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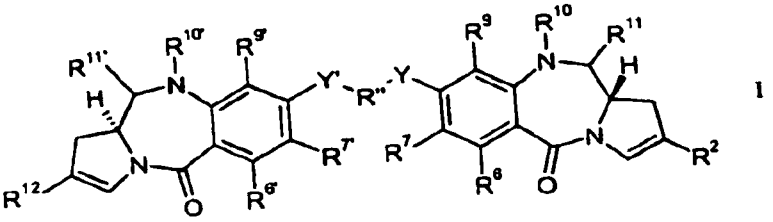

I

(57) Abstract: Pyrrolobenzodiazepine dimers I having a C2-C3 double bond and an aryl group at the C2 position on one monomer unit, and a C2-C3 double bond and either a conjugated double or triple bond at the C2 position or an alkyl group at the C2 position on the other monomer unit, and conjugates of these compounds.

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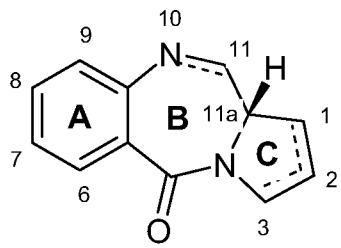
# PYRROLOBENZODIAZEPINES USED TO TREAT PROLIFERATIVE DISEASES

The present invention relates to pyrrolobenzodiazepines (PBDs), in particular pyrrolobenzodiazepine dimers having a C2-C3 double bond and an aryl group at the C2 position on one monomer unit, and a C2-C3 double bond and either a conjugated double or triple bond at the C2 position or an alkyl group at the C2 position on the other monomer unit.

## Background to the invention

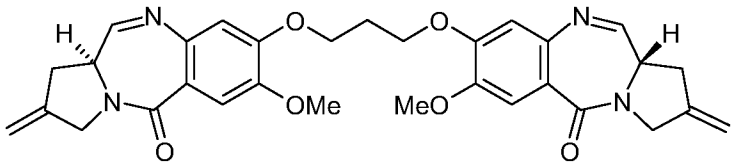
Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5793-5795 (1965); Leimgruber, *et al.*, *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

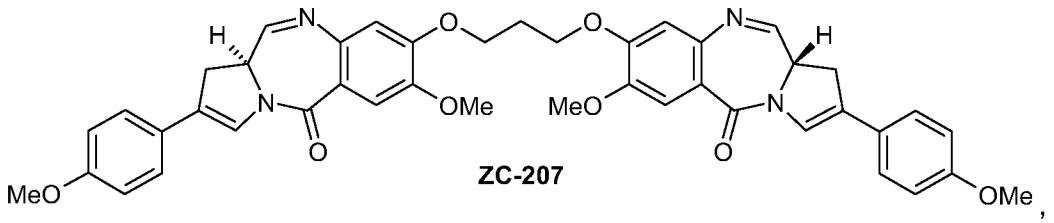
alkylating DNA. All of the known natural products have an (*S*)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form 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 this 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 dimmer, SG2000 (SJG-136):



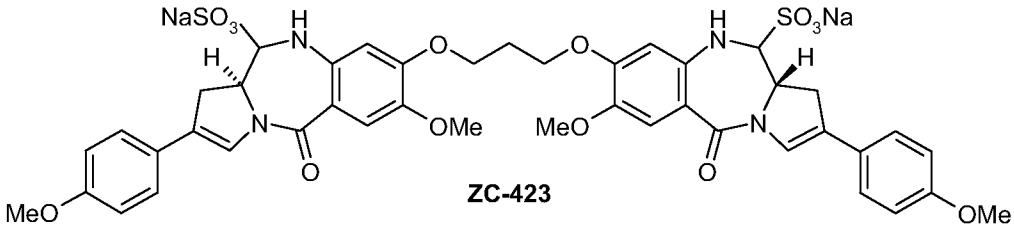

has recently completed Phase I clinical trials in the oncology area and is about to enter Phase II (Gregson, S., *et al.*, *J. Med. Chem.*, **44**, 737-748 (2001); Alley, M.C., *et al.*, *Cancer Research*, **64**, 6700-6706 (2004); Hartley, J.A., *et al.*, *Cancer Research*, **64**, 6693-6699 (2004)).

More recently, the present inventors have previously disclosed in WO 2005/085251, dimeric PBD compounds bearing C2 aryl substituents, such as SG2202 (ZC-207):




**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).

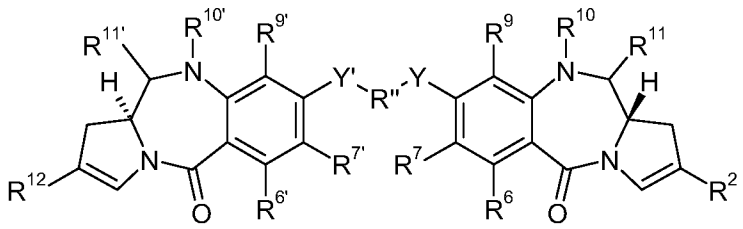
Due to the manner in which these highly potent compounds act in cross-linking DNA, these molecules have been made symmetrically. This provides for straightforward synthesis, either by constructing the PBD moieties simultaneously having already formed the dimer linkage, or by reacting already constructed PBD moieties with the dimer linking group.

Co-pending International Application PCT/GB2009/002498, filed 16 October 2009, discloses unsymmetrical dimeric PBD compound bearing aryl groups in the C2 position of each monomer, where one of these groups bears a substituent designed to provide an anchor for linking the compound to another moiety.

**Disclosure of the invention**

The present inventors have developed further unsymmetrical dimeric PBD compounds bearing an aryl group in the C2 position of one monomer, said aryl group bearing a substituent designed to provide an anchor for linking the compound to another moiety, and either a unsaturated bond conjugated to the C2-C3 double bond or an alkyl group in the other monomer unit.

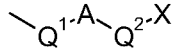
The present invention comprises a compound with the formula **I**:



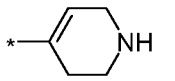
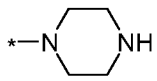

I

wherein:

$R^2$ is of formula **II**:




II

where A is a $C_{5-7}$ aryl group, X is selected from the group comprising: OH, SH, $CO_2H$, COH, N=C=O, $NHNH_2$, $CONHNH_2$,  ,  , $NHR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl, and either:

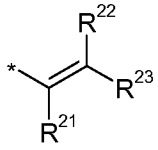
(i) $Q^1$ is a single bond, and $Q^2$ is selected from a single bond and -Z-$(CH_2)_n$-, where Z is selected from a single bond, O, S and NH and n is from 1 to 3; or

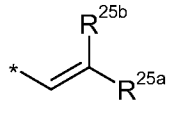
(ii) $Q^1$ is -CH=CH-, and $Q^2$ is a single bond;

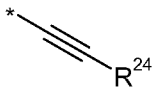
$R^{12}$ is selected from:

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

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

(iic)  , wherein each of $R^{21}$, $R^{22}$ 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 $R^{12}$ group is no more than 5;

(iid)  , 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

(iie)  , 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;

$R^6$ 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, NHRR', nitro, $Me_3Sn$ and halo;

either:

(a) $R^{10}$ is H, and $R^{11}$ is OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl;

(b) $R^{10}$ and $R^{11}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) $R^{10}$ is H and $R^{11}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

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;

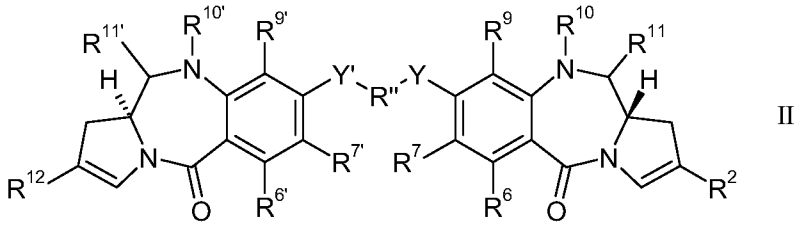
Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as $R^6$, $R^7$ and $R^9$ respectively and $R^{10'}$ and $R^{11'}$ are the same as $R^{10}$ and $R^{11}$, wherein if $R^{11}$ and $R^{11'}$ are $SO_zM$, M may represent a divalent pharmaceutically acceptable cation.

A second aspect of the present invention provides the use of a compound of the first aspect of the invention in the manufacture of a medicament for treating a proliferative disease. The second aspect also provides a compound of the first aspect of the invention for use in the treatment of a proliferative disease.

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.

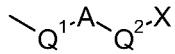
A third aspect of the present invention comprises a compound of formula **II**:



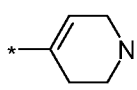
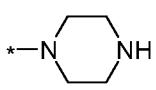
II

wherein:

$R^2$ is of formula II:



II

where A is a $C_{5-7}$ aryl group, X is selected from the group comprising: OH, SH, $CO_2H$, COH, N=C=O, $NHNH_2$, $CONHNH_2$,  ,  , $NHR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl, and either:

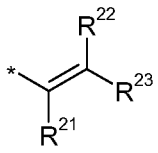
(i) $Q^1$ is a single bond, and $Q^2$ is selected from a single bond and -Z-$(CH_2)_n$-, where Z is selected from a single bond, O, S and NH and n is from 1 to 3; or

(ii) $Q^1$ is -CH=CH-, and $Q^2$ is a single bond;

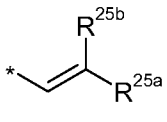
$R^{12}$ is selected from:

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

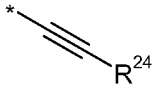
(iib) $C_{3-6}$ saturated cycloalkyl;



(iic) , wherein each of $R^{21}$, $R^{22}$ 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 $R^{12}$ group is no more than 5;



(iid) , 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



(iie) , 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;

$R^6$ 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, NHRR', nitro, $Me_3Sn$ and halo;

either:

(a) $R^{10}$ is carbamate nitrogen protecting group, and $R^{11}$ is O-$Prot^O$, wherein $Prot^O$ is an oxygen protecting group;

(b) $R^{10}$ is a hemi-aminal nitrogen protecting group and $R^{11}$ is an oxo group;

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 and $R^{10'}$ and $R^{11'}$ are the same as $R^{10}$ and $R^{11}$.

A fourth aspect of the present invention comprises a method of making a compound of formula I from a compound of formula II by deprotection of the imine bond.

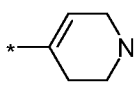
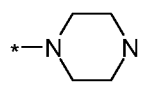
The unsymmetrical dimeric PBD compounds of the present invention are made by different strategies to those previously employed in making symmetrical dimeric PBD compounds. In particular, the present inventors have developed a method which involves adding each each C2 substituent to a symmetrical PBD dimer core in separate method steps. Accordingly, a fifth aspect of the present invention provides a method of making a compound of the first or third aspect of the invention, comprising at least one of the method steps set out below.

In a sixth aspect, the present invention relates to Conjugates comprising dimers of PBDs linked to a targeting agent, wherein a PBD is a dimer of formula I (supra).

In some embodiments, the Conjugates have the following formula III:

$$L - (LU-D)_p \qquad (III)$$

wherein L is a Ligand unit (i.e., a targeting agent), LU is a Linker unit and D is a Drug unit comprising a PBD dimer. The subscript p is an integer of from 1 to 20. Accordingly, the Conjugates comprise a Ligand unit covalently linked to at least one Drug unit by a Linker unit. 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. Accordingly, the present invention also provides methods for the treatment of, for example, various cancers and autoimmune disease. These methods encompass the use of the Conjugates wherein the Ligand unit is a targeting agent that specifically binds to a target molecule. 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.

The PBD dimer D is of formula I, except that X is selected from the group comprising: O, S, C(=O), C=, NH(C=O), NHNH, CONHNH,  ,  , $NR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl.

Brief Description of Figure

Figure 1 shows the effect of a conjugate of the invention on a tumour.

Definitions

*Pharmaceutically acceptable cations*

Examples of pharmaceutically acceptable monovalent and divalent cations are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977), which is incorporated herein by reference.

The pharmaceutically acceptable cation may be inorganic or organic.

Examples of pharmaceutically acceptable monovalent inorganic cations include, but are not limited to, alkali metal ions such as $Na^+$ and $K^+$. Examples of pharmaceutically acceptable divalent inorganic cations include, but are not limited to, alkaline earth cations such as $Ca^{2+}$ and $Mg^{2+}$. Examples of pharmaceutically acceptable organic cations include, but are not limited to, ammonium ion (i.e. $NH_4^+$) and substituted ammonium ions (e.g. $NH_3R^+$, $NH_2R_2^+$, $NHR_3^+$, $NR_4^+$). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

*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). 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. Preferably, each ring has from 5 to 7 ring atoms.

In this context, the prefixes (e.g. $C_{3-20}$, $C_{5-7}$, $C_{5-6}$, 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 $-OC(=O)CH_2Ph$.

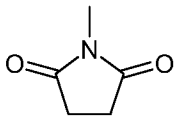
Oxycarboyloxy: -OC(=O)OR, wherein R is an ester substituent, for example, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably a $C_{1-7}$ alkyl group. Examples of ester groups include, but are not limited to, $-OC(=O)OCH_3$, $-OC(=O)OCH_2CH_3$, $-OC(=O)OC(CH_3)_3$,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

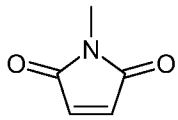
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein $R^1$ and $R^2$ are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, 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)$NHCH_2CH_3$.

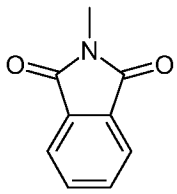
Acylamido (acylamino): -$NR^1$C(=O)$R^2$, wherein $R^1$ is an amide substituent, for example, hydrogen, a $C_{1-7}$ alkyl group, a $C_{3-20}$ heterocyclyl group, or a $C_{5-20}$ aryl group, preferably hydrogen or a $C_{1-7}$ alkyl group, and $R^2$ is an acyl substituent, for example, a $C_{1-7}$ alkyl 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



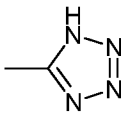
phthalimidyl

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)$_2$R, 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)$_2$OR; 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)$_2$OCH$_3$ and -SO(=O)$_2$OCH$_2$CH$_3$.

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): -S(=O)NR$^1$R$^2$, 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$_2$CH$_3$), -S(=O)N(CH$_2$CH$_3$)$_2$, and -S(=O)NHPh.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): -S(=O)$_2$NR$^1$R$^2$, 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)$_2$NH$_2$, -S(=O)$_2$NH(CH$_3$), -S(=O)$_2$N(CH$_3$)$_2$, -S(=O)$_2$NH(CH$_2$CH$_3$), -S(=O)$_2$N(CH$_2$CH$_3$)$_2$, and -S(=O)$_2$NHPh.

Sulfamino: -NR$^1$S(=O)$_2$OH, wherein R$^1$ is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, -NHS(=O)$_2$OH and -N(CH$_3$)S(=O)$_2$OH.

Sulfonamino: -NR$^1$S(=O)$_2$R, 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)$_2$CH$_3$ and -N(CH$_3$)S(=O)$_2$C$_6$H$_5$.

Sulfinamino: -NR$^1$S(=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$_6$H$_5$.

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-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-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-t-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-t-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-t-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-Pr)$_2$, and -OP($OCH_2CH_2CN$)-N(i-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-Pr)$_2$, and -OP(=O)($OCH_2CH_2CN$)-N(i-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 -$CH_2CH_2CH_2CH_2CH_2CH_2$- (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≡C-$CH_2$-.

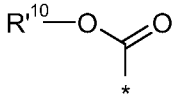
Examples of branched partially unsaturated $C_{3-12}$ alkylene groups ($C_{3-12}$ alkenylene and alkynylene groups) include, but are not limited to, -C($CH_3$)=CH-, -C($CH_3$)=CH-$CH_2$-, -CH=CH-CH($CH_3$)- and -C≡C-CH($CH_3$)-.

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).

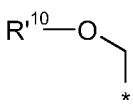
Oxygen protecting group: the term "oxygen protecting group" refers to a moiety which masks a hydroxy group, and these are well known in the art. A large number of suitable groups are described on pages 23 to 200 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference. Classes of particular interest include silyl ethers (e.g. TMS, TBDMS), substituted methyl ethers (e.g. THP) and esters (e.g. acetate).

Carbamate nitrogen protecting group: the term "carbamate nitrogen protecting group" pertains to a moiety which masks the nitrogen in the imine bond, and these are well known in the art. These groups have the following structure:



wherein R'10 is R as defined above. A large number of suitable groups are described on pages 503 to 549 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Hemi-aminal nitrogen protecting group: the term "hemi-aminal nitrogen protecting group" pertains to a group having the following structure:



wherein $R'^{10}$ is R as defined above. A large number of suitable groups are described on pages 633 to 647 as amide protecting groups of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Conjugates

The present invention provides Conjugates comprising a PBD dimer connected to a Ligand unit via a Linker Unit. In one embodiment, the Linker unit includes a Stretcher unit (A), a Specificity unit ($L^1$), and a Spacer unit ($L^2$). The Linker unit is connected at one end to the Ligand unit and at the other end to the PBD dimer compound.

In one aspect, such a Conjugate is shown below in formula IIIa:

$$L\text{-}(A^1_a\text{-}L^1_s\text{-}L^2_y\text{-}D)_p \qquad \text{(IIIa)}$$

wherein:

L is the Ligand unit; and

$-A^1_a-L^1_s-L^2_y-$ is a Linker unit (LU), wherein:

$-A^1-$ is a Stretcher unit,

a is 1 or 2,

$L^1$ - is a Specificity unit,

s is an integer ranging from 1 to 12,

$-L^2-$ is a Spacer unit,

y is 0, 1 or 2;

-D is an PBD dimer; and

p is from 1 to 20.

In another aspect, such a Conjugate is shown below in formula IIIb:

$$\begin{array}{l} L^1_s \\ | \\ L - (A^1_a - L^2_y - D)_p \end{array} \qquad \text{(IIIb)}$$

Also illustrated as:

$$L - (A^1_a - L^2_y\ (- L^1_s) - D)_p \qquad \text{(Ib)}$$

wherein:

L is the Ligand unit; and

$-A^1_a-L^1_s(L^2_y)-$ is a Linker unit (LU), wherein:

$-A^1-$ is a Stretcher unit linked to a Stretcher unit ($L^2$),

a is 1 or 2,

$L^1$ - is a Specificity unit linked to a Stretcher unit ($L^2$),

s is an integer ranging from 0 to 12,

$-L^2-$ is a Spacer unit,

y is 0, 1 or 2;

-D is a PBD dimer; and

p is from 1 to 20.

Preferences

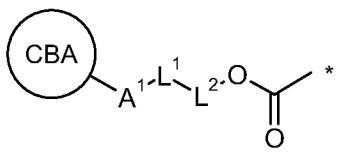
The following preferences may apply to all aspects of the invention as described above, or may relate to a single aspect. The preferences may be combined together in any combination.

In one embodiment, the Conjugate has the formula:

$$L-(A^1_a-L^1_s-L^2_y-D)_p$$

wherein L, $A^1$, a, $L^1$, s, $L^2$, D and p are as described above.

In one embodiment, the Ligand unit (L) is a Cell Binding Agent (CBA) that specifically binds to a target molecule on the surface of a target cell. An exemplary formula is illustrated below:




where the asterisk indicates the point of attachment to the Drug unit (D), CBA is the Cell Binding Agent, $L^1$ is a Specificity unit, $A^1$ is a Stretcher unit connecting $L^1$ to the Cell Binding Agent, $L^2$ is a Spacer unit, which is a covalent bond, a self-immolative group or together with -OC(=O)- forms a self-immolative group, and $L^2$ optional.

In another embodiment, the Ligand unit (L) is a Cell Binding Agent (CBA) that specifically binds to a target molecule on the surface of a target cell. An exemplary formula is illustrated below:

$$CBA - A^1_a - L^1_s - L^2_y - *$$

where the asterisk indicates the point of attachment to the Drug unit (D), CBA is the Cell Binding Agent, $L^1$ is a Specificity unit, $A^1$ is a Stretcher unit connecting $L^1$ to the Cell Binding Agent, $L^2$ is a Spacer unit which is a covalent bond or a self-immolative group, and a is 1 or 2, s is 0, 1 or 2, and y is 0 or 1 or 2.

In the embodiments illustrated above, $L^1$ can be a cleavable Specificity unit, and may be referred to as a "trigger" that when cleaved activates a self-immolative group (or self-immolative groups) $L^2$, when a self-immolative group(s) is present. When the Specificity unit $L^1$ is cleaved, or the linkage (i.e., the covalent bond) between $L^1$ and $L^2$ is cleaved, the self-immolative group releases the Drug unit (D).

In another embodiment, the Ligand unit (L) is a Cell Binding Agent (CBA) that specifically binds to a target molecule on the surface of a target cell. An exemplary formula is illustrated below:

$$\begin{array}{l} L^1_s \\ | \\ CBA - A^1_a - L^2_y - * \end{array}$$

where the asterisk indicates the point of attachment to the Drug (D), CBA is the Cell Binding Agent, $L^1$ is a Specificity unit connected to $L^2$, $A^1$ is a Stretcher unit connecting $L^2$ to the Cell Binding Agent, $L^2$ is a self-immolative group, and a is 1 or 2, s is 1 or 2, and y is 1 or 2.

In the various embodiments discussed herein, the nature of $L^1$ and $L^2$ can vary widely. These groups are chosen on the basis of their characteristics, which may be dictated in part, by the conditions at the site to which the conjugate is delivered. Where the Specificity unit $L^1$ is cleavable, the structure and/or sequence of $L^1$ is selected such that it is cleaved by the action of enzymes present at the target site (e.g., the target cell). $L^1$ units that are cleavable by changes in pH (e.g. acid or base labile), temperature or upon irradiation (e.g. photolabile) may also be used. $L^1$ units that are cleavable under reducing or oxidising conditions may also find use in the Conjugates.

