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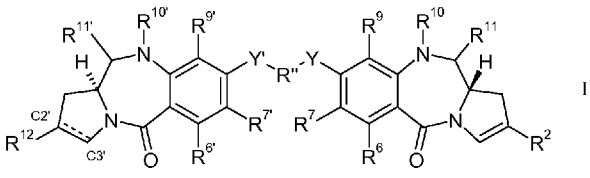
(54) Title: PYRROLOBENZODIAZEPINES AS UNSYMMETRICAL DIMERIC PBD COMPOUNDS FOR INCLUSION IN TARGETED CONJUGATES



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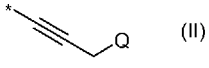


(II)

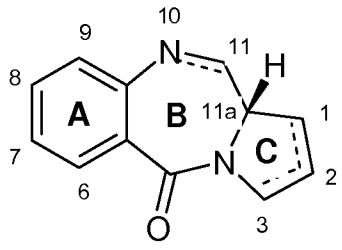
(57) Abstract: A compound with the formula I wherein: R^2 is of formula II where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl, as well as drug-linkers and drug-conjugates made from this compound.

PYRROLOBENZODIAZEPINES AS UNSYMMETRICAL DIMERIC PBD COMPOUNDS FOR INCLUSION IN TARGETED CONJUGATES

The present invention relates to pyrrolobenzodiazepines (PBDs), in particular pyrrolobenzodiazepine dimers having a C2-C3 double bond and a propargyl group at the C2 position on one monomer unit, and their inclusion in targeted conjugates.

Background to the invention

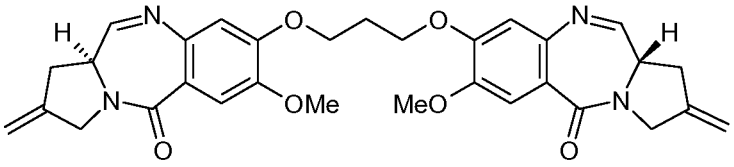
Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al., J. Am. Chem. Soc,* **87,** 5793-5795 (1965); Leimgruber, *et al., J. Am. Chem. Soc,* **87,** 5791-5793 (1965)). Since then, a number of naturally occurring PBDs have been reported, and numerous synthetic routes have been developed to a variety of analogues (Thurston, *et al., Chem. Rev.* **1994,** 433-465 (1994); Antonow, D. and Thurston, D.E., *Chem. Rev.* **2011** 111 (4), 2815-2864). 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, *etal, 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-C1 1 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (S)-configuration at the chiral

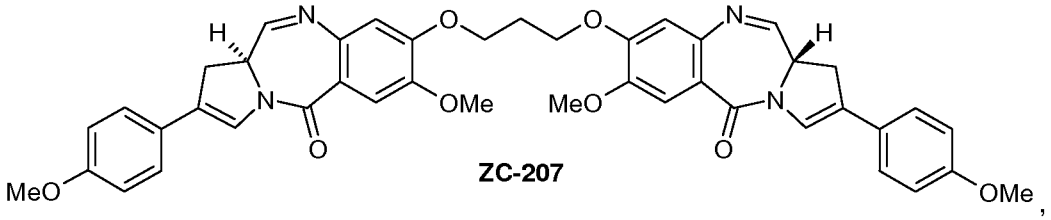
C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III.* Springer-Verlag, 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 ai, Biochemistry,* **44,** 4135-4147) which is thought to be mainly responsible for their biological activity. One example of a PBD dimmer, SG2000 (SJG-136):



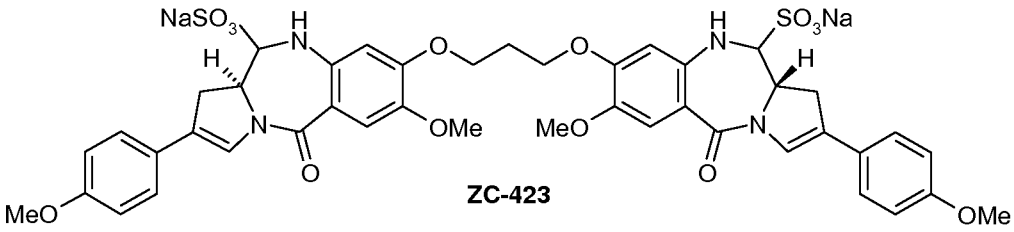
has recently entered Phase II clinical trials in the oncology area (Gregson, S., *et al., J. Med. Chem.,* **44,** 737-748 (2001); Alley, M.C., *et al., Cancer Research,* **64,** 6700-6706 (2004); Hartley, J.A., *et al., Cancer Research,* **64,** 6693-6699 (2004)).

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), 19 (22), 6463-6466, doi: 10.1016/j.bmcl.2009.09.012).

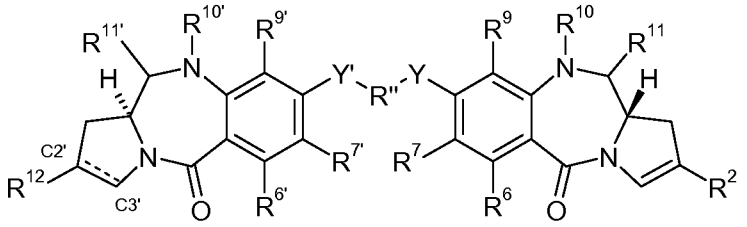
Due to the manner in which these highly potent compounds act in cross-linking DNA, these molecules have been made symmetrically. This provides for straightforward synthesis, either by constructing the PBD moieties simultaneously having already formed the dimer linkage, or by reacting already constructed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

WO 2010/043880 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. Co-pending International application PCT/US201 1/032664, filed 15 April 201 1, published as WO 201 1/130613, discloses the inclusion of these compounds in targeted conjugates. Co-pending International application PCT/US201 1/032668, filed 15 April 201 1, published as WO 201 1/130616, discloses unsymmetrical dimeric PBD compound bearing an aryl group in the C2 position of one monomer bearing a substituent designed to provide an anchor for linking the compound to another moiety, the other monomer bearing a non-aromatic group in the C2 position. The inclusion of these compounds in targeted conjugates is also disclosed.

Disclosure of the invention

The present inventors have developed further unsymmetrical dimeric PBD compounds for inclusion in targeted conjugates, eliminating the need for an aryl group bearing an anchor. The C2 group bearing a substituent designed to provide an anchor for linking the compound to another moiety in the present invention is a propargyl group.

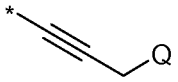
The present invention comprises a compound with the formula I:



I

wherein :

R^2 is of formula II:



(II)

,

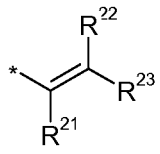
where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl;

when there is a double bond present between C2' and C3', R^{12} is selected from :

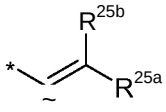
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C1-7 alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

(ib) C1-5 saturated aliphatic alkyl;

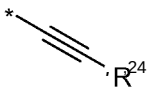
(ic) C3-6 saturated cycloalkyl;



(id) , wherein each of R^{21}, R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C2-3 alkenyl, C2-3 alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5;

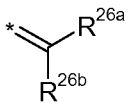


(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



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

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

R^{12} is  , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group

selected from C1-4 alkyl amido and C1-4 alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

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

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C3-20 heterocyclyl and C5-20 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 C3-12 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;

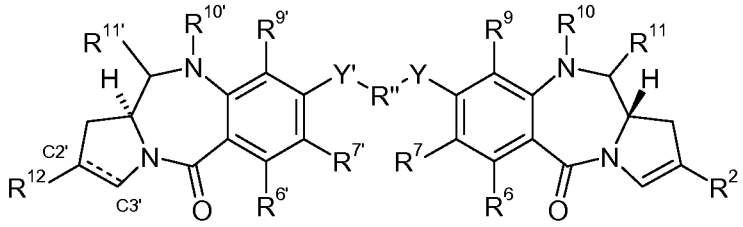
Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ 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.

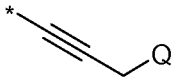
A third aspect of the present invention comprises a compound of formula II:



II

wherein :

R^2 is of formula II:



(II)

,

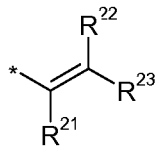
where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl;

when there is a double bond present between C2' and C3', R^{12} is selected from :

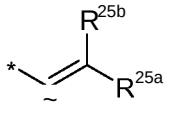
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C1-7 alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

(ib) C1-5 saturated aliphatic alkyl;

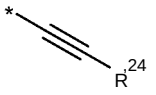
(ic) C3-6 saturated cycloalkyl;



(id) , wherein each of R^{21}, R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C2-3 alkenyl, C2-3 alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5;

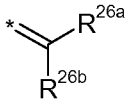


(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;and



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

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



R^{12} is , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group

selected from C1-4 alkyl amido and C1-4 alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

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

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

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

either:

(a) R^{10} is carbamate nitrogen protecting group, and R^{11} is 0-Prot°, wherein Prot° 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 the PBD is a dimer of formula I (supra).

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

L - (LU-D)p (III)

wherein L is a Ligand unit (i.e., a targeting agent), LU is a Linker unit and D is a Drug unit that is a PBD dimer (see below). 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 Q is selected from the group comprising: O, S, NR^N, wherein R^N is selected from the group comprising H and C_{1-2} alkyl.

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-i-$_{12}$ 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 (Ci), 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 (Ci), 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).

C2-12 alkynyl: The term "C2-12 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).

C3-12 cycloalkyl: The term "C3-12 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 "C3-20 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. C3-20, 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-1 H-indene) (C_9), indene (C_9), isoindene (C_9), tetraline (1,2,3,4-tetrahydronaphthalene (C10), acenaphthene (C12), fluorene (C13), phenalene (C13), acephenanthrene (C15), and aceanthrene (Ci_6).

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);

NiOi : 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 (O1), isobenzofuran (O1), indole (N^, isoindole (N^, indolizine (N^, indoline (N^, isoindoline (N_1), purine (N_4) (e.g., adenine, guanine), benzimidazole (N_2), indazole (N_2), benzoxazole (NiOi), benzisoxazole (NiOi),

benzodioxole (O_2), benzofurazan (N_2Oi), benzotriazole (N_3), benzothiofuran (Si), benzothiazole (N_1S_1), benzothiadiazole (N_2S);

Cio (with 2 fused rings) derived from chromene (Oi), isochromene (Oi), chroman (Oi), isochroman (Oi), benzodioxan (O_2), quinoline (Ni), isoquinoline (Ni), quinolizine (Ni), benzoxazine (NiOi), 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-i), dibenzofuran (O-i), dibenzothiophene (S^, carboline (N_2), perimidine (N_2), pyridoindole (N_2); and,

C_{14} (with 3 fused rings) derived from acridine (N-i), xanthene (O-i), thioxanthene (S-i), oxanthrene (O_2), phenoxathiin (O-iS-i), phenazine (N_2), phenoxazine (N_1O_1), phenothiazine (N-IS-I), thianthrene (S_2), phenanthridine (N-i), 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_{3-2}oheterocyclyl group (also referred to as a C_{3-2}oheterocyclyloxy group), or a C_{5-2}oaryl group (also referred to as a C_{5-2}oaryloxy 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_{5-2}oaryl group, preferably a Ci_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): =0.

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(=0)H.

Acyl (keto): -C(=0)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 Ci-7 alkyl group. Examples of acyl groups include, but are not limited to, -C(=0)CH $_3$

(acetyl), -C(=0)CH $_2$CH$_3$ (propionyl), -C(=0)C(CH $_3$)$_3$ (t-butyryl), and -C(=0)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -C(=0)OH .

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

Thiolocarboxy (thiolocarboxylic acid): -C(=0)SH .

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

Imidic acid: -C(=NH)OH .

Hydroxamic acid: -C(=NOH)OH .

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=0)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C3-20 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(=0)OCH $_3$, -C(=0)OCH $_2$CH$_3$, -C(=0)OC(CH $_3$)$_3$, and -C(=0)OPh.

Acyloxy (reverse ester): -OC(=0)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(=0)CH $_3$ (acetoxy), -OC(=0)CH $_2$CH$_3$, -OC(=0)C(CH $_3$)$_3$, -OC(=0)Ph, and -OC(=0)CH $_2$Ph.

Oxycarboyloxy: -OC(=0)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(=0)OCH $_3$, -OC(=0)OCH $_2$CH$_3$, -OC(=0)OC(CH $_3$)$_3$, and -OC(=0)OPh .

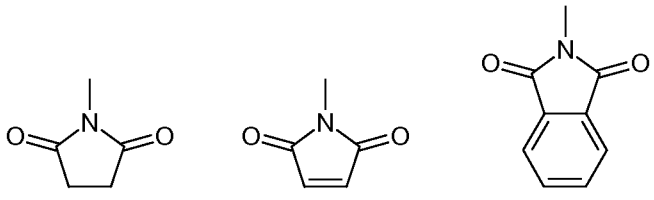
Amino: -NR^{1}R^2, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case 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, -NHCH3, -$NHC(CH_3)_2$, -$N(CH_3)_2$, -$N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

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

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

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



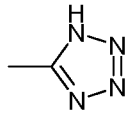
succinimidyl maleimidyl phthalimidyl

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)CON R^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 C1-7 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: -N0$_2$.

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_{3-2}oheterocyclyl group, or a C_{5-2}oaryl 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_{3-2_0} 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(=0)R, wherein R is a sulfine substituent, for example, a C_{1-7} alkyl group, a C_{3-2}oheterocyclyl 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, $-S(=0)CH_3$ and $-S(=0)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=0)_2R$, 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, $-S(=0)_2CH_3$ (methanesulfonyl, mesyl), $-S(=0)_2CF_3$ (triflyl), $-S(=0)_2CH_2CH_3$ (esyl), $-S(=0)_2C_4F_9$ (nonaflyl), $-S(=0)_2CH_2CF_3$ (tresyl), $-S(=0)_2CH_2CH_2NH_2$ (tauryl), $-S(=0)_2Ph$ (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(=0)OH, $-S0_2H$.

Sulfonic acid (sulfo): -S(=0) $_2$OH, -S0 $_3$H.

Sulfinate (sulfinic acid ester): -S(=0)OR; wherein R is a sulfinate substituent, for example, a C_{1-7} alkyl group, a $_{C3-20}$ 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(=0)OCH $_3$ (methoxysulfinyl; methyl sulfinate) and -S(=0)OCH $_2$CH $_3$ (ethoxysulfinyl; ethyl sulfinate).

Sulfonate (sulfonic acid ester): -S(=0) $_2$OR, 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(=0) $_2$OCH $_3$ (methoxysulfonyl; methyl sulfonate) and -S(=0) $_2$OCH $_2$CH $_3$ (ethoxysulfonyl; ethyl sulfonate).

