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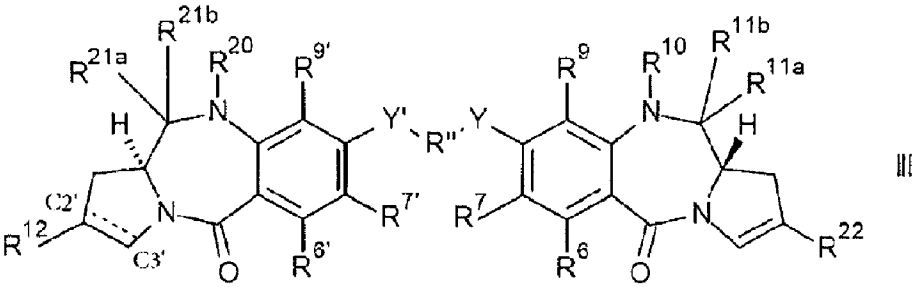
(54) Titre : DIMERES DE PYRROLOBENZODIAZEPINES ASYMETRIQUES A UTILISER DANS LE TRAITEMENT DE MALADIES PROLIFERATIVES ET AUTO-IMMUNES

(54) Title: UNSYMMETRICAL PYRROLOBENZODIAZEPINES-DIMERS FOR USE IN THE TREATMENT OF PROLIFERATIVE AND AUTOIMMUNE DISEASES



III

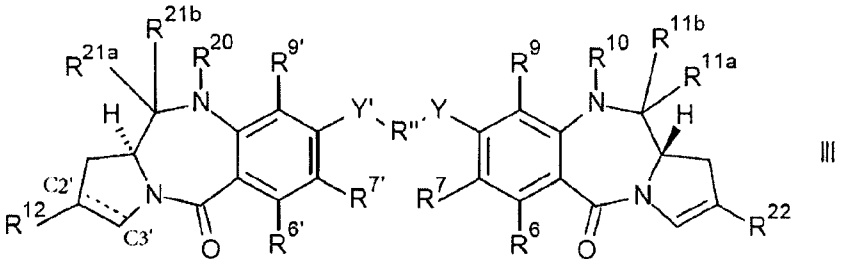
(57) Abrégé/Abstract:
Disclosed are compounds of formula III:

(see formula III)

or pharmaceutically acceptable salts or solvates thereof, as defined herein, as well as their use in the treatment of diseases and conditions including proliferative diseases.

ABSTRACT

Disclosed are compounds of formula III:



III

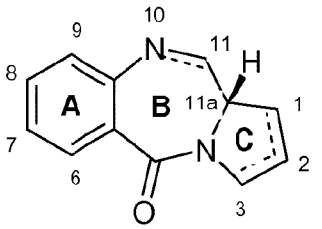
or pharmaceutically acceptable salts or solvates thereof, as defined herein, as well as their use in the treatment of diseases and conditions including proliferative diseases.

UNSYMMETRICAL PYRROLOBENZODIAZEPINES-DIMERS FOR USE IN THE TREATMENT OF PROLIFERATIVE AND AUTOIMMUNE DISEASES

The present invention relates to pyrrolobenzodiazepines (PBDs), and their inclusion in targeted conjugates. The PBDs of the present invention are in a mixed dimer where one PBD moiety compises an imine or equivalent group and the other moiety comprises either an amine or amido group. The PBDs are linked to a cell binding agent via a substituent on the C2 position.

Background to the invention

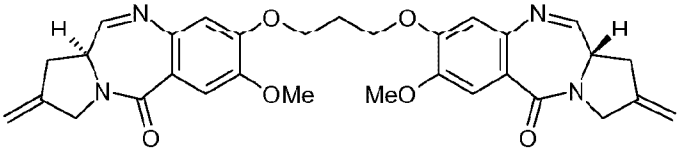
Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5793-5795 (1965); Leimgruber, *et al.*, *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, *et al*, *J. Antibiotics*, **29**, 2492-2503 (1982); Langley and Thurston, *J. Org. Chem.*, **52**, 91-97 (1987)), sibanomicin (DC-102)(Hara, *et al.*, *J. Antibiotics*, **41**, 702-704 (1988); Itoh, *et al.*, *J. Antibiotics*, **41**, 1281-1284 (1988)), sibiromycin (Leber, *et al.*, *J. Am. Chem. Soc.*, **110**, 2992-2993 (1988)) and tomamycin (Arima, *et al.*, *J. Antibiotics*, **25**, 437-444 (1972)). PBDs are of the general structure:



They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-

CH(OMe)) at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (*S*)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III*. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem. Res.*, **19**, 230-237 (1986)). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

It has been previously disclosed that the biological activity of these molecules can be potentiated by joining two PBD units together through their C8/C'-hydroxyl functionalities via a flexible alkylene linker (Bose, D.S., *et al.*, *J. Am. Chem. Soc.*, **114**, 4939-4941 (1992); Thurston, D.E., *et al.*, *J. Org. Chem.*, **61**, 8141-8147 (1996)). The PBD dimers are thought to form sequence-selective DNA lesions such as the palindromic 5'-Pu-GATC-Py-3' interstrand cross-link (Smellie, M., *et al.*, *Biochemistry*, **42**, 8232-8239 (2003); Martin, C., *et al.*, *Biochemistry*, **44**, 4135-4147) which is thought to be mainly responsible for their biological activity. One example of a PBD dimmer, SG2000 (SJG-136):

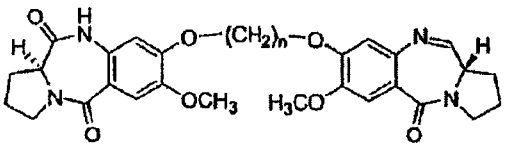


has recently entered Phase II clinical trials in the oncology area (Gregson, S., *et al.*, *J. Med. Chem.*, **44**, 737-748 (2001); Alley, M.C., *et al.*, *Cancer Research*, **64**, 6700-6706 (2004); Hartley, J.A., *et al.*, *Cancer Research*, **64**, 6693-6699 (2004)).

WO 2010/043880 discloses unsymmetrical dimeric PBD compound bearing aryl groups in the C2 position of each monomer, where one of these aryl groups bears a substituent designed to provide an anchor for linking the compound to another moiety. WO 2011/130613, discloses the inclusion of these PBD dimer compounds in targeted conjugates. WO 2011/130616, 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. Co-pending International application PCT/EP2012/070233,

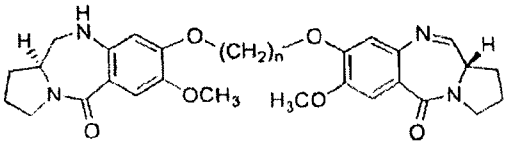
filed 12 October 2012, discloses further unsymmetrical dimeric PBD compound bearing an propylenyl group in the C2 position of one monomer bearing a substituent designed to provide an anchor for linking the compound to another moiety, the other monomer bearing an aromatic or non-aromatic group in the C2 position.

In 2002, Kamal described the synthesis and evaluation of PBD dimers having an imine bond in one PBD and an amide group in the other PBD (Kamal, A, *et al.*, *J. Med. Chem.*, **2002**, 4679-4688), such as:



n = 3-5,8

In 2004, he described the synthesis and evaluation of PBD dimers having an imine bond in one PBD and an amine bond in the other PBD (Kamal, A, *et al.*, *Bioorg. Med. Chem.*, 12 (2004) 5427-5436), such as:

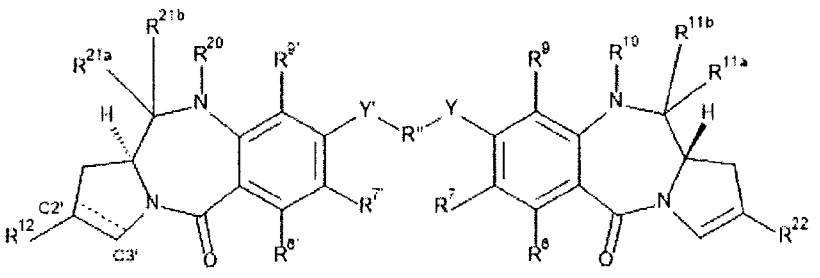


n = 3
n = 5

These compounds are unable to cross-link DNA but were shown to possess some cytotoxicity.

Summary

Certain exemplary embodiments provide a compound of formula III:



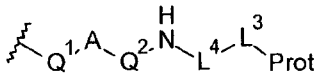
III

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

R^{22} is selected from the group consisting of:

(a) formula IVa:



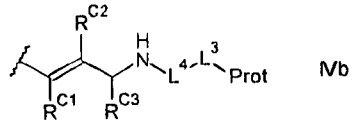
IVa

wherein A is a C_{5-7} aryl group, and either

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

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

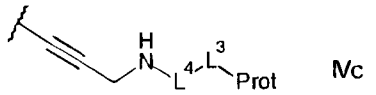
(b) formula IVb:



IVb

wherein R^{C1}, R^{C2} and R^{C3} are independently selected from the group consisting of H and unsubstituted C_{1-2} alkyl; and

(c) formula IVc:

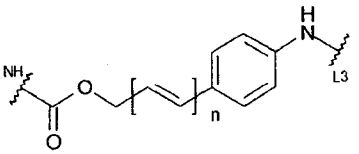


IVc

;

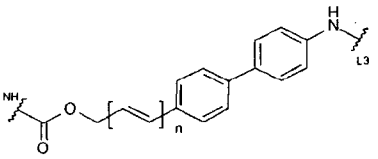
L^4 is selected from the group consisting of a single bond and a group of:

(a):



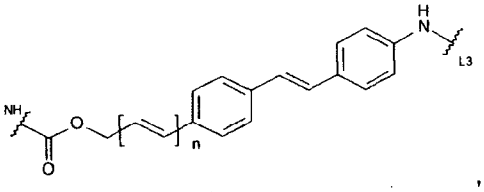
wherein n is 0 to 3;

(b)



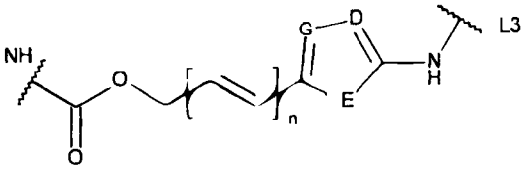
, wherein n is 0 to 3;

(c)



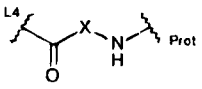
, wherein n is 0 to 3; and

(d)



, wherein n is 0 to 3, E is O, S or NR, D is N, CH, or CR, and G is N, CH, or CR;

L^3 is:



, where X is such that L^3 is an amino-acid residue, a dipeptide residue or a tripeptide residue;

Prot is selected from the group consisting of Fmoc (fluorenylmethyloxycarbonyl), Teoc (2-(trimethylsilyl)ethoxycarbonyl) and Boc (t-butoxycarbonyl);

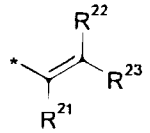
and either:

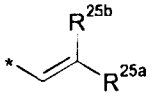
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

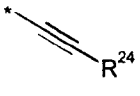
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group consiting of: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

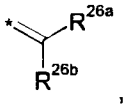
(ic) C_{3-6} saturated cycloalkyl;

(id)  , wherein each of R^{21}, R^{22} and R^{23} are independently selected from the group consisting of 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;

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

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

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

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

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

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

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

R'' is a C_{3-12} alkylene group, which chain is interrupted by one or more aromatic rings, selected from the group consisting of benzene and pyridine;

Y and Y' are selected from the group consisting of O, S, and NH;

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

either:

(A) R^{20} is H or Me and R^{21a} and R^{21b} are both H or together form =O and either:

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

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

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

(B) R^{10} is H or Me and R^{11a} and R^{11b} are both H or together form =O and either:

(i) R^{20} is H, R^{21a} is H and R^{21b} is OH or OR^A, where R^A is C_{1-4} alkyl; or

(ii) R^{20} and R^{21b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{11a} is H; or

(iii) R^{20} is H, R^{21a} is H and R^{21b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation.

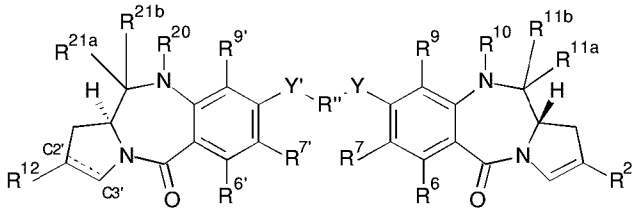
Other exemplary embodiments provide a conjugate having formula V:

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

wherein L is an antibody or antibody fragment,

p is 1 to 20; and

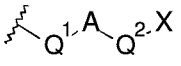
D is a Drug unit according to formula I:



I

wherein R^6, $R^{6'}$, R^7, $R^{7'}$, R^9, $R^{9'}$, R^{10}, R^{11a}, R^{11b}, R^{12}, R^{20}, R^{21a}, R^{21b}, Y, Y' and R" are as defined herein; and

R^2 is of formula IIa, formula IIb or formula IIc:

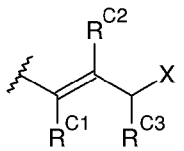
(a) 

IIa

where A is a C_{5-7} aryl group, and either

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

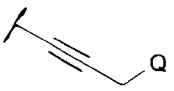
(ii) Q^1 is -CH=CH-, and Q^2 is a single bond;

(b) 

IIb

where;

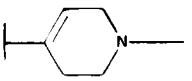
R^{C1}, R^{C2} and R^{C3} are independently selected from the group consisting of H and unsubstituted C_{1-2} alkyl;



IIc

(c)

where Q is selected from the group consisting of -O-, -S- and $-N(R^N)-$, and R^N is selected from the group consisting of H, methyl and ethyl

X is selected from the group consisting of: *-O-, *-S-, *-CO_2-, *-CO-, *-NH(C=O)-, *-NHNH-, *-CONHNH-,  and $-N(R^N)-$, wherein R^N is selected from the group consisting of H and C_{1-4} alkyl; wherein the asterix or wavy line indicates the point of attachment to the remainder of the drug unit;

wherein LU is connected to D via the X or Q substituent of R^2, wherein LU has the formula (Va):

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

wherein:

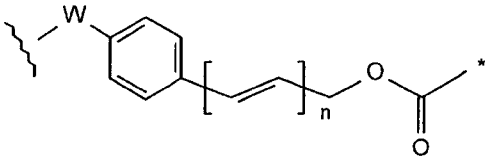
a is 1 or 2,

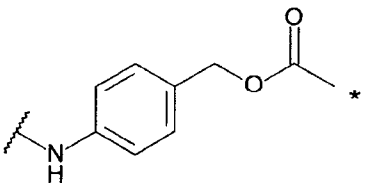
s is an integer ranging from 0 to 12,

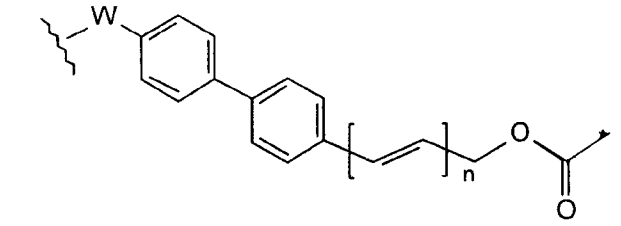
y is 0, 1 or 2, and

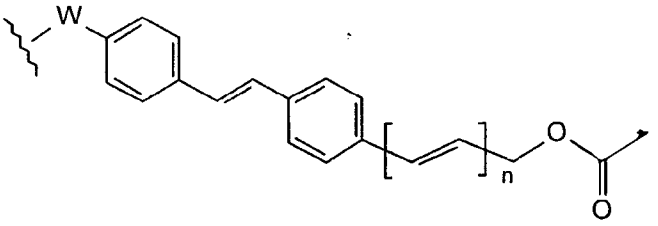
L^1 comprises an amino acid sequence;

L^2 is selected from the groups consisting of

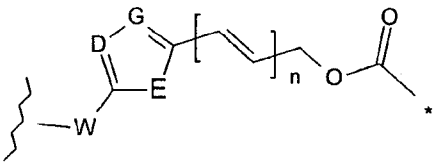








and



wherein the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to L^1,

W is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-,

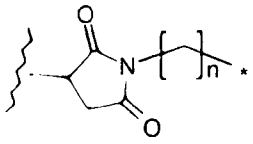
n is 0 to 3

E is O, S or NH,

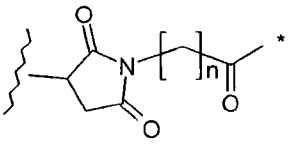
D is N or CH,

G is N or CH;

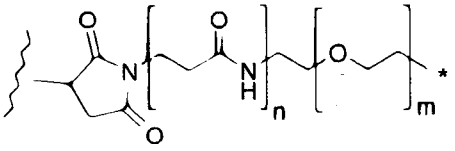
$-A^1-$ is selected from the group consisting of:



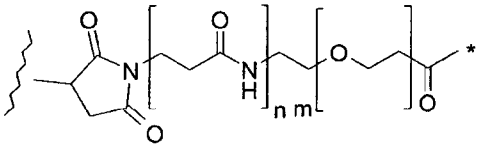
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to L, and n is 0 to 6;



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



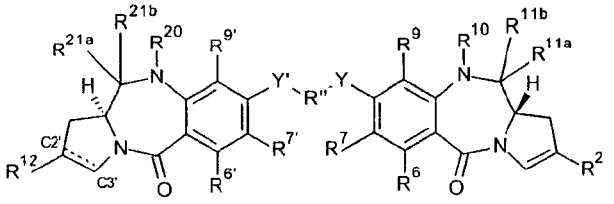
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to L, n is 0 or 1, and m is 0 to 30; and



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

Disclosure of the invention

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

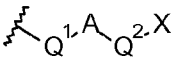


I

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

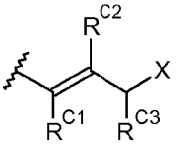
R^2 is of formula IIa, formula IIb or formula IIc:

(a)  IIa

where A is a C_{5-7} aryl group, and either

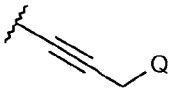
(i) Q^1 is a single bond, and Q^2 is selected from a single bond and -Z-$(CH_2)_n$-, where Z is selected from a single bond, O, S and NH and n is from 1 to 3; or

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

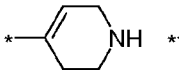
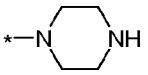
(b)  IIb

where;

R^{C1}, R^{C2} and R^{C3} are independently selected from H and unsubstituted C_{1-2} alkyl;

(c)  IIc

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

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

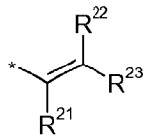
and either:

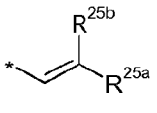
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

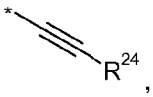
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

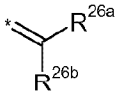
(ic) C_{3-6} saturated cycloalkyl;

(id)  , wherein each of R^{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;

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

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;

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;

either:

(A) R^{20} is H or Me and R^{21a} and R^{21b} are both H or together form =O and either:

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

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

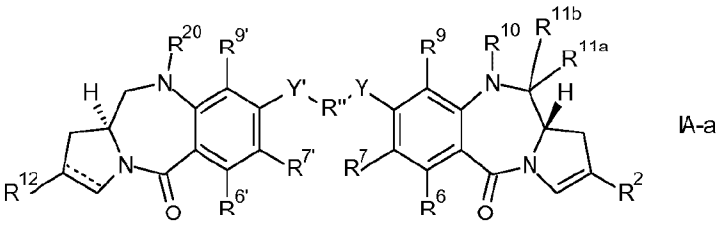
(iii) R^{10} is H, R^{11a} is H and R^{11b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation; or

(B) R^{10} is H or Me and R^{11a} and R^{11b} are both H or together form =O and either:

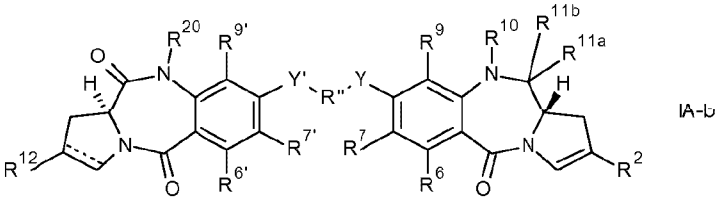
(i) R^{20} is H, R^{21a} is H and R^{21b} is OH or OR^A, where R^A is C_{1-4} alkyl; or

(ii) R^{20} and R^{21b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{11a} is H; or
(iii) R^{20} is H, R^{21a} is H and R^{21b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation.