In some embodiments, $L^1$ may comprise one amino acid or a contiguous sequence of amino acids. The amino acid sequence may be the target substrate for an enzyme.

In one embodiment, $L^1$ is cleavable by the action of an enzyme. In one embodiment, the enzyme is an esterase or a peptidase. For example, $L^1$ may be cleaved by a lysosomal protease, such as a cathepsin.

In one embodiment, $L^2$ is present and together with -C(=O)O- forms a self-immolative group or self-immolative groups. In some embodiments, -C(=O)O- also is a self-immolative group.

In one embodiment, where $L^1$ is cleavable by the action of an enzyme and $L^2$ is present, the enzyme cleaves the bond between $L^1$ and $L^2$, whereby the self-immolative group(s) release the Drug unit.

$L^1$ and $L^2$, where present, may be connected by a bond selected from:

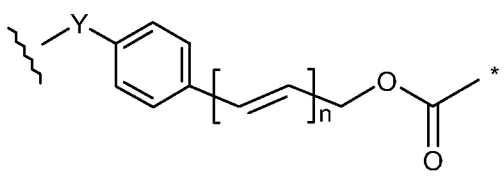
-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-,
-NHC(=O)NH, and
-O- (a glycosidic bond).

An amino group of $L^1$ that connects to $L^2$ may be the N-terminus of an amino acid or may be derived from an amino group of an amino acid side chain, for example a lysine amino acid side chain.

A carboxyl group of $L^1$ that connects to $L^2$ may be the C-terminus of an amino acid or may be derived from a carboxyl group of an amino acid side chain, for example a glutamic acid amino acid side chain.

A hydroxy group of $L^1$ that connects to $L^2$ may be derived from a hydroxy group of an amino acid side chain, for example a serine amino acid side chain.

In one embodiment, -C(=O)O- and $L^2$ together form the group:

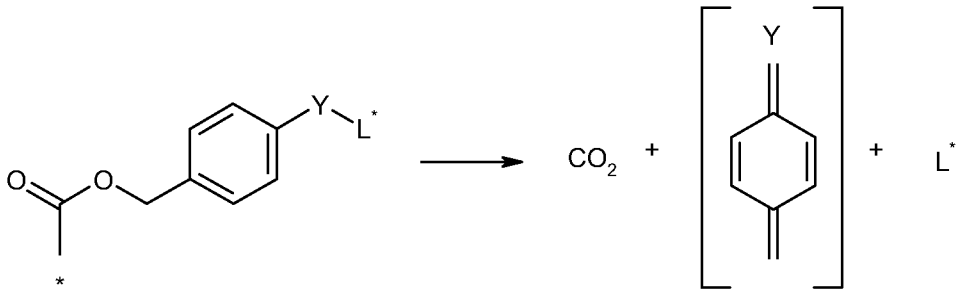


where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to the $L^1$, Y is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-, and n is 0 to 3. The phenylene ring is optionally substituted with one, two or three substituents as described herein.

In one embodiment, Y is NH.
In one embodiment, n is 0 or 1. Preferably, n is 0.

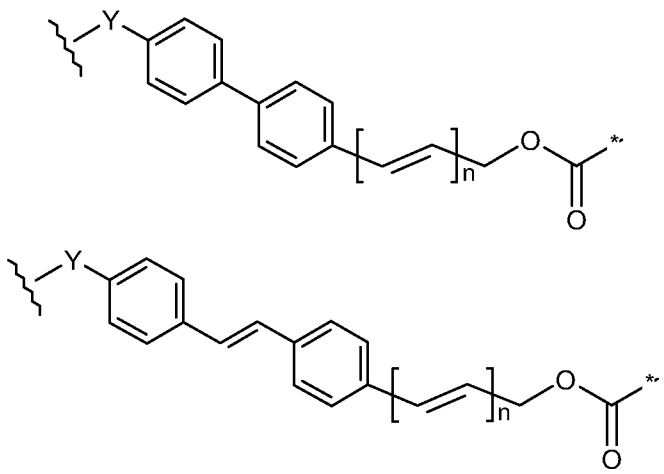
Where Y is NH and n is 0, the self-immolative group may be referred to as a p-aminobenzylcarbonyl linker (PABC).

The self-immolative group will allow for release of the Drug unit (i.e., the asymmetric PBD) when a remote site in the linker is activated, proceeding along the lines shown below (for n=0):




where the asterisk indicates the attachment to the Drug, $L^*$ is the activated form of the remaining portion of the linker and the released Drug unit is not shown. These groups have the advantage of separating the site of activation from the Drug.

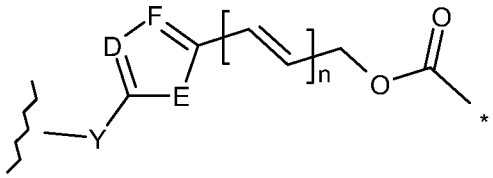
In another embodiment, -C(=O)O- and $L^2$ together form a group selected from:




where the asterisk, the wavy line, Y, and n are as defined above. Each phenylene ring is optionally substituted with one, two or three substituents as described herein. In one

embodiment, the phenylene ring having the Y substituent is optionally substituted and the phenylene ring not having the Y substituent is unsubstituted.

In another embodiment, -C(=O)O- and $L^2$ together form a group selected from:




where the asterisk, the wavy line, Y, and n are as defined above, E is O, S or NR, D is N, CH, or CR, and F is N, CH, or CR.

In one embodiment, D is N.
In one embodiment, D is CH.
In one embodiment, E is O or S.
In one embodiment, F is CH.

In a preferred embodiment, the covalent bond between $L^1$ and $L^2$ is a cathepsin labile (e.g., cleavable) bond.

In one embodiment, $L^1$ comprises a dipeptide. 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, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

where Cit is citrulline. In such a dipeptide, -NH- is the amino group of $X_1$, and CO is the carbonyl group of $X_2$.

Preferably, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

Most preferably, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is -Phe-Lys-, Val-Cit or -Val-Ala-.

Other dipeptide combinations of interest include:
-Gly-Gly-,
-Pro-Pro-, and
-Val-Glu-.

Other dipeptide combinations may be used, including those described by Dubowchik et al., which is incorporated herein by reference.

In one embodiment, the amino acid side chain is chemically protected, where appropriate. The side chain protecting group may be a group as discussed below. Protected amino acid sequences are cleavable by enzymes. For example, 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;
Ser: Bzl, TBDMS, TBDPS;
Thr: Bz;
Trp: Boc;
Tyr: Bzl, Z, Z-Br.

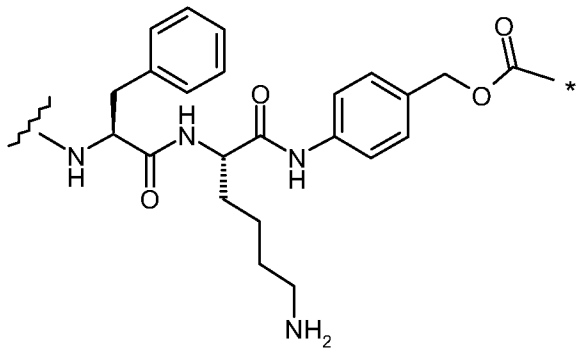
In one embodiment, -$X_2$- is connected indirectly to the Drug unit. In such an embodiment, the Spacer unit $L^2$ is present.

In one embodiment, the dipeptide is used in combination with a self-immolative group(s) (the Spacer unit). The self-immolative group(s) may be connected to -$X_2$-.

Where a self-immolative group is present, -$X_2$- is connected directly to the self-immolative group. In one embodiment, -$X_2$- is connected to the group Y of the self-immolative group. Preferably the group -$X_2$-CO- is connected to Y, where Y is NH.

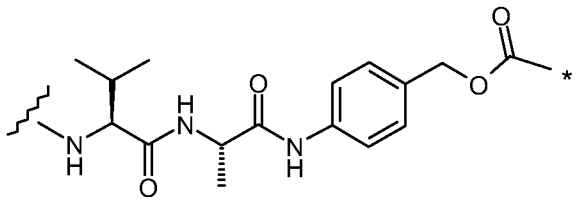
-$X_1$- is connected directly to $A^1$. In one embodiment, -$X_1$- is connected directly to $A^1$. Preferably the group NH-$X_1$- (the amino terminus of $X_1$) is connected to $A^1$. $A^1$ may comprise the functionality -CO- thereby to form an amide link with -$X_1$-.

In one embodiment, $L^1$ and $L^2$ together with -OC(=O)- comprise the group -$X_1$-$X_2$-PABC-. The PABC group is connected directly to the Drug unit. In one example, the self-immolative group and the dipeptide together form the group -Phe-Lys-PABC-, which is illustrated below:



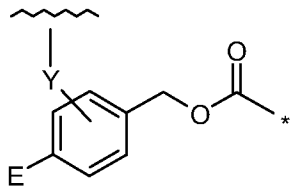
where the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of $L^1$ or the point of attachment to $A^1$. Preferably, the wavy line indicates the point of attachment to $A^1$.

Alternatively, the self-immolative group and the dipeptide together form the group -Val-Ala-PABC-, which is illustrated below:

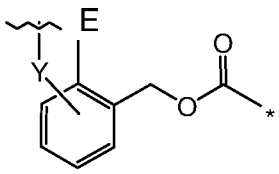


where the asterisk and the wavy line are as defined above.

In another embodiment, $L^1$ and $L^2$ together with -OC(=O)- represent:




or




,

where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to $A^1$, Y is a covalent bond or a functional group, and E is a group that is susceptible to cleavage thereby to activate a self-immolative group.

E is selected such that the group is susceptible to cleavage, e.g., by light or by the action of an enzyme. E may be $-NO_2$ or glucuronic acid (e.g., β-glucuronic acid). The former may be susceptible to the action of a nitroreductase, the latter to the action of a β-glucuronidase.

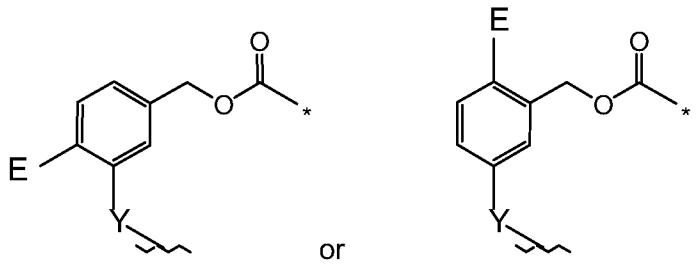
The group Y may be a covalent bond.

The group Y may be a functional group selected from:

-C(=O)-
-NH-
-O-
-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-,
-NHC(=O)NH-,
-NHC(=O)NH,
-C(=O)NHC(=O)-,
$SO_2$, and
-S-.

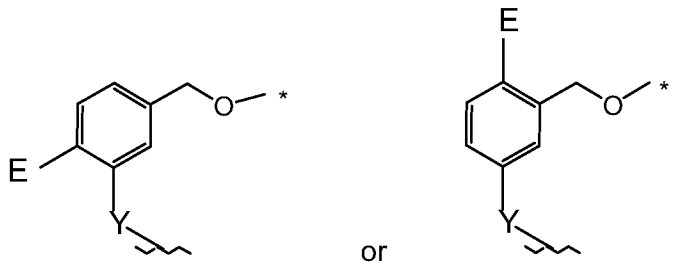
The group Y is preferably –NH-, $-CH_2-$, -O-, and -S-.

In some embodiments, $L^1$ and $L^2$ together with -OC(=O)- represent:



where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to A, Y is a covalent bond or a functional group and E is glucuronic acid (e.g., β-glucuronic acid). Y is preferably a functional group selected from –NH-.

In some embodiments, $L^1$ and $L^2$ together represent:



where the asterisk indicates the point of attachment to the remainder of $L^2$ or the Drug unit, the wavy line indicates the point of attachment to $A^1$, Y is a covalent bond or a functional group and E is glucuronic acid (e.g., β-glucuronic acid). Y is preferably a functional group selected from –NH-, -$CH_2$–, -O-, and -S-.

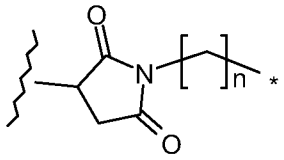
In some further embodiments, Y is a functional group as set forth above, the functional group is linked to an amino acid, and the amino acid is linked to the Stretcher unit $A^1$. In some embodiments, amino acid is β-alanine. In such an embodiment, the amino acid is equivalently considered part of the Stretcher unit.

The Specificity unit $L^1$ and the Ligand unit are indirectly connected via the Stretcher unit.

$L^1$ and $A^1$ may be connected by a bond selected from:

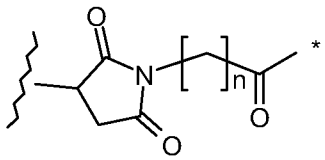
-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-, and
-NHC(=O)NH-.

In one embodiment, the group $A^1$ is:



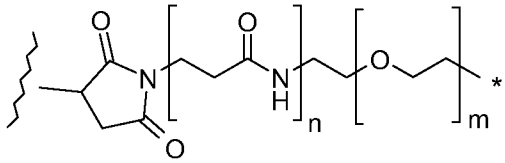
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $A^1$ is:



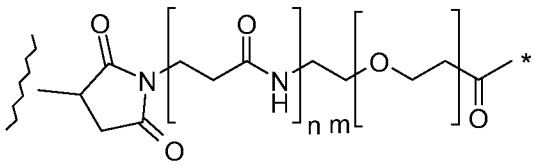
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $A^1$ is:



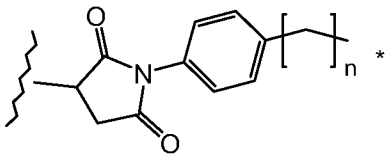
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group $A^1$ is:



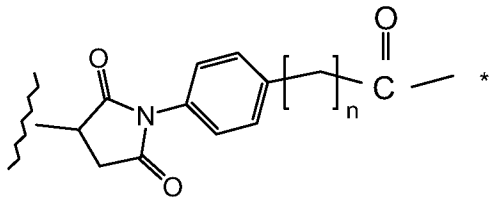
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group $A^1$ is:



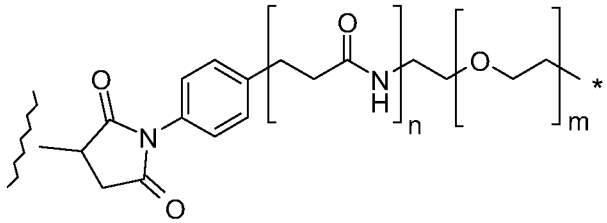
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $A^1$ is:



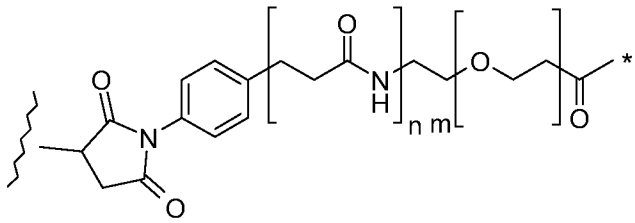
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $A^1$ is:



where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

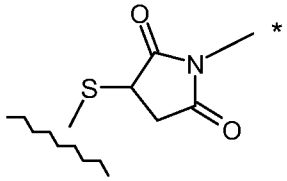
In one embodiment, the group $A^1$ is:



where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

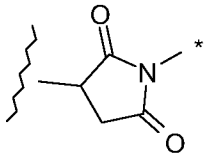
In one embodiment, the connection between the Ligand unit and $A^1$ is through a thiol residue of the Ligand unit and a maleimide group of $A^1$.

In one embodiment, the connection between the Ligand unit and $A^1$ is:



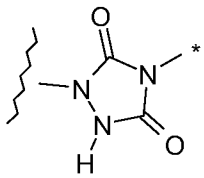
where the asterisk indicates the point of attachment to the remaining portion of $A^1$, $L^1$, $L^2$ or D, and the wavy line indicates the point of attachment to the remaining portion of the Ligand unit. In this embodiment, the S atom is typically derived from the Ligand unit.

In each of the embodiments above, an alternative functionality may be used in place of the malemide-derived group shown below:



where the wavy line indicates the point of attachment to the Ligand unit as before, and the asterisk indicates the bond to the remaining portion of the $A^1$ group, or to $L^1$, $L^2$ or D.

In one embodiment, the maleimide-derived group is replaced with the group:



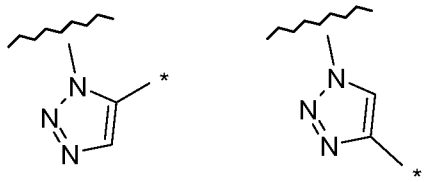
where the wavy line indicates point of attachment to the Ligand unit, and the asterisk indicates the bond to the remaining portion of the $A^1$ group , or to $L^1$, $L^2$ or D.

In one embodiment, the maleimide-derived group is replaced with a group, which optionally together with a Ligand unit (e.g., a Cell Binding Agent), is selected from:

-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-,
-NHC(=O)NH-,
-NHC(=O)NH,

-C(=O)NHC(=O)-,
-S-,
-S-S-,
-$CH_2$C(=O)-
-C(=O)$CH_2$-,
=N-NH-, and
-NH-N=.

In one embodiment, the maleimide-derived group is replaced with a group, which optionally together with the Ligand unit, is selected from:



where the wavy line indicates either the point of attachment to the Ligand unit or the bond to the remaining portion of the $A^1$ group, and the asterisk indicates the other of the point of attachment to the Ligand unit or the bond to the remaining portion of the $A^1$ group.

Other groups suitable for connecting $L^1$ to the Cell Binding Agent are described in WO 2005/082023.

In one embodiment, the Stretcher unit $A^1$ is present, the Specificity unit $L^1$ is present and Spacer unit $L^2$ is absent. Thus, $L^1$ and the Drug unit are directly connected via a bond. Equivalently in this embodiment, $L^2$ is a bond.

$L^1$ and D may be connected by a bond selected from:
-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-, and
-NHC(=O)NH-.

In one embodiment, $L^1$ and D are preferably connected by a bond selected from:
-C(=O)NH-, and
-NHC(=O)-.

In one embodiment, $L^1$ comprises a dipeptide and one end of the dipeptide is linked to D. As described above, 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, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

where Cit is citrulline. In such a dipeptide, -NH- is the amino group of $X_1$, and CO is the carbonyl group of $X_2$.

Preferably, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

Most preferably, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is -Phe-Lys- or -Val-Ala-.

Other dipeptide combinations of interest include:

-Gly-Gly-,
-Pro-Pro-, and
-Val-Glu-.

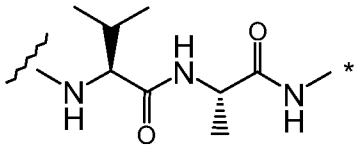
Other dipeptide combinations may be used, including those described above.

In one embodiment, $L^1$-D is:


-NH-$X_1$-$X_2$-CO-NH- *

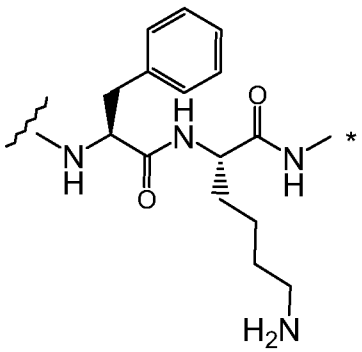
where -NH-$X_1$-$X_2$-CO is the dipeptide, -NH- is part of the Drug unit, the asterisk indicates the point of attachment to the remainder of the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of $L^1$ or the point of attachment to $A^1$. Preferably, the wavy line indicates the point of attachment to $A^1$.

In one embodiment, the dipeptide is valine-alanine and $L^1$-D is:



where the asterisk, -NH- and the wavy line are as defined above.

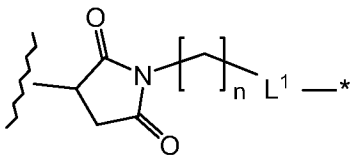
In one embodiment, the dipeptide is phenylalnine-lysine and $L^1$-D is:



where the asterisk, -NH- and the wavy line are as defined above.

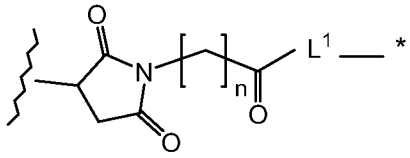
In one embodiment, the dipeptide is valine-citrulline.

In one embodiment, the groups $A^1$-$L^1$ are:



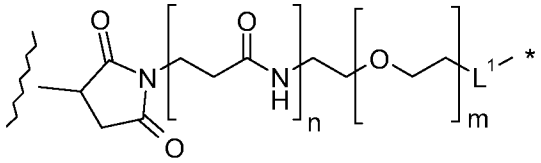
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups $A^1$-$L^1$ are:



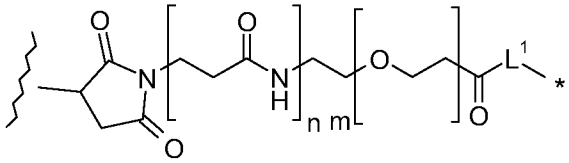
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups $A^1$-$L^1$ are:



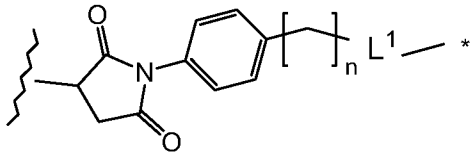
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups $A^1$-$L^1$ are:



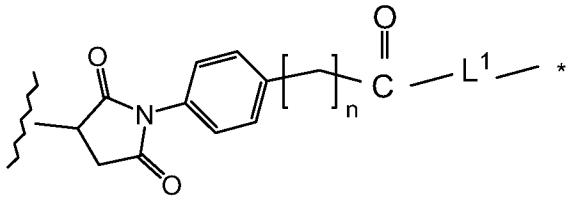
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 3 to 7, most preferably 3 or 7.