Sulfinyloxy: -OS(=0)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(=0)CH $_3$ and -OS(=0)CH $_2$CH $_3$.

Sulfonyloxy: -OS(=0) $_2$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(=0) $_2$CH $_3$ (mesylate) and -OS(=0) $_2$CH $_2$CH $_3$ (esylate).

Sulfate: -OS(=0) $_2$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(=0) $_2$OCH $_3$ and -SO(=0) $_2$OCH $_2$CH $_3$.

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): -S(=0)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(=0)NH $_2$, -S(=0)NH(CH $_3$), -S(=0)N(CH $_3$)$_2$, -S(=0)NH(CH $_2$CH $_3$), -S(=0)N(CH $_2$CH $_3$)$_2$, and -S(=0)NHPh.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): -S(=0) $_2$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(=0) $_2$NH $_2$, -S(=0) $_2$NH(CH $_3$), -S(=0) $_2$N(CH $_3$)$_2$, -S(=0) $_2$NH(CH $_2$CH $_3$), -S(=0) $_2$N(CH $_2$CH $_3$)$_2$, and -S(=0) $_2$NHPh.

Sulfamino: -$NR^1S(=0)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, -$NHS(=0)_2OH$ and -$N(CH_3)S(=0)_2OH$.

Sulfonamino: -$NR^1S(=0)_2R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-2}oaryl group, preferably a C_{1-7} alkyl group. Examples of sulfonamino groups include, but are not limited to, -$NHS(=0)_2CH_3$ and -$N(CH_3)S(=0)_2C_6H_5$.

Sulfinamino: -$NR^1S(=0)R$, 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_{5-2}oaryl group, preferably a C_{1-7} alkyl group. Examples of sulfinamino groups include, but are not limited to, -$NHS(=0)CH_3$ and -$N(CH_3)S(=0)C_6H_5$.

Phosphino (phosphine): -PR_2, wherein R is a phosphino substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-2}oaryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-2}oaryl group. Examples of phosphino groups include, but are not limited to, -PH_2, -$P(CH_3)_2$, -$P(CH_2CH_3)_2$, -$P(t\text{-}Bu)_2$, and -$P(Ph)_2$.

Phospho: -$P(=0)_2$.

Phosphinyl (phosphine oxide): -$P(=0)R_2$, wherein R is a phosphinyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-2}oaryl 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, -$P(=0)(CH_3)_2$, -$P(=0)(CH_2CH_3)_2$, -$P(=0)(t\text{-}Bu)_2$, and -$P(=0)(Ph)_2$.

Phosphonic acid (phosphono): -$P(=0)(OH)_2$.

Phosphonate (phosphono ester): -$P(=0)(OR)_2$, where R is a phosphonate substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-2}oaryl group, preferably -H, a Ci-7 alkyl group, or a C_{5-2}oaryl group. Examples of phosphonate groups include, but are not limited to, -$P(=0)(OCH_3)_2$, -$P(=0)(OCH_2CH_3)_2$, -$P(=0)(0\text{-}t\text{-}Bu)_2$, and -$P(=0)(OPh)_2$.

Phosphoric acid (phosphonooxy): -$OP(=0)(OH)_2$.

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$_2$CH$_3$)$_2$, -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$_2$CH$_3$)$_2$, -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$_2$CH$_3$)-N(CH$_3$)$_2$, -OP(OCH$_2$CH$_3$)-N(i-Pr)$_2$, and -OP(OCH$_2$CH$_2$CN)-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$_2$CH$_3$)-N(CH$_3$)$_2$, -OP(=O)(OCH$_2$CH$_3$)-N(i-Pr)$_2$, and -OP(=O)(OCH$_2$CH$_2$CN)-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, **-CH2CH2CH2-** (propylene), **-CH2CH2CH2CH2-** (butylene), **-CH2CH2CH2CH2CH2-** (pentylene) and **-CH2CH2CH2CH-2CH2CH2CH2-** (heptylene).

Examples of branched saturated **C_{3-12}** alkylene groups include, but are not limited to, $-CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH(CH_3)CH_2CH_2$**CH2-,** $-CH_2CH(CH_3)CH_2-$, $-CH_2CH(CH_3)CH_2$**CH2-,** $-CH(CH_2CH_3)-$, $-CH(CH_2CH_3)CH_2-$, and $-CH_2CH(CH_2CH_3)CH_2-$.

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

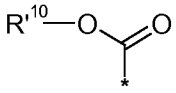
Examples of branched partially unsaturated **C_{3-12}** alkylene groups (**C_{3-12}** alkenylene and alkynylene groups) include, but are not limited to, $-C(CH_3)=CH-$, $-C(CH_3)=CH-CH_2-$, $-CH=CH-CH(CH_3)-$ and $-C\equiv C-CH(CH_3)-$.

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

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

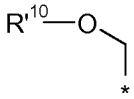
Oxygen protecting group: the term "oxygen protecting group" refers to a moiety which masks a hydroxy group, and these are well known in the art. A large number of suitable groups are described on pages 23 to 200 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John 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,

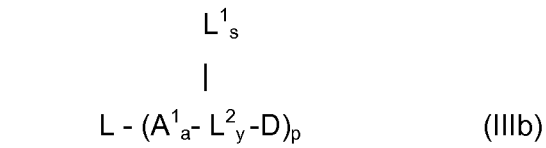
-L^2- is a Spacer unit,

y is 0, 1 or 2;

-D is a PBD dimer; and

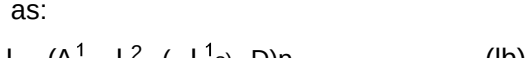
p is from 1 to 20.

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



(IIIb)

Also illustrated as:



(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

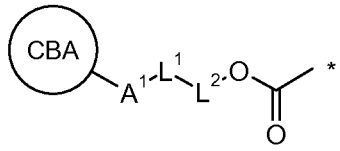
The following preferences may apply to all aspects of the invention as described above, or may relate to a single aspect. The preferences may be combined together in any combination.

In one embodiment, the Conjugate has the formula:

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

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(=0)- forms a self-immolative group, and L^2 optional.

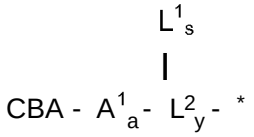
In another embodiment, the Ligand unit (L) is a Cell Binding Agent (CBA) that specifically binds to a target molecule on the surface of a target cell. An exemplary formula is illustrated below:

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

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:



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(=0)0- forms a self-immolative group or self-immolative groups. In some embodiments, -C(=0)0- 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(=0)NH-,
-C(=0)0- ,
-NHC(=0)-,
-OC(=0)-,
-OC(=0)0- ,
-NHC(=0)0- ,
-OC(=0)NH-,

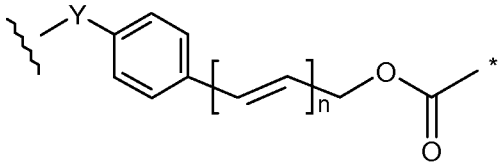
-NHC(=0)NH, and
-O- (a glycosidic bond).

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(=0)O- and L^2 together form the group:

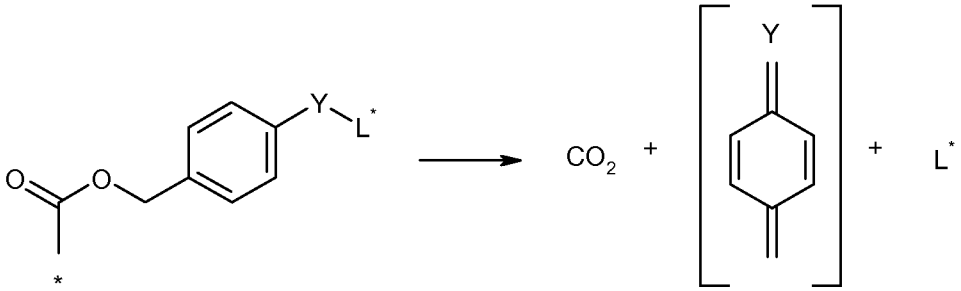


where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to the L^1, Y is -N(H)-, -0-, -C(=0)N(H)- or -C(=0)0-, and n is 0 to 3. The phenylene ring is optionally substituted with one, two or three substituents as described herein.

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

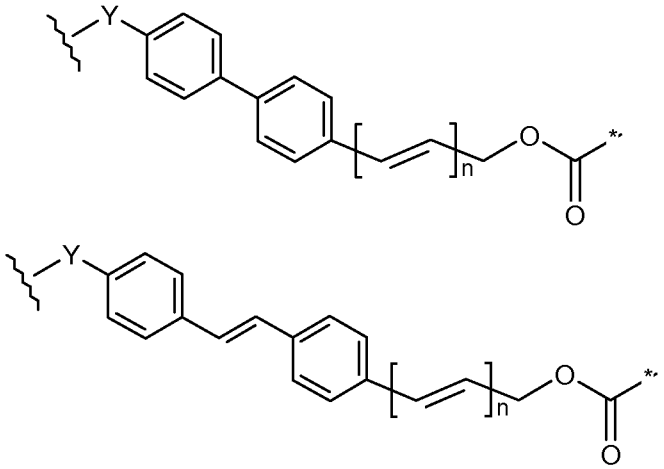
Where Y is NH and n is 0, the self-immolative group may be referred to as a p-aminobenzylcarbonyl linker (PABC).

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):



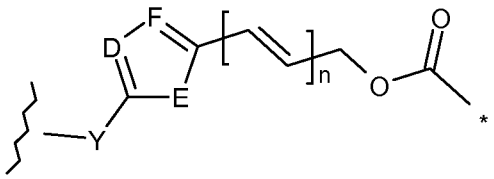
where the asterisk indicates the attachment to the Drug, L^* is the activated form of the remaining portion of the linker and the released Drug unit is not shown. These groups have the advantage of separating the site of activation from the Drug.

In another embodiment, -C(=0)0- 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(=0)0- 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 **-XrX$_2$-** in dipeptide, -NH**-XrX**$_2$-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 **Xi,** and CO is the carbonyl group of **X$_2$.**

Preferably, the group **-X$_1$-X$_2$-** in dipeptide, -NH**-XrX**^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**-XrX**^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;
Gin: Trt, Xan;
His: Boc, Dnp, Tos, Trt;
Lys: Boc, Z-CI, Fmoc, Z;
Ser: Bzl, TBDMS, TBDPS;
Thr: Bz;
Trp: Boc;
Tyr: Bzl, Z, Z-Br.

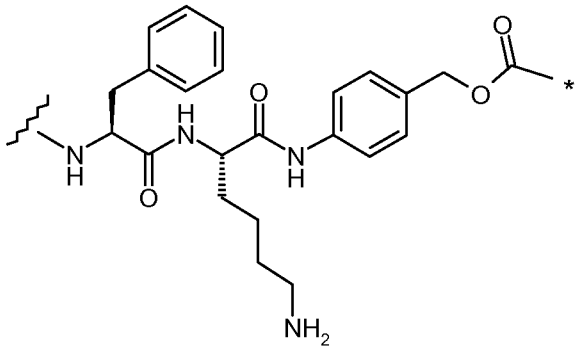
In one embodiment, $-X_2-$ is connected indirectly to the Drug unit. In such an embodiment, the Spacer unit L^2 is present.

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.

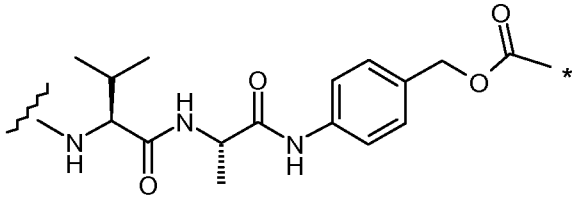
-Xr is connected directly to A^1. In one embodiment, $-X_1-$ is connected directly to A^1. Preferably the group NH-X-r (the amino terminus of X_1) is connected to A^1. A^1 may comprise the functionality -CO- thereby to form an amide link with $-X_1-$.

In one embodiment, L^1 and L^2 together with -OC(=0)- 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:



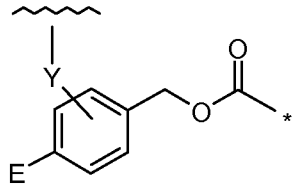
where the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of L^1 or the point of attachment to A^1. Preferably, the wavy line indicates the point of attachment to A^1.

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

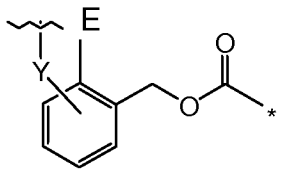


where the asterisk and the wavy line are as defined above.

In another embodiment, L^1 and L^2 together with -OC(=0)- 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.

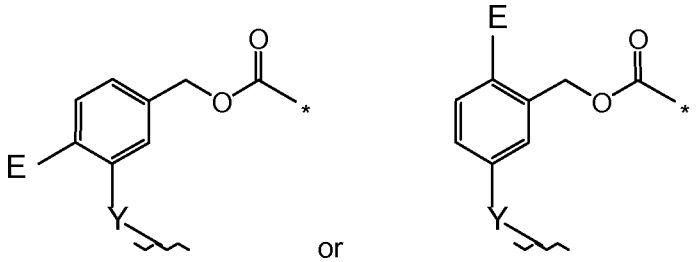
The group Y may be a functional group selected from:

-C(=0)-

-NH-
-O-
-C(=0)NH-,
-C(=0)0-,
-NHC(=0)-,
-OC(=0)-,
-OC(=0)0-,
-NHC(=0)0-,
-OC(=0)NH-,
-NHC(=0)NH-,
-NHC(=0)NH,
-C(=0)NHC(=0)-,
$S0_2$, and
-S-.

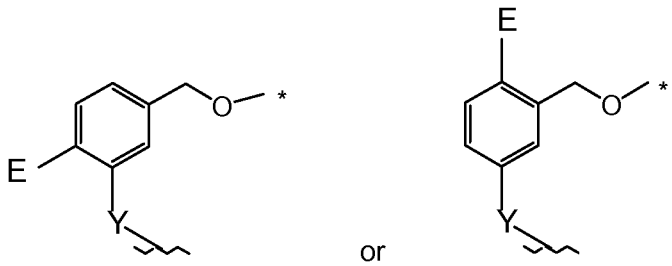
The group Y is preferably -NH-, $-CH_2-$, -0-, and -S-.

In some embodiments, L^1 and L^2 together with -OC(=0)- 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-, $-CH_2-$, -0-, and -S-.