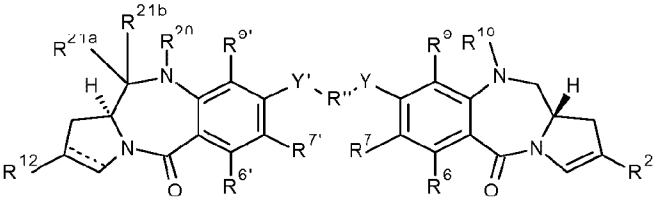
Thus, the options (A) and (B) above can result in compounds of the following formulae (IA-a, IA-b, IB-a, IB-b):



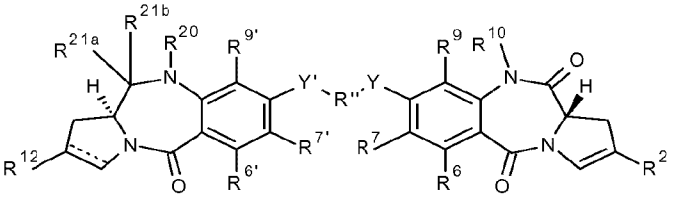
IA-a



IA-b



IB-a



IB-b

Dimers of the invention therefore have an imine bond in one monomer, that may be present as a carbinolamine, carbinolamine ether or bisulphite form, and either a second/tertiary amine or (methyl)amido functionality in the other monomer.

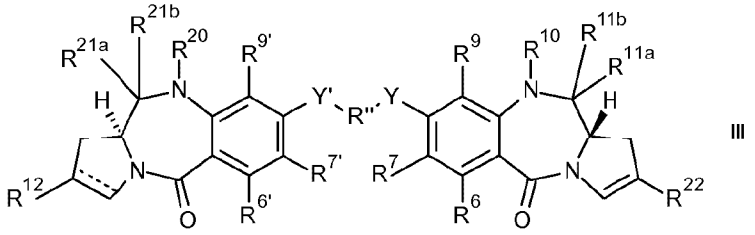
A second aspect of the present invention provides the use of a compound of the first aspect of the invention in the manufacture of a medicament for treating a proliferative

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 compound 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 provides a method of making a compound of the first aspect of the invention, comprising at least one of the method steps set out below.

A fourth aspect of the present invention provides compounds of formula III:



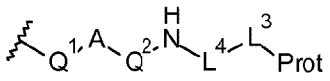
III

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

R^{22} is selected from:

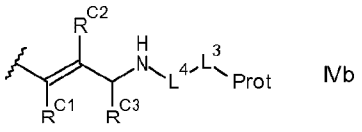
(a) formula IVa:



IVa

where A, Q^1, Q^2 are as defined in the first aspect of the invention;

(b) formula IVb:

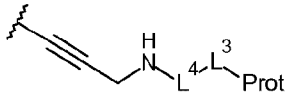


IVb

;

where R^{C1}, R^{C2} and R^{C3} are as defined in the first aspect of the invention;

(c) formula IVc:

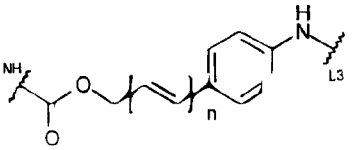


IVc

;

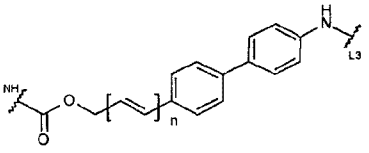
L^4 is selected from a single bond and a group of:

(a):



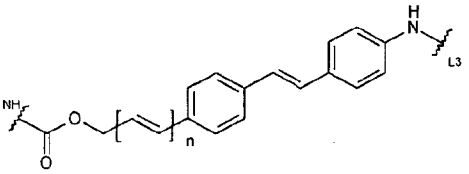
wherein n is 0 to 3;

(b)



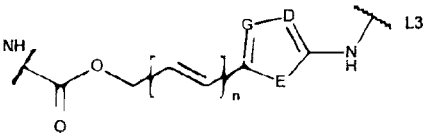
, wherein n is as defined above;

(c)



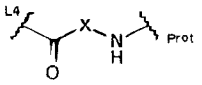
, wherein n is as defined above; and

(d)



, wherein n is 0 to 3, E is O, S or NR, D is N, CH, or CR, and G is N, CH, or CR;

L^3 is:



, where X is such that L^3 is an amino-acid residue, a dipeptide residue or a tripeptide residue;

Prot is selected from Fmoc (fluorenylmethyloxycarbonyl), Teoc (2-(trimethylsilyl)ethoxycarbonyl), Boc (t-butoxycarbonyl) and Alloc (allyloxycarbonyl);

and R^6, R^7, R^9, $R^{6'}$, $R^{7'}$, $R^{9'}$, R^{12}, R'', Y, Y', R^{10}, R^{11a}, R^{11b}, R^{20}, R^{21a} and R^{21b} are as defined in the first aspect of the invention.

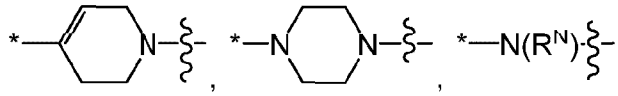
In a fifth aspect, the present invention relates to Conjugates comprising dimers of PBDs linked to a targeting agent, wherein the PBD dimer is of formula I, or a pharmaceutically acceptable salt or solvate thereof (supra).

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

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

or a pharmaceutically acceptable salt or solvate thereof, 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.

In conjugates of the present invention, the PBD dimer D is of formula I, or a pharmaceutically acceptable salt or solvate thereof, except that X is selected from the group comprising: *-O-$^+$, *-S-$^+$, *-CO_2-$^+$, *-CO-$^+$, *-NH(C=O)-$^+$, *-NHNH-$^+$, *-CONHNH-$^+$,



, wherein R^N is selected from the group comprising H and C_{1-4} alkyl, and the asterix indicates the point of attachment to the remainder of the Drug unit and the wavy line or $^+$ indicates the point of attachment to the Linker Unit.

The drug loading is represented by p, the number of drug molecules per Ligand unit (e.g., an antibody). Drug loading may range from 1 to 20 Drug units (D) per Ligand unit (e.g., Ab or mAb). For compositions, p represents the average drug loading of the Conjugates in the composition, and p ranges from 1 to 20.

A sixth aspect of the present invention provides the use of a conjugate of the fifth aspect of the invention in the manufacture of a medicament for treating a proliferative disease. The sixth aspect also provides a conjugate of the fifth 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.

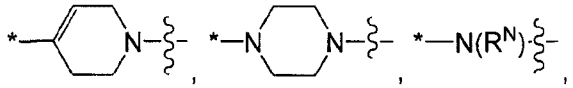
In a seventh aspect, the present invention relates to Linker-Drug compounds (i.e., Drug-Linkers) comprising dimers of PBDs (see above) linked to a linking unit. These Drug-linkers can be used as intermediates for the synthesis of Conjugates comprising dimers of PBDs linked to a targeting agent.

These Drug-Linkers have the following formula VI:

LU-D (VI)

or a pharmaceutically acceptable salt or solvate thereof, wherein LU is a Linker unit and D is a Drug unit that is a PBD dimer.

In the Drug-Linkers of the present invention, the PBD dimer D is of formula I, or a pharmaceutically acceptable salt or solvate thereof, except that X is selected from the group comprising: *-O-^{q}, *-S-^{q}, *-CO_2-^{q}, *-CO-^{q}, *-NH(C=O)-^{q}, *-NHNH-^{q}, *-CONHNH-^{q},



, wherein R^N is selected from the group comprising H and C_{1-4} alkyl, and the asterix indicates the point of attachment to the remainder of the Drug unit and the wavy line or ^{q} indicates the point of attachment to the Linker Unit.

In some embodiments, the drug linkers are of formula III as defined above.

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).

The pharmaceutically acceptable cation may be inorganic or organic.

Examples of pharmaceutically acceptable monovalent inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+. Examples of pharmaceutically

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

Substituents

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

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

Examples of substituents are described in more detail below.

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

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

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

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

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

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, -CH=CH_2), 1-propenyl (-CH=CH-CH_3), 2-propenyl (allyl, -CH-CH=CH_2), isopropenyl (1-methylvinyl, -C(CH_3)=CH_2), butenyl (C_4), pentenyl (C_5), and hexenyl (C_6).

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

Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (-C≡CH) and 2-propynyl (propargyl, -CH_2-C≡CH).

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

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

saturated monocyclic hydrocarbon compounds:

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

unsaturated monocyclic hydrocarbon compounds:

cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclopropene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7) and methylcyclohexene (C_7); and

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

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

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

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:
N_1: aziridine (C_3), azetidine (C_4), pyrrolidine (tetrahydropyrrole) (C_5), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C_5), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C_5), piperidine (C_6), dihydropyridine (C_6), tetrahydropyridine (C_6), azepine (C_7);
O_1: oxirane (C_3), oxetane (C_4), oxolane (tetrahydrofuran) (C_5), oxole (dihydrofuran) (C_5), oxane (tetrahydropyran) (C_6), dihydropyran (C_6), pyran (C_6), oxepin (C_7);
S_1: thiirane (C_3), thietane (C_4), thiolane (tetrahydrothiophene) (C_5), thiane (tetrahydrothiopyran) (C_6), thiepane (C_7);
O_2: dioxolane (C_5), dioxane (C_6), and dioxepane (C_7);
O_3: trioxane (C_6);
N_2: imidazolidine (C_5), pyrazolidine (diazolidine) (C_5), imidazoline (C_5), pyrazoline (dihydropyrazole) (C_5), piperazine (C_6);
N_1O_1: tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);
N_1S_1: thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);
N_2O_1: oxadiazine (C_6);
O_1S_1: oxathiole (C_5) and oxathiane (thioxane) (C_6); and,
$N_1O_1S_1$: oxathiazine (C_6).

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

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

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

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

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

Alternatively, the ring atoms may include one or more heteroatoms, as in "heteroaryl groups". Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

N_1: pyrrole (azole) (C_5), pyridine (azine) (C_6);

O_1: furan (oxole) (C_5);

S_1: thiophene (thiole) (C_5);

N_1O_1: oxazole (C_5), isoxazole (C_5), isoxazine (C_6);

N_2O_1: oxadiazole (furazan) (C_5);

N_3O_1: oxatriazole (C_5);

N_1S_1: thiazole (C_5), isothiazole (C_5);

N_2: imidazole (1,3-diazole) (C_5), pyrazole (1,2-diazole) (C_5), pyridazine (1,2-diazine) (C_6), pyrimidine (1,3-diazine) (C_6) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (C_6);

N_3: triazole (C_5), triazine (C_6); and,

N_4: tetrazole (C_5).

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

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

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

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

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

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

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

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

Hydroxy: -OH.

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

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

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

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

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

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

Oxo (keto, -one): =O.

Thione (thioketone): =S.

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

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

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

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

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

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

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

Imidic acid: -C(=NH)OH.

Hydroxamic acid: -C(=NOH)OH.

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

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

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

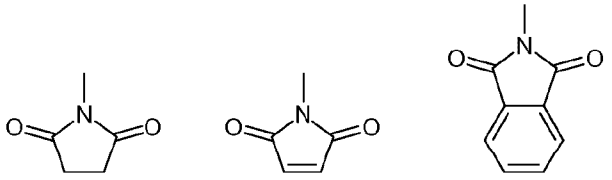
Amino: -NR^1R^2, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of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

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

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

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

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



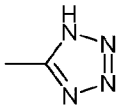
succinimidyl maleimidyl phthalimidyl

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

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

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

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



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

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

Nitro: -NO_2.

Nitroso: -NO.

Azido: -N_3.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

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

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

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

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

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

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

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

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

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

Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ (mesylate) and $-OS(=O)_2CH_2CH_3$ (esylate).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Phosphoramidate: -OP(=O)(OR^1)-NR$^2_2$, where R^1 and R^2 are phosphoramidate substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group.

Examples of phosphoramidate groups include, but are not limited to, -OP(=O)(OCH$_2$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, -CH$_2$CH$_2$CH$_2$- (propylene), -CH$_2$CH$_2$CH$_2$CH$_2$- (butylene), -CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$- (pentylene) and -CH$_2$CH$_2$CH$_2$CH-$_2$CH$_2$CH$_2$CH$_2$- (heptylene).

Examples of branched saturated C_{3-12} alkylene groups include, but are not limited to, -CH(CH$_3$)CH$_2$-, -CH(CH$_3$)CH$_2$CH$_2$-, -CH(CH$_3$)CH$_2$CH$_2$CH$_2$-, -CH$_2$CH(CH$_3$)CH$_2$-, -CH$_2$CH(CH$_3$)CH$_2$CH$_2$-, -CH(CH$_2$CH$_3$)-, -CH(CH$_2$CH$_3$)CH$_2$-, and -CH$_2$CH(CH$_2$CH$_3$)CH$_2$-.

Examples of linear partially unsaturated C_{3-12} alkylene groups (C_{3-12} alkenylene, and alkynylene groups) include, but are not limited to, -CH=CH-CH$_2$-, -CH$_2$-CH=CH$_2$-, -CH=CH-CH$_2$-CH$_2$-, -CH=CH-CH$_2$-CH$_2$-CH$_2$-, -CH=CH-CH=CH-, -CH=CH-CH=CH-CH$_2$-, -CH=CH-CH=CH-CH$_2$-CH$_2$-, -CH=CH-CH$_2$-CH=CH-, -CH=CH-CH$_2$-CH$_2$-CH=CH-, and -CH$_2$-C≡C-CH$_2$-.

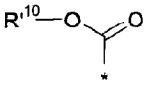
Examples of branched partially unsaturated C_{3-12} alkylene groups (C_{3-12} alkenylene and alkynylene groups) include, but are not limited to, -C(CH$_3$)=CH-, -C(CH$_3$)=CH-CH$_2$-, -CH=CH-CH(CH$_3$)- and -C≡C-CH(CH$_3$)-.

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

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

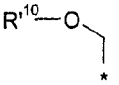
Oxygen protecting group: the term "oxygen protecting group" refers to a moiety which masks a hydroxy group, and these are well known in the art. A large number of suitable groups are described on pages 23 to 200 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999. 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.

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.

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 (L) and at the other end to the PBD dimer compound (D).

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

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

or a pharmaceutically acceptable salt or solvate thereof, 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 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.

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

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

Also illustrated as:

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

or a pharmaceutically acceptable salt or solvate thereof, 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 Spacer unit (L^2),

a is 1 or 2,

-L^1- is a Specificity unit linked to a Spacer 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:

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

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

$$L\text{-}(A^1\text{-}L^1\text{-}D)_p \quad \text{or}$$

$$L\text{-}(A^1\text{-}D)_p$$

or a pharmaceutically acceptable salt or solvate thereof, wherein L, A^1, a, L^1, s, L^2, D, y and p are as described above.

The present invention is suitable for use in providing a PBD compound to a preferred site in a subject. In the preferred embodiments, the conjugate allows the release of an active PBD compound that does not retain any part of the linker. There is no stub present that could affect the reactivity of the PBD compound.

In certain embodiments, the invention provides conjugates comprising a PBD dimer group having a linker connected to a cell binding agent. The present inventors describe herein methods of synthesis that enable such dimer conjugates to be prepared.

The linker attaches the Ligand Unit (L), e.g. antibody, to the PBD drug moiety D through covalent bond(s). The linker is a bifunctional or multifunctional moiety which can be used to link one or more drug moiety (D) and an antibody unit (Ab) to form antibody-drug conjugates (ADC). The linker may be stable outside a cell, i.e. extracellular, or it may be cleavable by enzymatic activity, hydrolysis, or other metabolic conditions. Antibody-drug conjugates (ADC) can be conveniently prepared using a linker having reactive functionality for binding to the drug moiety and to the antibody. A cysteine thiol, or an amine, e.g. N-terminus or amino acid side chain such as lysine, of the antibody (Ab) can form a bond with a functional group of a linker or spacer reagent, PBD drug moiety (D) or drug-linker reagent ($D\text{-}R^L$).

The linkers of the ADC preferably prevent aggregation of ADC molecules and keep the ADC freely soluble in aqueous media and in a monomeric state.

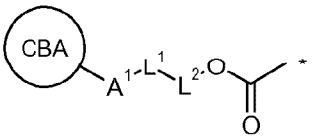
The linkers of the ADC are preferably stable extracellularly. Before transport or delivery into a cell, the antibody-drug conjugate (ADC) is preferably stable and remains intact, i.e. the antibody remains linked to the drug moiety. The linkers are stable outside the target cell and may be cleaved at some efficacious rate inside the cell. An effective linker will: (i) maintain the specific binding properties of the antibody; (ii) allow intracellular delivery of the conjugate or drug moiety; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its targetted site; and (iv) maintain a cytotoxic, cell-killing effect or a cytostatic effect of the PBD drug moiety. Stability of the ADC may be measured by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS.

Covalent attachment of the antibody and the drug moiety requires the linker to have two reactive functional groups, i.e. bivalency in a reactive sense. Bivalent linker reagents which are useful to attach two or more functional or biologically active moieties, such as peptides, nucleic acids, drugs, toxins, antibodies, haptens, and reporter groups are known, and methods have been described their resulting conjugates (Hermanson, G.T. (1996) Bioconjugate Techniques; Academic Press: New York, p 234-242).

In another embodiment, the linker may be substituted with groups which modulate aggregation, solubility or reactivity. For example, a sulfonate substituent may increase

water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody or the drug moiety, or facilitate the coupling reaction of Ab-L with D, or D-L with Ab, depending on the synthetic route employed to prepare the ADC.

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



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

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:

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

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

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

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

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

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

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

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

-C(=O)NH-,

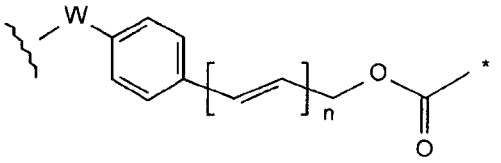
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-,
-NHC(=O)NH, and
-O- (a glycosidic bond).