In one embodiment, the groups $A^1$-$L^1$ are:



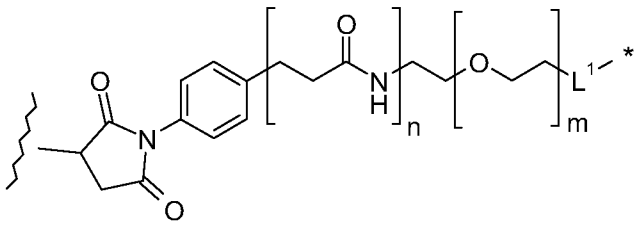
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups $A^1$-$L^1$ are:



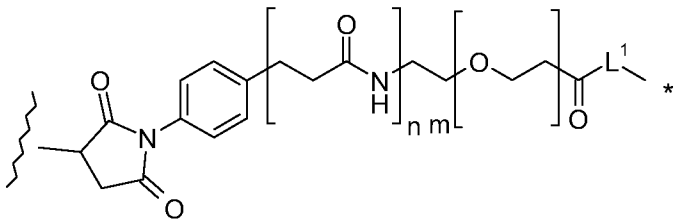
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups $A^1$-$L^1$ are:



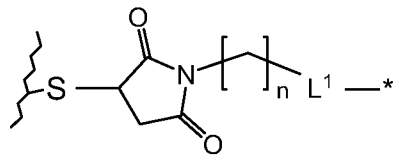
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups $A^1$-$L^1$ is:



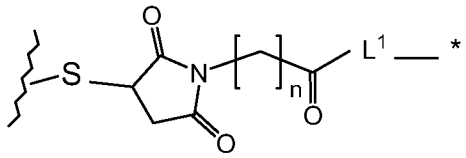
where the asterisk indicates the point of attachment to $L^2$ orD, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups L- $A^1$-$L^1$ are:



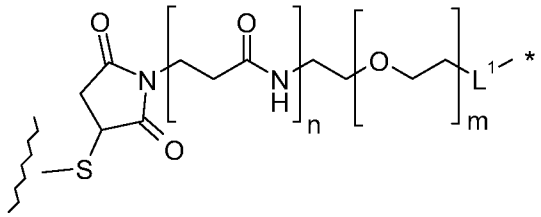
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the rest of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group L-$A^1$-$L^1$ are:



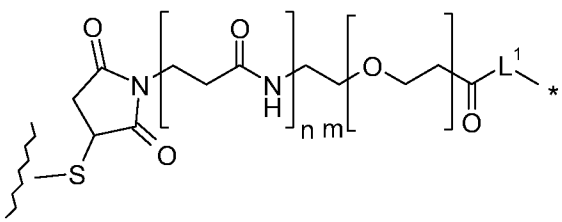
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups L-$A^1$-$L^1$ are:



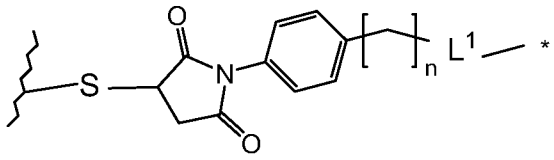
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups L-$A^1$-$L^1$ are:



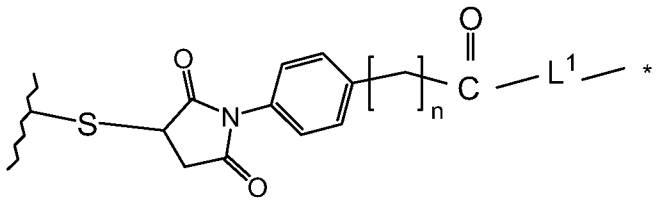
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups L-$A^1$-$L^1$ are:



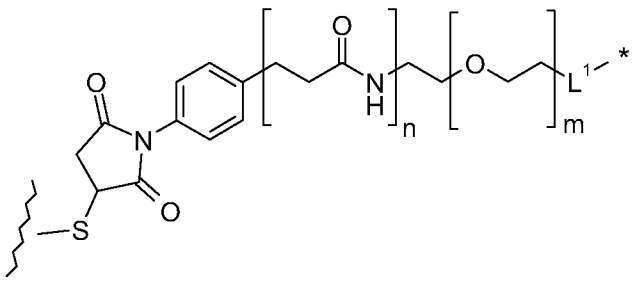
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups L-$A^1$-$L^1$ are:



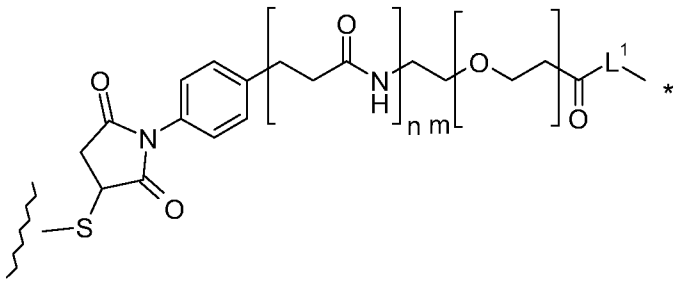
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the groups L-$A^1$-$L^1$ are:



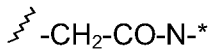
where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the groups L-$A^1$-$L^1$ are:



where the asterisk indicates the point of attachment to $L^2$ or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

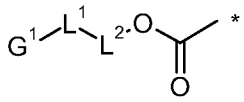
In one embodiment, the Stretcher unit is an acetamide unit, having the formula:



where the asterisk indicates the point of attachment to the remainder of the Stretcher unit, $L^1$ or D, and the wavy line indicates the point of attachment to the Ligand unit.

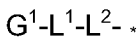
In other embodiments, Linker-Drug compounds are provided for conjugation to a Ligand unit. In one embodiment, the Linker-Drug compounds are designed for connection to a Cell Binding Agent.

In one embodiment, the Drug Linker compound has the formula:



where the asterisk indicates the point of attachment to the Drug unit, $G^1$ is a Stretcher group ($A^1$) to form a connection to a Ligand unit, $L^1$ is a Specificity unit, $L^2$ (a Spacer unit) is a covalent bond or together with -OC(=O)- forms a self-immolative group(s).

In another embodiment, the Drug Linker compound has the formula:



where the asterisk indicates the point of attachment to the Drug unit, $G^1$ is a Stretcher unit ($A^1$) to form a connection to a Ligand unit, $L^1$ is a Specificity unit, $L^2$ (a Spacer unit) is a covalent bond or a self-immolative group(s).

$L^1$ and $L^2$ are as defined above. References to connection to $A^1$ can be construed here as referring to a connection to $G^1$.

In one embodiment, where $L^1$ comprises an amino acid, the side chain of that amino acid may be protected. Any suitable protecting group may be used. In one embodiment, the side chain protecting groups are removable with other protecting groups in the compound, where present. In other embodiments, the protecting groups may be orthogonal to other protecting groups in the molecule, where present.

Suitable protecting groups for amino acid side chains include those groups described in the Novabiochem Catalog 2006/2007. Protecting groups for use in a cathepsin labile linker are also discussed in Dubowchik et al.

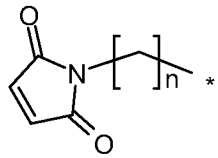
In certain embodiments of the invention, the group $L^1$ includes a Lys amino acid residue. The side chain of this amino acid may be protected with a Boc or Alloc protected group. A Boc protecting group is most preferred.

The functional group $G^1$ forms a connecting group upon reaction with a Ligand unit (e.g., a cell binding agent.

In one embodiment, the functional group $G^1$ is or comprises an amino, carboxylic acid, hydroxy, thiol, or maleimide group for reaction with an appropriate group on the Ligand unit. In a preferred embodiment, $G^1$ comprises a maleimide group.

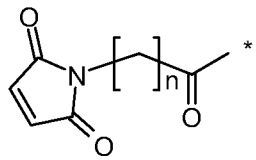
In one embodiment, the group $G^1$ is an alkyl maleimide group. This group is suitable for reaction with thiol groups, particularly cysteine thiol groups, present in the cell binding agent, for example present in an antibody.

In one embodiment, the group $G^1$ is:



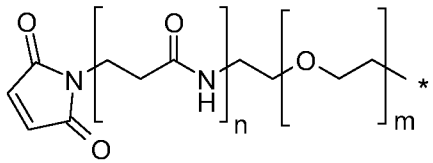
where the asterisk indicates the point of attachment to $L^1$, $L^2$ or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $G^1$ is:



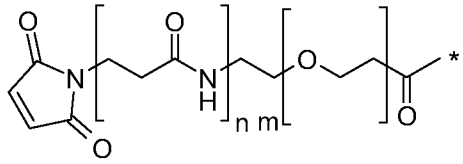
where the asterisk indicates the point of attachment to $L^1$, $L^2$ or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $G^1$ is:



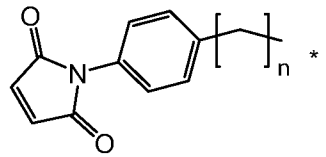
where the asterisk indicates the point of attachment to $L^1$, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group $G^1$ is:



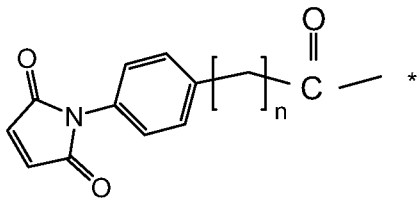
where the asterisk indicates the point of attachment to $L^1$, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group $G^1$ is:



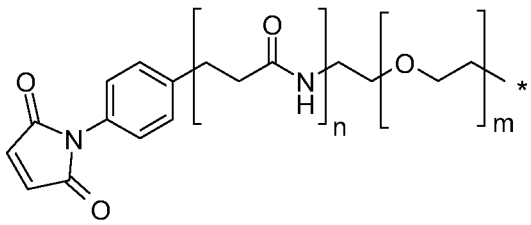
where the asterisk indicates the point of attachment to $L^1$, $L^2$ or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $G^1$ is:



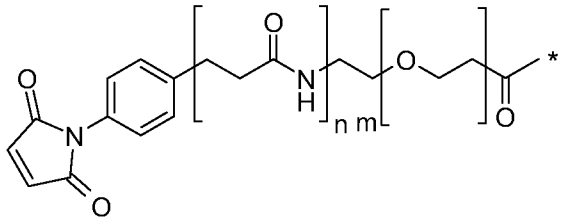
where the asterisk indicates the point of attachment to $L^1$, $L^2$ or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group $G^1$ is:



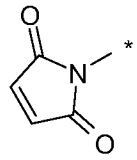
where the asterisk indicates the point of attachment to $L^1$, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group $G^1$ is:



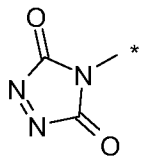
where the asterisk indicates the point of attachment to $L^1$, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8.

In each of the embodiments above, an alternative functionality may be used in place of the malemide group shown below:



where the asterisk indicates the bond to the remaining portion of the G group.

In one embodiment, the maleimide-derived group is replaced with the group:



where the asterisk indicates the bond to the remaining portion of the G group.

In one embodiment, the maleimide group is replaced with a group selected from:

-C(=O)OH,
-OH,
-$NH_2$,
-SH,
-$C(=O)CH_2X$, where X is Cl, Br or I,
-CHO,
-$NHNH_2$
-C≡CH, and
-$N_3$ (azide).

In one embodiment, $L^1$ is present, and $G^1$ is -$NH_2$, -NHMe, -COOH, -OH or -SH.

In one embodiment, where $L^1$ is present, $G^1$ is $-NH_2$ or -NHMe. Either group may be the N-terminal of an $L^1$ amino acid sequence.

In one embodiment, $L^1$ is present and $G^1$ is $-NH_2$, and $L^1$ is an amino acid sequence $-X_1-X_2-$, as defined above.

In one embodiment, $L^1$ is present and $G^1$ is COOH. This group may be the C-terminal of an $L^1$ amino acid sequence.

In one embodiment, $L^1$ is present and $G^1$ is OH.
In one embodiment, $L^1$ is present and $G^1$ is SH.

The group $G^1$ may be convertable from one functional group to another. In one embodiment, $L^1$ is present and $G^1$ is $-NH_2$. This group is convertable to another group $G^1$ comprising a maleimide group. For example, the group $-NH_2$ may be reacted with an acids or an activated acid (e.g., N-succinimide forms) of those $G^1$ groups comprising maleimide shown above.

The group $G^1$ may therefore be converted to a functional group that is more appropriate for reaction with a Ligand unit.

As noted above, in one embodiment, $L^1$ is present and $G^1$ is $-NH_2$, -NHMe, -COOH, -OH or -SH. In a further embodiment, these groups are provided in a chemically protected form. The chemically protected form is therefore a precursor to the linker that is provided with a functional group.

In one embodiment, $G^1$ is $-NH_2$ in a chemically protected form. The group may be protected with a carbamate protecting group. The carbamate protecting group may be selected from the group consisting of:

Alloc, Fmoc, Boc, Troc, Teoc, Cbz and PNZ.

Preferably, where $G^1$ is $-NH_2$, it is protected with an Alloc or Fmoc group.

In one embodiment, where $G^1$ is $-NH_2$, it is protected with an Fmoc group.

In one embodiment, the protecting group is the same as the carbamate protecting group of the capping group.

In one embodiment, the protecting group is not the same as the carbamate protecting group of the capping group. In this embodiment, it is preferred that the protecting group is removable under conditions that do not remove the carbamate protecting group of the capping group.

The chemical protecting group may be removed to provide a functional group to form a connection to a Ligand unit. Optionally, this functional group may then be converted to another functional group as described above.

In one embodiment, the active group is an amine. This amine is preferably the N-terminal amine of a peptide, and may be the N-terminal amine of the preferred dipeptides of the invention.

The active group may be reacted to yield the functional group that is intended to form a connection to a Ligand unit.

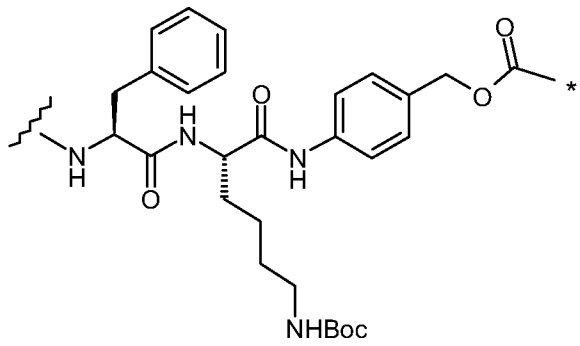
In other embodiments, the Linker unit is a precursor to the Linker uit having an active group. In this embodiment, the Linker unit comprises the active group, which is protected by way of a protecting group. The protecting group may be removed to provide the Linker unit having an active group.

Where the active group is an amine, the protecting group may be an amine protecting group, such as those described in Green and Wuts.

The protecting group is preferably orthogonal to other protecting groups, where present, in the Linker unit.

In one embodiment, the protecting group is orthogonal to the capping group. Thus, the active group protecting group is removable whilst retaining the capping group. In other embodiments, the protecting group and the capping group is removable under the same conditions as those used to remove the capping group.

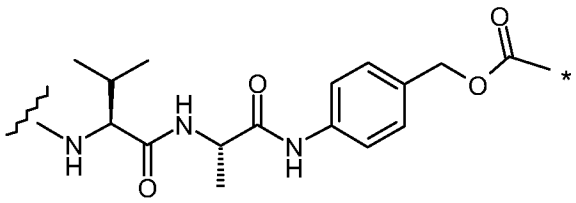
In one embodiment, the Linker unit is:




where the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of the Linker unit, as

applicable or the point of attachment to $G^1$. Preferably, the wavy line indicates the point of attachment to $G^1$.

In one embodiment, the Linker unit is:



where the asterisk and the wavy line are as defined above.

Other functional groups suitable for use in forming a connection between $L^1$ and the Cell Binding Agent are described in WO 2005/082023.

*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).

In one embodiment the antibody is a monoclonal antibody; chimeric antibody; humanized antibody; fully human antibody; or a single chain antibody. One embodiment the antibody is a fragment of one of these antibodies having biological activity. Examples of such fragments include Fab, Fab', $F(ab')_2$ and Fv fragments.

The antibody may be a diabody, a domain antibody (DAB) or a single chain antibody.

In one embodiment, the antibody is a monoclonal antibody.

Antibodies for use in the present invention include those antibodies described in WO 2005/082023 which is incorporated herein. Particularly preferred are those antibodies for tumour-associated antigens. Examples of those antigens known in the art include, but are not limited to, those tumour-associated antigens set out in WO 2005/082023. See, for instance, pages 41-55.

In some embodiments, the conjugates are designed to target tumour cells via their cell surface antigens. The antigens may be cell surface antigens which are either over-expressed or expressed at abnormal times or cell types. Preferably, the target antigen is expressed only on proliferative cells (preferably tumour cells); however this is rarely observed in practice. As a result, target antigens are usually selected on the basis of differential expression between proliferative and healthy tissue.

Antibodies have been raised to target specific tumour related antigens including:
Cripto, CD19, CD20, CD22, CD30, CD33, Glycoprotein NMB, CanAg, Her2 (ErbB2/Neu), CD56 (NCAM), CD70, CD79, CD138, PSCA, PSMA (prostate specific membrane antigen), BCMA, E-selectin, EphB2, Melanotransferin, Muc16 and TMEFF2.

The Ligand unit is connected to the Linker unit. In one embodiment, the Ligand unit is connected to A, where present, of the Linker unit.

In one embodiment, the connection between the Ligand unit and the Linker unit is through a thioether bond.
In one embodiment, the connection between the Ligand unit and the Linker unit is through a disulfide bond.
In one embodiment, the connection between the Ligand unit and the Linker unit is through an amide bond.
In one embodiment, the connection between the Ligand unit and the Linker unit is through an ester bond.

In one embodiment, the connection between the Ligand unit and the Linker is formed between a thiol group of a cysteine residue of the Ligand unit and a maleimide group of the Linker unit.

The cysteine residues of the Ligand unit may be available for reaction with the functional group of the Linker unit to form a connection. In other embodiments, for example where the Ligand unit is an antibody, the thiol groups of the antibody may participate in interchain disulfide bonds. These interchain bonds may be converted to free thiol groups by e.g. treatment of the antibody with DTT prior to reaction with the functional group of the Linker unit.

In some embodiments, the cysteine residue is an introduced into the heavy or light chain of an antibody. Positions for cysteine insertion by substitution in antibody heavy or light chains include those described in Published U.S. Application No. 2007-0092940 and International Patent Publication WO2008070593, which are incorporated herein.

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 compound of formula **I.** 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 compound 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 compound 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 a 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 Compounds and 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.

Includes Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms.

Salts

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of

pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as $Na^+$ and $K^+$, alkaline earth cations such as $Ca^{2+}$ and $Mg^{2+}$, and other cations such as $Al^{+3}$. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. $NH_4^+$) and substituted ammonium ions (e.g. $NH_3R^+$, $NH_2R_2^+$, $NHR_3^+$, $NR_4^+$). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

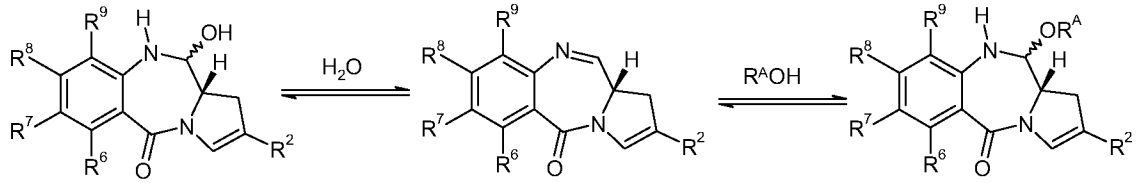
Solvates

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term “solvate” is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If

the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

Carbinolamines

The invention includes compounds where a solvent adds across the imine bond of the PBD moiety, which is illustrated below where the solvent is water or an alcohol ($R^AOH$, where $R^A$ is $C_{1-4}$ alkyl):




These forms can be called the carbinolamine and carbinolamine ether forms of the PBD. The balance of these equilibria depend on the conditions in which the compounds are found, as well as the nature of the moiety itself.

These particular compounds may be isolated in solid form, for example, by lyophilisation.

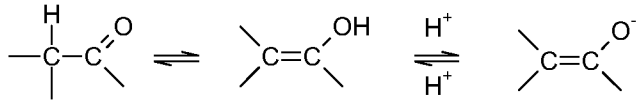
Isomers

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as “isomers” (or “isomeric forms”).

Note that, except as discussed below for tautomeric forms, specifically excluded from the term “isomers”, as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. $C_{1-7}$ alkyl includes n-propyl and iso-propyl; butyl

includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.



keto enol enolate

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including $^{1}H$, $^{2}H$ (D), and $^{3}H$ (T); C may be in any isotopic form, including $^{12}C$, $^{13}C$, and $^{14}C$; O may be in any isotopic form, including $^{16}O$ and $^{18}O$; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

<u>General synthetic routes</u>

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);

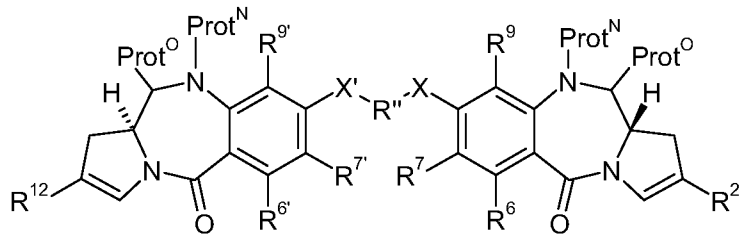
b) WO 2005/023814 (pages 3 to 10);

c) WO 2004/043963 (pages 28 to 29); and

d) WO 2005/085251 (pages 30 to 39).

