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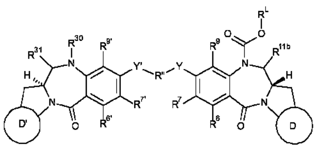
(54) Titre : CONJUGUES DE PYRROLOBENZODIAZEPINE
(54) Title: PYRROLOBENZODIAZEPINE CONJUGATES



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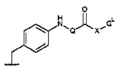

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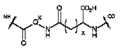
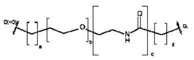


(A)



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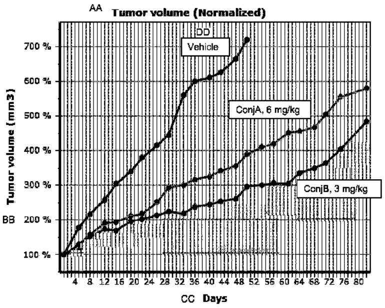

Fig. 1

AA
BB
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DD

(57) Abrégé/Abstract:
A compound of formula (I) and salts and solvates thereof, wherein $R^L$ is a linker for connection to a cell binding agent, which is formula (IIa) wherein Q is a tripeptide residue of formula (A), where x is 1 or 2, $-C(=O)-Q^X-NH-$ is a dipeptide residue; X is: formula (B), where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5; and $G^L$ is a linker for connecting to a Ligand Unit.

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| 1817110.8 | 19 October 2018 (19.10.2018) | GB |
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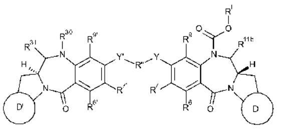
## (54) Title: PYRROLOBENZODIAZEPINE CONJUGATES

**(57) Abstract:** A compound of formula (I) and salts and solvates thereof, wherein $R^L$ is a linker for connection to a cell binding agent, which is formula (IIa) wherein Q is a tripeptide residue of formula (A), where x is 1 or 2, -C(=O)-$Q^X$-NH- is a dipeptide residue; X is: formula (B), where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5; and $G^L$ is a linker for connecting to a Ligand Unit.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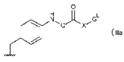
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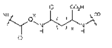
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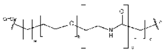
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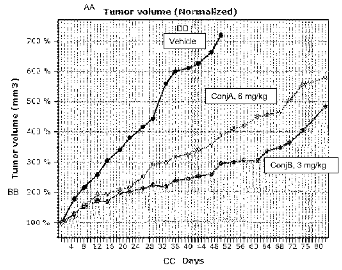

Fig. 1

AA
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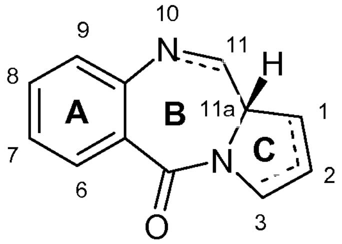
# PYRROLOBENZODIAZEPINE CONJUGATES

The present invention relates to conjugates comprising pyrrolobenzodiazepines and related dimers (PBDs), and the precursor drug linkers used to make such conjugates.

## Background to the invention

Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5793-5795 (1965); Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5791-5793 (1965)). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston, *et al.*, *Chem. Rev.* **1994**, 433-465 (1994)). Family members include abbeymycin (Hochlowski, *et al.*, *J. Antibiotics*, **40**, 145-148 (1987)), chicamycin (Konishi, *et al.*, *J. Antibiotics,* **37**, 200-206 (1984)), DC-81 (Japanese Patent 58-180 487; Thurston, *et al.*, *Chem. Brit.*, **26**, 767-772 (1990); Bose, *et al.*, *Tetrahedron*, **48**, 751-758 (1992)), mazethramycin (Kuminoto, *et al.*, *J. Antibiotics*, **33**, 665-667 (1980)), neothramycins A and B (Takeuchi, *et al.*, *J. Antibiotics*, **29**, 93-96 (1976)), porothramycin (Tsunakawa, *et al.*, *J. Antibiotics*, **41**, 1366-1373 (1988)), prothracarcin (Shimizu, *et al*, *J. Antibiotics*, **29**, 2492-2503 (1982); Langley and Thurston, *J. Org. Chem.*, **52**, 91-97 (1987)), sibanomicin (DC-102)(Hara, *et al.*, *J. Antibiotics*, **41**, 702-704 (1988); Itoh, *et al.*, *J. Antibiotics*, **41**, 1281-1284 (1988)), sibiromycin (Leber, *et al.*, *J. Am. Chem. Soc.*, **110**, 2992-2993 (1988)) and tomamycin (Arima, *et al.*, *J. Antibiotics*, **25**, 437-444 (1972)). PBDs are of the general structure:

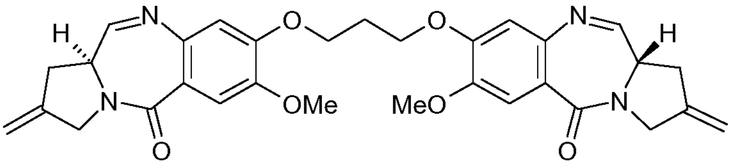


They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (*S*)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In

*Antibiotics III*. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem. Res.*, **19**, 230-237 (1986)). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

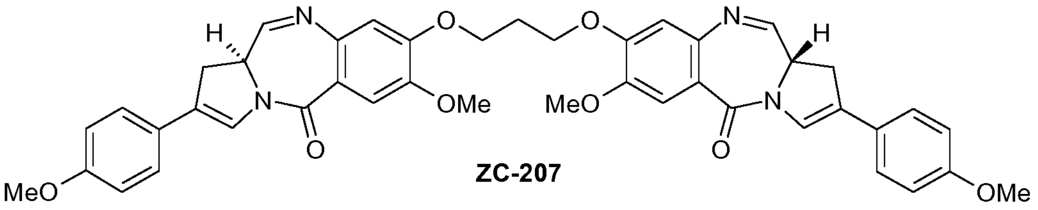
It has been previously disclosed that the biological activity of these molecules can be potentiated by joining two PBD units together through their C8/C'-hydroxyl functionalities via a flexible alkylene linker (Bose, D.S., *et al.*, *J. Am. Chem. Soc.*, **114**, 4939-4941 (1992); Thurston, D.E., *et al.*, *J. Org. Chem.*, **61**, 8141-8147 (1996)). The PBD dimers are thought to form sequence-selective DNA lesions such as the palindromic 5'-Pu-GATC-Py-3' interstrand cross-link (Smellie, M., *et al.*, *Biochemistry*, **42**, 8232-8239 (2003); Martin, C., *et al.*, *Biochemistry*, **44**, 4135-4147) which is thought to be mainly responsible for their biological activity.

One example of a PBD dimer is SG2000 (SJG-136):



(Gregson, S., *et al.*, *J. Med. Chem.*, **44**, 737-748 (2001); Alley, M.C., *et al.*, *Cancer Research*, **64**, 6700-6706 (2004); Hartley, J.A., *et al.*, *Cancer Research*, **64**, 6693-6699 (2004)) which has been involved in clinical trials as a standalone agent, for example, NCT02034227 investigating its use in treating Acute Myeloid Leukemia and Chronic Lymphocytic Leukemia (see: https://www.clinicaltrials.gov/ct2/show/NCT02034227).

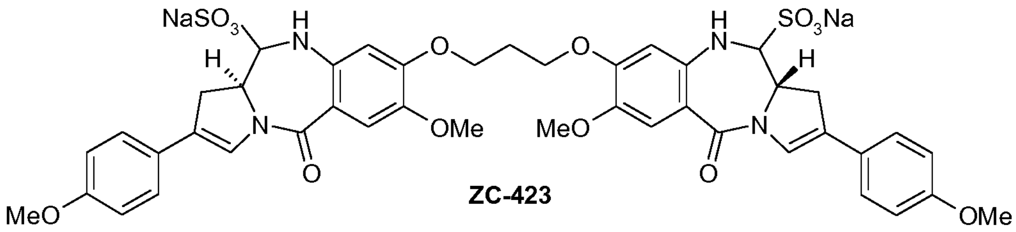
Dimeric PBD compounds bearing C2 aryl substituents, such as SG2202 (ZC-207), are disclosed in WO 2005/085251:



**ZC-207**

,

and in WO2006/111759, bisulphites of such PBD compounds, for example SG2285 (ZC-423):



**ZC-423**

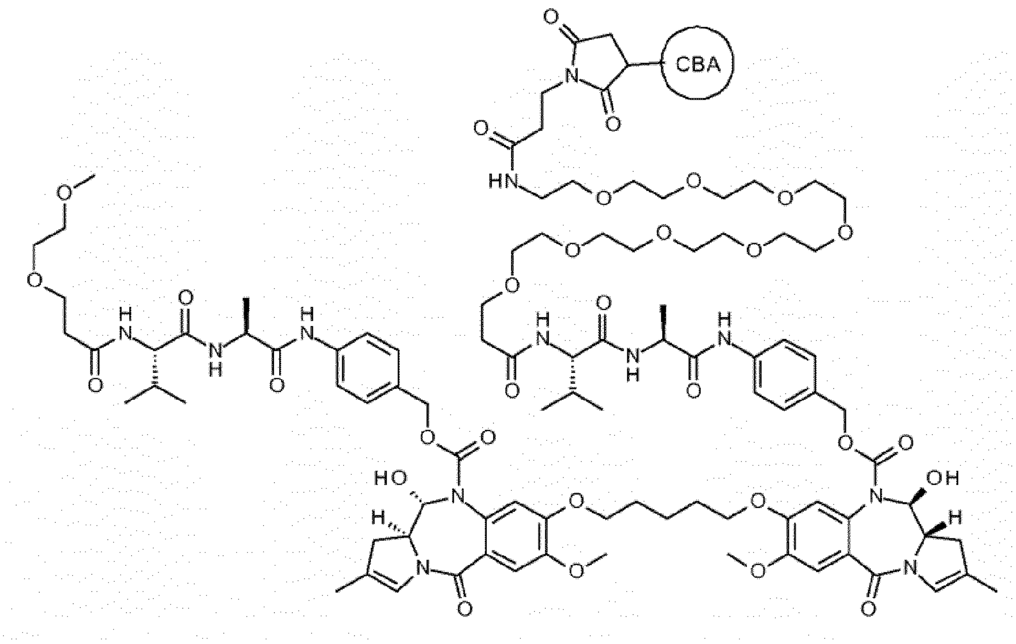
These compounds have been shown to be highly useful cytotoxic agents (Howard, P.W., *et al.*, *Bioorg. Med. Chem.* (2009), doi: 10.1016/j.bmcl.2009.09.012).

WO 2007/085930 describes the preparation of dimer PBD compounds having linker groups for connection to a cell binding agent, such as an antibody. The linker is present in the bridge linking the monomer PBD units of the dimer.

Dimer PBD compounds having linker groups for connection to a cell binding agent, such as an antibody, are described in WO 2011/130598. The linker in these compounds is attached to one of the available N10 positions, and are generally cleaved by action of an enzyme on the linker group. If the non-bound N10 position is protected with a capping group, the capping groups exemplified have the same cleavage trigger as the linker to the antibody.

WO 2014/057074 describes two specific PBD dimer conjugates bound via the N10 position on one monomer, the other PBD monomer being in imine form.

WO 2015/052322 describes a specific PBD dimer conjugate bound via the N10 position on one monomer, the other PBD monomer being in imine form. It also describes a specific PBD dimer conjugate bound via the N10 position on one monomer, the other PBD monomer having a capping group with the same cleavage trigger as the linker to the antibody:



ConjC

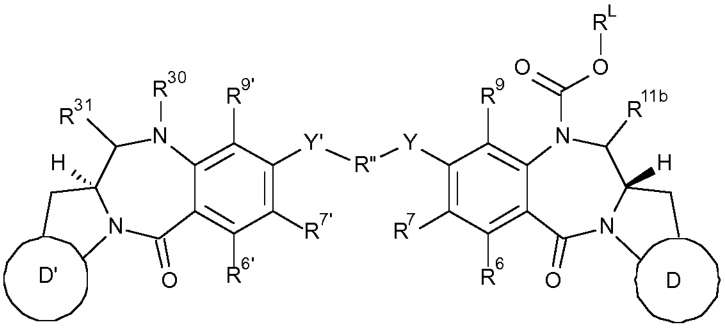
The linker most commonly used in those PBD-ADCs undergoing clinical trial comprises the Val-Ala dipeptide group which is cathepsin cleavable (Beck, A., *et al.*, *Nature Reviews Drug Discovery*, **16**, 315–337 (2017) – doi: 10.1038/nrd.2016.268).

**Disclosure of the invention**

The present invention provides PBD and related dimer conjugates where the conjugate linker comprises a tripeptide, where one of the amino acid groups has a carboxy side group.

The present invention also provides PBD and related dimer drug linkers, suitable for conjugating to a ligand unit, where the group which is intended to form the conjugate linker comprises a tripeptide, where one of the amino acid groups has a carboxy side group.

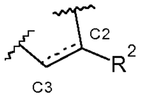
A first aspect of the present invention comprises a compound with the formula **I**:



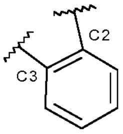
I

and salts and solvates thereof, wherein:

D represents either group D1 or D2:



D1



D2

;

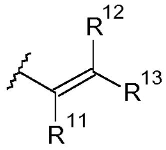
the dotted line indicates the optional presence of a double bond between C2 and C3;

when there is a double bond present between C2 and C3, $R^2$ is selected from the group consisting of:

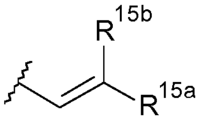
(ia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(ib) $C_{1-5}$ saturated aliphatic alkyl;

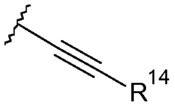
(ic) $C_{3-6}$ saturated cycloalkyl;

(id) 

, wherein each of $R^{11}$, $R^{12}$ and $R^{13}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^2$ group is no more than 5;

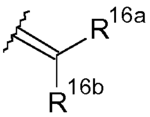
(ie) 

, wherein one of $R^{15a}$ and $R^{15b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(if) 

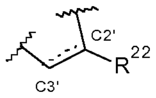
, where $R^{14}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond present between C2 and C3,

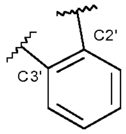
$R^2$ is selected from H, OH, F, diF and 

, where $R^{16a}$ and $R^{16b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{16a}$ and $R^{16b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

D' represents either group D'1 or D'2:



D'1



D'2

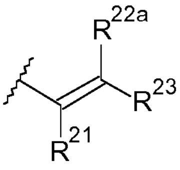
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

when there is a double bond present between C2' and C3', $R^{22}$ is selected from the group consisting of:

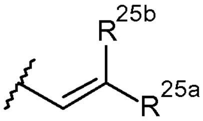
(iia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(iib) $C_{1-5}$ saturated aliphatic alkyl;

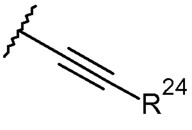
(iic) $C_{3-6}$ saturated cycloalkyl;

(iid) 

, wherein each of $R^{21}$, $R^{22a}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{22}$ group is no more than 5;

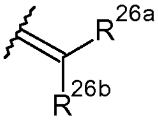
(iie) 

, wherein one of $R^{25a}$ and $R^{25b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(iif) 

, where $R^{24}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond present between C2' and C3',

$R^{22}$ is selected from H, OH, F, diF and 

, where $R^{26a}$ and $R^{26b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{26a}$ and $R^{26b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

$R^6$ and $R^9$ are independently selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

where R and R' are independently selected from optionally substituted $C_{1-12}$ alkyl, $C_{3-20}$ heterocyclyl and $C_{5-20}$ aryl groups;

$R^7$ is selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

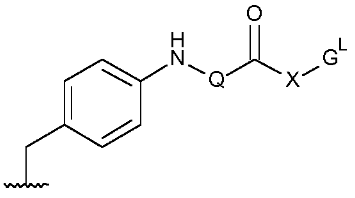
R" is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, $NR^{N2}$ (where $R^{N2}$ is H or $C_{1-4}$ alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as $R^6$, $R^7$ and $R^9$ respectively;

$R^{11b}$ is selected from OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl; and

$R^L$ is a linker for connection to a cell binding agent, which is

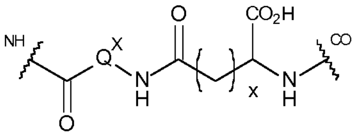


IIIa

,

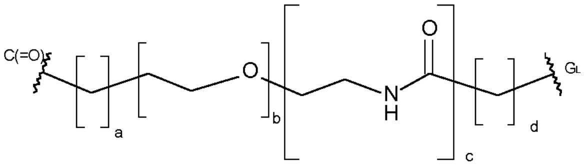
wherein

Q is a tripeptide residue of formula:



, where x is 1 or 2, and -C(=O)-$Q^X$-NH- is a dipeptide residue;

X is:



,

where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5;

$G^L$ is a linker for connecting to a Ligand Unit;

either

(a) $R^{30}$ is H, and $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1-4}$ alkyl; or

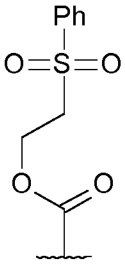
(b) $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) $R^{30}$ is H and $R^{31}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation; or

(d) $R^{30}$ is H and $R^{31}$ is H or =O; or

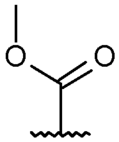
(e) $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1-4}$ alkyl and $R^{30}$ is selected from:

(e-i)



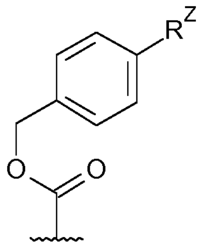
;

(e-ii)



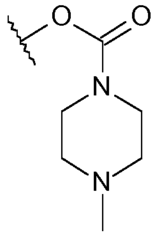
;

(e-iii)



, where $R^Z$ is selected from:

(z-i)



;

(z-ii) OC(=O)$CH_3$;

(z-iii) $NO_2$;

(z-iv) OMe;

(z-v) glucoronide;

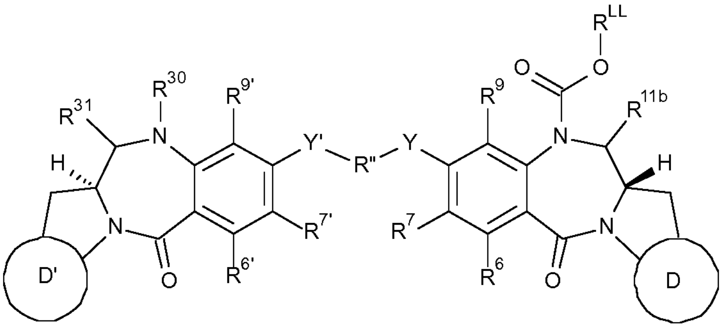
(z-vi) NH-C(=O)-X$_1$-NHC(=O)X$_2$-NH-C(=O)-R$^{ZC}$, where -C(=O)-X$_1$-NH- and -C(=O)-X$_2$-NH- represent natural amino acid residues and R$^{ZC}$ is selected from Me, OMe, CH$_2$CH$_2$OMe, and (CH$_2$CH$_2$O)$_2$Me.

In alternative embodiments, R$^7$ and R$^{7'}$ may together form a group which is: (i) -O-(CH$_2$)$_n$-O-, where n is from 7 to 16; or (ii) -O-(CH$_2$CH$_2$O)$_m$-, where m is 2 to 5.

A second aspect of the present invention provides Conjugates of formula II:

$$L - (D^L)_p \qquad (II)$$

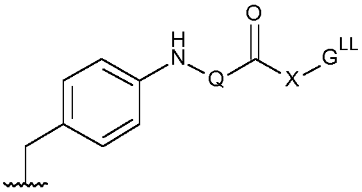
wherein L is a Ligand unit (i.e., a targeting agent), D$^L$ is a Drug Linker unit of formula I':



I'

wherein D, R$^2$, R$^6$, R$^7$, R$^9$, R$^{11b}$, Y, R", Y', D', R$^{6'}$, R$^{7'}$, R$^{9'}$,R$^{22}$, R$^{30}$ and R$^{31}$ (including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, and the NO$_2$ group) are as defined in the first aspect of the invention;

R$^{LL}$ is a linker for connection to a cell binding agent, which is



IIIa'

,

where Q and X are as defined in the first aspect and G$^{LL}$ is a linker connected to a Ligand Unit;

wherein p is an integer of from 1 to 20.

The Ligand unit, described more fully below, is a targeting agent that binds to a target moiety. The Ligand unit can, for example, specifically bind to a cell component (a Cell Binding Agent) or to other target molecules of interest. The Ligand unit can be, for example, a protein, polypeptide or peptide, such as an antibody, an antigen-binding fragment of an antibody, or other binding agent, such as an Fc fusion protein.

A third aspect of the present invention provides the use of a conjugate of the second aspect of the invention in the manufacture of a medicament for treating a proliferative disease. The third aspect also provides a conjugate of the second aspect of the invention for use in the treatment of a proliferative disease. The third aspect also provides a method of treating a proliferative disease comprising administering a therapeutically effective amount of a conjugate of the second aspect of the invention to a patient in need thereof.

One of ordinary skill in the art is readily able to determine whether or not a candidate conjugate treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

A fourth aspect of the present invention provides the synthesis of a conjugate of the second aspect of the invention comprising conjugating a compound (drug linker) of the first aspect of the invention with a Ligand Unit.

## Definitions

### *Substituents*

The phrase “optionally substituted” as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

Unless otherwise specified, the term “substituted” as used herein, pertains to a parent group which bears one or more substituents. The term “substituent” is used herein in the conventional sense and refers to a chemical moiety which is covalently attached to, or if appropriate, fused to, a parent group. A wide variety of substituents are well known, and methods for their formation and introduction into a variety of parent groups are also well known.

Examples of substituents are described in more detail below.

$C_{1-12}$ alkyl: The term “$C_{1-12}$ alkyl” as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 12 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). The term “$C_{1-4}$ alkyl” as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 4 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term “alkyl” includes the sub-classes alkenyl, alkynyl, cycloalkyl, etc., discussed below.

Examples of saturated alkyl groups include, but are not limited to, methyl ($C_1$), ethyl ($C_2$), propyl ($C_3$), butyl ($C_4$), pentyl ($C_5$), hexyl ($C_6$) and heptyl ($C_7$).

Examples of saturated linear alkyl groups include, but are not limited to, methyl ($C_1$), ethyl ($C_2$), n-propyl ($C_3$), n-butyl ($C_4$), n-pentyl (amyl) ($C_5$), n-hexyl ($C_6$) and n-heptyl ($C_7$).

Examples of saturated branched alkyl groups include iso-propyl ($C_3$), iso-butyl ($C_4$), sec-butyl ($C_4$), tert-butyl ($C_4$), iso-pentyl ($C_5$), and neo-pentyl ($C_5$).

$C_{2-12}$ Alkenyl: The term “$C_{2-12}$ alkenyl” as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds.

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, -CH=CH$_2$), 1-propenyl (-CH=CH-CH$_3$), 2-propenyl (allyl, -CH-CH=CH$_2$), isopropenyl (1-methylvinyl, -C(CH$_3$)=CH$_2$), butenyl ($C_4$), pentenyl ($C_5$), and hexenyl ($C_6$).

$C_{2-12}$ alkynyl: The term “$C_{2-12}$ alkynyl” as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (-C≡CH) and 2-propynyl (propargyl, -CH$_2$-C≡CH).

$C_{3-12}$ cycloalkyl: The term “$C_{3-12}$ cycloalkyl” as used herein, pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a cyclic hydrocarbon (carbocyclic) compound, which moiety has from 3 to 7 carbon atoms, including from 3 to 7 ring atoms.

Examples of cycloalkyl groups include, but are not limited to, those derived from:

saturated monocyclic hydrocarbon compounds:

cyclopropane ($C_3$), cyclobutane ($C_4$), cyclopentane ($C_5$), cyclohexane ($C_6$), cycloheptane ($C_7$), methylcyclopropane ($C_4$), dimethylcyclopropane ($C_5$), methylcyclobutane ($C_5$), dimethylcyclobutane ($C_6$), methylcyclopentane ($C_6$), dimethylcyclopentane ($C_7$) and methylcyclohexane ($C_7$);

unsaturated monocyclic hydrocarbon compounds:
cyclopropene ($C_3$), cyclobutene ($C_4$), cyclopentene ($C_5$), cyclohexene ($C_6$), methylcyclopropene ($C_4$), dimethylcyclopropene ($C_5$), methylcyclobutene ($C_5$), dimethylcyclobutene ($C_6$), methylcyclopentene ($C_6$), dimethylcyclopentene ($C_7$) and methylcyclohexene ($C_7$); and

saturated polycyclic hydrocarbon compounds:
norcarane ($C_7$), norpinane ($C_7$), norbornane ($C_7$).

$C_{3-20}$ heterocyclyl: The term "$C_{3-20}$ heterocyclyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

In this context, the prefixes (e.g. $C_{3-20}$, $C_{3-7}$, $C_{5-6}$, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "$C_{5-6}$heterocyclyl", as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms.

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:

$N_1$: aziridine ($C_3$), azetidine ($C_4$), pyrrolidine (tetrahydropyrrole) ($C_5$), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) ($C_5$), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) ($C_5$), piperidine ($C_6$), dihydropyridine ($C_6$), tetrahydropyridine ($C_6$), azepine ($C_7$);

$O_1$: oxirane ($C_3$), oxetane ($C_4$), oxolane (tetrahydrofuran) ($C_5$), oxole (dihydrofuran) ($C_5$), oxane (tetrahydropyran) ($C_6$), dihydropyran ($C_6$), pyran ($C_6$), oxepin ($C_7$);

$S_1$: thiirane ($C_3$), thietane ($C_4$), thiolane (tetrahydrothiophene) ($C_5$), thiane (tetrahydrothiopyran) ($C_6$), thiepane ($C_7$);

$O_2$: dioxolane ($C_5$), dioxane ($C_6$), and dioxepane ($C_7$);

$O_3$: trioxane ($C_6$);

$N_2$: imidazolidine ($C_5$), pyrazolidine (diazolidine) ($C_5$), imidazoline ($C_5$), pyrazoline (dihydropyrazole) ($C_5$), piperazine ($C_6$);

$N_1O_1$: tetrahydrooxazole ($C_5$), dihydrooxazole ($C_5$), tetrahydroisoxazole ($C_5$), dihydroisoxazole ($C_5$), morpholine ($C_6$), tetrahydrooxazine ($C_6$), dihydrooxazine ($C_6$), oxazine ($C_6$);
$N_1S_1$: thiazoline ($C_5$), thiazolidine ($C_5$), thiomorpholine ($C_6$);
$N_2O_1$: oxadiazine ($C_6$);
$O_1S_1$: oxathiole ($C_5$) and oxathiane (thioxane) ($C_6$); and,
$N_1O_1S_1$: oxathiazine ($C_6$).

Examples of substituted monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses ($C_5$), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranse, and pyranoses ($C_6$), such as allopyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

$C_{5-20}$ aryl: The term “$C_{5-20}$ aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms. The term “$C_{5-7}$ aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 5 to 7 ring atoms and the term “$C_{5-10}$ aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 5 to 10 ring atoms. Preferably, each ring has from 5 to 7 ring atoms.

In this context, the prefixes (e.g. $C_{3-20}$, $C_{5-7}$, $C_{5-6}$, $C_{5-10}$, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term “$C_{5-6}$ aryl” as used herein, pertains to an aryl group having 5 or 6 ring atoms.

The ring atoms may be all carbon atoms, as in “carboaryl groups”.
Examples of carboaryl groups include, but are not limited to, those derived from benzene (i.e. phenyl) ($C_6$), naphthalene ($C_{10}$), azulene ($C_{10}$), anthracene ($C_{14}$), phenanthrene ($C_{14}$), naphthacene ($C_{18}$), and pyrene ($C_{16}$).

Examples of aryl groups which comprise fused rings, at least one of which is an aromatic ring, include, but are not limited to, groups derived from indane (e.g. 2,3-dihydro-1H-indene) ($C_9$), indene ($C_9$), isoindene ($C_9$), tetraline (1,2,3,4-tetrahydronaphthalene ($C_{10}$), acenaphthene ($C_{12}$), fluorene ($C_{13}$), phenalene ($C_{13}$), acephenanthrene ($C_{15}$), and aceanthrene ($C_{16}$).

Alternatively, the ring atoms may include one or more heteroatoms, as in "heteroaryl groups". Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:
$N_1$: pyrrole (azole) ($C_5$), pyridine (azine) ($C_6$);
$O_1$: furan (oxole) ($C_5$);
$S_1$: thiophene (thiole) ($C_5$);
$N_1O_1$: oxazole ($C_5$), isoxazole ($C_5$), isoxazine ($C_6$);
$N_2O_1$: oxadiazole (furazan) ($C_5$);
$N_3O_1$: oxatriazole ($C_5$);
$N_1S_1$: thiazole ($C_5$), isothiazole ($C_5$);
$N_2$: imidazole (1,3-diazole) ($C_5$), pyrazole (1,2-diazole) ($C_5$), pyridazine (1,2-diazine) ($C_6$), pyrimidine (1,3-diazine) ($C_6$) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) ($C_6$);
$N_3$: triazole ($C_5$), triazine ($C_6$); and,
$N_4$: tetrazole ($C_5$).