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

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

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

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

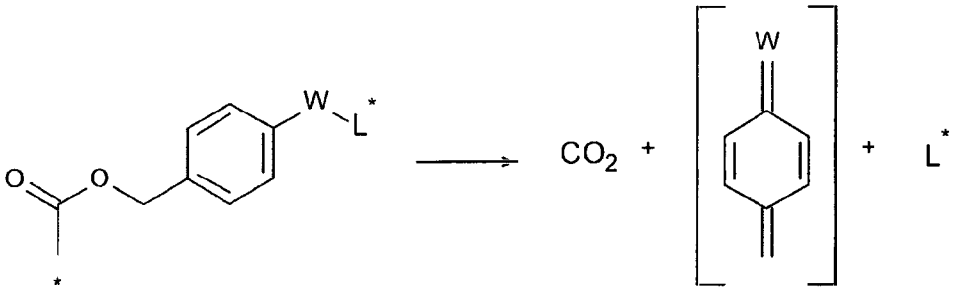


where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to the L^1, W is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-, and n is 0 to 3. The phenylene ring is optionally substituted with one, two or three substituents as described herein.

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

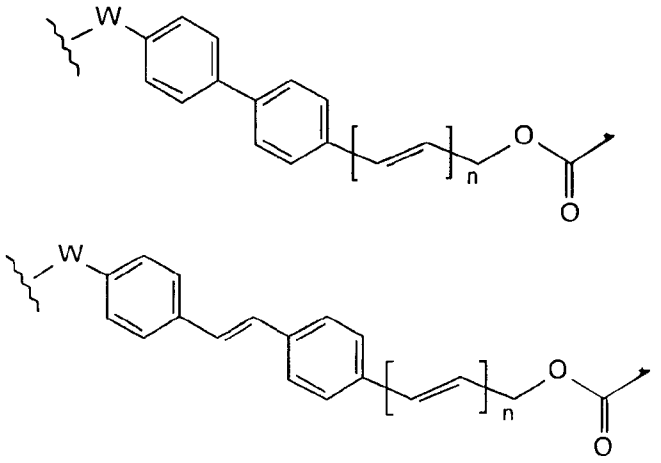
Where W is NH and n is 0, the self-immolative group may be referred to as a p-aminobenzylcarbonyl linker (PABC).

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



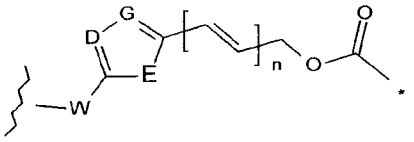
where the asterisk indicates the attachment to the Drug, L* is the activated form of the remaining portion of the linker and the released Drug unit is not shown. These groups have the advantage of separating the site of activation from the Drug.

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



where the asterisk, the wavy line, W, 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 W substituent is optionally substituted and the phenylene ring not having the W substituent is unsubstituted.

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



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

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

In one embodiment, L^1 comprises a dipeptide. The amino acids in the dipeptide may be any combination of natural amino acids and non-natural amino acids. In some embodiments, the dipeptide comprises natural amino acids. Where the linker is a cathepsin labile linker, the dipeptide is the site of action for cathepsin-mediated cleavage. The dipeptide then is a recognition site for cathepsin.

In one embodiment, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

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

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

-Phe-Lys-,

-Val-Ala-,

-Val-Lys-,

-Ala-Lys-, and

-Val-Cit-.

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

Other dipeptide combinations of interest include:

-Gly-Gly-,

-Pro-Pro-, and

-Val-Glu-.

Other dipeptide combinations may be used, including those described by Dubowchik et al.

In one embodiment, the amino acid side chain is chemically protected, where appropriate. The side chain protecting group may be a group as discussed below. Protected amino acid sequences are cleavable by enzymes. For example, a dipeptide sequence comprising a Boc side chain-protected Lys residue is cleavable by cathepsin.

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

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

Arg: Z, Mtr, Tos;

Asn: Trt, Xan;

Asp: Bzl, t-Bu;

Cys: Acm, Bzl, Bzl-OMe, Bzl-Me, Trt;

Glu: Bzl, t-Bu;

Gln: Trt, Xan;
His: Boc, Dnp, Tos, Trt;
Lys: Boc, Z-Cl, Fmoc, Z;
Ser: Bzl, TBDMS, TBDPS;
Thr: Bz;
Trp: Boc;
Tyr: Bzl, Z, Z-Br.

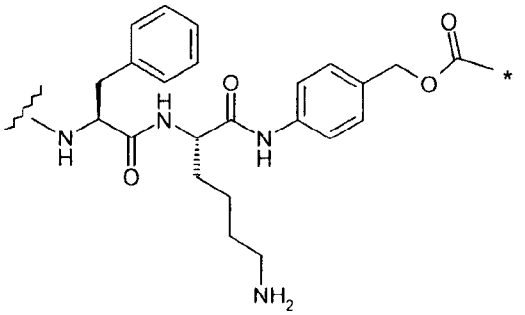
In one embodiment, $-X_2-$ is connected indirectly to the Drug unit. In such an embodiment, the Spacer unit L^2 is present.

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

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

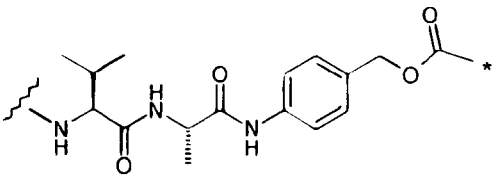
In one embodiment, $-X_1-$ is connected directly to A^1. Preferably the group $NH-X_1-$ (the amino terminus of X_1) is connected to A^1. A^1 may comprise the functionality -CO- thereby to form an amide link with $-X_1-$.

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



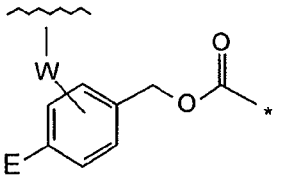
where the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of L^1 or the point of attachment to A^1. Preferably, the wavy line indicates the point of attachment to A^1.

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

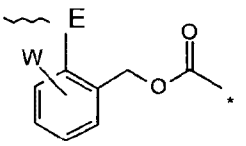


where the asterisk and the wavy line are as defined above.

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



or



,

where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to A^1, W 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 W may be a covalent bond.

The group W may be a functional group selected from:

-C(=O)-

-NH-

-O-

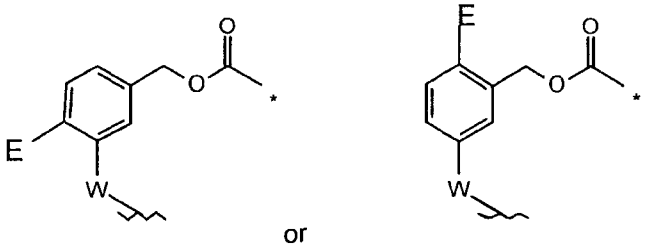
-C(=O)NH-,

-C(=O)O-,

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

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

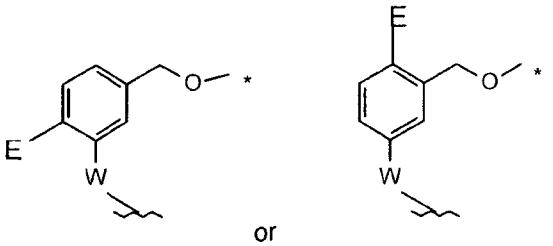
In some embodiments, L^1 and L^2 together with -OC(=O)- represent:



or

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

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



or

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, W is a covalent bond or a functional group and E is glucuronic acid (e.g., β-glucuronic acid). W is preferably a functional group selected from –NH-, -CH_2–, -O-, and -S-.

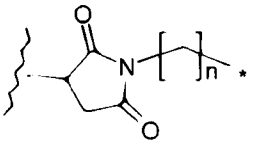
In some further embodiments, W is a functional group as set forth above, the functional group is linked to an amino acid, and the amino acid is linked to the Stretcher unit A^1. In some embodiments, amino acid is β-alanine. In such an embodiment, the amino acid is equivalently considered part of the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

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

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

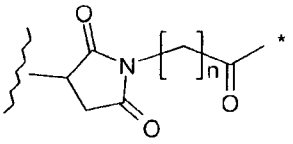
-C(=O)NH-,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)NH-, and
-NHC(=O)NH-.

In one embodiment, the group A^1 is:



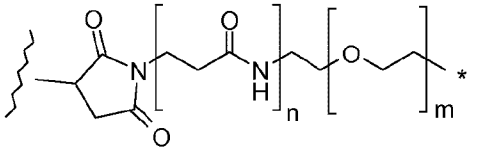
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



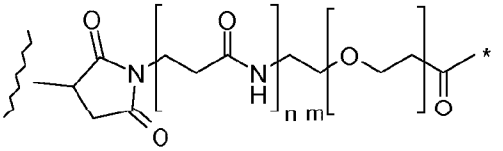
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



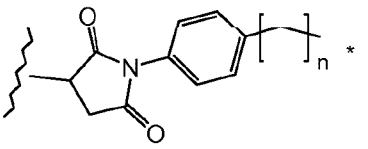
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group A^1 is:



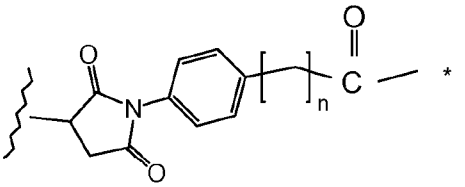
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

In one embodiment, the group A^1 is:



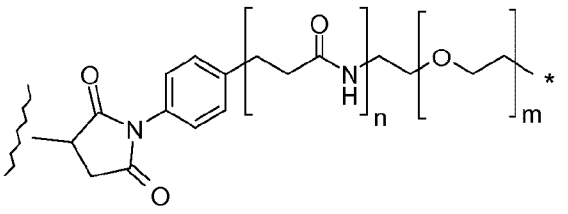
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



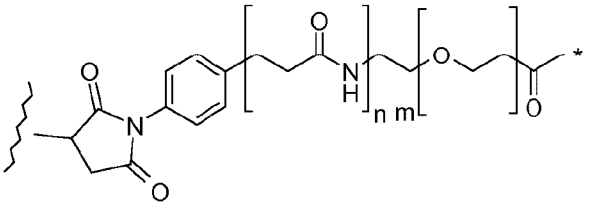
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A^1 is:



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

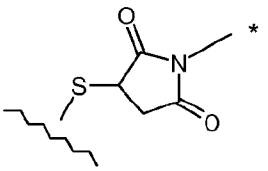
In one embodiment, the group A^1 is:



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

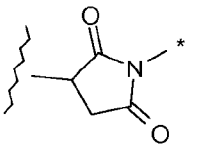
In one embodiment, the connection between the Ligand unit and A^1 is through a thiol residue of the Ligand unit and a maleimide group of A^1.

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



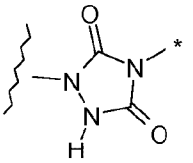
where the asterisk indicates the point of attachment to the remaining portion of A^1, L^1, L^2 or D, and the wavy line indicates the point of attachment to the remaining portion of the Ligand unit. In this embodiment, the S atom is typically derived from the Ligand unit.

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



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

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



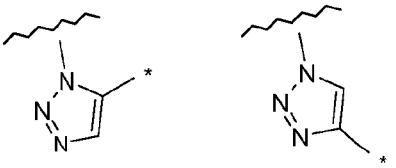
where the wavy line indicates point of attachment to the Ligand unit, and the asterisk indicates the bond to the remaining portion of the A^1 group , or to L^1, L^2 or D.

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

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

Of these -C(=O)CH_2- may be preferred especially when the carbonyl group is bound to –NH-.

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



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

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

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

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

-C(=O)N<,
-C(=O)O-,
-NHC(=O)-,
-OC(=O)-,
-OC(=O)O-,
-NHC(=O)O-,
-OC(=O)N<, and
-NHC(=O)N<,

where N< or O- are part of D.

In one embodiment, L^1 and D are preferably connected by a bond selected from:

-C(=O)N<, and
-NHC(=O)-.

In one embodiment, L^1 comprises a dipeptide and one end of the dipeptide is linked to D. As described above, the amino acids in the dipeptide may be any combination of natural amino acids and non-natural amino acids. In some embodiments, the dipeptide comprises natural amino acids. Where the linker is a cathepsin labile linker, the dipeptide is the site of action for cathepsin-mediated cleavage. The dipeptide then is a recognition site for cathepsin.

In one embodiment, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is selected from:

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

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

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

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

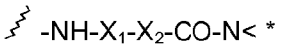
Most preferably, the group $-X_1-X_2-$ in dipeptide, $-NH-X_1-X_2-CO-$, is -Phe-Lys- or -Val-Ala-.

Other dipeptide combinations of interest include:

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

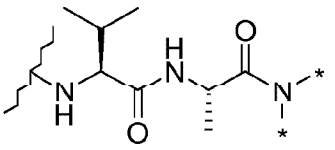
Other dipeptide combinations may be used, including those described above.

In one embodiment, L^1-D is:



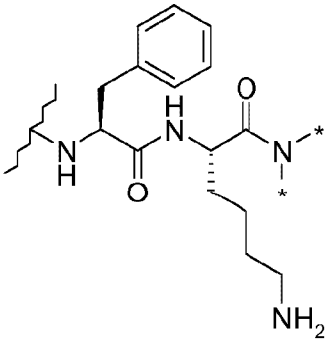
where -NH-X_1-X_2-CO is the dipeptide, -N< is part of the Drug unit, the asterisk indicates the points of attachment to the remainder of the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of L^1 or the point of attachment to A^1. Preferably, the wavy line indicates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

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



where the asterisks, -N< and the wavy line are as defined above.

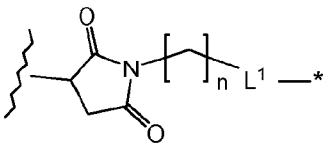
In one embodiment, the dipeptide is phenylalnine-lysine and L^1-D is:



where the asterisks, -N< and the wavy line are as defined above.

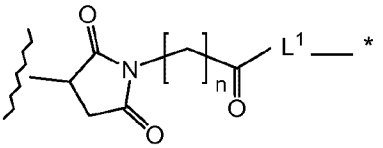
In one embodiment, the dipeptide is valine-citrulline.

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



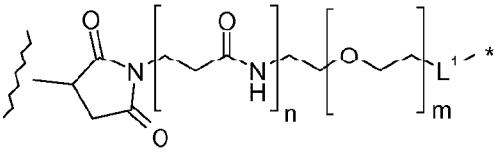
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



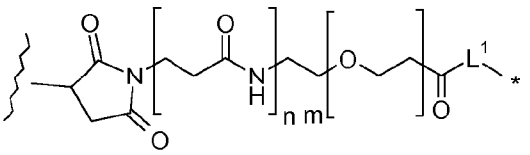
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



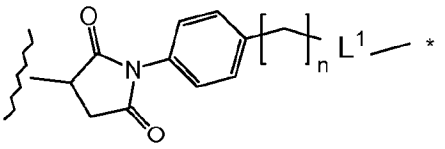
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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



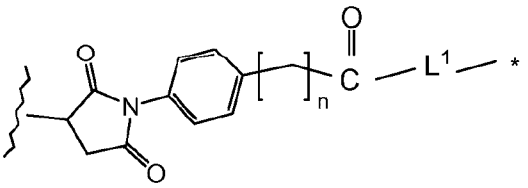
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 3 to 7, most preferably 3 or 7.

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



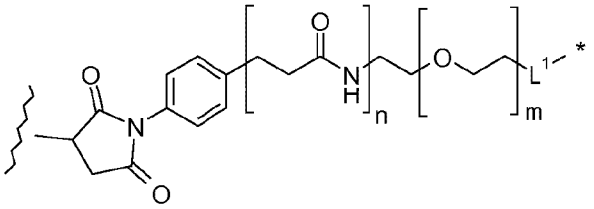
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



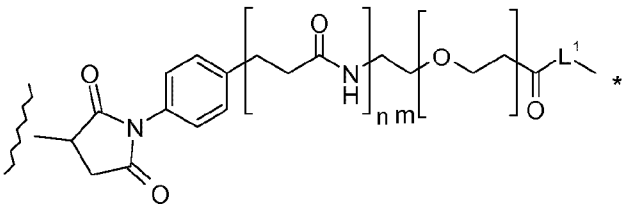
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



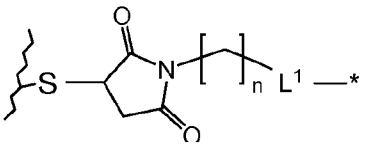
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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



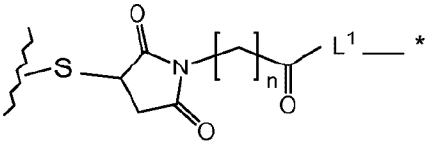
where the asterisk indicates the point of attachment to L^2 orD, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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



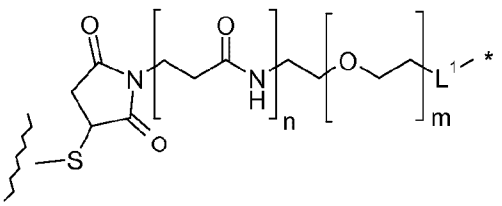
where the asterisk indicates the point of attachment to L^2 or D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the rest of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



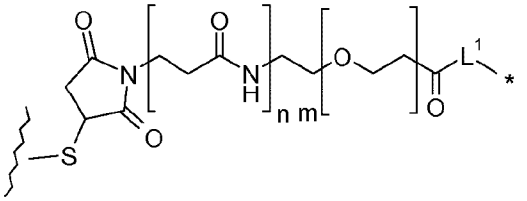
where the asterisk indicates the point of attachment to L^2 or D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



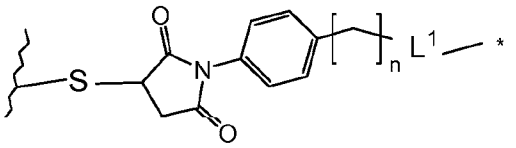
where the asterisk indicates the point of attachment to L^2 or D, S is a sulfur group of the Ligand unit, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8,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

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



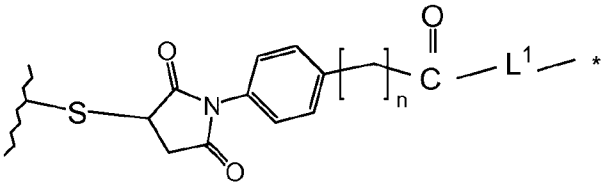
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 7, preferably 4 to 8, most preferably 4 or 8.

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



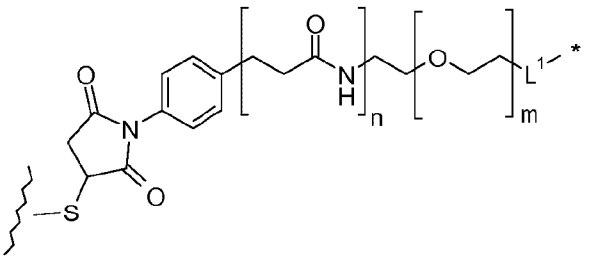
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



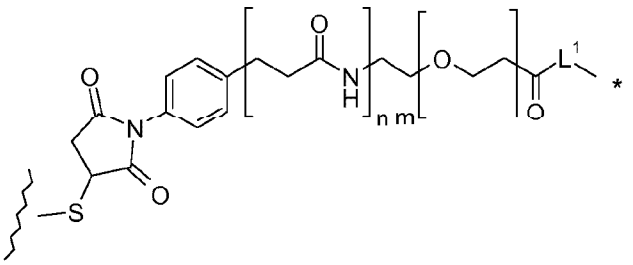
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, and n is 0 to 6. In one embodiment, n is 5.