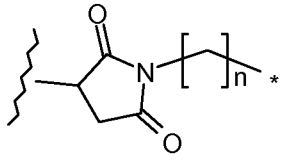
In some further embodiments, Y is a functional group as set forth above, the functional group is linked to an amino acid, and the amino acid is linked to the Stretcher unit A^1. In some embodiments, amino acid is β-alanine. In such an embodiment, the amino acid is equivalently considered part of the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

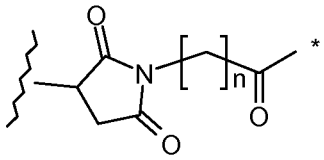
-C(=0)NH-,
-C(=0)0- ,
-NHC(=0)-,
-OC(=0)-,
-OC(=0)0- ,
-NHC(=0)0- ,
-OC(=0)NH-, and
-NHC(=0)NH-.

In one embodiment, the group A^1 is:



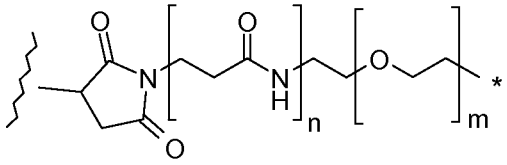
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



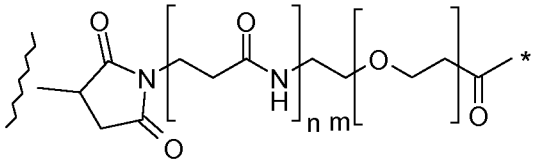
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



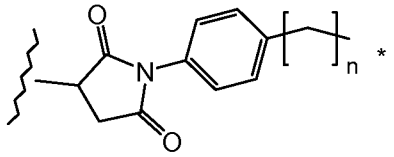
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group A^1 is:



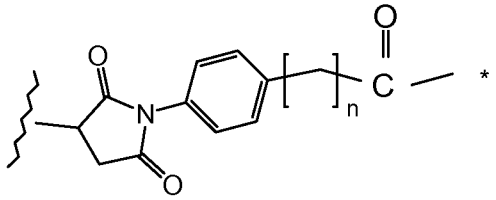
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group A^1 is:



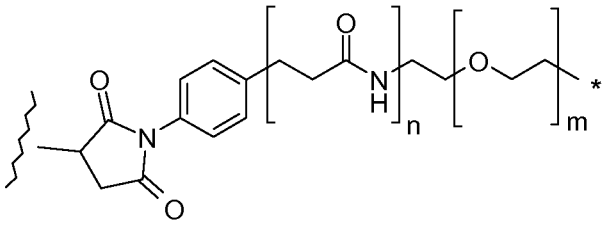
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



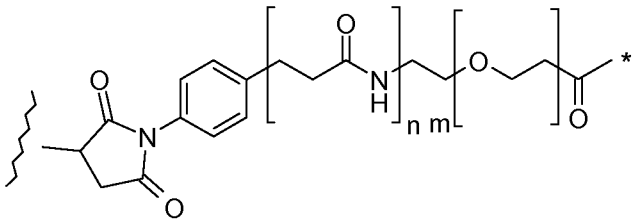
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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.

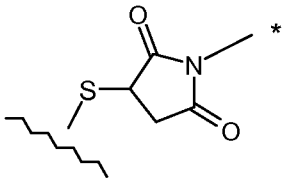
In one embodiment, the group A^1 is:



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.

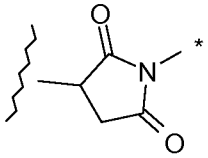
In one embodiment, the connection between the Ligand unit and A^1 is through a thiol residue of the Ligand unit and a maleimide group of A^1.

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



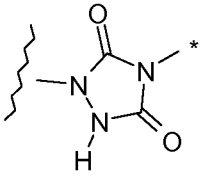
where the asterisk indicates the point of attachment to the remaining portion of A^1, L^1, L^2 or D, and the wavy line indicates the point of attachment to the remaining portion of the Ligand unit. In this embodiment, the S atom is typically derived from the Ligand unit.

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:

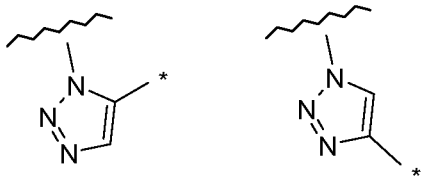


where the wavy line indicates point of attachment to the Ligand unit, and the asterisk indicates the bond to the remaining portion of the A^1 group , or to L^1, L^2 or D.

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(=0)NH-,
-C(=0)0-,
-NHC(=0)-,
-OC(=0)-,
-OC(=0)0-,
-NHC(=0)0-,
-OC(=0)NH-,
-NHC(=0)NH-,
-NHC(=0)NH,
-C(=0)NHC(=0)-,
-S-,
-S-S-,
$-CH_2C(=0)-$
$-C(=0)CH_2-$,
=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(=0)NH-,
-C(=0)0- ,
-NHC(=0)-,
-OC(=0)-,
-OC(=0)0- ,
-NHC(=0)0- ,
-OC(=0)NH-, and
-NHC(=0)NH-.

In one embodiment, L^1 and D are preferably connected by a bond selected from:
-C(=0)NH-, and
-NHC(=0)-.

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 **-Xi-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 **Xi,** 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:

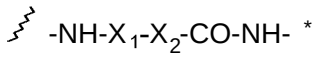
-Gly-Gly-,

-Pro-Pro-, and

-Val-Glu-.

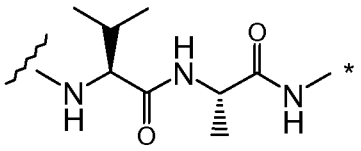
Other dipeptide combinations may be used, including those described above.

In one embodiment, L^1-D is:



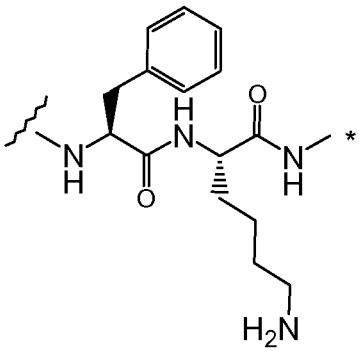
where $-NH-X_1-X_2-CO$ is the dipeptide, -NH- is part of the Drug unit, the asterisk indicates the point of attachment to the remainder of the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of L^1 or the point of attachment to A^1. Preferably, the wavy line indicates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

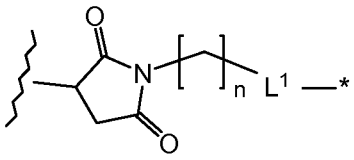
In one embodiment, the dipeptide is phenylalnine-lysine and L^1-D is:



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

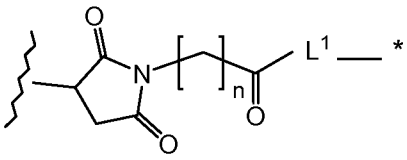
In one embodiment, the dipeptide is valine-citrulline.

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



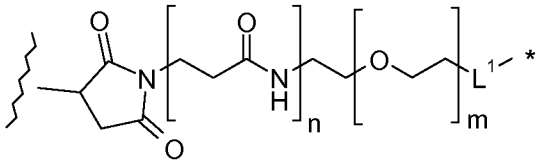
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



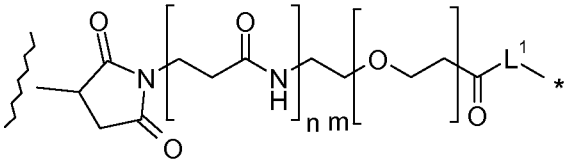
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



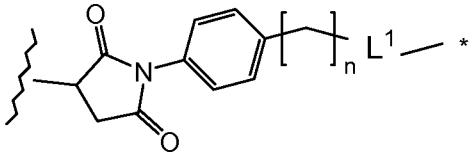
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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



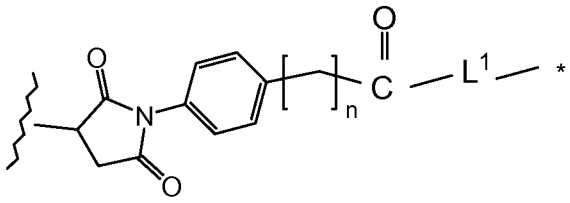
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 3 to 7, most preferably 3 or 7.

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



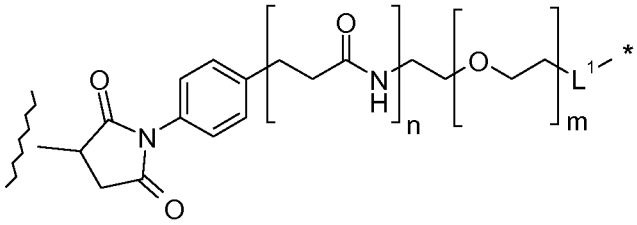
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



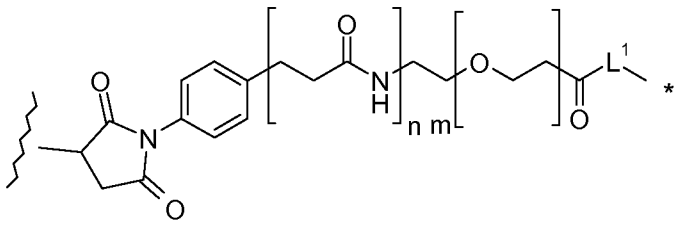
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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.

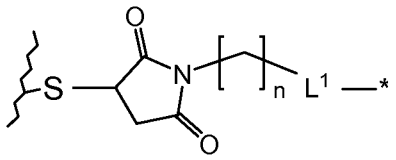
In one embodiment, the groups A^1-L^1 is:



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

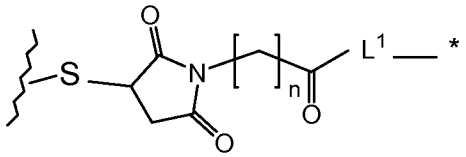
preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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



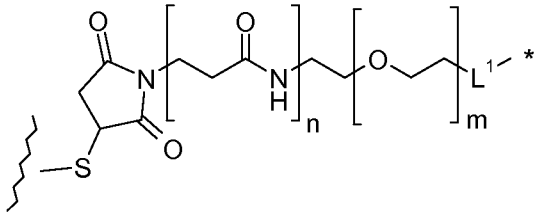
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the rest of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



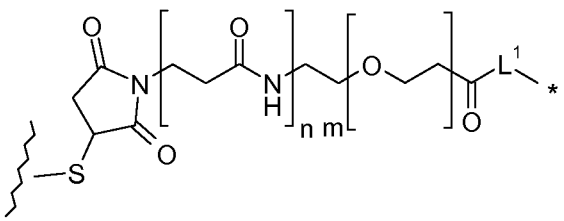
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



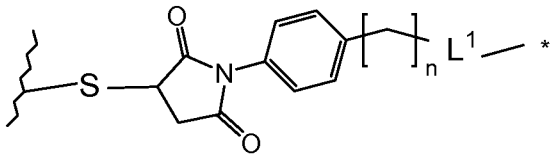
where the asterisk indicates the point of attachment to D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably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

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



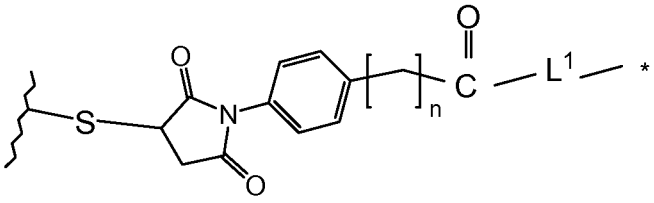
where the asterisk indicates the point of attachment to D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 4 to 8, most preferably 4 or 8.

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



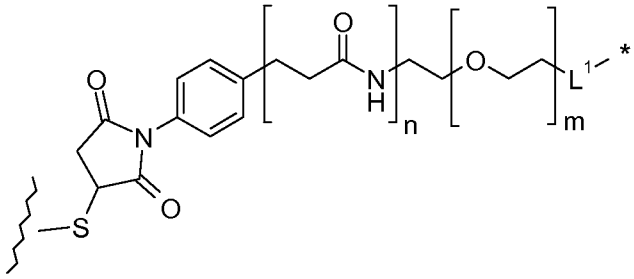
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



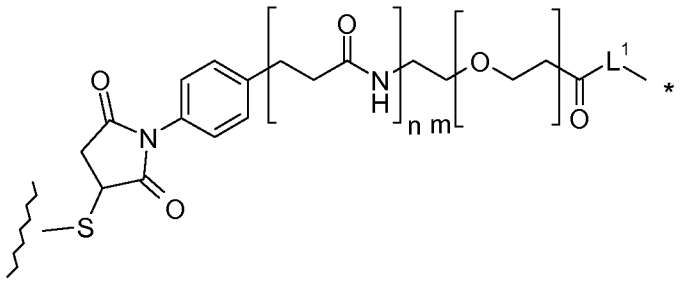
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



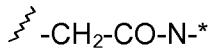
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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:

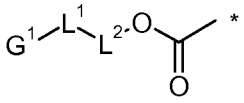


where the asterisk indicates the point of attachment to the remainder of the Stretcher unit, L^1 or D, and the wavy line indicates the point of attachment to the Ligand unit.

Linker-Drugs

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 (D, as defined above), 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(=0)- forms a self-immolative group(s).

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

$$G^1\text{-}L^1\text{-}L^2\text{-}.$$

where the asterisk indicates the point of attachment to the Drug unit (D), 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.

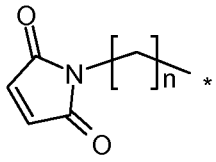
In certain embodiments of the invention, the group L^1 includes a Lys amino acid residue. The side chain of this amino acid may be protected with a Boc or Alloc protected group. A Boc protecting group is most preferred.

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.

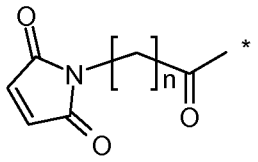
In one embodiment, the group G^1 is an alkyl maleimide group. This group is suitable for reaction with thiol groups, particularly cysteine thiol groups, present in the cell binding agent, for example present in an antibody.