Examples of heteroaryl which comprise fused rings, include, but are not limited to:

$C_9$ (with 2 fused rings) derived from benzofuran ($O_1$), isobenzofuran ($O_1$), indole ($N_1$), isoindole ($N_1$), indolizine ($N_1$), indoline ($N_1$), isoindoline ($N_1$), purine ($N_4$) (e.g., adenine, guanine), benzimidazole ($N_2$), indazole ($N_2$), benzoxazole ($N_1O_1$), benzisoxazole ($N_1O_1$), benzodioxole ($O_2$), benzofurazan ($N_2O_1$), benzotriazole ($N_3$), benzothiofuran ($S_1$), benzothiazole ($N_1S_1$), benzothiadiazole ($N_2S$);

$C_{10}$ (with 2 fused rings) derived from chromene ($O_1$), isochromene ($O_1$), chroman ($O_1$), isochroman ($O_1$), benzodioxan ($O_2$), quinoline ($N_1$), isoquinoline ($N_1$), quinolizine ($N_1$), benzoxazine ($N_1O_1$), benzodiazine ($N_2$), pyridopyridine ($N_2$), quinoxaline ($N_2$), quinazoline ($N_2$), cinnoline ($N_2$), phthalazine ($N_2$), naphthyridine ($N_2$), pteridine ($N_4$);

$C_{11}$ (with 2 fused rings) derived from benzodiazepine ($N_2$);

$C_{13}$ (with 3 fused rings) derived from carbazole ($N_1$), dibenzofuran ($O_1$), dibenzothiophene ($S_1$), carboline ($N_2$), perimidine ($N_2$), pyridoindole ($N_2$); and,

$C_{14}$ (with 3 fused rings) derived from acridine ($N_1$), xanthene ($O_1$), thioxanthene ($S_1$), oxanthrene ($O_2$), phenoxathiin ($O_1S_1$), phenazine ($N_2$), phenoxazine ($N_1O_1$), phenothiazine ($N_1S_1$), thianthrene ($S_2$), phenanthridine ($N_1$), phenanthroline ($N_2$), phenazine ($N_2$).

The above groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a $C_{1-7}$ alkyl group (also referred to as a $C_{1-7}$ alkoxy group, discussed below), a $C_{3-20}$ heterocyclyl group (also referred to as a $C_{3-20}$ heterocyclyloxy group), or a $C_{5-20}$ aryl group (also referred to as a $C_{5-20}$ aryloxy group), preferably a $C_{1-7}$alkyl group.

Alkoxy: -OR, wherein R is an alkyl group, for example, a $C_{1-7}$ alkyl group. Examples of $C_{1-7}$ alkoxy groups include, but are not limited to, -OMe (methoxy), -OEt (ethoxy), -O(nPr) (n-propoxy), -O(iPr) (isopropoxy), -O(nBu) (n-butoxy), -O(sBu) (sec-butoxy), -O(iBu) (isobutoxy), and -O(tBu) (tert-butoxy).

Acetal: $-CH(OR^1)(OR^2)$, wherein $R^1$ and $R^2$ are independently acetal substituents, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group, or, in the case of a “cyclic” acetal group, $R^1$ and $R^2$, taken together with the two oxygen atoms to which they are attached, and the carbon atoms to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of acetal groups include, but are not limited to, $-CH(OMe)_2$, $-CH(OEt)_2$, and -CH(OMe)(OEt).

Hemiacetal: $-CH(OH)(OR^1)$, wherein $R^1$ is a hemiacetal substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of hemiacetal groups include, but are not limited to, -CH(OH)(OMe) and -CH(OH)(OEt).

Ketal: $-CR(OR^1)(OR^2)$, where $R^1$ and $R^2$ are as defined for acetals, and R is a ketal substituent other than hydrogen, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples ketal groups include, but are not limited to, $-C(Me)(OMe)_2$, $-C(Me)(OEt)_2$, -C(Me)(OMe)(OEt), $-C(Et)(OMe)_2$, $-C(Et)(OEt)_2$, and -C(Et)(OMe)(OEt).

Hemiketal: -CR(OH)(OR$^1$), where R$^1$ is as defined for hemiacetals, and R is a hemiketal substituent other than hydrogen, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of hemiacetal groups include, but are not limited to, -C(Me)(OH)(OMe), -C(Et)(OH)(OMe), -C(Me)(OH)(OEt), and -C(Et)(OH)(OEt).

Oxo (keto, -one): =O.

Thione (thioketone): =S.

Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably hydrogen or a $C_{1-7}$ alkyl group. Examples of ester groups include, but are not limited to, =NH, =NMe, =NEt, and =NPh.

Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a $C_{1-7}$ alkyl group (also referred to as $C_{1-7}$ alkylacyl or $C_{1-7}$ alkanoyl), a $C_{3-20}$ heterocyclyl group (also referred to as $C_{3-20}$ heterocyclylacyl), or a $C_{5-20}$ aryl group (also referred to as $C_{5-20}$ arylacyl), preferably a $C_{1-7}$ alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (t-butyryl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -C(=O)OH.

Thiocarboxy (thiocarboxylic acid): -C(=S)SH.

Thiolocarboxy (thiolocarboxylic acid): -C(=O)SH.

Thionocarboxy (thionocarboxylic acid): -C(=S)OH.

Imidic acid: -C(=NH)OH.

Hydroxamic acid: -C(=NOH)OH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and -C(=O)OPh.

Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, -OC(=O)Ph, and $-OC(=O)CH_2Ph$.

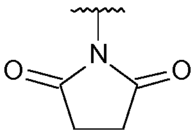
Oxycarboyloxy: -OC(=O)OR, wherein R is an ester substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of ester groups include, but are not limited to, $-OC(=O)OCH_3$, $-OC(=O)OCH_2CH_3$, $-OC(=O)OC(CH_3)_3$, and -OC(=O)OPh.

Amino: $-NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, for example, hydrogen, a $C_{1-7}$ alkyl group (also referred to as $C_{1-7}$ alkylamino or di-$C_{1-7}$ alkylamino), a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably H or a $C_{1-7}$ alkyl group, or, in the case of a “cyclic” amino group, $R^1$ and $R^2$, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Amino groups may be primary ($-NH_2$), secondary ($-NHR^1$), or tertiary ($-NHR^1R^2$), and in cationic form, may be quaternary ($-^+NR^1R^2R^3$). Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

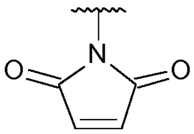
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which $R^1$ and $R^2$, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Thioamido (thiocarbamyl): $-C(=S)NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)N(CH_3)_2$, and $-C(=S)NHCH_2CH_3$.

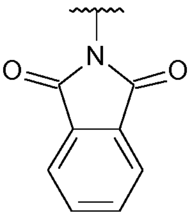
Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein $R^1$ is an amide substituent, for example, hydrogen, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably hydrogen or a $C_{1-7}$ alkyl group, and $R^2$ is an acyl substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$aryl group, preferably hydrogen or a $C_{1-7}$ alkyl group. Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$ , $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. $R^1$ and $R^2$ may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl, and phthalimidyl:



succinimidyl



maleimidyl



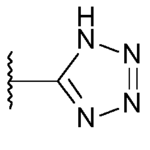
phthalimidyl

Aminocarbonyloxy: $-OC(=O)NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of aminocarbonyloxy groups include, but are not limited to, $-OC(=O)NH_2$, $-OC(=O)NHMe$, $-OC(=O)NMe_2$, and $-OC(=O)NEt_2$.

Ureido: $-N(R^1)CONR^2R^3$ wherein $R^2$ and $R^3$ are independently amino substituents, as defined for amino groups, and $R^1$ is a ureido substituent, for example, hydrogen, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably hydrogen or a $C_{1-7}$ alkyl group. Examples of ureido groups include, but are not limited to, $-NHCONH_2$, $-NHCONHMe$, $-NHCONHEt$, $-NHCONMe_2$, $-NHCONEt_2$, $-NMeCONH_2$, $-NMeCONHMe$, $-NMeCONHEt$, $-NMeCONMe_2$, and $-NMeCONEt_2$.

Guanidino: $-NH-C(=NH)NH_2$.

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,



Imino: =NR, wherein R is an imino substituent, for example, for example, hydrogen, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably H or a $C_{1-7}$alkyl group. Examples of imino groups include, but are not limited to, =NH, =NMe, and =NEt.

Amidine (amidino): $-C(=NR)NR_2$, wherein each R is an amidine substituent, for example, hydrogen, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably H or a $C_{1-7}$ alkyl group. Examples of amidine groups include, but are not limited to, $-C(=NH)NH_2$, $-C(=NH)NMe_2$, and $-C(=NMe)NMe_2$.

Nitro: $-NO_2$.

Nitroso: -NO.

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a $C_{1-7}$ alkyl group (also referred to as a $C_{1-7}$alkylthio group), a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of $C_{1-7}$ alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group (also referred to herein as $C_{1-7}$ alkyl disulfide). Examples of $C_{1-7}$ alkyl disulfide groups include, but are not limited to, $-SSCH_3$ and $-SSCH_2CH_3$.

Sulfine (sulfinyl, sulfoxide): -S(=O)R, wherein R is a sulfine substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfine groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group, including, for example, a fluorinated or perfluorinated $C_{1-7}$ alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$ (triflyl), $-S(=O)_2CH_2CH_3$ (esyl), $-S(=O)_2C_4F_9$ (nonaflyl), $-S(=O)_2CH_2CF_3$ (tresyl), $-S(=O)_2CH_2CH_2NH_2$ (tauryl), $-S(=O)_2Ph$ (phenylsulfonyl, besyl), 4-methylphenylsulfonyl (tosyl), 4-chlorophenylsulfonyl (closyl), 4-bromophenylsulfonyl (brosyl), 4-nitrophenyl (nosyl), 2-naphthalenesulfonate (napsyl), and 5-dimethylamino-naphthalen-1-ylsulfonate (dansyl).

Sulfinic acid (sulfino): -S(=O)OH, $-SO_2H$.

Sulfonic acid (sulfo): $-S(=O)_2OH$, $-SO_3H$.

Sulfinate (sulfinic acid ester): -S(=O)OR; wherein R is a sulfinate substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfinate groups include, but are not limited to, $-S(=O)OCH_3$ (methoxysulfinyl; methyl sulfinate) and $-S(=O)OCH_2CH_3$ (ethoxysulfinyl; ethyl sulfinate).

Sulfonate (sulfonic acid ester): $-S(=O)_2OR$, wherein R is a sulfonate substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfonate groups include, but are not limited to, $-S(=O)_2OCH_3$ (methoxysulfonyl; methyl sulfonate) and $-S(=O)_2OCH_2CH_3$ (ethoxysulfonyl; ethyl sulfonate).

Sulfinyloxy: -OS(=O)R, wherein R is a sulfinyloxy substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group.

Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ (mesylate) and $-OS(=O)_2CH_2CH_3$ (esylate).

Sulfate: $-OS(=O)_2OR$; wherein R is a sulfate substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfate groups include, but are not limited to, $-OS(=O)_2OCH_3$ and $-SO(=O)_2OCH_2CH_3$.

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): $-S(=O)NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, $-S(=O)NH_2$, $-S(=O)NH(CH_3)$, $-S(=O)N(CH_3)_2$, $-S(=O)NH(CH_2CH_3)$, $-S(=O)N(CH_2CH_3)_2$, and $-S(=O)NHPh$.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): $-S(=O)_2NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of sulfonamido groups include, but are not limited to, $-S(=O)_2NH_2$, $-S(=O)_2NH(CH_3)$, $-S(=O)_2N(CH_3)_2$, $-S(=O)_2NH(CH_2CH_3)$, $-S(=O)_2N(CH_2CH_3)_2$, and $-S(=O)_2NHPh$.

Sulfamino: $-NR^1S(=O)_2OH$, wherein $R^1$ is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

Sulfonamino: $-NR^1S(=O)_2R$, wherein $R^1$ is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfonamino groups include, but are not limited to, $-NHS(=O)_2CH_3$ and $-N(CH_3)S(=O)_2C_6H_5$.

Sulfinamino: $-NR^1S(=O)R$, wherein $R^1$ is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of sulfinamino groups include, but are not limited to, $-NHS(=O)CH_3$ and $-N(CH_3)S(=O)C_6H_5$.

Phosphino (phosphine): $-PR_2$, wherein R is a phosphino substituent, for example, -H, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphino groups include, but are not limited to, $-PH_2$, $-P(CH_3)_2$, $-P(CH_2CH_3)_2$, $-P(t\text{-}Bu)_2$, and $-P(Ph)_2$.

Phospho: $-P(=O)_2$.

Phosphinyl (phosphine oxide): $-P(=O)R_2$, wherein R is a phosphinyl substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group or a $C_{5-20}$ aryl group. Examples of phosphinyl groups include, but are not limited to, $-P(=O)(CH_3)_2$, $-P(=O)(CH_2CH_3)_2$, $-P(=O)(t\text{-}Bu)_2$, and $-P(=O)(Ph)_2$.

Phosphonic acid (phosphono): $-P(=O)(OH)_2$.

Phosphonate (phosphono ester): $-P(=O)(OR)_2$, where R is a phosphonate substituent, for example, -H, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphonate groups include, but are not limited to, $-P(=O)(OCH_3)_2$, $-P(=O)(OCH_2CH_3)_2$, $-P(=O)(O\text{-}t\text{-}Bu)_2$, and $-P(=O)(OPh)_2$.

Phosphoric acid (phosphonooxy): $-OP(=O)(OH)_2$.

Phosphate (phosphonooxy ester): $-OP(=O)(OR)_2$, where R is a phosphate substituent, for example, -H, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphate groups include, but are not limited to, $-OP(=O)(OCH_3)_2$, $-OP(=O)(OCH_2CH_3)_2$, $-OP(=O)(O\text{-}t\text{-}Bu)_2$, and $-OP(=O)(OPh)_2$.

Phosphorous acid: $-OP(OH)_2$.

Phosphite: $-OP(OR)_2$, where R is a phosphite substituent, for example, -H, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphite groups include, but are not limited to, $-OP(OCH_3)_2$, $-OP(OCH_2CH_3)_2$, $-OP(O\text{-}t\text{-}Bu)_2$, and $-OP(OPh)_2$.

Phosphoramidite: $-OP(OR^1)-NR^2_2$, where $R^1$ and $R^2$ are phosphoramidite substituents, for example, -H, a (optionally substituted) $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$

aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphoramidite groups include, but are not limited to, $-OP(OCH_2CH_3)-N(CH_3)_2$, $-OP(OCH_2CH_3)-N(i\text{-}Pr)_2$, and $-OP(OCH_2CH_2CN)-N(i\text{-}Pr)_2$.

Phosphoramidate: $-OP(=O)(OR^1)-NR^2_2$, where $R^1$ and $R^2$ are phosphoramidate substituents, for example, -H, a (optionally substituted) $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably -H, a $C_{1-7}$ alkyl group, or a $C_{5-20}$ aryl group. Examples of phosphoramidate groups include, but are not limited to, $-OP(=O)(OCH_2CH_3)-N(CH_3)_2$, $-OP(=O)(OCH_2CH_3)-N(i\text{-}Pr)_2$, and $-OP(=O)(OCH_2CH_2CN)-N(i\text{-}Pr)_2$.

*Alkylene*

$C_{3-12}$ alkylene: The term "$C_{3-12}$ alkylene", as used herein, pertains to a bidentate moiety obtained by removing two hydrogen atoms, either both from the same carbon atom, or one from each of two different carbon atoms, of a hydrocarbon compound having from 3 to 12 carbon atoms (unless otherwise specified), which may be aliphatic or alicyclic, and which may be saturated, partially unsaturated, or fully unsaturated. Thus, the term "alkylene" includes the sub-classes alkenylene, alkynylene, cycloalkylene, etc., discussed below.

Examples of linear saturated $C_{3-12}$ alkylene groups include, but are not limited to, $-(CH_2)_n-$ where n is an integer from 3 to 12, for example, $-CH_2CH_2CH_2-$ (propylene), $-CH_2CH_2CH_2CH_2-$ (butylene), $-CH_2CH_2CH_2CH_2CH_2-$ (pentylene) and $-CH_2CH_2CH_2CH\text{-}_2CH_2CH_2CH_2-$ (heptylene).

Examples of branched saturated $C_{3-12}$ alkylene groups include, but are not limited to, $-CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH(CH_3)CH_2CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-CH_2CH(CH_3)CH_2CH_2-$, $-CH(CH_2CH_3)-$, $-CH(CH_2CH_3)CH_2-$, and $-CH_2CH(CH_2CH_3)CH_2-$.

Examples of linear partially unsaturated $C_{3-12}$ alkylene groups ($C_{3-12}$ alkenylene, and alkynylene groups) include, but are not limited to, $-CH=CH-CH_2-$, $-CH_2-CH=CH_2-$, $-CH=CH-CH_2-CH_2-$, $-CH=CH-CH_2-CH_2-CH_2-$, $-CH=CH-CH=CH-$, $-CH=CH-CH=CH-CH_2-$, $-CH=CH-CH=CH-CH_2-CH_2-$, $-CH=CH-CH_2-CH=CH-$, $-CH=CH-CH_2-CH_2-CH=CH-$, and $-CH_2-C\equiv C-CH_2-$.

Examples of branched partially unsaturated $C_{3-12}$ alkylene groups ($C_{3-12}$ alkenylene and alkynylene groups) include, but are not limited to, $-C(CH_3)=CH-$, $-C(CH_3)=CH-CH_2-$, $-CH=CH-CH(CH_3)-$ and $-C\equiv C-CH(CH_3)-$.

Examples of alicyclic saturated $C_{3-12}$ alkylene groups ($C_{3-12}$ cycloalkylenes) include, but are not limited to, cyclopentylene (e.g. cyclopent-1,3-ylene), and cyclohexylene (e.g. cyclohex-1,4-ylene).

Examples of alicyclic partially unsaturated $C_{3-12}$ alkylene groups ($C_{3-12}$ cycloalkylenes) include, but are not limited to, cyclopentenylene (e.g. 4-cyclopenten-1,3-ylene), cyclohexenylene (e.g. 2-cyclohexen-1,4-ylene; 3-cyclohexen-1,2-ylene; 2,5-cyclohexadien-1,4-ylene).

The symbols * and  are used interchangably to represent the attachment point of the chemical group.

*Ligand Unit*

The Ligand Unit may be of any kind, and include a protein, polypeptide, peptide and a non-peptidic agent that specifically binds to a target molecule. In some embodiments, the Ligand unit may be a protein, polypeptide or peptide. In some embodiments, the Ligand unit may be a cyclic polypeptide. These Ligand units can include antibodies or a fragment of an antibody that contains at least one target molecule-binding site, lymphokines, hormones, growth factors, or any other cell binding molecule or substance that can specifically bind to a target.

The terms “specifically binds” and “specific binding” refer to the binding of an antibody or other protein, polypeptide or peptide to a predetermined molecule (e.g., an antigen). Typically, the antibody or other molecule binds with an affinity of at least about $1x10^7$ $M^{-1}$, and binds to the predetermined molecule with an affinity that is at least two-fold greater than its affinity for binding to a non-specific molecule (e.g., BSA, casein) other than the predetermined molecule or a closely-related molecule.

Examples of Ligand units include those agents described for use in WO 2007/085930, which is incorporated herein.

In some embodiments, the Ligand unit is a Cell Binding Agent that binds to an extracellular target on a cell. Such a Cell Binding Agent can be a protein, polypeptide, peptide or a non-peptidic agent. In some embodiments, the Cell Binding Agent may be a protein, polypeptide or peptide. In some embodiments, the Cell Binding Agent may be a cyclic polypeptide. The Cell Binding Agent also may be antibody or an antigen-binding fragment of an antibody. Thus, in one embodiment, the present invention provides an antibody-drug conjugate (ADC).

## Cell Binding Agent

A cell binding agent may be of any kind, and include peptides and non-peptides. These can include antibodies or a fragment of an antibody that contains at least one binding site, lymphokines, hormones, hormone mimetics, vitamins, growth factors, nutrient-transport molecules, or any other cell binding molecule or substance.

### *Peptides*

In one embodiment, the cell binding agent is a linear or cyclic peptide comprising 4-30, preferably 6-20, contiguous amino acid residues. In this embodiment, it is preferred that one cell binding agent is linked to one monomer or dimer pyrrolobenzodiazepine compound.

In one embodiment the cell binding agent comprises a peptide that binds integrin $\alpha_v\beta_6$. The peptide may be selective for $\alpha_v\beta_6$ over XYS.

In one embodiment the cell binding agent comprises the A20FMDV-Cys polypeptide. The A20FMDV-Cys has the sequence: NAVPNLRGDLQVLAQKVARTC. Alternatively, a variant of the A20FMDV-Cys sequence may be used wherein one, two, three, four, five, six, seven, eight, nine or ten amino acid residues are substituted with another amino acid residue. Furthermore, the polypeptide may have the sequence NAVXXXXXXXXXXXXXXXXRTC.

### *Antibodies*

The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (*e.g.,* bispecific antibodies), multivalent antibodies and antibody fragments, so long as they exhibit the desired biological activity (Miller *et al* (2003) *Jour. of Immunology* 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, *i.e.*, a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin can be of any type (e.g. IgG, IgE, IgM, IgD, and IgA), class (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species, including human, murine, or rabbit origin.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and scFv fragments; diabodies; 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 which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be 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 or from transgenic mice carrying a fully human immunoglobulin system (Lonberg (2008) Curr. Opinion 20(4):450-459).

The monoclonal antibodies herein specifically include chimeric antibodies, humanized antibodies and human antibodies.

Examples of cell binding agents include those agents described for use in WO 2007/085930, which is incorporated herein.

Tumour-associate antigens and cognate antibodies for use in embodiments of the present invention are listed below, and are described in more detail on pages 14 to 86 of WO 2017/186894, which is incorporated herein.

**(1)** BMPR1B (bone morphogenetic protein receptor-type IB)
**(2)** E16 (LAT1, SLC7A5)
**(3)** STEAP1 (six transmembrane epithelial antigen of prostate)
**(4)** 0772P (CA125, MUC16)
**(5)** MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin)
**(6)** Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b)
**(7)** Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, 25 sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B)
**(8)** PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA
2700050C12 gene)
**(9)** ETBR (Endothelin type B receptor)
**(10)** MSG783 (RNF124, hypothetical protein FLJ20315)
**(11)** STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer
associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein)
**(12)** TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation 5 channel, subfamily M, member 4)
**(13)** CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor)
**(14)** CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792)
**(15)** CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29)

**(16)** FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C)

**(17)** HER2 (ErbB2)

**(18)** NCA (CEACAM6)

**(19)** MDP (DPEP1)

**(20)** IL20R-alpha (IL20Ra, ZCYTOR7)

**(21)** Brevican (BCAN, BEHAB)

**(22)** EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5)

**(23)** ASLG659 (B7h)

**(24)** PSCA (Prostate stem cell antigen precursor)

**(25)** GEDA

**(26)** BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3)

**(27)** CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814)

**(27a)** CD22 (CD22 molecule)

**(28)** CD79a (CD79A, CD79alpha), immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2).

**(29)** CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a

10 role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia); 372 aa, pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3,

**(30)** HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and 20 presents them to CD4+ T lymphocytes); 273 aa, pI: 6.56, MW: 30820.TM: 1 [P] Gene Chromosome: 6p21.3)

**(31)** P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability); 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3).

**(32)** CD72 (B-cell differentiation antigen CD72, Lyb-2); 359 aa, pI: 8.66, MW: 40225, TM: 1 5 [P] Gene Chromosome: 9p13.3).

**(33)** LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated

with increased disease activity in patients with systemic lupus erythematosis); 661 aa, pI:

6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12).

**(34)** FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain

that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte 20 differentiation); 429 aa, pl: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22)

**(35)** IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies); 977 aa, pl: 6.88, MW: 106468, TM: 1 [P] Gene Chromosome: 1q21)

**(36)** TENB2 (TMEFF2, tomoregulin, TPEF, HPP1, TR, putative transmembrane 35 proteoglycan, related to the EGF/heregulin family of growth factors and follistatin); 374 aa)

**(37)** PSMA – FOLH1 (Folate hydrolase (prostate-specific membrane antigen) 1)

**(38)** SST ( Somatostatin Receptor; note that there are5 subtypes)

(38.1) SSTR2 (Somatostatin receptor 2)

(38.2) SSTR5 (Somatostatin receptor 5)

(38.3) SSTR1

(38.4) SSTR3

(38.5) SSTR4

**AvB6 – Both subunits (39+40)**

**(39)** ITGAV (Integrin, alpha V)

**(40)** ITGB6 (Integrin, beta 6)

**(41)** CEACAM5 (Carcinoembryonic antigen-related cell adhesion molecule 5)

**(42)** MET (met proto-oncogene; hepatocyte growth factor receptor)

**(43)** MUC1 (Mucin 1, cell surface associated)

**(44)** CA9 (Carbonic anhydrase IX)

**(45)** EGFRvIII ( Epidermal growth factor receptor (EGFR), transcript variant 3,

**(46)** CD33 (CD33 molecule)

**(47)** CD19 (CD19 molecule)

**(48)** IL2RA (Interleukin 2 receptor, alpha); NCBI Reference Sequence: NM_000417.2);

**(49)** AXL (AXL receptor tyrosine kinase)

**(50)** CD30 - TNFRSF8 (Tumor necrosis factor receptor superfamily, member 8)

**(51)** BCMA (B-cell maturation antigen) - TNFRSF17 (Tumor necrosis factor receptor superfamily, member 17)

**(52)** CT Ags – CTA (Cancer Testis Antigens)

**(53)** CD174 (Lewis Y) - FUT3 (fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group)

**(54)** CLEC14A (C-type lectin domain family 14, member A; Genbank accession no. NM175060)

**(55)** GRP78 – HSPA5 (heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)

**(56)** CD70 (CD70 molecule) L08096

**(57)** Stem Cell specific antigens. For example:

- 5T4 (see entry (63) below)
- CD25 (see entry (48) above)
- CD32
- LGR5/GPR49
- Prominin/CD133

**(58)** ASG-5

**(59)** ENPP3 (Ectonucleotide pyrophosphatase/phosphodiesterase 3)

**(60)** PRR4 (Proline rich 4 (lacrimal))

**(61)** GCC – GUCY2C (guanylate cyclase 2C (heat stable enterotoxin receptor)

**(62)** Liv-1 – SLC39A6 (Solute carrier family 39 (zinc transporter), member 6)

**(63)** 5T4, Trophoblast glycoprotein, TPBG – TPBG (trophoblast glycoprotein)

**(64)** CD56 – NCMA1 (Neural cell adhesion molecule 1)

**(65)** CanAg (Tumor associated antigen CA242)

**(66)** FOLR1 (Folate Receptor 1)

**(67)** GPNMB (Glycoprotein (transmembrane) nmb)

**(68)** TIM-1 – HAVCR1 (Hepatitis A virus cellular receptor 1)

**(69)** RG-1/Prostate tumor target Mindin – Mindin/RG-1

**(70)** B7-H4 – VTCN1 (V-set domain containing T cell activation inhibitor 1

**(71)** PTK7 (PTK7 protein tyrosine kinase 7)

**(72)** CD37 (CD37 molecule)

**(73)** CD138 – SDC1 (syndecan 1)

**(74)** CD74 (CD74 molecule, major histocompatibility complex, class II invariant chain)

**(75)** Claudins – CLs (Claudins)

**(76)** EGFR (Epidermal growth factor receptor)

**(77)** Her3 (ErbB3) – ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian))

**(78)** RON - MST1R (macrophage stimulating 1 receptor (c-met-related tyrosine kinase))

**(79)** EPHA2 (EPH receptor A2)

**(80)** CD20 – MS4A1 (membrane-spanning 4-domains, subfamily A, member 1)

**(81)** Tenascin C – TNC (Tenascin C)
**(82)** FAP (Fibroblast activation protein, alpha)
**(83)** DKK-1 (Dickkopf 1 homolog (Xenopus laevis)
**(84)** CD52 (CD52 molecule)
**(85)** CS1 - SLAMF7 (SLAM family member 7)
**(86)** Endoglin – ENG (Endoglin)
**(87)** Annexin A1 – ANXA1 (Annexin A1)
**(88)** V-CAM (CD106) - VCAM1 (Vascular cell adhesion molecule 1)

An additional tumour-associate antigen and cognate antibodies of interest are:
(**89**) ASCT2 (ASC transporter 2, also known as SLC1A5).
ASCT2 antibodies are described in WO 2018/089393, which is incorporated herein by reference.

The cell binding agent may be labelled, for example to aid detection or purification of the agent either prior to incorporation as a conjugate, or as part of the conjugate. The label may be a biotin label. In another embodiment, the cell binding agent may be labelled with a radioisotope.

Methods of Treatment

The compounds of the present invention may be used in a method of therapy. Also provided is a method of treatment, comprising administering to a subject in need of treatment a therapeutically-effective amount of a conjugate of formula **II**. The term “therapeutically effective amount” is an amount sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.

A conjugate may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g. drugs; surgery; and radiation therapy.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a conjugate of formula **I**, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

The Conjugates can be used to treat proliferative disease and autoimmune disease. The term “proliferative disease” pertains to an unwanted or uncontrolled cellular proliferation of excessive or abnormal cells which is undesired, such as, neoplastic or hyperplastic growth, whether *in vitro* or *in vivo*.

Examples of proliferative conditions include, but are not limited to, benign, pre-malignant, and malignant cellular proliferation, including but not limited to, neoplasms and tumours (e.g., histocytoma, glioma, astrocyoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, gastrointestinal cancer, bowel cancer, colon cancer, breast carinoma, ovarian carcinoma, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreatic cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis. Other cancers of interest include, but are not limited to, haematological; malignancies such as leukemias and lymphomas, such as non-Hodgkin lymphoma, and subtypes such as DLBCL, marginal zone, mantle zone, and follicular, Hodgkin lymphoma, AML, and other cancers of B or T cell origin.