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



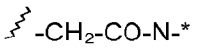
where the asterisk indicates the point of attachment to L^2 or D, the wavy line indicates the point of attachment to the remainder of the Ligand unit, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, most preferably 4 or 8.

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:

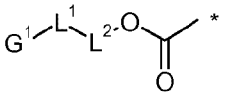


where the asterisk indicates the point of attachment to the remainder of the Stretcher unit, L^1 or D, and the wavy line indicates the point of attachment to the Ligand unit.

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(=O)- 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.

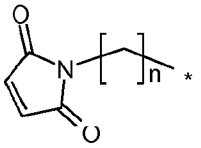
In certain embodiments of the invention, the group L^1 includes a Lys amino acid residue. The side chain of this amino acid may be protected with a Boc or Alloc protected group. A Boc protecting group is most preferred.

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.

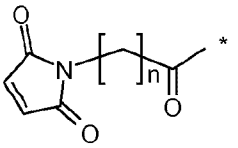
In one embodiment, the group G^1 is an alkyl maleimide group. This group is suitable for reaction with thiol groups, particularly cysteine thiol groups, present in the cell binding agent, for example present in an antibody.

In one embodiment, the group G^1 is:



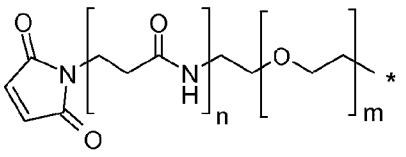
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



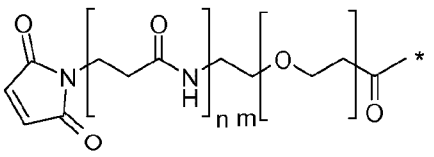
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



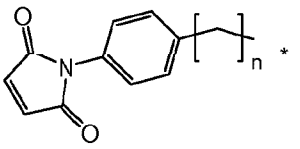
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

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:

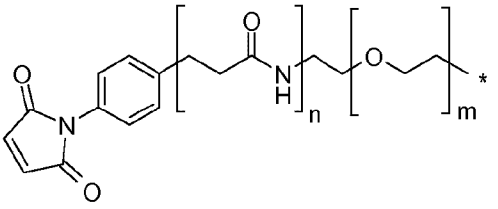


where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:

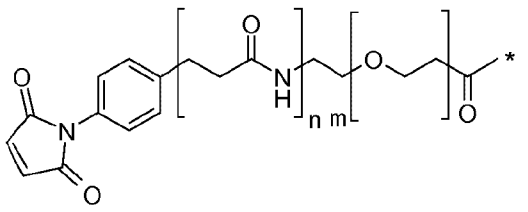
where the asterisk indicates the point of attachment to L^1, L^2 or D, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group G^1 is:



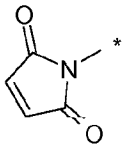
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8.

In one embodiment, the group G^1 is:



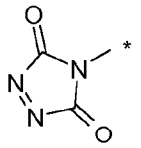
where the asterisk indicates the point of attachment to L^1, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8.

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).

Of these, $-C(=O)CH_2X$ may be preferred, especially when the carbonyl group is bound to –NH-.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

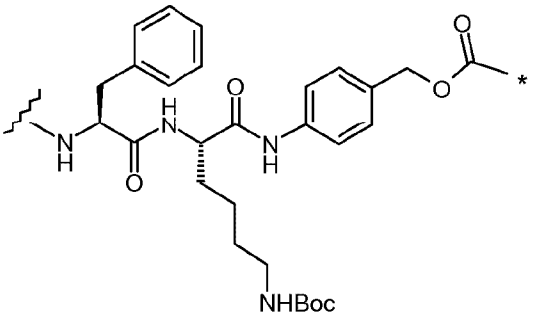
In other embodiments, the Linker unit is a precursor to the Linker uit having an active group. In this embodiment, the Linker unit comprises the active group, which is protected by way of a protecting group. The protecting group may be removed to provide the Linker unit having an active group.

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

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

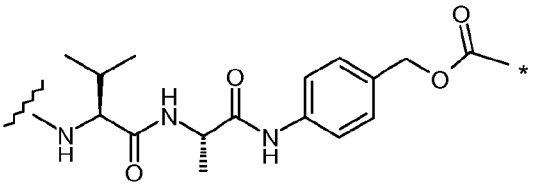
In one embodiment, the protecting group is orthogonal to the capping group. Thus, the active group protecting group is removable whilst retaining the capping group. In other embodiments, the protecting group and the capping group is removable under the same conditions as those used to remove the capping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

In one embodiment, the Linker unit is:



where the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to the remaining portion of the Linker unit, as applicable or the point of attachment to G^1. Preferably, the wavy line indicates the point of attachment to G^1.

In one embodiment, the Linker unit is:



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

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

Ligand Unit

The Ligand Unit may be of any kind, and include a protein, polypeptide, peptide and a non-peptidic agent that specifically binds to a target molecule. In some embodiments, the

Ligand unit may be a protein, polypeptide or peptide. In some embodiments, the Ligand unit may be a cyclic polypeptide. These Ligand units can include antibodies or a fragment of an antibody that contains at least one target molecule-binding site, lymphokines, hormones, growth factors, or any other cell binding molecule or substance that can specifically bind to a target. The ligand Unit is also referred to herein as a "binding agent" or "targeting agent".

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.

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).

Peptides

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

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

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

Antibodies

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

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and scFv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597 or from transgenic mice carrying a fully human immunoglobulin system (Lonberg (2008) Curr. Opinion 20(4):450-459).

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4816567; and Morrison *et al* (1984) *Proc. Natl. Acad. Sci.* USA, 81:6851-6855). Chimeric antibodies include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey or Ape) and human constant region sequences.

An "intact antibody" herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence

constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.,* IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

Humanisation

Techniques to reduce the *in vivo* immunogenicity of a non-human antibody or antibody fragment include those termed "humanisation".

A "humanized antibody" refers to a polypeptide comprising at least a portion of a modified variable region of a human antibody wherein a portion of the variable region, preferably a portion substantially less than the intact human variable domain, has been substituted by the corresponding sequence from a non-human species and wherein the modified variable region is linked to at least another part of another protein, preferably the constant region of a human antibody. The expression "humanized antibodies" includes human antibodies in which one or more complementarity determining region ("CDR") amino acid residues and/or one or more framework region ("FW" or "FR") amino acid residues are substituted by amino acid residues from analogous sites in rodent or other non-human antibodies. The expression "humanized antibody" also includes an immunoglobulin amino acid sequence variant or fragment thereof that comprises an FR having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. Or, looked at another way, a humanized antibody is a human antibody that also contains selected sequences from non-human (e.g. murine) antibodies in place of the human sequences. A humanized antibody can include conservative amino acid substitutions or non-natural residues from the same or different species that do not significantly alter its binding and/or biologic activity. Such antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulins.

There are a range of humanisation techniques, including 'CDR grafting', 'guided selection', 'deimmunization', 'resurfacing' (also known as 'veneering'), 'composite antibodies', 'Human String Content Optimisation' and framework shuffling.

CDR grafting

In this technique, the humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, camel, bovine, goat, or rabbit having the desired properties (in effect, the non-human CDRs are 'grafted' onto the human framework). In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues (this may happen when, for example, a particular FR residue has significant effect on antigen binding).

Furthermore, humanized antibodies can comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. Thus, in general, a humanized antibody will comprise all of at least one, and in one aspect two, variable domains, in which all or all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), or that of a human immunoglobulin.

Guided selection

The method consists of combining the V_H or V_L domain of a given non-human antibody specific for a particular epitope with a human V_H or V_L library and specific human V

domains are selected against the antigen of interest. This selected human VH is then combined with a VL library to generate a completely human VHxVL combination. The method is described in Nature Biotechnology (N.Y.) 12, (1994) 899-903.

Composite antibodies

In this method, two or more segments of amino acid sequence from a human antibody are combined within the final antibody molecule. They are constructed by combining multiple human VH and VL sequence segments in combinations which limit or avoid human T cell epitopes in the final composite antibody V regions. Where required, T cell epitopes are limited or avoided by, exchanging V region segments contributing to or encoding a T cell epitope with alternative segments which avoid T cell epitopes. This method is described in US 2008/0206239 A1.

Deimmunization

This method involves the removal of human (or other second species) T-cell epitopes from the V regions of the therapeutic antibody (or other molecule). The therapeutic antibodies V-region sequence is analysed for the presence of MHC class II- binding motifs by, for example, comparison with databases of MHC-binding motifs (such as the "motifs" database hosted at www.wehi.edu.au). Alternatively, MHC class II- binding motifs may be identified using computational threading methods such as those devised by Altuvia et al. (J. Mol. Biol. 249 244-250 (1995)); in these methods, consecutive overlapping peptides from the V-region sequences are testing for their binding energies to MHC class II proteins. This data can then be combined with information on other sequence features which relate to successfully presented peptides, such as amphipathicity, Rothbard motifs, and cleavage sites for cathepsin B and other processing enzymes.

Once potential second species (e.g. human) T-cell epitopes have been identified, they are eliminated by the alteration of one or more amino acids. The modified amino acids are usually within the T-cell epitope itself, but may also be adjacent to the epitope in terms of the primary or secondary structure of the protein (and therefore, may not be adjacent in the primary structure). Most typically, the alteration is by way of substitution but, in some circumstances amino acid addition or deletion will be more appropriate.

All alterations can be accomplished by recombinant DNA technology, so that the final molecule may be prepared by expression from a recombinant host using well established

methods such as Site Directed Mutagenesis. However, the use of protein chemistry or any other means of molecular alteration is also possible.

Resurfacing

This method involves:

(a) determining the conformational structure of the variable region of the non-human (e.g. rodent) antibody (or fragment thereof) by constructing a three-dimensional model of the non-human antibody variable region;

(b) generating sequence alignments using relative accessibility distributions from x-ray crystallographic structures of a sufficient number of non-human and human antibody variable region heavy and light chains to give a set of heavy and light chain framework positions wherein the alignment positions are identical in 98% of the sufficient number of non-human antibody heavy and light chains;

(c) defining for the non-human antibody to be humanized, a set of heavy and light chain surface exposed amino acid residues using the set of framework positions generated in step (b);

(d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to the set of surface exposed amino acid residues defined in step (c), wherein the heavy and light chain from the human antibody are or are not naturally paired;

(e) substituting, in the amino acid sequence of the non-human antibody to be humanized, the set of heavy and light chain surface exposed amino acid residues defined in step (c) with the set of heavy and light chain surface exposed amino acid residues identified in step (d);

(f) constructing a three-dimensional model of the variable region of the non-human antibody resulting from the substituting specified in step (e);

(g) identifying, by comparing the three-dimensional models constructed in steps (a) and (f), any amino acid residues from the sets identified in steps (c) or (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of the non-human antibodt to be humanized; and

(h) changing any residues identified in step (g) from the human to the original non-human amino acid residue to thereby define a non-human antibody humanizing set of surface exposed amino acid residues; with the proviso that step (a) need not be conducted first, but must be conducted prior to step (g).

Superhumanization

The method compares the non-human sequence with the functional human germline gene repertoire. Those human genes encoding canonical structures identical or closely related to the non-human sequences are selected. Those selected human genes with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these human FRs. This method is described in patent WO 2005/079479 A2.

Human String Content Optimization

This method compares the non-human (e.g. mouse) sequence with the repertoire of human germline genes and the differences are scored as Human String Content (HSC) that quantifies a sequence at the level of potential MHC/T-cell epitopes. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants (described in Molecular Immunology, 44, (2007) 1986–1998).

Framework Shuffling

The CDRs of the non-human antibody are fused in-frame to cDNA pools encompassing all known heavy and light chain human germline gene frameworks. Humanised antibodies are then selected by e.g. panning of the phage displayed antibody library. This is described in *Methods* **36**, 43-60 (2005).

Examples of cell binding agents include those agents described for use in WO 2007/085930.

Tumour-associate antigens and cognate antibodies for use in embodiments of the present invention are listed below.

TUMOR-ASSOCIATED ANTIGENS AND COGNATE ANTIBODIES

(1) *BMPR1B (bone morphogenetic protein receptor-type IB)*

Nucleotide

Genbank accession no. NM_001203

Genbank version no. NM_001203.2 GI:169790809

Genbank record update date: Sep 23, 2012 02:06 PM

Polypeptide

Genbank accession no. NP_001194
Genbank version no. NP_001194.1 GI:4502431
Genbank record update date: Sep 23, 2012 02:06 PM

Cross-references
ten Dijke,P., *et al Science* 264 (5155): 101-104 (1994), *Oncogene* 14 10 (11):1377-1382 (1997)); WO2004/063362 (Claim 2); WO2003/042661 (Claim 12); US2003/134790-A1 (Page 38-39); WO2002/102235 (Claim 13; Page 296); WO2003/055443
(Page 91-92); WO2002/99122 (Example 2; Page 528-530); WO2003/029421 (Claim 6); WO2003/024392 (Claim 2; Fig 112); WO2002/98358 (Claim 1; Page 183); WO2002/54940 (Page 100-101); WO2002/59377(Page 349-350); WO2002/30268 (Claim 27; Page 376); WO2001/48204 (Example; Fig 4); NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1.; MIM:603248; AY065994

(2) *E16 (LAT1, SLC7A5)*

Nucleotide
Genbank accession no. NM_003486
Genbank version no. NM_003486.5 GI:71979931
Genbank record update date: Jun 27, 2012 12:06 PM

Polypeptide
Genbank accession no. NP_003477
Genbank version no. NP_003477.4 GI:71979932
Genbank record update date: Jun 27, 2012 12:06 PM

Cross references
Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), *Nature* 395 (6699):288-291 (1998), Gaugitsch, H.W., *et al* (1992) *J. Biol. Chem.* 267 (16):11267-11273); WO2004/048938 (Example 2); WO2004/032842 (Example IV); WO2003/042661 (Claim 12); WO2003/016475 (Claim 1); WO2002/78524 (Example 2); WO2002/99074 (Claim 19; Page 127-129); WO2002/86443 (Claim 27; Pages 222, 393); WO2003/003906 (Claim 10; Page 293); WO2002/64798 (Claim 33; Page 93-95); WO2000/14228 (Claim 5; Page 133-136); US2003/224454 (Fig 3);

25 WO2003/025138 (Claim 12; Page 150); NP_003477 solute carrier family 7 (cationic amino acid transporter, y+system), member 5 /pid=NP_003477.3 - Homo sapiens; MIM:600182;; NM_015923.

(3) STEAP1 (six transmembrane epithelial antigen of prostate)

Nucleotide

Genbank accession no. NM_012449

Genbank version no. NM_012449.2 GI:22027487

Genbank record update date: Sep 9, 2012 02:57 PM

Polypeptide

Genbank accession no. NP_036581

Genbank version no. NP_036581.1 GI:9558759

Genbank record update date: Sep 9, 2012 02:57 PM

Cross references

Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., *et al* (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96 (25):14523-14528); WO2004/065577 (Claim 6); WO2004/027049 (Fig 1L); EP1394274 (Example 11); WO2004/016225 (Claim 2); WO2003/042661 (Claim 12); US2003/157089 (Example 5); US2003/185830 (Example 5); US2003/064397 (Fig 2); WO2002/89747 (Example 5; Page 618-619); WO2003/022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A); six transmembrane epithelial antigen of the prostate; MIM:604415.

(4) 0772P (CA125, MUC16)

Nucleotide

Genbank accession no. AF361486

Genbank version no. AF361486.3 GI:34501466

Genbank record update date: Mar 11, 2010 07:56 AM

Polypeptide

Genbank accession no. AAK74120

Genbank version no. AAK74120.3 GI:34501467

Genbank record update date: Mar 11, 2010 07:56 AM

Cross references
J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004/045553 (Claim 14); WO2002/92836 (Claim 6; Fig 12); WO2002/83866 (Claim 15; Page 116-121); US2003/124140 (Example 16); GI:34501467;

(5) *MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin)*
Nucleotide
Genbank accession no. NM_005823
Genbank version no. NM_005823.5 GI:293651528
Genbank record update date: Sep 2, 2012 01:47 PM

Polypeptide
Genbank accession no. NP_005814
Genbank version no. NP_005814.2 GI:53988378
Genbank record update date: Sep 2, 2012 01:47 PM

Cross references
Yamaguchi, N., *et al Biol. Chem.* 269 (2), 805-808 (1994), *Proc. Natl. Acad. Sci. U.S.A.* 96 (20):11531-11536 (1999), *Proc. Natl. Acad. Sci. U.S.A.* 93 10 (1):136-140 (1996), *J. Biol. Chem.* 270 (37):21984-21990 (1995)); WO2003/101283 (Claim 14); (WO2002/102235 (Claim 13; Page 287-288); WO2002/101075 (Claim 4; Page 308- 309); WO2002/71928 (Page 320-321); WO94/10312 (Page 52-57); IM:601051.

(6) *Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b)*
Nucleotide
Genbank accession no. NM_006424
Genbank version no. NM_006424.2 GI:110611905
Genbank record update date: Jul 22, 2012 03:39 PM

Polypeptide
Genbank accession no. NP_006415
Genbank version no. NP_006415.2 GI:110611906
Genbank record update date: Jul 22, 2012 03:39 PM

Cross references

J. Biol. Chem. 277 (22):19665-19672 (2002), *Genomics* 62 (2):281-284 (1999), Feild, J.A., *et al* (1999) *Biochem. Biophys. Res. Commun.* 258 (3):578-582); WO2004/022778 (Claim 2); EP1394274 (Example 11); WO2002/102235 (Claim 13; Page 20 326); EP0875569 (Claim 1; Page 17-19); WO2001/57188 (Claim 20; Page 329); WO2004/032842 (Example IV); WO2001/75177 (Claim 24; Page 139-140); MIM:604217.

(7) *Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, 25 sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B)*

Nucleotide

Genbank accession no. AB040878

Genbank version no. AB040878.1 GI:7959148

Genbank record update date: Aug 2, 2006 05:40 PM

Polypeptide

Genbank accession no. BAA95969

Genbank version no. BAA95969.1 GI:7959149

Genbank record update date: Aug 2, 2006 05:40 PM

Cross references

Nagase T., *et al* (2000) *DNA Res.* 7 (2):143-150); WO2004/000997 (Claim 1); WO2003/003984 (Claim 1); WO2002/06339 (Claim 1; Page 50); WO2001/88133 (Claim 1; Page 41-43, 48-58); WO2003/054152 (Claim 20); WO2003/101400 (Claim 11); Accession: 30 Q9P283; Genew; HGNC:10737

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA
2700050C12 gene)

Nucleotide

Genbank accession no. AY358628

Genbank version no. AY358628.1 GI:37182377

Genbank record update date: Dec 1, 2009 04:15 AM

Polypeptide

Genbank accession no. AAQ88991
Genbank version no. AAQ88991.1 GI:37182378
Genbank record update date: Dec 1, 2009 04:15 AM

Cross references
Ross *et al* (2002) *Cancer Res.* 62:2546-2553; US2003/129192 (Claim 2); US2004/044180 (Claim 12); US2004/044179 35 (Claim 11); US2003/096961 (Claim 11); US2003/232056 (Example 5); WO2003/105758 16 (Claim 12); US2003/206918 (Example 5); EP1347046 (Claim 1); WO2003/025148 (Claim 20); GI:37182378.

