be observed as a function of CD13 expression.

Table 34. Summary of the *in vitro* cytotoxicity of ADC 7

	CD13 positive		CD13 negative		IC50 in CD13+ (geom. mean)	IC50 in CD13- (geom. mean	Selec. ratio
	HT-1080	NB-4	Raji	RPMI8226			
IC_{50} (µg/mL)	1.30E-01	2.70E-01	2.21E+01	4.10E+01	1.87E-01	3.00E+01	160.4
IC_{50} (M)	8.67E-10	1.80E-09	1.47E-07	2.73E-07	1.25E-09	2.00E-07	

Bioactivity Example 17 – Cytotoxicity of the conjugate ADC 8 against CD13 positive and negative cancer cells.

The cytotoxicity of the ADC **8** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 35 summarizes the deduced IC_{50} values. **ADC 8** has shown a cytotoxic activity in the low nM range comparable to that of the parental drug **11-R** but only in CD13-expressing cells, whereas its activity in CD13-negative cells is rather modest. Consequently, an outstanding selectivity close to 200 was obtained as a function of CD13 expression.

Table 35. Summary of the *in vitro* cytotoxicity of ADC 8

	CD13 positive		CD13 negative		IC50 in CD13+ (geom. mean)	IC50 in CD13- (geom. Mean)	Selec. ratio
	HT-1080	NB-4	Raji	RPMI8226			
IC_{50} (µg/mL)	1.61E-01	2.51E-01	4.31E+01	3.30E+01	2.01E-01	3.77E+01	188
IC_{50} (M)	1.07E-09	1.67E-09	2.87E-07	2.20E-07	1.34E-09	2.51E-07	

Bioactivity Example 18 – Cytotoxicity of the conjugate ADC 9 and related reagents against CD13 positive and negative cancer cells.

Cytotoxicity of 12-R

The cytotoxicity of the intermediate compound 12-R was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 to 0.03 ng/mL (1.28E-07 – 3.83E-11 M).

As shown in Table 36, where results corresponding to the geometric mean of the IC_{50} values obtained in three independent experiments are presented, the cytotoxicity of this compound was similar in all the tumor cell lines regardless of their CD13 expression, with IC_{50} values in the low nanomolar range, from 0.3 to 1.1 ng/mL (4.21E-10 to 1.40E-09 M). The geometric mean IC_{50} value across the whole cell panel was 0.6 ng/mL (7.8E-10 M), with the standard geometric deviation being 1.7 in agreement with the homogeneity of results across the four cell lines.

Table 36. Summary of the in vitro cytotoxicity of 12-R.

	CD13 positive		CD13 negative	
	HT-1080	NB-4	Raji	RPMI8226
IC_{50}, µg/mL	1.10E-03	3.30E-04	4.70E-04	8.30E-04

IC_{50}, M	1.40E-09	4.21E-10	6.00E-10	1.06E-09

Cytotoxicity of ADC-9

The cytotoxicity of the ADC **9** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 37 summarizes the deduced IC_{50} values. **ADC 9** has shown a cytotoxic activity in the low nM range comparable to that of the parental drug **12-R** but only in CD13-expressing cells, whereas its activity in CD13-negative cells is rather modest. Consequently, an outstanding selectivity ratio above 200 was yielded as a function of CD13 expression.

Table 37. Summary of the *in vitro* cytotoxicity of ADC 9

	CD13 positive		CD13 negative		IC50 in CD13+ (geom. mean)	IC50 in CD13- (geom. Mean)	Selec. ratio
	HT-1080	NB-4	Raji	RPMI8226			
IC_{50} (µg/mL)	1.20E-01	5.21E-02	1.70E+01	1.70E+01	7.90E-02	1.70E+01	214
IC_{50} (M)	8.00E-10	3.47E-10	1.13E-07	1.13E-07	5.27E-10	1.13E-07	

Bioactivity Example 19 – Cytotoxicity of the conjugate ADC 10 againsts CD13 positive and negative cancer cells.

The cytotoxicity of the ADC **10** was evaluated against the different tumor cell lines by performing triplicate 10-points, 2.5-fold dilution DR curves ranging from 100 µg/mL to 26 ng/mL (6.67E-07 – 1.75E-10 M). Table 38 summarizes the deduced IC_{50} values. **ADC 10** has shown a cytotoxic activity in the low nM range comparable to that of the parental drug **11-R** but only in CD13-expressing cells, whereas its activity in CD13-negative cells is rather modest. Consequently, a remarkable selectivity close to 100 was obtained as a function of CD13 expression.

Table 38. Summary of the *in vitro* cytotoxicity of ADC 10

	CD13 positive		CD13 negative		IC50 in CD13+ (geom. mean)	IC50 in CD13- (geom. mean	Selec. ratio
	HT1080	NB-4	Raji	RPMI 8226			
IC_{50} (µg/mL)	9.71E-02	9.71E-01	3.30E+01	2.81E+01	3.07E-01	3.04E+01	99.14
IC_{50} (M)	6.47E-10	6.47E-09	2.20E-07	1.87E-07	2.05E-09	2.03E-07	

Example 4: Demonstrating the *in vivo* efficacy of the Antibody-Drug Conjugates of the Present Invention

Trastuzumab-based antibody drug conjugate **ADC 1** was tested in several in vivo models. ADC-1 batches used in these studies were prepared using 2.2 eq of TCEP (ADC 1 2.2 TCEP) or 3 eq of TCEP (ADC-1), These batches were manufactured using the procedures described above with the exception that the final purification by size exclusion chromatography (SEC) was carried out using a Hi Load 26/600 SuperdexTM 200 pg column and PBS (pH 7.4) as eluent.

ADC-1 and ADC 1 2.2 TCEP were evaluated in a breast HER2 positive model, BT-474 together with their payload, compound **11-R**. Of note, in spite of the low dose (suboptimal) administered to mice bearing tumors in this experiment, encouraging positive result was obtained (See Figure 12). Therefore, a set of new experiments, aimed at evaluating the antitumor activity in breast and non-breast Her2 positive models were performed. ADC-1 was evaluated at a higher dose level in the breast tumor model, JIMT-1 (See Figure 13), in one ovarian tumor model, SK-OV-3 (Figure 14) and in a gastric model, Gastric-008, a patient derived xenograft (PDX), (Figure 16). Additionally ADC-1 2.2 TCEP) was evaluated in another gastric model, N87 (Figure 15).

Briefly, 4 to 6 week-old athymic nu/nu (N87, Gastric-008 or SK-OV-3) or SCID (BT-474 or JIMT-1) mice were subcutaneously implanted with either tumor cell suspension (JIMT-1 or N87) or tumor fragments (BT-474, Gastric-008 or SK-OV3) previously generated in donor mice.

Tumor dimension and body weight was recorded 3 times per week starting from the first day of treatment (Day 0). Treatments producing >20% lethality and/or 20% net body weight loss were considered toxic. Tumor volume was calculated using the equation $(a \cdot b^2)/2$, where *a* and *b* were the longest and shortest diameters, respectively. Animals were euthanized when their tumors reached *ca.* 2,000 mm^3 and/or severe necrosis was seen. Median was calculated for tumor volume on each measurement day. Complete tumor regression (CR) was defined when tumor volume < 63 mm^3 for 2 or more consecutive measurements.

The animals were implanted as above described and when tumors reached *ca.* 150-200 mm^3, tumor bearing animals (*N* = 8-10/group) were randomly allocated into treatment groups according to the following experimental design:

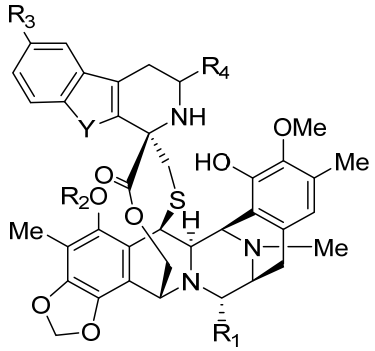
Tumor	Group	Dose (mg/kg)
JIMT-1	Control	0.0

Tumor	Group	Dose (mg/kg)
	11-R	5.0
	ADC-1	30.0
	ADC-1	10.0
	ADC-1	5.0
N87	Control	0.0
	11-R	5.0
	ADC-1 (2.2 TCEP)	30.0
	ADC-1 (2.2 TCEP)	10.0
	ADC-1 (2.2 TCEP)	5.0
SK-OV-3	Control	0.0
	11-R	5.0
	ADC-1	30.0
	ADC-1	10.0
	ADC-1	5.0
Gastric-008	Control	0.0
	11-R	5.0
	ADC-1	30.0
	ADC-1	10.0
	ADC-1	5.0
BT-474	Control	0.0
	11-R	5.0
	ADC-1 (2.2 TCEP)	6.5
	ADC-1	2.24
	ADC-1 (2.2 TCEP)	1.6

Claims

1. A drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the drug conjugate having formula $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]_n\text{-}Ab$ wherein:

D is a drug moiety having the following formula (I) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof,



(I)

wherein:

D is covalently attached via a hydroxy or amine group to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_4 is selected from $-CH_2O-$ and $-CH_2NH-$;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

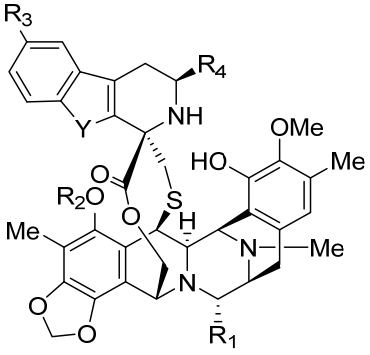
g is an integer of 0 or 1;

Ab is a moiety comprising at least one antigen binding site; and

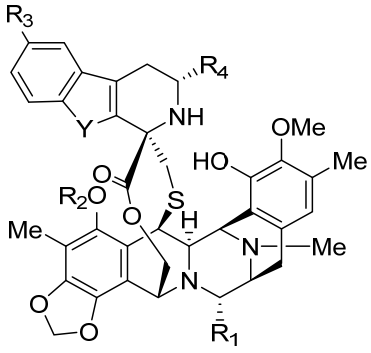
n is the ratio of the group $[D\text{-}(X)_b\text{-}(AA)_w\text{-}(T)_g\text{-}(L)\text{-}]$ to the moiety comprising at least one antigen binding site and is in the range from 1 to 20;

wherein the drug moiety D is conjugated via the R_4 position.

2. The drug conjugate according to claim 1, wherein D is selected from formula Ia or Ib, or a pharmaceutically acceptable salt or ester thereof:



Ia



Ib

wherein:

Y is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O) R_a group;

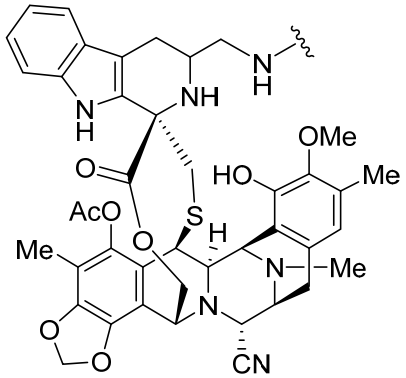
R_3 is hydrogen or a $–OR_b$ group;

R_4 is selected from $-CH_2O-$ and $-CH_2NH-$;

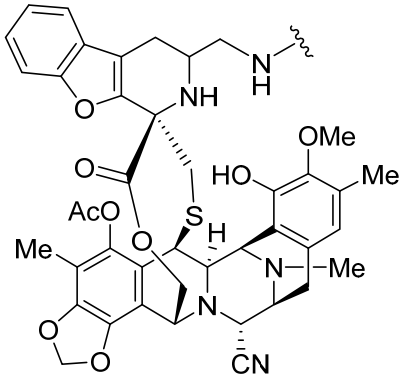
R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl.