Examples of autoimmune disease include the following: rheumatoid arthritis, autoimmune demyelinative diseases (e.g., multiple sclerosis, allergic encephalomyelitis), psoriatic arthritis, endocrine ophthalmopathy, uveoretinitis, systemic lupus erythematosus, myasthenia gravis, Graves' disease, glomerulonephritis, autoimmune hepatological disorder, inflammatory bowel disease (e.g., Crohn's disease), anaphylaxis, allergic reaction, Sjögren's syndrome, type I diabetes mellitus, primary biliary cirrhosis, Wegener's granulomatosis, fibromyalgia, polymyositis, dermatomyositis, multiple endocrine failure, Schmidt's syndrome, autoimmune uveitis, Addison's disease, adrenalitis, thyroiditis, Hashimoto's thyroiditis, autoimmune thyroid disease, pernicious anemia, gastric atrophy, chronic hepatitis, lupoid hepatitis, atherosclerosis, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler's syndrome, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus, dermatitis herpetiformis, alopecia arcata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, ulcerative colitis, mixed connective tissue disease, polyarteritis nedosa, systemic necrotizing vasculitis, atopic dermatitis, atopic rhinitis, Goodpasture's syndrome, Chagas' disease, sarcoidosis, rheumatic fever, asthma, recurrent abortion, anti-phospholipid syndrome, farmer's lung, erythema multiforme, post cardiotomy syndrome, Cushing's syndrome, autoimmune chronic active hepatitis, bird-fancier's lung, toxic epidermal necrolysis, Alport's syndrome, alveolitis, allergic alveolitis, fibrosing alveolitis, interstitial lung disease, erythema nodosum, pyoderma gangrenosum, transfusion reaction, Takayasu's arteritis, polymyalgia rheumatica, temporal arteritis, schistosomiasis, giant cell arteritis, ascariasis, aspergillosis, Sampter's syndrome, eczema, lymphomatoid granulomatosis, Behcet's disease, Caplan's syndrome, Kawasaki's disease, dengue, encephalomyelitis, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, psoriasis, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, filariasis, cyclitis, chronic cyclitis, heterochronic cyclitis, Fuch's cyclitis, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, cardiomyopathy, Eaton-Lambert syndrome, relapsing polychondritis, cryoglobulinemia, Waldenstrom's macroglobulemia, Evan's syndrome, and autoimmune gonadal failure.

In some embodiments, the autoimmune disease is a disorder of B lymphocytes (e.g., systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes), Th1-lymphocytes (e.g., rheumatoid arthritis, multiple sclerosis, psoriasis, Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease), or Th2-lymphocytes (e.g., atopic dermatitis, systemic lupus erythematosus, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, or chronic graft versus host disease). Generally, disorders involving dendritic cells involve disorders of Th1-lymphocytes or Th2-lymphocytes. In some embodiments, the autoimmunie disorder is a T cell-mediated immunological disorder.

In some embodiments, the amount of the Conjugate administered ranges from about 0.01 to about 10 mg/kg per dose. In some embodiments, the amount of the Conjugate administered ranges from about 0.01 to about 5 mg/kg per dose. In some embodiments, the amount of the Conjugate administerd ranges from about 0.05 to about 5 mg/kg per dose. In some embodiments, the amount of the Conjugate administerd ranges from about 0.1 to about 5 mg/kg per dose. In some embodiments, the amount of the Conjugate administered ranges from about 0.1 to about 4 mg/kg per dose. In some embodiments, the amount of the Conjugate administered ranges from about 0.05 to about 3 mg/kg per dose. In some embodiments, the amount of the Conjugate administered ranges from about 0.1 to about 3 mg/kg per dose. In some embodiments, the amount of the Conjugate administered ranges from about 0.1 to about 2 mg/kg per dose.

*Drug loading*

The drug loading (p) is the average number of PBD drugs per cell binding agent, e.g. antibody. Where the compounds of the invention are bound to cysteines, drug loading may range from 1 to 8 drugs (D) per cell binding agent, i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the cell binding agent. Compositions of conjugates include collections of cell binding agents, e.g. antibodies, conjugated with a range of drugs, from 1 to 8. Where the compounds of the invention are bound to lysines, drug loading may range from 1 to 80 drugs (D) per cell binding agent, although an upper limit of 40, 20, 10 or 8 may be preferred. Compositions of conjugates include collections of cell binding agents, e.g. antibodies, conjugated with a range of drugs, from 1 to 80, 1 to 40, 1 to 20, 1 to 10 or 1 to 8.

The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as UV, reverse phase HPLC, HIC, mass spectroscopy, ELISA assay, and electrophoresis. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Sanderson et al (2005) Clin. Cancer Res. 11:843-852). However, the distribution of p (drug) values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also, ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. Such techniques are also applicable to other types of conjugates.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. Higher drug loading, e.g. $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the Drug Linker. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of Drug Linker relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut’s reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by engineering one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues). US 7521541 teaches engineering antibodies by introduction of reactive cysteine amino acids.

Cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249). The engineered cysteine thiols may react with linker reagents or the drug-linker reagents of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form ADC with cysteine engineered antibodies and the PBD drug moieties. The location of the drug moiety can thus be designed, controlled, and known. The drug loading can be controlled since the engineered cysteine thiol groups typically react with thiol-reactive linker reagents or drug-linker reagents in high yield. Engineering an IgG antibody to introduce a cysteine amino acid by substitution at a single site on the heavy or light chain gives two new cysteines on the symmetrical antibody. A drug loading near 2 can be achieved with near homogeneity of the conjugation product ADC.

Where more than one nucleophilic or electrophilic group of the antibody reacts with a drug-linker intermediate, or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (p) may be isolated, however, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody.

Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more PBD drug moieties and where the drug moieties may be attached to the antibody at various amino acid residues.

In one embodiment, the average number of dimer pyrrolobenzodiazepine groups per cell binding agent is in the range 1 to 20. In some embodiments the range is selected from 1 to 8, 2 to 8, 2 to 6, 2 to 4, and 4 to 8.

In some embodiments, there is one dimer pyrrolobenzodiazepine group per cell binding agent.

Brief Description of Figures

Figure 1 shows the effect of conjugates of the invention on the growth of a tumour *in vivo*;
Figure 2 shows the effect of a conjugate of the invention on the growth of a tumour *in vivo.*

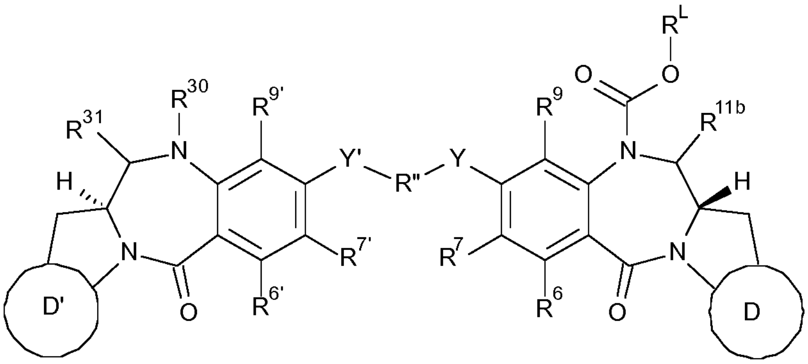
General synthetic routes

The synthesis of PBD compounds is extensively discussed in the following references, which discussions are incorporated herein by reference:

a) WO 00/12508 (pages 14 to 30);
b) WO 2005/023814 (pages 3 to 10);
c) WO 2004/043963 (pages 28 to 29); and
d) WO 2005/085251 (pages 30 to 39).

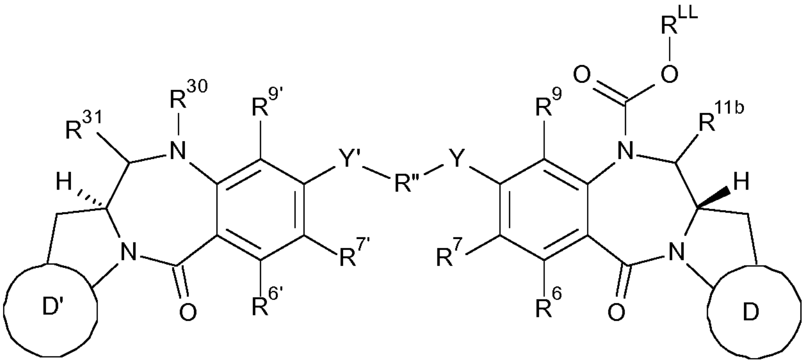
*Synthesis route*

Compounds of the present invention of formula I:



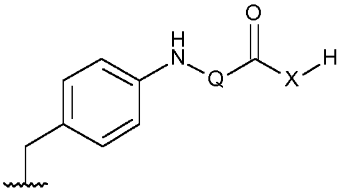
I

can be synthesised from a compound of Formula 2:



Formula 2

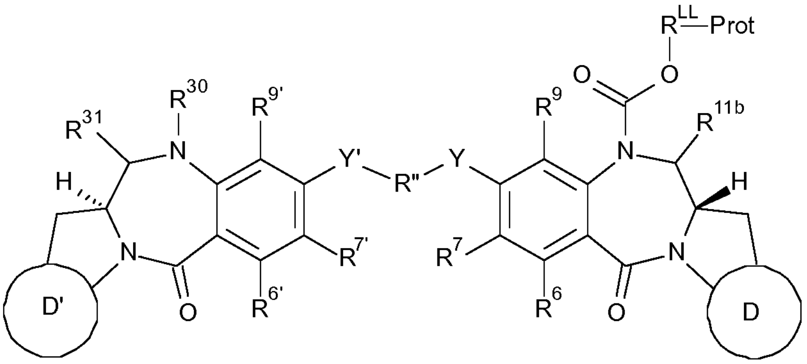
where $R^2$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{11b}$, $R^{22}$, $R^{30}$, $R^{31}$, Y, Y' and R" are as defined for compounds of formula I, and $R^{LL}$ is a precursor of $R^L$. $R^{LL}$ will typically be a portion of $R^L$, such as a group of formula IIIa':



IIIa'

. In such a case, the reaction involves adding the group $G^L$.

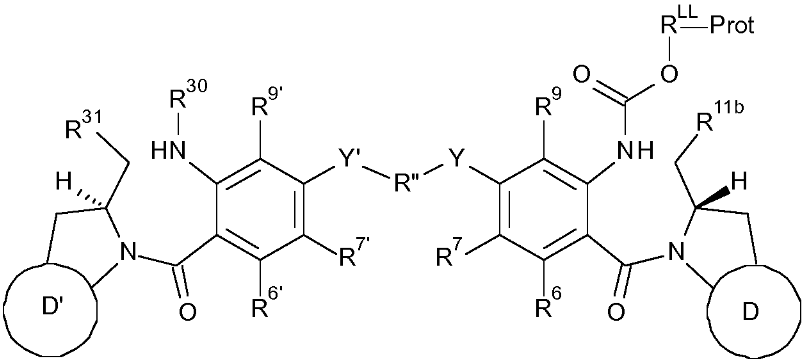
The compounds of Formula 2 may be made by deprotecting compounds of Formula 3:



Formula 3

where $R^2$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{11b}$, $R^{22}$, $R^{30}$, $R^{31}$, Y, Y' and R" are as defined for compounds of formula I, $R^{LL\text{-}Prot}$ is a protected version of $R^{LL}$, and the Prot represents an appropriate carboxy/hydroxy protecting group. Depending on the nature of $R^{30}$ and $R^{31}$, these may need to be in protected form at points during the synthesis.

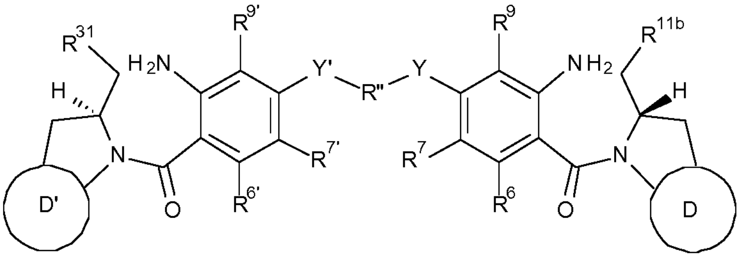
Compounds of formula 3 may be made by ring-closure of compounds of Formula 4:



Formula 4

where the ring closure is carried out by oxidation, e.g. Swern.

Compounds of formula 4 can be synthesised from compounds of formula 5:



Formula 5

by step-wise addition of the two amino protecting groups. Step-wise addition can be achieved by simple protection of one amino group (e.g. by Fmoc), followed by installation of a desired protecting group at the other amino group. This can be followed by removal of the simple protecting group, and then installation of the other desired amino protecting group.

Compounds of Formula 5 can be synthesised by known methods, such as those disclosed in WO 2011/130598.

Synthesis of Drug Conjugates

Conjugates can be prepared as previously described. Antibodies can be conjugated to the Drug Linker compound as described in Doronina et al., Nature Biotechnology, 2003, 21, 778-784). Briefly, antibodies (4-5 mg/mL) in PBS containing 50 mM sodium borate at pH 7.4 are reduced with tris(carboxyethyl)phosphine hydrochloride (TCEP) at 37 °C. The progress of the reaction, which reduces interchain disulfides, is monitored by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) and allowed to proceed until the desired level of thiols/mAb is achieved. The reduced antibody is then cooled to 0°C and alkylated with 1.5 equivalents of maleimide drug-linker per antibody thiol. After 1 hour, the reaction is quenched by the addition of 5 equivalents of N-acetyl cysteine. Quenched drug-linker is removed by gel filtration over a PD-10 column. The ADC is then sterile-filtered through a

0.22 µm syringe filter. Protein concentration can be determined by spectral analysis at 280 nm and 329 nm, respectively, with correction for the contribution of drug absorbance at 280 nm. Size exclusion chromatography can be used to determine the extent of antibody aggregation, and RP-HPLC can be used to determine the levels of remaining NAC-quenched drug-linker.

## Further Preferences

The following preferences may apply to all aspects of the invention as described above, or may relate to a single aspect. The preferences may be combined together in any combination.

In some embodiments, $R^{6'}$, $R^{7'}$, $R^{9'}$, and Y' are selected from the same groups as $R^6$, $R^7$, $R^9$, and Y respectively. In some of these embodiments, $R^{6'}$, $R^{7'}$, $R^{9'}$, and Y' are the same as $R^6$, $R^7$, $R^9$, and Y respectively.

In some embodiments, $R^{22}$ is the same as $R^2$.

### *N10'-C11'*

In some embodiment, $R^{30}$ is H, and $R^{31}$ is OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl. In some of these embodiments, $R^{31}$ is OH. In others of these embodiments, $R^{31}$ is $OR^A$, where $R^A$ is $C_{1-4}$ alkyl. In some of these embodiments, $R^A$ is methyl.

In some embodiments, $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound.

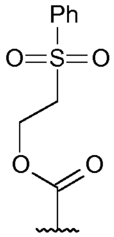
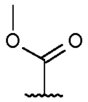
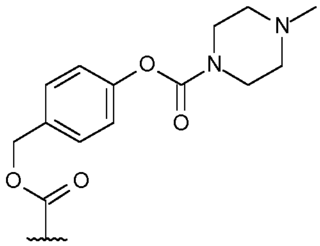
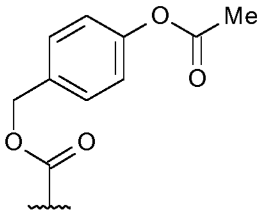
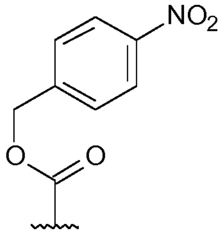
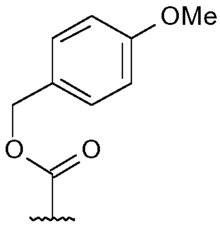
In some embodiments, $R^{30}$ is H and $R^{31}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation. In some of these embodiments, M is a monovalent pharmaceutically acceptable cation, and may be $Na^+$. Furthermore, in some embodiments z is 3.

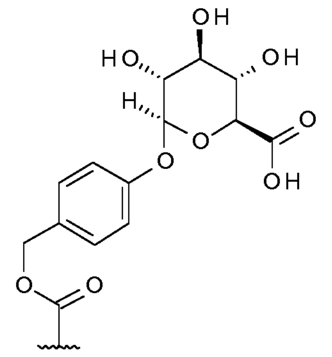
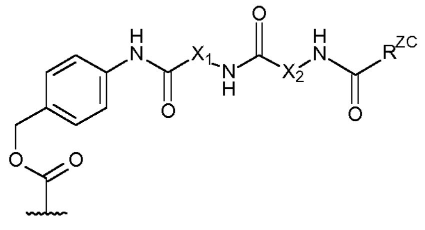
In some embodiments, $R^{30}$ is H and $R^{31}$ is H.

In some embodiments, $R^{30}$ is H and $R^{31}$ is =O.

In some embodiments where $R^{30}$ is (e-iii), there may be an additional ntiro group on the benzene ring, e.g. ortho to $R^Z$.

In some embodiments, $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1-4}$ alkyl and $R^{30}$ is selected from:

| | |
|---|---|
| $R^{30a}$ |  |
| $R^{30b}$ |  |
| $R^{30c}$ |  |
| $R^{30d}$ |  |
| $R^{30e}$ |  |
| $R^{30f}$ |  |

| | |
|---|---|
| $R^{30g}$ |  |
| $R^{30h}$ |  |

where -C(=O)-$X_1$-NHC(=O)$X_2$-NH- represent a dipeptide. The amino acids in the dipeptide may be any combination of natural amino acids. The dipeptide may be the site of action for cathepsin-mediated cleavage.

In one embodiment, the dipeptide, -C(=O)-$X_1$-NHC(=O)$X_2$-NH-, is selected from:

-Phe-Lys-,
-Val-Ala-,
-Val-Lys-,
-Ala-Lys-,
-Val-Cit-,
-Phe-Cit-,
-Leu-Cit-,
-Ile-Cit-,
-Phe-Arg-,
-Trp-Cit-

where Cit is citrulline.

Preferably, the dipeptide, -C(=O)-$X_1$-NHC(=O)$X_2$-NH-, is selected from:

-Phe-Lys-,
-Val-Ala-,
-Val-Lys-,
-Ala-Lys-,
-Val-Cit-.

Most preferably, the dipeptide, -C(=O)-$X_1$-NHC(=O)$X_2$-NH-, is -Phe-Lys- or -Val-Ala-.

Other dipeptide combinations may be used, including those described by Dubowchik *et al.*, *Bioconjugate Chemistry*, 2002, 13,855-869, which is incorporated herein by reference.

In one embodiment, the amino acid side chain is derivatised, where appropriate. For example, an amino group or carboxy group of an amino acid side chain may be derivatised.

In one embodiment, an amino group $NH_2$ of a side chain amino acid, such as lysine, is a derivatised form selected from the group consisting of NHR and NRR'.
In one embodiment, a carboxy group COOH of a side chain amino acid, such as aspartic acid, is a derivatised form selected from the group consisting of COOR, $CONH_2$, CONHR and CONRR'.

In one embodiment, the amino acid side chain is chemically protected, where appropriate. The side chain protecting group may be a group as discussed above. The present inventors have established that protected amino acid sequences are cleavable by enzymes. For example, it has been established that a dipeptide sequence comprising a Boc side chain-protected Lys residue is cleavable by cathepsin.

Protecting groups for the side chains of amino acids are well known in the art and are described in the Novabiochem Catalog. Additional protecting group strategies are set out in Protective Groups in Organic Synthesis, Greene and Wuts.

Possible side chain protecting groups are shown below for those amino acids having reactive side chain functionality:

Arg: Z, Mtr, Tos;
Asn: Trt, Xan;
Asp: Bzl, t-Bu;
Cys: Acm, Bzl, Bzl-OMe, Bzl-Me, Trt;
Glu: Bzl, t-Bu;
Gln: Trt, Xan;
His: Boc, Dnp, Tos, Trt;
Lys: Boc, Z-Cl, Fmoc, Z, Alloc;

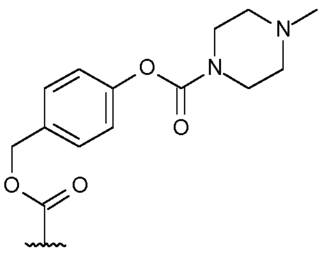
Ser: Bzl, TBDMS, TBDPS;
Thr: Bz;
Trp: Boc;
Tyr: Bzl, Z, Z-Br.

In one embodiment, the side chain protection is selected to be orthogonal to a group provided as, or as part of, a capping group, where present. Thus, the removal of the side chain protecting group does not remove the capping group, or any protecting group functionality that is part of the capping group.

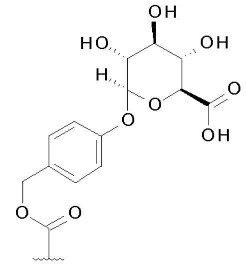
In other embodiments of the invention, the amino acids selected are those having no reactive side chain functionality. For example, the amino acids may be selected from: Ala, Gly, Ile, Leu, Met, Phe, Pro, and Val.

It is particularly preferred in the present invention, that -C(=O)-$X_1$-NHC(=O)$X_2$-NH- is the same dipeptide comprised in $R^L$.

Other preferred $R^{30}$ groups include:



and

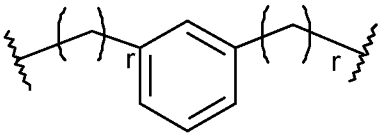


.

*Dimer link*
In some embodiments, Y and Y' are both O.

In some embodiments, R'' is a $C_{3-7}$ alkylene group with no substituents. In some of these embodiments, R'' is a $C_3$, $C_5$ or $C_7$ alkylene. In particulae, R'' may be a $C_3$ or $C_5$ alkylene.

In other embodiments, R'' is a group of formula:



where r is 1 or 2.

*$R^6$ to $R^9$*

In some embodiments, $R^9$ is H.

In some embodiments, $R^6$ is selected from H, OH, OR, SH, $NH_2$, nitro and halo, and may be selected from H or halo. In some of these embodiments $R^6$ is H.

In some embodiments, $R^7$ is selected from H, OH, OR, SH, SR, $NH_2$, NHR, NRR', and halo. In some of these embodiments $R^7$ is selected from H, OH and OR, where R is selected from optionally substituted $C_{1-7}$ alkyl, $C_{3-10}$ heterocyclyl and $C_{5-10}$ aryl groups. R may be more preferably a $C_{1-4}$ alkyl group, which may or may not be substituted. A substituent of interest is a $C_{5-6}$ aryl group (e.g. phenyl). Particularly preferred substituents at the 7- positions are OMe and $OCH_2Ph$. Other substituents of particular interest are dimethylamino (i.e. $-NMe_2$); $-(OC_2H_4)_qOMe$, where q is from 0 to 2; nitrogen-containing $C_6$ heterocyclyls, including morpholino, piperidinyl and N-methyl-piperazinyl.

These embodiments and preferences apply to $R^{9'}$, $R^{6'}$ and $R^{7'}$ respectively.

*D and D'*

In some embodiments, D and D' are D1 and D'1 respectively.

In some embodiments, D and D' are D2 and D'2 respectively.

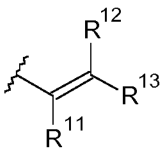
*$R^2$*

When there is a double bond present between C2 and C3, $R^2$ is selected from:

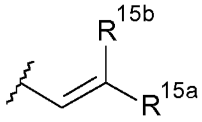
(a) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

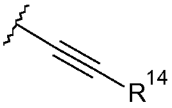
(b) $C_{1-5}$ saturated aliphatic alkyl;

(c) $C_{3-6}$ saturated cycloalkyl;

(d) 

, wherein each of $R^{11}$, $R^{12}$ and $R^{13}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^2$ group is no more than 5;

(e)  , wherein one of $R^{15a}$ and $R^{15b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl; and

(f)  , where $R^{14}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl.

When $R^2$ is a $C_{5-10}$ aryl group, it may be a $C_{5-7}$ aryl group. A $C_{5-7}$ aryl group may be a phenyl group or a $C_{5-7}$ heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, $R^2$ is preferably phenyl. In other embodiments, $R^2$ is preferably thiophenyl, for example, thiophen-2-yl and thiophen-3-yl.

When $R^2$ is a $C_{5-10}$ aryl group, it may be a $C_{8-10}$ aryl, for example a quinolinyl or isoquinolinyl group. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. Of these quinolin-3-yl and quinolin-6-yl may be preferred. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. Of these isoquinolin-3-yl and isoquinolin-6-yl may be preferred.

When $R^2$ is a $C_{5-10}$ aryl group, it may bear any number of substituent groups. It preferably bears from 1 to 3 substituent groups, with 1 and 2 being more preferred, and singly substituted groups being most preferred. The substituents may be any position.

Where $R^2$ is $C_{5-7}$ aryl group, a single substituent is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the $C_{5-7}$ aryl group is phenyl, the substituent is preferably in the meta- or para- positions, and more preferably is in the para-position.

Where $R^2$ is a $C_{8-10}$ aryl group, for example quinolinyl or isoquinolinyl, it may bear any number of substituents at any position of the quinoline or isoquinoline rings. In some embodiments, it bears one, two or three substituents, and these may be on either the proximal and distal rings or both (if more than one substituent).

*$R^2$ substituents, when $R^2$ is a $C_{5\text{-}10}$ aryl group*

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is halo, it is preferably F or Cl, more preferably Cl.

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is ether, it may in some embodiments be an alkoxy group, for example, a $C_{1\text{-}7}$ alkoxy group (e.g. methoxy, ethoxy) or it may in some embodiments be a $C_{5\text{-}7}$ aryloxy group (e.g phenoxy, pyridyloxy, furanyloxy). The alkoxy group may itself be further substituted, for example by an amino group (e.g. dimethylamino).

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is $C_{1\text{-}7}$ alkyl, it may preferably be a $C_{1\text{-}4}$ alkyl group (e.g. methyl, ethyl, propryl, butyl).

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is $C_{3\text{-}7}$ heterocyclyl, it may in some embodiments be $C_6$ nitrogen containing heterocyclyl group, e.g. morpholino, thiomorpholino, piperidinyl, piperazinyl. These groups may be bound to the rest of the PBD moiety via the nitrogen atom. These groups may be further substituted, for example, by $C_{1\text{-}4}$ alkyl groups. If the $C_6$ nitrogen containing heterocyclyl group is piperazinyl, the said further substituent may be on the second nitrogen ring atom.

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is bis-oxy-$C_{1\text{-}3}$ alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

If a substituent on $R^2$ when $R^2$ is a $C_{5\text{-}10}$ aryl group is ester, this is preferably methyl ester or ethyl ester.

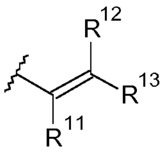
Particularly preferred substituents when $R^2$ is a $C_{5\text{-}10}$ aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl. Other particularly preferred substituents for $R^2$ are dimethylaminopropyloxy and carboxy.

Particularly preferred substituted $R^2$ groups when $R^2$ is a $C_{5\text{-}10}$ aryl group include, but are not limited to, 4-methoxy-phenyl, 3-methoxyphenyl, 4-ethoxy-phenyl, 3-ethoxy-phenyl, 4-

fluoro-phenyl, 4-chloro-phenyl, 3,4-bisoxymethylene-phenyl, 4-methylthiophenyl, 4-cyanophenyl, 4-phenoxyphenyl, quinolin-3-yl and quinolin-6-yl, isoquinolin-3-yl and isoquinolin-6-yl, 2-thienyl, 2-furanyl, methoxynaphthyl, and naphthyl. Another possible substituted $R^2$ group is 4-nitrophenyl. $R^2$ groups of particular interest include 4-(4-methylpiperazin-1-yl)phenyl and 3,4-bisoxymethylene-phenyl.

When $R^2$ is $C_{1-5}$ saturated aliphatic alkyl, it may be methyl, ethyl, propyl, butyl or pentyl. In some embodiments, it may be methyl, ethyl or propyl (n-pentyl or isopropyl). In some of these embodiments, it may be methyl. In other embodiments, it may be butyl or pentyl, which may be linear or branched.

When $R^2$ is $C_{3-6}$ saturated cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.

When $R^2$ is , each of $R^{11}$, $R^{12}$ and $R^{13}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^2$ group is no more than 5. In some embodiments, the total number of carbon atoms in the $R^2$ group is no more than 4 or no more than 3.

In some embodiments, one of $R^{11}$, $R^{12}$ and $R^{13}$ is H, with the other two groups being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

In other embodiments, two of $R^{11}$, $R^{12}$ and $R^{13}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

In some embodiments, the groups that are not H are selected from methyl and ethyl. In some of these embodiments, the groups that re not H are methyl.

In some embodiments, $R^{11}$ is H.

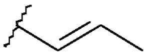
In some embodiments, $R^{12}$ is H.

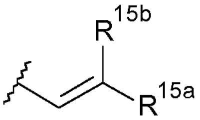
In some embodiments, $R^{13}$ is H.

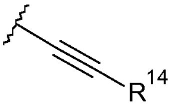
In some embodiments, $R^{11}$ and $R^{12}$ are H.

In some embodiments, $R^{11}$ and $R^{13}$ are H.

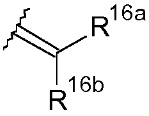
In some embodiments, $R^{12}$ and $R^{13}$ are H.

An $R^2$ group of particular interest is:  .

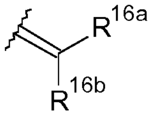
When $R^2$ is  , one of $R^{15a}$ and $R^{15b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl. In some embodiments, the group which is not H is optionally substituted phenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted.

When $R^2$ is  , $R^{14}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted.
In some embodiments, $R^{14}$ is selected from H, methyl, ethyl, ethenyl and ethynyl. In some of these embodiments, $R^{14}$ is selected from H and methyl.

When there is a single bond present between C2 and C3,

$R^2$ is H, OH, F, diF or  , where $R^{16a}$ and $R^{16b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{16a}$ and $R^{16b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester.