*Synthesis route*

The compounds of the present invention, where $R^{10}$ and $R^{11}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound, can be synthesised from a compound of Formula 2:

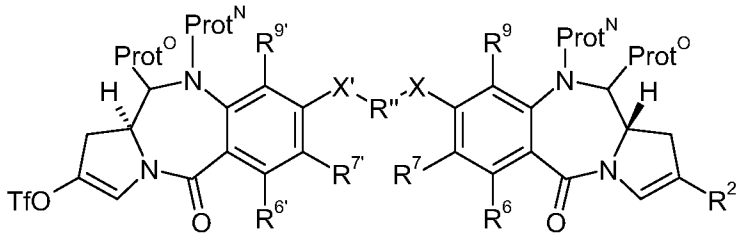


Formula 2

where $R^2$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{12}$, X, X' and R" are as defined for compounds of formula I, $Prot^N$ is a nitrogen protecting group for synthesis and $Prot^O$ is a protected oxygen group for synthesis or an oxo group, by deprotecting the imine bond by standard methods.

The compound produced may be in its carbinolamine or carbinolamine ether form depending on the solvents used. For example if $Prot^N$ is Alloc and $Prot^O$ is an oxygen protecting group for synthesis, then the deprotection is carried using palladium to remove the N10 protecting group, followed by the elimination of the oxygen protecting group for synthesis. If $Prot^N$ is Troc and $Prot^O$ is an oxygen protecting group for synthesis, then the deprotection is carried out using a Cd/Pb couple to yield the compound of formula (I). If $Prot^N$ is SEM, or an analogous group, and $Prot^O$ is an an oxo group, then the oxo group can be removed by reduction, which leads to a protected carbinolamine intermediate, which can then be treated to remove the SEM protecting group, followed by the elimination of water. The reduction of the compound of Formula 2 can be accomplished by, for example, lithium tetraborohydride, whilst a suitable means for removing the SEM protecting group is treatment with silica gel.

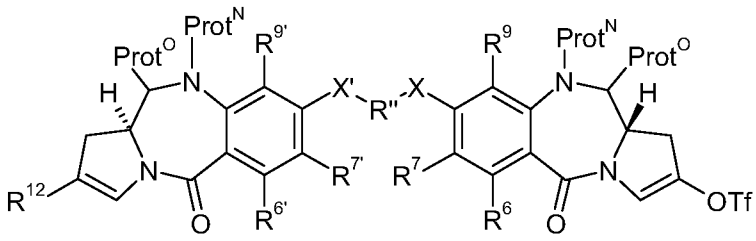
Compounds of formula 2 can be synthesised from a compound of formula 3a:



Formula 3a

where $R^2$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, X, X' and R" are as defined for compounds of formula 2, by coupling an organometallic derivative comprising $R^{12}$, such as an organoboron derivative. The organoboron derivative may be a boronate or boronic acid.

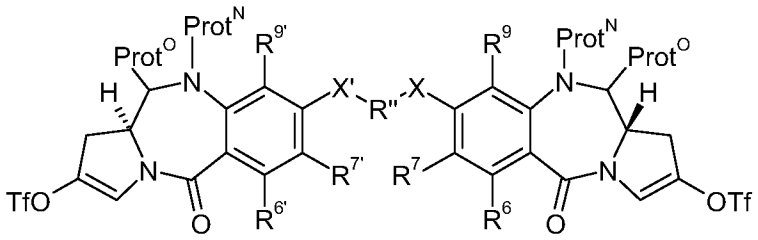
Compounds of formula 2 can be synthesised from a compound of formula 3b:



Formula 3b

where $R^{12}$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, X, X' and R" are as defined for compounds of formula 2, by coupling an organometallic derivative comprising $R^2$, such as an organoboron derivative. The organoboron derivative may be a boronate or boronic acid.

Compounds of formulae 3a and 3b can be synthesised from a compound of formula 4:



Formula 4

where $R^2$, $R^6$, $R^7$, $R^9$, $R^{6'}$, $R^{7'}$, $R^{9'}$, X, X' and R" are as defined for compounds of formula 2, by coupling about a single equivalent (e.g. 0.9 or 1 to 1.1 or 1.2) of an organometallic derivative, such as an organoboron derivative, comprising $R^2$ or $R^{12}$.

The couplings described above are usually carried out in the presence of a palladium catalyst, for example $Pd(PPh_3)_4$, $Pd(OCOCH_3)_2$, $PdCl_2$, $Pd_2(dba)_3$. The coupling may be carried out under standard conditions, or may also be carried out under microwave conditions.

The two coupling steps are usually carried out sequentially. They may be carried out with or without purification between the two steps. If no purification is carried out, then the two steps may be carried out in the same reaction vessel. Purification is usually required after the second coupling step. Purification of the compound from the undesired by-products may be carried out by column chromatography or ion-exchange separation.

The synthesis of compounds of formula 4 where $Prot^O$ is an oxo group and $Prot^N$ is SEM are described in detail in WO 00/12508, which is incorporated herein by reference. In particular, reference is made to scheme 7 on page 24, where the above compound is designated as intermediate **P**. This method of synthesis is also described in WO 2004/043963.

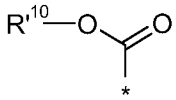
The synthesis of compounds of formula 4 where $Prot^O$ is a protected oxygen group for synthesis are described in WO 2005/085251, which synthesis is herein incorporated by reference.

Compounds of formula I where $R^{10}$ and $R^{10'}$ are H and $R^{11}$ and $R^{11'}$ are $SO_zM$, can be synthesised from compounds of formula I where $R^{10}$ and $R^{11}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound, by the addition of the appropriate bisulphite salt or sulphinate salt, followed by an appropriate purification step. Further methods are described in GB 2 053 894, which is herein incorporated by reference.

Nitrogen protecting groups for synthesis

Nitrogen protecting groups for synthesis are well known in the art. In the present invention, the protecting groups of particular interest are carbamate nitrogen protecting groups and hemi-aminal nitrogen protecting groups.

Carbamate nitrogen protecting groups have the following structure:



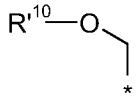
wherein $R'^{10}$ is R as defined above. A large number of suitable groups are described on pages 503 to 549 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Particularly preferred protecting groups include Troc, Teoc, Fmoc, BOC, Doc, Hoc, TcBOC, 1-Adoc and 2-Adoc.

Other possible groups are nitrobenzyloxycarbonyl (e.g. 4- nitrobenzyloxycarbonyl) and 2-(phenylsulphonyl)ethoxycarbonyl.

Those protecting groups which can be removed with palladium catalysis are not preferred, e.g. Alloc.

Hemi-aminal nitrogen protecting groups have the following structure:



wherein R'$^{10}$ is R as defined above. A large number of suitable groups are described on pages 633 to 647 as amide protecting groups of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference. The groups disclosed herein can be applied to compounds of the present invention. Such groups include, but are not limited to, SEM, MOM, MTM, MEM, BOM, nitro or methoxy substituted BOM, $Cl_3CCH_2OCH_2$-.

Protected oxygen group for synthesis

Protected oxygen group for synthesis are well known in the art. A large number of suitable oxygen protecting groups are described on pages 23 to 200 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Classes of particular interest include silyl ethers, methyl ethers, alkyl ethers, benzyl ethers, esters, acetates, benzoates, carbonates, and sulfonates.

Preferred oxygen protecting groups include acetates, TBS and THP.

Synthesis of Drug Conjugates

Conjugates can be prepared as previously described. Linkers having a maleimidyl group (A), a peptide group ($L^1$) and self-immolative group ($L^2$) can be prepared as described in U.S. Patent No. 6,214,345. Linkers having a maleimidyl group (A) and a peptide group ($L^1$) can be prepared as described in WO 2009-0117531. Other linkers can be prepared according to the references cited herein or as known to the skilled artisan.

Linker-Drug compounds can be prepared according to methods known in the art. Linkage of amine-based X substituents (of the PDB dimer Drug unit) to active groups of the Linker units can be performed according to methods generally described in U.S. Patent Nos. 6,214,345 and 7,498,298; and WO 2009-0117531, or as otherwise known to the skilled artisan.

Antibodies can be conjugated to Linker-Drug compounds 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 ºC. 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, $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{10'}$, $R^{11'}$ and Y' are preferably the same as $R^6$, $R^7$, $R^9$, $R^{10}$, $R^{11}$ and Y respectively.

*Dimer link*

Y and Y' are preferably O.

R'' is preferably a $C_{3-7}$ alkylene group with no substituents. More preferably R'' is a $C_3$, $C_5$ or $C_7$ alkylene. Most preferably, R'' is a $C_3$ or $C_5$ alkylene.

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

$R^9$ is preferably H.

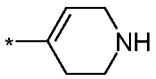
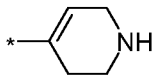
$R^6$ is preferably selected from H, OH, OR, SH, $NH_2$, nitro and halo, and is more preferably H or halo, and most preferably is H.

$R^7$ is preferably selected from H, OH, OR, SH, SR, $NH_2$, NHR, NRR', and halo, and more preferably independently selected from H, OH and OR, where R is preferably 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 preferences apply to $R^{9'}$, $R^{6'}$ and $R^{7'}$ respectively.

*$R^2$*

A in $R^2$ may be phenyl group or a $C_{5-7}$ heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, A is preferably phenyl.

X is a group selected from the list comprising: OH, SH, $CO_2H$, COH, N=C=O, $NHNH_2$, $CONHNH_2$,  ,  and $NHR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl. X may preferably be: OH, SH, $CO_2H$, -N=C=O or $NHR^N$, and may more preferably be: OH, SH, $CO_2H$, -N=C=O or $NH_2$. Particularly preferred groups include: OH, SH and $NH_2$, with $NH_2$ being the most preferred group.

$Q^2$-X may be on any of the available ring atoms of the $C_{5-7}$ aryl group, but 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 (A) is phenyl, the substituent ($Q^2$-X) is preferably in the meta- or para- positions, and more preferably is in the para- position.

In some embodiments, $Q^1$ is a single bond. In these embodiments, $Q^2$ is selected from a single bond and $-Z-(CH_2)_n-$, where Z is selected from a single bond, O, S and NH and is from 1 to 3. In some of these embodiments, $Q^2$ is a single bond. In other embodiments, $Q^2$ is $-Z-(CH_2)_n-$. In these embodiments, Z may be O or S and n may be 1 or n may be 2. In other of these embodiments, Z may be a single bond and n may be 1.

In other embodiments, $Q^1$ is -CH=CH-.

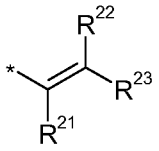
In some embodiments, $R^2$ may be -A-$CH_2$-X and -A-X. In these embodiments, X may be OH, SH, $CO_2H$, COH and $NH_2$. In particularly preferred embodiments, X may be $NH_2$.

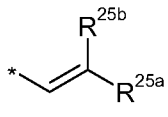
*R*$^{12}$

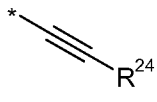
$R^{12}$ is selected from:

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

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

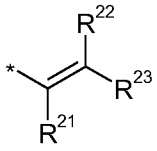
(c)  , wherein each of $R^{21}$, $R^{22}$ and $R^{23}$ are independently selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl, where the total number of carbon atoms in the $R^{12}$ group is no more than 5;

(d)  , 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

(e)  , 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 $R^{12}$ 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 $R^{12}$ is $C_{3-6}$ saturated cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.



When $R^{12}$ is , each of $R^{21}$, $R^{22}$ 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 $R^{12}$ group is no more than 5. In some embodiments, the total number of carbon atoms in the $R^{12}$ group is no more than 4 or no more than 3.

In some embodiments, one of $R^{21}$, $R^{22}$ 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 $R^{21}$, $R^{22}$ 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.

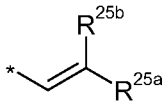
In some embodiments, $R^{22}$ is H.

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

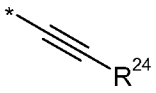
In some embodiments, $R^{21}$ and $R^{22}$ are H.

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

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



When $R^{12}$ 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 $R^{12}$ 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.

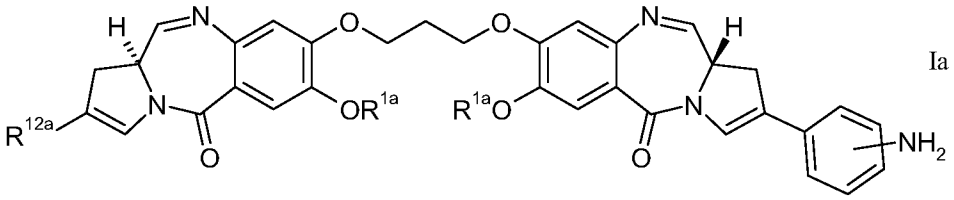
In some embodiments, $R^{24}$ is selected from H, methyl, ethyl, ethenyl and ethynyl. In some of these embodiments, $R^{24}$ is selected from H and methyl.

*M and z*

It is preferred that M and M' are monovalent pharmaceutically acceptable cations, and are more preferably $Na^+$.

z is preferably 3.

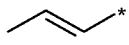
Particularly preferred compounds of the present invention are of formula Ia:

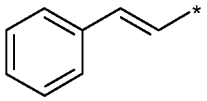


Ia

where $R^{12a}$ is selected from:

(a) 

(b) 

(c)  ;

(d)  ;

(e)  ; and

the amino group is at either the meta or para positions of the phenyl group.

3rd aspect

The preferences expressed above for the first aspect may apply to the compounds of this aspect, where appropriate.

When $R^{10}$ is carbamate nitrogen protecting group, it may preferably be Teoc, Fmoc and Troc, and may more preferably be Troc.

When $R^{11}$ is O-$Prot^{O}$, wherein $Prot^{O}$ is an oxygen protecting group, $Prot^{O}$ may preferably be TBS or THP, and may more preferably be TBS.

When $R^{10}$ is a hemi-aminal nitrogen protecting group, it may preferably be MOM, BOM or SEM, and may more preferably be SEM.

The preferences for compounds of formula I apply as appropriate to D in the sixth aspect of the invention.

## Examples

### General Experimental Methods

Optical rotations were measured on an ADP 220 polarimeter (Bellingham Stanley Ltd.) and concentrations (*c*) are given in g/100mL. Melting points were measured using a digital melting point apparatus (Electrothermal). IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were acquired at 300 K using a Bruker Avance NMR spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported relative to TMS ($\delta$ = 0.0 ppm), and signals are designated as s (singlet), d (doublet), t (triplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets) or m (multiplet), with coupling constants given in Hertz (Hz). Mass spectroscopy (MS) data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; De-solvation flow rate (L/h), 250. High-resolution mass spectroscopy (HRMS) data were recorded on a Waters Micromass QTOF Global in positive W-mode using metal-coated borosilicate glass tips to introduce the samples into the instrument. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, $F_{254}$), and flash chromatography utilised silica gel (Merck 60, 230-400 mesh ASTM). Except for the HOBt (NovaBiochem)

and solid-supported reagents (Argonaut), all other chemicals and solvents were purchased from Sigma-Aldrich and were used as supplied without further purification. Anhydrous solvents were prepared by distillation under a dry nitrogen atmosphere in the presence of an appropriate drying agent, and were stored over 4Å molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

Compound 1b was synthesised as described in WO 00/012508 (compound 210), which is herein incorporated by reference.

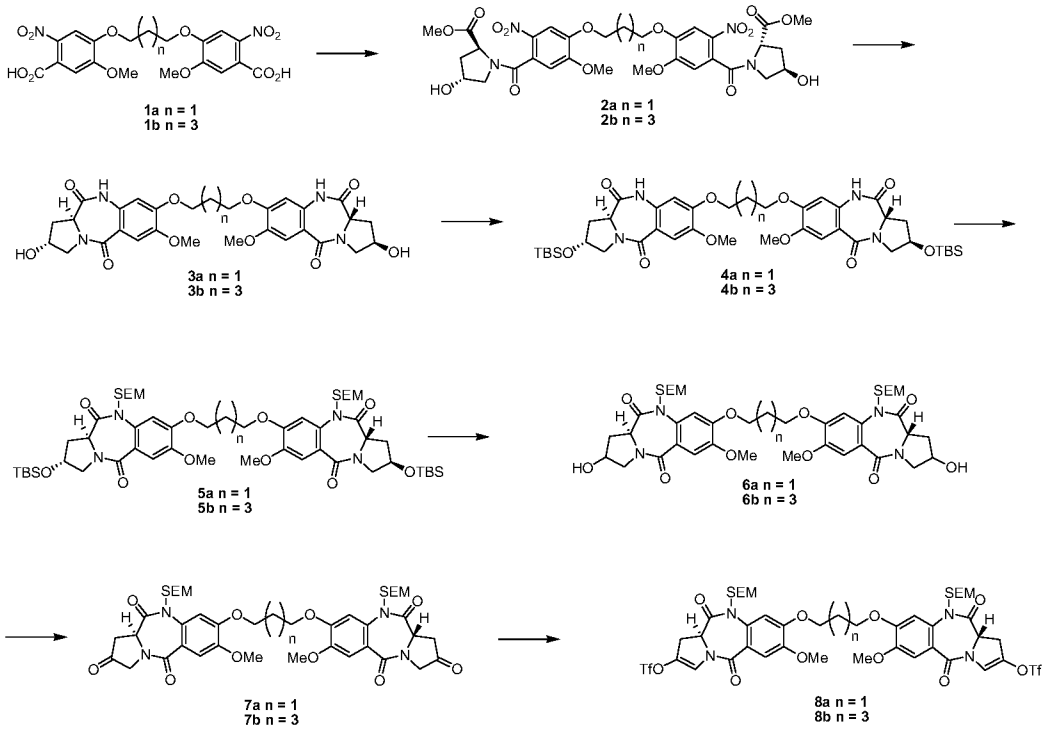
General LC/MS conditions: The HPLC (Waters Alliance 2695) was run using a mobile phase of water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B over 1.0 min then 5% B to 95% B within 3 min. The composition was held for 0.5 min at 95% B, and then returned to 5% B in 0.3 minutes. Total gradient run time equals 5 min. Flow rate 3.0 mL/min, 400µL was split *via* a zero dead volume tee piece which passes into the mass spectrometer. Wavelength detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex® Onyx Monolithic C18 50 x 4.60 mm

LC/MS conditions specific for compounds protected by both a Troc and a TBDMs group: Chromatographic separation of Troc and TBDMS protected compounds was performed on a Waters Alliance 2695 HPLC system utilizing a Onyx Monolitic reversed-phase column (3 µm particles, 50 x 4.6 mm) from Phenomenex Corp. Mobile-phase A consisted of 5% acetonitrile – 95 % water containing 0.1% formic acid, and mobile phase B consisted of 95% acetonitrile – 5% water containing 0.1% formic acid. After 1 min at 5% B, the proportion of B was raised to 95% B over the next 2.5 min and maintained at 95% B for a further 1 min, before returning to 95% A in 10 s and re-equilibration for a further 50 sec, giving a total run time of 5.0 min. The flow rate was maintained at 3.0 mL/min.

LC/MS conditions for Example 4: The HPLC (Waters Alliance 2695) was run using a mobile phase of water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B for 2.0 min rising to 50% B over 3 min. The composition was held for 1 min at 50% B, before rising to 95% B over 1 minute. The gradient composition then dropped to 5% B over 2.5 minutes and was held at this percentage for 0.5 minutes.Total gradient run time equals 10 min. Flow rate 1.5 mL/min, 400µL was split *via* a zero dead volume tee piece which passes into the mass spectrometer. Wavelength

detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex® Onyx Monolithic C18 50 x 4.60 mm

**Synthesis of key intermediates**




*(a) 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(5-methoxy-2-nitro-1,4-phenylene)carbonyl]]bis[(2S,4R)-methyl-4-hydroxypyrrolidine-2-carboxylate] (**2a**)*

**Method A**: A catalytic amount of DMF (2 drops) was added to a stirred solution of the nitro-acid **1a** (1.0 g, 2.15 mmol) and oxalyl chloride (0.95 mL, 1.36 g, 10.7 mmol) in dry THF (20 mL). The reaction mixture was allowed to stir for 16 hours at room temperature and the solvent was removed by evaporation *in vacuo*. The resulting residue was re-dissolved in dry THF (20 mL) and the acid chloride solution was added dropwise to a stirred mixture of (2*S*,4*R*)-methyl-4-hydroxypyrrolidine-2-carboxylate hydrochloride (859 mg, 4.73 mmol) and TEA (6.6 mL, 4.79 g, 47.3 mmol) in THF (10 mL) at -30°C (dry ice/ethylene glycol) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for a further 3 hours after which time TLC (95:5 v/v $CHCl_3$/MeOH) and LC/MS (2.45 min (ES+) *m/z* (relative intensity) 721 ($[M + H]^{+\cdot}$, 20)) revealed formation of product. Excess THF was removed by rotary evaporation and the resulting residue was dissolved in DCM (50 mL). The organic layer was washed with 1N

HCl (2 x 15 mL), saturated $NaHCO_3$ (2 x 15 mL), $H_2O$ (20 mL), brine (30 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent gave the crude product as a dark coloured oil. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) isolated the pure amide **2a** as an orange coloured glass (840 mg, 54%).

**Method B**: Oxalyl chloride (9.75 mL, 14.2 g, 111 mmol) was added to a stirred suspension of the nitro-acid **1a** (17.3 g, 37.1 mmol) and DMF (2 mL) in anhydrous DCM (200 mL). Following initial effervescence the reaction suspension became a solution and the mixture was allowed to stir at room temperature for 16 hours. Conversion to the acid chloride was confirmed by treating a sample of the reaction mixture with MeOH and the resulting bis-methyl ester was observed by LC/MS. The majority of solvent was removed by evaporation *in vacuo*, 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 filtration, washed with cold diethyl ether and dried for 1 hour in a vacuum oven at 40°C. The solid acid chloride was added portionwise over a period of 25 minutes to a stirred suspension of (2*S*,4*R*)-methyl-4-hydroxypyrrolidine-2-carboxylate hydrochloride (15.2 g, 84.0 mmol) and TEA (25.7 mL, 18.7 g, 185 mmol) in DCM (150 mL) at -40°C (dry ice/$CH_3CN$). Immediately, the reaction was complete as judged by LC/MS (2.47 min (ES+) *m/z* (relative intensity) 721 ($[M + H]^{+\cdot}$, 100)), the mixture was diluted with DCM (150 mL) and washed with 1N HCl (300 mL), saturated $NaHCO_3$ (300 mL), brine (300 mL), filtered (through a phase separator) and the solvent evaporated *in vacuo* to give the pure product **2a** as an orange solid (21.8 g, 82%).