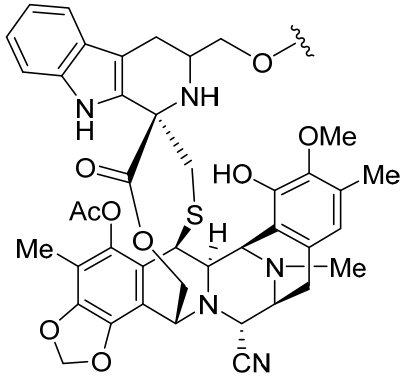
3. The drug conjugate according to claim 1, wherein D is a compound of formula:



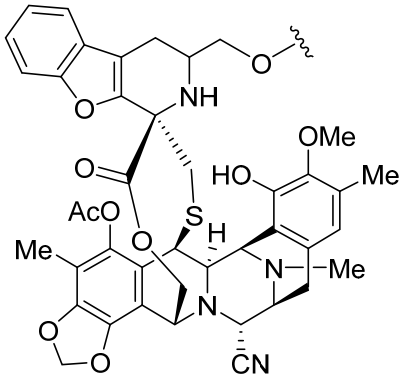
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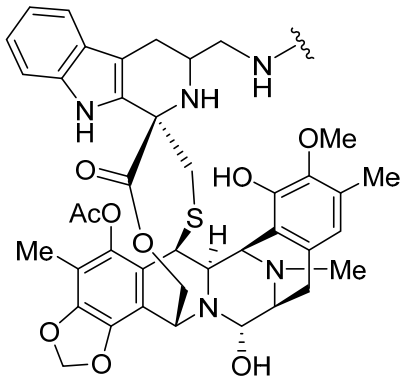
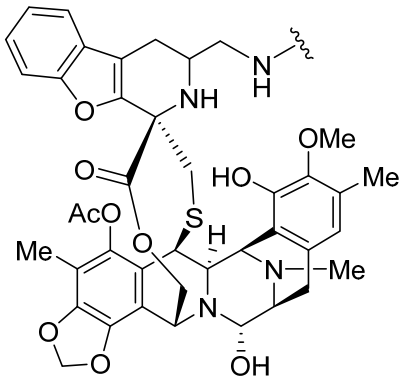
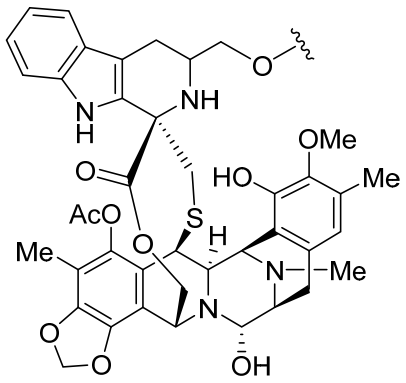
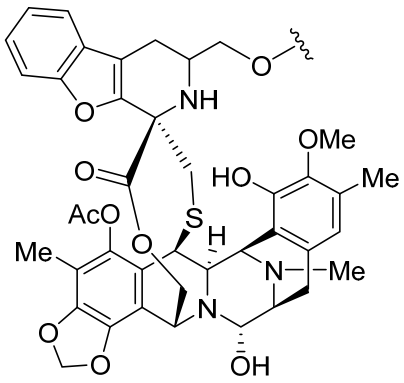
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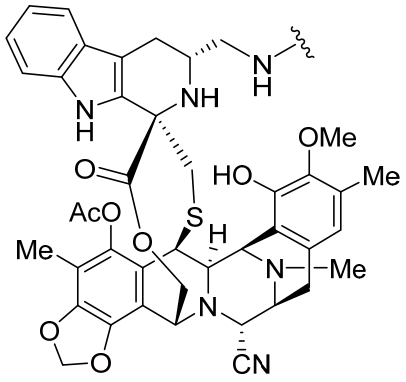
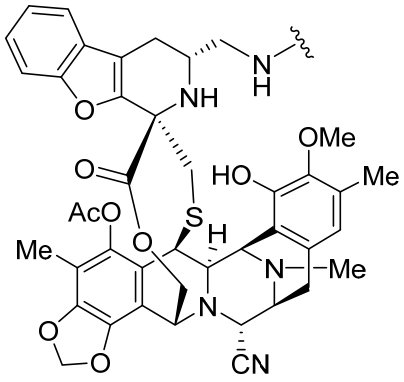
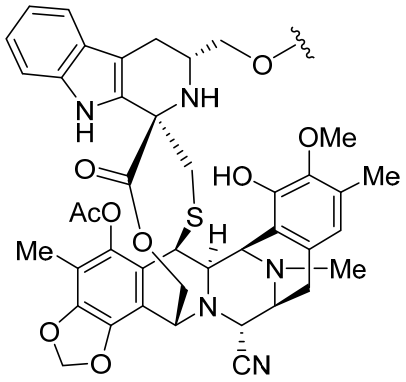
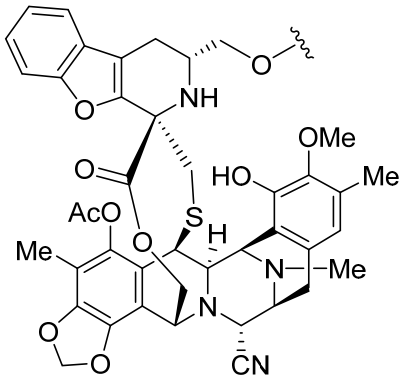
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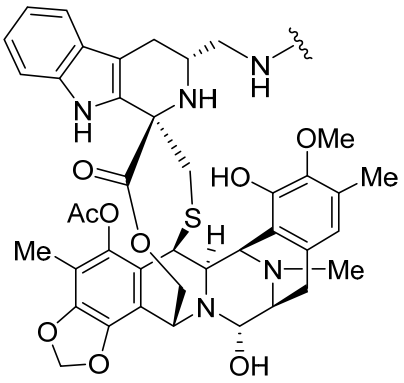
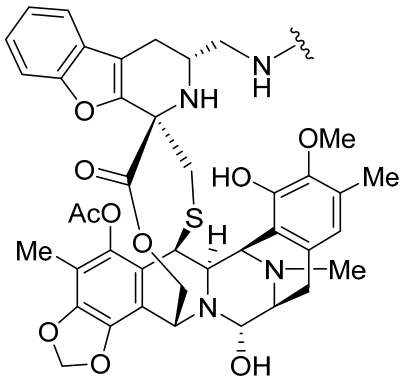
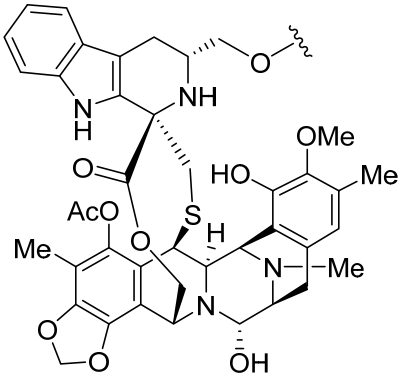
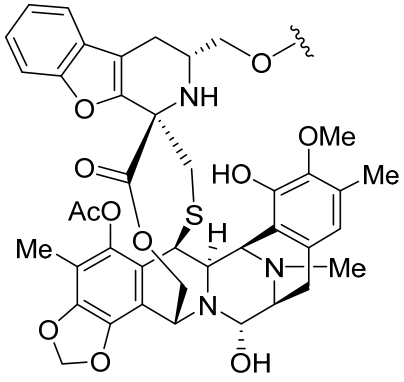
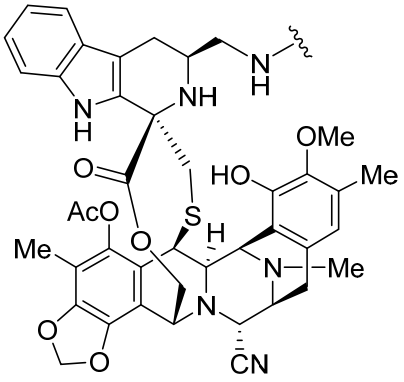
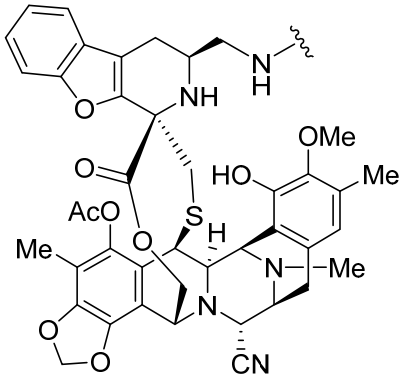
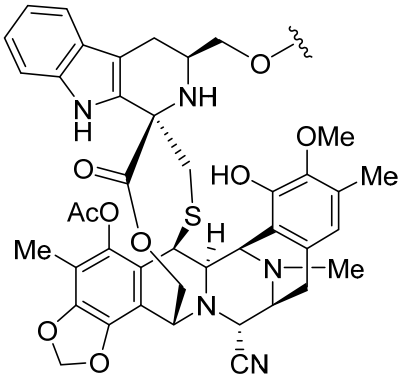
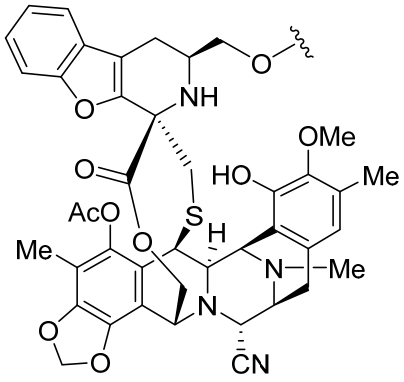





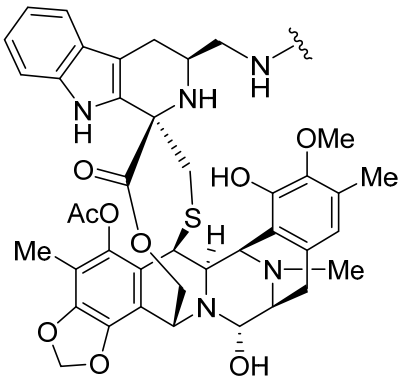
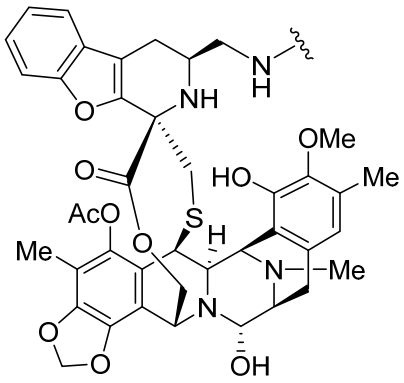
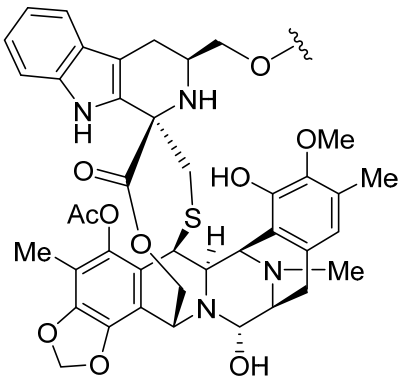
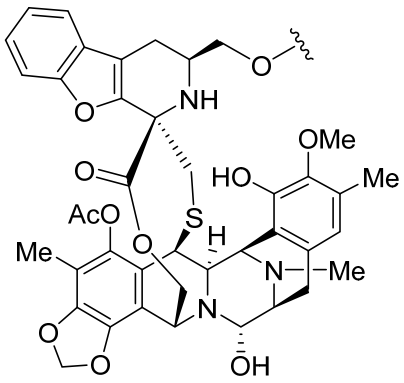
or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

preferably of formula:

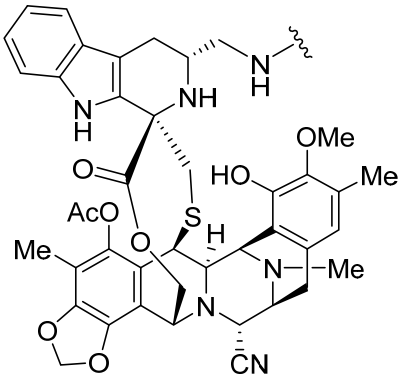
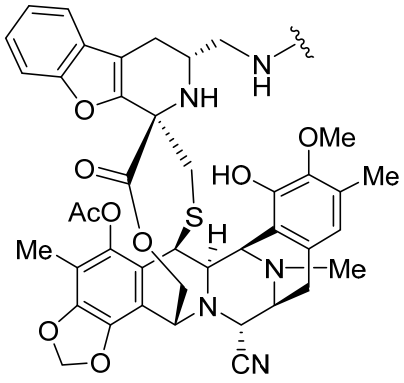
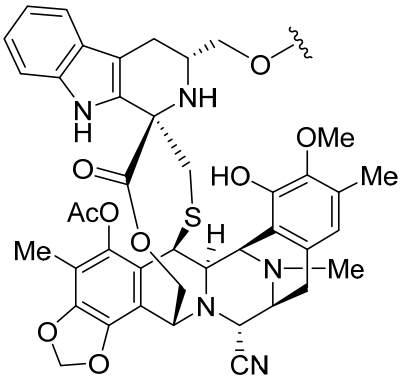
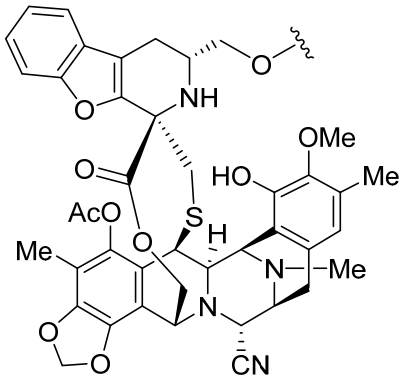