In some embodiments, $R^2$ is H.
In some embodiments, $R^2$ is OH.

In some embodiments, $R^2$ is  .

In some embodiments, it is preferred that $R^{16a}$ and $R^{16b}$ are both H.

In other embodiments, it is preferred that $R^{16a}$ and $R^{16b}$ are both methyl.

In further embodiments, it is preferred that one of $R^{16a}$ and $R^{16b}$ is H, and the other is selected from $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted. In these further embodiment, it may be further preferred that the group which is not H is selected from methyl and ethyl.

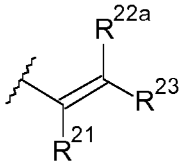
### *$R^{22}$*

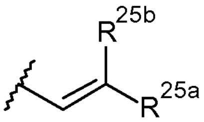
When there is a double bond present between C2' and C3', $R^{22}$ is selected from:

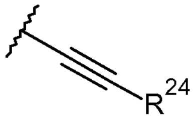
(a) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(b) $C_{1-5}$ saturated aliphatic alkyl;

(c) $C_{3-6}$ saturated cycloalkyl;

(d)  , wherein each of $R^{21}$, $R^{22a}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{22}$ group is no more than 5;

(e)  , wherein one of $R^{25a}$ and $R^{25b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl; and

(f)  , where $R^{24}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl.

When $R^{22}$ is a $C_{5-10}$ aryl group, it may be a $C_{5-7}$ aryl group. A $C_{5-7}$ aryl group may be a phenyl group or a $C_{5-7}$ heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, $R^{22}$ is preferably phenyl. In other embodiments, $R^{22}$ is preferably thiophenyl, for example, thiophen-2-yl and thiophen-3-yl.

When $R^{22}$ is a $C_{5-10}$ aryl group, it may be a $C_{8-10}$ aryl, for example a quinolinyl or isoquinolinyl group. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. Of these quinolin-3-yl and quinolin-6-yl may be preferred. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. Of these isoquinolin-3-yl and isoquinolin-6-yl may be preferred.

When $R^{22}$ is a $C_{5-10}$ aryl group, it may bear any number of substituent groups. It preferably bears from 1 to 3 substituent groups, with 1 and 2 being more preferred, and singly substituted groups being most preferred. The substituents may be any position.

Where $R^{22}$ is $C_{5-7}$ aryl group, a single substituent is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the $C_{5-7}$ aryl group is phenyl, the substituent is preferably in the meta- or para- positions, and more preferably is in the para-position.

Where $R^{22}$ is a $C_{8-10}$ aryl group, for example quinolinyl or isoquinolinyl, it may bear any number of substituents at any position of the quinoline or isoquinoline rings. In some embodiments, it bears one, two or three substituents, and these may be on either the proximal and distal rings or both (if more than one substituent).

*$R^{22}$ substituents, when $R^{22}$ is a $C_{5-10}$ aryl group*

If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is halo, it is preferably F or Cl, more preferably Cl.

If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is ether, it may in some embodiments be an alkoxy group, for example, a $C_{1-7}$ alkoxy group (e.g. methoxy, ethoxy) or it may in some embodiments be a $C_{5-7}$ aryloxy group (e.g phenoxy, pyridyloxy, furanyloxy). The alkoxy group may itself be further substituted, for example by an amino group (e.g. dimethylamino).

If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is $C_{1-7}$ alkyl, it may preferably be a $C_{1-4}$ alkyl group (e.g. methyl, ethyl, propryl, butyl).

If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is $C_{3-7}$ heterocyclyl, it may in some embodiments be $C_6$ nitrogen containing heterocyclyl group, e.g. morpholino, thiomorpholino, piperidinyl, piperazinyl. These groups may be bound to the rest of the PBD moiety via the nitrogen atom. These groups may be further substituted, for example, by $C_{1-4}$ alkyl groups. If the $C_6$ nitrogen containing heterocyclyl group is piperazinyl, the said further substituent may be on the second nitrogen ring atom.

If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is bis-oxy-$C_{1-3}$ alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

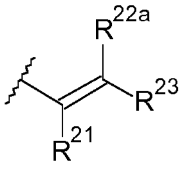
If a substituent on $R^{22}$ when $R^{22}$ is a $C_{5-10}$ aryl group is ester, this is preferably methyl ester or ethyl ester.

Particularly preferred substituents when $R^{22}$ is a $C_{5-10}$ aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl. Other particularly preferred substituents for $R^{22}$ are dimethylaminopropyloxy and carboxy.

Particularly preferred substituted $R^{22}$ groups when $R^{22}$ is a $C_{5-10}$ aryl group include, but are not limited to, 4-methoxy-phenyl, 3-methoxyphenyl, 4-ethoxy-phenyl, 3-ethoxy-phenyl, 4-fluoro-phenyl, 4-chloro-phenyl, 3,4-bisoxymethylene-phenyl, 4-methylthiophenyl, 4-cyanophenyl, 4-phenoxyphenyl, quinolin-3-yl and quinolin-6-yl, isoquinolin-3-yl and isoquinolin-6-yl, 2-thienyl, 2-furanyl, methoxynaphthyl, and naphthyl. Another possible substituted $R^{22}$ group is 4-nitrophenyl. $R^{22}$ groups of particular interest include 4-(4-methylpiperazin-1-yl)phenyl and 3,4-bisoxymethylene-phenyl.

When $R^{22}$ is $C_{1-5}$ saturated aliphatic alkyl, it may be methyl, ethyl, propyl, butyl or pentyl. In some embodiments, it may be methyl, ethyl or propyl (n-pentyl or isopropyl). In some of these embodiments, it may be methyl. In other embodiments, it may be butyl or pentyl, which may be linear or branched.

When $R^{22}$ is $C_{3-6}$ saturated cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.

When $R^{22}$ is  , each of $R^{21}$, $R^{22a}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{22}$ group is no more than 5. In some embodiments, the total number of carbon atoms in the $R^{22}$ group is no more than 4 or no more than 3.

In some embodiments, one of $R^{21}$, $R^{22a}$ and $R^{23}$ is H, with the other two groups being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

In other embodiments, two of $R^{21}$, $R^{22a}$ and $R^{23}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

In some embodiments, the groups that are not H are selected from methyl and ethyl. In some of these embodiments, the groups that re not H are methyl.

In some embodiments, $R^{21}$ is H.

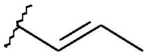
In some embodiments, $R^{22a}$ is H.

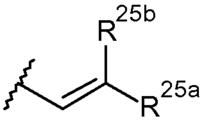
In some embodiments, $R^{23}$ is H.

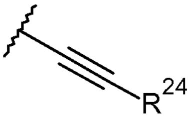
In some embodiments, $R^{21}$ and $R^{22a}$ are H.

In some embodiments, $R^{21}$ and $R^{23}$ are H.

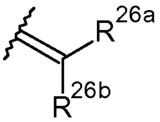
In some embodiments, $R^{22a}$ and $R^{23}$ are H.

An $R^{22}$ group of particular interest is:  .

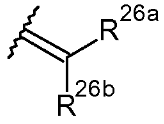
When $R^{22}$ is , one of $R^{25a}$ and $R^{25b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl. In some embodiments, the group which is not H is optionally substituted phenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted.

When $R^{22}$ is , $R^{24}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted.
In some embodiments, $R^{24}$ is selected from H, methyl, ethyl, ethenyl and ethynyl. In some of these embodiments, $R^{24}$ is selected from H and methyl.

When there is a single bond present between C2' and C3',

$R^{22}$ is H, OH, F, diF or , where $R^{26a}$ and $R^{26b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{26a}$ and $R^{26b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester.

In some embodiments, $R^{22}$ is H.
In some embodiments, $R^{22}$ is OH.

In some embodiments, $R^{22}$ is  .

In some embodiments, it is preferred that $R^{26a}$ and $R^{26b}$ are both H.

In other embodiments, it is preferred that $R^{26a}$ and $R^{26b}$ are both methyl.

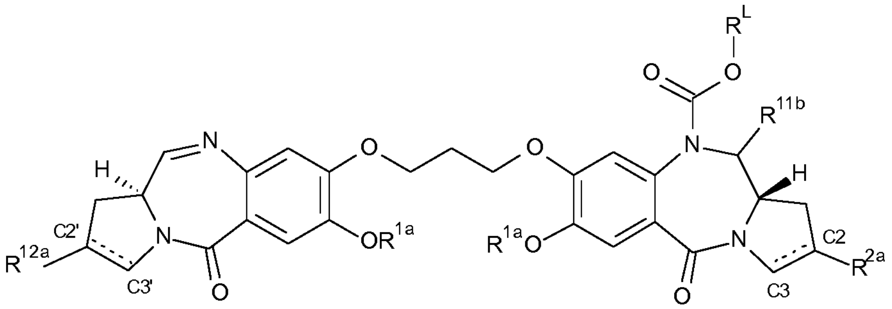
In further embodiments, it is preferred that one of $R^{26a}$ and $R^{26b}$ is H, and the other is selected from $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted. In these further embodiment, it may be further preferred that the group which is not H is selected from methyl and ethyl.

*$R^{11b}$*

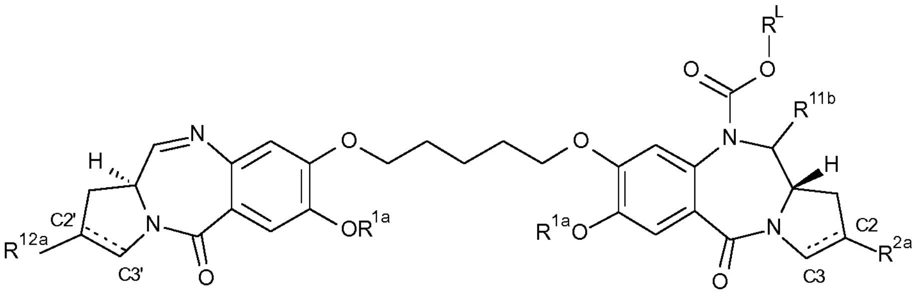
In some embodiments, $R^{11b}$ is OH.

In some embodiments, $R^{11b}$ is $OR^A$, where $R^A$ is $C_{1-4}$ alkyl. In some of these embodiments, $R^A$ is methyl.

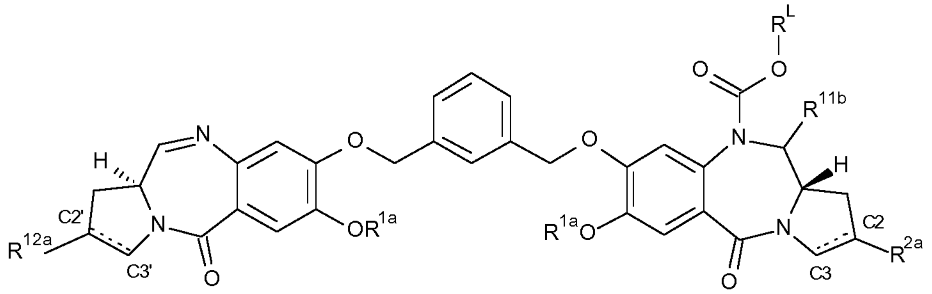
In some embodiments of the first aspect of the present invention are of formula Ia-1, Ia-2 or Ia-3:



Ia

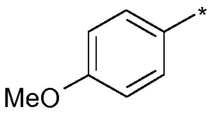


Ib



Ic

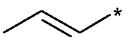
where $R^{2a}$ and $R^{12a}$ are the same and are selected from:

(a) 

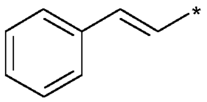
;

(b) 

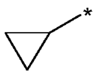
;

(c) 

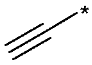
;

(d) 

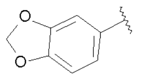
;

(e) 

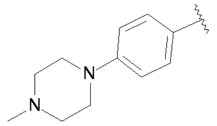
;

(f) 

;

(g) 

; and

(h) 

;

$R^{1a}$ is selected from methyl and benzyl;

$R^L$ and $R^{11b}$ are as defined above.

In some embodiments of the present invention both $R^2$ and $R^{22}$ comprise no more than 3 carbon atoms.

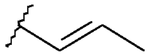
Thus in these embodiments where there is a double bond present between C2 and C3, $R^2$ may be selected from:

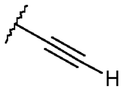
(i) Methyl;

(ii) Ethyl;

(iii) Propyl;

(iv) Cyclopropyl;

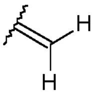
(v)  ;

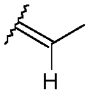
(vi)  ; and

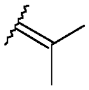
(vi) 

Thus in these embodiments where there is no double bond present between C2 and C3, $R^2$ may be selected from:

(i) H;

(ii)  ;

(iii)  ; and

(iv)  .

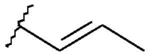
Thus in these embodiments where there is a double bond present between C2' and C3', $R^{22}$ may be selected from:

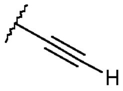
(i) Methyl;

(ii) Ethyl;

(iii) Propyl;

(iv) Cyclopropyl;

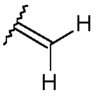
(v)  ;

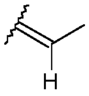
(vi)  ; and

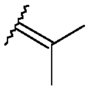
(vi) 

Thus in these embodiments where there is no double bond present between C2' and C3', $R^{22}$ may be selected from:

(i) H;

(ii)  ;

(iii)  ; and

(iv)  .

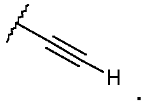
In some of these embodiments both $R^2$ and $R^{22}$ comprise no more than 2 carbon atoms.

Thus in these embodiments where there is a double bond present between C2 and C3, $R^2$ may be selected from:

(i) Methyl;

(ii) Ethyl; and

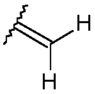
(vi)



Thus in these embodiments where there is no double bond present between C2 and C3, $R^2$ may be selected from:

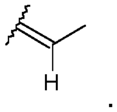
(i) H;

(ii)



; and

(iii)

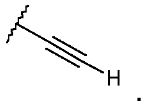


Thus in these embodiments where there is a double bond present between C2' and C3', $R^{22}$ may be selected from:

(i) Methyl;

(ii) Ethyl; and

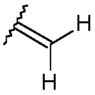
(vi)



Thus in these embodiments where there is no double bond present between C2' and C3', $R^{22}$ may be selected from:

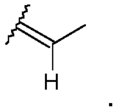
(i) H;

(ii)



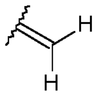
; and

(iii)

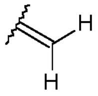


In further of these embodiments both $R^2$ and $R^{22}$ comprise no more than 1 carbon atom.

Thus in these embodiments where there is a double bond present between C2 and C3, $R^2$ may be methyl. Thus in these embodiments where there is no double bond present between C2 and C3, $R^2$ may be selected from:

(i) H; and (ii)  .

Thus in these embodiments where there is a double bond present between C2' and C3', $R^{22}$ may be methyl. Thus in these embodiments where there is no double bond present between C2' and C3', $R^{22}$ may be selected from:

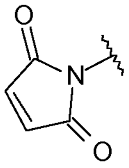
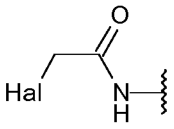
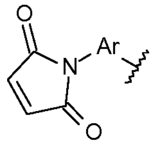
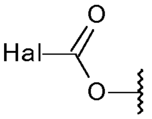
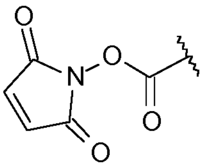
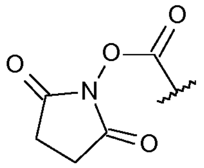
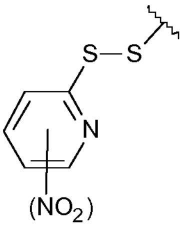
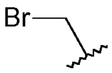
(i) H; and (ii)  .

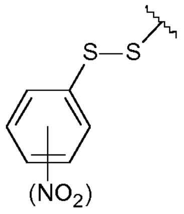
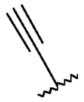
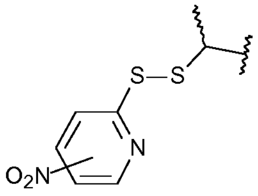
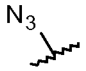
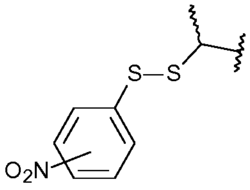
These embodiments and preferences also apply to the second aspect of the invention.

*Linker ($R^L$/$R^{LL}$)*

*$G^L$*

$G^L$ may be selected from

| | | | |
|---|---|---|---|
| ($G^{L1\text{-}1}$) |  | ($G^{L4}$) | <br>Where Hal = I, Br, Cl |
| ($G^{L1\text{-}2}$) |  | ($G^{L5}$) |  |
| ($G^{L2}$) |  | ($G^{L6}$) |  |
| ($G^{L3\text{-}1}$) | <br>where the $NO_2$ group is optional | ($G^{L7}$) |  |

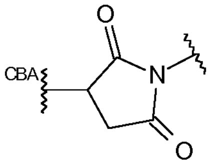
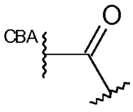
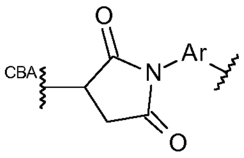
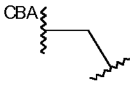
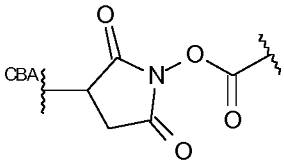
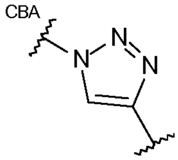
| | | | |
|---|---|---|---|
| ($G^{L3-2}$) | <br>where the $NO_2$ group is optional | ($G^{L8}$) |  |
| ($G^{L3-3}$) | <br>where the $NO_2$ group is optional | ($G^{L9}$) |  |
| ($G^{L3-4}$) | <br>where the $NO_2$ group is optional | | |

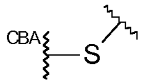
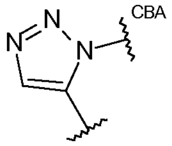
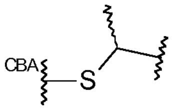
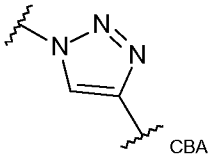
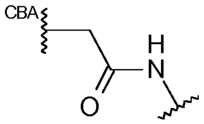
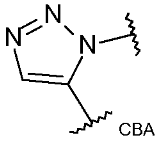
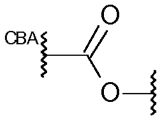
where Ar represents a $C_{5-6}$ arylene group, e.g. phenylene.

In some embodiments, $G^L$ is selected from $G^{L1-1}$ and $G^{L1-2}$. In some of these embodiments, $G^L$ is $G^{L1-1}$.

*$G^{LL}$*

$G^{LL}$ may be selected from:

| | | | |
|---|---|---|---|
| ($G^{LL1-1}$) |  | ($G^{LL6}$) |  |
| ($G^{LL1-2}$) |  | ($G^{LL7}$) |  |
| ($G^{LL2}$) |  | ($G^{LL8-1}$) |  |

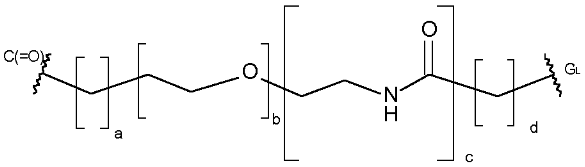
| | | | |
|---|---|---|---|
| ($G^{LL3-1}$) |  | ($G^{LL8-2}$) |  |
| ($G^{LL3-2}$) |  | ($G^{LL9-1}$) |  |
| ($G^{LL4}$) |  | ($G^{LL9-2}$) |  |
| ($G^{LL5}$) |  | | |

where Ar represents a $C_{5-6}$ arylene group, e.g. phenylene.

In some embodiments, $G^{LL}$ is selected from $G^{LL1-1}$ and $G^{LL1-2}$. In some of these embodiments, $G^{LL}$ is $G^{LL1-1}$.

*X*

X is:



,

where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5.

a may be 0, 1, 2, 3, 4 or 5. In some embodiments, a is 0 to 3. In some of these embodiments, a is 0 or 1. In further embodiments, a is 0.

b may be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16. In some embodiments, b is 0 to 12. In some of these embodiments, b is 0 to 8, and may be 0, 2, 4 or 8.

c may be 0 or 1.

d may be 0, 1, 2, 3, 4 or 5. In some embodiments, d is 0 to 3. In some of these embodiments, d is 1 or 2. In further embodiments, d is 2.

In some embodiments of X, a is 0, c is 1 and d is 2, and b may be from 0 to 8. In some of these embodiments, b is 0, 4 or 8.

In some embodiments of X, a, b and c are 0 and d is 2 or 5.

### $Q^X$

$Q^X$ comprises a dipeptide residue. The amino acids in the dipeptide may be any combination of natural amino acids and non-natural amino acids. In some embodiments, the dipeptide comprises natural amino acids. Where the linker is a cathepsin labile linker, the dipeptide is the site of action for cathepsin-mediated cleavage. The dipeptide then is a recognition site for cathepsin.

In one embodiment, -C(=O)-$Q^X$-NH- is selected from:

$^{CO}$-Phe-Lys-$^{NH}$,
$^{CO}$-Val-Ala-$^{NH}$,
$^{CO}$-Val-Lys-$^{NH}$,
$^{CO}$-Ala-Lys-$^{NH}$,
$^{CO}$-Val-Cit-$^{NH}$,
$^{CO}$-Phe-Cit-$^{NH}$,
$^{CO}$-Leu-Cit-$^{NH}$,
$^{CO}$-Ile-Cit-$^{NH}$,
$^{CO}$-Phe-Arg-$^{NH}$, and
$^{CO}$-Trp-Cit-$^{NH}$;

where Cit is citrulline.

Preferably, -C(=O)-$Q^X$-NH- is selected from:

$^{CO}$-Phe-Lys-$^{NH}$,
$^{CO}$-Val-Ala-$^{NH}$,
$^{CO}$-Val-Lys-$^{NH}$,
$^{CO}$-Ala-Lys-$^{NH}$,
$^{CO}$-Val-Cit-$^{NH}$.

Most preferably, -C(=O)-$Q^X$-NH- is selected from $^{CO}$-Phe-Lys-$^{NH}$, $^{CO}$-Val-Cit-$^{NH}$ and $^{CO}$-Val-Ala-$^{NH}$.

Other dipeptide combinations of interest include:

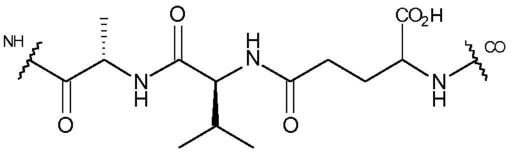
$^{CO}$-Gly-Gly-$^{NH}$,

$^{CO}$-Pro-Pro-$^{NH}$, and
$^{CO}$-Val-Glu-$^{NH}$.

Other dipeptide combinations may be used, including those described by Dubowchik et al., *Bioconjugate Chemistry*, 2002, 13,855-869, which is incorporated herein by reference.

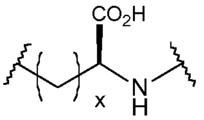
In Q, x can be 1 or 2.
In some embodiments, x in Q is 1.
In some embodiments, x in Q is 2

Thus, in some embodiments Q is:



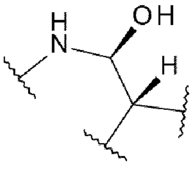
.

In Q, the carboxy group may be in the following stereochemical arrangement relative to neighbouring groups:

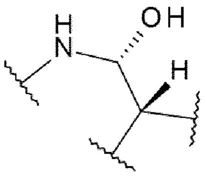


, such that it is derived from the appropriate natural amino acid.

In some embodiments of the present invention, the C11 substituent may be in the following stereochemical arrangement relative to neighbouring groups:



In other embodiments, the C11 substituent may be in the following stereochemical arrangement relative to neighbouring groups:



Compounds of particular interest include those of the examples.

## Examples

Flash chromatography was performed using a Biotage Isolera 1™ using gradient elution starting from either 88% hexane/EtOAc or 99.9% DCM/MeOH until all UV active components (detection at 214 and 254 nm) eluted from the column. The gradient was manually held whenever substantial elution of UV active material was observed. Fractions were checked for purity using thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel, with fluorescent indicator on aluminium plates. Visualisation of TLC was achieved with UV light or iodine vapour unless otherwise stated. Extraction and chromatography solvents were bought and used without further purification from VWR U.K. All fine chemicals were purchased from Sigma-Aldrich or TCI Europe unless otherwise stated. Pegylated reagents were obtained from Quanta biodesign US via Stratech UK.

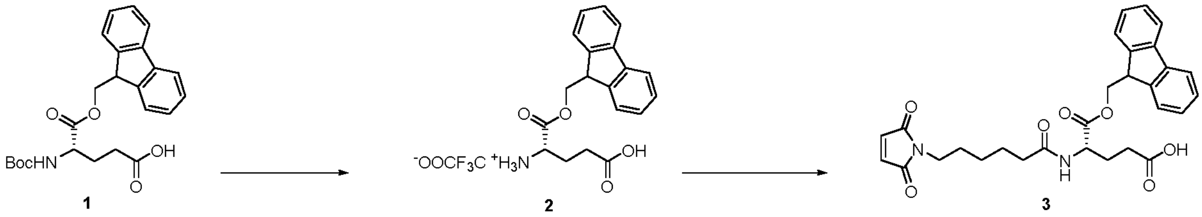
The analytical LC/MS conditions (for reaction monitoring and purity determination) were as follows: Positive mode electrospray mass spectrometry was performed using a Shimadzu Nexera®/Prominence® LCMS-2020. Mobile phases used were solvent A ($H_2O$ with 0.1% formic acid) and solvent B ($CH_3CN$ with 0.1% formic acid). Gradient for routine 3-minute run: Initial composition 5% B held over 25 seconds, then increased from 5% B to 100% B over a 1 minute 35 seconds' period. The composition was held for 50 seconds at 100% B, then returned to 5% B in 5 seconds and held there for 5 seconds. The total duration of the gradient run was 3.0 minutes. Gradient for 15-minute run: Initial composition 5% B held over 1 minute, then increased from 5% B to 100% B over a 9 minute period. The composition was held for 2 minutes at 100% B, then returned to 5% B in 10 seconds and held there for 2 minutes 50 seconds. The total duration of the gradient run was 15.0 minutes. Flow rate was 0.8 mL/minute (for 3-minute run) and 0.6 mL/minute (for 15-minute run). Detection was at 254 nm. Columns: Waters Acquity UPLC® BEH Shield RP18 1.7μm 2.1 x 50 mm at 50 °C fitted with Waters Acquity UPLC® BEH Shield RP18 VanGuard Pre-column, 130A, 1.7μm, 2.1 mm x 5 mm (routine 3-minute run); and ACE Excel 2 C18-AR, 2 μ, 3.0 x 100mm fitted with Waters Acquity UPLC® BEH Shield RP18 VanGuard Pre-column, 130A, 1.7μm, 2.1 mm x 5 mm (15-minute run).

The preparative HPLC conditions were as follows: Reverse-phase ultra-fast high-performance liquid chromatography (UFLC) was carried out on a Shimazdzu Prominence® machine using a Phenomenex® Gemini NX 5μ C18 column (at 50 °C) dimensions: 150 x 21.2 mm. Eluents used were solvent A ($H_2O$ with 0.1% formic acid) and solvent B ($CH_3CN$

with 0.1% formic acid). All UFLC experiments were performed with gradient conditions: Initial composition 13% B increased to 60% B over a 15 minute period then increased to 100% B over 2 minutes. The composition was held for 1 minute at 100% B, then returned to 13% B in 0.1 minute and held there for 1.9 minutes. The total duration of the gradient run was 20.0 minutes. Flow rate was 20.0 mL/minute and detection was at 254 and 280 nm.