**Analytical Data:** $[\alpha]^{22}_D$ = -46.1° (*c* = 0.47, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) (rotamers) $\delta$ 7.63 (s, 2H), 6.82 (s, 2H), 4.79-4.72 (m, 2H), 4.49-4.28 (m, 6H), 3.96 (s, 6H), 3.79 (s, 6H), 3.46-3.38 (m, 2H), 3.02 (d, 2H, *J* = 11.1 Hz), 2.48-2.30 (m, 4H), 2.29-2.04 (m, 4H); $^{13}C$ NMR (100 MHz, $CDCl_3$) (rotamers) $\delta$ 172.4, 166.7, 154.6, 148.4, 137.2, 127.0, 109.7, 108.2, 69.7, 65.1, 57.4, 57.0, 56.7, 52.4, 37.8, 29.0; IR (ATR, $CHCl_3$) 3410 (br), 3010, 2953, 1741, 1622, 1577, 1519, 1455, 1429, 1334, 1274, 1211, 1177, 1072, 1050, 1008, 871 $cm^{-1}$; MS ($ES^+$) *m/z* (relative intensity) 721 ($[M + H]^{+\cdot}$, 47), 388 (80); HRMS $[M + H]^{+\cdot}$ theoretical $C_{31}H_{36}N_4O_{16}$ *m/z* 721.2199, found ($ES^+$) *m/z* 721.2227.

*(a) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis[(5-methoxy-2-nitro-1,4-phenylene)carbonyl]]bis[(2S,4R)-methyl-4-hydroxypyrrolidine-2-carboxylate] (**2b**)*

Preparation from **1b** according to **Method B** gave the pure product as an orange foam (75.5 g, 82%).

**Analytical Data:** ($ES^+$) *m/z* (relative intensity) 749 ($[M + H]^{+\cdot}$, 100).

*(b) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(hydroxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**3a**)*

**Method A**: A suspension of 10% Pd/C (7.5 g, 10% w/w) in DMF (40 mL) was added to a solution of the nitro-ester **2a** (75 g, 104 mmol) in DMF (360 mL). The suspension was hydrogenated in a Parr hydrogenation apparatus over 8 hours. Progress of the reaction was monitored by LC/MS (2.12 min (ES+) *m/z* (relative intensity) 597 ($[M + H]^{+\cdot}$, 100), (ES-) *m/z* (relative intensity) 595 ($[M + H]^{+\cdot}$, 100) after the hydrogen uptake had stopped. Solid Pd/C was removed by filtration and the filtrate was concentrated by rotary evaporation under vacuum (below 10 mbar) at 40°C to afford a dark oil containing traces of DMF and residual charcoal. The residue was digested in EtOH (500 mL) at 40°C on a water bath (rotary evaporator bath) and the resulting suspension was filtered through celite and washed with ethanol (500 mL) to give a clear filtrate. Hydrazine hydrate (10 mL, 321 mmol) was added to the solution and the reaction mixture was heated at reflux. After 20 minutes the formation of a white precipitate was observed and reflux was allowed to continue for a further 30 minutes. The mixture was allowed to cool down to room temperature and the precipitate was retrieved by filtration, washed with diethyl ether (2*1 volume of precipitate) and dried in a vacuum desiccator to provide **3a** (50 g, 81%).

**Method B**: A solution of the nitro-ester **2a** (6.80 g, 9.44 mmol) in MeOH (300 mL) was added to Raney™ nickel (4 large spatula ends of a ~ 50% slurry in $H_2O$) and anti-bumping granules in a 3-neck round bottomed flask. The mixture was heated at reflux and then treated dropwise with a solution of hydrazine hydrate (5.88 mL, 6.05 g, 188 mmol) in MeOH (50 mL) at which point vigorous effervescence was observed. When the addition was complete (~ 30 minutes) additional Raney™ nickel was added carefully until effervescence had ceased and the initial yellow colour of the reaction mixture was discharged. The mixture was heated at reflux for a further 30 minutes at which point the reaction was deemed complete by TLC (90:10 v/v $CHCl_3$/MeOH) and LC/MS (2.12 min (ES+) *m/z* (relative intensity) 597 ($[M + H]^{+\cdot}$, 100)). The reaction mixture was allowed to cool to around 40°C and then excess nickel removed by filtration through a sinter funnel without vacuum suction. The filtrate was reduced in volume by evaporation *in vacuo* at

which point a colourless precipitate formed which was collected by filtration and dried in a vacuum desiccator to provide **3a** (5.40 g, 96%).

**Analytical Data:** $[\alpha]^{27}_{D}$ = +404° (*c* = 0.10, DMF); $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.2 (s, 2H, N*H*), 7.26 (s, 2H), 6.73 (s, 2H), 5.11 (d, 2H, *J* = 3.98 Hz, O*H*), 4.32-4.27 (m, 2H), 4.19-4.07 (m, 6H), 3.78 (s, 6H), 3.62 (dd, 2H, *J* = 12.1, 3.60 Hz), 3.43 (dd, 2H, *J* = 12.0, 4.72 Hz), 2.67-2.57 (m, 2H), 2.26 (p, 2H, *J* = 5.90 Hz), 1.99-1.89 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.1, 164.0, 149.9, 144.5, 129.8, 117.1, 111.3, 104.5, 54.8, 54.4, 53.1, 33.5, 27.5; IR (ATR, neat) 3438, 1680, 1654, 1610, 1605, 1516, 1490, 1434, 1379, 1263, 1234, 1216, 1177, 1156, 1115, 1089, 1038, 1018, 952, 870 cm$^{-1}$; MS (ES$^+$) *m/z* (relative intensity) 619 ([*M* + Na]$^{+\cdot}$, 10), 597 ([*M* + H]$^{+\cdot}$, 52), 445 (12), 326 (11); HRMS [*M* + H]$^{+\cdot}$ theoretical $C_{29}H_{32}N_4O_{10}$ *m/z* 597.2191, found (ES$^+$) *m/z* 597.2205.

*(b) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis(11aS,2R)-2-(hydroxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**3b**)*

Preparation from **2b** according to **Method A** gave the product as a white solid (22.1 g, 86%).

**Analytical Data:** MS (ES$^-$) *m/z* (relative intensity) 623.3 ([*M* - H]$^{-\cdot}$, 100);

*(c) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**4a**)*

TBSCl (317 mg, 2.1 mmol) and imidazole (342 mg, 5.03 mmol) were added to a cloudy solution of the tetralactam **3a** (250 mg, 0.42 mmol) in anhydrous DMF (6 mL). The mixture was allowed to stir under a nitrogen atmosphere for 3 hours after which time the reaction was deemed complete as judged by LC/MS (3.90 min (ES+) *m/z* (relative intensity) 825 ([*M* + H]$^{+\cdot}$, 100)). The reaction mixture was poured onto ice (~ 25 mL) and allowed to warm to room temperature with stirring. The resulting white precipitate was collected by vacuum filtration, washed with $H_2O$, diethyl ether and dried in the vacuum desiccator to provide pure **4a** (252 mg, 73%).

**Analytical Data:** $[\alpha]^{23}_{D}$ = +234° (*c* = 0.41, $CHCl_3$); $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 2H, N*H*), 7.44 (s, 2H), 6.54 (s, 2H), 4.50 (p, 2H, *J* = 5.38 Hz), 4.21-4.10 (m, 6H), 3.87 (s, 6H), 3.73-3.63 (m, 4H), 2.85-2.79 (m, 2H), 2.36-2.29 (m, 2H), 2.07-1.99 (m, 2H), 0.86 (s, 18H), 0.08 (s, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.4, 165.7, 151.4, 146.6, 129.7,

118.9, 112.8, 105.3, 69.2, 65.4, 56.3, 55.7, 54.2, 35.2, 28.7, 25.7, 18.0, -4.82 and -4.86; IR (ATR, $CHCl_3$) 3235, 2955, 2926, 2855, 1698, 1695, 1603, 1518, 1491, 1446, 1380, 1356, 1251, 1220, 1120, 1099, 1033 $cm^{-1}$; MS ($ES^+$) *m/z* (relative intensity) 825 ($[M + H]^{+\cdot}$, 62), 721 (14), 440 (38); HRMS $[M + H]^{+\cdot}$ theoretical $C_{41}H_{60}N_4O_{10}Si_2$ *m/z* 825.3921, found ($ES^+$) *m/z* 825.3948.

*(c) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**4b**)*

Preparation from **3b** according to the above method gave the product as a white solid (27.3 g, 93%).

**Analytical Data:** MS ($ES^+$) *m/z* (relative intensity) 853.8 ($[M + H]^{+\cdot}$, 100), ($ES^-$) *m/z* (relative intensity) 851.6 ($[M - H]^{-\cdot}$, 100.

*(d) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**5a**)*

A solution of *n*-BuLi (4.17 mL of a 1.6 M solution in hexane, 6.67 mmol) in anhydrous THF (10 mL) was added dropwise to a stirred suspension of the tetralactam **4a** (2.20 g, 2.67 mmol) in anhydrous THF (30 mL) at -30°C (dry ice/ethylene glycol) under a nitrogen atmosphere. The reaction mixture was allowed to stir at this temperature for 1 hour (now a reddish orange colour) at which point a solution of SEMCl (1.18 mL, 1.11 g, 6.67 mmol) in anhydrous THF (10 mL) was added dropwise. The reaction mixture was allowed to slowly warm to room temperature and was stirred for 16 hours under a nitrogen atmosphere. The reaction was deemed complete as judged by TLC (EtOAc) and LC/MS (4.77 min (ES+) *m/z* (relative intensity) 1085 ($[M + H]^{+\cdot}$, 100)). The THF was removed by evaporation *in vacuo* and the resulting residue dissolved in EtOAc (60 mL), washed with $H_2O$ (20 mL), brine (20 mL), dried ($MgSO_4$) filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (80:20 v/v Hexane/EtOAc) gave the pure N10-SEM-protected tetralactam **5a** as an oil (2.37 g, 82%).

**Analytical Data:** $[\alpha]^{23}_D$ = +163° (*c* = 0.41, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.33 (s, 2H), 7.22 (s, 2H), 5.47 (d, 2H, *J* = 9.98 Hz), 4.68 (d, 2H, *J* = 9.99 Hz), 4.57 (p, 2H, *J* = 5.77 Hz), 4.29-4.19 (m, 6H), 3.89 (s, 6H), 3.79-3.51 (m, 8H), 2.87-2.81 (m, 2H), 2.41 (p, 2H, *J* = 5.81 Hz), 2.03-1.90 (m, 2H), 1.02-0.81 (m, 22H), 0.09 (s, 12H), 0.01 (s, 18H); $^{13}C$ NMR

(100 MHz, $CDCl_3$) δ 170.0, 165.7, 151.2, 147.5, 133.8, 121.8, 111.6, 106.9, 78.1, 69.6, 67.1, 65.5, 56.6, 56.3, 53.7, 35.6, 30.0, 25.8, 18.4, 18.1, -1.24, -4.73; IR (ATR, $CHCl_3$) 2951, 1685, 1640, 1606, 1517, 1462, 1433, 1360, 1247, 1127, 1065 $cm^{-1}$; MS ($ES^+$) *m/z* (relative intensity) 1113 ([*M* + Na]$^{+\cdot}$, 48), 1085 ([*M* + H]$^{+\cdot}$, 100), 1009 (5), 813 (6); HRMS [*M* + H]$^{+\cdot}$ theoretical $C_{53}H_{88}N_4O_{12}Si_4$ *m/z* 1085.5548, found ($ES^+$) *m/z* 1085.5542.

*(d) 1,1'-[[(Pentane1,5-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**5b**)*

Preparation from **4b** according to the above method gave the product as a pale orange foam (46.9 g, 100%), used without further purification.

**Analytical Data:** MS ($ES^+$) *m/z* (relative intensity) 1114 ([M + H]$^{+\cdot}$, 90), ($ES^-$) *m/z* (relative intensity) 1158 ([*M* + 2Na]$^{-\cdot}$, 100).

*(e) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-hydroxy-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**6a**)*

A solution of TBAF (5.24 mL of a 1.0 M solution in THF, 5.24 mmol) was added to a stirred solution of the bis-silyl ether **5a** (2.58 g, 2.38 mmol) in THF (40 mL) at room temperature. After stirring for 3.5 hours, analysis of the reaction mixture by TLC (95:5 v/v $CHCl_3$/MeOH) revealed completion of reaction. The reaction mixture was poured into a solution of saturated $NH_4Cl$ (100 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash chromatography (gradient elution: 100% $CHCl_3$ to 96:4 v/v $CHCl_3$/MeOH) gave the pure tetralactam **6a** as a white foam (1.78 g, 87%).

**Analytical Data:** $[\alpha]^{23}_D$ = +202° (*c* = 0.34, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 2H), 7.20 (s, 2H), 5.44 (d, 2H, *J* = 10.0 Hz), 4.72 (d, 2H, *J* = 10.0 Hz), 4.61-4.58 (m, 2H), 4.25 (t, 4H, *J* = 5.83 Hz), 4.20-4.16 (m, 2H), 3.91-3.85 (m, 8H), 3.77-3.54 (m, 6H), 3.01 (br s, 2H, O*H*), 2.96-2.90 (m, 2H), 2.38 (p, 2H, *J* = 5.77 Hz), 2.11-2.05 (m, 2H), 1.00-0.91 (m, 4H), 0.00 (s, 18H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 169.5, 165.9, 151.3, 147.4, 133.7, 121.5, 111.6, 106.9, 79.4, 69.3, 67.2, 65.2, 56.5, 56.2, 54.1, 35.2, 29.1, 18.4, -1.23; IR (ATR, $CHCl_3$) 2956, 1684, 1625, 1604, 1518, 1464, 1434, 1361, 1238, 1058, 1021 $cm^{-1}$; MS ($ES^+$)

*m/z* (relative intensity) 885 ([*M* + 29]$^{+\cdot}$, 70), 857 ([*M* + H]$^{+\cdot}$, 100), 711 (8), 448 (17); HRMS [*M* + H]$^{+\cdot}$ theoretical $C_{41}H_{60}N_4O_{12}Si_2$ *m/z* 857.3819, found (ES$^+$) *m/z* 857.3826.

*(e) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis(11aS,2R)-2-hydroxy-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**6b**)*

Preparation from **5b** according to the above method gave the product as a white foam (15.02 g).

**Analytical Data:** MS (ES$^+$) *m/z* (relative intensity) 886 ([*M* + H]$^{+\cdot}$, 10), 739.6 (100), (ES$^-$) *m/z* (relative intensity) 884 ([M - H]$^{-\cdot}$, 40).

*(f) 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-11-sulpho-7-methoxy-2-oxo-10-((2-(trimethylsilyl)ethoxy)methyl)1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]] (**7a**)*

**Method A:** A 0.37 M sodium hypochlorite solution (142.5 mL, 52.71 mmol, 2.4 eq) was added dropwise to a vigorously stirred mixture of the diol **6a** (18.8 g, 21.96 mmol, 1 eq), TEMPO (0.069 g, 0.44 mmol, 0.02 eq) and 0.5 M potassium bromide solution (8.9 mL, 4.4 mmol, 0.2 eq) in DCM (115 mL) at 0°C. The temperature was maintained between 0°C and 5°C by adjusting the rate of addition. The resultant yellow emulsion was stirred at 0°C to 5°C for 1 hour. TLC (EtOAc) and LC/MS [3.53 min. (ES+) *m/z* (relative intensity) 875 ([M + Na]$^{+\cdot}$, 50), (ES-) *m/z* (relative intensity) 852 ([M – H]$^{-\cdot}$, 100)] indicated that reaction was complete.

The reaction mixture was filtered, the organic layer separated and the aqueous layer was backwashed with DCM (x 2). The combined organic portions were washed with brine (x 1), dried ($MgSO_4$) and evaporated to give a yellow foam. Purification by flash column chromatography (gradient elution 35/65 v/v *n*-hexane/EtOAC, 30/70 to 25/75 v/v *n*-hexane/EtOAC) afforded the bis-ketone **7a** as a white foam (14.1 g, 75%).

Sodium hypochlorite solution, reagent grade, available at chlorine 10-13%, was used. This was assumed to be 10% (10 g NaClO in 100 g) and calculated to be 1.34 M in NaClO. A stock solution was prepared from this by diluting it to 0.37 M with water. This gave a solution of approximately pH 14. The pH was adjusted to 9.3 to 9.4 by the addition of solid $NaHCO_3$. An aliquot of this stock was then used so as to give 2.4 mol eq. for the reaction.

On addition of the bleach solution an initial increase in temperature was observed. The rate of addition was controlled, to maintain the temperature between 0°C to 5°C. The reaction mixture formed a thick, lemon yellow coloured, emulsion.

The oxidation was an adaptation of the procedure described in Thomas Fey *et al*, *J. Org. Chem.*, **2001**, *66*, 8154-8159.

**Method B:** Solid TCCA (10.6 g, 45.6 mmol) was added portionwise to a stirred solution of the alcohol **6a** (18.05 g, 21.1 mmol) and TEMPO (123 mg, 0.78 mmol) in anhydrous DCM (700 mL) at 0°C (ice/acetone). The reaction mixture was stirred at 0°C under a nitrogen atmosphere for 15 minutes after which time TLC (EtOAc) and LC/MS [3.57 min (ES+) *m/z* (relative intensity) 875 ([*M* + Na]$^{+\cdot}$, 50)] revealed completion of reaction. The reaction mixture was filtered through celite and the filtrate was washed with saturated aqueous $NaHCO_3$ (400mL), brine (400mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by flash column chromatography (80:20 v/v EtOAc/Hexane) afforded the bis-ketone **7a** as a foam (11.7 g, 65%).

**Method C:** A solution of anhydrous DMSO (0.72 mL, 0.84 g, 10.5 mmol) in dry DCM (18 mL) was added dropwise over a period of 25 min to a stirred solution of oxalyl chloride (2.63 mL of a 2.0 M solution in DCM, 5.26 mmol) under a nitrogen atmosphere at -60°C (liq $N_2$/$CHCl_3$). After stirring at -55°C for 20 minutes, a slurry of the substrate **6a** (1.5 g, 1.75 mmol) in dry DCM (36 mL) was added dropwise over a period of 30 min to the reaction mixture. After stirring for a further 50 minutes at -55°C, a solution of TEA (3.42 mL, 2.49 g; 24.6 mmol) in dry DCM (18 mL) was added dropwise over a period of 20 min to the reaction mixture. The stirred reaction mixture was allowed to warm to room temperature (~ 1.5 h) and then diluted with DCM (50 mL). The organic solution was washed with 1 N HCl (2 x 25 mL), $H_2O$ (30 mL), brine (30 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent *in vacuo* afforded the crude product which was purified by flash column chromatography (80:20 v/v EtOAc/Hexane) to afford bis-ketone **7a** as a foam (835 mg, 56%)

**Analytical Data:** $[\alpha]^{20}_D$ = +291° (*c* = 0.26, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 2H), 7.25 (s, 2H), 5.50 (d, 2H, *J* = 10.1 Hz), 4.75 (d, 2H, *J* = 10.1 Hz), 4.60 (dd, 2H, *J* = 9.85, 3.07 Hz), 4.31-4.18 (m, 6H), 3.89-3.84 (m, 8H), 3.78-3.62 (m, 4H), 3.55 (dd, 2H, *J* =

19.2, 2.85 Hz), 2.76 (dd, 2H, *J* = 19.2, 9.90 Hz), 2.42 (p, 2H, *J* = 5.77 Hz), 0.98-0.91 (m, 4H), 0.00 (s, 18H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 206.8, 168.8, 165.9, 151.8, 148.0, 133.9, 120.9, 111.6, 107.2, 78.2, 67.3, 65.6, 56.3, 54.9, 52.4, 37.4, 29.0, 18.4, -1.24; IR (ATR, $CHCl_3$) 2957, 1763, 1685, 1644, 1606, 1516, 1457, 1434, 1360, 1247, 1209, 1098, 1066, 1023 $cm^{-1}$; MS ($ES^+$) *m/z* (relative intensity) 881 ($[M + 29]^{+\cdot}$, 38), 853 ($[M + H]^{+\cdot}$, 100), 707 (8), 542 (12); HRMS $[M + H]^{+\cdot}$ theoretical $C_{41}H_{56}N_4O_{12}Si_2$ *m/z* 853.3506, found ($ES^+$) *m/z* 853.3502.

*(f) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis[(11aS)-11-sulpho-7-methoxy-2-oxo-10-((2-(trimethylsilyl)ethoxy)methyl)1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5,11-dione]] (**7b**)*

Preparation from **6b** according to **Method C** gave the product as a white foam (10.5 g, 76%).

**Analytical Data:** MS ($ES^+$) *m/z* (relative intensity) 882 ($[M + H]^{+\cdot}$, 30), 735 (100), ($ES^-$) *m/z* (relative intensity) 925 ($[M + 45]^{-\cdot}$, 100), 880 ($[M - H]^{-\cdot}$, 70).