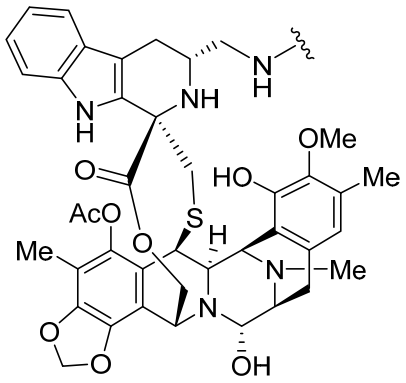
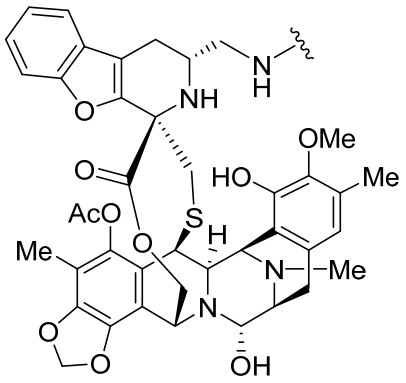
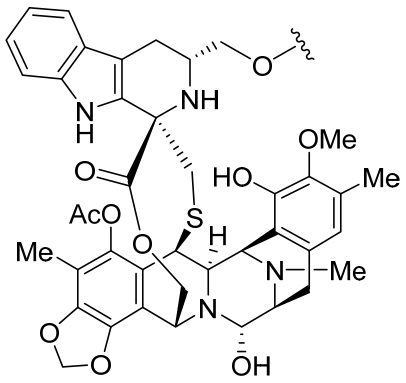
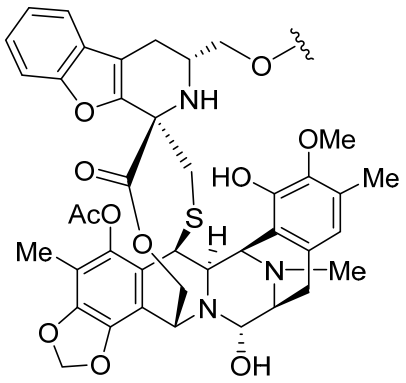

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or a pharmaceutically acceptable salt or ester thereof;

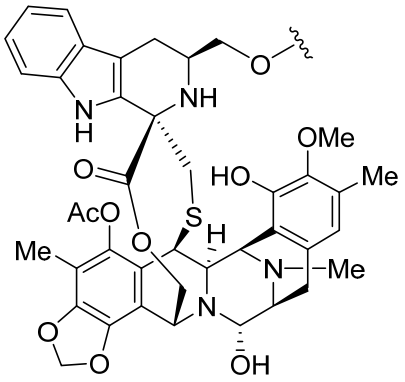
preferably of formula:


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or a pharmaceutically acceptable salt or ester thereof.

4. The drug conjugate according to claim 1, wherein D is a compound of formula:

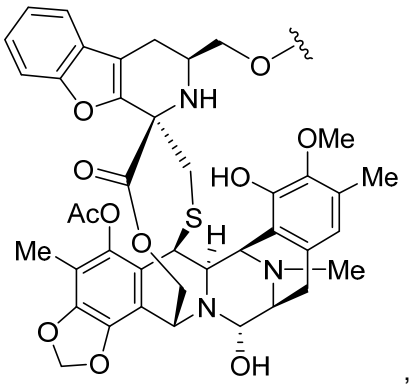

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or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

or

wherein D is a compound of formula:

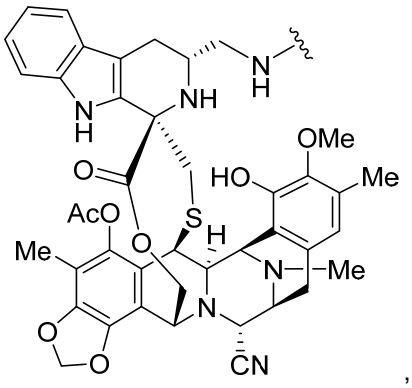


or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

or

wherein D is a compound of formula:

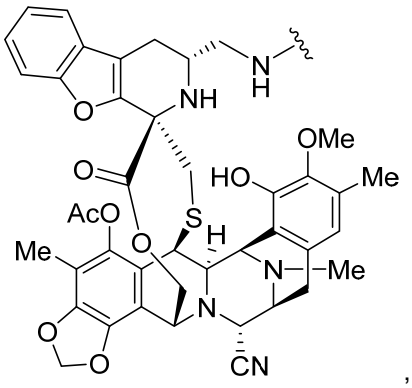


or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

or

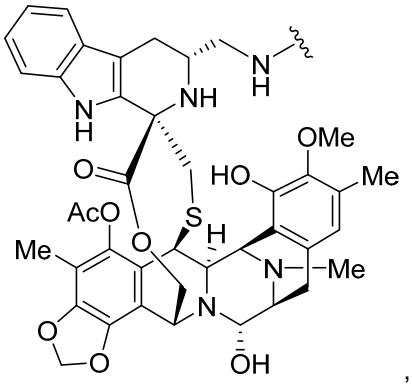
wherein D is a compound of formula:



or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L); or

wherein D is a compound of formula:

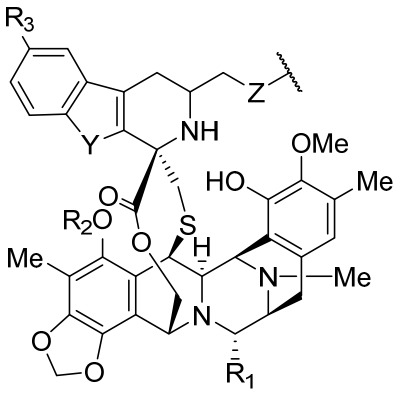


or a pharmaceutically acceptable salt or ester thereof;

wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L).

5. A drug conjugate comprising a drug moiety covalently attached to the rest of the drug conjugate, the drug conjugate having formula $[D-(X)_b-(AA)_w-(T)_g-(L)-]_n$-Ab wherein:

D is a drug moiety having the following formula (IH) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof:



(IH)

wherein:

the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or (L);

each Y and Z is independently selected from -NH- and -O-;

R_1 is -OH or -CN;

R_2 is a $-C(=O)R_a$ group;

R_3 is hydrogen or a $-OR_b$ group;

R_a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl, wherein the optional substituents are one or more substituents R_x; and

R_b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl, wherein the optional substituents are one or more substituents R_x;

substituents R_x are selected from the group consisting of C_1-C_{12} alkyl groups which may be optionally substituted with at least one group R_y, C_2-C_{12} alkenyl groups which may be optionally substituted with at least one group R_y, C_2-C_{12} alkynyl groups which may be optionally substituted with at least one group R_y, halogen atoms, oxo groups, thio groups, cyano groups, nitro groups, OR_y, $OCOR_y$, $OCOOR_y$, COR_y, $COOR_y$, $OCONR_yR_z$, $CONR_yR_z$, $S(O)R_y$, SO_2R_y, $P(O)(R_y)OR_z$, NR_yR_z, NR_yCOR_z, $NR_yC(=O)NR_yR_z$, $NR_yC(=NR_y)NR_yR_z$, aryl groups having from 6 to 18 carbon atoms in one or more rings which may optionally be substituted with one or more substituents which may be the same or different selected from the group consisting of R_y, OR_y, $OCOR_y$, $OCOOR_y$, NR_yR_z, NR_yCOR_z, and $NR_yC(=NR_y)NR_yR_z$, aralkyl groups comprising an alkyl group having from 1 to 12 carbon atoms substituted with an optionally substituted aryl group as defined above, aralkyloxy groups comprising an alkoxy group having from 1 to 12 carbon atoms substituted with an optionally substituted aryl group as defined above, and a 5-

to 14- membered saturated or unsaturated heterocyclic group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said heterocyclic group optionally being substituted with one or more substituents R_y, and where there is more than one optional substituents on any given group the optional substituents R_y may be the same or different;

each R_y and R_z is independently selected from the group consisting of hydrogen, C_1-C_{12} alkyl groups, C_1-C_{12} alkyl groups that are substituted with at least one halogen atom, aralkyl groups comprising a C_1-C_{12} alkyl group that is substituted with an aryl group having from 6 to 18 carbon atoms in one or more rings and heterocycloalkyl groups comprising a C_1-C_{12} alkyl group that is substituted with a 5- to 14- membered saturated or unsaturated heterocyclic group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s);

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

L is a linker group;

w is an integer ranging from 0 to 12;

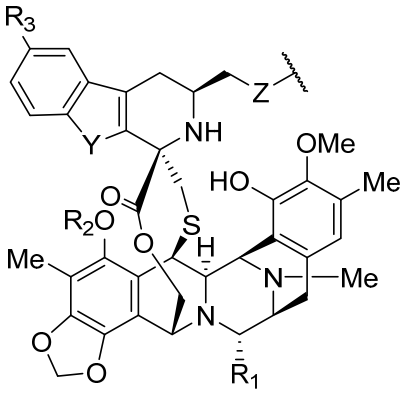
b is an integer of 0 or 1;

g is an integer of 0 or 1;

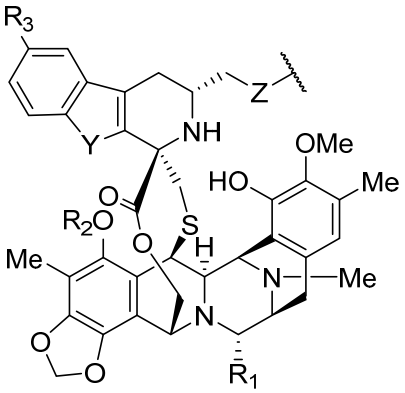
Ab is a moiety comprising at least one antigen binding site; and

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] to the moiety comprising at least one antigen binding site and is in the range from 1 to 20.

6. A drug conjugate according to claim 5, or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein D is a drug moiety selected from formulas (IHa) and (IHb):



(IHa)

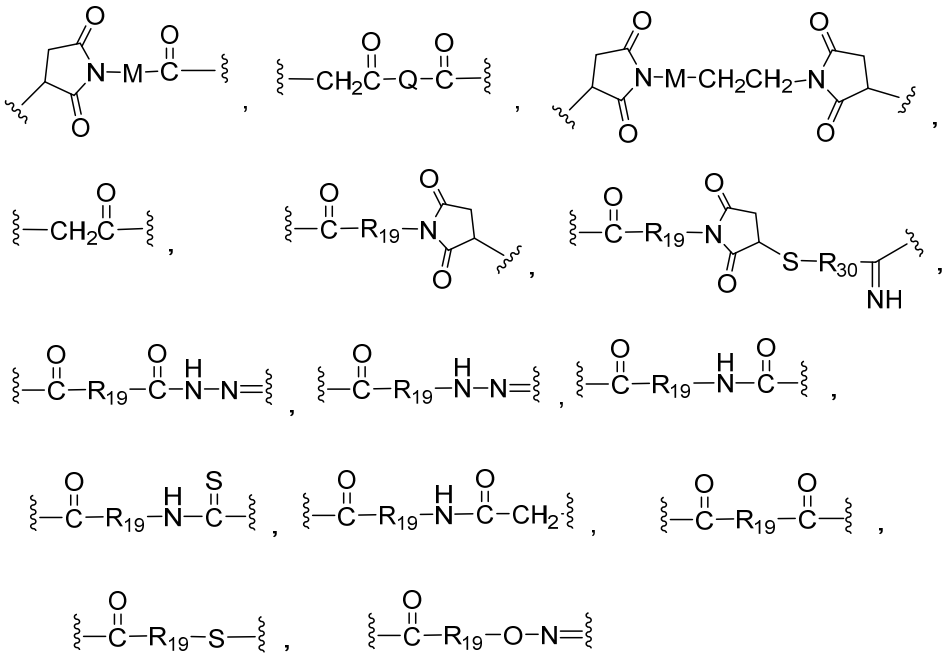


(IHb)

where the wavy lines, R_1, R_2, R_3, Y, and Z are as defined for formula (IH).

7. The drug conjugate according to any one of claims 1 to 6, wherein the salt is selected from hydrochloride, hydrobromide, hydroiodide, sulfate, nitrate, phosphate, acetate, trifluoroacetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulfonate, *p*-toluenesulfonate, sodium, potassium, calcium, ammonium, ethylenediamine, ethanolamine, *N*,*N*-dialkylenethanolamine, triethanolamine and basic aminoacids.