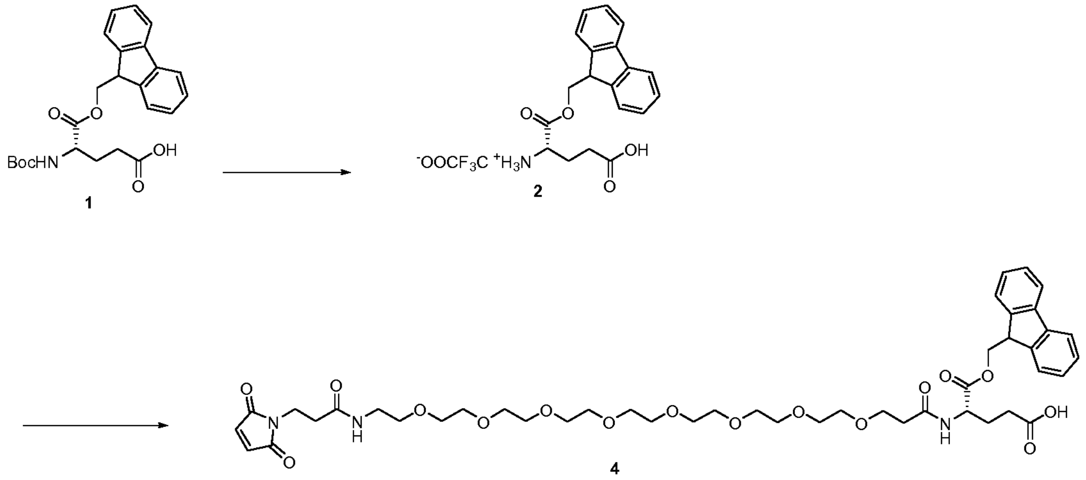
**Intermediate synthesis**

*(**S**)-5-((9H-fluoren-9-yl)methoxy)-4-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-5-oxopentanoic acid **3***



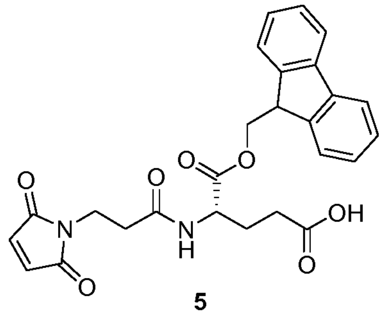
(R)-5-((9H-fluoren-9-yl)methoxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid **1** (0.271 g, 0.64 mmol, 1 eq.) was dissolved in a solution of trifluoroacetic acid in DCM (4 mL, 20% v/v). Stirred at room temperature for 2h. The solution was evaporated, azeotroping with toluene (x3) to remove trifluoroacetic acid. The resulting TFA salt **2** was suspended in DCM (10 mL) and 6-maleimidohexanoic acid N-hydroxysuccinimide ester (0.196 g, 0.64 mmol, 1 eq.) and DIPEA (0.25 g, 333 µl, 1.9 mmol, 3 eq.) were added. The mixture was stirred at room temperature for 18h, diluted with DCM (50 mL) and extracted with 1M citric acid solution (2 x 50 mL), brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give the product **3** as a white foam (0.313 g, 95%). Analytical Data: RT 1.49 min; MS ($ES^+$) *m/z* (relative intensity) 519 ([*M* + H]$^{+\cdot}$,100)

*(**S**)-33-(((9H-fluoren-9-yl)methoxy)carbonyl)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,31-dioxo-7,10,13,16,19,22,25,28-octaoxa-4,32-diazahexatriacontan-36-oic acid **4***



(**S**)-5-((9H-fluoren-9-yl)methoxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid **1** (1.254 g, 2.9 mmol, 1 eq.) was dissolved in a solution of trifluoroacetic acid in DCM (18 mL, 20% v/v). Stirred at room temperature for 4h. The solution was evaporated, azeotroping with toluene (x3) to remove trifluoroacetic acid giving the product as a white solid. A portion of the resulting glutamic acid TFA salt **2** (0.313 g, 0.61 mmol, 1eq.) was suspended in DCM (30 mL) and 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22,25,28-octaoxa-4-azahentriacontan-31-oate (0.418 g, 0.61 mmol, 1 eq.) and DIPEA (0.234 g, 317 µl, 1.8 mmol, 3 eq.) were added. The mixture was stirred at room temperature for 18h. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography [$CHCl_3$/MeOH 0% to 8% in 1% increments] to give the product as a colourless oil (0.22 g) along with fractions mixed with unreacted TFA salt. The mixed fractions were dissolved in DCM, washed with water, brine, dried ($MgSO_4$) and evaporated under reduced pressure to give further product (0.109 g). Combined yield of **4** (0.329 g, 51%).

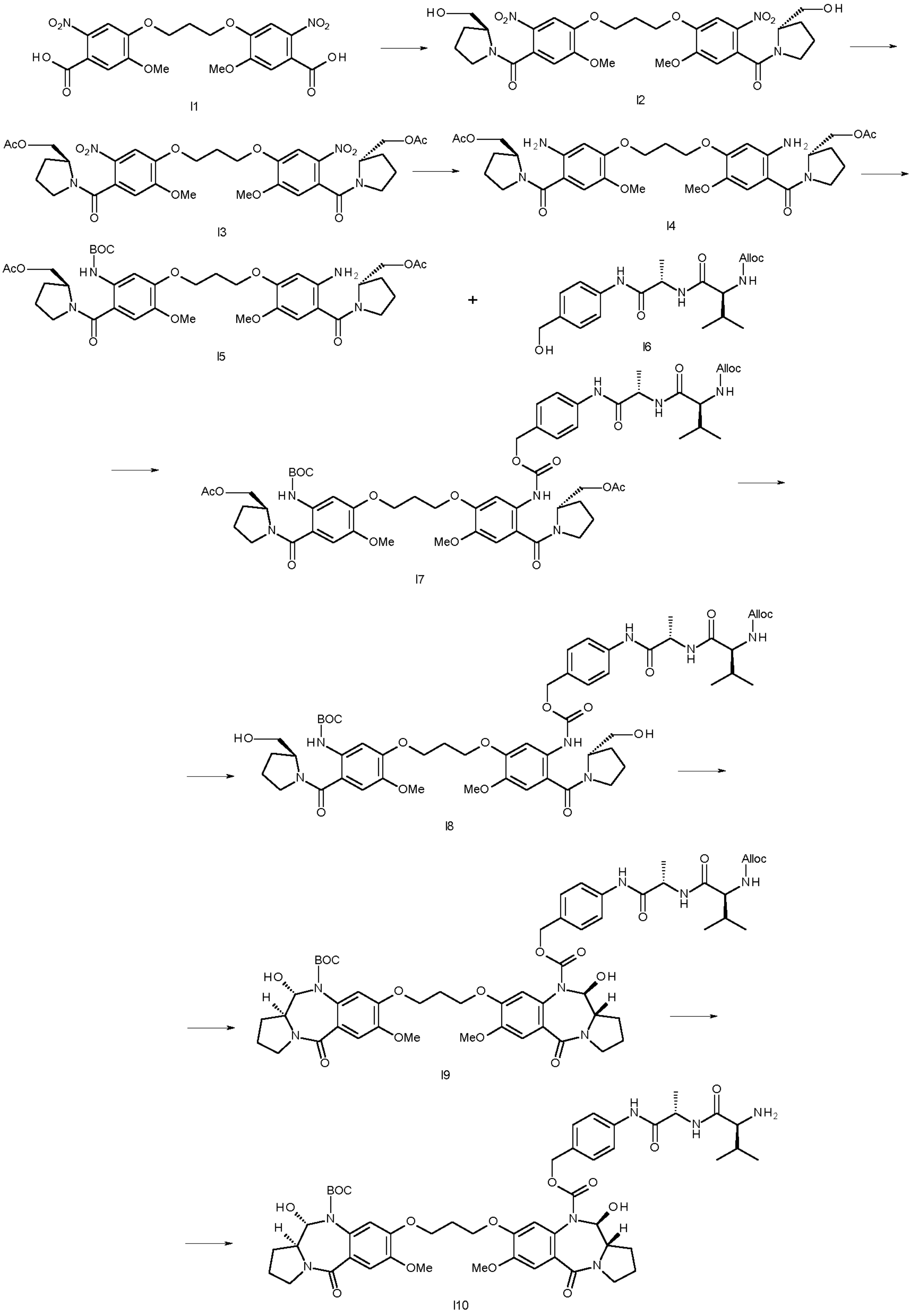
*(4S)-4-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]-5-(9H-fluoren-9-ylmethoxy)-5-oxo-pentanoic acid **5***



**5**

**5** was synthesised by an analogous method to **3** and **4** above.

*4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl(11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c[1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate* ***(I10)***



*(a) ((Propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(hydroxymethyl)pyrrolidin-1-yl)methanone) (**I2**)*

DMF (12 drops) was added to a stirred suspension of the *bis*-nitrobenzoic acid **I1** (10 g, 21.5 mmol) and oxalyl chloride (5.6 mL, 8.2 g, 64.5 mmol) in anhydrous DCM (150 mL). Following initial effervescence the reaction suspension became a solution and the mixture was allowed to stir at room temperature for 16 hours. The majority of solvent was removed by evaporation *in vacuo* and the resulting concentrated solution was re-dissolved in a minimum amount of dry DCM and triturated with diethyl ether. The resulting yellow precipitate was collected by vacuum filtration, washed with cold diethyl ether and dried for 1 hour in a vacuum oven at 40°C. The solid acid chloride was added portion-wise to a stirred suspension of (*S*)-(+)-2-pyrrolidinemethanol (5.0 g, 4.9 mL, 49.5 mmol) and TEA (15.0 mL, 10.9 g, 108 mmol) in DCM (100mL) at -40°C (dry ice/$CH_3CN$). After 1 hour stirring, the reaction was complete as judged by LC/MS with exclusively desired product at retention time 1.33 minutes, ES+ *m/z* 655 $[M+\text{Na}]^{+\cdot}$, 633 $[M+\text{H}]^{+\cdot}$. The mixture was diluted with DCM (100 mL) and washed with 1N HCl (2 x 50 mL), saturated $NaHCO_3$ (3 x 40 mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the pure product **I2** as a yellow solid (13.6 g, 100% yield).

*(b) ((2S,2'S)-(4,4'-(propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitrobenzoyl))bis(pyrrolidine-1,2-diyl))bis(methylene) diacetate (**I3**)*

A solution of $Ac_2O$ (4.47 mL, 4.83 g, 47.3 mmol) in dry DCM (25 mL) was added drop-wise to a stirred solution of the *bis*-alcohol **I2** (13.6 g, 21.5 mmol), DMAP (263 mg, 2.15 mmol) and pyridine (4.17 mL, 4.08 g, 51.6 mmol) in dry DCM (125mL) at 0°C (ice/acetone) under an argon atmosphere. The reaction mixture was allowed to warm-up and after 1 hour at room temperature analysis by LC/MS revealed completion of reaction and clean conversion to desired product at retention time 1.55 minutes, ES+ *m/z* 740 $[M+\text{Na}]^{+\cdot}$, 717 $[M+\text{H}]^{+\cdot}$. The mixture was diluted with DCM (20 mL) and washed with 1N HCl (2 x 100 mL), $H_2O$ (25 mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the crude *bis*-acetate **I3** as a yellow solid (14.4 g, 94% yield) which was of satisfactory purity to be carried through to the next step without further purification.

*(c) ((2S,2'S)-(4,4'-(propane-1,3-diylbis(oxy))bis(2-amino-5-methoxybenzoyl))bis(pyrrolidine-1,2-diyl))bis(methylene) diacetate (**I4**)*

A sample of 10% Pd-C (132 mg) was treated carefully with EtOAc (10 mL) to give a slurry which was added to a solution of the nitro compound **I3** (1.32 g, 1.84 mmol) in EtOAc (20 mL) and EtOH (30 mL) in a hydrogenation vessel. Using Parr® apparatus, the mixture was treated with hydrogen gas to 10 psi and shaken at room temperature then degassed *in vacuo*, this process was repeated a further two times. The vessel was filled with hydrogen gas to 45 psi, shaken and the pressure maintained upon consumption of hydrogen. Analysis by LC/MS showed the reaction was incomplete after 3 hours and was left shaking at 45 psi for 3 days (the weekend) after which time satisfactory conversion to product was achieved, retention time = 1.32 minutes, ES+ *m/z* 657 [*M* + H]$^{+\cdot}$. The reaction mixture was degassed *in vacuo* and then filtered through a celite® pad. The filtrate was evaporated *in vacuo*, the resulting residue re-dissolved in DCM (30 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the crude *bis*-aniline **I4** as a yellowish foam (1.1 g, 91% yield) which contained an 8% impurity but was carried through to the next step without further purification.

*(d) ((S)-1-(4-(3-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-((tert-butoxycarbonyl)amino)-2-methoxyphenoxy)propoxy)-2-amino-5-methoxybenzoyl)pyrrolidin-2-yl)methyl acetate (**I5**)*

$Boc_2O$ (330 mg, 1.51 mmol) was added to a stirred solution of the *bis*-aniline **I4** (1.1 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was heated and stirred at 75°C for 16 hours. Analysis by LC/MS revealed desired mono Boc product **I5** at retention time 1.58 minutes, I% = 50, ES+ *m/z* 779 [*M*+ Na]$^{+\cdot}$, 757 [*M*+ H]$^{+\cdot}$ along with unreacted starting material at retention time 1.32 minutes, I% = 30, and bis-Boc material at retention time 1.81 minutes, I% = 21, ES+ *m/z* 879 [*M*+ Na]$^{+\cdot}$,857 [*M*+ H]$^{+\cdot}$. The reaction mixture was allowed to cool to room temperature and the THF removed by evaporation *in vacuo*. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 50 g, 100 mL per minute) provided the mono Boc product **I5** as an orange foam (519 mg, 46% yield based on $Boc_2O$, eluting at 97%DCM/MeOH) unreacted *bis*-aniline **I4** (285 mg, eluting at 95% DCM/MeOH) and *bis*-Boc (248 mg, eluting at 98% DCM/MeOH).

*(e) ((S)-1-(4-(3-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-2-((tert-butoxycarbonyl)amino)-5-methoxybenzoyl)pyrrolidin-2-yl)methyl acetate (**I7**)*

Triphosgene (380 mg, 1.28 mmol) was added to a stirred solution of the mono Boc product **I5** (2.69 g, 3.56 mmol) and TEA (1.09 mL, 791 mg, 7.83 mmol) in dry DCM (30 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 1.66 minutes, ES+ *m/z* 837 [*M*+ Na]$^{+\cdot}$, 815 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional TEA (740 µL, 539 mg, 5.34 mmol) followed by the addition of linker **I6** (1.34 g, 3.56 mmol). After 2 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **I7** (retention time 1.74 minutes, (ES+) *m/z* 1182 [*M*+ Na]$^{+\cdot}$, 1160 [*M*+ H]$^{+\cdot}$). The mixture was diluted with DCM (80 mL) and washed with saturated $NH_4Cl$ (2 x 30 mL), $H_2O$ (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **I7** (eluting at 65% Hexane/EtOAc) as a yellow foam (2.95 g, 71% yield).

*(f) tert-butyl (5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**I8**)*

Solid $K_2CO_3$ (1.75 g, 12.7 mmol) was added to a stirred solution of the acetate-protected compound **I7** (2.93 g, 2.53 mmol) in MeOH (60 mL) and $H_2O$ (12 mL). After 1 hour stirring at room temperature the reaction was deemed to be complete as judged by LC/MS with desired product at retention time 1.57 minutes, ES+ *m/z* 1098 [*M*+ Na]$^{+\cdot}$, 1076 [*M*+ H]$^{+\cdot}$. The MeOH was removed by evaporation *in vacuo* and the resulting residue was partitioned between water (75 mL) and DCM (75 mL). The layers were separated and the aqueous phase was extracted with DCM (3 x 25 mL). The combined organic layers were washed with water (3 x 50 mL), brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) the *bis*-alcohol **I8** (eluting at 97% DCM/MeOH) as a white foam (2.44 g, 90% yield).

*(g) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl(11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**I9**)*

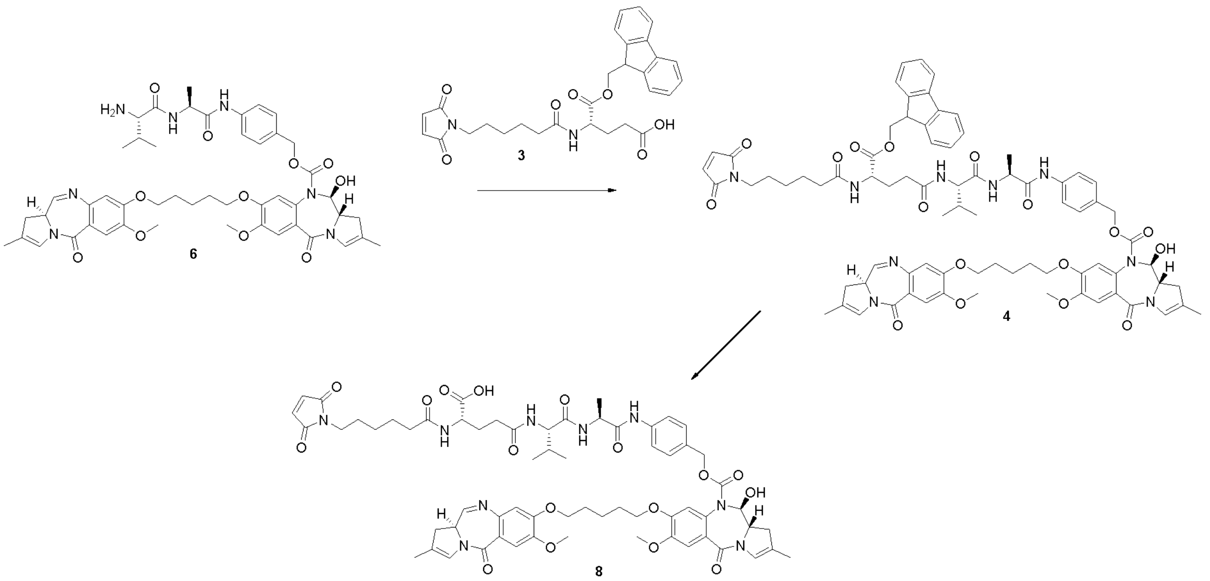
A solution of anhydrous DMSO (710 µL, 780 mg, 9.99 mmol) in dry DCM (20 mL) was added drop-wise to a stirred solution of oxalyl chloride (2.72 mL of a 2.0M solution in DCM, 5.44 mmol) in dry DCM (20 mL) at -45°C (dry ice/$CH_3CN$) under an argon atmosphere. After 15 minutes stirring at -45°C, the reaction mixture was treated drop-wise with a solution of the *bis*-alcohol **I8** (2.44 g, 2.27 mmol) in dry DCM (30 mL). After stirring at -45°C for a further 1 hour, the reaction mixture was treated drop-wise with a solution of TEA (3.16

mL, 2.29 g, 22.7 mmol) in dry DCM (20 mL). The reaction mixture was allowed to warm to room temperature over a period of 1.5 hours and diluted with DCM (100 mL) then washed with saturated $NH_4Cl$ (2 x 50 mL), saturated $NaHCO_3$ (50 mL), water (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the cyclised compound **I9** (eluting at 95.7% DCM/MeOH) as a yellowish foam (1.61 g, 66% yield): LC/MS **I9** at retention time 1.46 minutes, ES+ *m/z* 1072 [*M*+ H]$^{+\cdot}$, 1094 [*M*+ Na]$^{+\cdot}$.

*(h) 4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl(11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c[1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**I10**)*

$Pd(PPh_3)_4$ (6.47 mg, 5.6 µmol) was added to a stirred solution of pyrrolidine (29 µL, 25 mg, 0.35 mmol) and the Alloc compound **I9** (300 mg, 0.28 mmol) in dry DCM (10 mL). After stirring for 4 hours under argon at room temperature, analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.10 minutes, ES+, *m/z* 1010 [*M*+ Na]$^{+\cdot}$ , 988 [*M*+ H]$^{+\cdot}$. The reaction mixture was diluted with DCM (30 mL) then washed with saturated $NH_4Cl$ (2 x 20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Trituration with diethyl ether followed by evaporation *in vacuo* gave the crude amine **I10** (261 mg, 95% yield) which was carried through to the next step without further purification or analysis.

**Example 1**



Compound **6** is Compound 23 of WO2014/057074

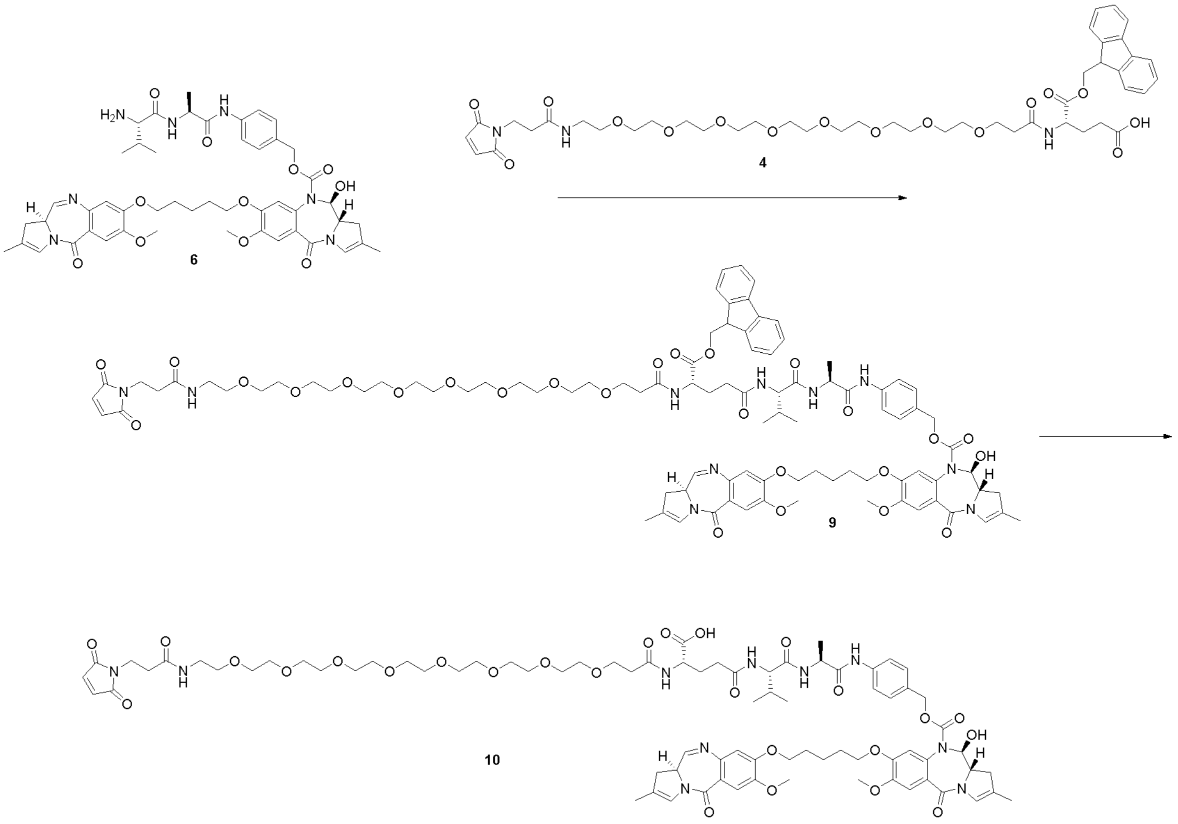
*a) 4-((**S**)-2-((**S**)-2-((**S**)-5-((9H-fluoren-9-yl)methoxy)-4-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-5-oxopentanamido)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-11-hydroxy-7-methoxy-8-((5-(((**S**)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **7***

Acid **6** (0.153 g, 0.295 mmol, 1.1 eq.) and EDCI hydrochloride (0.077 g, 0.4 mmol, 1.5 eq.) were added to a solution of SG3305 (0.247 g, 0.27 mmol, 1 eq.) in $CHCl_3$ (20 mL). The solution was stirred at room temperature for 3h when reaction was shown to be complete by LCMS. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by flash column chromatography [$CHCl_3$/MeOH 0% to 4% in 1% increments] to give the product **7** as a yellow solid (0.275 g, 72%). Analytical Data: RT 1.68 min; MS (ES$^+$) *m/z* (relative intensity) 1422 ([*M* + H]$^{+\cdot}$,2)

*b) N2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)-N5-((**S**)-1-(((**S**)-1-((4-((((11S,11a**S**)-11-hydroxy-7-methoxy-8-((5-(((**S**)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-L-glutamine **8***

A solution of N-Methyl pyrrolidine in $CHCl_3$ (2 mL, 10% v/v, 30 eq.) was added to a solution of Fmoc protected compound **7** (0.130 g, 91 μmol, 1eq) in $CHCl_3$ (2 mL). The solution was stirred at room temperature for 5h. The reaction mixture was evaporated under reduced pressure triturating with diethyl ether (x3). Purification by prep HPLC followed by lyophilisation gave the product as a white foam (0.041 g, 36%). Analytical Data: RT 1.48 min; MS (ES$^+$) *m/z* (relative intensity) 1245 ([*M* + H]$^{+\cdot}$)

**Example 2**



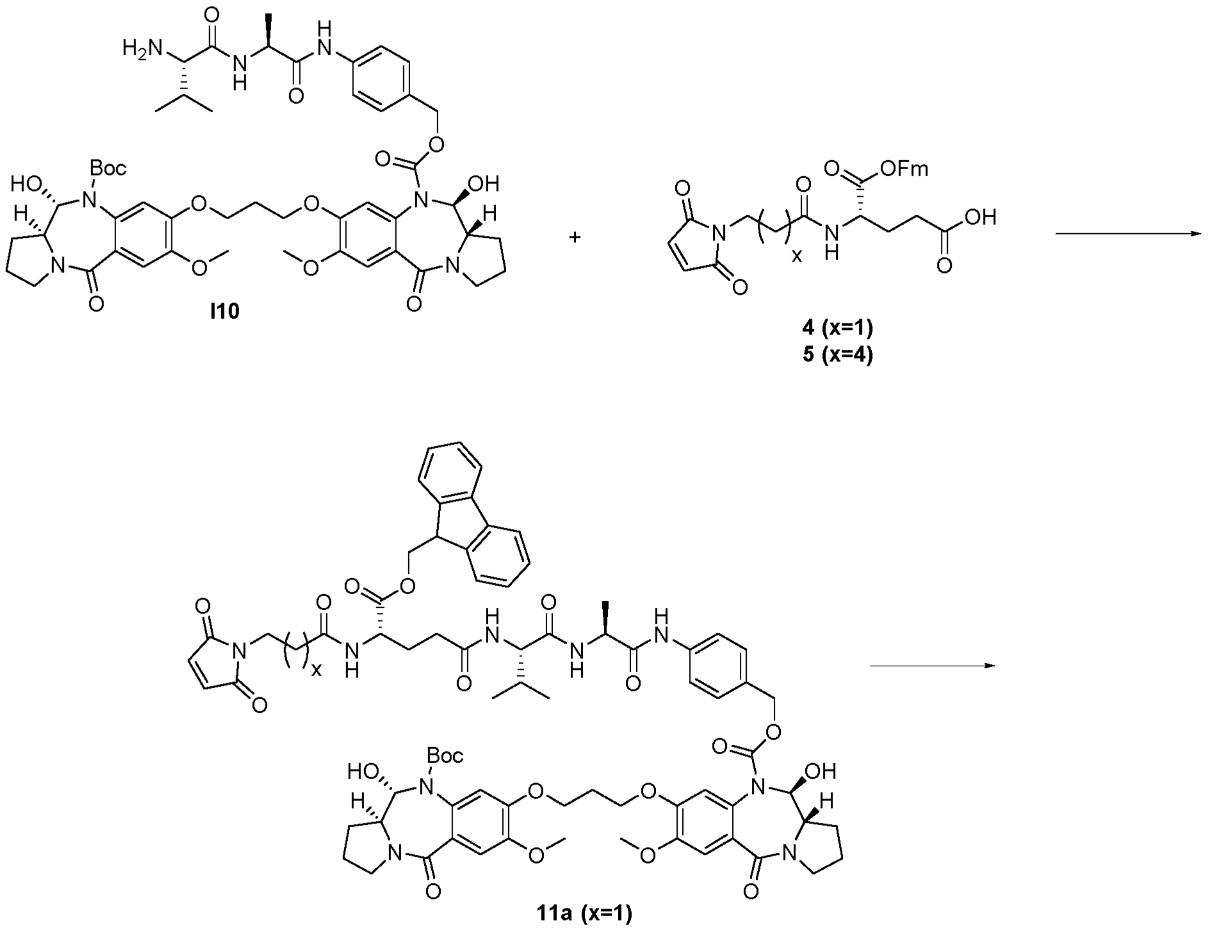
*a) 4-((2**S**,5**S**,10**S**)-10-(((9H-fluoren-9-yl)methoxy)carbonyl)-42-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,12,40-tetraoxo-15,18,21,24,27,30,33,36-octaoxa-3,6,11,39-tetraazadotetracontanamido)benzyl (11**S**,11a**S**)-11-hydroxy-7-methoxy-8-((5-(((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **9***

Acid **4** (0.108 g, 0.2 mmol, 1 eq.) and EDCI hydrochloride (0.057 g, 0.3 mmol, 1.5 eq.) were added to a solution of **6** (0.184 g, 0.2 mmol, 1 eq.) in $CHCl_3$ (10 mL). The solution was stirred at room temperature for 18h when reaction was shown to be complete by LCMS. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by flash column chromatography [$CHCl_3$/MeOH 0%] to give the product as a colourless oil (0.064 g, 18%).