*(g) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS)-7-methoxy-2-[[(trifluoromethyl)sulfonyl]oxy]-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**8a**)*

Anhydrous 2,6-lutidine (5.15 mL, 4.74 g, 44.2 mmol) was injected in one portion to a vigorously stirred solution of bis-ketone **7a** (6.08 g, 7.1 mmol) in dry DCM (180 mL) at -45°C (dry ice/acetonitrile cooling bath) under a nitrogen atmosphere. Anhydrous triflic anhydride, taken from a freshly opened ampoule (7.2 mL, 12.08 g, 42.8 mmol), was injected rapidly dropwise, while maintaining the temperature at -40°C or below. The reaction mixture was allowed to stir at -45°C for 1 hour at which point TLC (50/50 v/v *n*-hexane/EtOAc) revealed the complete consumption of starting material. The cold reaction mixture was immediately diluted with DCM (200 mL) and, with vigorous shaking, washed with water (1 x 100 mL), 5% citric acid solution (1 x 200 mL) saturated $NaHCO_3$ (200 mL), brine (100 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent *in vacuo* afforded the crude product which was purified by flash column chromatography (gradient elution: 90:10 v/v *n*-hexane/EtOAc to 70:30 v/v *n*-hexane/EtOAc) to afford bis-enol triflate **8a** as a yellow foam (5.5 g, 70%).

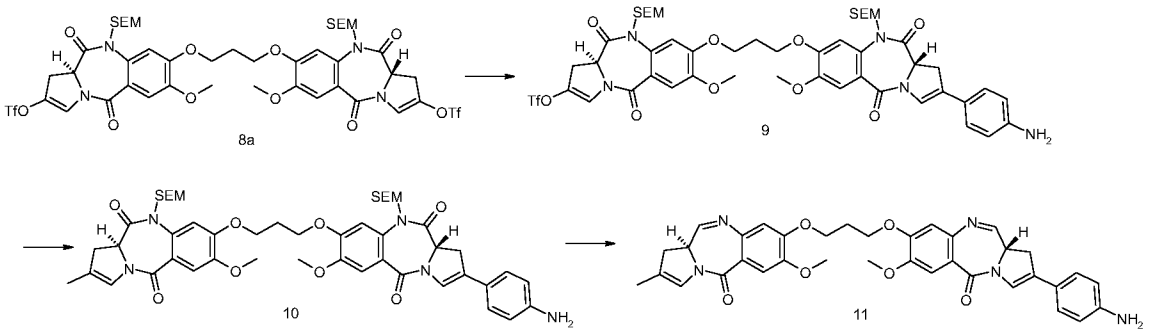
**Analytical Data:** $[\alpha]^{24}_{D}$ = +271° (*c* = 0.18, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 2H), 7.26 (s, 2H), 7.14 (t, 2H, *J* = 1.97 Hz), 5.51 (d, 2H, *J* = 10.1 Hz), 4.76 (d, 2H, *J* = 10.1 Hz), 4.62 (dd, 2H, *J* = 11.0, 3.69 Hz), 4.32-4.23 (m, 4H), 3.94-3.90 (m, 8H), 3.81-3.64 (m, 4H), 3.16 (ddd, 2H, *J* = 16.3, 11.0, 2.36 Hz), 2.43 (p, 2H, *J* = 5.85 Hz), 1.23-0.92 (m, 4H), 0.02 (s, 18H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 167.1, 162.7, 151.9, 148.0, 138.4, 133.6, 120.2, 118.8, 111.9, 107.4, 78.6, 67.5, 65.6, 56.7, 56.3, 30.8, 29.0, 18.4, -1.25; IR (ATR, $CHCl_3$) 2958, 1690, 1646, 1605, 1517, 1456, 1428, 1360, 1327, 1207, 1136, 1096, 1060, 1022, 938, 913 $cm^{-1}$; MS ($ES^+$) *m/z* (relative intensity) 1144 ($[M + 28]^{+\cdot}$, 100), 1117 ($[M + H]^{+\cdot}$, 48), 1041 (40), 578 (8); HRMS $[M + H]^{+\cdot}$ theoretical $C_{43}H_{54}N_4O_{16}Si_2S_2F_6$ *m/z* 1117.2491, found ($ES^+$) *m/z* 1117.2465.

*(g) 1,1'-[[(Pentane-1,5-diyl)dioxy]bis(11aS)-7-methoxy-2-[[(trifluoromethyl)sulfonyl]oxy]-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] (**8b**)*

Preparation from **7b** according to the above method gave the bis-enol triflate as a pale yellow foam (6.14 g, 82%).

**Analytical Data:** (ES+) *m/z* (relative intensity) 1146 ($[M + H]^{+\cdot}$, 85).

**Example 1**




*(a) (S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(trifluoromethylsulfonyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5,11(10H,11aH)-dione (**9**)*

Solid $Pd(PPh_3)_4$ (20.18 mg, 17.46 μmol) was added to a stirred solution of the triflate **8a** (975 mg, 0.87 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)aniline (172 mg, 0.79 mmol) and $Na_2CO_3$ (138 mg, 1.30 mmol) in toluene (13 mL) EtOH (6.5 mL) and $H_2O$ (6.5 mL). The dark solution was allowed to stir under a nitrogen atmosphere for 24 hours, after

which time analysis by TLC (EtOAc) and LC/MS revealed the formation of the desired mono-coupled product and as well as the presence of unreacted starting material. The solvent was removed by rotary evaporation under reduced pressure and the resulting residue partitioned between $H_2O$ (100 mL) and EtOAc (100 mL), after eventual separation of the layers the aqueous phase was extracted again with EtOAc (2 x 25 mL). The combined organic layers were washed with $H_2O$ (50 mL), brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude Suzuki product. The crude Suzuki product was subjected to flash chromatography (40%EtOAc/60% Hexane →70% EtOAc, 30% Hexane). Removal of the excess eluent by rotary evaporation under reduced pressure afforded the desired product **9** (399 mg) in 43% yield.

$^1$H–NMR: ($CDCl_3$, 400 MHz) δ 7.40 (s, 1H), 7.33 (s, 1H), 7.27 (bs, 3H), 7.24 (d, 2H, *J* = 8.5 Hz), 7.15 (t, 1H, *J* = 2.0 Hz), 6.66 (d, 2H, *J* = 8.5 Hz), 5.52 (d, 2H, *J* = 10.0 Hz), 4.77 (d, 1H, *J* = 10.0 Hz), 4.76 (d, 1H, *J* = 10.0 Hz), 4.62 ( dd, 1H, *J* = 3.7, 11.0 Hz), 4.58 (dd, 1H, *J* = 3.4, 10.6 Hz), 4.29 (t, 4H, *J* = 5.6 Hz), 4.00-3.85 (m, 8H), 3.80 – 3.60 (m, 4H), 3.16 (ddd, 1H, *J* = 2.4, 11.0, 16.3 Hz), 3.11 (ddd, 1H, *J* = 2.2, 10.5, 16.1 Hz), 2.43 (p, 2H, *J* = 5.9 Hz), 1.1-0.9 (m, 4H), 0.2 (s, 18H). $^{13}$C-NMR: ($CDCl_3$, 100 MHz) δ 169.8, 168.3, 164.0, 162.7, 153.3, 152.6, 149.28, 149.0, 147.6, 139.6, 134.8, 134.5, 127.9 (methine), 127.5, 125.1, 123.21, 121.5, 120.5 (methine), 120.1 (methine), 116.4 (methine), 113.2 (methine), 108.7 (methine), 79.8 (methylene), 79.6 (methylene), 68.7 (methylene), 68.5 (methylene), 67.0 (methylene), 66.8 (methylene), 58.8 (methine), 58.0 (methine), 57.6 (methoxy), 32.8 (methylene), 32.0 (methylene), 30.3 (methylene), 19.7 (methylene), 0.25 (methyl).

*(b) (S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-methyl-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione (**10**)*

A suspension of the 4-anilino triflate [see Patent 33], (210 mg, 0.198 mmol), methylboronic acid (50 mg, 0.835 mmol, 4.2 eq.), silver I oxide (139 mg, 0.600 mmol., 3 eq.), potassium phosphate tribasic (252 mg, 1.2 eq w/w), triphenylarsine (36.7 mg, 0.12 mmol, 0.6 eq.) and bis(benzonitrile)dichloro-palladium II (11.5 mg, 0.030 mmol, 0.15 eq.) was heated at 75°C in dry dioxane (8 mL) in a sealed tube under an inert atmosphere for 1.5 hrs. The reaction mixture was filtered through cotton-wool and the filter pad rinsed with ethylacetate and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with 80% EtOAc: 20% Hexane. Removal of excess eluent by

rotary evaporation under reduced pressure gave the product as an off-white foam (100 mg, 0.11 mmol, 54% yield).

LC-MS RT 3.87 mins, 926 (M + H)

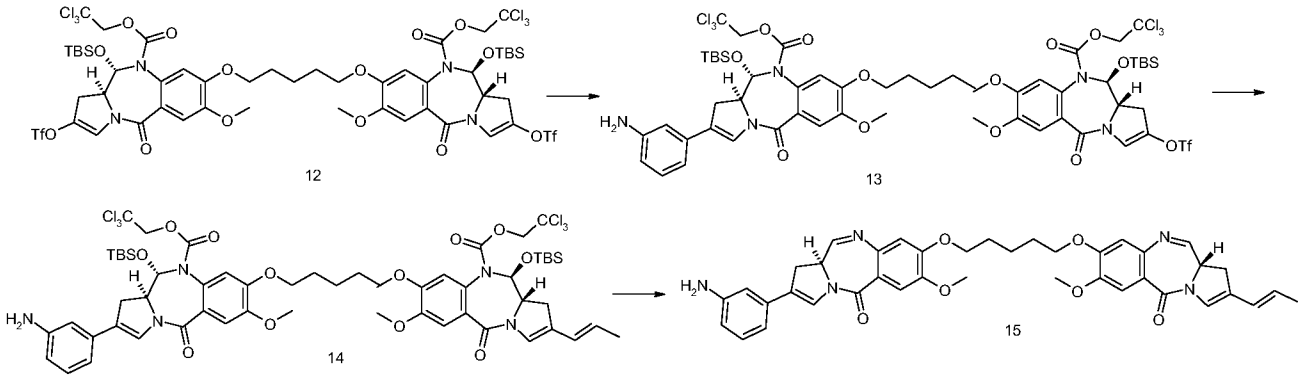
*(c) (S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-8-yloxy)propoxy)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5(11aH)-one (**11**)*

Fresh $LiBH_4$ (44 mg, 2.0 mmol, 20 eq.) was added to a stirred solution of the SEM-dilactam (90 mg, 0.1 mmol) in THF (8 mL) at room temperature. The reaction mixture was allowed to stir for 0.5 hr, at which time LC-MS revealed complete reaction. The reaction mixture was partitioned between water (50 mL) and chloroform (100 mL). The organic phase was washed with brine (50 mL), dried over magnesium sulphate and concentrated *in vacuo*. The resulting residue was treated with DCM (5 mL), EtOH (14 mL), $H_2O$ (7 mL) and silica gel (10 g). The viscous mixture was allowed to stir at room temperature for 5 days. The mixture was filtered slowly through a sinter funnel and the silica residue washed with 90% $CHCl_3$: 10% MeOH (~250 mL) until UV activity faded completely from the eluent. The organic phase was washed with $H_2O$ (50 mL), brine 60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude material. The crude product was purified by flash chromatography (gradient from 100% $CHCl_3$ : 0% MeOH to 96% $CHCl_3$ : 4% MeOH) to provide the PBD dimer (5 mg 8 % yield).

LC-MS RT 2.30 mins, 634 (M + H)

$^1$H-NMR (400 MHZ, $CDCl_3$) $\delta$ 7.80 (d, *J* = 4.0 Hz, 1H), 7.73 (d, *J* = 4.0 Hz, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 7.26 (bs, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.79 (s, 1H), 6.77 (s, 1H), 6.71-6.64 (m, 1H), 4.34-4.03 (m, 6H), 3.86 (s, 3H), 3.85 (s 3H), 3.55-3.37 (m, 1H), 3.36-3.19 (m, 1H), 3.17-3.00 (m, 1H), 2.96-2.80(m, 1H), 1.75 (s 3H).

**Example 2**



*(a) (11S,11aS)-2,2,2-trichloroethyl 2-(3-aminophenyl)-11-(tert-butyldimethylsilyloxy)-8-(5-((11S,11aS)-11-(tert-butyldimethylsilyloxy)-7-methoxy-5-oxo-10-((2,2,2-trichloroethoxy)carbonyl)-2-(trifluoromethylsulphonyloxy)-5,10,11,11a-tetrahydro-1H-pyrrolo [2,1-c][1,4] benzodiazepindiazepin-8-yloxy)pentyloxy)-7-methoxy-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate **13***

Solid 3-aminobenzeneboronic acid (60.3 mg) was added to a solution of the Troc protected *bis* triflate **12**(Compound 44, WO 2006/111759) (600 mg, 0.41 mmol), sodium carbonate (65 mg, 0.61 mmoml) and palladium *tetrakis* triphenylphosphine (0.012 mmol) in toluene (10.8 mL), ethanol (5.4 mL) and water (5.4 mL). The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was then partitioned between ethylacetate and water. The organic layer was washed with water and brine and dried over magnesium sulphate. Excess solvent was removed by rotary evaporation under reduced pressure and the resulting residue was subjected to flash column chromatography (silica gel; gradient elution EtOAc/hexane 20/80→30/70→40/60→60/40) to remove unreacted *bis*-triflate. Removal of excess eluent from selected fractions to afford the desired compound in 41% yield (230 mg, 0.163 mmol)

LC-MS RT 4.28 mins, 1411 (M + H); $^1$H-NMR (400 MHZ, $CDCl_3$) $\delta$ 7.44 (bs, 1H), 7.29 (s, 1H), 7.25 (s, 1H), 7.20 (s, 1H), 7.16 (t, *J* = 7.9 Hz, 1H), 6.84 – 6.73 (m, 3H), 6.70 (bs, 1H), 6.62 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.66 – 6.58 (m, 2H), 5.25 (d, *J* = 12.0 Hz, 1H), 5.24 (d, *J* = 12.0 Hz, 1H), 4.24 (d, *J* = 12.0 Hz, 1H), 4.22 (d, *J* = 12.0 Hz, 1H), 4.17 – 4.07 (m, 2H), 4.08 – 3.89 (m, 10H), 3.43 – 3.28 (m, 2H), 2.85 (d, *J* = 1.65 Hz, 2H), 2.07 – 1.90 (m, 4H), 1.78 – 1.63 (m, 2H), 0.94 (s, 9H), 0.90 (s, 9H), 0.30 (s, 6H), 0.27 (s, 6H).

*(b) (11S,11aS)-2,2,2-trichloroethyl 2-(3-aminophenyl)-11-(tert-butyldimethylsilyloxy)-8-(5-((11S,11aS)-11-(tert-butyldimethylsilyloxy)-2-propenyl-7-methoxy-5-oxo-10-((2,2,2-trichloroethoxy)carbonyl)-5,10,11,11a-tetrahydro-1H-_pyrrolo [2,1-c][1,4]*

*benzodiazepindiazepin-8-yloxy)pentyloxy)-7-methoxy-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate* ***14***

Solid 1-propenyl boronic acid (7.1 mg, 0.084 mmol) was added to a solution of the Troc protected triflate **13** (73 mg, 0.052 mmol), sodium carbonate (18 mg, 0.17 mmol) and palladium *tetrakis* triphenylphosphine (3 mg) in toluene (1 mL), ethanol (0.5 mL) and water (0.5 mL). The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was then partitioned between ethyl acetate and water. The organic layer was washed with water and brine and dried over magnesium sulphate. Excess solvent was removed by rotary evaporation under reduced pressure and the resulting residue was eluted through a plug of silica gel with ethylacetate. Removal of excess eluent from selected fractions afforded the coupled product **14** (40 mg, 0.031 mmol, 59%).

LC-MS RT 4.38 mins, (1301, 1305, 1307, 1308, 1310 multiple masses due to chlorine isotopes)

*(c) (S)-2-(3-aminophenyl)-8-(5-((S)-2-propenyl-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-8-yloxy)pentyloxy)-7-methoxy-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5(11aH)-one* ***15***

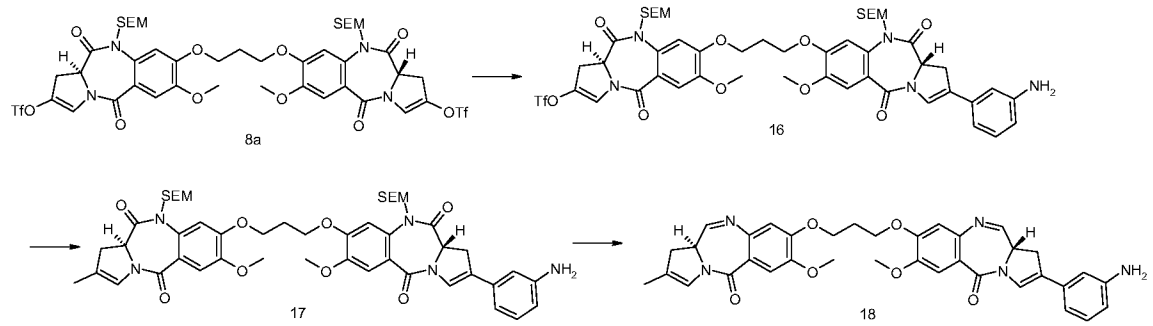
Cadmium/lead couple (100 mg, Q Dong *et al*. Tetrahedron Letters vol **36**, issue *32*, 5681-5682, 1995) was added to a solution of the Suzuki product **14** (40 mg, 0.029 mmol) in THF (1 mL) and ammonium acetate (1N, 1 mL) and the reaction mixture was allowed to stir for 1 hour. The reaction was filtered through cotton wool to remove particulates and break-up the emulsion. The reaction mixture was partitioned between chloroform and water, the phases separated and the aqueous phase extracted with chloroform. The combined organic layers were washed with brine and dried over magnesium sulphate. Rotary evaporation under reduced pressure yielded the crude product which was subjected to column chromatography (silica gel, 1 → 5% MeOH/$CHCl_3$). Removal of excess eluent by rotary evaporation under reduced pressure afforded the desired imine product **15** (9 mg 0.013 mmol 43%)

LC-MS RT 2.80 mins, 689 (M + H)

$^1$H-NMR (400 MHZ, $CDCl_3$) $\delta$ 7.88 (d, *J* = 3.9 Hz, 1H), 7.82 (d, *J* = 3.9Hz, 1H), 7.52 (s, 1H), 7.49 (s, 1H), 7.45 (bs, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.92 (bs, 1H), 6.84-6.76 (m, 3H), 6.72 (bs, 1H), 6.60 (dd, *J* = 7.9, 1.9 Hz, 1H), 6.26 (d, *J* = 15.3 Hz, 1H), 5.67-5.51 (m, 1H), 4.46-

4.35 (m, 1H), 4.34-4.24 (m, 1H), 4.20-4.00 (m, 4H), 3.94 (s, 3H), 3.93 (s 3H), 3.62-3.44 (m, 1H), 3.43-3.23 (m, 2H), 3.19-3.02 (m, 1H), 2.06-1.89(m, 4H), 1.84 (d, *J* = 6.5 Hz ,3H), 1.76-1.62 (m, 2H).

**Example 3**




*(a) (S)-2-(3-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(trifluoromethylsulfonyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5,11(10H,11aH)-dione* ***16***

Solid $Pd(PPh_3)_4$ (20 mg, 17.8 μmol) was added to a stirred solution of the triflate **8a** (2.5 g, 2.24 mmol), 3-aminobenzeneboronic acid (291 mg, 2.12 mmol) and $Na_2CO_3$ (356 mg, 3.35 mmol) in toluene (20 mL), EtOH (10 mL) and $H_2O$ (10 mL). The solution was allowed to stir under a nitrogen atmosphere for 3 hours at room temperature, after which time analysis by TLC (EtOAc) and LC/MS revealed the formation of the desired mono-coupled product and as well as the presence of unreacted starting material. The solvent was removed by rotary evaporation under reduced pressure and the resulting residue partitioned between $H_2O$ (100 mL) and EtOAc (100 mL), after eventual separation of the layers the aqueous phase was extracted again with EtOAc (2 x 25 mL). The combined organic layers were washed with $H_2O$ (50 mL), brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude Suzuki product. The crude Suzuki product was subjected to flash chromatography (30%EtOAc/70% Hexane →80% EtOAc, 20% Hexane). Removal of the excess eluent by rotary evaporation under reduced pressure afforded the desired product (1 g) in 42% yield.

LC-MS, 4.17 minutes, $ES^+$ 1060.19.

*(b) (S)-2-(3-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-methyl-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-*

*8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5,11(10H,11aH)-dione* ***17***

A suspension of the 3-anilino triflate, (50 mg, 47.2 μmol), methylboronic acid (8.47 mg, 141 μmol, 3 eq.), silver(I)oxide (21.8 mg, 94.3 μmol., 2 eq.), potassium phosphate tribasic (60 mg, 1.2 eq w/w), triphenylarsine (5.78 mg, 18.9 μmol, 0.4 eq.) and *bis*(benzonitrile)dichloro-palladium II (1.81 mg, 4.7 μmol, 0.1 eq.) was heated at 67°C in dry dioxane (2 mL) in a sealed tube under an inert atmosphere for 3 hrs. The reaction mixture was filtered through cotton-wool and the filter pad rinsed with ethylacetate and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with 80% EtOAc: 20% Hexane. Removal of excess eluent by rotary evaporation under reduced pressure gave the product as an off-white foam (18 mg, 19.4 μmol, 41% yield). The reaction was subsequently repeated on a larger scale to afford 250 mg of the 2-methyl product.