In one embodiment, the group G^1 is:



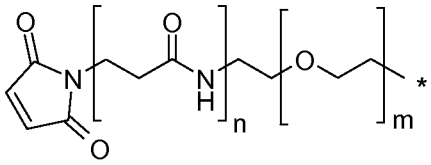
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



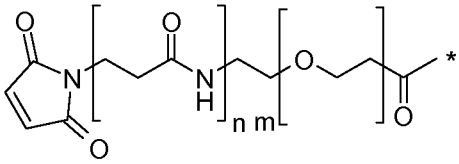
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



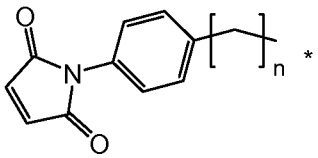
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group G^1 is:



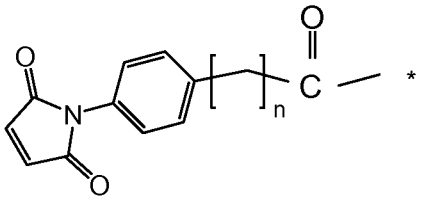
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group G^1 is:



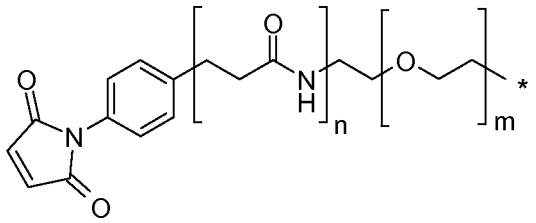
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



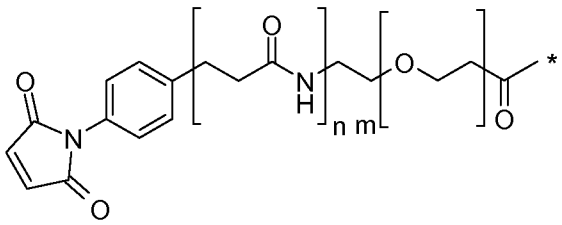
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



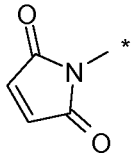
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group G^1 is:



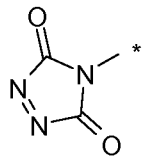
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8.

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 convertible from one functional group to another. In one embodiment, L^1 is present and G^1 is -NH_2. This group is convertible 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.

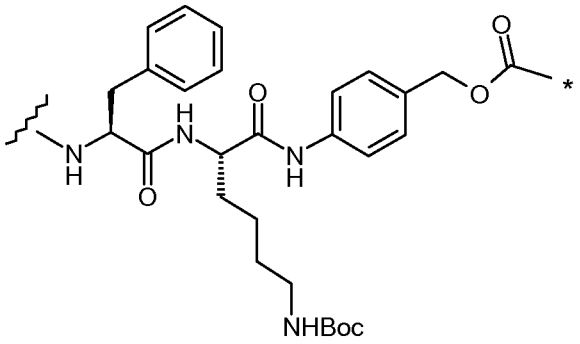
In other embodiments, the Linker unit is a precursor to the Linker uit having an active group. In this embodiment, the Linker unit comprises the active group, which is protected by way of a protecting group. The protecting group may be removed to provide the Linker unit having an active group.

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.

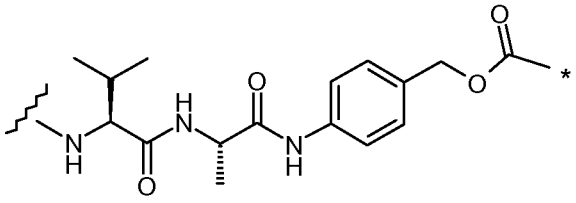
In one embodiment, the protecting group is orthogonal to the capping group. Thus, the active group protecting group is removable whilst retaining the capping group. In other embodiments, the protecting group and the capping group is removable under the same conditions as those used to remove the capping 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, Sjogren'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 areata, 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, Sjogren's syndrome, Hashimoto's thyroiditis, Graves' disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease), or Th2-lymphocyt.es (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, *etal., 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.

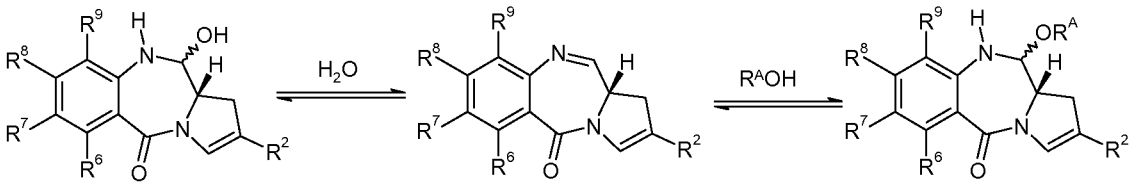
Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, 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 C1-4 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.

Isolation of compounds of formula I

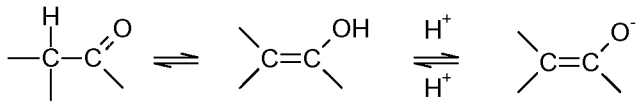
Without wishing to be bound by theory, it has been found that compounds of formula I where R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound, have a tendency to reversibly dimerise on isolation. This reversible dimerisation is thought to occur due to reversible addition of Q on one molecule with the imine bond of another compound. Treatment with methanol, e.g. for 1 hour, can assist with isolation of the monomeric form of compounds of formula I.

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; a- 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.

General synthetic routes

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

a) WO 00/12508 (pages 14 to 30);

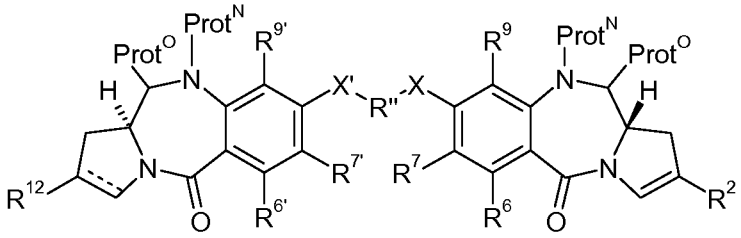
b) WO 2005/023814 (pages 3 to 10);

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

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

Synthesis route

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:

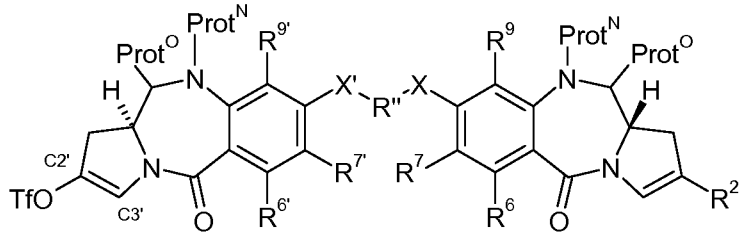


Formula 2

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° 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 Troc and Prot° 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° 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, superhydride or lithium tetraborohydride, whilst a suitable means for removing the SEM protecting group is treatment with silica gel.

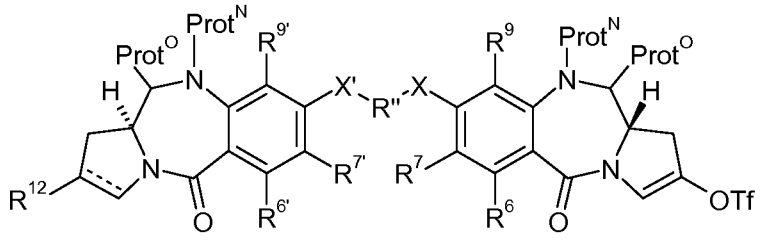
Compounds of formula 2, where there is double bond between C2' and C3', can be synthesised from a compound of formula 3a:



Formula 3a

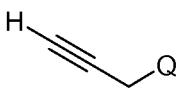
where R^2, R^6, R^7, R^9, $R^{6'}$, $R^{7'}$, $R^{9'}$, X, X' and R" are as defined for compounds of formula 2, by coupling an organometallic derivative comprising R^{12}, such as an organoboron derivative. The organoboron derivative may be a boronate or boronic acid.

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



Formula 3b

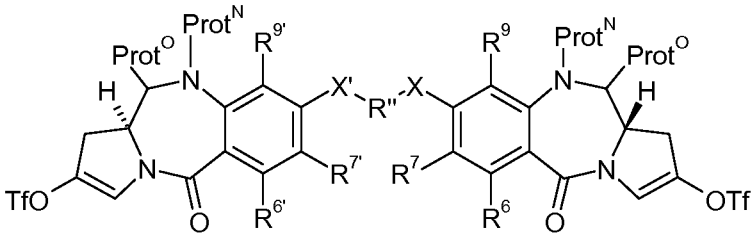
where R^{12}, R^6, R^7, R^9, $R^{6'}$, $R^{7'}$, $R^{9'}$, X, X' and R" are as defined for compounds of formula 2, by coupling an alkyne of formula IIa:



(IIa)

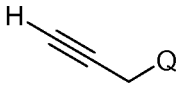
by a Sonogashira coupling.

Compounds of formulae 3a and 3b, where there is double bond between C2' and C3', 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 either 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^{12} to make a compound of formula 3b, or by coupling about a single equivalent (e.g. 0.9 or 1 to 1.1 or 1.2) an alkyne of formula IIa:



(IIa)

by a Sonogashira coupling to make a compound of Formula 3a.

The couplings of an organometallic derivative, such as an organoboron derivative, 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 Sonogashira coupling is carried out using two catalysts: a zerovalent palladium complex and a halide salt of copper(I). Phosphine-palladium complexes such as tetrakis(triphenylphosphine)palladium(0) are used for this reaction, but palladium(II) complexes can also be used because they are reduced to the palladium(O) species by the consumption of the terminal alkynes in the reaction medium. The oxidation of triphenylphosphine to triphenylphosphine oxide can also lead to the formation of Pd(0) in situ when catalysts such as bis(triphenylphosphine)palladium(II) chloride are used. In contrast, copper(I) halides react with the terminal alkyne and produce copper(I) acetylide, which acts as an activated species for the coupling reactions.

The reaction medium must be basic to neutralize the hydrogen halide produced as the byproduct of this coupling reaction, so alkylamine compounds such as triethylamine, diethylamine or piperidine are sometimes used as solvents, but also DMF or ether can be

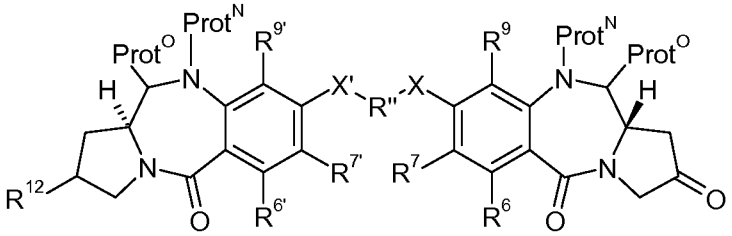
used as solvent. Other bases such as potassium carbonate or cesium carbonate are occasionally used.

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° 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° is a protected oxygen group for synthesis are described in WO 2005/085251, which synthesis is herein incorporated by reference.

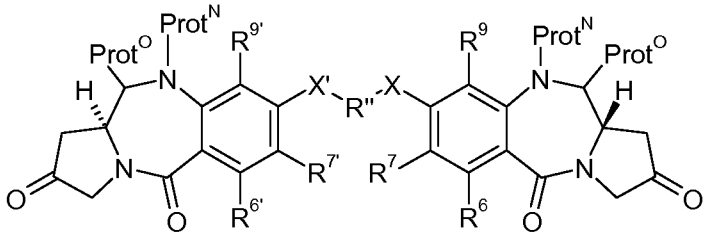
Compounds of Formula 3b, where there is single bond between C2' and C3' can be synthesised from a compound of Formula 5a:



Formula 5a

by an adaption of the methods described in the above references for the synthesis of compounds of formula 4 by triflation of the C2 keto group.

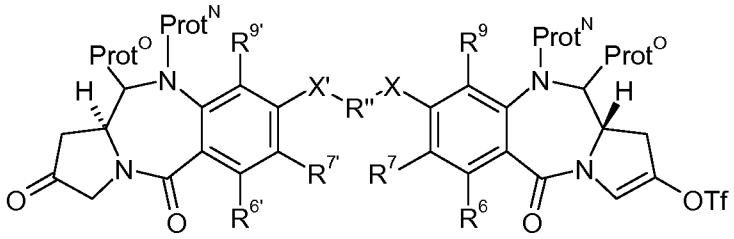
Compounds of Formula 5a may be synthesised from a compound of Formula 6:



Formula 6

by reaction with about a single equivalent (e.g. 0.9 or 1 to 1.1 or 1.2) of an appropriate alkene forming reagent, for example Wittig reagents (such as ylides), Tebbe reagents and Horner-Emmons-Wadworth reagents. Reference is made to the discussion on page 16 of WO 2010/043877, which is incorporated herein by reference.

Compounds of Formula 3b, where there is single bond between C2' and C3' can also be synthesised from a compound of Formula 5b:

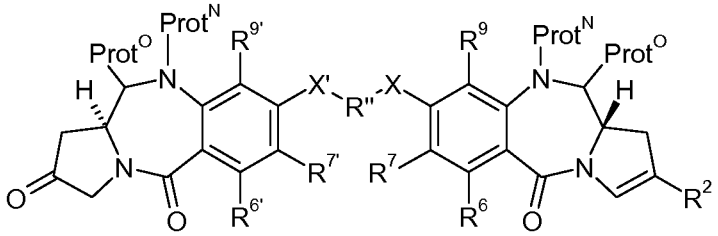


Formula 5b

by reaction with an appropriate alkene forming reagent, as discussed above.

Compounds of Formula 5b can be synthesised from compounds of formula 6 by a selective triflation using a single equivalent (e.g. 0.9 or 1 to 1.1 or 1.2) of the triflating agent.

Compounds of Formula 5b can also be used to synthesise a compound of formula 3c:



Formula 3c

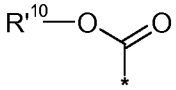
by the Sonogashira coupling an alkyne of formula IIa. These compounds can then be used to synthesise a compound of Formula 2, where there is single bond between C2' and C3', by reaction with an appropriate alkene forming reagent, as discussed above.

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:



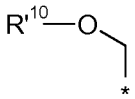
wherein R'^{10} is R as defined above. A large number of suitable groups are described on pages 503 to 549 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

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, which is incorporated herein by reference. Linkers having a maleimidyl group (A) and a peptide group (L^1) can be prepared as described in WO 2009/01 17531 , which is incorporated herein by reference. 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 2009-01 17531 , 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 °C. The progress of the reaction, which reduces interchain disulfides, is monitored by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) and allowed to proceed until the desired level of thiols/mAb is achieved. The reduced antibody is then cooled to 0°C and alkylated with 1.5 equivalents of maleimide drug-linker per antibody thiol. After 1 hour, the reaction is quenched by the addition of 5 equivalents of N-acetyl cysteine. Quenched drug-linker is removed by gel filtration over a PD-10 column. The ADC is then sterile-filtered through a 0.22 μη 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.

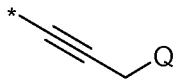
R^6 is preferably selected from H, OH, OR, SH, NH_2, nitro and halo, and is more preferably H or halo, and most preferably is H.

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.

R2

R^2 is of formula II:



(II)

,

where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl.

In some embodiments, it is preferred that Q is NR^N. In other embodiments, Q is OH. In further embodiments, R is SH.

R^N is preferably selected from H and methyl. In some embodiment, R^N is H. In other embodiments, R^N is methyl.