8. A drug conjugate according to any one of the preceding claims, wherein L is a linker group selected from the group consisting of:



wherein

the wavy lines indicate the point of covalent attachments to an Ab (the wavy line to the right) and to $(T)_g$ if any, or $(AA)_w$ if any, or $(X)_b$ if any, or D (the wavy line to the left);

R_{19} is selected from -C_1-C_{12} alkylene-, -C_3-C_8 carbocyclo, -O-(C_1-C_{12} alkylene), -C_6-C_{18} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{18} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{18} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_3-C_8 carbocyclo)-, -(C_3-C_8 carbocyclo)-C_1-C_{12} alkylene-, -C_5-C_{14} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{14} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{14} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$- and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a -C_1-C_6 alkylene- group;

M is selected from the group consisting of -C_1-C_6 alkylene-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)-, -$(CH_2CH_2O)_s$-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)-CON(H or C_1-C_6 alkyl)-C_1-C_6 alkylene-, phenylene which may optionally be substituted with one or more substituents R_x, phenylene-C_1-C_6 alkylene- wherein the phenylene moiety may optionally be substituted with one or more substituents R_x and -C_1-C_6 alkylene-CON(H or C_1-C_6 alkyl)C_1-C_6 alkylene-;

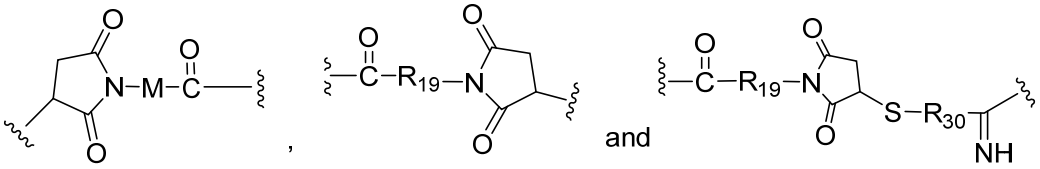
Q is selected from the group consisting of -N(H or C_1-C_6 alkyl)phenylene- and -N(H or C_1-C_6 alkyl)-$(CH_2)_s$;

r is an integer ranging from 1 to 10; and

s is an integer ranging from 1 to 10;

or

wherein L is a linker group selected from the group consisting of:



wherein:

the wavy lines indicate the point of covalent attachments to an Ab (the wavy line to the right) and to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ (the wavy line to the left);

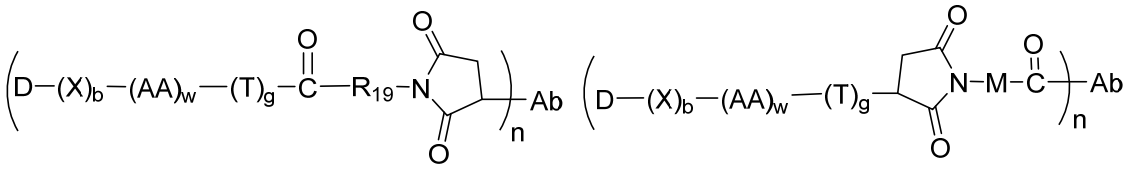
R_{19} is selected from -C_1-C_{12} alkylene-, -O-(C_1-C_{12} alkylene), -C_6-C_{12} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{12} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_5-C_{12} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{12} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{12} heterocyclo)-C_1-C_{12} alkylene- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$- and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a -C_1-C_6 alkylene- group;

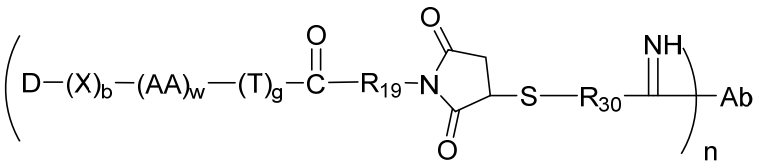
M is selected from the group consisting of -C_1-C_6 alkylene-, -C_1-C_6 alkylene-(C_3-C_8 carbocyclo)- and phenylene which may optionally be substituted with one or more substituents R_x; and

r is an integer ranging from 1-6.

9. A drug conjugate according to any one of claims 1 to 8, selected from the formulas (IV), (V) and (VI):



(IV) (V)



(VI)

wherein:

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

g is an integer of 0 or 1;

D is a drug moiety;

Ab is a moiety comprising at least one antigen binding site;

n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in formula (IV), (V) or (VI) to the moiety comprising at least one antigen binding site and is in the range from 1 to 20;

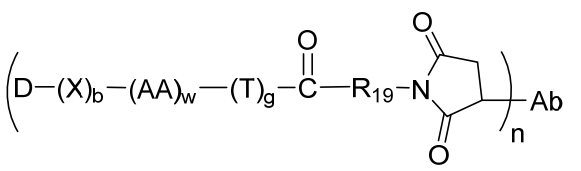
R_{19} is selected from -C_1-C_8 alkylene-, -O-(C_1-C_8 alkylene), -C_1-C_8 alkylene-C_6-C_{12} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, and -C_6-C_{12} arylene-C_1-C_8 alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{30} is a –C_2-C_4 alkylene- group; and

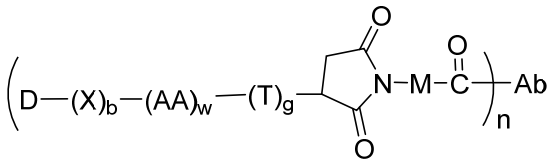
M is selected from the group consisting of -C_1-C_3 alkylene- and -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-;

or

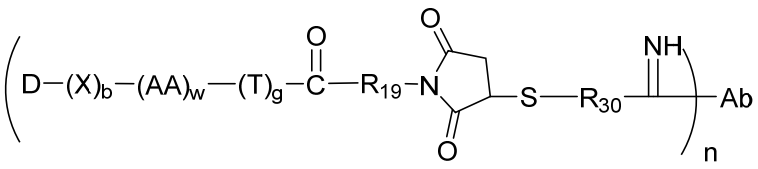
selected from the formulas (IV), (V) and (VI):



(IV)



(V)



(VI)

wherein:

X and T are extending groups that may be the same or different;

each AA is independently an amino acid unit;

w is an integer ranging from 0 to 12;

b is an integer of 0 or 1;

g is an integer of 0 or 1;

D is a drug moiety;

Ab is a moiety comprising at least one antigen binding site;

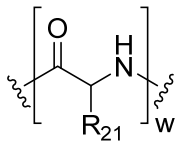
n is the ratio of the group [D-$(X)_b$-$(AA)_w$-$(T)_g$-(L)-] wherein L is as defined in (IV), (V) or (VI) to the moiety comprising at least one antigen binding site and is in the range from 1 to 20;

R_{19} is selected from -C_1-C_6 alkylene-, phenylene-C_1-C_6 alkylene- wherein the phenylene group may optionally be substituted with one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups, wherein each of the above alkylene substituents whether alone or attached to another moiety in the carbon chain may optionally be substituted by one or more substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, aryl groups having from 6 to 12 carbon atoms, halogen atoms, nitro groups and cyano groups, and preferably R_{19} is a -C_1-C_6 alkylene group;

R_{30} is a –C_2-C_4 alkylene- group; and

M is -C_1-C_3 alkylene-(C_5-C_7 carbocyclo)-.

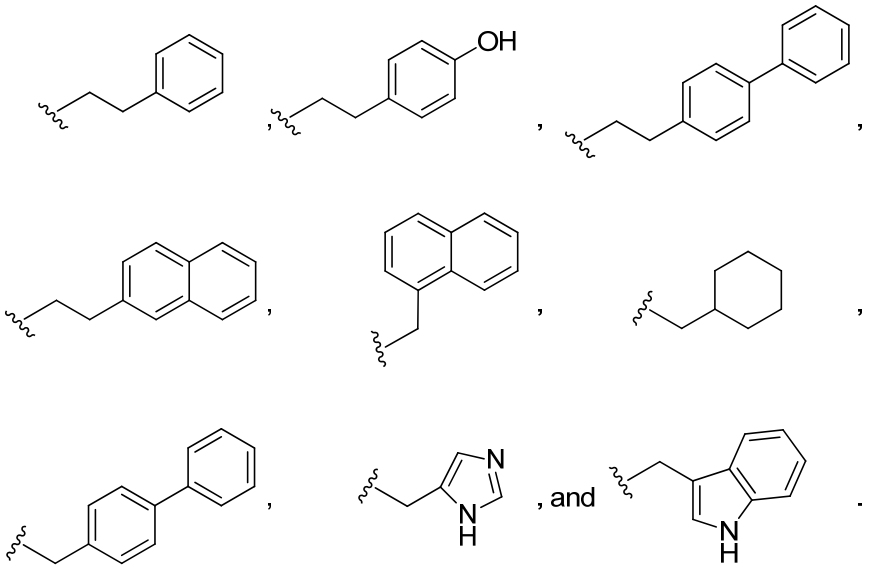
10. A drug conjugate according to any one of claims 1 to 9, wherein $(AA)_w$ is of formula (II):



(II)

wherein the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right); and

R_{21} is, at each occurrence, selected from the group consisting of hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, $-CH_2OH$, $-CH(OH)CH_3$, $-CH_2CH_2SCH_3$, $-CH_2CONH_2$, $-CH_2COOH$, $-CH_2CH_2CONH_2$, $-CH_2CH_2COOH$, $-(CH_2)_3NHC(=NH)NH_2$, $-(CH_2)_3NH_2$, $-(CH_2)_3NHCOCH_3$, $-(CH_2)_3NHCHO$, $-(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_4NHCOCH_3$, $-(CH_2)_4NHCHO$, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NHCONH_2$, $-CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



and w is an integer ranging from 0 to 12;

or

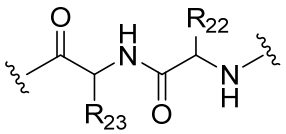
wherein $(AA)_w$ is of formula (II) wherein:

R_{21} is selected, at each occurrence, from the group consisting of hydrogen, methyl, isopropyl, sec-butyl, benzyl, indolylmethyl, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_3NHC(=NH)NH_2$ and $-(CH_2)_4NHC(=NH)NH_2$; and

w is an integer ranging from 0 to 6;

or

wherein w is 0 or 2, and where w is 2, then $(AA)_w$ is of formula (III):



(III)

wherein:

the wavy lines indicate the point of covalent attachments to $(X)_b$ if any, or to the drug moiety (the wavy line to the left) and to $(T)_g$ if any, or to the linker (the wavy line to the right);

R_{22} is selected from methyl, benzyl, isopropyl, *sec*-butyl and indolylmethyl; and

R_{23} is selected from methyl, $-(CH_2)_4NH_2$, $-(CH_2)_3NHCONH_2$ and $-(CH_2)_3NHC(=NH)NH_2$.

11. A drug conjugate according to any one of claims 1 to 10, wherein X is an extending group selected from:
where D is covalently attached via an amine group (for example where Z is -NH-):

-COO-(C_1-C_6 alkylene)NH-;

-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

-COO-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

-$COCH_2NH$-$COCH_2$-NH-;

-$COCH_2NH$-;

-COO-(C_1-C_6 alkylene)S-;

-COO-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-; and

where D is covalently attached via an hydroxy group (for example where Z is -O-):

-CONH-(C_1-C_6 alkylene)NH-;

-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

-CONH-(C_1-C_6 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with one or more substituents R_x)-NH-;

-$COCH_2NH$-$COCH_2$-NH-;

-$COCH_2NH$-;

-CONH-(C_1-C_6 alkylene)S-;

-CONH-(C_1-C_6 alkylene)NHCO(C_1-C_6 alkylene)S-; and

b is 0 or 1, preferably 1;

or

wherein X is an extending group selected from the group consisting of:

where D is covalently attached via an amine group (for example where Z is -NH-):

-COO-(C_2-C_4 alkylene)NH-;

-COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups;

-COO-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-;

-$COCH_2NH$-$COCH_2$-NH-;

-COO-(C_2-C_4 alkylene)S-;

-COO-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-; or

where D is covalently attached via an hydroxy group (for example where Z is -O-):

-CONH-(C_2-C_4 alkylene)NH-;

-COO-CH_2-phenylene-NH-, wherein said phenylene group may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups;

-CONH-(C_2-C_4 alkylene)NH-COO-CH_2-(phenylene which may optionally be substituted with from one to four substituents R_x selected from the group consisting of alkyl groups having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, nitro groups and cyano groups)-NH-;

-$COCH_2NH$-$COCH_2$-NH-;

-CONH-(C_2-C_4 alkylene)S-;

-CONH-(C_2-C_4 alkylene)NHCO(C_1-C_3 alkylene)S-; and

b is 0 or 1, preferably 1;

or

wherein X is an extending group selected from the group consisting of:

where D is covalently attached via an amine group (for example where Z is -NH-):

-COO-CH_2-phenylene-NH-

-$COO(CH_2)_3NHCOOCH_2$-phenylene-NH-;

-$COO(CH_2)_3$NH-;

-$COO(CH_2)_3$-S-;

-$COO(CH_2)_3NHCO(CH_2)_2$S-; or

where D is covalently attached via an hydroxy group (for example where Z is -O-):

-COO-CH_2-phenylene-NH-

-$CONH(CH_2)_3NHCOOCH_2$-phenylene-NH-;

-$CONH(CH_2)_3$NH-;

-$CONH(CH_2)_3$-S-;

-$CONH(CH_2)_3NHCO(CH_2)_2$S-; and

b is 0 or 1, preferably 1.

12. A drug conjugate according to any one of claims 1 to 11, wherein T is an extending group selected from the group consisting of -CO-(C_1-C_6 alkylene)-NH-,-CO-(C_1-C_6 alkylene)-

[O-(C_2-C_6 alkylene)]$_j$-NH-, -COO-(C_1-C_6 alkylene)-[O-(C_2-C_6 alkylene)]$_j$-NH-; where j is an integer from 1 to 25, and g is 0 or 1;
or

wherein T is an extending group selected from the group consisting of -CO-(C_1-C_4 alkylene)NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-,where j is an integer from 1 to 10; and g is 0 or 1;

or

wherein T is an extending group selected from the group consisting of -CO-(C_1-C_4 alkylene)NH-, -CO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-, -COO-(C_1-C_4 alkylene)-[O-(C_2-C_4 alkylene)]$_j$-NH-; where j is an integer from 1 to 5; and g is 0 or 1.