(9) *ETBR (Endothelin type B receptor)*
Nucleotide
Genbank accession no. AY275463
Genbank version no. AY275463.1 GI:30526094
Genbank record update date: Mar 11, 2010 02:26 AM

Polypeptide
Genbank accession no. AAP32295
Genbank version no. AAP32295.1 GI:30526095
Genbank record update date: Mar 11, 2010 02:26 AM

Cross references
Nakamuta M., *et al Biochem. Biophys. Res. Commun.* 177, 34-39, 1991; Ogawa Y., *et al Biochem. Biophys. Res. Commun.* 178, 248-255, 1991; Arai H., *et al Jpn. Circ. J.* 56, 1303-1307, 1992; Arai H., *et al J. Biol. Chem.* 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., *et al Biochem. Biophys. Res. Commun.* 178, 656-663, 1991; Elshourbagy N.A., *et al J. Biol. Chem.* 268, 3873-3879, 1993; Haendler B., *et al J. Cardiovasc. Pharmacol.* 20, s1-S4, 1992; Tsutsumi M., *et al Gene* 228, 43-49, 1999; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002; Bourgeois C., *et al J. Clin. Endocrinol. Metab.* 82, 3116-3123, 1997;
Okamoto Y., *et al Biol. Chem.* 272, 21589-21596, 1997; Verheij J.B., *et al Am. J. Med. Genet.* 108, 223-225, 2002; Hofstra R.M.W., *et al Eur. J. Hum. Genet.* 5, 180-185, 1997; Puffenberger E.G., *et al Cell* 79, 1257-1266, 1994; Attie T., *et al, Hum. Mol. Genet.* 4, 2407-

15 2409, 1995; Auricchio A., *et al Hum. Mol. Genet.* 5:351-354, 1996; Amiel J., *et al Hum. Mol.*
Genet. 5, 355-357, 1996; Hofstra R.M.W., *et al Nat. Genet.* 12, 445-447, 1996; Svensson P.J., *et al Hum. Genet.* 103, 145-148, 1998; Fuchs S., *et al Mol. Med.* 7, 115-124, 2001; Pingault V., *et al (2002) Hum. Genet.* 111, 198-206; WO2004/045516 (Claim 1); WO2004/048938 (Example 2); WO2004/040000 (Claim 151); WO2003/087768 (Claim 1); WO2003/016475 (Claim 1); WO2003/016475 (Claim 1); WO2002/61087 (Fig 1); WO2003/016494 (Fig 6); WO2003/025138 (Claim 12; Page 144); WO2001/98351 (Claim 1;
Page 124-125); EP0522868 (Claim 8; Fig 2); WO2001/77172 (Claim 1; Page 297-299); US2003/109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004/001004.

(10) MSG783 (RNF124, hypothetical protein FLJ20315)

Nucleotide

Genbank accession no. NM_017763
Genbank version no. NM_017763.4 GI:167830482
Genbank record update date: Jul 22, 2012 12:34 AM

Polypeptide

Genbank accession no. NP_060233
Genbank version no. NP_060233.3 GI:56711322
Genbank record update date: Jul 22, 2012 12:34 AM

Cross references

WO2003/104275 (Claim 1); WO2004/046342 (Example 2); WO2003/042661 (Claim 12); WO2003/083074 (Claim 14; Page 61); WO2003/018621 (Claim 1); WO2003/024392 (Claim 2; Fig 93); WO2001/66689 (Example 6); LocusID:54894.

(11) *STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer*
associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein)

Nucleotide

Genbank accession no. AF455138
Genbank version no. AF455138.1 GI:22655487

Genbank record update date: Mar 11, 2010 01:54 AM

Polypeptide

Genbank accession no. AAN04080

Genbank version no. AAN04080.1 GI:22655488

Genbank record update date: Mar 11, 2010 01:54 AM

Cross references

Lab. Invest. 82 (11):1573-1582 (2002)); WO2003/087306; US2003/064397 (Claim 1; Fig 1); WO2002/72596 (Claim 13; Page 54-55); WO2001/72962 (Claim 1; Fig 4B); 35 WO2003/104270 (Claim 11); WO2003/104270 (Claim 16); US2004/005598 (Claim 22); WO2003/042661 (Claim 12); US2003/060612 (Claim 12; Fig 10); WO2002/26822 (Claim 23; Fig 2); WO2002/16429 (Claim 12; Fig 10); GI:22655488.

(12) *TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation 5 channel, subfamily M, member 4)*

Nucleotide

Genbank accession no. NM_017636

Genbank version no. NM_017636.3 GI:304766649

Genbank record update date: Jun 29, 2012 11:27 AM

Polypeptide

Genbank accession no. NP_060106

Genbank version no. NP_060106.2 GI:21314671

Genbank record update date: Jun 29, 2012 11:27 AM

Cross references

Xu, X.Z., *et al Proc. Natl. Acad. Sci. U.S.A.* 98 (19):10692-10697 (2001), *Cell* 109 (3):397-407 (2002), *J. Biol. Chem.* 278 (33):30813-30820 (2003)); US2003/143557 (Claim 4); WO2000/40614 (Claim 14; Page 100-103); WO2002/10382 (Claim 1; Fig 9A); WO2003/042661 (Claim 12); WO2002/30268 (Claim 27; Page 391); US2003/219806 (Claim 4); WO2001/62794 (Claim 10 14; Fig 1A-D); MIM:606936.

(13) *CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor)*

Nucleotide
Genbank accession no. NM_003212
Genbank version no. NM_003212.3 GI:292494881
Genbank record update date: Sep 23, 2012 02:27 PM

Polypeptide
Genbank accession no. NP_003203
Genbank version no. NP_003203.1 GI:4507425
Genbank record update date: Sep 23, 2012 02:27 PM

Cross references
Ciccodicola, A., *et al EMBO J.* 8 (7):1987-1991 (1989), *Am. J. Hum. Genet.* 49 (3):555-565 (1991)); US2003/224411 (Claim 1); WO2003/083041 (Example 1); WO2003/034984 (Claim 12); WO2002/88170 (Claim 2; Page 52-53); WO2003/024392 (Claim 2; Fig 58); WO2002/16413 (Claim 1; Page 94-95, 105); WO2002/22808 (Claim 2; Fig 1); US5854399 (Example 2; Col 17-18); US5792616 (Fig 2); MIM:187395.

(14) *CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792)*

Nucleotide
Genbank accession no M26004
Genbank version no. M26004.1 GI:181939
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide
Genbank accession no. AAA35786
Genbank version no. AAA35786.1 GI:181940
Genbank record update date: Jun 23, 2010 08:47 AM

Cross references
Fujisaku *et al* (1989) *J. Biol. Chem.* 264 (4):2118-2125); Weis J.J., *et al J. Exp. Med.* 167, 1047-1066, 1988; Moore M., *et al Proc. Natl. Acad. Sci. U.S.A.* 84, 9194-9198, 1987; Barel M., *et al Mol. Immunol.* 35, 1025-1031, 1998; Weis J.J., *et al Proc. Natl. Acad. Sci. U.S.A.* 83, 5639-5643, 1986; Sinha S.K., *et al* (1993) *J. Immunol.* 150, 5311-5320; WO2004/045520 (Example 4); US2004/005538 (Example 1); WO2003/062401 (Claim 9);

WO2004/045520 (Example 4); WO91/02536 (Fig 9.1-9.9); WO2004/020595 (Claim 1); Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) *CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29)*

Nucleotide

Genbank accession no NM_000626

Genbank version no. NM_000626.2 GI:90193589

Genbank record update date: Jun 26, 2012 01:53 PM

Polypeptide

Genbank accession no. NP_000617

Genbank version no. NP_000617.1 GI:11038674

Genbank record update date: Jun 26, 2012 01:53 PM

Cross references

Proc. Natl. Acad. Sci. U.S.A. (2003) 100 (7):4126-4131, *Blood* (2002) 100 (9):3068-3076, Muller *et al* (1992) *Eur. J. Immunol.* 22 (6):1621-1625); WO2004/016225 (claim 2, Fig 140); WO2003/087768, US2004/101874 (claim 1, page 102); WO2003/062401 (claim 9); WO2002/78524 (Example 2); US2002/150573 (claim 35 5, page 15); US5644033; WO2003/048202 (claim 1, pages 306 and 309); WO 99/58658, US6534482 (claim 13, Fig 17A/B); WO2000/55351 (claim 11, pages 1145-1146); MIM:147245

(16) *FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 5 1a), SPAP1B, SPAP1C)*

Nucleotide

Genbank accession no NM_030764

Genbank version no. NM_030764.3 GI:227430280

Genbank record update date: Jun 30, 2012 12:30 AM

Polypeptide

Genbank accession no. NP_110391

Genbank version no. NP_110391.2 GI:19923629

Genbank record update date: Jun 30, 2012 12:30 AM

Cross references

AY358130); *Genome Res.* 13 (10):2265-2270 (2003), *Immunogenetics* 54 (2):87-95 (2002), *Blood* 99 (8):2662-2669 (2002), *Proc. Natl. Acad. Sci. U.S.A.* 98 (17):9772-9777 (2001), Xu, M.J., *et al* (2001) *Biochem. Biophys. Res. Commun.* 280 (3):768-775; WO2004/016225 (Claim 2); WO2003/077836; WO2001/38490 (Claim 5; Fig 18D-1-18D-2); WO2003/097803 (Claim 12); WO2003/089624 (Claim 25);: MIM:606509.

(17) *HER2 (ErbB2)*

Nucleotide

Genbank accession no M11730

Genbank version no. M11730.1 GI:183986

Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

Genbank accession no. AAA75493

Genbank version no. AAA75493.1 GI:306840

Genbank record update date: Jun 23, 2010 08:47 AM

Cross references

Coussens L., *et al Science* (1985) 230(4730):1132-1139); Yamamoto T., *et al Nature* 319, 230-234, 1986; Semba K., *et al Proc. Natl. Acad. Sci. U.S.A.* 82, 6497-6501, 1985; Swiercz J.M., *et al J. Cell Biol.* 165, 869- 15 880, 2004; Kuhns J.J., *et al J. Biol. Chem.* 274, 36422-36427, 1999; Cho H.-S., *et al Nature* 421, 756-760, 2003; Ehsani A., *et al* (1993) *Genomics* 15, 426-429; WO2004/048938 (Example 2); WO2004/027049 (Fig 1I); WO2004/009622; WO2003/081210; WO2003/089904 (Claim 9); WO2003/016475 (Claim 1); US2003/118592; WO2003/008537 (Claim 1); WO2003/055439 (Claim 29; Fig 1A-B); WO2003/025228 (Claim 37; Fig 5C); WO2002/22636 (Example 13; Page 95-107); WO2002/12341 (Claim 68; Fig 7); WO2002/13847 (Page 71-74); WO2002/14503 (Page 114-117); WO2001/53463 (Claim 2; Page 41-46); WO2001/41787 (Page 15); WO2000/44899 (Claim 52; Fig 7); WO2000/20579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61);

EP1439393 (Claim 7); WO2004/043361 (Claim 7); WO2004/022709; WO2001/00244 25 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1

ANTIBODIES

Abbott: US20110177095

For example, an antibody comprising CDRs having overall at least 80% sequence identity to CDRs having amino acid sequences of SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:104 and/or SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2), and SEQ ID NO:8 (CDR-L3), wherein the anti-HER2 antibody or anti-HER2 binding fragment has reduced immunogenicity as compared to an antibody having a VH of SEQ ID NO:1 and a VL of SEQ ID NO:2.

Biogen: US20100119511

For example, ATCC accession numbers: PTA-10355, PTA-10356, PTA-10357, PTA-10358

For example, a purified antibody molecule that binds to HER2 comprising a all six CDR's from an antibody selected from the group consisting of BIIB71F10 (SEQ ID NOs:11, 13), BIIB69A09 (SEQ ID NOs:15, 17); BIIB67F10 (SEQ ID NOs:19, 21); BIIB67F11 (SEQ ID NOs:23, 25), BIIB66A12 (SEQ ID NOs:27, 29), BIIB66C01 (SEQ ID NOs:31, 33), BIIB65C10 (SEQ ID NOs:35, 37), BIIB65H09 (SEQ ID NOs:39, 41) and BIIB65B03 (SEQ ID NOs:43, 45), or CDRs which are identical or which have no more than two alterations from said CDRs.

Herceptin (Genentech) - US6,054,297; ATCC accession no. CRL-10463 (Genentech)

Pertuzumab (Genentech)

US20110117097

for example, see SEQ IDs No. 15&16, SEQ IDs No. 17&18, SEQ IDs No. 23&24 & ATCC accession numbers HB-12215, HB-12216, CRL 10463, HB-12697.

US20090285837

US20090202546

for example, ATCC accession numbers: HB-12215, HB-12216, CRL 10463, HB-12698.

US20060088523

- for example, ATCC accession numbers: HB-12215, HB-12216
- for example, an antibody comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.
- for example, an antibody comprising a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24

US20060018899

- for example, ATCC accession numbers: (7C2) HB-12215, (7F3) HB-12216, (4D5) CRL-10463, (2C4) HB-12697.
- for example, an antibody comprising the amino acid sequence in SEQ ID No. 23, or a deamidated and/or oxidized variant thereof.

US2011/0159014

- for example, an antibody having a light chain variable domain comprising the hypervariable regions of SEQ ID NO: 1".
- For example, an antibody having a heavy chain variable domain comprising the hypervariable regions of SEQ ID NO: 2.

US20090187007

Glycotope: TrasGEX antibody http://www.glycotope.com/pipeline

For example, see International Joint Cancer Institute and Changhai Hospital Cancer Cent: HMTI-Fc Ab - Gao J., et al *BMB Rep*. 2009 Oct 31;42(10):636-41.

Symphogen: US20110217305

Union Stem Cell &Gene Engineering, China - Liu HQ., et al *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2010 May;26(5):456-8.

(18) *NCA (CEACAM6)*

Nucleotide

Genbank accession no M18728

Genbank version no. M18728.1 GI:189084

Genbank record update date: Jun 23, 2010 08:48 AM

Polypeptide

Genbank accession no. AAA59907

Genbank version no. AAA59907.1 GI:189085

Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

Barnett T., *et al Genomics* 3, 59-66, 1988; Tawaragi Y., *et al Biochem. Biophys. Res. Commun.* 150, 89-96, 1988; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99:16899-16903, 2002; WO2004/063709; EP1439393 (Claim 7); WO2004/044178 (Example 4); WO2004/031238; WO2003/042661 (Claim 12); WO2002/78524 (Example 2); WO2002/86443 (Claim 27; Page 427); WO2002/60317 (Claim 2); Accession: P40199; Q14920; EMBL; M29541; AAA59915.1.

EMBL; M18728.

(19) *MDP (DPEP1)*

Nucleotide

Genbank accession no BC017023

Genbank version no. BC017023.1 GI:16877538

Genbank record update date: Mar 6, 2012 01:00 PM

Polypeptide

Genbank accession no. AAH17023

Genbank version no. AAH17023.1 GI:16877539

Genbank record update date: Mar 6, 2012 01:00 PM

Cross references

Proc. Natl. Acad. Sci. U.S.A. 99 (26):16899-16903 (2002)); WO2003/016475 (Claim 1); WO2002/64798 (Claim 33; Page 85- 87); JP05003790 (Fig 6-8); WO99/46284 (Fig 9); MIM:179780.

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

Nucleotide

Genbank accession no AF184971

Genbank version no. AF184971.1 GI:6013324

Genbank record update date: Mar 10, 2010 10:00 PM

Polypeptide

Genbank accession no. AAF01320

Genbank version no. AAF01320.1 GI:6013325

Genbank record update date: Mar 10, 2010 10:00 PM

Cross references

Clark H.F., *et al Genome Res.* 13, 2265-2270, 2003; Mungall A.J., *et al Nature* 425, 805-811, 2003; Blumberg H., *et al Cell* 104, 9-19, 2001; Dumoutier L., *et al J. Immunol.* 167, 3545-3549,

2001; Parrish-Novak J., *et al J. Biol. Chem.* 277, 47517-47523, 2002; Pletnev S., *et al* (2003)

10 *Biochemistry* 42:12617-12624; Sheikh F., *et al* (2004) *J. Immunol.* 172, 2006-2010;

EP1394274 (Example 11); US2004/005320 (Example 5); WO2003/029262 (Page 74-75); WO2003/002717 (Claim 2; Page 63); WO2002/22153 (Page 45-47); US2002/042366 (Page

20-21); WO2001/46261 (Page 57-59); WO2001/46232 (Page 63-65); WO98/37193 (Claim 1;

Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) *Brevican (BCAN, BEHAB)*

Nucleotide

Genbank accession no AF229053

Genbank version no. AF229053.1 GI:10798902

Genbank record update date: Mar 11, 2010 12:58 AM

Polypeptide

Genbank accession no. AAG23135

Genbank version no. AAG23135.1 GI:10798903

Genbank record update date: Mar 11, 2010 12:58 AM

Cross references

Gary S.C., *et al Gene* 256, 139-147, 2000; Clark H.F., *et al Genome Res.* 13, 2265-2270, 2003; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002;

US2003/186372 (Claim 11); US2003/186373 (Claim 11); US2003/119131 (Claim 1; Fig 52); US2003/119122 (Claim 1; 20 Fig 52); US2003/119126 (Claim 1); US2003/119121 (Claim 1; Fig 52); US2003/119129 (Claim 1); US2003/119130 (Claim 1); US2003/119128 (Claim 1; Fig 52); US2003/119125 (Claim 1); WO2003/016475 (Claim 1); WO2002/02634 (Claim 1)

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

Nucleotide

Genbank accession no NM_004442

Genbank version no. NM_004442.6 GI:111118979

Genbank record update date: Sep 8, 2012 04:43 PM

Polypeptide

Genbank accession no. NP_004433

Genbank version no. NP_004433.2 GI:21396504

Genbank record update date: Sep 8, 2012 04:43 PM

Cross references

Chan,J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000)); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42); MIM:600997.

(23) *ASLG659 (B7h)*

Nucleotide

Genbank accession no. AX092328

Genbank version no. AX092328.1 GI:13444478

Genbank record update date: Jan 26, 2011 07:37 AM

Cross references

US2004/0101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); US2003/165504 (Claim 1); US2003/124140 (Example 2); US2003/065143 (Fig 60); WO2002/102235 (Claim 13; Page 299); US2003/091580 (Example 2); WO2002/10187

(Claim 6; Fig 10); WO2001/94641 (Claim 12; Fig 7b); WO2002/02624 (Claim 13; Fig 1A-1B); US2002/034749 (Claim 54; Page 45-46); WO2002/06317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO2002/71928 (Page 468-469); WO2002/02587 (Example 1; Fig 1); WO2001/40269 (Example 3; Pages 190-192); WO2000/36107 (Example 2; Page 205-207); WO2004/053079 (Claim 12); WO2003/004989 (Claim 1); WO2002/71928 (Page 233-234, 452-453); WO 01/16318.