LC-MS 3.88 mins, 925.86 (M + H)

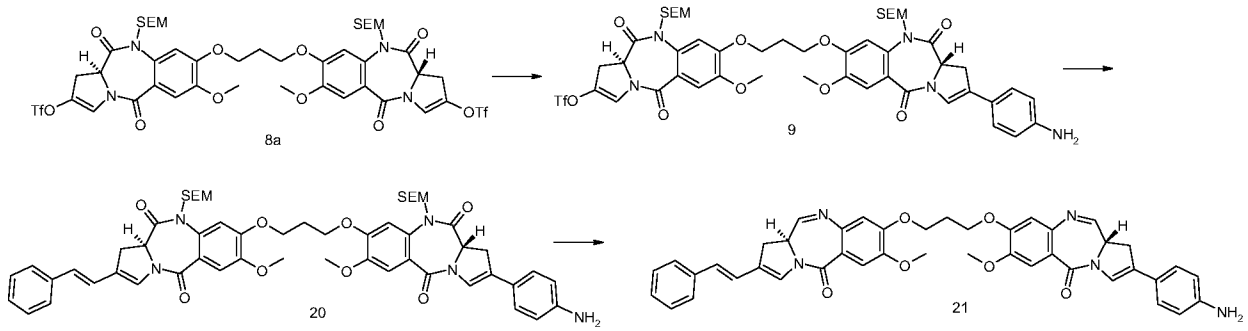
*(c) (S)-2-(3-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-8-yloxy)propoxy)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5(11aH)-one* ***18***

Fresh $LiBH_4$ (20.6 mg, 0.95 mmol, 3.5 eq.) was added to a stirred solution of the SEM-dilactam (250 mg, 0.27 mmol) in THF (4 mL) at room temperature. The reaction mixture was allowed to stir for 1.0 hr, at which time LC-MS revealed complete reaction. Excess $LiBH_4$ was quenched with acetone (c. 1 mL) at 0°C (ice bath). The reaction mixture was partitioned between water (50 mL) and 10% methanol in chloroform (100 mL). The organic phase was washed with brine (50 mL), dried over magnesium sulphate and concentrated *in vacuo*.

The resulting residue was treated with 10% methanol in chloroform (c. 50 mL) and silica gel (20 g). The viscous mixture was allowed to stir at room temperature for 5 days. The mixture was filtered slowly through a sinter funnel and the silica residue washed with 90% $CHCl_3$: 10% MeOH (~250 mL) until UV activity faded completely from the eluent. The organic phase was washed with $H_2O$ (50 mL), brine 60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude material. The crude product was purified by flash chromatography (gradient from 100% $CHCl_3$ : 0% MeOH to 96% $CHCl_3$ : 4% MeOH) to provide the PBD dimer **18**.

**Example 4**

*Part (i)*




*(a) Alternate Synthesis of (S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(trifluoromethylsulfonyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5,11(10H,11aH)-dione (9)*

Tetrakis(triphenylphosphine)palladium(0) (208 mg) was added to triflate **(8a)(**5 g), 4-anilineboronic acid (0.93 g) and sodium carbonate (0.62 g) in a mixture of toluene (60 mL), ethanol (30 mL) and water (10 mL). The reaction mixture was allowed to stir for 3 days at room temperature. The reaction mixture was washed with water, brine and dried over magnesium sulphate. After filtration excess solvent was removed by rotary evaporation under reduced pressure. The crude coupling product was purified by flash column chromatography (silica gel; gradient: 100% hexane to 100% ethyl acetate). Pure fractions were combined and removal of excess eluent afforded the pure product as a solid (2.2 g, 93 % yield, LC/MS 8.05 mins, m/z $ES^+$ 1060).

(b) *(S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(phenyl-vinyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5,11(10H,11aH)-dione (**20**)*

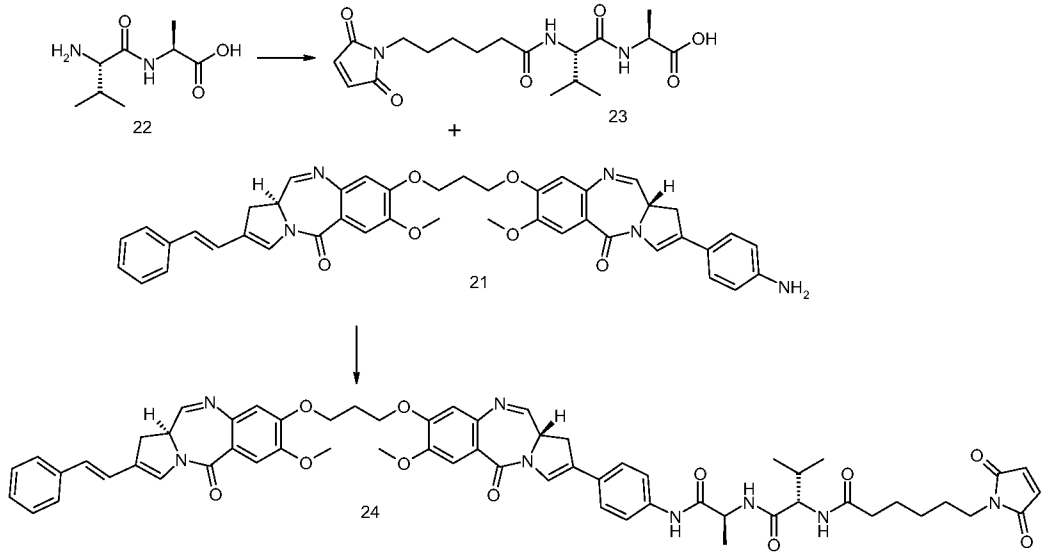
*A* mixture of triflate **9** (0.5 g), *trans*-2-phenylvinylboronic acid (0.091 g), triethylamine (0.38 g) and tetrakis(triphenylphosphine)palladium(0) (30 mL) in ethanol (3 mL), toluene (6 mL) and water (1mL) was irradiated with microwaves for 8 minutes at 80°C in a sealed microwave vial. The reaction mixture was diluted with dichloromethane washed with water and dried over magnesium sulphate. Excess solvent was removed by rotary evaporation

under reduced pressure to afford the crude product which was used without further purification in the next reaction. Retention time 8.13 mins, $ES^+$1014.13.

(c) *(S)-2-(4-aminophenyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(phenyl-vinyl)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-8-yloxy)propoxy)-1H-pyrrolo[2,1-c] [1,4]benzodiazepine-5(11aH)-one (**21**)*

A solution of superhydride in THF (1 M, 1.2 mL) was added by syringe to a solution of the crude Suzuki product (0.477 g) in THF (10 mL) at -78°C (acetone/dry ice bath). The reaction mixture was allowed to stir at -78°C for 20 minutes, after which time the reaction was quenched with water. The reaction mixture was extracted with ethyl acetate and the organic layer washed with brine and dried over magnesium sulphate. Removal of excess solvent by rotary evaporation under reduced pressure afforded the crude SEM-carbinolamine which was dissolved in dichloromethane (3 mL), ethanol (6 mL) and water (1 mL) and stirred with silica gel for 2 days. The reaction mixture was filtered excess solvent evaporated by rotary evaporation under reduced pressure and the residue subjected to flash column chromatography (3 % methanol in chloroform). Pure fractions were combined and excess eluent removed by rotary evaporation under reduced pressure to afford compound **21** (0.75 mg, 22 % yield over 3 steps). Retention time 5.53 mins, $ES^+$721.99.

*Part (ii)*




*(a) (S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)propanoic acid (**23**)*

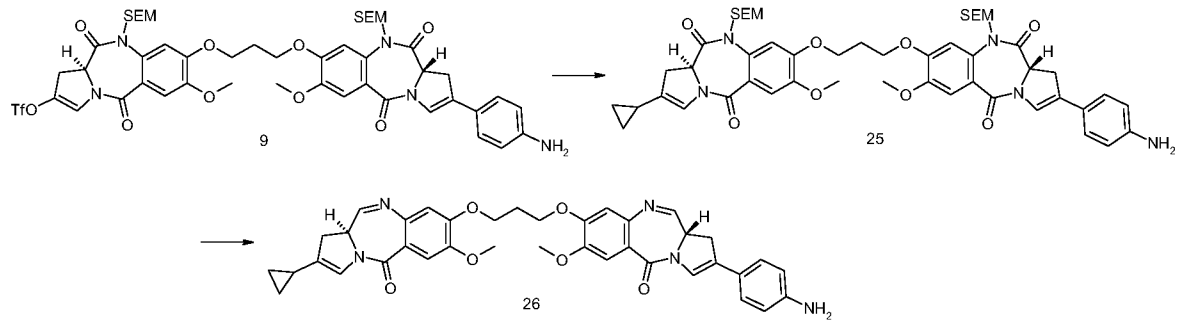
A suspension of dipeptide (**22**) (0.1 g, 0.54 mmol, 1 eq.) and 6-maleimidohexanoic acid succinimide ester (0.165 g, 0.54 mmol, 1 eq.) in anhydrous DMF (5 mL) was stirred at

room temperature for 24 hours at which time LCMS indicated 50% conversion to a new product. The reaction mixture was diluted with anhydrous DMF (5 mL) and the reaction was allowed to continue for a further 24 hours. The solvent was evaporated under reduced pressure to give a colourless residue. Diethyl ether (60 mL) was added and the mixture was sonicated for 5 min, the ether was decanted and the process was repeated (x 2). The final ethereal portion was filtered to isolate the product (**23**) as a white powder which was dried under vacuum (0.105 g, 52%). Analytical Data: RT 2.28 min; MS ($ES^+$) *m/z* (relative intensity) 382 ($[M + H]^{+\cdot}$, 90), MS ($ES^-$) *m/z* (relative intensity) 380 ($[M - H])^{-\cdot}$, 100).

*(b) 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((S)-7-methoxy-8-(3-(((S)-7-methoxy-5-oxo-2-((E)-styryl)-5,11a-dihydro-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)hexanamide (**24**)*

The unsymmetrical PBD dimer (**21**) (0.019 g, 26 µmol, 1 eq.) was added to a solution of the linker (**23**) (0.0121 g, 31.6 µmol, 1.2 eq.) and EEDQ (0.0098 g, 39.6 µmol, 1.5 eq.) in a mixture of anhydrous DCM/MeOH (3 mL/0.5 mL) under an argon atmosphere. The resultant solution was stirred at room temperature for 5 hours at which time LCMS indicated 50% conversion to a new product. The reaction mixture was diluted with anhydrous DCM (2 mL) and the reaction was allowed to continue for a further 18 hours. The solvent was evaporated under reduced pressure and the residue purified by flash column chromatography [DCM 100% to DCM 94%/MeOH 6% in 1% increments] to give the product as a yellow solid (5.2 mg, 18%). Analytical Data: RT 3.10 min; MS ($ES^+$) *m/z* (relative intensity) 1085 ($[M + H]^{+\cdot}$, 90).

**Example 5**



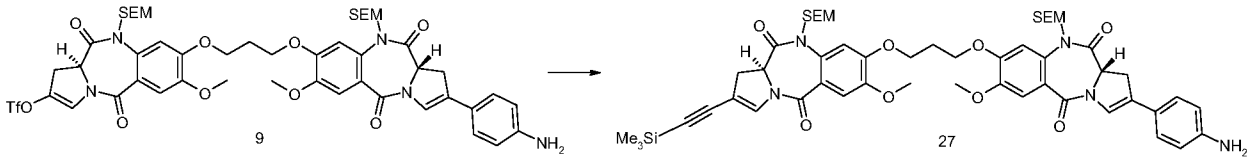

*(a) (S)-2-(4-aminophenyl)-8-(3-(((S)-2-cyclopropyl-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione (**25**)*

A suspension of silver (I) oxide (0.441 g), potassium phosphate tribasic (1.187 g), triphenylarsine (0.116 g), cyclopropylboronic acid (0.206 g) and starting material **9** (0.5 g) in dioxane (15 mL) under and an argon atmosphere was heated to 71°C. A catalytic amount of palladium (II) *bis* (benzonitrile chloride) (0.036 g) was added and the reaction mixture was allowed to stir for 2 hours and 10 mins at 71°C. The reaction mixture was filtered through celite and the filter pad washed with ethyl acetate (400 mL). The organic solution was extracted with water (2 x 600 mL) and brine (600 mL) and dried over magnesium sulphate. Removal of organic solvent by rotary evaporation under reduced pressure afforded the crude product which was purified via gravity silica gel chromatography (ethyl acetate only as eluent). Removal of excess eluent by rotary evaporation under reduced pressure afforded the product **25** as a yellow solid (145 mg, 32 % yield). LCMS RT 3.92 mins, $ES^+$952.06

*(b) (S)-2-(4-aminophenyl)-8-(3-(((S)-2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-pyrrolo[2,1-c][1,4]benzodiazepin-5(11aH)-one (**26**)*

A solution of super hydride (0.361 mL, 1M in THF) was added drop wise over 5 minutes to a solution of the SEM dilictam **25** (0.137 g) in anhydrous tetrahydrofuran (5 mL) under an argon atmosphere at -78°C. LCMS after 35 minutes revealed that the reaction was complete and excess super hydride was quenched with water (4 mL) followed by brine (4 mL). The aqueous solution was extracted with a mixture of dichloromethane/methanol (9:1, 2 x 16 mL) and the organic layer dried over magnesium sulphate. Solvent was removed by rotary evaporation under reduced pressure and the crude product was taken up in a mixture of ethanol, dichloromethane and water (8:3:1, 15 mL) and treated with silica gel. The thick suspension was allowed to stir for 4 days. The mixture was filtered through a sinter, washing with dichloromethane/methanol (9:1, 140 mL) until product ceased to be eluted. The organic layer was washed with brine (2x 250 mL) and then dried over magnesium sulphate. Rotary evaporation under reduced pressure afforded the crude product which was subjected to flash column chromatography (silica gel; gradient 100% to 5% methanol/dichloromethane). Removal of excess eluent afforded the product **26** (23 mg, 25 % yield). LCMS RT 2.42, $ES^+$ 659.92

**Example 6**

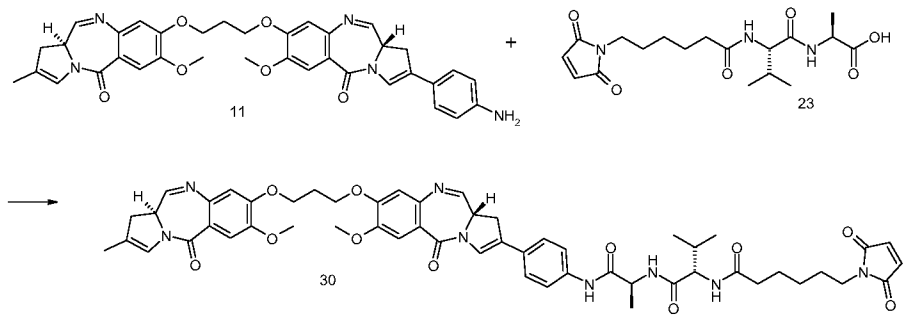


*(S)-2-(4-aminophenyl)-7-methoxy-8-(3-(((S)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-2-((trimethylsilyl)ethynyl)-5,10,11,11a-tetrahydro-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione (**27**)*

A mixture of **9** (0.150 g, 0.14 mmol), CuI (0.003 g, 0.014 mmol, 0.1 eq), $Pd(PPh_3)_4$ (0.0162 g, 0.014 mmol, 0.1 eq) and $PPh_3$ (0.007 g, 0.028 mmol, 0.2 eq) was dissolved in piperidine (9 mL) in presence of molecular sieves under an argon atmosphere. Ethynyltrimethylsilane (0.06 ml, 0.42 mmol, 3 eq) was added to the mixture at 70°C and the reaction mixture was allowed to stir overnight. The solvent was removed by rotary evaporation under reduced pressure and the resulting brown solid purified by flash column chromatography (silica gel, 90% EtOAc, 10% hexane). Compound **27** was obtained as an orange solid (0.043 g, 30%); **Rf** 0.69 [EtOAc]; **LC-MS** (5 min) 4.28 min, $ES^+$ 1008.28.

**Example 7**

(a)

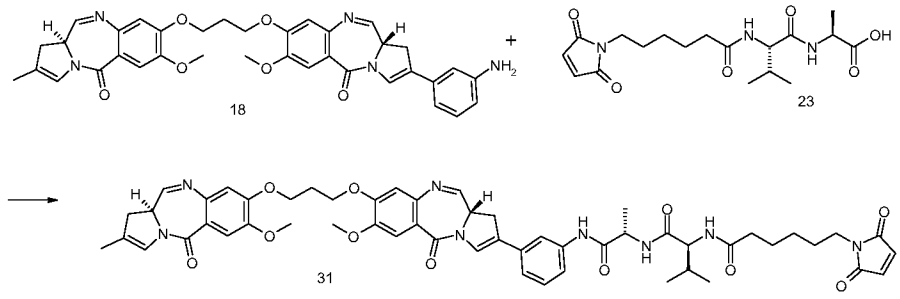


*6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-((S)-7-methoxy-8-(3-(((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)hexanamide (**30**)*

To a mixture of carboxylic acid **23** (8 mg, 21 umol) in 5% methanol/dichloromethane was added EEDQ (6.1 mg, 24.6 umol) and the mixture was stirred for 15 minutes under nitrogen at an ambient temperature. The resulting mixture was added to **11** (12 mg, 18.9 umol) and stirred for 3 hours under nitrogen. The reaction mixture was aspirated directly onto a 1 mm radial chromatotron plate and eluted with a gradient of 1 to 4% methanol in

dichloromethane. Product containing fractions were concentrated under reduced pressure to give 9.4 mg (50%) of **30** as a yellow solid**:** MS ($ES^-$) *m/z* 997.18 $(M+H)^+$.

(b)




*6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((3-((S)-7-methoxy-8-(3-(((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-pyrrolo [2,1-c][1,4]benzo diazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)hexanamide (**31**)*

Compound 31 was synthesised from compound 18 using the same method as in part (a) with a yield of 25%.

**Example 8: Determination of Free Drug *In Vitro* Cytotoxicity**

Cells as detailed below were collected and plated in 96 well black-sided plates at a density of 10,000 cells/well in 150 μL of medium. Serial dilutions of the test article (50 μL) were added, and incubation was carried out for 92 hours at 37°C. After addition of test compound, cultures were incubated to 96 hours at 37°C. Resazurin (0.25 mM, 50 μL, Sigma, St. Louis, MO) in medium was added and incubation was continued for 4 h. The plates were read on a Fusion HT microplate reader (Packard, Meriden, CT) using an excitation wavelength of 525 nm and an emission wavelength of 590 nm. Data from all assays were reduced using GraphPad Prism Version 4 for Windows (GraphPad Software, San Diego, CA). The $IC_{50}$ concentrations compared to untreated control cells were determined using a 4 parameter curve fits.

The $IC_{50}$ (nM) values for compound 15 are:

| | **L428** | **786-O** | **HEL** | **HL-60** | **MCF-7** |
|---|---|---|---|---|---|
| $IC_{50}$ (nm) | <0.00001 | <0.00001 | <0.00001 | <0.00001 | 0.03 |

The same method was also used to determine the activity of compounds 11 and 18:

| $IC_{50}$ (nM) | **Caki-1** | **786-O** | **TF1a** | **MCF-7** |
|---|---|---|---|---|
| 11 | 0.06 | 0.1 | 0.07 | 0.2 |
| 18 | 0.6 | 1 | 0.7 | 2 |

*Alternative cell assay*

Cells were plated in 150 µL growth media per well into black-sided clear-bottom 96-well plates (Costar, Corning) and allowed to settle for 1 hour in the biological cabinet before placing in the incubator at 37°C, 5% $CO_2$. The following day, 4X concentration of drug stocks were prepared, and then titrated as 10-fold serial dilutions producing 8-point dose curves and added at 50µl per well in duplicate. Cells were then incubated for 48 hours at 37°C, 5% $CO_2$. Cytotoxicity was measure by incubating with 100µL Cell Titer Glo (Promega) solution for 1 hour, and then luminescence was measured on a Fusion HT plate reader (Perkin Elmer). Data was processed with Excel (Microsoft) and GraphPad (Prism) to produce dose response curves and IC50 values were generated and data collected.

| $IC_{50}$ (nM) | **786-O** | **Caki-1** | **MCF-7** | **BxPC-3** | **HL-60** | **HEL** |
|---|---|---|---|---|---|---|
| 11 | 0.85 | 0.4 | 7 | 3 | 0.1 | 0.06 |

**Example 9: Preparation of PDB Dimer Conjugates**

Antibody-drug conjugates were prepared as previously described (see Doronina et al., *Nature Biotechnology*, 21, 778-784 (2003)) or as described below.

Engineered hIgG1 antibodies with introduced cysteines: CD70 antibodies containing a cysteine residue at position 239 of the heavy chain (h1F6d) were fully reduced by adding 10 equivalents of TCEP and 1 mM EDTA and adjusting the pH to 7.4 with 1M Tris buffer (pH 9.0). Following a 1 hour incubation at 37 °C, the reaction was cooled to 22 °C and 30 equivalents of dehydroascorbic acid were added to selectively reoxidize the native disulfides, while leaving cysteine 239 in the reduced state. The pH was adjusted to 6.5 with 1M Tris buffer (pH 3.7) and the reaction was allowed to proceed for 1 hour at 22 °C. The pH of the solution was then raised again to 7.4 by addition of 1 M Tris buffer (pH 9.0). 3.5 equivalents of the PBD drug linker in DMSO were placed in a suitable container for dilution with propylene glycol prior to addition to the reaction. To maintain solubility of the PBD drug linker, the antibody itself was first diluted with propylene glycol to a final concentration of 33% (e.g., if the antibody solution was in a 60 mL reaction volume, 30 mL

of propylene glycol was added). This same volume of propylene glycol (30 mL in this example) was then added to the PBD drug linker as a diluent. After mixing, the solution of PBD drug linker in propylene glycol was added to the antibody solution to effect the conjugation; the final concentration of propylene glycol is 50%. The reaction was allowed to proceed for 30 minutes and then quenched by addition of 5 equivalents of N-acetyl cysteine. The ADC was then purified by ultrafiltration through a 30 kD membrane. (Note that the concentration of propylene glycol used in the reaction can be reduced for any particular PBD, as its sole purpose is to maintain solubility of the drug linker in the aqueous media.)