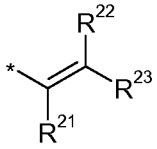
R12

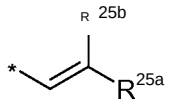
When there is a double bond present between C2' and C3', R^{12} is selected from:

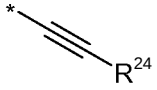
(a) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

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

(d)  , wherein each of R^{21}, R^{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;

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

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

When R^{12} is a C_{5-10} aryl group, it may be a C_{5-7} aryl group. A C_{5-7} aryl group may be a phenyl group or a C_{5-7} heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, R^{12} is preferably phenyl. In other embodiments, R^{12} is preferably thiophenyl, for example, thiophen-2-yl and thiophen-3-yl.

When R^{12} is a C_{5-10} aryl group, it may be a C_{8-10} aryl, for example a quinolinyl or isoquinolinyl group. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. Of these quinolin-3-yl and quinolin-6-yl may be preferred. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. Of these isoquinolin-3-yl and isoquinolin-6-yl may be preferred.

When R^{12} is a C_{5-10} aryl group, it may bear any number of substituent groups. It preferably bears from 1 to 3 substituent groups, with 1 and 2 being more preferred, and singly substituted groups being most preferred. The substituents may be any position.

Where R^{12} is C_{5-7} aryl group, a single substituent is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the C_{5-7} aryl group is phenyl, the substituent is preferably in the meta- or para- positions, and more preferably is in the para-position.

Where R^{12} is a C_{8-10} aryl group, for example quinolinyl or isoquinolinyl, it may bear any number of substituents at any position of the quinoline or isoquinoline rings. In some embodiments, it bears one, two or three substituents, and these may be on either the proximal and distal rings or both (if more than one substituent).

R^{12} substituents, when R^{12} is a C_{5-10} aryl group

If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is halo, it is preferably F or Cl, more preferably Cl.

If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is ether, it may in some embodiments be an alkoxy group, for example, a C_{1-7} alkoxy group (e.g. methoxy, ethoxy) or it may in

some embodiments be a C_{5-7} aryloxy group (e.g phenoxy, pyridyloxy, furanyloxy). The alkoxy group may itself be further substituted, for example by an amino group (e.g. dimethylamino).

If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is C_{1-7} alkyl, it may preferably be a C_{1-4} alkyl group (e.g. methyl, ethyl, propryl, butyl).

If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is C_{3-7} heterocyclyl, it may in some embodiments be C_6 nitrogen containing heterocyclyl group, e.g. morpholino, thiomorpholino, piperidinyl, piperazinyl. These groups may be bound to the rest of the PBD moiety via the nitrogen atom. These groups may be further substituted, for example, by C_{1-4} alkyl groups. If the C_6 nitrogen containing heterocyclyl group is piperazinyl, the said further substituent may be on the second nitrogen ring atom.

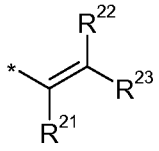
If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is bis-oxy -Ci_{-3} alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

Particularly preferred substituents when R^{12} is a C_{5-10} aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl. Another particularly preferred substituent for R^{12} is dimethylaminopropyloxy.

Particularly preferred substituted R^{12} groups when R^{12} is a C_{5-10} aryl group include, but are not limited to, 4-methoxy-phenyl, 3-methoxyphenyl, 4-ethoxy-phenyl, 3-ethoxy-phenyl, 4-fluoro-phenyl, 4-chloro-phenyl, 3,4-bisoxymethylene-phenyl, 4-methylthiophenyl, 4-cyanophenyl, 4-phenoxyphenyl, quinolin -3-yl and quinolin -6-yl, isoquinolin -3-yl and isoquinolin -6-yl, 2-thienyl, 2-furanyl, methoxynaphthyl, and naphthyl. Another possible substituted R^{12} group is 4-nitrophenyl. R^{12} groups of particular interest include 4-(4-methylpiperazin -1-yl)phenyl and 3,4-bisoxymethylene-phenyl.

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 cycloalkyi, 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, C2-3 alkenyl, C2-3 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, C2-3 alkenyl, C2-3 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.

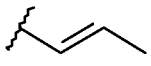
In some embodiments, R^{22} is H.

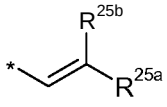
In some embodiments, R^{23} is H.

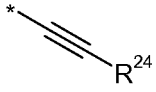
In some embodiments, R^{21} and R^{22} are H.

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

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

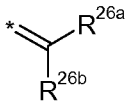
An R^{12} group of particular interest is:  .

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.

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

R^{12} is  , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C1-4 alkyl amido and C1-4 alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester.

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

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

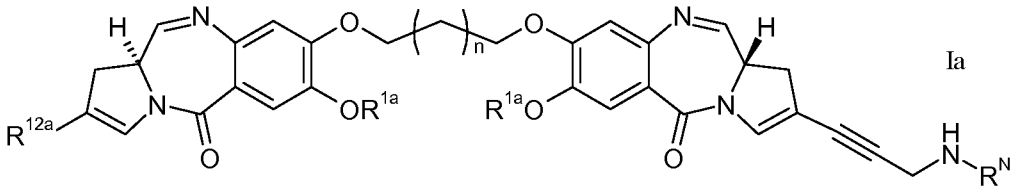
In further embodiments, it is preferred that one of R^{26a} and R^{26b} is H, and the other is selected from C1-4 saturated alkyl, C2-3 alkenyl, which alkyl and alkenyl groups are optionally substituted. In these further embodiment, it may be further preferred that the group which is not H is selected from methyl and ethyl.

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

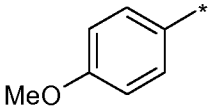
where

n is 1 or 3;

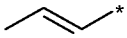
R^{1a} is methyl or phenyl;

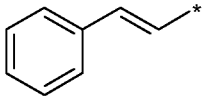
R^N is H, methyl or ethyl;

R^{12a} is selected from:

(a)  ;

(b)  ;

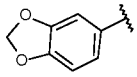
(c)  ;

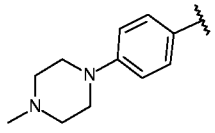
(d)  ;

(e)  ;

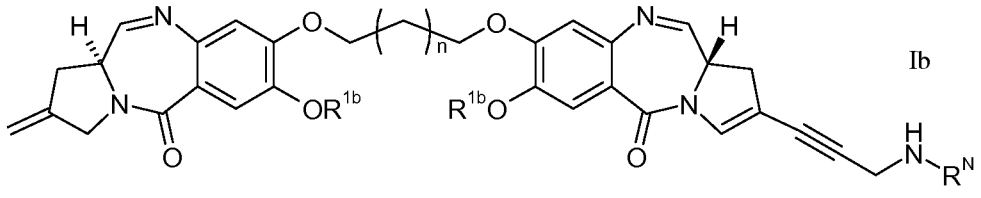
(f)  .

Further group for R^{12a} may be:

(g)  ; and

(h)  .

Other particularly preferred compounds of the present invention are of formula Ib:



Ib

where

n is 1 or 3;

R^{1b} is methyl or phenyl; and

R^N is H, methyl or ethyl.

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 0-Prot°, wherein Prot° is an oxygen protecting group, Prot° 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 for Example 1

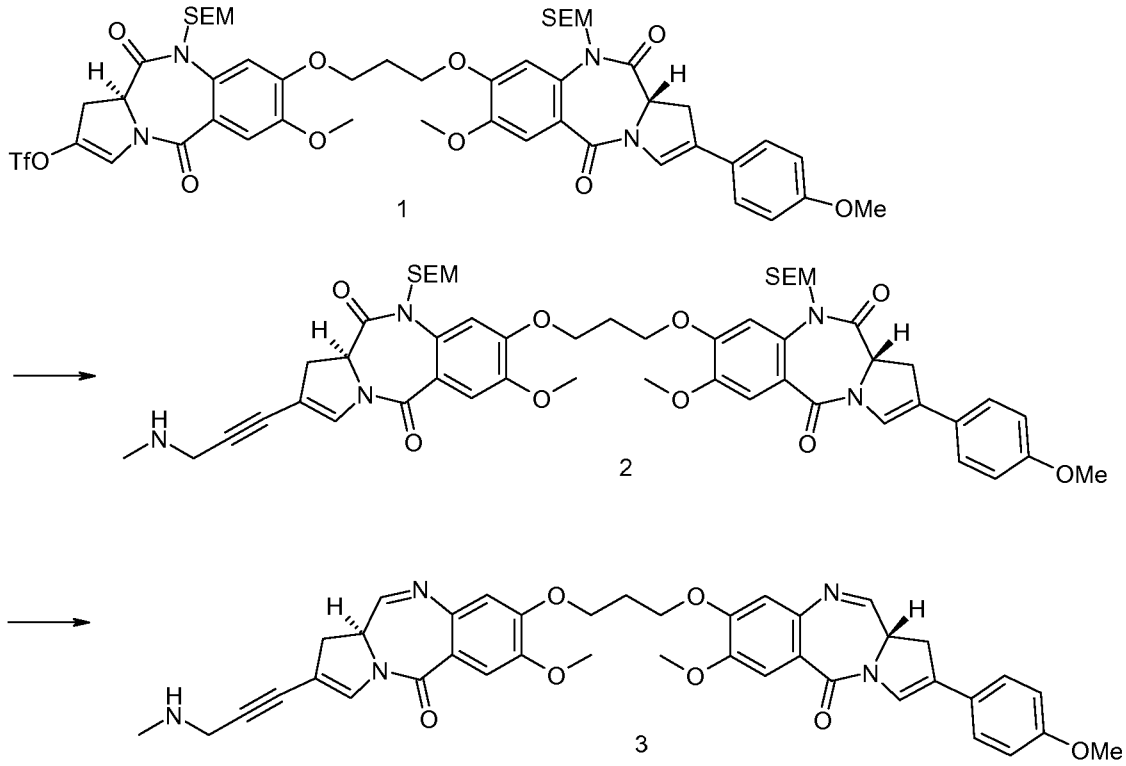
Optical rotations were measured on an ADP 220 polarimeter (Bellingham Stanley Ltd.) and concentrations (c) are given in g/100ml_. Melting points were measured using a digital melting point apparatus (Electrothermal). IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. ^1H and ^{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 (δ = 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, $_{F254)}$, 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 4A molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

Compound 1 was synthesised as described in WO 2010/043880 (Compound 17), 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, 400 µL 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

Example 1



(a) (S)-7-methoxy-8-(3-(((S)-7-methoxy-2-(3-(methylamino)prop-1-yn-^ -yl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1 H-benzo[e]pyrrolo[1 ,2-a][1,4]diazepin-8-yl)oxy)propoxy)-2-(4-methoxyphenyl)-1 0-((2-(trimethylsilyl)ethoxy)methyl)-1 H-benzo[e]pyrrolo[1 ,2-a][1,4]diazepine-5, 11(1 OH, 11aH)-dione ***(2)***

A mixture of **1** (0.433 g, 0.40 mmol), Cul (0.008 g, 0.04 mmol, 0.1 eq), N-methyl-N-(2-propynyl)amine (0.10 ml, 1.20 mmol, 3 eq) and PPh_3 (0.021 g, 0.08 mmol, 0.2 eq) was dissolved in anhydrous piperidine (5 ml.) in the presence of molecular sieves under an argon atmosphere. $Pd(PPh_3)_4$ (0.046 g, 0.04 mmol, 0.1 eq) was added to the mixture, and the reaction was warmed to 60°C and stirred overnight. The solvent was removed by rotary evaporation under reduced pressure and the resulting brown solid purified by flash column chromatography (silica gel, gradient 96% DCM-4% methanol). Compound **2** was obtained as a brown solid (0.213 g, 53%); Rf 0.32 [10% methanol in DCM]; LC-MS (5 min) 3.35 min, ES^+ 995.35.

(b) (S)-2-((Z)-(((E)-4-methoxy-3-(3-(((S)-7-methoxy-2-(4-methoxyphenyl)-5-oxo -5,11a-dihydro-1 H-benzo[e]pyrrolo[1 ,2-a][1,4]diazepin-8-yl)oxy)propoxy)but-2-en-1 -yl)imino)methyl)-4-(3-(methylamino)prop-1 -yn-1 -yl)-2,3-dihydro-1 H-pyrrole-1 -carbaldehyde ***(3)***

Compound **2** (0.041 g, 0.04 mmol) was dissolved in anhydrous THF (3.8 mL) under an argon atmosphere and cooled to -78°C in an acetone-dry ice bath. $LiEt_3BH$ (0.25 mL of a 1M solution in THF, 0.35 mmol, 6 eq) was added to the mixture, and the reaction was stirred at -78°C for 40 minutes. The reaction was quenched with H_20 (3 mL) and after addition of brine (3 mL) the aqueous phase was extracted (DCM, 9mL- methanol 1 mL), dried ($MgS0_4$) and the solvent was removed by rotary evaporation under reduced pressure. The crude was dissolved in a mixture of ethanol (3 mL), DCM (1.5 mL) and H_20 (1.5 mL) and silica gel was added to the mixture. The reaction was stirred for four days at room temperature under an atmosphere of Argon. The mixture was filtered over a sinter funnel washing with a mixture of DMC (140 mL) and methanol (15 mL). The organic phase was washed with brine (200 mL), dried ($MgS0_4$) and the solvent was removed by rotary evaporation under reduced pressure. The resulting brown solid was purified by flash column chromatography (silica gel, gradient 90% DCM-10% methanol). Compound **3** was obtained as a brown solid (0.029 g, 59%); Rf 0.11 [8% methanol in DCM]; LC-MS (5 min) 2.47 min, ES^+ 702.28.

General Experimental Methods for following examples

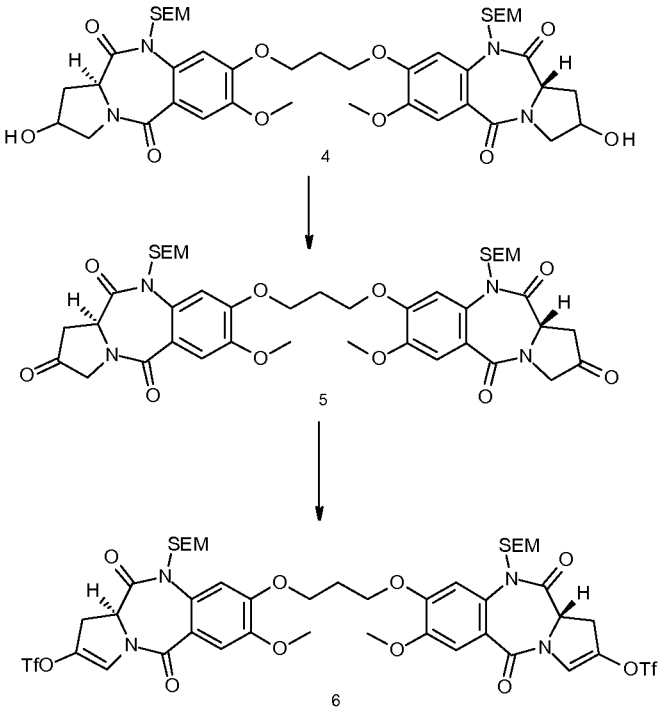
LCMS data were obtained using an Agilent 1200 series LC/MS with an Agilent 6110 quadrupole MS, with Electrospray ionisation. Mobile phase A - 0.1% Acetic acid in water. Mobile Phase B - 0.1% in acetonitrile. Flow rate of 1.00ml/min. Gradient from 5% B rising up to 95% B over 3 minutes, remaining at 95% B for 1 minute and then back down to 5% B over 6 seconds. The total run time is 5 minutes. Column: Phenomenex Gemini-NX 3μη C18, 30 x 2.00mm. Chromatograms based on UV detection at 254nm. Mass Spectra were achieved using the MS in positive mode. Proton NMR chemical shift values were measured on the delta scale at 400 MHz using a Bruker AV400. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are reported in Hz. Unless otherwise stated, column chromatography (by the flash procedure) were performed on Merck Kieselgel silica (Art. 9385). Mass spectroscopy (MS) data were collected using a Waters Micromass LCT instrument coupled to a Waters 2795 HPLC separations module. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F_{254}). All other chemicals and solvents were purchased from Sigma-Aldrich or Fisher Scientific and were used as supplied without further purification.