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

Nucleotide

Genbank accession no AJ297436

Genbank version no. AJ297436.1 GI:9367211

Genbank record update date: Feb 1, 2011 11:25 AM

Polypeptide

Genbank accession no. CAB97347

Genbank version no. CAB97347.1 GI:9367212

Genbank record update date: Feb 1, 2011 11:25 AM

Cross references

Reiter R.E., *et al Proc. Natl. Acad. Sci. U.S.A.* 95, 1735-1740, 1998; Gu Z., *et al Oncogene* 19,

1288-1296, 2000; *Biochem. Biophys. Res. Commun.* (2000) 275(3):783-788; WO2004/022709; EP1394274 (Example 11); US2004/018553 (Claim 17); WO2003/008537 (Claim 1); WO2002/81646 (Claim 1; Page 164); WO2003/003906 (Claim 10; Page 288); WO2001/40309 (Example 1; Fig 17); US2001/055751 (Example 1; Fig 1b); WO2000/32752 (Claim 18; Fig 1); WO98/51805 (Claim 17; Page 97); WO98/51824 (Claim 10; Page 94); WO98/40403 (Claim 2; Fig 1B); Accession: O43653; EMBL; AF043498; AAC39607.1

(25) *GEDA*

Nucleotide

Genbank accession no AY260763

Genbank version no. AY260763.1 GI:30102448

Genbank record update date: Mar 11, 2010 02:24 AM

Polypeptide

Genbank accession no. AAP14954
Genbank version no. AAP14954.1 GI:30102449
Genbank record update date: Mar 11, 2010 02:24 AM

Cross references
AP14954 lipoma HMGIC fusion-partnerlike protein /pid=AAP14954.1 - Homo sapiens (human); WO2003/054152 (Claim 20); WO2003/000842 (Claim 1); WO2003/023013 (Example 3, Claim 20); US2003/194704 (Claim 45); GI:30102449;

(26) *BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3)*
Nucleotide
Genbank accession no AF116456
Genbank version no. AF116456.1 GI:4585274
Genbank record update date: Mar 10, 2010 09:44 PM

Polypeptide
Genbank accession no. AAD25356
Genbank version no. AAD25356.1 GI:4585275
Genbank record update date: Mar 10, 2010 09:44 PM

Cross references
BAFF receptor /pid=NP_443177.1 - Homo sapiens: Thompson, J.S., *et al Science* 293 (5537), 2108-2111 (2001); WO2004/058309; WO2004/011611; WO2003/045422 (Example; Page 32-33); WO2003/014294 (Claim 35; Fig 6B); WO2003/035846 (Claim 70; Page 615-616); WO2002/94852 (Col 136-137); WO2002/38766 25 (Claim 3; Page 133); WO2002/24909 (Example 3; Fig 3); MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) *CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814)*
Nucleotide
Genbank accession no AK026467
Genbank version no. AK026467.1 GI:10439337
Genbank record update date: Sep 11, 2006 11:24 PM

Polypeptide

Genbank accession no. BAB15489
Genbank version no. BAB15489.1 GI:10439338
Genbank record update date: Sep 11, 2006 11:24 PM

Cross references
Wilson *et al* (1991) *J. Exp. Med.* 173:137-146; 30 WO2003/072036 (Claim 1; Fig 1); IM:107266; NP_001762.1; NM_001771_1.

(27a) *CD22 (CD22 molecule)*

Nucleotide
Genbank accession no X52785
Genbank version no. X52785.1 GI:29778
Genbank record update date: Feb 2, 2011 10:09 AM

Polypeptide
Genbank accession no. CAA36988
Genbank version no. CAA36988.1 GI:29779
Genbank record update date: Feb 2, 2011 10:09 AM

Cross references
Stamenkovic I. et al., *Nature* 345 (6270), 74-77 (1990)??

Other information
Official Symbol: CD22
Other Aliases: SIGLEC-2, SIGLEC2
Other Designations: B-cell receptor CD22; B-lymphocyte cell adhesion molecule; BL-CAM; CD22 antigen; T-cell surface antigen Leu-14; sialic acid binding Ig-like lectin 2; sialic acid-binding Ig-like lectin 2

ANTIBODIES
G5/44 (Inotuzumab): DiJoseph JF.,et al *Cancer Immunol Immunother.* 2005 Jan;54(1):11-24.

Epratuzumab- Goldenberg DM., et al *Expert Rev Anticancer Ther.* 6(10): 1341-53, 2006.

(28) *CD79a (CD79A, CD79alpha), immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M*
35 molecules, transduces a signal involved in B-cell differentiation), pI: 4.84, MW: 25028 TM: 2
[P] Gene Chromosome: 19q13.2).
Nucleotide
Genbank accession no NM_001783
Genbank version no. NM_001783.3 GI:90193587
Genbank record update date: Jun 26, 2012 01:48 PM

Polypeptide
Genbank accession no. NP_001774
Genbank version no. NP_001774.1 GI:4502685
Genbank record update date: Jun 26, 2012 01:48 PM

Cross references
WO2003/088808, US2003/0228319; WO2003/062401 (claim 9); US2002/150573 (claim 4, pages 13-14); WO99/58658 (claim 13, Fig 16); WO92/07574 (Fig 1); US5644033; Ha *et al* (1992) *J. Immunol.* 148(5):1526-1531; Müller *et al* (1992) *Eur. J. Immunol..* 22:1621-1625; Hashimoto *et al* (1994) *Immunogenetics* 40(4):287-295; Preud'homme *et al* (1992) *Clin. Exp.*
5 *Immunol.* 90(1):141-146; Yu *et al* (1992) *J. Immunol.* 148(2) 633-637; Sakaguchi *et al* (1988)
EMBO J. 7(11):3457-3464

(29) *CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a*
10 role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia); 372 aa, pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3,
Nucleotide
Genbank accession no NM_001716
Genbank version no. NM_001716.4 GI:342307092

Genbank record update date: Sep 30, 2012 01:49 PM

Polypeptide

Genbank accession no. NP_001707

Genbank version no. NP_001707.1 GI:4502415

Genbank record update date: Sep 30, 2012 01:49 PM

Cross references

WO2004/040000; WO2004/015426; US2003/105292 (Example 2); US6555339 (Example 2); WO2002/61087 (Fig 1); WO2001/57188 (Claim 20, page 269); WO2001/72830 (pages 12-13); WO2000/22129 (Example 1, pages 152-153, 15 Example 2, pages 254-256); WO99/28468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO94/28931 (pages 56-58); WO92/17497 (claim 7, Fig 5); Dobner *et al* (1992) *Eur. J. Immunol.* 22:2795-2799; Barella *et al* (1995) *Biochem. J.* 309:773-779

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

Nucleotide

Genbank accession no NM_002120

Genbank version no. NM_002120.3 GI:118402587

Genbank record update date: Sep 8, 2012 04:46 PM

Polypeptide

Genbank accession no. NP_002111

Genbank version no. NP_002111.1 GI:4504403

Genbank record update date: Sep 8, 2012 04:46 PM

Cross references

Tonnelle *et al* (1985) *EMBO J.* 4(11):2839-2847; Jonsson *et al* (1989) *Immunogenetics* 29(6):411-413; Beck *et al* (1992) *J. Mol. Biol.* 228:433-441; Strausberg *et al* (2002) *Proc. Natl. Acad. Sci USA* 99:16899- 16903; Servenius *et al* (1987) *J. Biol. Chem.* 262:8759-8766; Beck *et al* (1996) *J. Mol. Biol.* 25 255:1-13; Naruse *et al* (2002) *Tissue Antigens* 59:512-519; WO99/58658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col

168-170); US6011146 (col 145-146); Kasahara *et al* (1989) *Immunogenetics* 30(1):66-68; Larhammar *et al* (1985) *J. Biol. Chem.* 260(26):14111-14119

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

Nucleotide

Genbank accession no NM_002561

Genbank version no. NM_002561.3 GI:325197202

Genbank record update date: Jun 27, 2012 12:41 AM

Polypeptide

Genbank accession no. NP_002552

Genbank version no. NP_002552.2 GI:28416933

Genbank record update date: Jun 27, 2012 12:41 AM

Cross references

Le *et al* (1997) *FEBS Lett.* 418(1-2):195-199; WO2004/047749; WO2003/072035 (claim 10); Touchman *et al* (2000) *Genome Res.* 10:165-173; WO2002/22660 (claim 20); WO2003/093444 (claim 1); WO2003/087768 (claim 1); WO2003/029277 (page 82)

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

Nucleotide

Genbank accession no NM_001782

Genbank version no. NM_001782.2 GI:194018444

Genbank record update date: Jun 26, 2012 01:43 PM

Polypeptide

Genbank accession no. NP_001773

Genbank version no. NP_001773.1 GI:4502683

Genbank record update date: Jun 26, 2012 01:43 PM

Cross references

WO2004042346 (claim 65); WO2003/026493 (pages 51-52, 57-58); WO2000/75655 (pages 105-106); Von Hoegen *et al* (1990) *J. Immunol.* 144(12):4870-4877; Strausberg *et al* (2002) *Proc. Natl. Acad. Sci USA* 99:16899-16903.

(33) *LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated*
with increased disease activity in patients with systemic lupus erythematosis); 661 aa, pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12).

Nucleotide

Genbank accession no NM_005582
Genbank version no. NM_005582.2 GI:167555126
Genbank record update date: Sep 2, 2012 01:50 PM

Polypeptide

Genbank accession no. NP_005573
Genbank version no. NP_005573.2 GI:167555127
Genbank record update date: Sep 2, 2012 01:50 PM

Cross references

US2002/193567; WO97/07198 (claim 11, pages 39-42); Miura *et al* (1996) 15 *Genomics* 38(3):299-304; Miura *et al* (1998) *Blood* 92:2815-2822; WO2003/083047; WO97/44452 (claim 8, pages 57-61); WO2000/12130 (pages 24-26).

(34) *FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain*
that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte 20 differentiation); 429 aa, pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22)

Nucleotide

Genbank accession no NM_052938
Genbank version no. NM_052938.4 GI:226958543
Genbank record update date: Sep 2, 2012 01:43 PM

Polypeptide

Genbank accession no. NP_443170

Genbank version no. NP_443170.1 GI:16418419
Genbank record update date: Sep 2, 2012 01:43 PM

Cross references

WO2003/077836; WO2001/38490 (claim 6, Fig 18E-1-18-E-2); Davis *et al* (2001) *Proc. Natl. Acad. Sci USA* 98(17):9772-9777; WO2003/089624 (claim 8); EP1347046 (claim 1); WO2003/089624 (claim 7).

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

Nucleotide

Genbank accession no AF343662
Genbank version no. AF343662.1 GI:13591709
Genbank record update date: Mar 11, 2010 01:16 AM

Polypeptide

Genbank accession no. AAK31325
Genbank version no. AAK31325.1 GI:13591710
Genbank record update date: Mar 11, 2010 01:16 AM

Cross references

AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse:AK089756, AY158090, AY506558; NP_112571.1; WO2003/024392 (claim 2, Fig 97); Nakayama *et al* (2000) *Biochem. Biophys. Res. Commun.* 277(1):124-127; WO2003/077836; WO2001/38490 (claim 3, Fig 18B-1-18B-2).

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

Nucleotide

Genbank accession no AF179274
Genbank version no. AF179274.2 GI:12280939

Genbank record update date: Mar 11, 2010 01:05 AM

Polypeptide

Genbank accession no. AAD55776

Genbank version no. AAD55776.2 GI:12280940

Genbank record update date: Mar 11, 2010 01:05 AM

Cross references

NCBI Accession: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; AY358907, CAF85723, CQ782436; WO2004/074320; JP2004113151; WO2003/042661; WO2003/009814; EP1295944 (pages 69-70); WO2002/30268 (page 329); WO2001/90304; US2004/249130; US2004/022727; WO2004/063355; US2004/197325; US2003/232350; 5 US2004/005563; US2003/124579; Horie *et al* (2000) *Genomics* 67:146-152; Uchida *et al* (1999) *Biochem. Biophys. Res. Commun.* 266:593-602; Liang *et al* (2000) *Cancer Res.* 60:4907-12; Glynne-Jones *et al* (2001) *Int J Cancer.* Oct 15; 94(2):178-84.

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

Nucleotide

Genbank accession no M99487

Genbank version no. M99487.1 GI:190663

Genbank record update date: Jun 23, 2010 08:48 AM

Polypeptide

Genbank accession no. AAA60209

Genbank version no. AAA60209.1 GI:190664

Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

Israeli R.S., et al *Cancer Res.* 53 (2), 227-230 (1993)

Other information

Official Symbol: FOLH1

Other Aliases: GIG27, FGCP, FOLH, GCP2, GCPII, NAALAD1, NAALAdase, PSM, PSMA, mGCP

Other Designations: N-acetylated alpha-linked acidic dipeptidase 1; N-acetylated-alpha-linked acidic dipeptidase I; NAALADase I; cell growth-inhibiting gene 27 protein; folylpoly-gamma-glutamate carboxypeptidase; glutamate carboxylase II; glutamate carboxypeptidase 2; glutamate carboxypeptidase II; membrane glutamate carboxypeptidase; prostate specific membrane antigen variant F; pteroylpoly-gamma-glutamate carboxypeptidase

ANTIBODIES

US 7,666,425:

Antibodies produces by Hybridomas having the following ATCC references:ATCC accession No. HB-12101, ATCC accession No. HB-12109, ATCC accession No. HB-12127 and ATCC accession No. HB-12126.

Proscan: a monoclonal antibody selected from the group consisting of 8H12, 3E11, 17G1, 29B4, 30C1 and 20F2 (US 7,811,564; Moffett S., et al *Hybridoma (Larchmt).* 2007 Dec;26(6):363-72).

Cytogen: monoclonal antibodies 7E11-C5 (ATCC accession No. HB 10494) and 9H10-A4 (ATCC accession No. HB11430) – US 5,763,202

GlycoMimetics: NUH2 - ATCC accession No. HB 9762 (US 7,135,301)

Human Genome Science: HPRAJ70 - ATCC accession No. 97131 (US 6,824,993); Amino acid sequence encoded by the cDNA clone (HPRAJ70) deposited as American Type Culture Collection ("ATCC") Deposit No. 97131

Medarex: Anti-PSMA antibodies that lack fucosyl residues - US 7,875,278

Mouse anti-PSMA antibodies include the 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6, 4C8B9, and monoclonal antibodies. Hybridomas secreting 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6 or 4C8B9 have been publicly deposited and are described in U.S. Pat. No. 6,159,508. Relevant hybridomas have been publicly deposited

and are described in U.S. Pat. No. 6,107,090. Moreover, humanized anti-PSMA antibodies, including a humanized version of J591, are described in further detail in PCT Publication WO 02/098897.

Other mouse anti-human PSMA antibodies have been described in the art, such as mAb 107-1A4 (Wang, S. et al. (2001) Int. J. Cancer 92:871-876) and mAb 2C9 (Kato, K. et al. (2003) Int. J. Urol. 10:439-444).

Examples of human anti-PSMA monoclonal antibodies include the 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 antibodies, isolated and structurally characterized as originally described in PCT Publications WO 01/09192 and WO 03/064606 and in U.S. Provisional Application Ser. No. 60/654,125, entitled "Human Monoclonal Antibodies to Prostate Specific Membrane Antigen (PSMA)", filed on Feb. 18, 2005. The V.sub.H amino acid sequences of 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 are shown in SEQ ID NOs: 1-9, respectively. The V.sub.L amino acid sequences of 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 are shown in SEQ ID NOs: 10-18, respectively.

Other human anti-PSMA antibodies include the antibodies disclosed in PCT Publication WO 03/034903 and US Application No. 2004/0033229.

NW Biotherapeutics: A hybridoma cell line selected from the group consisting of 3F5.4G6 having ATCC accession number HB12060, 3D7-1.I. having ATCC accession number HB12309, 4E10-1.14 having ATCC accession number HB12310, 3E11 (ATCC HB12488), 4D8 (ATCC HB12487), 3E6 (ATCC HB12486), 3C9 (ATCC HB12484), 2C7 (ATCC HB12490), 1G3 (ATCC HB12489), 3C4 (ATCC HB12494), 3C6 (ATCC HB12491), 4D4 (ATCC HB12493), 1G9 (ATCC HB12495), 5C8B9 (ATCC HB12492) and 3G6 (ATCC HB12485) – see US 6,150,508

PSMA Development Company / Progenics / Cytogen – Seattle Genetics: mAb 3.9, produced by the hybridoma deposited under ATCC Accession No. PTA-3258 or mAb 10.3, produced by the hybridoma deposited under ATCC Accession No. PTA-3347 - US 7,850,971

PSMA Development Company– Compositions of PSMA antibodies (US 20080286284, Table 1)

This application is a divisional of U.S. patent application Ser. No. 10/395,894, filed on Mar. 21, 2003 (US 7,850,971)

University Hospital Freiburg, Germany - mAbs 3/A12, 3/E7, and 3/F11 (Wolf P., et al *Prostate*. 2010 Apr 1;70(5):562-9).

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

(38.1) SSTR2 (Somatostatin receptor 2)

Nucleotide

Genbank accession no NM_001050

Genbank version no. NM_001050.2 GI:44890054

Genbank record update date: Aug 19, 2012 01:37 PM

Polypeptide

Genbank accession no. NP_001041

Genbank version no. NP_001041.1 GI:4557859

Genbank record update date: Aug 19, 2012 01:37 PM

Cross references

Yamada Y., et al Proc. Natl. Acad. Sci. U.S.A. 89 (1), 251-255 (1992); Susini C., et al Ann Oncol. 2006 Dec;17(12):1733-42

Other information

Official Symbol: SSTR2

Other Designations: SRIF-1; SS2R; somatostatin receptor type 2

(38.2) SSTR5 (Somatostatin receptor 5)

Nucleotide

Genbank accession no D16827

Genbank version no. D16827.1 GI:487683

Genbank record update date: Aug 1, 2006 12:45 PM

Polypeptide

Genbank accession no. BAA04107

Genbank version no. BAA04107.1 GI:487684

Genbank record update date: Aug 1, 2006 12:45 PM

Cross references

Yamada,Y., et al *Biochem. Biophys. Res. Commun.* 195 (2), 844-852 (1993)

Other information

Official Symbol: SSTR5

Other Aliases: SS-5-R

Other Designations: Somatostatin receptor subtype 5; somatostatin receptor type 5

(38.3) SSTR1

(38.4)SSTR3

(38.5) SSTR4

AvB6 – Both subunits (39+40)

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

Nucleotide

Genbank accession no M14648 J02826 M18365

Genbank version no. M14648.1 GI:340306

Genbank record update date: Jun 23, 2010 08:56 AM

Polypeptide

Genbank accession no. AAA36808

Genbank version no. AAA36808.1 GI:340307

Genbank record update date: Jun 23, 2010 08:56 AM

Cross references

Suzuki S., et al *Proc. Natl. Acad. Sci. U.S.A.* 83 (22), 8614-8618 (1986)

Other information

Official Symbol: ITGAV

Other Aliases: CD51, MSK8, VNRA, VTNR

Other Designations: antigen identified by monoclonal antibody L230; integrin alpha-V; integrin alphaVbeta3; integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51); vitronectin receptor subunit alpha

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

Nucleotide

Genbank accession no NM_000888

Genbank version no. NM_000888.3 GI:9966771

Genbank record update date: Jun 27, 2012 12:46 AM

Polypeptide

Genbank accession no. NP_000879

Genbank version no. NP_000879.2 GI:9625002

Genbank record update date: Jun 27, 2012 12:46 AM

Cross references

Sheppard D.J., et al *Biol. Chem.* 265 (20), 11502-11507 (1990)

Other information

Official Symbol: ITGB6

Other Designations: integrin beta-6

ANTIBODIES

Biogen: US 7,943,742 - Hybridoma clones 6.3G9 and 6.8G6 were deposited with the ATCC, accession numbers ATCC PTA-3649 and -3645, respectively.