*b) N2-(1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22,25,28-octaoxa-4-azahentriacontan-31-oyl)-N5-((**S**)-1-(((**S**)-1-((4-((((11**S**,11a**S**)-11-hydroxy-7-methoxy-8-((5-(((**S**)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-L-glutamine **10***

A solution of N-Methyl pyrrolidine in $CHCl_3$ (0.9 mL, 10% v/v, 30 eq.) was added to a solution of Fmoc protected compound **9** (0.055 g, 30 μmol, 1eq) in $CHCl_3$ (2 mL). The solution was stirred at room temperature for 18h. The reaction mixture was evaporated under reduced pressure triturating with diethyl ether (x3). Purification by prep HPLC followed by lyophilisation gave the product as a white foam (0.0125 g, 35%). Analytical Data: RT 1.37 min; MS ($ES^+$) *m/z* (relative intensity) 1626 ($[M + H]^{+\cdot}$)

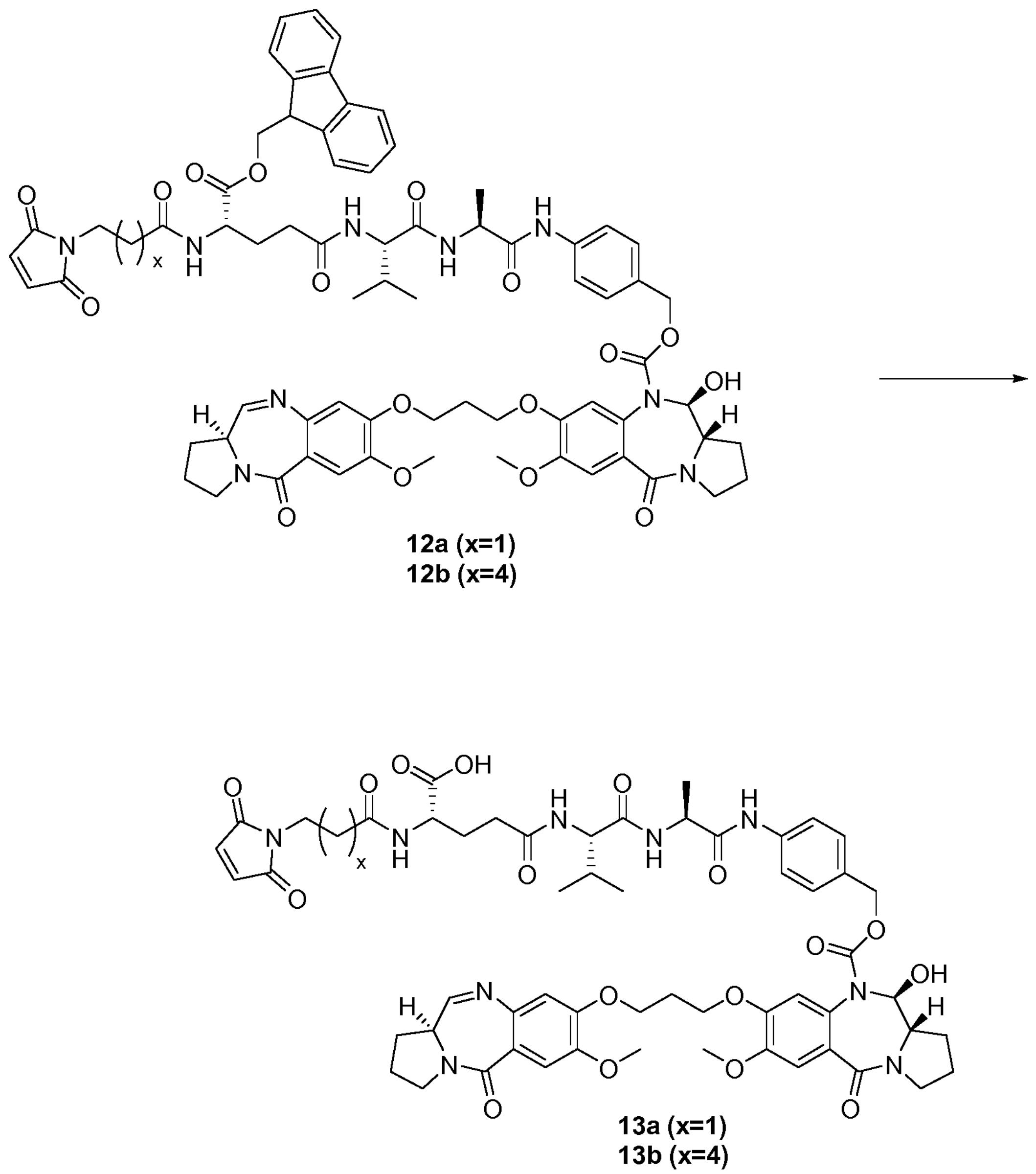
## Example 3



**I10**

**4 (x=1)**
**5 (x=4)**

**11a (x=1)**
**11b (x=4)**



**12a (x=1)**
**12b (x=4)**

**13a (x=1)**
**13b (x=4)**

*a) General method for amide coupling (**I10** to **11**)*

EDCI.HCl (0.28 mmol, 1.25eq) was added to a solution of **I10** (0.22 mmol, 1.0eq) and **2** (0.27 mmol, 1.2eq) in chloroform (10 mL) and the resulting mixture stirred at room temperature for 2 hrs. The reaction mixture was washed with water (20 mL), dried (biotage) and evaporated to leave a white solid which was purified by column.

*i) 4-((S)-2-((S)-2-((S)-5-((9H-fluoren-9-yl)methoxy)-4-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-5-oxopentanamido)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **11a*** (methanol / DCM, 4/96 v/v) to leave **11a** as a white solid. Yield = 275 mg (86%). LC/MS rt 1.80 min *m/z* (1448.0) M+H.

*ii) 4-((S)-2-((S)-2-((S)-5-((9H-fluoren-9-yl)methoxy)-4-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-5-oxopentanamido)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **11b***

(methanol / DCM, 4/96 v/v) to leave **11b** as a white solid. Yield = 256 mg (81%). LC/MS rt 1.85 min *m/z* (1489.7) M+H.

*b) General method for Boc deprotection of **11** to **12***

TFA (1.8 mL) and water (0.2 mL) were added to a solution of **3** (0.18 mmol) in water (0.2 mL) and stirred at 0°C for 30 mins. The solvent was removed under vacuum and saturated sodium hydrogen carbonate was added to neutralise the reaction, along with DCM to aid solubility. The organic phase was removed, dried (biotage) and evaporated. The resulting residue was purified by column.

*i) 4-((S)-2-((S)-2-((S)-5-((9H-fluoren-9-yl)methoxy)-4-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-5-oxopentanamido)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **12a***

(gradient: methanol / DCM, 2/98 to 7/93 v/v) to leave **12a** as a white solid. Yield = 256 mg (81%). LC/MS rt 1.85 min *m/z* (1489.7) M+H.

*ii) 4-((S)-2-((S)-2-((S)-5-((9H-fluoren-9-yl)methoxy)-4-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-5-oxopentanamido)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **13b***

(methanol / chloroform, 5/95 v/v) to leave **13b** as a white solid. Yield = 181 mg (79%). LC/MS rt 1.79 min *m/z* (1317.1) M+H.

*c) General method of acid deprotection of **12** to **13***

1-Methylpyrrolidine (250 µL) was added to a solution of **12** (0.13 mmol) in DMF (2.5 mL) and stirred at room temperature for 10 mins. The solvent was removed under vacuum and the residue purified by prep HPLC (30-35% MeCN / water + 0.1% formic acid over 10 mins). Fractions containing product were freeze dried to give an off-white solid.

*i) N2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)-N5-((S)-1-(((S)-1-((4-((((11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-L-glutamine **13a***

Yield = 23 mg (15%). LC/MS rt 1.46 min *m/z* (1151.3) M+H.

*ii) N2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)-N5-((S)-1-(((S)-1-((4-((((11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-L-glutamine **13b***

Yield = 60 mg (38%). LC/MS rt 1.50 min *m/z* (1193.3) M+H.

## Example 4 - Conjugation

*Conjugate Tratuzumab-13a (ConjA)*

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 16 micromoles, 320 µL) to a 7.5 mL solution of tratuzumab (30 mg, 200 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4.0 mg/mL. The reduction mixture was allowed to react at room temperature for 4 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. The reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 4 micromoles, 80 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (60 rpm) shaking at an antibody concentration of 3.5 mg/mL (or more DHAA added and reaction left for longer until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 3.0 mg/mL. Compound **13a** was added as a DMSO solution (10 molar equivalent/antibody, 2 micromoles, in 1.0 mL DMSO) to 9.0 mL of this reoxidised antibody solution (30 mg, 200 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (15 micromoles, 150 μL at 100 mM), then purified by spin filtration using a 15 mL Amicon Ultracell 30 kDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Thermo Scientific MAbPac 50 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjA at 214 nm and 330 nm (Compound **13a** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **13a**, consistent with a drug-per-antibody ratio (DAR) of 1.77 molecules of Compound **13a** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjA at 280 nm shows a monomer purity of 98%. UHPLC SEC analysis gives a concentration of final ConjA at 1.96 mg/mL in 11.5 mL, obtained mass of ConjA is 22.6 mg (75% yield).

*Conjugate Tratuzumab-13a (ConjB)*

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (180 molar equivalent/antibody, 36 micromoles, 720 μL) to a 7.5 mL solution of tratuzumab (30 mg, 200 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4 mg/mL. The reduction mixture was allowed to react at room temperature for 4 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. The reduced antibody solution was buffer exchanged (to remove all the excess reducing agent), *via* spin filter centrifugation, into a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 2.5 mg/mL. Compound **13a** was added as a DMSO solution (15 molar equivalent/antibody, 3 micromoles, in 1.2 mL DMSO) to 10.8 mL of this reduced antibody solution (30 mg, 200 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 16 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (15 micromoles, 150 μL at 100 mM), then purified *via* spin filter centrifugation using a 15mL Amicon Ultracell 30KDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Thermo Scientific MAbPac 50 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjB at 214 nm and 330 nm (Compound **13a** specific) shows a mixture of unconjugated light chains, light chains attached to a single molecule of Compound **13a**, unconjugated heavy chains and heavy chains attached to up to three molecules of Compound **13a**, consistent with a drug-per-antibody ratio (DAR) of 7.82 molecules of Compound **13a** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 μm 4.6 x 150 mm column (with a 4 μm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjB at 280 nm shows a monomer purity of 96.5%. UHPLC SEC analysis gives a concentration of final ConjA at 2.40 mg/mL in 11.9 mL, obtained mass of ConjB is 28.6 mg (95% yield).

*Herceptin-C239i antibody*

Herceptin antibodies were engineered to have cysteine inserted between the 239 and 240 positions were produced following the methods described in Dimasi, N., et al., Molecular Pharmaceutics, 2017, 14, 1501-1516 (DOI: 5 10.1021/acs.molpharmaceut.6b00995).

*Conjugate Herceptin-8 (ConjC)*

Herceptin-C239i antibody (30 mg) was loaded onto solid support and reduced, reoxidised, conjugated to Compound 8, purified, released from the resin and formulated onto 25 mM Histidine, 200 mM Sucrose, Tween-20 0.02%, pH 6.0 according to patent # US 2014/038041 A1.

UHPLC analysis on a Shimadzu Prominence system using a Thermo Scientific MAbPac 50 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjC at 214 nm and 330 nm (Compound 8 specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound 8, consistent with a drug-per-antibody ratio (DAR) of 1.9 molecules of Compound 8 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjC at 280 nm shows a monomer purity of 95%. UHPLC SEC analysis gives a concentration of ConjC at 2.2 mg/mL in 5.85 mL, obtained mass of ConjC is 12.9 mg (43% yield).

## Example 5 – Xenograft testing

NCI-N87 Xenografted Mice

Female severe combined immune-deficient mice (Fox Chase SCID®, C.B-17/Icr-*Prkdcscid*, Charles River) were ten weeks old with a body weight (BW) range of 16.5 to 21.1 grams on Day 1 of the study. The animals were fed *ad libitum* water (reverse osmosis, 1 ppm Cl), and NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% crude protein, 5.0% crude fat, and 5.0% crude fibre. The mice were housed on irradiated Enricho'cobs ™ Laboratory Animal Bedding in static micro-isolators on a 12-hour light cycle at 20–22°C (68–72°F) and 40–60% humidity. CR Discovery Services specifically complies with the recommendations of the *Guide for Care and Use of Laboratory Animals* with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care. The animal care and use program at CR Discovery Services is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), which assures compliance with accepted standards for the care and use of laboratory animals.

*Tumour Cell Culture*

Human NCI-N87 gastric carcinoma lymphoma cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin sulfate and 25 µg/mL gentamicin. The cells were grown in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% $CO_2$ and 95% air.

*In Vivo Implantation and Tumour Growth*

The NCI-N87 cells used for implantation were harvested during log phase growth and Re-suspended in phosphate buffered saline (PBS) containing 50% Matrigel™ (BD Biosciences). On the day of tumour implant, each test mouse was injected subcutaneously in the right flank with 1 x $10^7$ cells (0.1 mL cell suspension), and tumour growth was monitored as the average size approached the target range of 100 to 150 mm$^3$. Fourteen days later, designated as Day 1 of the study, mice were sorted according to calculated tumour size into groups each consisting of ten animals with individual tumour volumes ranging from 108 to 144 mm$^3$ and group mean tumour volumes of 115 mm$^3$.

Tumours were measured in two dimensions using calipers, and volume was calculated using the formula:

Tumour Volume (mm$^3$ ) = $\frac{w^2 \, x \, l}{2}$

where *w* = width and *l* = length, in mm, of the tumour. Tumour weight may be estimated with the assumption that 1 mg is equivalent to 1 mm$^3$ of tumour volume.

*Treatment*

Treatment began on Day 1 in groups of 10 mice (n=10) with established subcutaneous NCI-N87 tumours (108–144 mm$^3$). ConjA (6 mg/kg) and ConjB (3 mg/kg) were administered intravenously once on Day 1 (qd x 1). A vehicle-treated group served as the control group for efficacy analysis. Tumours were measured twice per week until the study was ended on Day 83. Each mouse was euthanized when its tumour reached the endpoint volume of 800 mm$^3$ or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

The results are illustrated in Figure 1 which shows the change in normalised tumour growth.

**Endpoint and Tumor Growth Delay (TGD) Analysis**

Tumors were measured using calipers twice per week, and each animal was euthanized when its tumor reached the endpoint volume of 800 mm$^3$ or at the end of the study (Day 82), whichever came first. Animals that exited the study for tumor volume endpoint were documented as euthanized for tumor progression (TP), with the date of euthanasia. The time to endpoint (TTE) for analysis was calculated for each mouse by the following equation:

$$\mathrm{TTE} = \frac{\log_{10}(\text{endpoint volume}) - \mathrm{b}}{\mathrm{m}}$$

where TTE is expressed in days, endpoint volume is expressed in mm$^3$, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set consisted of the first observation that exceeded the endpoint volume used in analysis and the three consecutive observations that immediately preceded the attainment of this endpoint volume. The calculated TTE is usually less than the TP date, the day on which the animal was euthanized for tumor size. Animals with tumors that did not reach the endpoint volume were assigned a TTE value equal to the last day of the study (Day 82). In instances in which the log-transformed calculated TTE preceded the day prior to reaching endpoint or exceeded the day of reaching tumor volume endpoint, a linear interpolation was performed to approximate the TTE. Any animal classified as having died from NTR (non-treatment-related) causes due to accident (NTRa) or due to unknown etiology (NTRu) were excluded from TTE calculations (and all further analyses). Animals classified as TR (treatment-related) deaths or NTRm (non-treatment-related death due to metastasis) were assigned a TTE value equal to the day of death. Treatment outcome was evaluated from tumor growth delay (TGD), which is defined as the increase in the median time to endpoint (TTE) in a treatment group compared to the control group:

$$\mathrm{TGD} = \mathrm{T} - \mathrm{C},$$

expressed in days, or as a percentage of the median TTE of the control group:

$$\%\mathrm{TGD} = \frac{\mathrm{T} - \mathrm{C}}{\mathrm{C}} \text{ x } 100$$

where:

T = median TTE for a treatment group, and

C = median TTE for the designated control group.

### Tumour growth inhibition

Tumor growth inhibition (TGI) analysis evaluates the difference in median tumor volumes (MTVs) of treated and control mice. For this study, the endpoint for determining TGI was Day 33, which was the last day that all evaluable control mice remained in the study. The MTV (n), the median tumor volume for the number of animals, n, on the day of TGI analysis, was determined for each group. Percent tumor growth inhibition (%TGI) was defined as the difference between the MTV of the designated control group and the MTV of the drug-treated group, expressed as a percentage of the MTV of the control group:

$$\%\mathrm{TGI} = \left(\frac{\mathrm{MTV}_{\mathrm{control}} - \mathrm{MTV}_{\mathrm{drug\text{-}treated}}}{\mathrm{MTV}_{\mathrm{control}}}\right) \mathrm{x}100 = [1 - (\mathrm{MTV}_{\mathrm{drug\text{-}treated}}/\mathrm{MTV}_{\mathrm{control}})] \text{ x } 100$$

The data set for TGI analysis included all animals in a group, except those that died due to treatment-related (TR) or non-treatment-related (NTR) causes prior to the day of TGI analysis.

## MTV and Criteria for Regression Responses

Treatment efficacy may be determined from the tumor volumes of animals remaining in the study on the last day. The MTV (n) was defined as the median tumor volume on the last day of the study in the number of animals remaining (n) whose tumors had not attained the endpoint volume. Treatment efficacy may also be determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 $mm^3$ for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 $mm^3$ for three consecutive measurements during the course of the study. Animals were scored only once during the study for a PR or CR event and only as CR if both PR and CR criteria were satisfied. An animal with a CR response at the termination of a study was additionally classified as a tumor-free survivor (TFS). Animals were monitored for regression responses.

## Toxicity

Animals were weighed daily on Days 1–5, then twice per week until the completion of the study. The mice were observed frequently for overt signs of any adverse, treatment-related (TR) side effects, and clinical signs were recorded when observed. Individual body weight was monitored as per protocol, and any animal with weight loss exceeding 30% for one measurement or exceeding 25% for three consecutive measurements was euthanized as a TR death. Group mean body weight loss was also monitored according to CR Discovery Services protocol. Acceptable toxicity was defined as a group mean body weight (BW) loss of less than 20% during the study and no more than 10% TR deaths. Dosing was suspended in any group where mean weight loss exceeded acceptable limits. If group mean body weight recovered to acceptable levels, then dosing was modified to lower levels and/or reduced frequency then resumed. Deaths were classified as TR if it was attributable to treatment side effects as evidenced by clinical signs and/or necropsy. A TR classification was also assigned to deaths by unknown causes during the dosing period or within 14 days of the last dose. A death was classified as non-treatment-related (NTR) if there was no evidence that death was related to treatment side effects. NTR deaths are further categorized as follows: NTRa describes deaths due to accidents or human error; NTRm is assigned to deaths thought to result from tumor dissemination by invasion and/or metastasis based on necropsy results; NTRu describes deaths of unknown causes that lack available evidence of death related to metastasis, tumor progression, accident or human error. It should be noted that treatment side effects cannot be excluded from deaths classified as NTRu.

**Statistical and Graphical Analyses**

GraphPad Prism 8.0 for Windows was used for all statistical analysis and graphical presentations. Study groups experiencing toxicity beyond acceptable limits (>20% group mean body weight loss or greater than 10% treatment-related deaths) or having fewer than five evaluable observations, were not included in the statistical analysis. The logrank test was employed to assess the significance of the difference between the overall survival experiences of two groups. The logrank test analyzes the individual TTEs for all animals in a group, except those lost to the study due to NTR death. Statistical analyses of the differences between Day 33 median tumor volumes (MTVs) of control and treated groups were accomplished using the Mann-Whitney *U*-test. For statistical analyses, two-tailed tests were conducted at significance level $P = 0.05$. Prism summarizes test results as not significant (ns) at $P > 0.05$, significant (symbolized by "*") at $0.01 < P \leq 0.05$, very significant ("**") at $0.001 < P \leq 0.01$, and extremely significant ("***") at $P \leq 0.001$. Because tests of statistical significance do not provide an estimate of the magnitude of the difference between groups, all levels of significance were described as either significant or not significant within the text of this report. A scatter plot was constructed to show TTE values for individual mice, by group. Tumor growth curves show group median, mean and individual tumor volumes as a function of time, with error bars (when present) indicating one standard error of the mean (SEM). When an animal exited the study due to tumor size, the final tumor volume recorded for the animal was included with the data used to calculate the mean volume at subsequent time points. Tumor growth curves were truncated when tumors in more than 50% of the assessable animals in the group grew to the endpoint volume and excluded the data for animals whose deaths were assessed as NTR. Kaplan-Meier plots show the percentage of animals in each group remaining in the study versus time. The Kaplan-Meier plot and logrank test share the same TTE data sets. Box and whisker plots were constructed to show the Day 33 tumor volume data by group, with the "box" representing the 25th and 75th percentile of observations, the "line" representing the median of observations, and the "whiskers" representing the extreme observations. Group body weight changes during the study were plotted as percent mean change from Day 1. Body weight plots were truncated after 50% of the assessable animals in a group had exited the study and excluded the data for animals whose deaths were assessed as NTR.

| | | n | Median TTE | T-C | %TGD | MTV (n), Day 82 |
|---|---|---|---|---|---|---|
| Vehicle | - | 10 | 45.7 | -- | - | -- |
| ConjA | 6 mg/kg | 10 | 77.8 | 32.1 | 70 | 446 (4) |
| ConjB | 3 mg/kg | 10 | 82 | 36.3 | 79 | 486 (7) |

| | PR | CR | TFS | BW Nadir | TR | NTRm | NTR |
|---|---|---|---|---|---|---|---|
| Vehicle | 0 | 0 | 0 | -0.5% (3) | 0 | 0 | 0 |
| ConjA | 0 | 0 | 0 | -- | 0 | 0 | 0 |
| ConjB | 0 | 0 | 0 | -- | 0 | 0 | 0 |

A further study was carried out with ConjB being dosed at 4 mg/kg, in an analogous manner to the above. The study completed on Day 79, and the results are shown in Figure 2, which shows the change in normalised tumour growth and the tables below.

| | | n | Median TTE | T-C | %TGD | MTV (n), Day 79 |
|---|---|---|---|---|---|---|
| Vehicle | - | 10 | 24.8 | -- | - | -- |
| ConjB | 4 mg/kg | 10 | 79 | 54.2 | 218 | - |

| | PR | CR | TFS | BW Nadir | TR | NTRm | NTR |
|---|---|---|---|---|---|---|---|
| Vehicle | 0 | 0 | 0 | -2.0% (2) | 0 | 0 | 0 |
| ConjA | 0 | 0 | 0 | -8.0% (50) | 0 | 0 | 0 |

**Example 6 -** ADC cytotoxicity method MTS

The concentration and viability of cells from a sub-confluent (80-90% confluency) T75 flask are measured by trypan blue staining, and counted using the LUNA-II™ Automated Cell Counter. Cells were diluted to 2x10$^5$/ml, dispensed (50 µl per well) into 96-well flat-bottom plates.

A stock solution (1 ml) of ConjC (20 µg/ml) was made by dilution of filter-sterilised ADC into cell culture medium. A set of 8x 10-fold dilutions of stock ADC were made in a 24-well plate by serial transfer of 100 µl into 900 µl of cell culture medium. ADC dilution was dispensed (50 µl per well) into 4 replicate wells of the 96-well plate, containing 50 µl cell suspension seeded the day previously. Control wells received 50 µl cell culture medium. The 96-well plate containing cells and ADCs was incubated at 37°C in a $CO_2$-gassed incubator for the exposure time.

At the end of the incubation period, cell viability was measured by MTS assay. MTS (Promega) was dispensed (20 µl per well) into each well and incubated for 4 hours at 37°C in the $CO_2$-gassed incubator. Well absorbance was measured at 490 nm. Percentage cell survival was calculated from the mean absorbance in the 4 ADC-treated wells compared to the mean absorbance in the 4 control untreated wells (100%). $IC_{50}$ was determined from the dose-response data using GraphPad Prism using the non-linear curve fit algorithm: sigmoidal dose-response curve with variable slope.

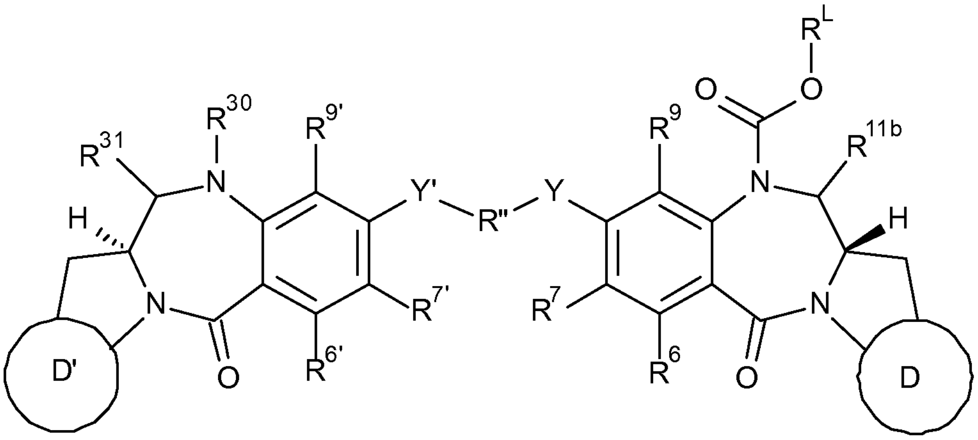
ADC incubation times were 4 days with MDA-MB-468 and 7 days for NCI-N87. MDA-MB-468 and NCI-N87 were cultured in RPMI 1640 with Glutamax + 10% (v/v) HyClone™ Fetal Bovine Serum.

| $EC_{50}$ (µM) | NCI-N87 | MDA-MB-468 |
|---|---|---|
| ConjC | 0.0003081 | 1.869 |

All documents and other references mentioned above are herein incorporated by reference.

## EMBODIMENTS OF INVENTION

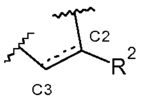
1. A compound of formula **I**:



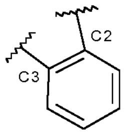
I

and salts and solvates thereof, wherein:

D represents either group D1 or D2:



D1



D2

;

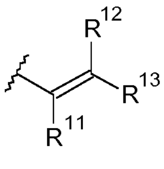
the dotted line indicates the optional presence of a double bond between C2 and C3;

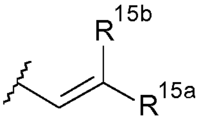
when there is a double bond present between C2 and C3, $R^2$ is selected from the group consisting of:

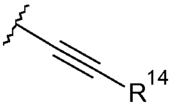
(ia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(ib) $C_{1-5}$ saturated aliphatic alkyl;

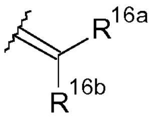
(ic) $C_{3-6}$ saturated cycloalkyl;

(id)  , wherein each of $R^{11}$, $R^{12}$ and $R^{13}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^2$ group is no more than 5;

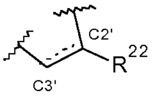
(ie)  , wherein one of $R^{15a}$ and $R^{15b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(if)  , where $R^{14}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

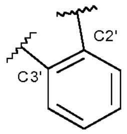
when there is a single bond present between C2 and C3,

$R^2$ is selected from H, OH, F, diF and  , where $R^{16a}$ and $R^{16b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{16a}$ and $R^{16b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

D' represents either group D'1 or D'2:



D'1



D'2

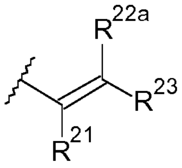
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

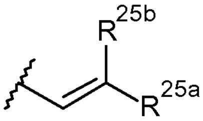
when there is a double bond present between C2' and C3', $R^{22}$ is selected from the group consisting of:

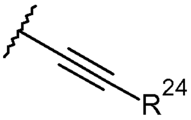
(iia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(iib) $C_{1-5}$ saturated aliphatic alkyl;

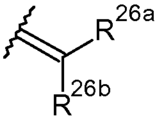
(iic) $C_{3-6}$ saturated cycloalkyl;

(iid) , wherein each of $R^{21}$, $R^{22a}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{22}$ group is no more than 5;

(iie) , wherein one of $R^{25a}$ and $R^{25b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(iif) , where $R^{24}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond present between C2' and C3',

$R^{22}$ is selected from H, OH, F, diF and , where $R^{26a}$ and $R^{26b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{26a}$ and $R^{26b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

$R^6$ and $R^9$ are independently selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

where R and R' are independently selected from optionally substituted $C_{1-12}$ alkyl, $C_{3-20}$ heterocyclyl and $C_{5-20}$ aryl groups;

$R^7$ is selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

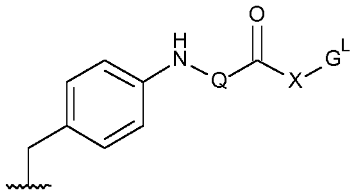
R″ is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, $NR^{N2}$ (where $R^{N2}$ is H or $C_{1-4}$ alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as $R^6$, $R^7$ and $R^9$ respectively;

$R^{11b}$ is selected from OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl; and

$R^L$ is a linker for connection to a cell binding agent, which is

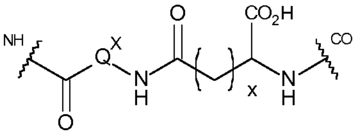


IIIa

,

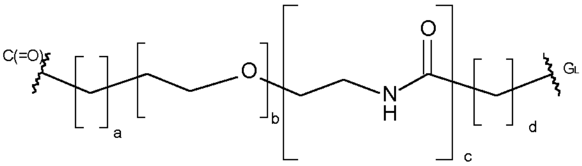
wherein

Q is a tripeptide residue of formula:



, where x is 1 or 2, -C(=O)-$Q^X$-NH- is a dipeptide residue;

X is:



,

where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5;

$G^L$ is a linker for connecting to a Ligand Unit;

either

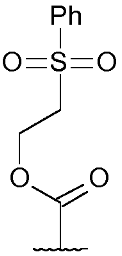
(a) $R^{30}$ is H, and $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1\text{-}4}$ alkyl; or

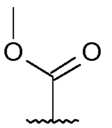
(b) $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

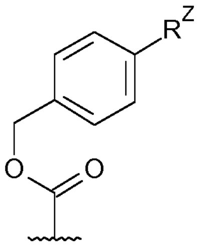
(c) $R^{30}$ is H and $R^{31}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation; or

(d) $R^{30}$ is H and $R^{31}$ is H or =O; or

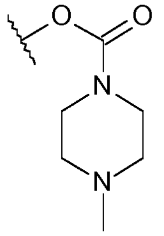
(e) $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1\text{-}4}$ alkyl and $R^{30}$ is selected from:

(e-i)  ;

(e-ii)  ;



(e-iii) , where $R^Z$ is selected from:



(z-i) ;

(z-ii) $OC(=O)CH_3$;

(z-iii) $NO_2$;

(z-iv) OMe;

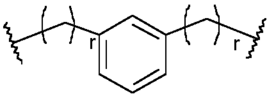
(z-v) glucoronide;

(z-vi) NH-C(=O)-$X_1$-NHC(=O)$X_2$-NH-C(=O)-$R^{ZC}$, where -C(=O)-$X_1$-NH- and -C(=O)-$X_2$-NH- represent natural amino acid residues and $R^{ZC}$ is selected from Me, OMe, $CH_2CH_2OMe$, and $(CH_2CH_2O)_2Me$.