**Example 10: Determination of Conjugate *In Vitro* Cytotoxicity**

Cells as detailed below were collected and plated in 96 well black-sided plates at a density of 10,000 cells/well in 150 μL of medium. Serial dilutions of the test article (50 μL) were added, and incubation was carried out for 92 hours at 37°C. After addition of test compound, cultures were incubated to 96 hours at 37°C. Resazurin (0.25 mM, 50 μL, Sigma, St. Louis, MO) in medium was added and incubation was continued for 4 h. The plates were read on a Fusion HT microplate reader (Packard, Meriden, CT) using an excitation wavelength of 525 nm and an emission wavelength of 590 nm. Data from all assays were reduced using GraphPad Prism Version 4 for Windows (GraphPad Software, San Diego, CA). The $IC_{50}$ concentrations compared to untreated control cells were determined using a 4 parameter curve fits. The antibody used was a CD70 antibody (humanized 1F6; see Published U.S. Application No. 2009-148942) having introduced cysteine residues at amino acid heavy chain position 239 (according to the EU numbering system) (indicated as h1F6d).

The $IC_{50}$ (nM) values for ADCs of compound **31** are:

| **ADCs** | **Caki-1** | **786-O** | **HL60** | **HEL** | **TF1a** |
|---|---|---|---|---|---|
| h1F6d-**31** (2dr/Ab) | 7 | 36 | 5371 | Max Inh=50% | Max Inh=40% |

Note: Maximum inhibition (Max Inhb) = % inhibition at top concentration out of 100% untreated.

**Example 11: Determination of In Vivo Cytotoxicity of Selected Conjugates**

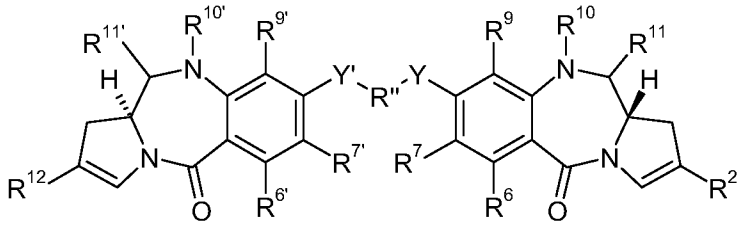
The following study was conducted in concordance with the Animal Care and Use Committee in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The antibodies used were an antibody having introduced cysteine residues at position 239 (S239C) in the heavy chains and conjugated to compound **31**, and a nonbinding control conjugated to the same compound **31**.

Treatment studies were conducted in an antigen+ xenograft model. Tumor cells were implanted subcutaneously into scid mice. Mice were randomized to study groups (n=6). The ADC-compound **31** or control ADCs were dosed ip according to a q4dx4 schedule (as shown by the triangles on the x axis). Tumor volume as a function of time was determined using the formula (L x W2)/2. Animals were euthanized when tumor volumes reached 1000 $mm^3$.

Referring to Figure 1, the ADC of compound **31** was dosed at 0.1 (□), 0.3 (▩) and 1 (■) mg/kg. A nonbinding control, conjugated to compound **31**, was administered at the same doses (0.1 (△), 0.3 (▲) and 1(▲) mg/kg). All three doses of the Ab-compound **31** conjugate had better activity than the nonbinding control conjugate. Untreated tumours are shown by *.

## CLAIMS

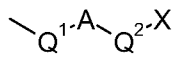
1. A compound with the formula **I**:



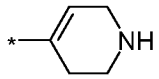
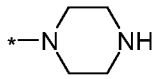
I

wherein:

$R^2$ is of formula **II**:



II

where A is a $C_{5-7}$ aryl group, X is selected from the group comprising: OH, SH, $CO_2H$, COH, N=C=O, $NHNH_2$, $CONHNH_2$,  ,  , $NHR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl, and either:

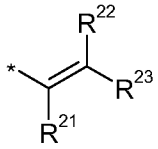
(i) $Q^1$ is a single bond, and $Q^2$ is selected from a single bond and -Z-$(CH_2)_n$-, where Z is selected from a single bond, O, S and NH and is from 1 to 3; or

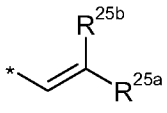
(ii) $Q^1$ is -CH=CH-, and $Q^2$ is a single bond;

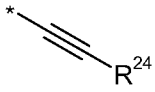
$R^{12}$ is selected from:

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

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

(iic)  , wherein each of $R^{21}$, $R^{22}$ 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 $R^{12}$ group is no more than 5;

(iid)  , 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

(iie)  , 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;

$R^6$ 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, NHRR', nitro, $Me_3Sn$ and halo;

either:

(a) $R^{10}$ is H, and $R^{11}$ is OH, $OR^A$, where $R^A$ is $C_{1-4}$ alkyl;

(b) $R^{10}$ and $R^{11}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) $R^{10}$ is H and $R^{11}$ is $SO_zM$, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

R″ is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms, and/or aromatic rings;

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 and $R^{10'}$ and $R^{11'}$ are the same as $R^{10}$ and $R^{11}$, wherein if $R^{11}$ and $R^{11'}$ are $SO_zM$, M may represent a divalent pharmaceutically acceptable cation.

2. A compound according to any claim 1, wherein $R^7$ is selected from H, OH and OR.

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

4. A compound according to any one of claims 1 to 3, wherein Y is O.

5. A compound according to any one of the preceding claims, wherein R'' is $C_{3-7}$ alkylene.

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

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

8. A compound according to any one of claims 1 to 7, wherein A is phenyl.

9. A compound according to any one of claims 1 to 8, wherein X is selected from OH, SH, or $NH_2$.

10. A compound according to any one of claims 1 to 9, wherein $Q^1$ is a single bond.

11. A compound according to claim 10, wherein $Q^2$ is a single bond.

12. A compound according to claim 10, wherein $Q^2$ is $-Z-(CH_2)_n-$, Z is O or S and n is 1 or 2.

13. A compound according to any one of claims 1 to 9, wherein $Q^1$ is -CH=CH-.

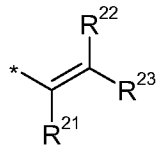
14. A compound according to any one of claims 1 to 13, wherein $R^{12}$ is a $C_{1-5}$ saturated aliphatic alkyl group.

15. A compound according to claim 14, wherein $R^{12}$ is methyl, ethyl or propyl.

16. A compound according to any one of claims 1 to 13, wherein $R^{12}$ is a $C_{3-6}$ saturated cycloalkyl group.

17. A compound according to claim 16, wherein $R^{12}$ is cyclopropyl.

18. A compound according to any one of claims 1 to 13, wherein $R^{12}$ is a group of formula:




.

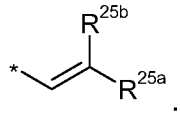
19. A compound according to claim 18, wherein the total number of carbon atoms in the $R^{12}$ group is no more than 4.

20. A compound according to claim 19, wherein the total number of carbon atoms in the $R^{12}$ group is no more than 3.

21. A compound according to any one of claims 18 to 20, wherein one of $R^{21}$, $R^{22}$ 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.

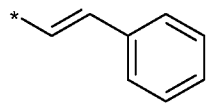
22. A compound according to any one of claims 18 to 20, wherein two of $R^{21}$, $R^{22}$ and $R^{23}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

23. A compound according to any one of claims 1 to 13, wherein $R^{12}$ is a group of formula:



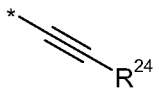
.

24. A compound according to claim 23, wherein $R^{12}$ is the group:



.

25. A compound according to any one of claims 1 to 13, wherein $R^{12}$ is a group of formula:



.

26. A compound according to claim 25, wherein $R^{24}$ is selected from H, methyl, ethyl, ethenyl and ethynyl.

27. A compound according to claim 26, wherein $R^{24}$ is selected from H and methyl.

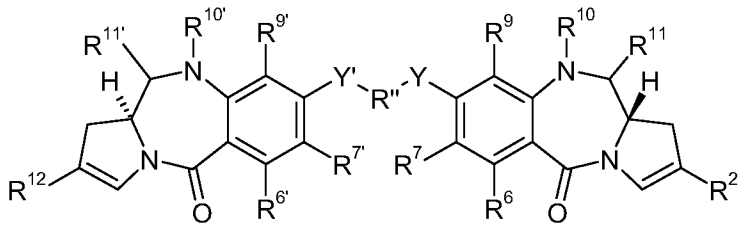
28. A compound according to any one of claims 1 to 27, wherein $R^{10}$ and $R^{11}$ and form a nitrogen-carbon double bond.

29. A compound according to any one of claims 1 to 28, wherein $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{10'}$, $R^{11'}$ and Y' are the same as $R^6$, $R^7$, $R^9$, $R^{10}$, $R^{11}$ and Y respectively.

30. The use of a compound according to any one of claims 1 to 29 in the manufacture of a medicament for treating a proliferative disease.

31. A compound according to any one of claims 1 to 29 for use in the treatment of a proliferative disease.

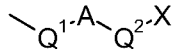
32. A compound of formula **II**:



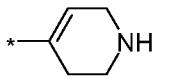
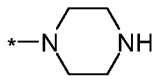
II

wherein:

$R^2$ is of formula II:



II

where A is a $C_{5-7}$ aryl group, X is selected from the group comprising: OH, SH, $CO_2H$, COH, N=C=O, $NHNH_2$, $CONHNH_2$,  ,  , $NHR^N$, wherein $R^N$ is selected from the group comprising H and $C_{1-4}$ alkyl, and either:

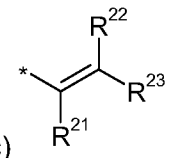
(i) $Q^1$ is a single bond, and $Q^2$ is selected from a single bond and -Z-$(CH_2)_n$-, where Z is selected from a single bond, O, S and NH and n is from 1 to 3; or

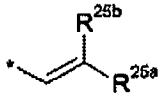
(ii) $Q^1$ is -CH=CH-, and $Q^2$ is a single bond;

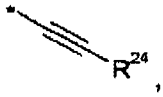
$R^{12}$ is selected from:

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

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

(iic)  , wherein each of $R^{21}$, $R^{22}$ 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 $R^{12}$ group is no more than 5;

(iid)  , 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

(iie)  , 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;

$R^6$ 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, NHRR', nitro, $Me_3Sn$ and halo;
either:
(a) $R^{10}$ is carbamate nitrogen protecting group, and $R^{11}$ is O-$Prot^O$, wherein $Prot^O$ is an oxygen protecting group;
(b) $R^{10}$ is a hemi-aminal nitrogen protecting group and $R^{11}$ is an oxo group;
R'' is a $C_{3-12}$ alkylene group, which chain may be interrupted by one or more heteroatoms, and/or aromatic rings;
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 and $R^{10'}$ and $R^{11'}$ are the same as $R^{10}$ and $R^{11}$.

33. A compound according to any claim 32, wherein $R^7$ is selected from H, OH and OR.

34. A compound according to claim 33, wherein $R^7$ is a $C_{1-4}$ alkyloxy group.

35. A compound according to any one of claims 32 to 34, wherein Y is O.

36. A compound according to any one of claims 32 to 35, wherein R'' is $C_{3-7}$ alkylene.

37. A compound according to any one of claims 32 to 36, wherein $R^9$ is H.

38. A compound according to any one of claims 32 to 37, wherein $R^6$ is selected from H and halo.

39. A compound according to any one of claims 32 to 38, wherein A is phenyl.

40. A compound according to any one of claims 32 to 39, wherein X is selected from OH, SH, or $NH_2$.

41. A compound according to any one of claims 32 to 40, wherein $Q^1$ is a single bond.

42. A compound according to claim 41, wherein $Q^2$ is a single bond.

43. A compound according to claim 41, wherein $Q^2$ is $-Z-(CH_2)_n-$, Z is O or S and n is 1 or 2.

44. A compound according to any one of claims 32 to 40, wherein $Q^1$ is -CH=CH-.

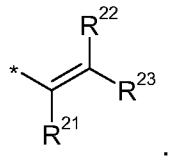
45. A compound according to any one of claims 32 to 44, wherein $R^{12}$ is a $C_{1-5}$ saturated aliphatic alkyl group.

46. A compound according to claim 45, wherein $R^{12}$ is methyl, ethyl or propyl.

47. A compound according to any one of claims 32 to 44, wherein $R^{12}$ is a $C_{3-6}$ saturated cycloalkyl group.

48. A compound according to claim 47, wherein $R^{12}$ is cyclopropyl.

49. A compound according to any one of claims 32 to 44, wherein $R^{12}$ is a group of formula:




.

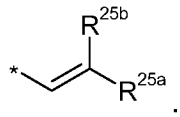
50. A compound according to claim 49, wherein the total number of carbon atoms in the $R^{12}$ group is no more than 4.

51. A compound according to claim 50, wherein the total number of carbon atoms in the $R^{12}$ group is no more than 3.

52. A compound according to any one of claims 49 to 51, wherein one of $R^{21}$, $R^{22}$ 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.

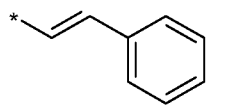
53. A compound according to any one of claims 49 to 51, wherein two of $R^{21}$, $R^{22}$ and $R^{23}$ are H, with the other group being selected from H, $C_{1-3}$ saturated alkyl, $C_{2-3}$ alkenyl, $C_{2-3}$ alkynyl and cyclopropyl.

54. A compound according to any one of claims 32 to 44, wherein $R^{12}$ is a group of formula:



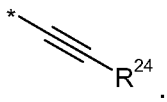
.

55. A compound according to claim 54, wherein $R^{12}$ is the group:



.

56. A compound according to any one of claims 32 to 44, wherein $R^{12}$ is a group of formula:



.

57. A compound according to claim 56 wherein $R^{24}$ is selected from H, methyl, ethyl, ethenyl and ethynyl.

58. A compound according to claim 57, wherein $R^{24}$ is selected from H and methyl.

59. A compound according to any one of claims 32 to 58, wherein $R^{10}$ is Troc.

60. A compound according to any one of claims 32 to 59, wherein $R^{11}$ is OTBS.

61. A compound according to any one of claims 32 to 59, wherein $R^{11}$ is oxo and $R^{10}$ is SEM.

62. A compound according to any one of claims 32 to 60, wherein $R^{6'}$, $R^{7'}$, $R^{9'}$, $R^{10'}$, $R^{11'}$ and Y' are the same as $R^6$, $R^7$, $R^9$, $R^{10}$, $R^{11}$ and Y respectively.

63 A Conjugate having formula III:

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

wherein L is a Ligand unit,

LU is a Linker unit,

p is 1 to 20; and

D is a Drug unit comprising a PBD dimer according to any one of claims 1-29, wherein LU is connected to D via the X substituent of $R^2$.

64. The Conjugate according to claim 63, wherein the Linker unit (LU) has the formula IIIa or IIIb:

$$-A^1{}_a-L^1{}_s-L^2{}_y-, \qquad (IIIa)$$

wherein:

$-A^1-$ is a Stretcher unit,

a is 1 or 2,

$L^1$ - is a Specificity unit,

s is an integer ranging from 0 to 12,

$-L^2-$ is a Spacer unit, and

y is 0, 1 or 2, and

p is from 1- 20; or

$$L - (A^1{}_a- L^2{}_y --D)_p \qquad (IIIb)$$

wherein:

-$A^1$- is a Stretcher unit linked to a Stretcher unit ($L^2$),

a is 1 or 2,

$L^1$ - is a Specificity unit linked to a Stretcher unit ($L^2$),

s is an integer ranging from 1 to 12,

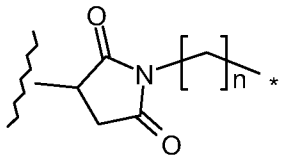
-$L^2$- is a Spacer unit,

y is 1 or 2, and

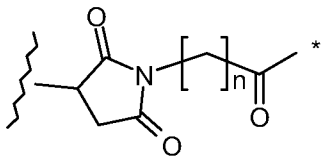
p is from 1 to 20.

65. The Conjugate of claim 64, wherein the Linker unit (LU) has formula IIIa.

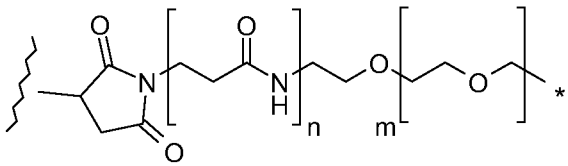
66. The Conjugate of claim 65, wherein $A^1$ is selected from:



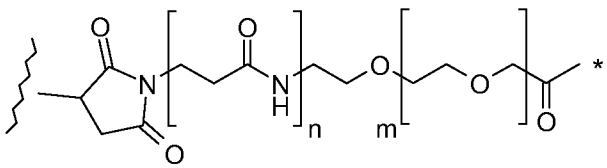
where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6;



where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6;

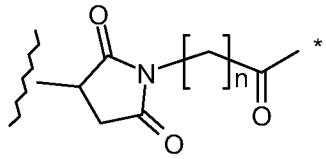


where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30; or



where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30.

67. The Conjugate of claim 65, wherein $A^1$ is:



where the asterisk indicates the point of attachment to $L^1$, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6.

68. The Conjugate of claim 67, wherein n is 5.

69. The Conjugate of any of claims 64-68, wherein $L^1$ comprises an amino acid sequence.

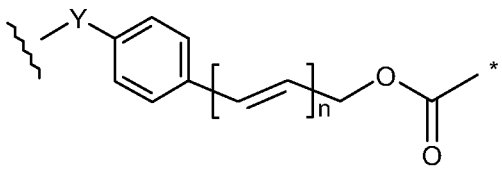
70. The Conjugate of claim 69, wherein $L^1$ is a dipeptide.

71. The Conjugate of claim 70, wherein $L^1$ is selected from the group consisting of valine-alanine, valine-citrulline and phenyalanine-lysine.

72. The Conjugate of any of claims 64-71, wherein y is 0.

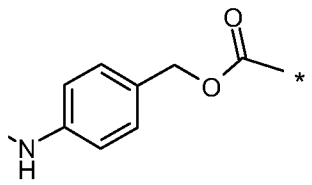
73. The Conjugate of any of claims 64-71, wherein y is 1 or 2.

74. The Conjugate of claim 73, wherein $L^2$ is:



where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to the $L^1$, Y is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-, and n is 0 to 3.

75. The Conjugate of claim 74, wherein $L^2$ is:



.

76. The use of a Conjugate of according to any one of claims 63-75, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease.

77. The use of a Conjugate according to any one of the claims 63-75 for treating a proliferative disease or an autoimmune disease.

78. A method of treating a mammal having a proliferative disease or an autoimmune disease, comprising administering an effective amount of the Conjugate of any one of claims 63-75.

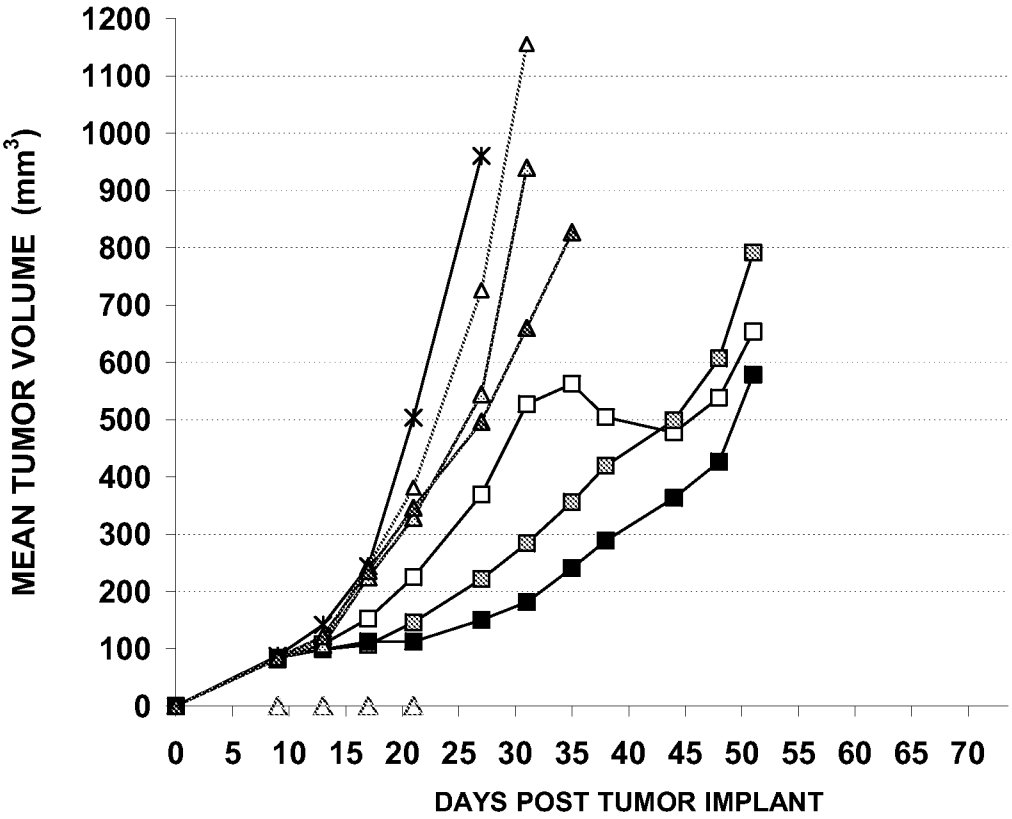

MEAN TUMOR VOLUME (mm3)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
DAYS POST TUMOR IMPLANT

Figure 1

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/032668

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D519/00 A61K31/5517 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | WO 93/18045 A1 (CANCER RES CAMPAIGN TECH [GB]) 16 September 1993 (1993-09-16) claims 1,13 | 1-62 |
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 May 2011 | 26/05/2011 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Gettins, Marc |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/032668

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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| X | WO 2006/111759 A1 (SPIROGEN LTD [GB]; GREGSON STEPHEN JOHN [GB]; HOWARD PHILIP WILSON [GB) 26 October 2006 (2006-10-26) cited in the application claims 1,8,17; example 7 | 1-62 |

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