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 (δ = 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, $_{F254)}$, 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 4A molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

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, 400 µL 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 Intermediate

Compound 4 was synthesised as described in WO 2010/043880 (Compound 6a), which is herein incorporated by reference.



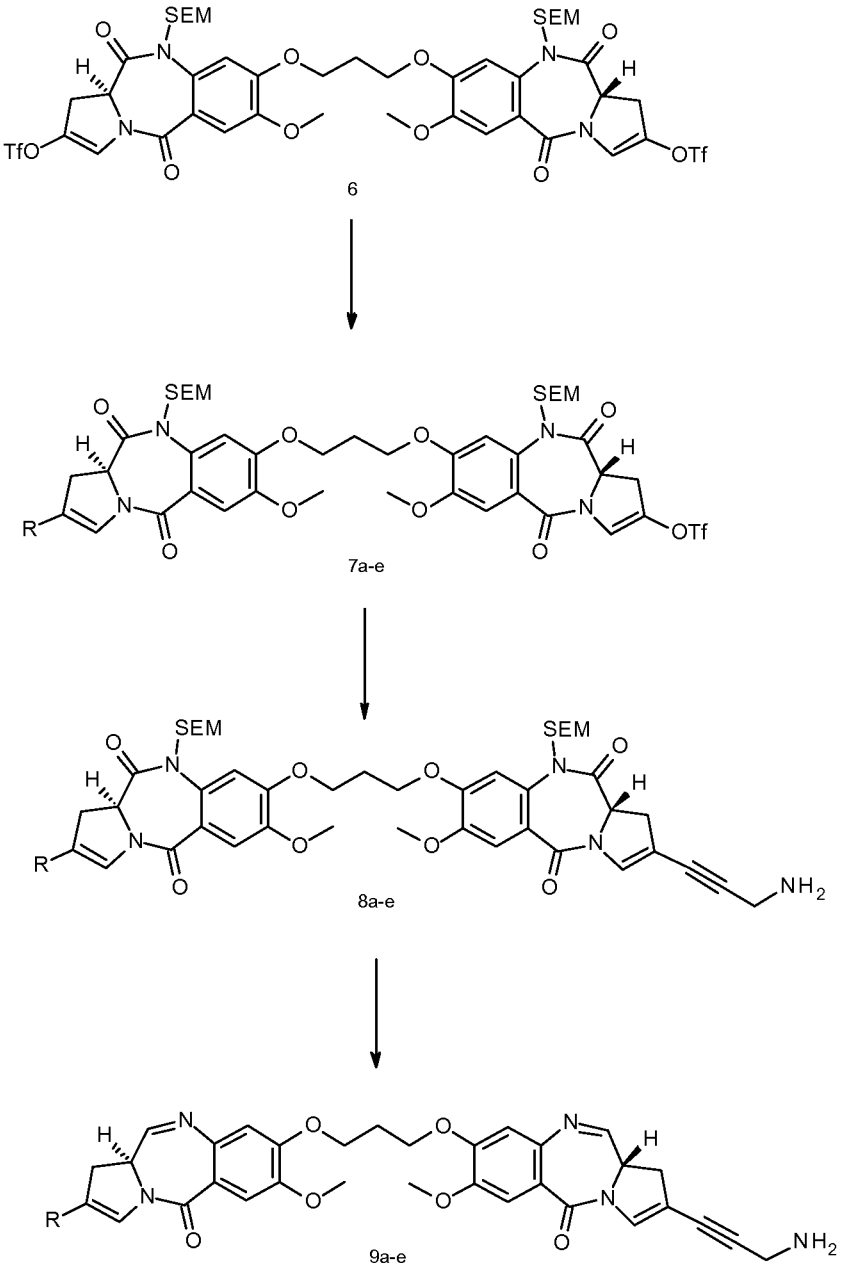
(a) 1, 1'-[[(Propane- 1,3-diyl)dioxy]bis[(1 1aS)- 11-sulpho-7-methoxy-2-oxo-10-((2-(trimethylsilyl)ethoxy)methyl) 1,2,3, 10, 11, 11a-hexahydro-5H-pyrrolo[2, 1-c][1,4]benzodiazepin-5, 11-dione]] (5)

Diol **4** (25.60 g, 29.9 mmol, 1.0 eq.), NaOAc (6.90 g, 84.1 mmol, 2.8 eq.) and TEMPO (188 mg, 1.2 mmol, 0.04 eq.) were dissolved in DCM (326 mL) under nitrogen. This was cooled to -8 °C and TCCA (9.70 g, 41.7 mmol, 1.40 eq.) was added portionwise over 20 min during which the solution turned dark brown which lightened as reaction proceeded. After 30 mins cold DCM (200 mL) was added and mixture was filtered through celite and then washed with a solution of saturdated sodium hydrogencarbonate/sodium thiosulphate (1:1 v/v; 200 mL x 2). The organic layer was dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield the bis ketone **5** as a yellow/orange sponge (25.5g, 100%). LC/MS (3.173 min (ES^+)). *m/z*: 854.20 $[M]^+$. ^1H 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* = 9.9, 3.1 Hz), 4.31-4.18 (m, 6H), 3.89-3.84 (m, 8H), 3.78-3.62 (m, 4H), 3.55 (dd, 2H, *J* = 19.3, 3.0 Hz), 2.76 (dd, 2H, *J* = 18.6, 10.2 Hz), 2.42 (p, 2H, *J* = 5.8 Hz), 0.98-0.91 (m, 4H), 0.00 (s, 18H).

(b) 1, 1'-[[(Propane- 1,3-diyl)dioxy]bis(11aS)-7-methoxy-2-[[(trifluoromethyl)sulfonyl]oxy]-1 0-((2-(trimethylsilyl)ethoxy)methyl)-1, 10, 11, 11a-tetrahydro-5H-pyrrolo[2, 1-c][1,4]-benzodiazepin-5, 11-dione] (6)

Anhydrous 2,6-lutidine (1.984 g, 17.864 mmol, 6.22 eq.) was injected in one portion to a vigorously stirred solution of bis-ketone 5 (2.45 g, 2.977 mmol, 1.00 eq.) in dry DCM (90 ml.) at -45 °C (dry ice/acetonitrile) under a nitrogen atmosphere. Anhydrous triflic anhydride (5.040 g, 17.864 mmol, 6.0 eq.) taken from a freshly opened ampule was injected rapidly dropwise while maintaining the temperature no higher than -40 °C. The reaction mixture was allowed to stir at -45 °C for 1 h at which point TLC (50/50 v/v n-hexane/EtOAc) and LCMS revealed complete consumption of the starting material. The cold reaction mixture was immediately diluted with DCM (100 ml.) and, with vigorous shaking, washed with water (1 x 50 ml_), 5% citric acid solution (1 x 100 ml_), saturated $NaHCO_3$ (100 ml_), brine (50 ml.) and dried over magnesium sulphate, filtered and concentrated under reduced pressure to give the crude product which was purified by flash chromatography (silica gel, gradient elution 90:10 n-hexane/EtOAc v/v to 60:40 n-hexane/EtOAc v/v) to afford the bis enol triflate **6** as a yellow foam (2.097g, 63%). LC/MS (3.916 min (ES^+)). *m/z*: 1117.24 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 2H), 7.26 (s, 2H), 7.14 (t, 2H, *J* = 2.0 Hz), 5.51 (d, 2H, *J* = 10.1 Hz), 4.76 (d, 2H, *J* = 10.1 Hz), 4.62 (dd, 2H, *J* = 11.0, 3.6 Hz), 4.32-4.23 (m, 4H), 3.94-3.90 (m, 8H), 3.81-3.64 (m, 4H), 3.16 (ddd, 2H, *J* = 16.4, 11.1, 2.3 Hz), 2.43 (p, 2H, *J* = 5.9 Hz), 1.23-0.92 (m, 4H), 0.02 (s, 18H).

Example 2



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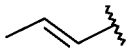
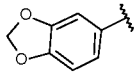
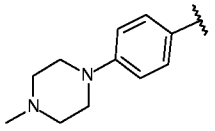
R					

*(a) Compounds **7a-e***

*(i) General preparation of compounds **7c-d***

Boronic acid (1.0 eq), triethylamine (4.0 eq) and (11aS,11a'S)-8,8'-(propane-1,3-diylbis(oxy))bis(7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-8,2-diyl) bis(trifluoromethanesulfonate) (6)(1.0 eq) were dissolved in a mixture of ethanol, toluene and water [3:6:1] (0.01 M) under a nitrogen atmosphere. The reaction mixture was degassed under nitrogen for 5 mins when tetrakis(triphenylphosphine)palladium(0) (0.04 eq) was added. The reaction mixture stirred at between 60°C and 70°C for 1 hour. Dichloromethane was added and washed with water. The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was adsorbed onto silica and purified by flash chromatography (silica gel, gradient elution, 80/20 to 20/80 v/v hexane/ ethyl acetate) to yield a mixture of recovered starting material, product and bis-substituted impurity.

*(S)-7-methoxy-8-(3-((S)-7-methoxy-5,11-dioxo-2-((E)-prop-1-enyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl trifluoromethanesulfonate **(7c)***

2.06 g (1.84 mmol) of **6** gave the following mixture:

0.446 g (24%) of **7c** as a brown solid. LC/MS (3.885 min (ES^+)). *m/z*: 1009.31 $[M]^+$.

0.057 g of **6.** LC/MS (3.920 min (ES^+)). *m/z*: 1117.24 $[M]^+$.

0.504 g of bis-substituted impurity as a brown solid. LC/MS (3.854 min (ES^+)). *m/z:* 901.41 $[M]^+$.

*(S)-8-(3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl trifluoromethanesulfonate **(7d)***

0.200 g (0.179 mmol) of **6** gave the following mixture:

0.075 g (38%) of **7d** as a brown solid. LC/MS (3.851 min (ES^+)). *m/z:* 1089.28 $[M]^+$.

0.024 g of **6.** LC/MS (3.920 min (ES^+)). *m/z:* 1117.24 $[M]^+$.

0.052 g of bis-substituted impurity as a brown solid. LC/MS (3.774 min (ES^+)). *m/z:* 1061 .38 $[M]^+$.

(ii)(S)-7-methoxy-8-(3-((S)-7-methoxy-2-(4-(4-methylpiperazi n-1-yl)phenyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-5, 11-dioxo-1 0-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro- 1H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-2-yl trifluoromethanesulfonate ***(7e)***

(1 1aS, 11a'S)-8,8'-(propane-1,3-diylbis(oxy))bis(7-methoxy-5, 11-dioxo-1 0-((2-(trimethylsilyl)ethoxy)methyl)-5, 10,1 1,1 1a-tetrahydro-1 H-benzo[e]pyrrolo[1 ,2-a][1,4]diazepine-8,2-diyl) bis(trifluoromethanesulfonate) **(6)** (2.00 g, 1.790 mmol, 1.0 eq), (4-methylpiperazin-1-yl)phenylboronic acid (0.394 g, 1.790 mmol, 1.0 eq) and triethylamine (0.574 mL, 4.1 17 mmol, 2.3 eq) were solubilised in a mixture of Toluene/Ethanol/H_20 6:3:1 (100 mL) under a nitrogen atmosphere. The reaction was flushed with nitrogen and palladium-tetrakistriphenylphosphine (20.7 mg, 0.0179 mmol, 0.01 eq) was added. The reaction was flushed with nitrogen and stirred at 30°C for 16 hours. All the volatiles were subsequently removed under reduced pressure and the solid residue was partitioned between H_20 (200 mL) and ethyl acetate (200 mL). The aqueous layer was extracted two more times with ethyl acetate (2 x 200 mL) before the combined organics were washed with brine (200 mL), dried with $MgS0_4$, filtered and the volatiles removed under reduced pressure. The crude product was purified by silica gel column chromatography ($CHCI_3$ 100 to 95:5 v/v $CHCI_3$/MeOH) to yield a mixture of recovered starting material, product and bis-substituted impurity.

0.669 g (33%) of **7e** as a brown solid. LC/MS (2.751 min (ES^+)), *m/z*: 1144.2 $[M+H]^+$. ^1H NMR (400 MHz, $CDCI_3$) δ 7.37 (s, 1H), 7.33 - 7.28 (m, 4H), 7.12 (m, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.51 - 5.49 (d, *J* = 10.0 Hz, 2H), 4.76 - 4.73 (dd, *J* = 10.0, 4.0 Hz, 2H), 4.62 - 4.56 (m, 2H), 4.30 - 4.22 (m, 4H), 3.94 - 3.86 (m, 9H), 3.81 - 3.71 (m, 3H), 3.70 - 3.62 (m, 2H), 3.26 - 3.22 (m, 4H), 3.17 - 3.07 (m, 2H), 2.66 - 2.55 (m, 4H), 2.46 - 2.38 (m, 2H), 2.35 (s, 3H), 0.99 - 0.93 (m, 4H), 0.00 (s, 18H).

1.093 g of **6.** LC/MS (3.917 min (ES^+)).

0.094 g of bis-substituted impurity as a brown solid. LC/MS (2.150 min (ES^+)). *m/z*: 1061 .38 $[M]^+$.