2. A compound according to statement 1, wherein both Y and Y' are O.

3. A compound according to either statement 1 or statement 2, wherein R'' is $C_{3-7}$ alkylene.

4. A compound according to either statement 1 or statement 2, wherein R" is a group of formula:



where r is 1 or 2.

5. A compound according to any one of statements 1 to 4, wherein $R^9$ is H.

6. A compound according to any one of statements 1 to 5, wherein $R^6$ is H.

7. A compound according to any one of statements 1 to 6, wherein $R^7$ is selected from H, OH and OR.

8. A compound according to statement 7, wherein $R^7$ is a $C_{1-4}$ alkyloxy group.

9. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a $C_{5-7}$ aryl group.

10. A compound according to statement 9, wherein $R^2$ is phenyl.

11. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a $C_{8-10}$ aryl group.

12. A compound according to any one of statements 9 to 11, wherein $R^2$ bears one to three substituent groups.

13. A compound according to any one of statements 9 to 12, wherein the substituents are selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl.

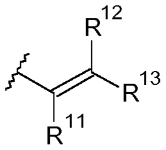
14. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a $C_{1-5}$ saturated aliphatic alkyl group.

15. A compound according to statement 14, wherein $R^2$ is methyl, ethyl or propyl.

16. A compound according to any one of statements 1 to 7, wherein there is a double bond between C2 and C3, and $R^2$ is a $C_{3-6}$ saturated cycloalkyl group.

17. A compound according to statement 16, wherein $R^2$ is cyclopropyl.

18. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a group of formula:



.

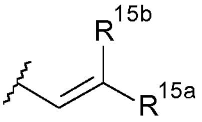
19. A compound according to statement 18, wherein the total number of carbon atoms in the $R^2$ group is no more than 4.

20. A compound according to statement 19, wherein the total number of carbon atoms in the $R^2$ group is no more than 3.

21. A compound according to any one of statements 18 to 20, wherein one of $R^{11}$, $R^{12}$ and $R^{13}$ is H, with the other two groups being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

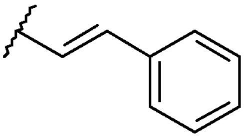
22. A compound according to any one of statements 18 to 20, wherein two of $R^{11}$, $R^{12}$ and $R^{13}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

23. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a group of formula:



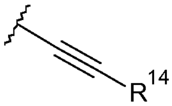
.

24. A compound according to statement 23, wherein $R^2$ is the group:



.

25. A compound according to any one of statements 1 to 8, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is a group of formula:

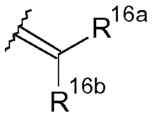


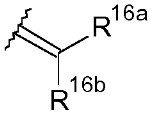
.

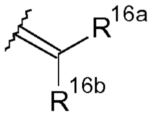
26. A compound according to statement 25, wherein $R^{14}$ is selected from H, methyl, ethyl, ethenyl and ethynyl.

27. A compound according to statement 26, wherein $R^{14}$ is selected from H and methyl.

28. A compound according to any one of statements 1 to 8, wherein D is D1, there is a single bond between C2 and C3, and $R^2$ is H.

29. A compound according to any one of statements 1 to 8, wherein D is D1, there is a single bond between C2 and C3, $R^2$ is  and $R^{16a}$ and $R^{16b}$ are both H.

30. A compound according to any one of statements 1 to 8, wherein D is D1, there is a single bond between C2 and C3, $R^2$ is , and $R^{16a}$ and $R^{16b}$ are both methyl.

31. A compound according to any one of statements 1 to 8, wherein D is D1, there is a single bond between C2 and C3, $R^2$ is , one of $R^{16a}$ and $R^{16b}$ is H, and the other is selected from $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted.

32. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a $C_{5-7}$ aryl group.

33. A compound according to statement 32, wherein $R^{22}$ is phenyl.

34. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a $C_{8-10}$ aryl group.

35. A compound according to any one of statements 32 to 34, wherein $R^{22}$ bears one to three substituent groups.

36. A compound according to any one of statements 32 to 35, wherein the substituents are selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl.

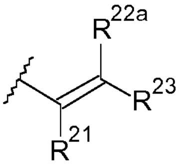
37. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a $C_{1-5}$ saturated aliphatic alkyl group.

38. A compound according to statement 37, wherein $R^{22}$ is methyl, ethyl or propyl.

39. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a $C_{3-6}$ saturated cycloalkyl group.

40. A compound according to statement 39, wherein $R^{22}$ is cyclopropyl.

41. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a group of formula:



.

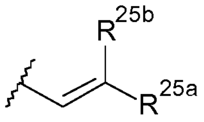
42. A compound according to statement 41, wherein the total number of carbon atoms in the $R^{22}$ group is no more than 4.

43. A compound according to statement 42, wherein the total number of carbon atoms in the $R^{22}$ group is no more than 3.

44. A compound according to any one of statements 41 to 43, wherein one of $R^{21}$, $R^{22a}$ and $R^{23}$ is H, with the other two groups being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

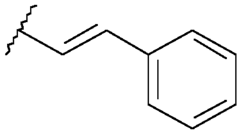
45. A compound according to any one of statements 41 to 43, wherein two of $R^{21}$, $R^{22a}$ and $R^{23}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

46. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a group of formula:



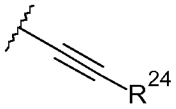
.

47. A compound according to statement 46, wherein $R^{22}$ is the group:



.

48. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is a group of formula:

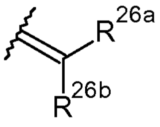


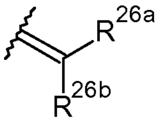
.

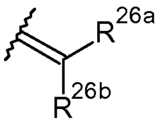
49. A compound according to statement 48, wherein $R^{24}$ is selected from H, methyl, ethyl, ethenyl and ethynyl.

50. A compound according to statement 49, wherein $R^{24}$ is selected from H and methyl.

51. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a single bond between C2' and C3', and $R^{22}$ is H.

52. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a single bond between C2' and C3', $R^{22}$ is  and $R^{26a}$ and $R^{26b}$ are both H.

53. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a single bond between C2' and C3', $R^{22}$ is , and $R^{26a}$ and $R^{26b}$ are both methyl.

54. A compound according to any one of statements 1 to 31, wherein D' is D'1, there is a single bond between C2' and C3', $R^{22}$ is , one of $R^{26a}$ and $R^{26b}$ is H, and the

other is selected from $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted.

55. A compound according to any one of statements 1 to 54, wherein $R^{6'}$ is selected from the same groups as $R^6$, $R^{7'}$ is selected from the same groups as $R^7$, $R^{9'}$ is selected from the same groups as $R^9$ and $Y'$ is selected from the same groups as Y.

56. A compound according to statement 55, wherein $R^{6'}$ is the same groups as $R^6$, $R^{7'}$ is the same groups as $R^7$, $R^{9'}$ is the same groups as $R^9$ and $Y'$ is the same groups as Y.

57. A compound according to any one of statements 1 to 56, wherein $R^{22}$ is the same group as $R^2$.

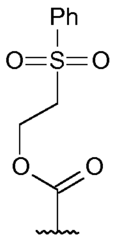
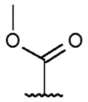
59. A compound according to any one of statements 1 to 57, wherein $R^{30}$ is H, and $R^{31}$ is OH.

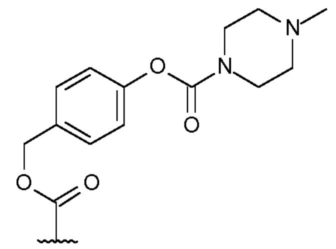
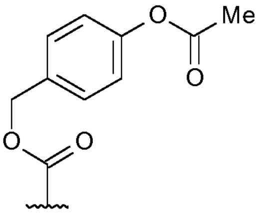
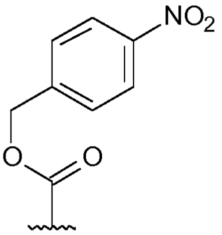
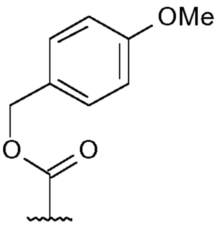
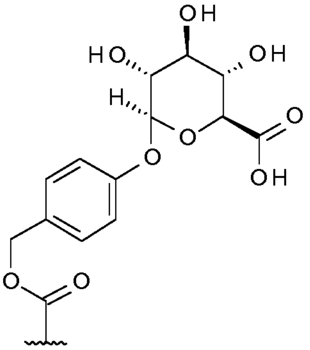
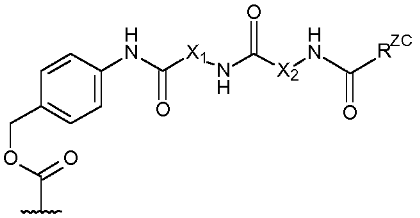
60. A compound according to any one of statements 1 to 57, wherein $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound.

61. A compound according to any one of statements 1 to 57, wherein $R^{30}$ is H, and $R^{31}$ is H.

62. A compound according to any one of statements 1 to 57, wherein $R^{30}$ is H, and $R^{31}$ is =O.

63. A compound according to any one of statements 1 to 57, wherein $R^{31}$ is OH or $OR^A$ and $R^{30}$ is selected from:

| | |
|---|---|
| $R^{30a}$ |  |
| $R^{30b}$ |  |

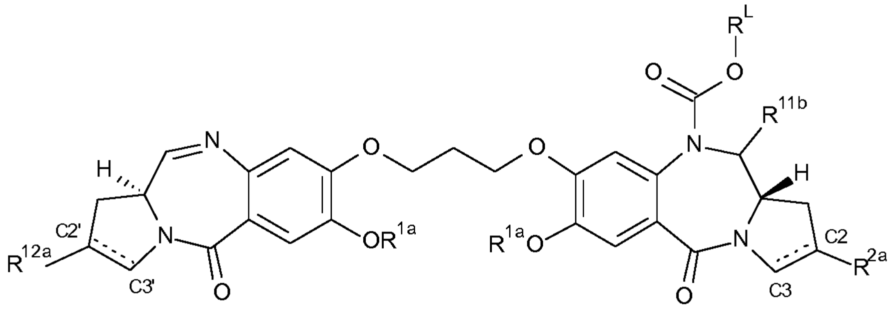
| | |
|---|---|
| $R^{30c}$ |  |
| $R^{30d}$ |  |
| $R^{30e}$ |  |
| $R^{30f}$ |  |
| $R^{30g}$ |  |
| $R^{30h}$ |  |

where -C(=O)-$X_1$-NHC(=O)$X_2$-NH- represent a dipeptide.

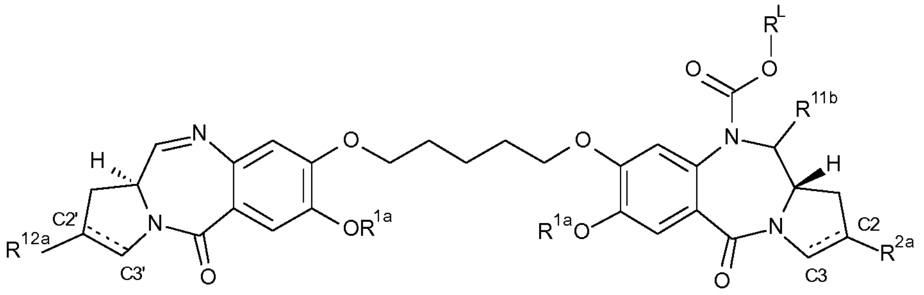
64. A compound according to statement 63, wherein -C(=O)-$X_1$-NHC(=O)$X_2$-NH-, is selected from: -Phe-Lys-, -Val-Ala-, -Val-Lys-, -Ala-Lys-, and -Val-Cit-.

65. The conjugate according to statement 64, wherein -C(=O)-$X_1$-NHC(=O)$X_2$-NH-, is selected from: -Phe-Lys-, and -Val-Ala-.

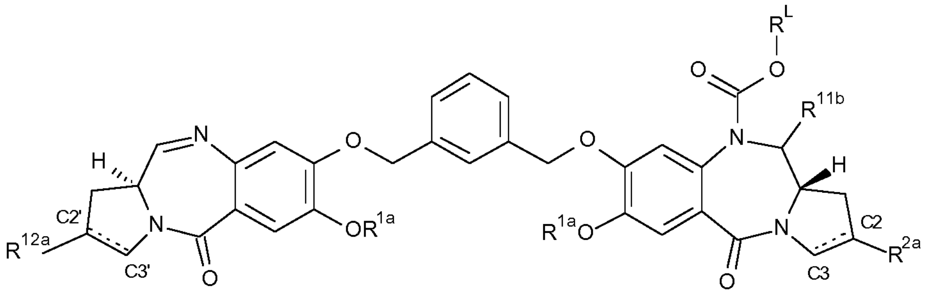
66. A compound according to statement 1, which is of formula Ia-1, Ia-2 or Ia-3:



Ia

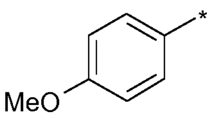


Ib



Ic

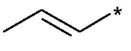
where $R^{2a}$ and $R^{12a}$ are the same and are selected from:

(a) 

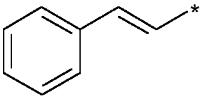
;

(b) 

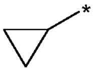
;

(c) 

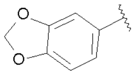
;

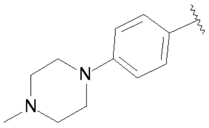
(d) 

;

(e)  ;

(f)  ;

(g)  ; and

(h)  ;

$R^{1a}$ is selected from methyl and benzyl;

$R^L$ and $R^{11b}$ are as defined in statement 1.

67. A compound according to any one of statements 1 to 66, wherein $R^{11b}$ is OH.

68. A compound according to any one of statements 1 to 66, wherein $R^{11b}$ is $OR^A$, where $R^A$ is $C_{1-4}$ alkyl.

69. A compound according to statement 68, wherein $R^A$ is methyl.

70. A compound according to any one of statements 1 to 69, wherein -C(=O)-$Q^x$-NH- is a dipeptide residue selected from:

$^{CO}$-Phe-Lys-$^{NH}$,
$^{CO}$-Val-Ala-$^{NH}$,
$^{CO}$-Val-Lys-$^{NH}$,
$^{CO}$-Ala-Lys-$^{NH}$,
$^{CO}$-Val-Cit-$^{NH}$,
$^{CO}$-Phe-Cit-$^{NH}$,
$^{CO}$-Leu-Cit-$^{NH}$,
$^{CO}$-Ile-Cit-$^{NH}$,
$^{CO}$-Phe-Arg-$^{NH}$, and
$^{CO}$-Trp-Cit-$^{NH}$.

71. A compound according to statement 70, wherein -C(=O)-$Q^X$-NH- is selected from $^{CO}$-Phe-Lys-$^{NH}$, $^{CO}$-Val-Cit-$^{NH}$ and $^{CO}$-Val-Ala-$^{NH}$.

72. A compound according to any one of statements 1 to 71 wherein x is 1.

73. A compound according to any one of statements 1 to 71 wherein x is 2.

74. A compound according to any one of statements 1 to 73, wherein a is 0 to 3.

75. A compound according to statement 74, wherein a is 0.

76. A compound according to any one of statements 1 to 75, wherein b is 0 to 12.

77. A compound according to statement 76, wherein b is 0 to 8.

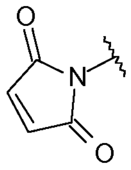
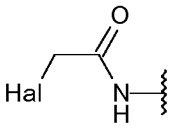
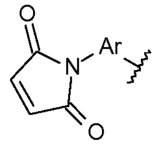
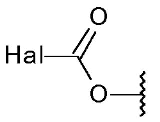
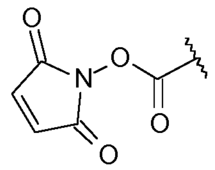
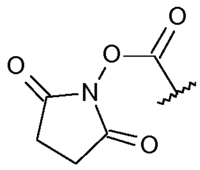
78. A compound according to any one of statements 1 to 77, wherein d is 0 to 3.

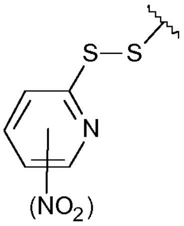
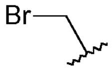
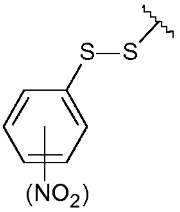
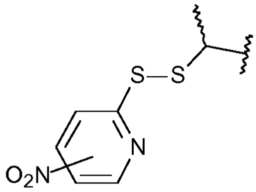
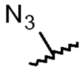
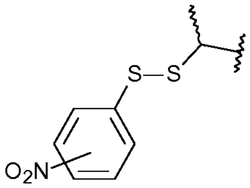
79. A compound according to statement 78, wherein d is 2.

80. A compound according to any one of statements 1 to 73, wherein a is 0, c is 1 and d is 2, and b is from 0, 4 or 8.

81. A compound according to any one of statements 1 to 73, wherein a, b and c are 0 and d is 2 or 5.

82. A compound according to any one of statements 1 to 81, wherein $G^L$ is selected from:

| | | | |
|---|---|---|---|
| ($G^{L1-1}$) |  | ($G^{L4}$) | <br>Where Hal = I, Br, Cl |
| ($G^{L1-2}$) |  | ($G^{L5}$) |  |
| ($G^{L2}$) |  | ($G^{L6}$) |  |

| | | | |
|---|---|---|---|
| ($G^{L3-1}$) |  <br>where the $NO_2$ group is optional | ($G^{L7}$) |  |
| ($G^{L3-2}$) |  <br>where the $NO_2$ group is optional | ($G^{L8}$) |  |
| ($G^{L3-3}$) |  <br>where the $NO_2$ group is optional | ($G^{L9}$) |  |
| ($G^{L3-4}$) |  <br>where the $NO_2$ group is optional | | |

where Ar represents a $C_{5-6}$ arylene group.

83. A compound according to statement 82, wherein Ar is a phenylene group.

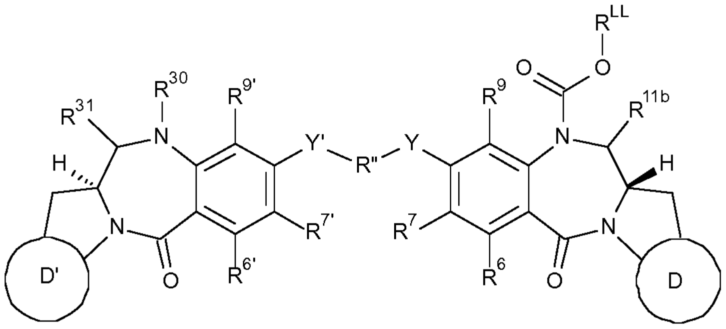
84. A compound according to either statement 82 or statement 83, wherein $G^L$ is selected from $G^{L1-1}$ and $G^{L1-2}$.

85. A compound according to statement 84, wherein $G^L$ is $G^{L1-1}$.

86. A conjugate of formula I:

$$L - (D^L)_p \qquad (I)$$

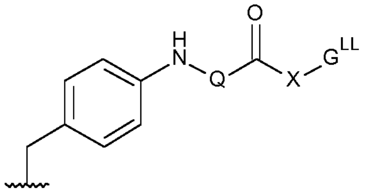
wherein L is a Ligand unit, D is a Drug Linker unit of formula I':



I'

wherein D, $R^2$, $R^6$, $R^7$, $R^9$, $R^{11b}$, Y, R", Y', D', $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{22}$, $R^{30}$ and $R^{31}$, including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, are as defined in any one of statements 1 to 69;

$R^{LL}$ is a linker for connection to a cell binding agent, which is:



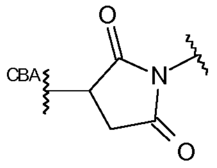
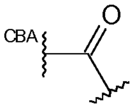
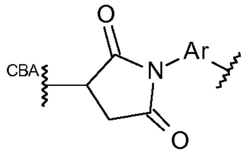
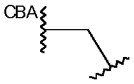
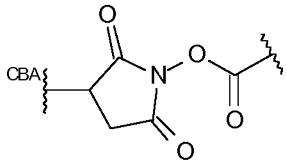
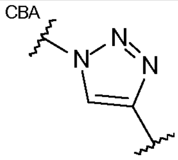
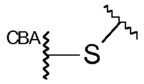
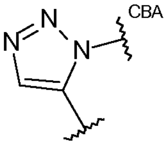
IIIa'

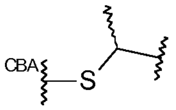
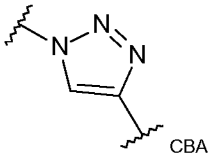
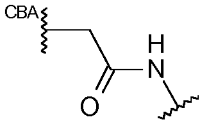
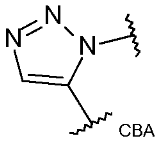
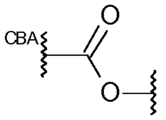
,

where Q and X are as defined in any one of statements 1 and 70 to 81 and $G^{LL}$ is a linker connected to the Ligand Unit;

wherein p is an integer of from 1 to 20.

87. A conjugate according to statement 86, wherein $G^{LL}$ is selected from:

| | | | |
|---|---|---|---|
| ($G^{LL1-1}$) |  | ($G^{LL6}$) |  |
| ($G^{LL1-2}$) |  | ($G^{LL7}$) |  |
| ($G^{LL2}$) |  | ($G^{LL8-1}$) |  |
| ($G^{LL3-1}$) |  | ($G^{LL8-2}$) |  |

| | | | |
|---|---|---|---|
| ($G^{LL3\text{-}2}$) |  | ($G^{LL9\text{-}1}$) |  |
| ($G^{LL4}$) |  | ($G^{LL9\text{-}2}$) |  |
| ($G^{LL5}$) |  | | |

where Ar represents a $C_{5\text{-}6}$ arylene group.

88. A conjugate according to statement 87, wherein Ar is a phenylene group.

89. A compound according to either statement 87 or statement 88, wherein $G^{LL}$ is selected from $G^{LL1\text{-}1}$ and $G^{LL1\text{-}2}$.

90. A compound according to statement 89, wherein $G^{LL}$ is $G^{LL1\text{-}1}$.

91. A conjugate according to any one of statements 86 to 90, wherein the Ligand Unit is an antibody or an active fragment thereof.

92. The conjugate according to statement 91, wherein the antibody or antibody fragment is an antibody or antibody fragment for a tumour-associated antigen.

93. The conjugate according to statement 94 wherein the antibody or antibody fragment is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(89):
**(1)** BMPR1B;
**(2)** E16;
**(3)** STEAP1;
**(4)** 0772P;
**(5)** MPF;
**(6)** Napi3b;
**(7)** Sema 5b;
**(8)** PSCA hlg;
**(9)** ETBR;

**(10)** MSG783;
**(11)** STEAP2;
**(12)** TrpM4;
**(13)** CRIPTO;
**(14)** CD21;
**(15)** CD79b;
**(16)** FcRH2;
**(17)** HER2;
**(18)** NCA;
**(19)** MDP;
**(20)** IL20R-alpha;
**(21)** Brevican;
**(22)** EphB2R;
**(23)** ASLG659;
**(24)** PSCA;
**(25)** GEDA;
**(26)** BAFF-R;
**(27)** CD22;
**(28)** CD79a;
**(29)** CXCR5;
**(30)** HLA-DOB;
**(31)** P2X5;
**(32)** CD72;
**(33)** LY64;
**(34)** FcRH1;
**(35)** IRTA2;
**(36)** TENB2;
**(37)** PSMA – FOLH1;
**(38)** SST;
(38.1) SSTR2;
(38.2) SSTR5;
(38.3) SSTR1;
(38.4)SSTR3;
(38.5) SSTR4;
**(39)** ITGAV;
**(40)** ITGB6;

**(41)** CEACAM5;

**(42)** MET;

**(43)** MUC1;

**(44)** CA9;

**(45)** EGFRvIII;

**(46)** CD33;

**(47)** CD19;

**(48)** IL2RA;

**(49)** AXL;

**(50)** CD30 - TNFRSF8;

**(51)** BCMA - TNFRSF17;

**(52)** CT Ags – CTA;

**(53)** CD174 (Lewis Y) - FUT3;

**(54)** CLEC14A;

**(55)** GRP78 – HSPA5;

**(56)** CD70;

**(57)** Stem Cell specific antigens;

**(58)** ASG-5;
**(59)** ENPP3;

**(60)** PRR4;

**(61)** GCC – GUCY2C;

**(62)** Liv-1 – SLC39A6;

**(63)** 5T4;

**(64)** CD56 – NCMA1;

**(65)** CanAg;

**(66)** FOLR1;

**(67)** GPNMB;

**(68)** TIM-1 – HAVCR1;

**(69)** RG-1/Prostate tumor target Mindin – Mindin/RG-1;

**(70)** B7-H4 – VTCN1;

**(71)** PTK7;

**(72)** CD37;

**(73)** CD138 – SDC1;

**(74)** CD74;

**(75)** Claudins – CLs;

**(76)** EGFR;

**(77)** Her3;

**(78)** RON - MST1R;
**(79)** EPHA2;
**(80)** CD20 – MS4A1;
**(81)** Tenascin C – TNC;
**(82)** FAP;
**(83)** DKK-1;
**(84)** CD52;
**(85)** CS1 - SLAMF7;
**(86)** Endoglin – ENG;
**(87)** Annexin A1 – ANXA1;
**(88)** V-CAM (CD106) - VCAM1;
**(89)** ASCT2 (SLC1A5).

94. The conjugate of any one of statements 91 to 93 wherein the antibody or antibody fragment is a cysteine-engineered antibody.

95. The conjugate according to any one of statements 86 to 94 wherein p is an integer from 1 to 8.

96. The conjugate according to statement 95, wherein p is 1, 2, 3, or 4.

97. A composition comprising a mixture of conjugates according to any one of statements 86 to 96, wherein the average p in the mixture of conjugate compounds is about 1 to about 8.

98. The conjugate according to any one of statements 86 to 96, for use in therapy.

99. A pharmaceutical composition comprising the conjugate of any one of statements 86 to 96 a pharmaceutically acceptable diluent, carrier or excipient.

100. The conjugate according to any one of statements 86 to 96 or the pharmaceutical composition according to statement 99, for use in the treatment of a proliferative disease in a subject.

101. The conjugate for use according to statement 100, wherein the disease treated is cancer.

102. Use of a conjugate according to any one of statements 86 to 96 or a pharmaceutical according to statement 99 in a method of medical treatment.

103. A method of medical treatment comprising administering to a patient the pharmaceutical composition of statement 99.

104. The method of statement 103 wherein the method of medical treatment is for treating cancer.

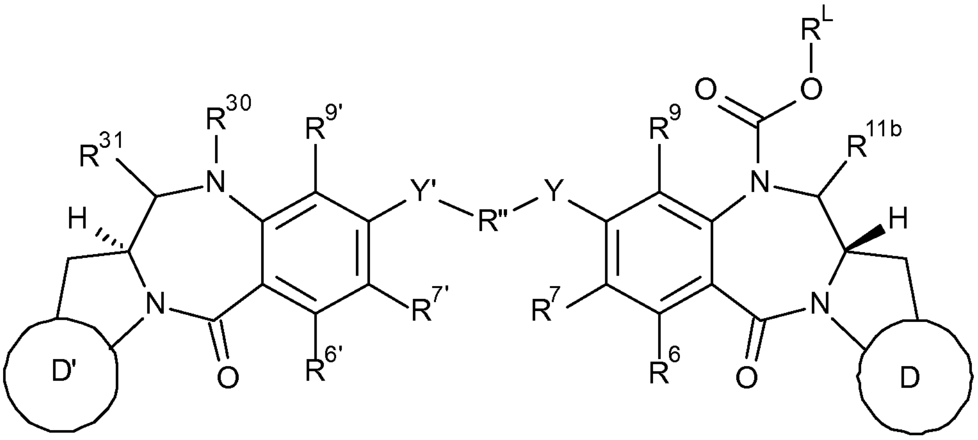
105. The method of statement 104, wherein the patient is administered a chemotherapeutic agent, in combination with the conjugate.

106. Use of a conjugate according to any one of statements 86 to 96 in a method of manufacture of a medicament for the treatment of a proliferative disease.

107. A method of treating a mammal having a proliferative disease, comprising administering an effective amount of a conjugate according to any one of statements 86 to 96 or a pharmaceutical composition according to statement 99.