13. A drug conjugate according to any one of claims 5 to 12, wherein D is a drug moiety of formula (IHa) or formula (IHb) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein:
R_1 is CN or OH;

R_2 is C(=O)R_a, wherein R_a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl, wherein the optional substituents are one or more substituents R_x;

R_3 is hydrogen or a -OR_b group wherein R_b is a substituted or unsubstituted C_1-C_6 alkyl group, wherein the optional substituents are one or more substituents R_x,

Y is -NH- or -O-; and

Z is -NH- or -O-;

or

wherein D is a drug moiety of formula (IHa) or formula (IHb) or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein:

R_1 is CN or OH;

R_2 is acetyl;

R_3 is hydrogen or methoxy, more preferably hydrogen;

Y is -NH- or -O-; and

Z is -NH- or -O-;

or

wherein D is a drug moiety of formula (IHa) or formula (IHb), or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof wherein:

R_1 is CN;

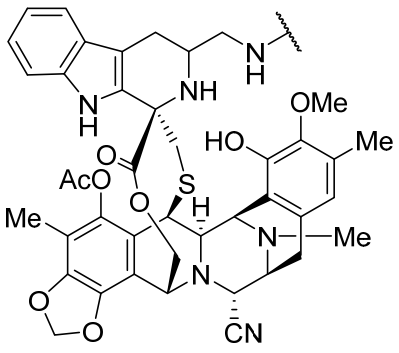
R_2 is acetyl:

R_3 is hydrogen;

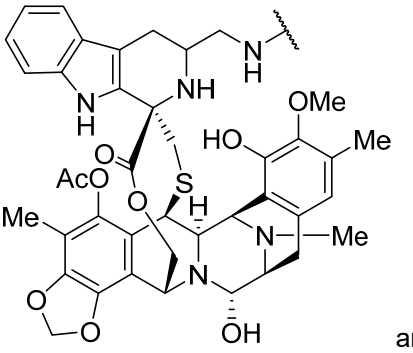
Y is -NH- or –O-; and

Z is -NH-.

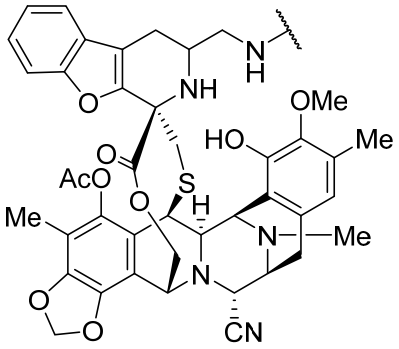
14. A drug conjugate according to any one of claims 1, 2, or 5 to 13, wherein D is selected from:



,



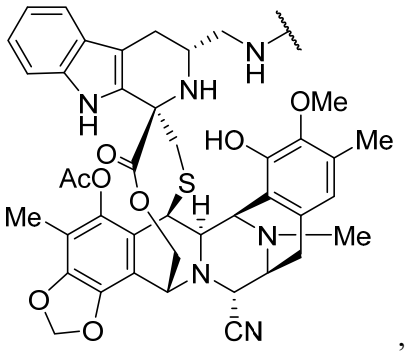
and



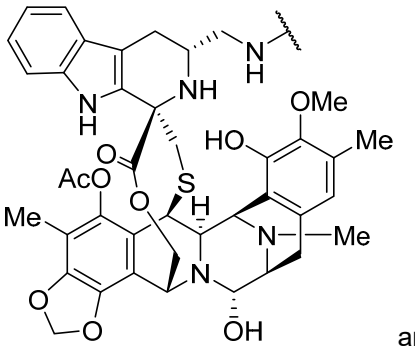
or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to (L);

or

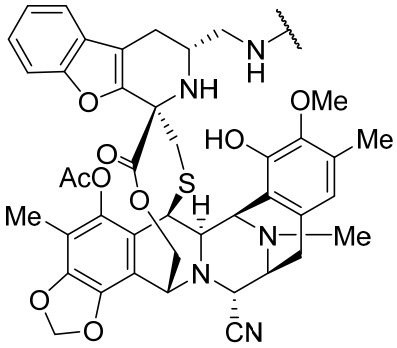
wherein D is



,



and



or a pharmaceutically acceptable salt, ester, solvate, tautomer or stereoisomer thereof, wherein the wavy line indicates the point of covalent attachment to $(X)_b$ if any, or $(AA)_w$ if any, or to $(T)_g$ if any, or to (L).

15. A drug conjugate according to any one of claims 1 to 14, wherein the moiety Ab comprising at least one antigen binding site is an antigen-binding peptide;
optionally

wherein the moiety Ab comprising at least one antigen binding site is an antibody, a single domain antibody or an antigen-binding fragment thereof;

optionally

wherein the moiety Ab comprising at least one antigen binding site is a monoclonal antibody, polyclonal antibody or bispecific antibody and wherein the antibody or an antigen-binding fragment thereof is derived from any species, preferably a human, mouse or rabbit;

optionally

wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a human antibody, an antigen-binding fragment of a human antibody, a humanized antibody, an antigen-binding fragment of a humanized antibody, a chimeric antibody, an antigen-binding fragment of a chimeric antibody, a glycosylated antibody and a glycosylated antigen binding fragment;

optionally

wherein the antibody or antigen-binding fragment thereof is an antigen-binding fragment selected from the group consisting of an Fab fragment, an Fab' fragment, an F(ab')2 fragment and an Fv fragment;

optionally

wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody which immunospecifically binds to cancer cell antigens, viral antigens, antigens of cells that produce autoimmune antibodies associated with autoimmune disease, microbial antigens, and preferably a monoclonal antibody which immunospecifically binds to cancer cell antigens;

optionally

wherein the moiety Ab comprising at least one antigen binding site is an antibody selected from the group consisting of Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Coltuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab, Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Lifastuzumab, Lorvotuzumab, Milatuzumab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Pinatuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab, Sofituzumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof;

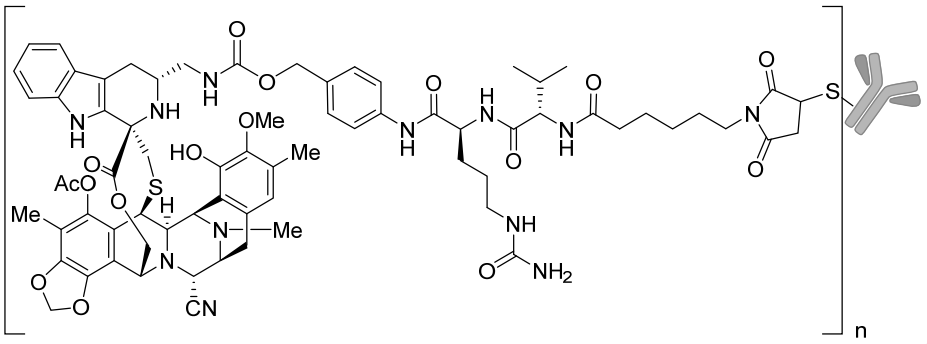
optionally

wherein the moiety Ab comprising at least one antigen binding site is an antibody selected from the group consisting of Abciximab, Alemtuzumab, Anetumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denintuzumab, Denosumab, Depatuxizumab, Dinutuximab, Durvalumab, Elotuzumab, Enfortumab, Glembatumumab, Gemtuzumab, Ibritumomab, Indatuximab, Indusatumab, Inotuzumab, Ipilimumab, Labetuzumab, Ladiratuzumab, Laprituximab, Mirvetuximab, Naratuximab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Polatuzumab, Ramucirumab, Rovalpituzumab, Sacituzumab, Siltuximab, Sirtratumab, Vadastuximab, Vorsetuzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof;

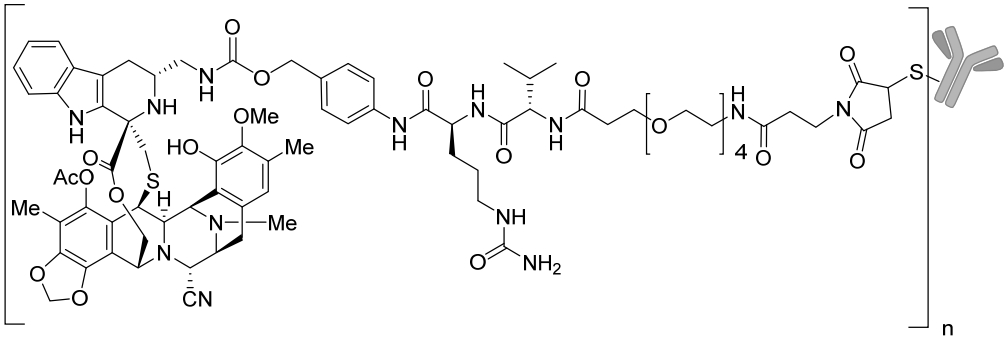
optionally

wherein the moiety Ab comprising at least one antigen binding site is an antibody selected from the group consisting of Abciximab, Alemtuzumab, Atezolizumab, Avelumab, Basiliximab, Bevacizumab, Blinatomumab, Brentuximab, Catumaxomab, Cetuximab, Daclizumab, Daratumumab, Denosumab, Dinutuximab, Durvalumab, Elotuzumab, Gemtuzumab, Ibritumomab, Inotuzumab, Ipilimumab, Labetuzumab, Necitumumab, Nimotuzumab, Nivolumab, Obinutuzumab, Ofatumumab, Olaratumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, Rovalpituzumab, Siltuximab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, more preferably Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologicallly active portion thereof, preferably Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, more preferably Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof.

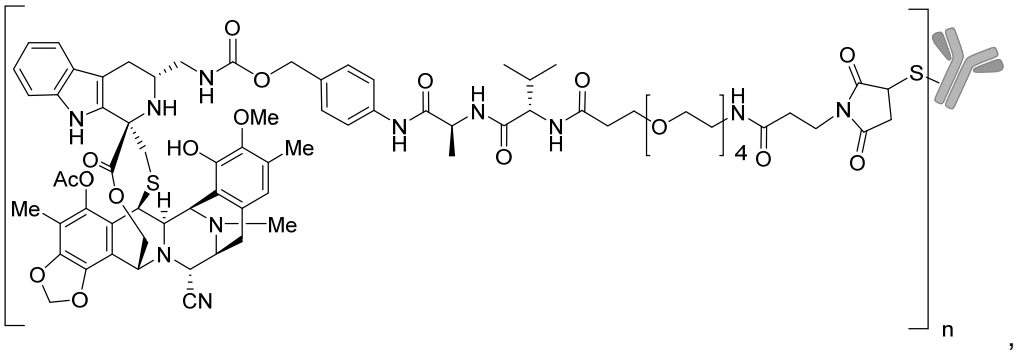
16. A drug conjugate according to claim 1, that is an antibody drug conjugate, selected from the group consisting of:



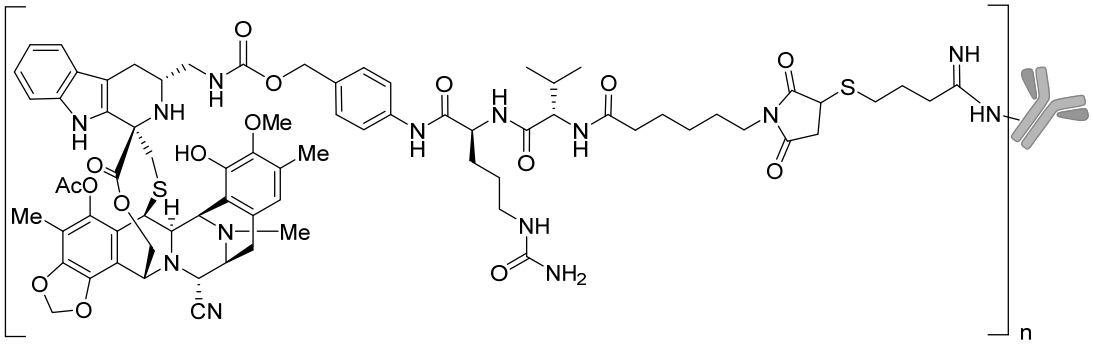
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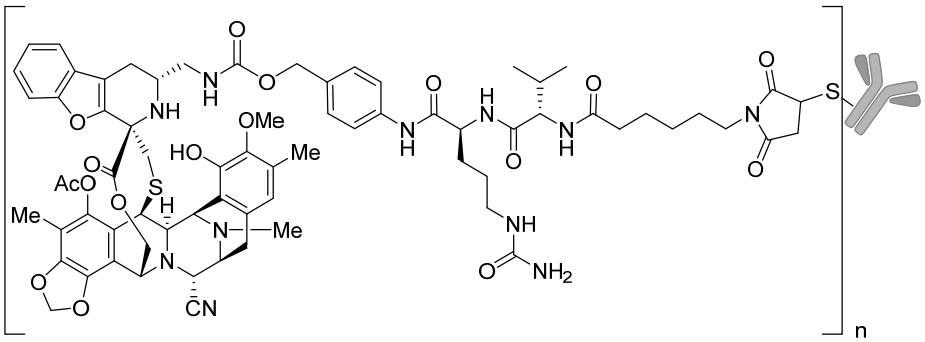
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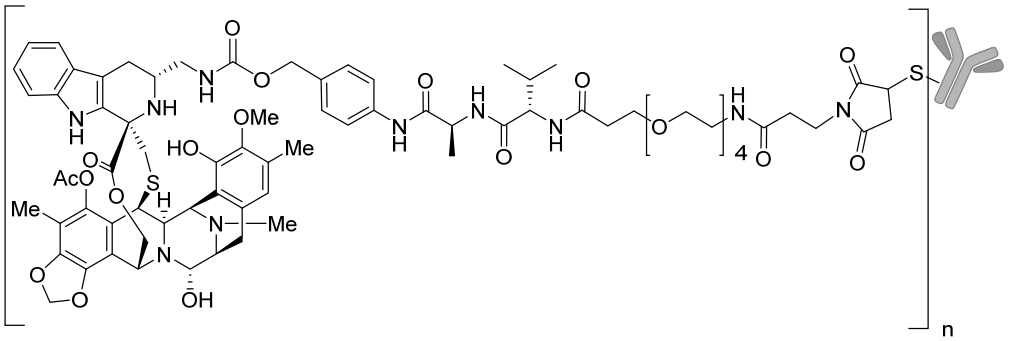
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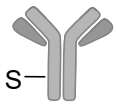
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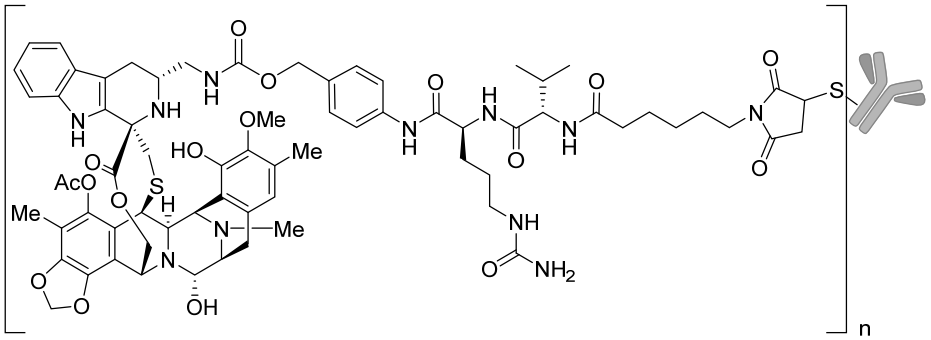
,