Biogen: US7,465,449 - In some embodiments, the antibody comprises the same heavy and light chain polypeptide sequences as an antibody produced by hybridoma 6.1A8, 6.3G9, 6.8G6, 6.2B1, 6.2B10, 6.2A1, 6.2E5, 7.1G10, 7.7G5, or 7.1C5.

Centocor (J&J): US7,550,142; US7,163,681

For example in US 7,550,142 - an antibody having human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 7 and SEQ ID NO: 8.

Seattle Genetics: 15H3 (Ryan MC., et al Cancer Res April 15, 2012; 72(8 Supplement): 4630)

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

Nucleotide

Genbank accession no M17303

Genbank version no. M17303.1 GI:178676

Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

Genbank accession no. AAB59513

Genbank version no. AAB59513.1 GI:178677

Genbank record update date: Jun 23, 2010 08:47 AM

Cross references

Beauchemin N., et al *Mol. Cell. Biol.* 7 (9), 3221-3230 (1987)

Other information

Official Symbol: CEACAM5

Other Aliases: CD66e, CEA

Other Designations: meconium antigen 100

ANTIBODIES

AstraZeneca-MedImmune:US 20100330103; US20080057063; US20020142359

- for example an antibody having complementarity determining regions (CDRs) with the following sequences: heavy chain; CDR1 - DNYMH, CDR2 - WIDPENGDTE YAPKFRG, CDR3 - LIYAGYLAMD Y; and light chain CDR1 - SASSSVTYMH, CDR2 - STSNLAS, CDR3 - QQRSTYPLT.
- Hybridoma 806.077 deposited as European Collection of Cell Cultures (ECACC) deposit no. 96022936.

Research Corporation Technologies, Inc.:US5,047,507

Bayer Corporation: US6,013,772

BioAlliance: US7,982,017; US7,674,605

- US 7,674,605
  - an antibody comprising the heavy chain variable region sequence from the amino acid sequence of SEQ ID NO: 1, and the light chain variable region sequence from the amino acid sequence of SEQ ID NO:2.
  - an antibody comprising the heavy chain variable region sequence from the amino acid sequence of SEQ ID NO:5, and the light chain variable region sequence from the amino acid sequence of SEQ ID NO:6.

Celltech Therapeutics Limited: US5,877,293

The Dow Chemical Company: US5,472,693; US6,417,337; US6,333,405

US5,472,693 – for example, ATCC No. CRL-11215

US6,417,337 – for example, ATCC CRL-12208

US6,333,405 – for example, ATCC CRL-12208

Immunomedics, Inc: US7,534,431; US7,230,084; US7,300,644; US6,730,300; US20110189085

- an antibody having CDRs of the light chain variable region comprise: CDR1 comprises KASQDVGTSVA (SEQ ID NO: 20); CDR2 comprises WTSTRHT (SEQ ID NO: 21); and CDR3 comprises QQYSLYRS (SEQ ID NO: 22);
  and the CDRs of the heavy chain variable region of said anti-CEA antibody comprise: CDR1 comprises TYWMS (SEQ ID NO: 23); CDR2 comprises EIHPDSSTINYAPSLKD (SEQ ID NO: 24); and CDR3 comprises LYFGFPWFAY (SEQ ID NO: 25).

US20100221175; US20090092598; US20070202044; US20110064653; US20090185974; US20080069775.

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

Nucleotide

Genbank accession no M35073

Genbank version no. M35073.1 GI:187553
Genbank record update date: Mar 6, 2012 11:12 AM

Polypeptide
Genbank accession no. AAA59589
Genbank version no. AAA59589.1 GI:553531
Genbank record update date: Mar 6, 2012 11:12 AM

Cross references
Dean M., et al *Nature* 318 (6044), 385-388 (1985)

Other information
Official Symbol: MET
Other Aliases: AUTS9, HGFR, RCCP2, c-Met
Other Designations: HGF receptor; HGF/SF receptor; SF receptor; hepatocyte growth factor receptor; met proto-oncogene tyrosine kinase; proto-oncogene c-Met; scatter factor receptor; tyrosine-protein kinase Met

ANTIBODIES

Abgenix/Pfizer: US20100040629

for example, the antibody produced by hybridoma 13.3.2 having American Type Culture Collection (ATCC) accession number PTA-5026; the antibody produced by hybridoma 9.1.2 having ATCC accession number PTA-5027; the antibody produced by hybridoma 8.70.2 having ATCC accession number PTA-5028; or the antibody produced by hybridoma 6.90.3 having ATCC accession number PTA-5029.

Amgen/Pfizer: US20050054019

for example, an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 2 where X2 is glutamate and X4 is serine and a light chain having the amino acid sequence set forth in SEQ ID NO: 4 where X8 is alanine, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 6 and a light chain having the amino acid sequence set forth in SEQ ID NO: 8, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 10 and a light chain having the amino acid

sequence set forth in SEQ ID NO: 12, without the signal sequences; or an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 14 and a light chain having the amino acid sequence set forth in SEQ ID NO: 16, without the signal sequences.

Agouron Pharmaceuticals (Now Pfizer): US20060035907

Eli Lilly: US20100129369

Genentech: US5,686,292; US20100028337; US20100016241; US20070129301; US20070098707; US20070092520, US20060270594; US20060134104; US20060035278; US20050233960; US20050037431

US 5,686,292 – for example, ATCC HB-11894 and ATCC HB-11895

US 20100016241 – for example, ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6)

National Defense Medical Center, Taiwan: Lu RM., et al Biomaterials. 2011 Apr;32(12):3265-74.

Novartis: US20090175860

- for example, an antibody comprising the sequences of CDR1, CDR2 and CDR3 of heavy chain 4687, wherein the sequences of CDR1, CDR2, and CDR3 of heavy chain 4687 are residues 26-35, 50-65, and 98-102, respectively, of SEQ ID NO: 58; and the sequences of CDR1, CDR2, and CDR3 of light chain 5097, wherein the sequences of CDR1, CDR2, and CDR3 oflight chain 5097 are residues 24-39,55-61, and 94-100 of SEQ ID NO: 37.

Pharmacia Corporation: US20040166544

Pierre Fabre: US20110239316, US20110097262, US20100115639

Sumsung: US 20110129481 – for example a monoclonal antibody produced from a hybridoma cell having accession number KCLRF-BP-00219 or accession number of KCLRF-BP-00223.

Samsung: US 20110104176 – for example an antibody produced by a hybridoma cell having Accession Number: KCLRF-BP-00220.

University of Turin Medical School: DN-30 Pacchiana G., et al *J Biol Chem*. 2010 Nov 12;285(46):36149-57

Van Andel Research Institute: Jiao Y., et al *Mol Biotechnol*. 2005 Sep;31(1):41-54.

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

Nucleotide

Genbank accession no J05581

Genbank version no. J05581.1 GI:188869

Genbank record update date: Jun 23, 2010 08:48 AM

Polypeptide

Genbank accession no. AAA59876

Genbank version no. AAA59876.1 GI:188870

Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

Gendler S.J., et al *J. Biol. Chem.* 265 (25), 15286-15293 (1990)

Other information

Official Symbol: MUC1

Other Aliases: RP11-263K19.2, CD227, EMA, H23AG, KL-6, MAM6, MUC-1, MUC-1/SEC, MUC-1/X, MUC1/ZD, PEM, PEMT, PUM

Other Designations: DF3 antigen; H23 antigen; breast carcinoma-associated antigen DF3; carcinoma-associated mucin; episialin; krebs von den Lungen-6; mucin 1, transmembrane; mucin-1; peanut-reactive urinary mucin; polymorphic epithelial mucin; tumor associated epithelial mucin; tumor-associated epithelial membrane antigen; tumor-associated mucin

ANTIBODIES

AltaRex- Quest Pharma Tech: US 6,716,966 – for example an Alt-1 antibody produced by the hybridoma ATCC No PTA-975.

AltaRex- Quest Pharma Tech: US7,147,850

CRT: 5E5 - Sørensen AL., et al *Glycobiology* vol. 16 no. 2 pp. 96–107, 2006; HMFG2 – Burchell J., et al *Cancer Res.*, 47, 5476–5482 (1987)

Glycotope GT-MAB: GT-MAB 2.5-GEX (Website: http://www.glycotope.com/pipeline/pankomab-gex)

Immunogen: US7,202,346

- for example, antibody MJ-170: hybridoma cell line MJ-170 ATCC accession no. PTA-5286Monoclonal antibody MJ-171: hybridoma cell line MJ-171 ATCC accession no. PTA-5287; monoclonal antibody MJ-172: hybridoma cell line MJ-172 ATCC accession no. PTA-5288; or monoclonal antibody MJ-173: hybridoma cell line MJ-173 ATCC accession no. PTA-5302

Immunomedics: US 6,653,104

Ramot Tel Aviv Uni: US7,897,351

Regents Uni. CA: US 7,183,388; US20040005647; US20030077676.

Roche GlycArt: US8,021,856

Russian National Cancer Research Center: Imuteran- Ivanov PK., et al *Biotechnol J.* 2007 Jul;2(7):863-70

Technische Univ Braunschweig: (IIB6, HT186-B7, HT186-D11, HT186-G2, HT200-3A-C1, HT220-M-D1, HT220-M-G8) - Thie H., et al *PLoS One*. 2011 Jan 14;6(1):e15921

(44) *CA9 (Carbonic anhydrase IX)*

Nucleotide

Genbank accession no . X66839

Genbank version no. X66839.1 GI:1000701

Genbank record update date: Feb 2, 2011 10:15 AM

Polypeptide

Genbank accession no. CAA47315

Genbank version no. CAA47315.1 GI:1000702

Genbank record update date: Feb 2, 2011 10:15 AM

Cross references

Pastorek J., et al *Oncogene* 9 (10), 2877-2888 (1994)

Other information

Official Symbol: CA9

Other Aliases: CAIX, MN

Other Designations: CA-IX; P54/58N; RCC-associated antigen G250; RCC-associated protein G250; carbonate dehydratase IX; carbonic anhydrase 9; carbonic dehydratase; membrane antigen MN; pMW1; renal cell carcinoma-associated antigen G250

ANTIBODIES

Abgenix/Amgen: US20040018198

Affibody: Anti-CAIX Affibody molecules
(http://www.affibody.com/en/Product-Portfolio/Pipeline/)

Bayer: US7,462,696

Bayer/Morphosys: 3ee9 mAb - Petrul HM., et al *Mol Cancer Ther.* 2012 Feb;11(2):340-9

Harvard Medical School: Antibodies G10, G36, G37, G39, G45, G57, G106, G119, G6, G27, G40 and G125. Xu C., et al *PLoS One*. 2010 Mar 10;5(3):e9625

Institute of Virology, Slovak Academy of Sciences (Bayer) - US5,955,075

- for example, M75- ATCC Accession No. HB 11128 or MN12 – ATCC Accession No. HB 11647

Institute of Virology, Slovak Academy of Sciences: US7,816,493

- for example the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128; or the V/10 monoclonal antibody secreted from the hybridoma V/10-VU, which was deposited at the International Depository Authority of the Belgian Coordinated Collection of Microorganisms (BCCM) at the Laboratorium voor Moleculaire Bioloqie-Plasmidencollectie (LMBP) at the Universeit Gent in Gent, Belgium, under Accession No. LMBP 6009CB.

Institute of Virology, Slovak Academy of Sciences US20080177046; US20080176310; US20080176258; US20050031623

Novartis: US20090252738

Wilex: US7,691,375 – for example the antibody produced by the hybridoma cell line DSM ASC 2526.

Wilex: US20110123537; Rencarex: Kennett RH., et al *Curr Opin Mol Ther.* 2003 Feb;5(1):70-5

Xencor: US20090162382

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

Nucleotide

Genbank accession no. NM_201283
Genbank version no. NM_201283.1 GI:41327733
Genbank record update date: Sep 30, 2012 01:47 PM

Polypeptide

Genbank accession no. NP_958440
Genbank version no. NP_958440.1 GI:41327734
Genbank record update date: Sep 30, 2012 01:47 PM

Cross-references

Batra SK., et al *Cell Growth Differ* 1995;6:1251–1259.

ANTIBODIES:

US7,628,986 and US7,736,644 (Amgen)

For example, a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 142 and variants & a light chain variable region amino acid sequence selected from the group consisting of: SEQ ID NO: 144 and variants.

US20100111979 (Amgen)

For example, an antibody comprising a heavy chain amino acid sequence comprising:

CDR1 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR1 region of antibodies 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17);

CDR2 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR2 region of antibodies 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17); and

CDR3 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR3 region of antibodies 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

US20090240038 (Amgen)

For example, an antibody having at least one of the heavy or light chain polypeptides comprises an amino acid sequence that is at least 90% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 19, SEQ ID NO: 142, SEQ ID NO: 144, and any combination thereof.

US20090175887 (Amgen)

For example, an antibody having a heavy chain amino acid sequence selected from the group consisting of the heavy chain amino acid sequence of antibody 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

US20090156790 (Amgen)

For example, antibody having heavy chain polypeptide and a light chain polypeptide, wherein at least one of the heavy or light chain polypeptides comprises an amino acid sequence that is at least 90% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 19, SEQ ID NO: 142, SEQ ID NO: 144, and any combination thereof.

US20090155282, US20050059087 and US20050053608 (Amgen)

For example, an antibody heavy chain amino acid sequence selected from the group consisting of the heavy chain amino acid sequence of antibody 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

MR1-1 (US7,129,332; Duke)

For example, a variant antibody having the sequence of SEQ ID NO.18 with the substitutions S98P-T99Y in the CDR3 VH, and F92W in CDR3 VL.

L8A4, H10, Y10 (Wikstrand CJ., et al *Cancer Res.* 1995 Jul 15;55(14):3140-8; Duke)

US20090311803 (Harvard University)

For example, SEQ ID NO:9 for antibody heavy chain variable region, and SEQ ID NO: 3 for light chain variable region amino acid sequences

US20070274991 (EMD72000, also known as matuzumab; Harvard University)

For example, SEQ ID NOs: 3 & 9 for light chain and heavy chain respectively

US6,129,915 (Schering)
For example, SEQ. ID NOs: 1, 2, 3, 4, 5 and 6.

mAb CH12 - Wang H., et al *FASEB J.* 2012 Jan;26(1):73-80 (Shanghai Cancer Institute).

RAbDMvIII - Gupta P., et al *BMC Biotechnol.* 2010 Oct 7;10:72 (Stanford University Medical Center).

mAb Ua30 - Ohman L., et al Tumour Biol. 2002 Mar-Apr;23(2):61-9 (Uppsala University).

Han DG., et al *Nan Fang Yi Ke Da Xue Xue Bao.* 2010 Jan;30(1):25-9 (Xi'an Jiaotong University).

(46) *CD33 (CD33 molecule)*

Nucleotide

Genbank accession no. M_23197

Genbank version no. NM_23197.1 GI:180097

Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

Genbank accession no. AAA51948

Genbank version no. AAA51948.1 GI:188098

Genbank record update date: Jun 23, 2010 08:47 AM

Cross-references

Simmons D., et al *J. Immunol.* 141 (8), 2797-2800 (1988)

Other information

Official Symbol: CD33

Other Aliases: SIGLEC-3, SIGLEC3, p67

Other Designations: CD33 antigen (gp67); gp67; myeloid cell surface antigen CD33; sialic acid binding Ig-like lectin 3; sialic acid-binding Ig-like lectin

ANTIBODIES

H195 (Lintuzumab)- Raza A., et al *Leuk Lymphoma*. 2009 Aug;50(8):1336-44;
US6,759,045 (Seattle Genetics/Immunomedics)

mAb OKT9: Sutherland, D.R. et al. *Proc Natl Acad Sci* USA 78(7): 4515-4519 1981,
Schneider,C., et al *J Biol Chem* 257, 8516-8522 (1982)

mAb E6: Hoogenboom,H.R., et al *J Immunol* 144, 3211-3217 (1990)

US6,590,088 (Human Genome Sciences)
For example, SEQ ID NOs: 1 and 2 and ATCC accession no. 97521

US7,557,189 (Immunogen)
For example, an antibody or fragment thereof comprising a heavy chain variable region which comprises three CDRs having the amino acid sequences of SEQ ID NOs:1-3 and a light chain variable region comprising three CDRs having the amino acid sequences of SEQ ID NOs:4-6.

(47) *CD19 (CD19 molecule)*

Nucleotide

Genbank accession no. NM_001178098
Genbank version no. NM_001178098.1 GI:296010920
Genbank record update date: Sep 10, 2012 12:43 AM

Polypeptide

Genbank accession no. NP_001171569
Genbank version no. NP_001171569.1 GI:296010921
Genbank record update date: Sep 10, 2012 12:43 AM

Cross-references

Tedder TF., et al J. Immunol. 143 (2): 712–7 (1989)

Other information

Official Symbol: CD19
Other Aliases: B4, CVID3

Other Designations: B-lymphocyte antigen CD19; B-lymphocyte surface antigen B4; T-cell surface antigen Leu-12; differentiation antigen CD19

ANTIBODIES

Immunogen: HuB4 - Al-Katib AM., et al *Clin Cancer Res.* 2009 Jun 15;15(12):4038-45.

4G7: Kügler M., et al *Protein Eng Des Sel.* 2009 Mar;22(3):135-47

For example, sequences in Fig. 3 of of Knappik, A. et al. J Mol Biol 2000 Feb;296(1):57-86

AstraZeneca /MedImmune: MEDI-551 - Herbst R., et al *J Pharmacol Exp Ther.* 2010 Oct;335(1):213-22

Glenmark Pharmaceuticals: GBR-401 - Hou S., et al Mol Cancer Ther November 2011 10 (Meeting Abstract Supplement) C164

US7,109,304 (Immunomedics)

For example, an antibody comprising the sequence of hA19Vk (SEQ ID NO:7) and the sequence of hA19VH (SEQ ID NO:10)

US7,902,338 (Immunomedics)

For example, an antibody or antigen-binding fragment thereof that comprises the light chain complementarity determining region CDR sequences CDR1 of SEQ ID NO: 16 (KASQSVDYDGDSYLN); CDR2 of SEQ ID NO: 17 (DASNLVS); and CDR3 of SEQ ID NO: 18 (QQSTEDPWT) and the heavy chain CDR sequences CDR1 of SEQ ID NO: 19 (SYWMN); CDR2 of SEQ ID NO: 20 (QIWPGDGDTNYNGKFKG) and CDR3 of SEQ ID NO: 21 (RETTTVGRYYYAMDY) and also comprises human antibody framework (FR) and constant region sequences with one or more framework region amino acid residues substituted from the corresponding framework region sequences of the parent murine antibody, and wherein said substituted FR residues comprise the substitution of serine for phenylalanine at Kabat residue 91 of the heavy chain variable region.

Medarex: MDX-1342 – Cardarelli PM., et al *Cancer Immunol Immunother.* 2010 Feb;59(2):257-65.

MorphoSys /Xencor: MOR-208/XmAb-5574 - Zalevsky J., et al *Blood*. 2009 Apr 16;113(16):3735-43

US7,968,687 (Seattle Genetics)
An antibody or antigen-binding fragment comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:9 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 24.