## CLAIMS

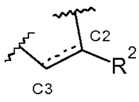
1. A compound of formula **I**:



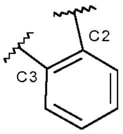
I

and salts and solvates thereof, wherein:

D represents either group D1 or D2:



D1



D2

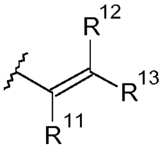
;

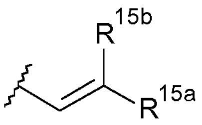
the dotted line indicates the optional presence of a double bond between C2 and C3; when there is a double bond present between C2 and C3, $R^2$ is selected from the group consisting of:

(ia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

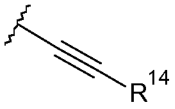
(ib) $C_{1-5}$ saturated aliphatic alkyl;

(ic) $C_{3-6}$ saturated cycloalkyl;

(id)  , wherein each of $R^{11}$, $R^{12}$ and $R^{13}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^2$ group is no more than 5;

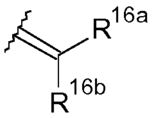
(ie) 

, wherein one of $R^{15a}$ and $R^{15b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(if) 

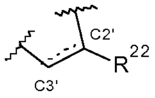
, where $R^{14}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond present between C2 and C3,

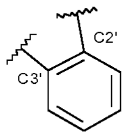
$R^2$ is selected from H, OH, F, diF and 

, where $R^{16a}$ and $R^{16b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{16a}$ and $R^{16b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

D' represents either group D'1 or D'2:



D'1



D'2

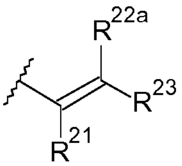
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

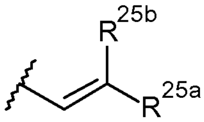
when there is a double bond present between C2' and C3', $R^{22}$ is selected from the group consisting of:

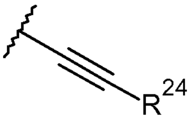
(iia) $C_{5-10}$ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, $C_{1-7}$ alkyl, $C_{3-7}$ heterocyclyl and bis-oxy-$C_{1-3}$ alkylene;

(iib) $C_{1-5}$ saturated aliphatic alkyl;

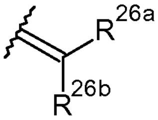
(iic) $C_{3-6}$ saturated cycloalkyl;

(iid)  , wherein each of $R^{21}$, $R^{22a}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{22}$ group is no more than 5;

(iie)  , wherein one of $R^{25a}$ and $R^{25b}$ is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(iif)  , where $R^{24}$ is selected from: H; $C_{1-3}$ saturated alkyl; $C_{2-3}$ alkenyl; $C_{2-3}$ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond present between C2' and C3',

$R^{22}$ is selected from H, OH, F, diF and  , where $R^{26a}$ and $R^{26b}$ are independently selected from H, F, $C_{1-4}$ saturated alkyl, $C_{2-3}$ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from $C_{1-4}$ alkyl amido and $C_{1-4}$ alkyl ester; or, when one of $R^{26a}$ and $R^{26b}$ is H, the other is selected from nitrile and a $C_{1-4}$ alkyl ester;

$R^6$ and $R^9$ are independently selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

where R and R' are independently selected from optionally substituted $C_{1-12}$ alkyl, $C_{3-20}$ heterocyclyl and $C_{5-20}$ aryl groups;

$R^7$ is selected from H, R, OH, OR, SH, SR, $NH_2$, NHR, NRR', nitro, $Me_3Sn$ and halo;

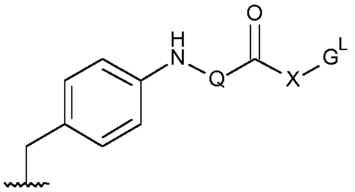
R″ is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, $NR^{N2}$ (where $R^{N2}$ is H or $C_{1-4}$ alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as $R^6$, $R^7$ and $R^9$ respectively;

$R^{11b}$ is selected from OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl; and

$R^L$ is a linker for connection to a cell binding agent, which is

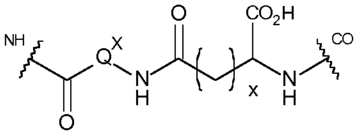


IIIa

,

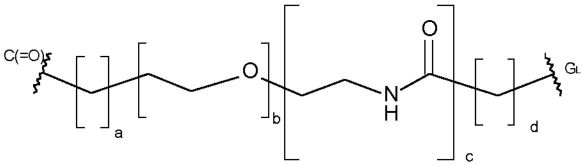
wherein

Q is a tripeptide residue of formula:



, where x is 1 or 2, and -C(=O)-$Q^X$-NH- is a dipeptide residue;

X is:



,

where a = 0 to 5, b = 0 to 16, c = 0 or 1, d = 0 to 5;

$G^L$ is a linker for connecting to a Ligand Unit;

either

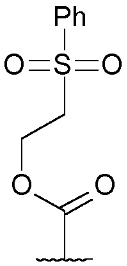
(a) $R^{30}$ is H, and $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1-4}$ alkyl; or

(b) $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

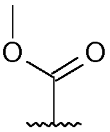
(c) $R^{30}$ is H and $R^{31}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation; or

(d) $R^{30}$ is H and $R^{31}$ is H or =O; or

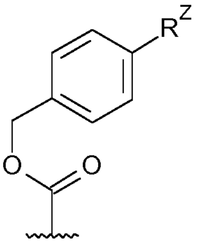
(e) $R^{31}$ is OH or $OR^A$, where $R^A$ is $C_{1-4}$ alkyl and $R^{30}$ is selected from:



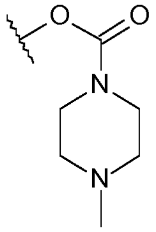
(e-i) ;



(e-ii) ;



(e-iii) , where $R^Z$ is selected from:



(z-i) ;

(z-ii) $OC(=O)CH_3$;

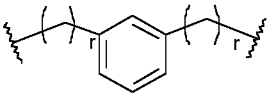
(z-iii) $NO_2$;

(z-iv) OMe;

(z-v) glucoronide;

(z-vi) NH-C(=O)-$X_1$-NHC(=O)$X_2$-NH-C(=O)-$R^{ZC}$, where -C(=O)-$X_1$-NH- and -C(=O)-$X_2$-NH- represent natural amino acid residues and $R^{ZC}$ is selected from Me, OMe, $CH_2CH_2OMe$, and $(CH_2CH_2O)_2Me$.

2. A compound according to claim 1, wherein both Y and Y' are O, R" is either $C_{3-7}$ alkylene or a group of formula:



where r is 1 or 2.

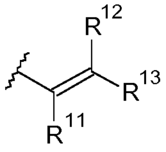
3. A compound according to either claim 1 or claim 2, wherein $R^6$ is H, $R^9$ is H and $R^7$ is a $C_{1-4}$ alkyloxy group.

4. A compound according to any one of claims 1 to 3, wherein D is D1, there is a double bond between C2 and C3, and $R^2$ is:

(a) phenyl, which bears one to three substituent groups, selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl;

(b) methyl; or

(c) a group of formula:

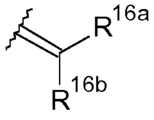


, wherein the total number of carbon atoms in the $R^2$ group is no more than 4.

5. A compound according to any one of claims 1 to 3, wherein D is D1, there is a single bond between C2 and C3, and $R^2$ is:

(a) H; or

(b)



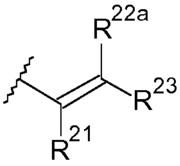
and $R^{16a}$ and $R^{16b}$ are both H.

6. A compound according to any one of claims 1 to 5, wherein D' is D'1, there is a double bond between C2' and C3', and $R^{22}$ is:

(a) phenyl, which bears one to three substituent groups, selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl;

(b) methyl; or

(c) a group of formula:

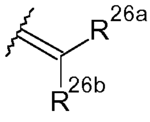


, wherein the total number of carbon atoms in the $R^{22a}$ group is no more than 4.

7. A compound according to any one of claims 1 to 5, wherein D' is D'1, there is a single bond between C2' and C3', and $R^{22}$ is:

(a) H; or

(b)



and $R^{26a}$ and $R^{26b}$ are both H.

8. A compound according to any one of claims 1 to 7, wherein $R^{6'}$ is the same group as $R^6$, $R^{7'}$ is the same group as $R^7$, $R^{9'}$ is the same group as $R^9$, $Y'$ is the same group as Y, $R^{22}$ (if present) is the same group as $R^2$ (if present).

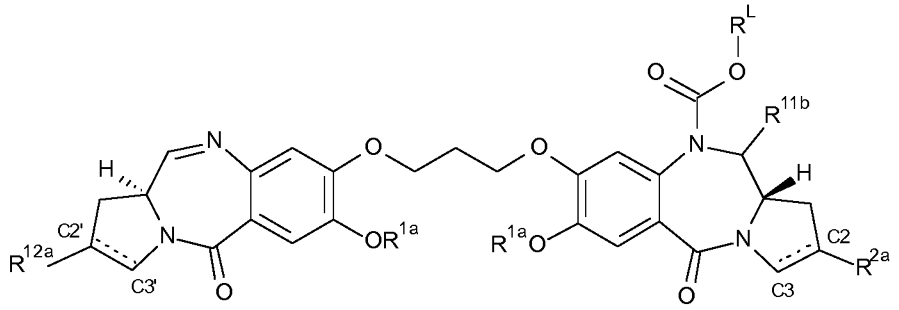
9. A compound according to any one of claims 1 to 8, wherein:

(a) $R^{30}$ is H, and $R^{31}$ is OH;

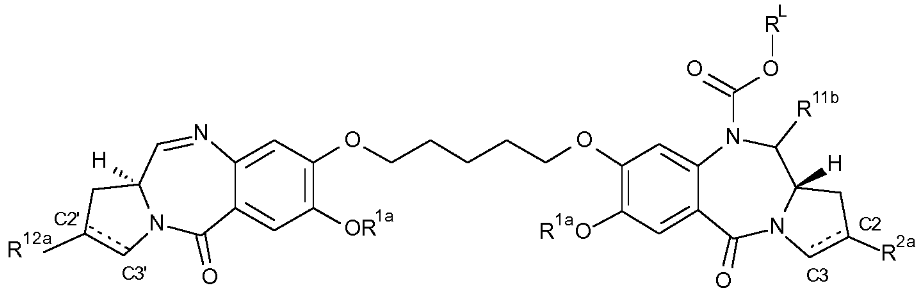
(b) $R^{30}$ and $R^{31}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) $R^{30}$ is H, and $R^{31}$ is H.

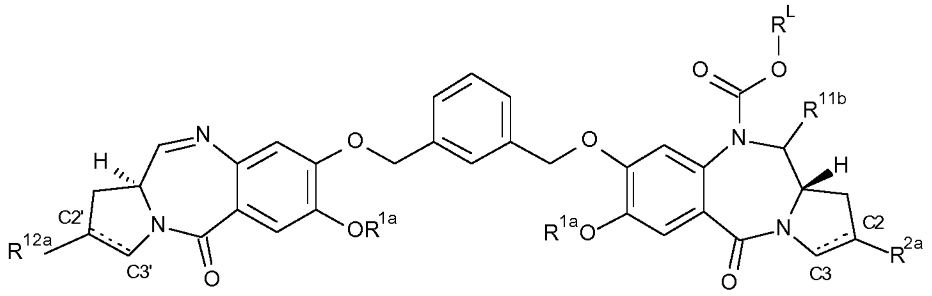
10. A compound according to claim 1, which is of formula Ia-1, Ia-2 or Ia-3:



Ia

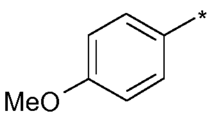


Ib



Ic

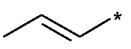
where $R^{2a}$ and $R^{12a}$ are the same and are selected from:

(a) 

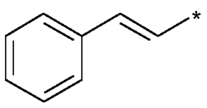
;

(b) 

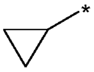
;

(c) 

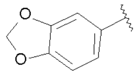
;

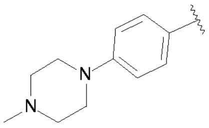
(d) 

;

(e)  ;

(f)  ;

(g)  ; and

(h)  ;

$R^{1a}$ is selected from methyl and benzyl;

$R^L$ and $R^{11b}$ are as defined in claim 1.

11. A compound according to any one of claims 1 to 10, wherein $R^{11b}$ is OH.

12. A compound according to any one of claims 1 to 11, wherein -C(=O)-$Q^x$-NH- is a dipeptide residue selected from $^{CO}$-Phe-Lys-$^{NH}$, $^{CO}$-Val-Cit-$^{NH}$ and $^{CO}$-Val-Ala-$^{NH}$.

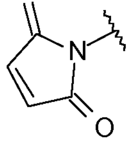
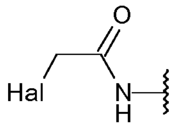
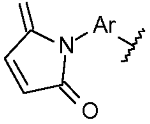
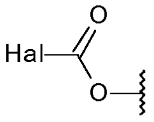
13. A compound according to any one of claims 1 to 12 wherein x is 1.

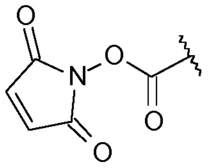
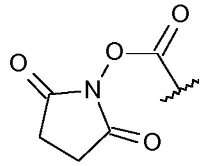
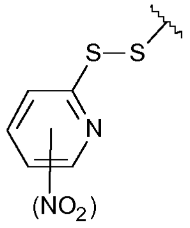
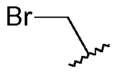
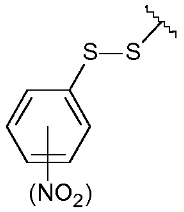
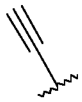
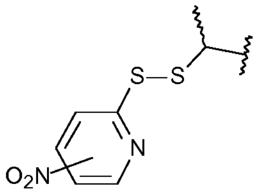
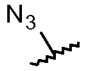
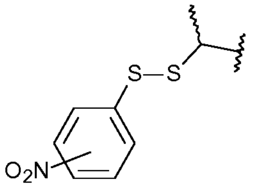
14. A compound according to any one of claims 1 to 12 wherein x is 2.

15. A compound according to any one of claims 1 to 14, wherein a is 0, c is 1 and d is 2, and b is 0, 4 or 8.

16. A compound according to any one of claims 1 to 14, wherein a, b and c are 0 and d is 2 or 5.

17. A compound according to any one of claims 1 to 16, wherein $G^L$ is selected from:

| | | | |
|---|---|---|---|
| ($G^{L1-1}$) |  | ($G^{L4}$) | <br>Where Hal = I, Br, Cl |
| ($G^{L1-2}$) |  | ($G^{L5}$) |  |

| | | | |
|---|---|---|---|
| ($G^{L2}$) |  | ($G^{L6}$) |  |
| ($G^{L3-1}$) | <br>where the $NO_2$ group is optional | ($G^{L7}$) |  |
| ($G^{L3-2}$) | <br>where the $NO_2$ group is optional | ($G^{L8}$) |  |
| ($G^{L3-3}$) | <br>where the $NO_2$ group is optional | ($G^{L9}$) |  |
| ($G^{L3-4}$) | <br>where the $NO_2$ group is optional | | |

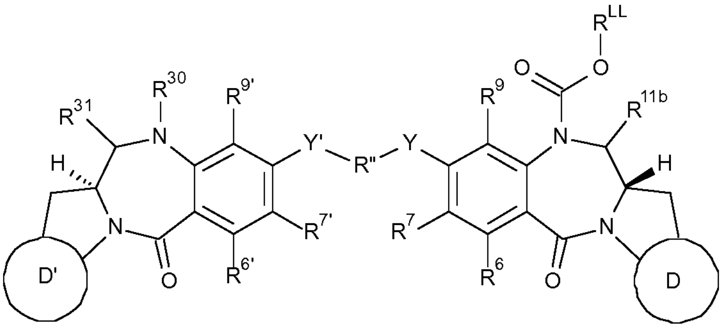
where Ar represents a $C_{5-6}$ arylene group.

18. A compound according to claim 17, wherein $G^L$ is $G^{L1-1}$.

19. A conjugate of formula I:

$$L - (D^L)_p \qquad (I)$$

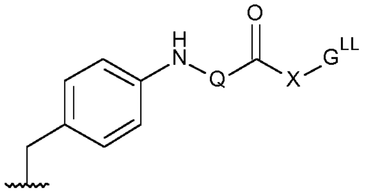
wherein L is a Ligand unit, D is a Drug Linker unit of formula I':



I'

wherein D, $R^2$, $R^6$, $R^7$, $R^9$, $R^{11b}$, Y, R", Y', D', $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{22}$, $R^{30}$ and $R^{31}$, including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, are as defined in any one of claims 1 to 11;

$R^{LL}$ is a linker for connection to a cell binding agent, which is:



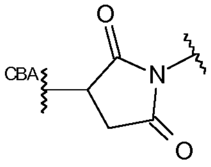
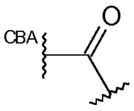
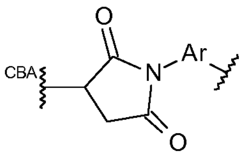
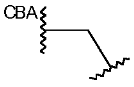
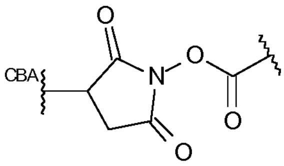
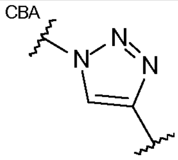
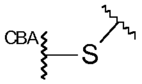
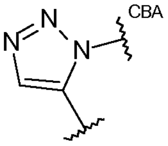
IIIa'

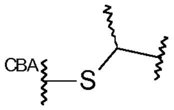
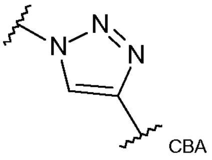
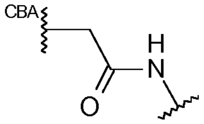
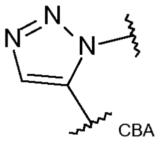
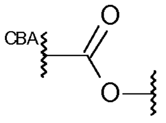
,

where Q and X are as defined in any one of claims 1 and 12 to 16 and $G^{LL}$ is a linker connected to the Ligand Unit;

wherein p is an integer of from 1 to 20.

20. A conjugate according to claim 19, wherein $G^{LL}$ is selected from:

| | | | |
|---|---|---|---|
| ($G^{LL1-1}$) |  | ($G^{LL6}$) |  |
| ($G^{LL1-2}$) |  | ($G^{LL7}$) |  |
| ($G^{LL2}$) |  | ($G^{LL8-1}$) |  |
| ($G^{LL3-1}$) |  | ($G^{LL8-2}$) |  |

| | | | |
|---|---|---|---|
| ($G^{LL3-2}$) |  | ($G^{LL9-1}$) |  |
| ($G^{LL4}$) |  | ($G^{LL9-2}$) |  |
| ($G^{LL5}$) |  | | |

where Ar represents a $C_{5-6}$ arylene group.

21. A compound according to claim 20, wherein $G^{LL}$ is $G^{LL1-1}$.

22. A conjugate according to any one of claims 19 to 21, wherein the Ligand Unit is an antibody or an active fragment thereof for a tumour-associated antigen.

23. The conjugate according to any one of claims 19 to 21, for use in therapy.

24. A pharmaceutical composition comprising the conjugate of any one of claims 19 to 21, and a pharmaceutically acceptable diluent, carrier or excipient.

25. The conjugate according to any one of claims 19 to 21 or the pharmaceutical composition according to claim 24, for use in the treatment of a proliferative disease in a subject.

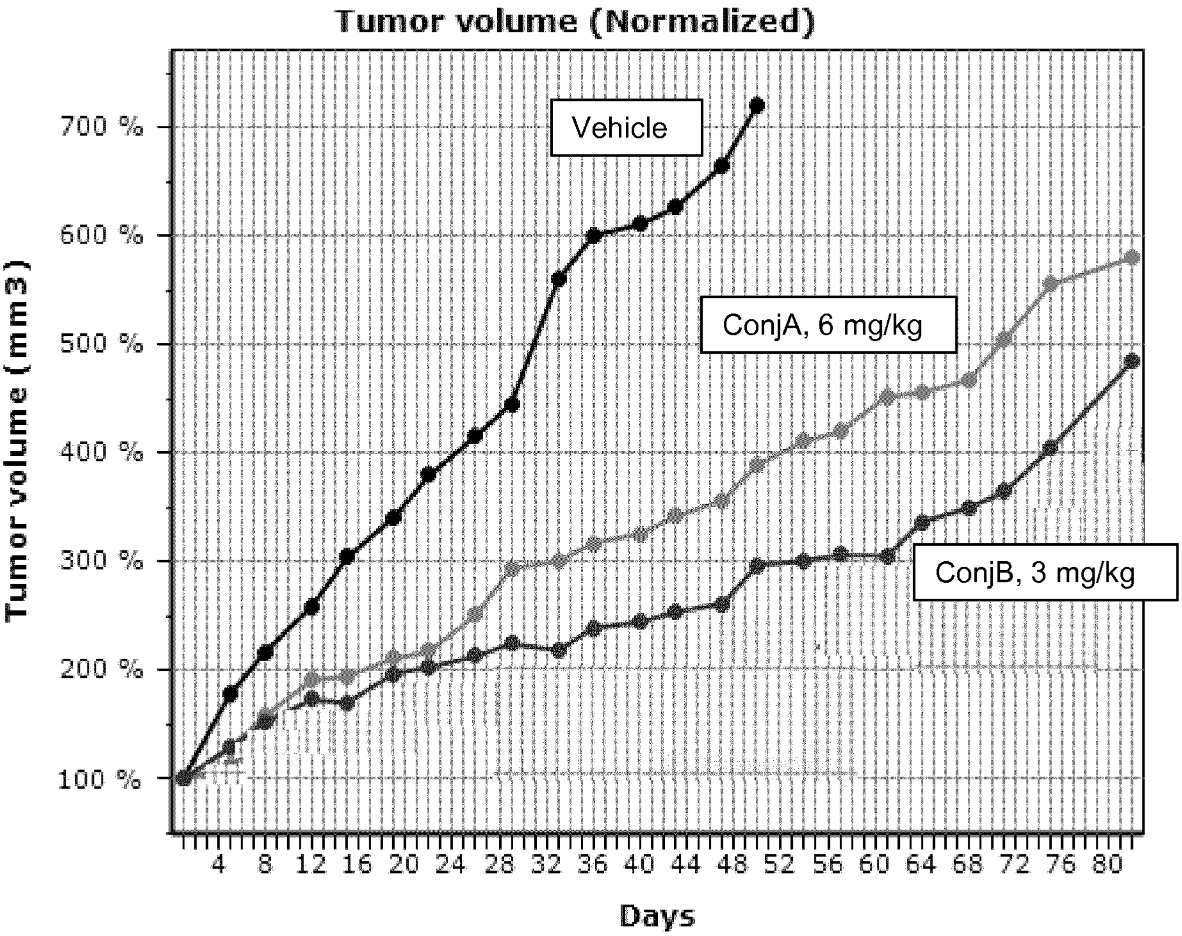

Tumor volume (Normalized)
Tumor volume (mm3)
700 %
600 %
500 %
400 %
300 %
200 %
100 %
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80
Days
Vehicle
ConjA, 6 mg/kg
ConjB, 3 mg/kg

Fig. 1

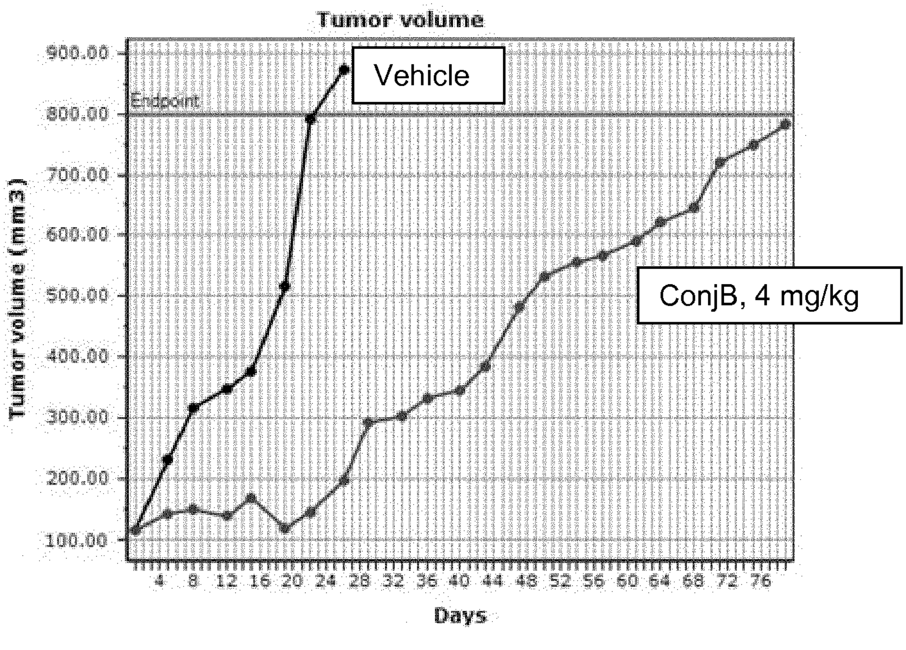
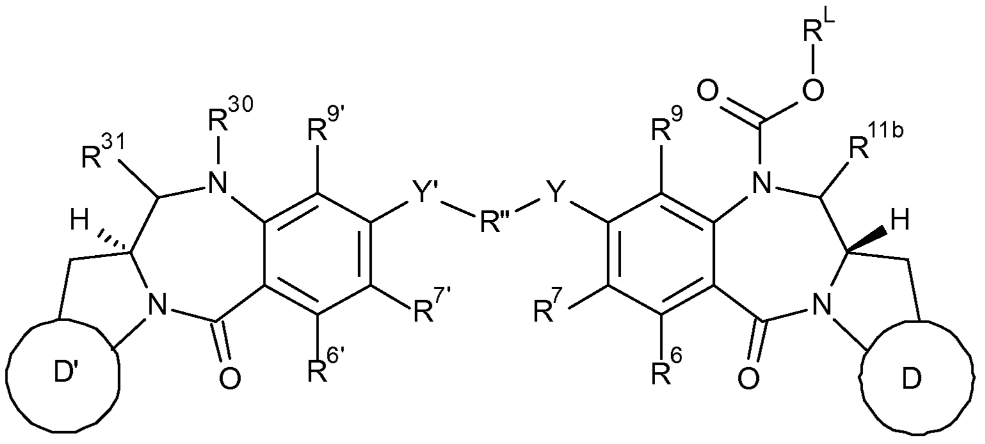
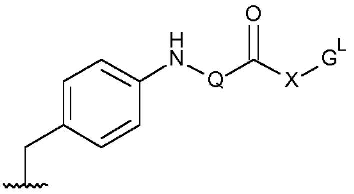

Tumor volume
Tumor volume (mm3)
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
Endpoint
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76
Days
Vehicle
ConjB, 4 mg/kg

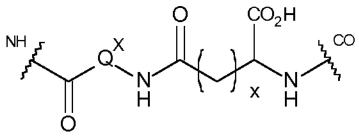
Fig. 2



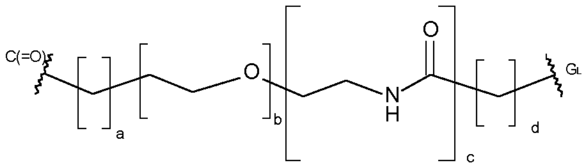
(I)



(IIIa)



(A)



(B)

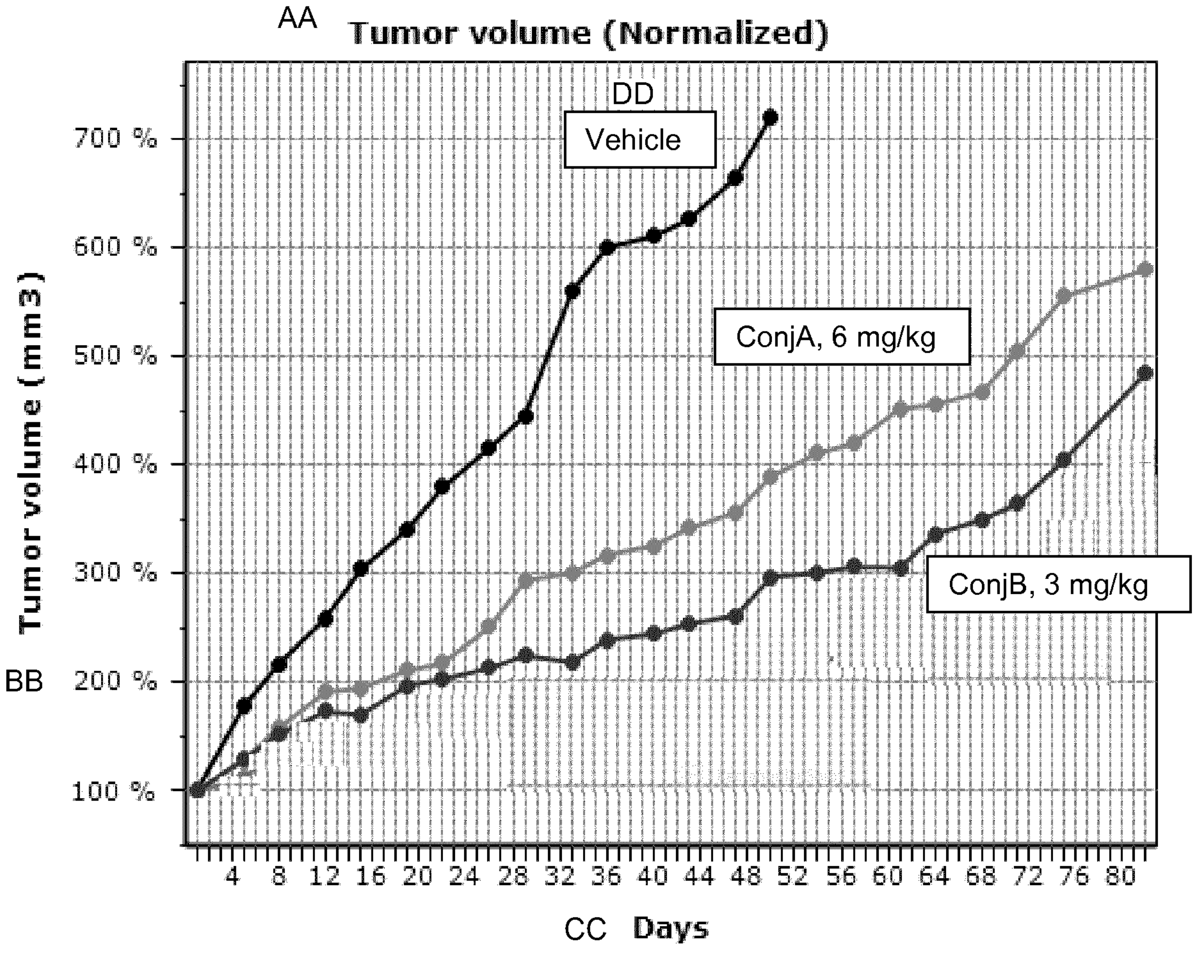


Fig. 1

AA
BB
CC
DD