wherein n is from 2 to 6, more preferably 3, 4, or 5 and each  and  is independently selected from is independently selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an

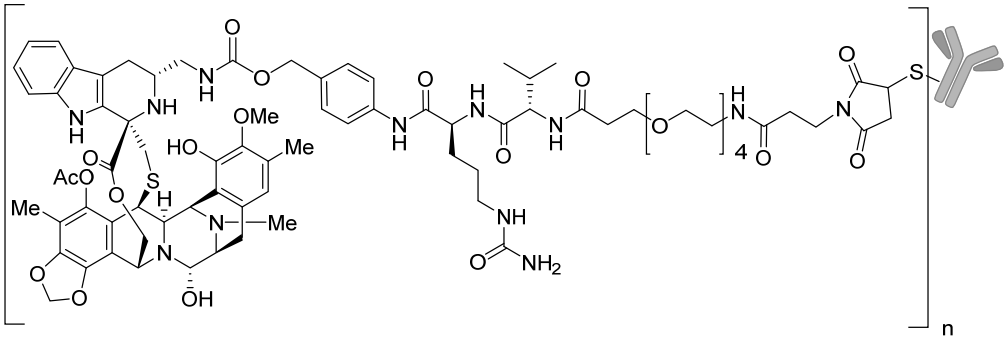
immunologically active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof;

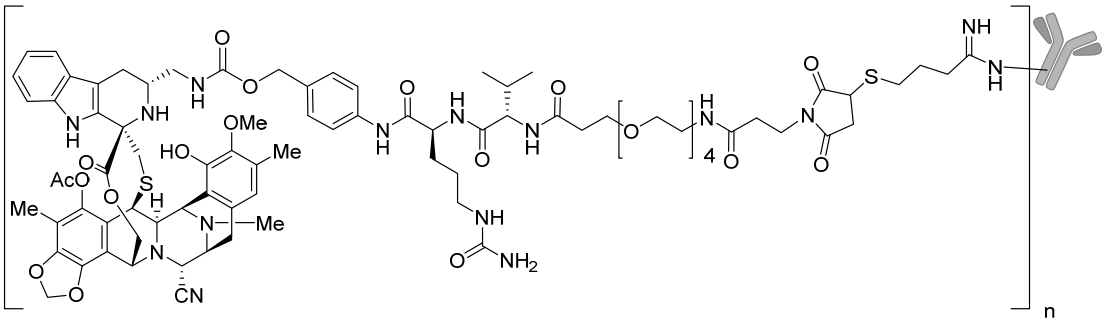
or

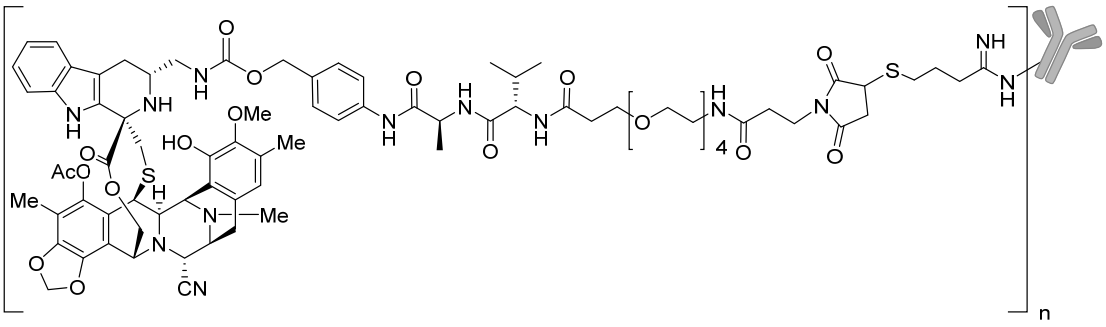
a drug conjugate, that is an antibody drug conjugate, selected from the group consisting of:

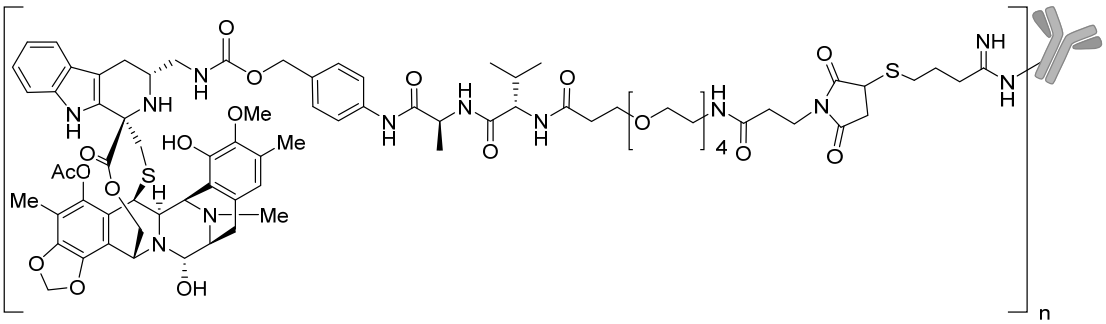


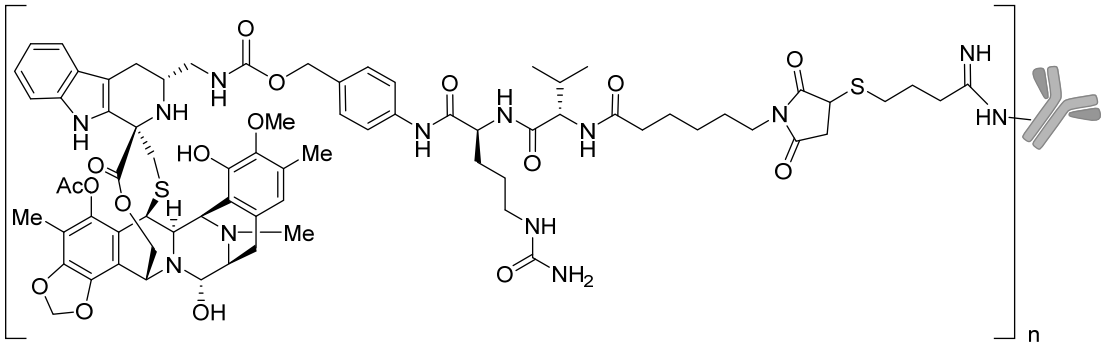
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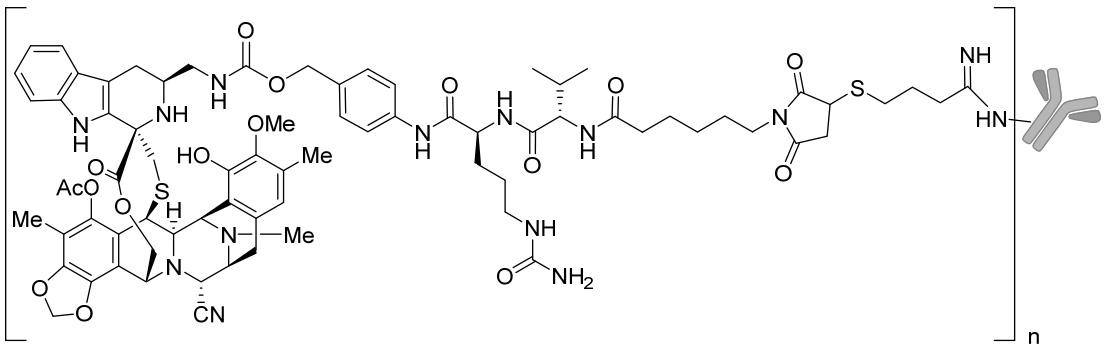