*(b) General preparation of compounds **8a-e***

A mixture of the appropriate mono-triflate from step (a)(1.0 eq), and triphenylphosphine (0.2 eq) was dissolved in anhydrous piperidine (0.05 M) in the presence of 4A molecular sieves under a nitrogen atmosphere. Propargylamine (6.0 eq), copper Iodide (0.1 eq), and tetrakis(triphenylphosphine)palladium(0) (0.1 eq) was then added to the mixture, and the reaction was warmed to between 50°C and 60°C for 0.5 hour to 1 hour. The mixture was cooled to room temperature, and the solvent was removed by rotary evaporation under reduced pressure. The resulting brown oil was purified by silica gel column chromatography (DCM 100 to 90:10 v/v DCM/MeOH to 85:15 v/v DCM/MeOH).

*(i) (S)-2-(3-aminoprop-1-ynyl)-7-methoxy-8-(3-((S)-7-methoxy-5, 11-dioxo-2-((E)-prop-1-enyl)-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5, 11(10H, 11aH)-dione **(8c)***

0.418 g (0.414 mmol) of **7c** yielded 0.150 g (40%) of product as a brown solid. LC/MS (2.576 min (ES^+)). *m/z*: 914.42 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.32 (m, 3H), 6.84 (s, 1H), 6.25 - 6.21 (d, *J* = 14.9 Hz, 1H), 5.72 - 5.65 (m, 1H), 5.50 - 5.48 (d, *J* = 9.9 Hz, 2H), 4.73 - 4.71 (m, 2H), 4.51 - 4.48 (m, 2H), 4.31 - 4.23 (m, 4H), 3.93 - 3.86 (m, 7H), 3.81 - 3.72 (m, 3H), 3.70 - 3.60 (m, 5H), 3.49 - 3.42 (m, 1H), 2.95 - 2.83 (m, 2H), 2.46 - 2.38 (m, 2H), 1.83 (d, *J* = 6.7 Hz, 3H), 1.76 - 1.69 (m, 2H), 1.00 - 0.92 (m, 4H), 0.00 (s, 18H).

*(ii) (S)-2-(3-aminoprop-1-ynyl)-8-(3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5, 11(10H, 11aH)-dione **(8d)***

1.120 g (1.03 mmol) of **7d** yielded 0.507 g (50%) of product as a brown solid. LC/MS (2.596 min (ES^+)). *m/z*: 944.2 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.34 (s, 1H), 7.30 (m, 1H), 7.09 (m, 1H), 6.94 (d, *J* = 1.6 Hz, 1H), 6.89 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 5.97 (s, 2H), 5.52 - 5.49 (dd, *J* = 10.0, 4.3 Hz, 2H), 4.77 - 4.73 (dd, *J* = 10.1, 6.1 Hz, 2H), 4.61 - 4.58 (dd, *J* = 10.6, 3.4 Hz, 1H), 4.53 - 4.49 (dd, *J* = 10.6, 3.4 Hz, 1H), 4.31 - 4.24 (m, 4H), 3.91 - 3.86 (m, 9H), 3.81 - 3.74 (m, 2H), 3.73 - 3.57 (m, 5H), 3.14 - 3.07 (m, 1H), 2.94 - 2.87 (m, 1H), 2.46 - 2.39 (m, 2H), 1.85 - 1.68 (m, 2H), 1.02 - 0.93 (m, 4H), 0.00 (s, 18H).

(Hi) *(S)-2-(3-aminoprop- 1-ynyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(4-(4-methylpiperazin- 1-yl)phenyl)-5, 11-dioxo- 10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro- 1H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-8-yloxy)propoxy)-1 0-((2-(trimethylsilyl)ethoxy)methyl)- 1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5, 11(1 OH, 11aH)-dione* ***(8e)***

0.812 g (0.710 mmol) of **7e** yielded 0.227 g (30%) of product as a brown solid. LC/MS (2.109 min (ES^+)). *m/z*: 1049.48 $[M+H]^+$.

(c) General preparation of compounds ***9a-e***

The appropriate SEM dilactam from step (b)(1 .0 eq.) was solubilised in THF (0.02 M) and cooled to -78°C under a nitrogen atmosphere. Super hydride solution (1M in THF, 2.04 eq.) was added dropwise over 5 minutes. After 20 minutes an aliquot was washed with water for LCMS and TLC analysis. After 30 minutes water was added and the cold bath removed. The organic layer was extracted with EtOAc (2 x) and the combined organic extracts washed with brine, dried with $MgS0_4$, filtered and the solvent removed under reduced pressure. The crude product was dissolved in MeOH/DCM/water (6:3:1) (0.01 M) and enough silica gel to form a thick stirring suspension.

After 5 days the suspension was filtered through a sintered funnel and washed with DCM/MeOH 9:1 until complete elution of product. The organic layer was washed with brine (2 x), dried with $MgS0_4$, filtered and the solvent removed under reduced pressure. Purification by silica gel column chromatography ($CHCl_3$ 100 to 90:10 v/v $CHCl_3$/MeOH) afforded the product.

(i) (S)-2-(3-aminoprop-1-ynyl)-7-methoxy-8-(3-((S)-7-methoxy-5-oxo-2-((E)-prop-1-enyl)- 5, 11a-dihydro-1 H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-8-yloxy)propoxy)-1 H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-5(1 1aH)-one ***(9c)***

0.150 g (0.164 mmol) of **8c** yielded 0.29 g (28%) of product as a brown solid. LC/MS (2.759 min (ES^+)). *m/z*: 1243.50 $[2M]^+$.

(ii) (S)-2-(3-aminoprop-1-ynyl)-8-(3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo- 5, 11a-dihydro-1 H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-8-yloxy)propoxy)-7-methoxy- 1H-benzo[e]pyrrolo[1 ,2-a][1 ,4]diazepin-5(1 1aH)-one ***(9d)***

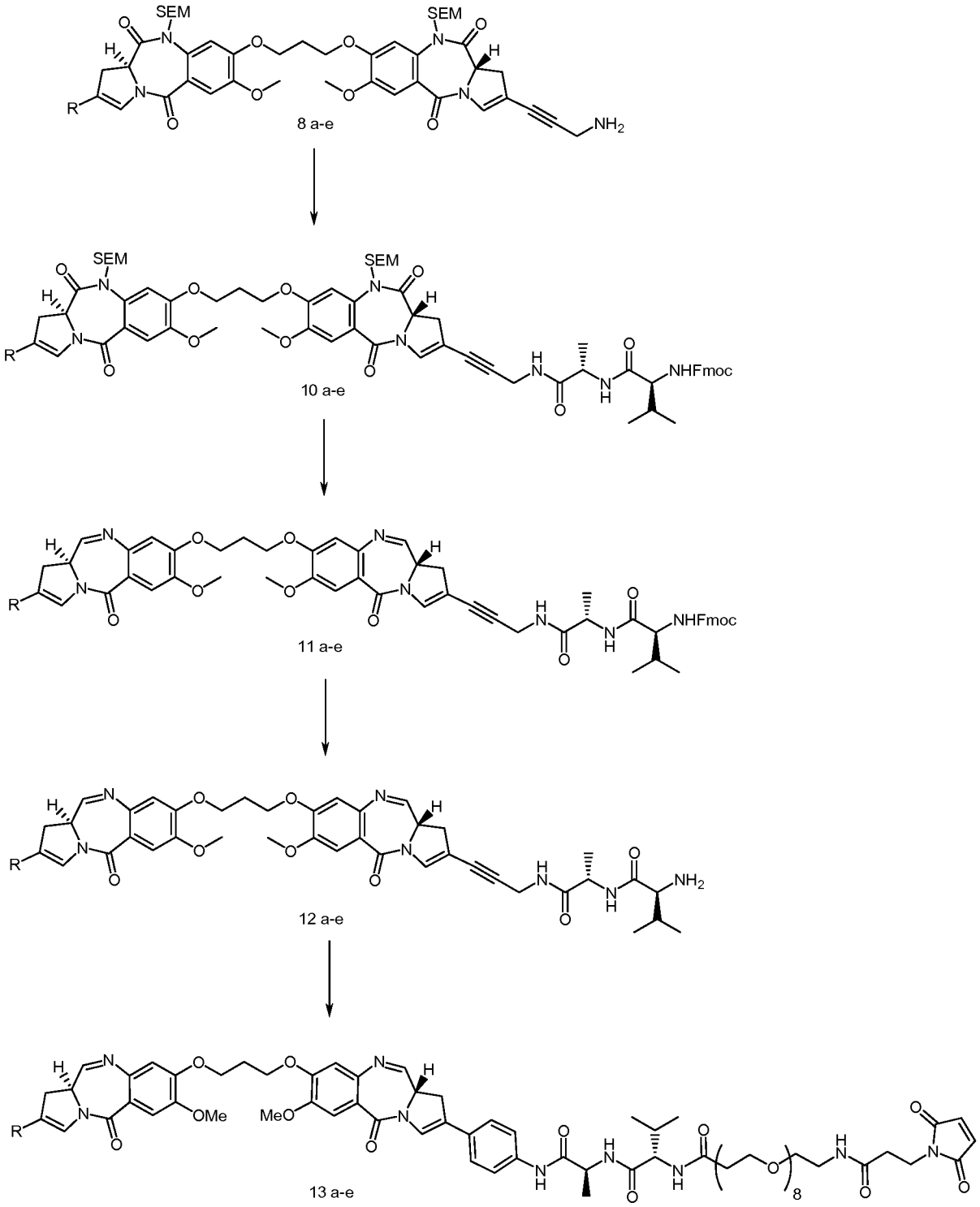
0.250 g (0.251 mmol) of **8d** yielded 0.026 g (15%) of product as a dark brown solid. LC/MS (2.758 min (ES^+)). *m/z*: 1402.79 $[2M]^+$.

(Hi) *(S)-2-(3-aminoprop- 1-ynyl)-7-methoxy-8-(3-((S)-7-methoxy-2-(4-(4-methylpiperazin- 1-yl)phenyl)-5-oxo-5, 11a-dihydro- 1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)- 1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(1 1aH)-one (9e)*

0.227 g (0.217 mmol) of **8e** yielded 0.028 g (17%) of product. LC/MS (2.759 min (ES^+)). *m/z*: 657.4 $[M\text{-piperazine}]^+$

These compounds were generally found to be in dimeric form when isolated (see discussion above). Treatment in methanol, by stirring for 1 hour, enabled detection of the monomeric form of the compounds. **9c:** LC/MS (1.52 min (ES^+)). *m/z*: 622.4 $[M+H]^+$; **9d:** LC/MS (1.51 min (ES^+)). *m/z*: 702.5 $[M+H]^+$.

Example 3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NH2
SEM
SEM
10 a-e
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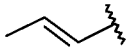
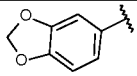
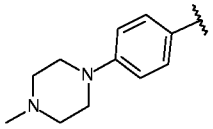
8

	a	b	c	d	e
R					

(a) Compounds ***10a-e***

(i) (9H-fluoren-9-yl)methyl (S)-1-((S)-1-((3-((S)-8-((3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5, 11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-5, 11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)prop-2-ynylamino)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate ***(10d)***

1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (0.0193 g, 0.101 mmol, 1.0 eq) was added to a solution of **8d** (0.100 g, 0.101 mmol, 1.0 eq) and HO-Ala-Val-Fmoc (0.0415 g, 0.101 mmol, 1.0 eq) in dry dichloromethane (6 mL) at room temperature. After 30 minutes the reaction was diluted with dichloromethane (20 mL) and washed sequentially with water (10 mL) and brine (10 mL). The organic phase was dried over magnesium sulphate filtered and excess dichloromethane removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 70:1 v/v DCM/MeOH to 50:1 v/v DCM/MeOH). Pure fractions were collected and combined and excess eluent was removed by rotary evaporation under reduced pressure to give product as a yellow solid (0.043 g, 31%).

LC/MS (3.785 min (ES^+)). *m/z*: 1385.01 $[M-H]^+$.

(b) Compounds ***11a-e***

(i) (9H-fluoren-9-yl)methyl (R)-1-((S)-1-(3-((S)-8-(3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)prop-2-ynylamino)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate ***(11d)***

The SEM dilactam **10d** (0.063g, 0.0455 mmol, 1.0 eq.) was solubilised in THF (2.7) and cooled to -78°C under a nitrogen atmosphere. Super hydride solution (1M in THF, 0.093 mL, 2.04 eq.) was added dropwise over 5 minutes. After 20 minutes an aliquot was washed with water for LCMS and TLC analysis. After 30 minutes water was added and the cold bath removed. The organic layer was extracted with EtOAc (2 x 10 mL) and the combined

organic extracts washed with brine (10 ml_), dried with $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was dissolved in MeOH/DCM/water (6:3:1) (0.01 M) and enough silica gel to form a thick stirring suspension.

After 5 days the suspension was filtered through a sintered funnel and washed with DCM/MeOH 9:1 until complete elution of product. The organic layer was washed with brine (2 x), dried with $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification by silica gel column chromatography ($CHCl_3$ 100 to 90:10 v/v $CHCl_3$/MeOH) afforded the product.

(c) Compounds ***12a-e***

(i) (S)-2-amino-N-((S)- 1-(3-((S)-8-(3-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-me^ γ-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)prop-2-ynylamino)- 1-oxopropan-2-yl)-3-methylbutanamide ***(12d)***

Excess piperidine was added (0.01 ml_, 0.1 mmol) to a solution of SEM-dilactam **11d** (1 mg, 0.0009 mmol) in DMF (0.1 ml_). The mixture was allowed to stir at room temperature for 20 min, at which point the reaction had gone to completion (monitored by LC/MS). The reaction mixture was diluted with CH_2Cl_2 (1 ml.) and the organic phase was washed with H_20 (3x1 ml.) until complete piperidine removal. The organic phase was dried over $MgSO_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure to afford crude material **12d.** LC/MS (1.917 min (ES^+)). *m/z*: 872.2 $[M+H]^+$

Example 4: Determination of *In Vitro* Cytotoxicity

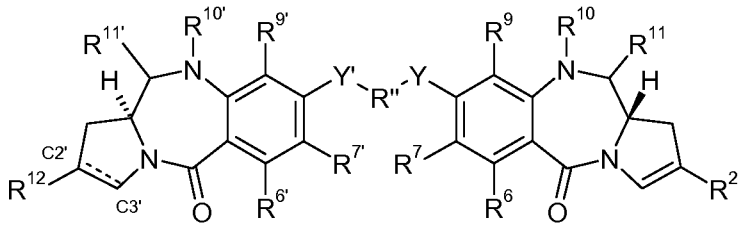
K562 human chronic myeloid leukaemia cells were maintained in RPM1 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37°C in a humidified atmosphere containing 5% CO_2 and were incubated with a specified dose of drug for 96 hours at 37°C in the dark. The incubation was terminated by centrifugation (5 min, 300 *g)* and the cells were washed once with drug-free medium. Following the appropriate drug treatment, the cells were transferred to 96-well microtiter plates (10^4 cells per well, 8 wells per sample). Plates were then kept in the dark at 37°C in a humidified atmosphere containing 5% CO_2. The assay is based on the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2/-/-tetrazolium bromide (MTT, Aldrich-Sigma), to an insoluble purple formazan precipitate. Following incubation of the plates for 4 days (to allow control cells to increase in number by approximately 10 fold), 20 μI_ of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and

the plates further incubated for 5 hours. The plates were then centrifuged for 5 minutes at 300 g and the bulk of the medium pipetted from the cell pellet leaving 10-20 μl per well. DMSO (200 μl) was added to each well and the samples agitated to ensure complete mixing. The optical density was then read at a wavelength of 550 nm on a Titertek Multiscan ELISA plate reader, and a dose-response curve was constructed. For each curve, an IC_{50} value was read as the dose required to reduce the final optical density to 50% of the control value.