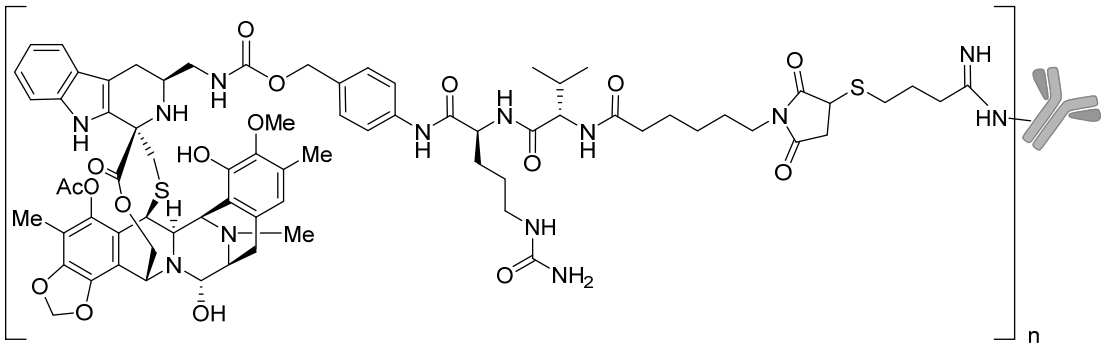


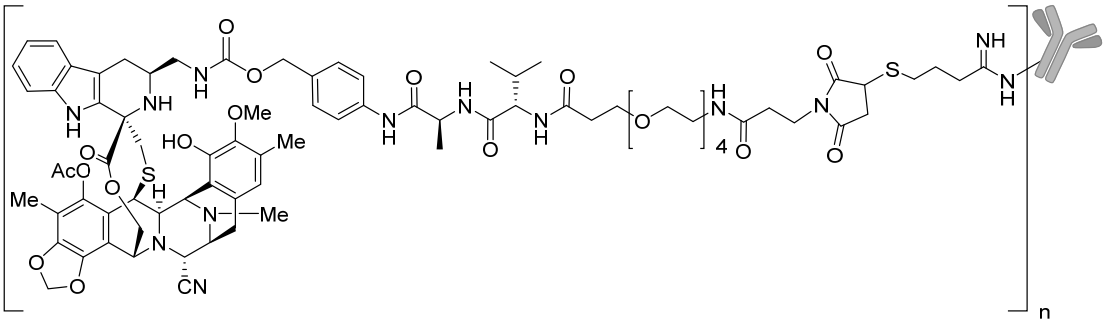


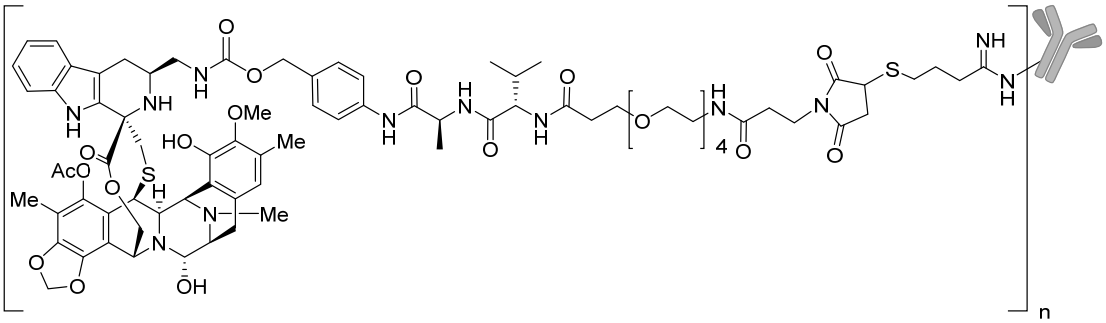


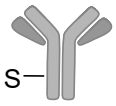


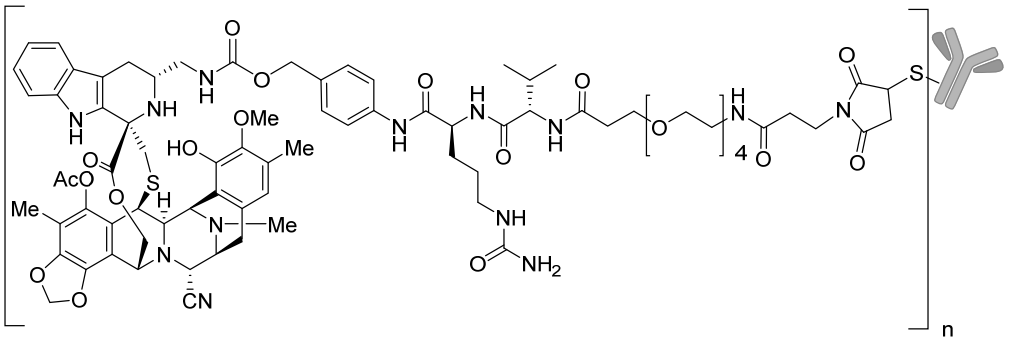


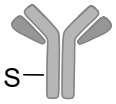






wherein n is from 2 to 6, more preferably 3, 4, or 5 and each  and  is independently selected from Brentuximab, Gemtuzumab, Inozutumab, Rovalpituzumab, Trastuzumab, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD13 antibody and an anti-CD 30 antibody, or an antigen-binding fragment or an immunologically active portion thereof, and more preferably its is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof, particularly Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof; or a drug conjugate of formula



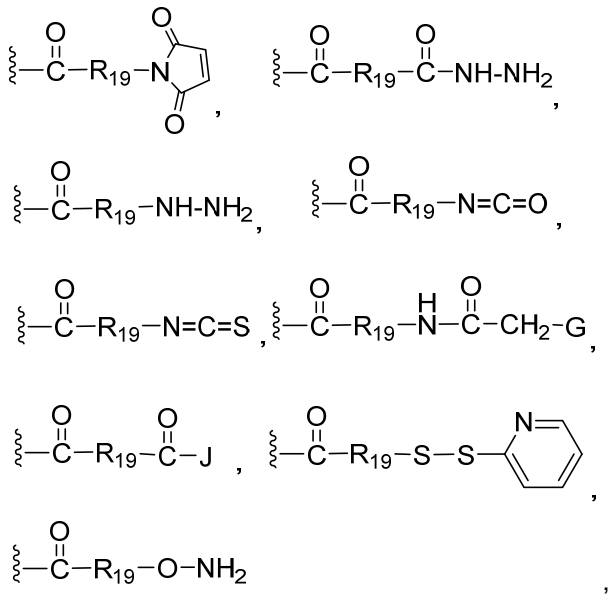
wherein n is from 2 to 6, more preferably 3, 4, or 5 and  is an anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof.

17. A drug conjugate according to claim 16, wherein the moiety Ab comprising at least one antigen binding site is selected from Trastuzumab and anti-CD13 antibody or an antigen-binding fragment or an immunologically active portion thereof;
or

wherein the moiety Ab comprising at least one antigen binding site is selected from Trastuzumab or an antigen-binding fragment or an immunologically active portion thereof.

18. A drug conjugate according to any one of claims 1 to 17 that is an antibody drug conjugate in isolated or purified form.

19. A compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1, wherein:
L_1 is a linker selected from the group of formulas consisting of:



wherein each of the the wavy lines indicates the point of covalent attachment to $(T)_g$ if any, or $(AA)_w$ if any, or to $(X)_b$ if any or to D;

G is selected from halo, -*O*-mesyl and -*O*-tosyl;

J is selected from halo, hydroxy, -*N*-succinimidoxy, -*O*-(4-nitrophenyl), -*O*-pentafluorophenyl, -*O*-tetrafluorophenyl and -O-C(O)-OR_{20};

R_{19} is selected from -C_1-C_{12} alkylene-, -C_3-C_8 carbocyclo, -O-(C_1-C_{12} alkylene), -C_6-C_{18} arylene in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-C_6-C_{18} arylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_6-C_{18} arylene-C_1-C_{12} alkylene- wherein the arylene group is in one or more rings which may optionally be substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_3-C_8 carbocyclo)-, -(C_3-C_8 carbocyclo)-C_1-C_{12} alkylene-, -C_5-C_{14} heterocyclo- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -C_1-C_{12} alkylene-(C_5-C_{14} heterocyclo)- wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -(C_5-C_{14} heterocyclo)-C_1-C_{12} alkylene-, wherein said heterocyclo group may be a saturated or unsaturated group having one or more rings and comprising at least one oxygen, nitrogen or sulphur atom in said ring(s), said group optionally being substituted with one or more substituents R_x, -$(OCH_2CH_2)_r$- and -CH_2-$(OCH_2CH_2)_r$-, wherein each of the above alkylene substituents whether alone or attached to another moiety the carbon chain may optionally be substituted by one or more substituents R_x;

R_{20} is a C_1-C_{12} alkyl or an aryl group having from 6 to 18 carbon atoms in one or more aromatic rings, said aryl groups optionally being substituted with one or more substituents R_x;

r is an integer ranging from 1-10;

b is an integer of 0 or 1;

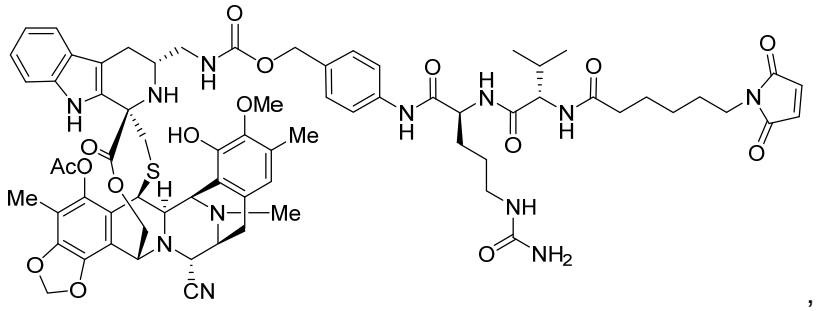
g is an integer of 0 or 1;

w is an integer ranging from 0 to 12;

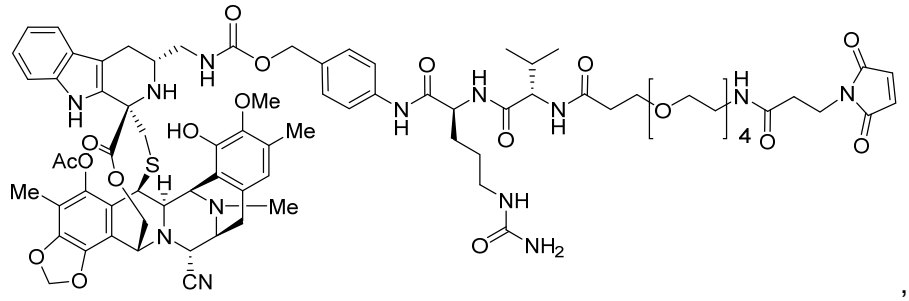
each of D, R_x, X, T, and AA is as defined in any one of claims 1 to 18;

optionally

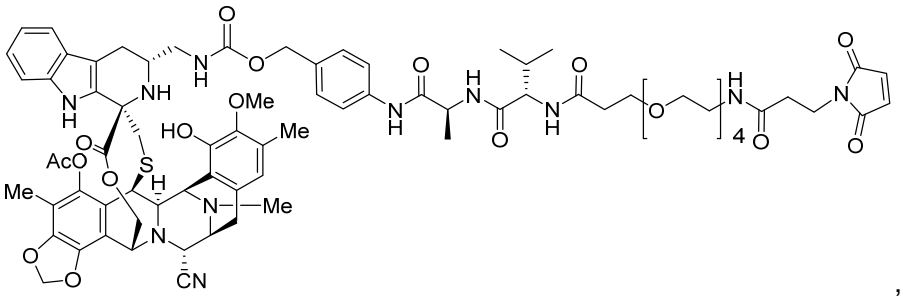
wherein the compound of formula D-X-$(AA)_w$-$(T)_g$-L_1 is selected from:



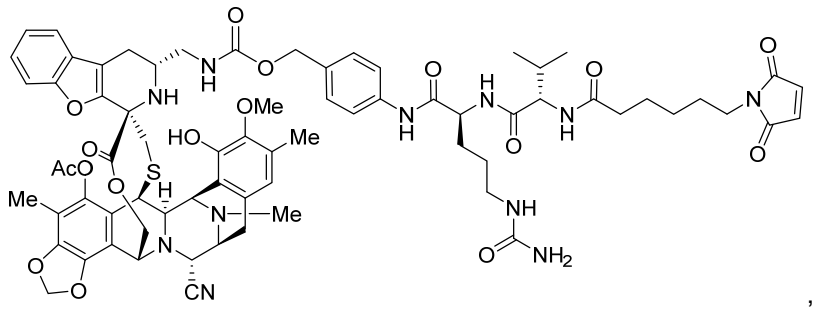
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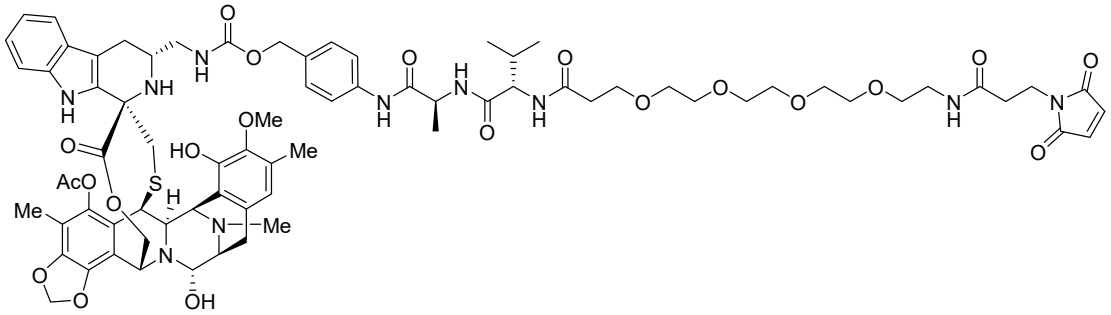


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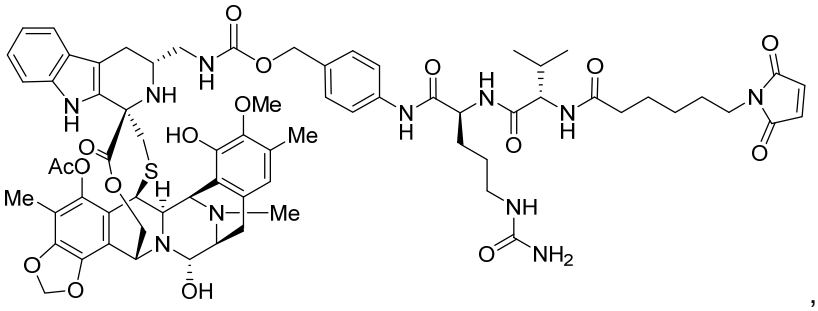


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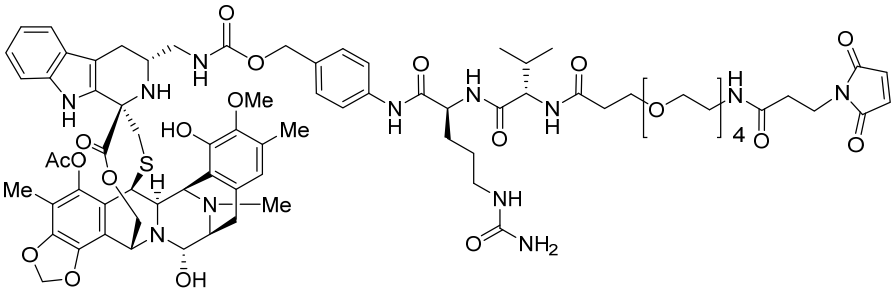
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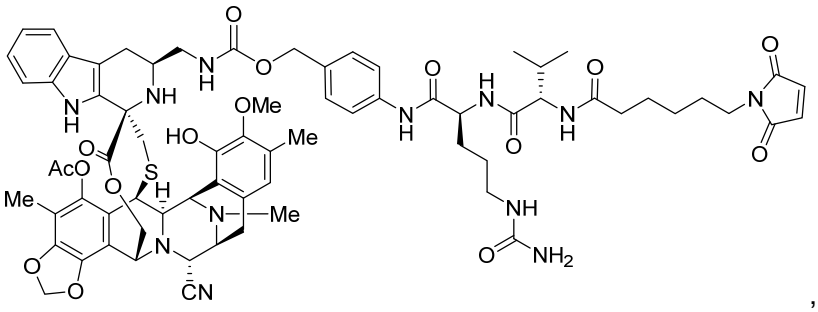
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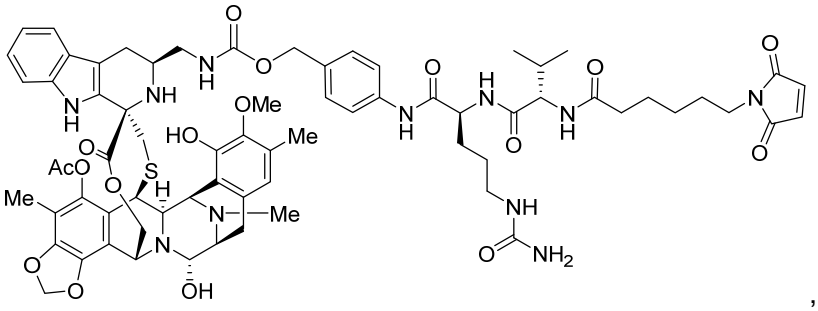
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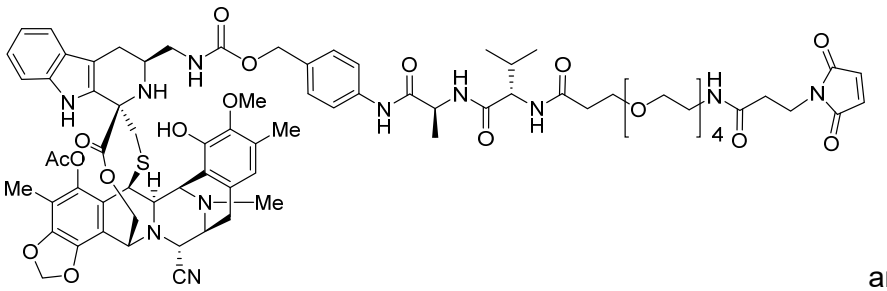
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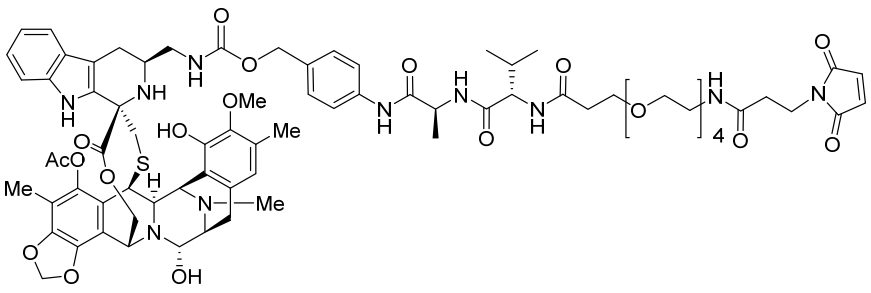
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and



20. A compound of formula D-$(X)_b$-$(AA)_w$-$(T)_g$-L_1, wherein each of D, X, AA, T, L_1, b, g and w are as defined in any one of claims 1 to 17.

21. A drug conjugate according to any one of claims 1 to 18, or a compound according to any one of claims 19 to 20, wherein b+g+w is not 0;

or

wherein b+w is not 0;

or

wherein when w is not 0, then b is 1.

22. Use of a drug moiety as described in any one of claims 1 to 7, as a payload in an antibody drug conjugate.

23. Use of a drug moiety as described in any one of claims 1 to 7, in the manufacture of a antibody drug conjugate.

24. A drug conjugate according to any one of claims 1 to 18, for use as a medicament.

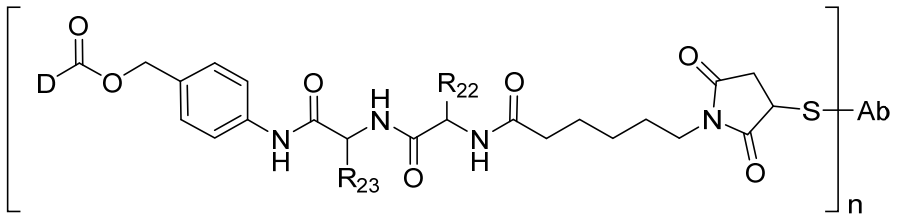
25. A pharmaceutical composition comprising a drug conjugate according to any one of claims 1 to 18 and a pharmaceutically acceptable carrier.

26. A kit comprising a therapeutically effective amount of a drug conjugate according to any one of claims 1 to 18 and a pharmaceutically acceptable carrier.

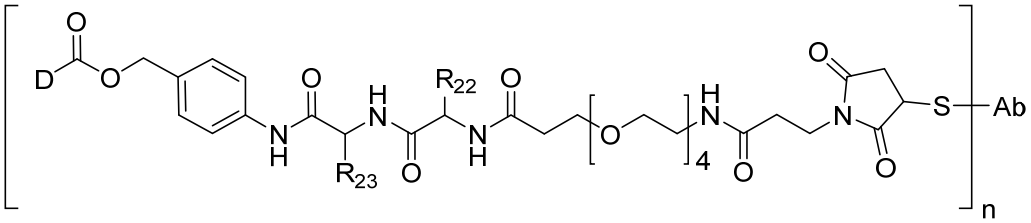
27. The kit according to claim 26 for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, colorectal cancer, breast cancer, pancreas carcinoma, kidney cancer, leukaemia, multiple myeloma, lymphoma, gastric and ovarian cancer.

28. The drug conjugate according to any one of claims 1 to 15, wherein n is in the range of from 1-12, 1-8, 3-8, 3-6, 3-5, 1, 2, 3, 4, 5 or 6; preferably 3, 4 or 5 or more preferably 4.

29. A process for the preparation of a drug antibody conjugate according to any one of claims 1 to 18, comprising conjugating a moiety Ab comprising at least one antigen binding site and a drug D, Ab and D being as defined in any one of claims 1 to 18;
optionally wherein the preparation of a drug antibody conjugate of formula (G) or (G'):



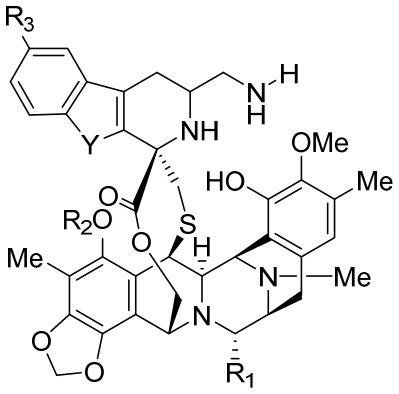
(G)



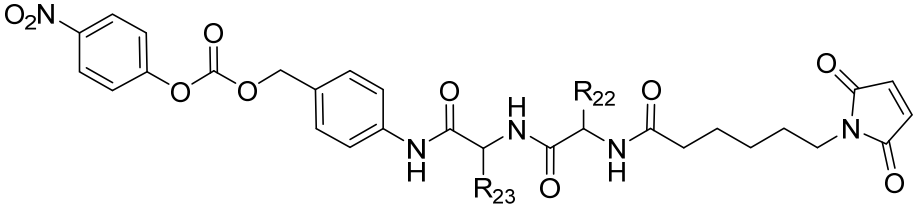
(G')

comprising the following steps:

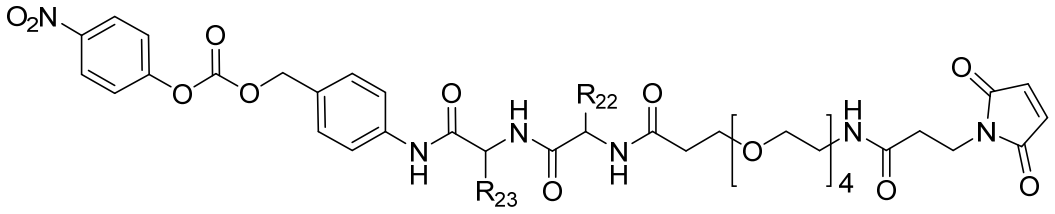
(i) reacting a drug D-H of formula (IH)-H:



wherein the substituents in the definitions of (IH)-H are as defined in any one of claims 1 to 18, with a compound of formula (D') or (E):

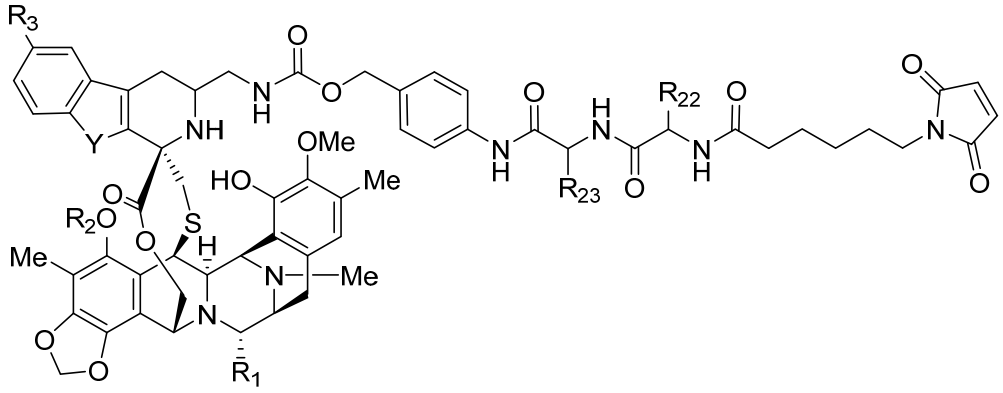


(D')

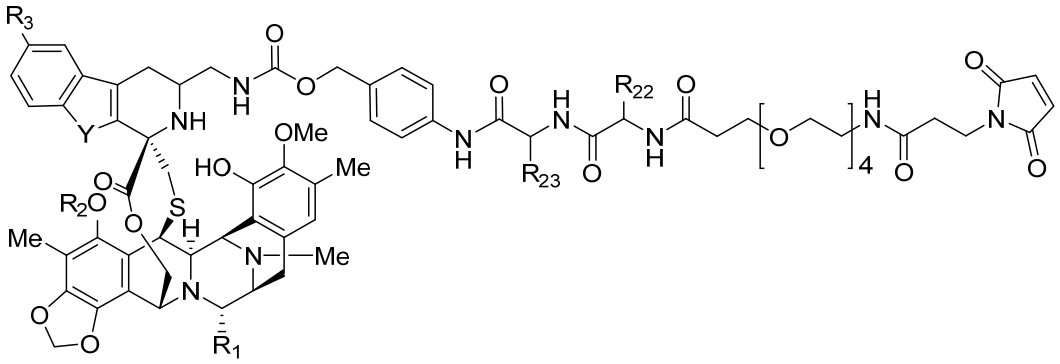


(E)

to give a compound of formula (F) or (F'),respectively:

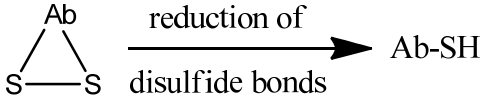


(F)



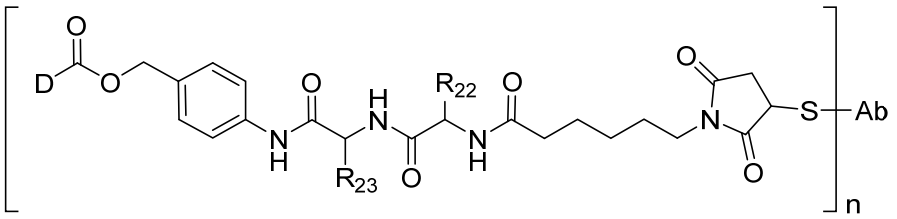
(F')

(ii)partial reduction of one or more disulfide bonds in the antibody to be conjugated to give a reduced antibody Ab-SH having free thiol groups:

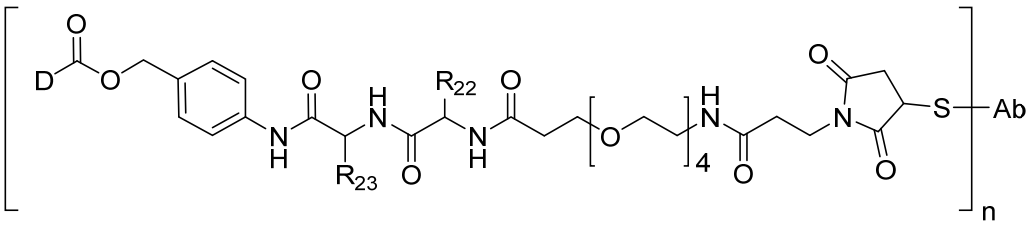


; and

(iii) reaction of the partially reduced antibody Ab-SH having free thiol groups with the compound of formula (F) or (F') produced in step (i) to give the desired drug antibody conjugate of formula (G) or (G') respectively:



(G)



(G')

5