Compound 13 has an IC_{50} of 0.5 nM in this assay.

CLAIMS

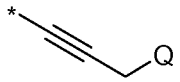
1. A compound with the formula I:



I

wherein:

R^2 is of formula II:



(II)

,

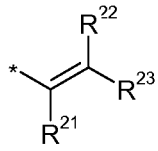
where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl;

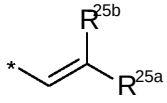
when there is a double bond present between **C2'** and C3', R^{12} is selected from:

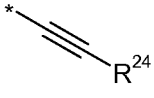
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, $\mathbf{C_{3-7}}$ heterocyclyl and bis-oxy-$\mathbf{Ci_{-3}}$ alkylene;

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

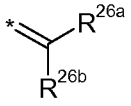
(ic) C_{3-6} saturated cycloalkyl;

(id)  , wherein each of R^{21}, R^{22} and R^{23} are independently selected from H, $\mathbf{C_{1-3}}$ 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**;

(ie)  , 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

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

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

R^{12} is  , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

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

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

R^7 is selected from H, R, OH, OR, SH, SR, NH_2, NHR, 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.

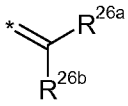
6. A compound according to any one of claims 1 to 5, wherein R^9 is H.

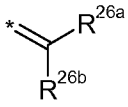
7. A compound according to any one of claims 1 to 6, wherein R^6 is selected from H and halo.

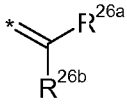
8. A compound according to any one of claims 1 to 7, wherein Q is NR^N.

9. A compound according to claim 8, wherein R^N is H or methyl.

10. A compound according to any one of claims 1 to 7, wherein Q is OH or SH.

11. A compound according to any one of claims 1 to 10, wherein there is a single bond between C2' and C3', R^{12} is  and R^{26a} and R^{26b} are both H.

12. A compound according to any one of claims 1 to 10, wherein there is a single bond between C2' and C3', R^{12} is  , and R^{26a} and R^{26b} are both methyl.

13. A compound according to any one of claims 1 to 10, wherein there is a single bond between C2' and C3', R^{12} is  , one of R^{26a} and R^{26b} is H, and the other is selected from C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted.

14. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a C_{5-7} aryl group.

15. A compound according to claim 14, wherein R^{12} is phenyl.

16. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a C_{8-10} aryl group.

17. A compound according to any one of claims 14 to 16, wherein R^{12} bears one to three substituent groups.

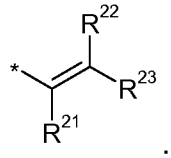
18. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a C_{1-5} saturated aliphatic alkyl group.

19. A compound according to claim 18, wherein R^{12} is methyl, ethyl or propyl.

20. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a C_{3-6} saturated cycloalkyl group.

21. A compound according to claim 20, wherein R^{12} is cyclopropyl.

22. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



.

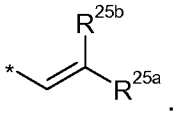
23. A compound according to claim 22, wherein the total number of carbon atoms in the R^{12} group is no more than 4.

24. A compound according to claim 23, wherein the total number of carbon atoms in the R^{12} group is no more than 3.

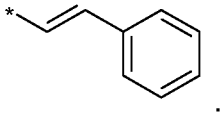
25. A compound according to any one of claims 22 to 24, wherein one of R^{21}, R^{22} and R^{23} is H, with the other two groups being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl.

26. A compound according to any one of claims 22 to 24, 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, C2-3 alkenyl, C2-3 alkynyl and cyclopropyl.

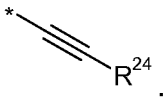
27. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



28. A compound according to claim 27, wherein R^{12} is the group:



29. A compound according to any one of claims 1 to 10, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



30. A compound according to claim 29, wherein R^{24} is selected from H, methyl, ethyl, ethenyl and ethynyl.

31. A compound according to claim 30, wherein R^{24} is selected from H and methyl.

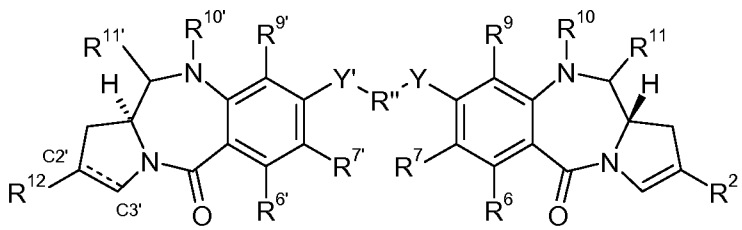
32. A compound according to any one of claims 1 to 31, wherein R^{10} and R^{11} and form a nitrogen-carbon double bond.

33. A compound according to any one of claims 1 to 32, 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.

34. The use of a compound according to any one of claims 1 to 33 in the manufacture of a medicament for treating a proliferative disease.

35. A compound according to any one of claims 1 to 33 for use in the treatment of a proliferative disease.

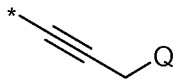
36. A compound of formula II:



II

wherein :

R^2 is of formula II:


(II)

,

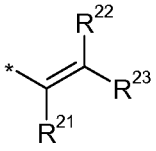
where Q is selected from OH, SH and NR^N, and R^N is selected from H, methyl and ethyl;

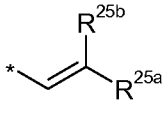
when there is a double bond present between C2' and C3', R^{12} is selected from :

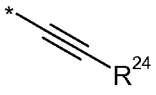
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C1-7 alkyl, C_{3-7} heterocyclyl and bis-oxy -Ci_{-3} alkylene;

(ib) C1-5 saturated aliphatic alkyl;

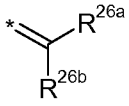
(ic) C3-6 saturated cycloalkyl;

(id)  , wherein each of R^{21}, R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C2-3 alkenyl, C2-3 alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5;

(ie)  , 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

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

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

R^{12} is  , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

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

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

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

either:

(a) R^{10} is carbamate nitrogen protecting group, and R^{11} is 0-Prot°, wherein Prot° 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}.

37. A compound according to any claim 36, wherein R^7 is selected from H, OH and OR.

38. A compound according to claim 37, wherein R^7 is a C_{1-4} alkyloxy group.

39. A compound according to any one of claims 36 to 38, wherein Y is O.

40. A compound according to any one of claims 36 to 39, wherein R" is C_{3-7} alkylene.

41. A compound according to any one of claims 36 to 40, wherein R^9 is H.

42. A compound according to any one of claims 36 to 41, wherein R^6 is selected from H and halo.

43. A compound according to any one of claims 36 to 42, wherein Q is NR^N.

44. A compound according to claim 43, wherein R^N is H or methyl.

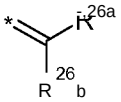
45. A compound according to any one of claims 36 to 42, wherein Q is OH or SH.

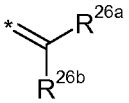
46. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a C_{5-7} aryl group.

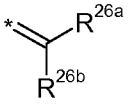
47. A compound according to claim 46, wherein R^{12} is phenyl.

48. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a C_{8-10} aryl group.

49. A compound according to any one of claims 46 to 48, wherein R^{12} bears one to three substituent groups.

50. A compound according to any one of claims 36 to 45, wherein there is a single bond between C2' and C3', R^{12} is  and R^{26a} and R^{26b} are both H.

51. A compound according to any one of claims 36 to 45, wherein there is a single bond between C2' and C3', R^{12} is  , and R^{26a} and R^{26b} are both methyl.

52. A compound according to any one of claims 36 to 45, wherein there is a single bond between C2' and C3', R^{12} is  , one of R^{26a} and R^{26b} is H, and the other is selected from C1-4 saturated alkyl, C2-3 alkenyl, which alkyl and alkenyl groups are optionally substituted.

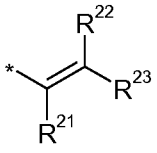
53. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a C_{1-5} saturated aliphatic alkyl group.

54. A compound according to claim 53, wherein R^{12} is methyl, ethyl or propyl.

55. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a C_{3-6} saturated cycloalkyl group.

56. A compound according to claim 55, wherein R^{12} is cyclopropyl.

57. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



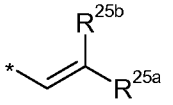
58. A compound according to claim 57, wherein the total number of carbon atoms in the R^{12} group is no more than 4.

59. A compound according to claim 58, wherein the total number of carbon atoms in the R^{12} group is no more than 3.

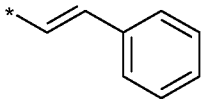
60. A compound according to any one of claims 57 to 59, wherein one of R^{21}, R^{22} and R^{23} is H, with the other two groups being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl.

61. A compound according to any one of claims 57 to 59, 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.

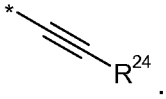
62. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



63. A compound according to claim 62, wherein R^{12} is the group:



64. A compound according to any one of claims 36 to 45, wherein there is a double bond between C2' and C3', and R^{12} is a group of formula:



65. A compound according to claim 64 wherein R^{24} is selected from H, methyl, ethyl, ethenyl and ethynyl.

66. A compound according to claim 65, wherein R^{24} is selected from H and methyl.

67. A compound according to any one of claims 36 to 66, wherein R^{10} is Troc.

68. A compound according to any one of claims 36 to 67, wherein R^{11} is OTBS.

69. A compound according to any one of claims 36 to 67, wherein R^{11} is oxo and R^{10} is SEM.

70. A compound according to any one of claims 36 to 68, 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.

71. 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 which is a PBD dimer according to any one of claims 1 to 33, wherein LU is connected to D via the Q substituent of R^2.

72. The Conjugate according to claim 71, wherein the Linker unit (LU) has the formula Ilia or 1Mb:

$$-A^1_a-L^1_s-L^2_y-, \qquad (Ilia)$$

wherein:

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

a is 1 or 2,

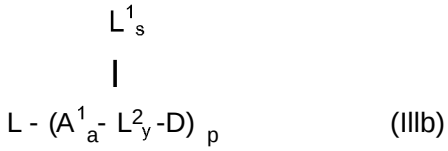
L^1- is a Specificity unit,

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



(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,

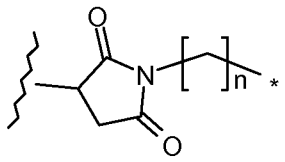
-L^2- is a Spacer unit,

y is 1 or 2, and

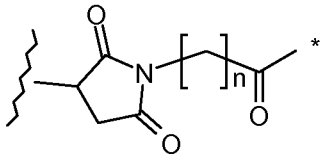
p is from 1 to 20.

73. The Conjugate of claim 72, wherein the Linker unit (LU) has formula IIia.

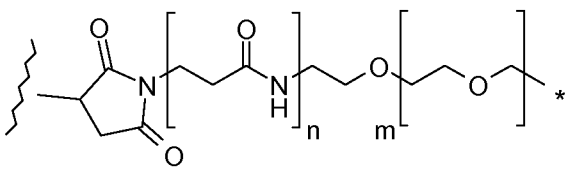
74. The Conjugate of claim 73, wherein A^1 is selected from:



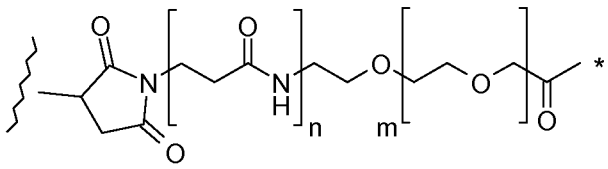
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6;



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;

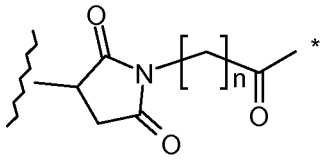


where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30; or



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.

75. The Conjugate of claim 73, 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.

76. The Conjugate of claim 75, wherein n is 5.

77. The Conjugate of any of claims 72 to 76, wherein L^1 comprises an amino acid sequence.

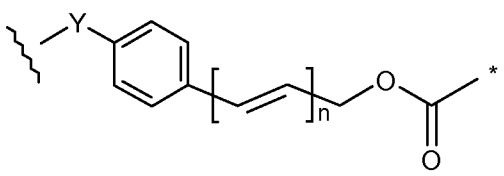
78. The Conjugate of claim 77, wherein L^1 is a dipeptide.

79. The Conjugate of claim 78, wherein L^1 is selected from the group consisting of valine-alanine, valine-citrulline and phenyalanine-lysine.

80. The Conjugate of any of claims 72 to 79, wherein y is 0.

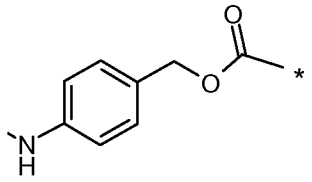
81. The Conjugate of any of claims 72 to 79, wherein y is 1 or 2.

82. The Conjugate of claim 81, 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)-, -0-, -C(=0)N(H)- or -C(=0)0-, and n is 0 to 3.

83. The Conjugate of claim 82, wherein L^2 is:



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84. The use of a Conjugate of according to any one of claims 71 to 83, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease.

85. The use of a Conjugate according to any one of the claims 71 to 83 for treating a proliferative disease or an autoimmune disease.

86. 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 71 to 83.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/068506

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D519/00 A61K31/5517 A61K47/48 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 A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2011/130616 A1 (SPIROGEN LTD [GB]; SEATTLE GENETICS INC [US]; HOWARD PHILIP WILSON [GB]) 20 October 2011 (2011-10-20) cited in the application page 89; example 7	1-86
A	WO 2010/043880 A1 (SPIROGEN LTD [GB]; HOWARD PHILIP WILSON [GB]; GREGSON STEPHEN JOHN [GB]) 22 April 2010 (2010-04-22) cited in the application examples	1-86
A	WO 2005/085251 A1 (SPIROGEN LTD [GB]; HOWARD PHILIP WILSON [GB]; GREGSON STEPHEN JOHN [GB]) 15 September 2005 (2005-09-15) cited in the application example 5	1-86

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"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
13 December 2012	20/12/2012

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Authorized officer
Fazzi , Raffael la

INTERNATIONAL SEARCH REPORT

Information on patent family members

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