4G7 chim - Lang P., et al Blood. 2004 May 15;103(10):3982-5 (University of Tübingen)
For example, fig. 6 and SEQ ID No: 80 of US20120082664

Zhejiang University School of Medicine: 2E8 - Zhang J., et al J Drug Target. 2010 Nov;18(9):675-8

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

Nucleotide

Genbank accession no. NM_000417
Genbank version no. NM_000417.2 GI:269973860
Genbank record update date: Sep 09, 2012 04:59 PM

Polypeptide

Genbank accession no. NP_000408
Genbank version no. NP_000408.1 GI:4557667
Genbank record update date: Sep 09, 2012 04:59 PM

Cross-references

Kuziel W.A., et al *J. Invest. Dermatol.* 94 (6 SUPPL), 27S-32S (1990)

Other information

Official Symbol: IL2RA
Other Aliases: RP11-536K7.1, CD25, IDDM10, IL2R, TCGFR
Other Designations: FIL-2 receptor subunit alpha; IL-2-RA; IL-2R subunit alpha; IL2-RA; TAC antigen; interleukin-2 receptor subunit alpha; p55

ANTIBODIES

US6,383,487 (Novartis/UCL: Baxilisimab [Simulect])

US6,521,230 (Novartis/UCL: Baxilisimab [Simulect])

For example, an antibody having an antigen binding site comprises at least one domain which comprises CDR1 having the amino acid sequence in SEQ. ID. NO: 7, CDR2 having the amino acid sequence in SEQ. ID. NO: 8, and CDR3 chaving the amino acid sequence in SEQ. ID. NO: 9; or said CDR1, CDR2 and CDR3 taken in sequence as a whole comprise an amino acid sequence which is at least 90% identical to SEQ. ID. NOs: 7, 8 and 9 taken in sequence as a whole.

Daclizumab – Rech AJ., et al *Ann N Y Acad Sci.* 2009 Sep;1174:99-106 (Roche)

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

Nucleotide

Genbank accession no. M76125

Genbank version no. M76125.1 GI:292869

Genbank record update date: Jun 23, 2010 08:53 AM

Polypeptide

Genbank accession no. AAA61243

Genbank version no. AAA61243.1 GI:29870

Genbank record update date: Jun 23, 2010 08:53 AM

Cross-references

O'Bryan J.P., et al *Mol. Cell. Biol.* 11 (10), 5016-5031 (1991); Bergsagel P.L., et al *J. Immunol.* 148 (2), 590-596 (1992)

Other information

Official Symbol: AXL

Other Aliases: JTK11, UFO

Other Designations: AXL oncogene; AXL transforming sequence/gene; oncogene AXL; tyrosine-protein kinase receptor UFO

ANTIBODIES

YW327.6S2 - Ye X., et al *Oncogene.* 2010 Sep 23;29(38):5254-64. (Genentech)

BergenBio: BGB324 (http://www.bergenbio.com/BGB324)

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

Nucleotide

Genbank accession no. M83554

Genbank version no. M83554.1 GI:180095

Genbank record update date: Jun 23, 2010 08:53 AM

Polypeptide

Genbank accession no. AAA51947

Genbank version no. AAA51947.1 GI:180096

Genbank record update date: Jun 23, 2010 08:53 AM

Cross-references

Durkop H., et al *Cell* 68 (3), 421-427 (1992)

Other information

Official Symbol: TNFRSF8

Other Aliases: CD30, D1S166E, Ki-1

Other Designations: CD30L receptor; Ki-1 antigen; cytokine receptor CD30; lymphocyte activation antigen CD30; tumor necrosis factor receptor superfamily member 8

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

Nucleotide

Genbank accession no. Z29574

Genbank version no. Z29574.1 GI:471244

Genbank record update date: Feb 02, 2011 10:40 AM

Polypeptide

Genbank accession no. CAA82690

Genbank version no. CAA82690.1 GI:471245

Genbank record update date: Feb 02, 2011 10:40 AM

Cross-references

Laabi Y., et al Nucleic Acids Res. 22 (7), 1147-1154 (1994)

Other information

Official Symbol: TNFRSF17

Other Aliases: BCM, BCMA, CD269

Other Designations: B cell maturation antigen; B-cell maturation factor; B-cell maturation protein; tumor necrosis factor receptor superfamily member 17

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

Cross-references

Fratta E., et al. *Mol Oncol.* 2011 Apr;5(2):164-82; Lim SH., at al *Am J Blood Res.* 2012;2(1):29-35.

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

Nucleotide

Genbank accession no. NM000149

Genbank version no. NM000149.3 GI:148277008

Genbank record update date: Jun 26, 2012 04:49 PM

Polypeptide

Genbank accession no. NP_000140

Genbank version no. NP_000140.1 GI:4503809

Genbank record update date: Jun 26, 2012 04:49 PM

Cross-references

Kukowska-Latallo,J.F., et al *Genes Dev.* 4 (8), 1288-1303 (1990)

Other information

Official Symbol: FUT3

Other Aliases: CD174, FT3B, FucT-III, LE, Les
Other Designations: Lewis FT; alpha-(1,3/1,4)-fucosyltransferase; blood group Lewis alpha-4-fucosyltransferase; fucosyltransferase III; galactoside 3(4)-L-fucosyltransferase

(54) *CLEC14A (C-type lectin domain family 14, member A; Genbank accession no. NM175060)*
Nucleotide
Genbank accession no. NM175060
Genbank version no. NM175060.2 GI:371123930
Genbank record update date: Apr 01, 2012 03:34 PM

Polypeptide
Genbank accession no. NP_778230
Genbank version no. NP_778230.1 GI:28269707
Genbank record update date: Apr 01, 2012 03:34 PM

Other information
Official Symbol: CLEC14A
Other Aliases: UNQ236/PRO269, C14orf27, CEG1, EGFR-5
Other Designations: C-type lectin domain family 14 member A; CIECT and EGF-like domain containing protein; epidermal growth factor receptor 5

(55) *GRP78 – HSPA5 (heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)*
Nucleotide
Genbank accession no. NM005347
Genbank version no. NM005347.4 GI:305855105
Genbank record update date: Sep 30, 2012 01:42 PM

Polypeptide
Genbank accession no. NP_005338
Genbank version no. NP_005338.1 GI:16507237
Genbank record update date: Sep 30, 2012 01:42 PM

Cross-references
Ting J., et al *DNA* 7 (4), 275-286 (1988)

Other infromation
Official Symbol: HSPA5
Other Aliases: BIP, GRP78, MIF2
Other Designations: 78 kDa glucose-regulated protein; endoplasmic reticulum lumenal Ca(2+)-binding protein grp78; immunoglobulin heavy chain-binding protein

(56) *CD70 (CD70 molecule) L08096*
Nucleotide
Genbank accession no. L08096
Genbank version no. L08096.1 GI:307127
Genbank record update date: Jun 23, 2012 08:54 AM

Polypeptide
Genbank accession no. AAA36175
Genbank version no. AAA36175.1 GI:307128
Genbank record update date: Jun 23, 2012 08:54 AM

Cross-references
Goodwin R.G., et al *Cell* 73 (3), 447-456 (1993)

Other information
Official Symbol: CD70
Other Aliases: CD27L, CD27LG, TNFSF7
Other Designations: CD27 ligand; CD27-L; CD70 antigen; Ki-24 antigen; surface antigen CD70; tumor necrosis factor (ligand) superfamily, member 7; tumor necrosis factor ligand superfamily member 7

ANTIBODIES
MDX-1411 against CD70 (Medarex)

h1F6 (Oflazoglu, E., et al, Clin Cancer Res. 2008 Oct 1;14(19):6171-80; Seattle Genetics)
For example, see US20060083736 SEQ ID NOs: 1, 2, 11 and 12 and Fig. 1.

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

- 5T4 (see entry (63) below)
- CD25 (see entry (48) above)
- CD32
  - Polypeptide
    - Genbank accession no. ABK42161
    - Genbank version no. ABK42161.1 GI:117616286
    - Genbank record update date: Jul 25, 2007 03:00 PM
- LGR5/GPR49
  - Nucleotide
    - Genbank accession no. NM_003667
    - Genbank version no. NM_003667.2 GI:24475886
    - Genbank record update date: Jul 22, 2012 03:38 PM
  - Polypeptide
    - Genbank accession no. NP_003658
    - Genbank version no. NP_003658.1 GI:4504379
    - Genbank record update date: Jul 22, 2012 03:38 PM
- Prominin/CD133
  - Nucleotide
    - Genbank accession no. NM_006017
    - Genbank version no. NM_006017.2 GI:224994187
    - Genbank record update date: Sep 30, 2012 01:47 PM
  - Polypeptide
    - Genbank accession no. NP_006008
    - Genbank version no. NP_006008.1 GI:5174387
    - Genbank record update date: Sep 30, 2012 01:47 PM

(58) *ASG-5*

Cross-references

(Smith L.M., et.al *AACR 2010 Annual Meeting* (abstract #2590); Gudas J.M., et.al. *AACR 2010 Annual Meeting* (abstract #4393)

ANTIBODIES

Anti- AGS-5 Antibody: M6.131 (Smith, L.M., et.al *AACR 2010 Annual Meeting* (abstract #2590)

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

Nucleotide

Genbank accession no. AF005632

Genbank version no. AF005632.2 GI:4432589

Genbank record update date: Mar 10, 2010 09:41 PM

Polypeptide

Genbank accession no. AAC51813

Genbank version no. AAC51813.1 GI:2465540

Genbank record update date: Mar 10, 2010 09:41 PM

Cross-references

Jin-Hua P., et al *Genomics* 45 (2), 412-415 (1997)

Other information

Official Symbol: ENPP3

Other Aliases: RP5-988G15.3, B10, CD203c, NPP3, PD-IBETA, PDNP3

Other Designations: E-NPP 3; dJ1005H11.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); dJ914N13.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); ectonucleotide pyrophosphatase/phosphodiesterase family member 3; gp130RB13-6; phosphodiesterase I beta; phosphodiesterase I/nucleotide pyrophosphatase 3; phosphodiesterase-I beta

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

Nucleotide

Genbank accession no. NM_007244

Genbank version no. NM_007244.2 GI:154448885

Genbank record update date: Jun 28, 2012 12:39 PM

Polypeptide

Genbank accession no. NP_009175

Genbank version no. NP_009175.2 GI:154448886

Genbank record update date: Jun 28, 2012 12:39 PM

Cross-references

Dickinson D.P., et al *Invest. Ophthalmol. Vis. Sci.* 36 (10), 2020-2031 (1995)

Other information

Official Symbol: PRR4

Other Aliases: LPRP, PROL4

Other Designations: lacrimal proline-rich protein; nasopharyngeal carcinoma-associated proline-rich protein 4; proline-rich polypeptide 4; proline-rich protein 4

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

Nucleotide

Genbank accession no. NM_004963

Genbank version no. NM_004963.3 GI:222080082

Genbank record update date: Sep 02, 2012 01:50 PM

Polypeptide

Genbank accession no. NP_004954

Genbank version no. NP_004954.2 GI:222080083

Genbank record update date: Sep 02, 2012 01:50 PM

Cross-references

De Sauvage F.J., et al *J. Biol. Chem.* 266 (27), 17912-17918 (1991); Singh S., et al *Biochem. Biophys. Res. Commun.* 179 (3), 1455-1463 (1991)

Other information

Official Symbol: GUCY2C

Other Aliases: DIAR6, GUC2C, MUCIL, STAR

Other Designations: GC-C; STA receptor; guanylyl cyclase C; hSTAR; heat-stable enterotoxin receptor; intestinal guanylate cyclase

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

Nucleotide

Genbank accession no. U41060

Genbank version no. U41060.2 GI:12711792

Genbank record update date: Nov 30, 2009 04:35 PM

Polypeptide

Genbank accession no. AAA96258

Genbank version no. AAA96258.2 GI:12711793

Genbank record update date: Nov 30, 2009 04:35 PM

Cross-references

Taylor KM., et al *Biochim Biophys Acta.* 2003 Apr 1;1611(1-2):16-30

Other information

Official Symbol: SLC39A6

Other Aliases: LIV-1

Other Designations: LIV-1 protein, estrogen regulated; ZIP-6; estrogen-regulated protein LIV-1; solute carrier family 39 (metal ion transporter), member 6; solute carrier family 39 member 6; zinc transporter ZIP6; zrt- and Irt-like protein 6

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

Nucleotide

Genbank accession no. AJ012159

Genbank version no. AJ012159.1 GI:3805946

Genbank record update date: Feb 01, 2011 10:27 AM

Polypeptide

Genbank accession no. CAA09930

Genbank version no. CAA09930.1 GI:3805947

Genbank record update date: Feb 01, 2011 10:27 AM

Cross-references

King K.W.,et al *Biochim. Biophys. Acta* 1445 (3), 257-270 (1999)

Other information

- Official Symbol: TPBG
- Other Aliases: 5T4, 5T4AG, M6P1
- Other Designations: 5T4 oncofetal antigen; 5T4 oncofetal trophoblast glycoprotein; 5T4 oncotrophoblast glycoprotein

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

Nucleotide

Genbank accession no. NM_000615

Genbank version no. NM_000615.6 GI:336285433

Genbank record update date: Sep 23, 2012 02:32 PM

Polypeptide

Genbank accession no. NP_000606

Genbank version no. NP_000606.3 GI:94420689

Genbank record update date: Sep 23, 2012 02:32 PM

Cross-references

Dickson,G., et al, *Cell* 50 (7), 1119-1130 (1987)

Other information

Official Symbol: NCAM1

Other Aliases: CD56, MSK39, NCAM

Other Designations: antigen recognized by monoclonal antibody 5.1H11; neural cell adhesion molecule, NCAM

ANTIBODIES

Immunogen: HuN901 (Smith SV., et al *Curr Opin Mol Ther.* 2005 Aug;7(4):394-401)

For example, see humanized from murine N901 antibody. See Fig. 1b and 1e of Roguska, M.A., et al. Proc Natl Acad Sci USA Feb 1994;91:969-973.

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

Cross-references

Haglund C., et al *Br J Cancer* 60:845-851, 1989;Baeckstrom D., et al *J Biol Chem* 266:21537-21547, 1991

ANTIBODIES

huC242 (Tolcher AW et al., *J Clin Oncol.* 2003 Jan 15;21(2):211-22; Immunogen)

For example, see US20080138898A1 SEQ ID NO: 1 and 2

(66) FOLR1 (Folate Receptor 1)

Nucleotide

Genbank accession no. J05013

Genbank version no. J05013.1 GI:182417

Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

Genbank accession no. AAA35823

Genbank version no. AAA35823.1 GI:182418

Genbank record update date: Jun 23, 2010 08:47 AM

Cross-references

Elwood P.C., et al *J. Biol. Chem.* 264 (25), 14893-14901 (1989)

Other information

Official Symbol: FOLR1

Other Aliases: FBP, FOLR

Other Designations: FR-alpha; KB cells FBP; adult folate-binding protein; folate binding protein; folate receptor alpha; folate receptor, adult; ovarian tumor-associated antigen MOv18

ANTIBODIES

M9346A - Whiteman KR., et al *Cancer Res* April 15, 2012; 72(8 Supplement): 4628 (Immunogen)

(67) GPNMB (Glycoprotein (transmembrane) nmb)

Nucleotide

Genbank accession no. X76534

Genbank version no. X76534.1 GI:666042

Genbank record update date: Feb 02, 2011 10:10 AM

Polypeptide

Genbank accession no. CAA54044

Genbank version no. CAA54044.1 GI:666043

Genbank record update date: Feb 02, 2011 10:10 AM

Cross-references

Weterman M.A., et al *Int. J. Cancer* 60 (1), 73-81 (1995)

Other information

Official Symbol: GPNMB

Other Aliases: UNQ1725/PRO9925, HGFIN, NMB

Other Designations: glycoprotein NMB; glycoprotein nmb-like protein; osteoactivin; transmembrane glycoprotein HGFIN; transmembrane glycoprotein NMB

ANTIBODIES

Celldex Therapeutics: CR011 (Tse KF., et al *Clin Cancer Res.* 2006 Feb 15;12(4):1373-82)

For example, see EP1827492B1 SEQ ID NO: 22, 24, 26, 31, 33 and 35

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

Nucleotide

Genbank accession no. AF043724

Genbank version no. AF043724.1 GI:2827453

Genbank record update date: Mar 10, 2010 06:24 PM

Polypeptide

Genbank accession no. AAC39862

Genbank version no. AAC39862.1 GI:2827454

Genbank record update date: Mar 10, 2010 06:24 PM

Cross-references

Feigelstock D., et al *J. Virol.* 72 (8), 6621-6628 (1998)

Other information

Official Symbol: HAVCR1

Other Aliases: HAVCR, HAVCR-1, KIM-1, KIM1, TIM, TIM-1, TIM1, TIMD-1, TIMD1

Other Designations: T cell immunoglobin domain and mucin domain protein 1; T-cell membrane protein 1; kidney injury molecule 1

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

Cross-references

Parry R., et al Cancer Res. 2005 Sep 15;65(18):8397-405

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

Nucleotide

Genbank accession no. BX648021

Genbank version no. BX648021.1 GI:34367180

Genbank record update date: Feb 02, 2011 08:40 AM

Cross-references

Sica GL., et al *Immunity.* 2003 Jun;18(6):849-61

Other information

Official Symbol: VTCN1

Other Aliases: RP11-229A19.4, B7-H4, B7H4, B7S1, B7X, B7h.5, PRO1291, VCTN1

Other Designations: B7 family member, H4; B7 superfamily member 1; T cell costimulatory molecule B7x; T-cell costimulatory molecule B7x; V-set domain-containing T-cell activation inhibitor 1; immune costimulatory protein B7-H4

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

Nucleotide

Genbank accession no. AF447176

Genbank version no. AF447176.1 GI:17432420

Genbank record update date: Nov 28, 2008 01:51 PM

Polypeptide

Genbank accession no. AAL39062

Genbank version no. AAL39062.1 GI:17432421

Genbank record update date: Nov 28, 2008 01:51 PM

Cross-references

Park S.K.,et al *J. Biochem.* 119 (2), 235-239 (1996)

Other information

Official Symbol: PTK7
Other Aliases: CCK-4, CCK4
Other Designations: colon carcinoma kinase 4; inactive tyrosine-protein kinase 7; pseudo tyrosine kinase receptor 7; tyrosine-protein kinase-like 7

(72) *CD37 (CD37 molecule)*

Nucleotide

Genbank accession no. NM_001040031
Genbank version no. NM_001040031.1 GI:91807109
Genbank record update date: Jul 29, 2012 02:08 PM

Polypeptide

Genbank accession no. NP_001035120
Genbank version no. NP_001035120.1 GI:91807110
Genbank record update date: Jul 29, 2012 02:08 PM

Cross-references

Schwartz-Albiez R., et al *J. Immunol.* 140 (3), 905-914 (1988)

Other information

Official Symbol: CD37
Other Aliases: GP52-40, TSPAN26
Other Designations: CD37 antigen; cell differentiation antigen 37; leukocyte antigen CD37; leukocyte surface antigen CD37; tetraspanin-26; tspan-26

ANTIBODIES

Boehringer Ingelheim: mAb 37.1 (Heider KH., et al *Blood*. 2011 Oct 13;118(15):4159-68)

Trubion: CD37-SMIP (G28-1 scFv-Ig) ((Zhao X., et al *Blood.* 2007;110: 2569-2577)
For example, see US20110171208A1 SEQ ID NO: 253

Immunogen: K7153A (Deckert J., et al Cancer Res April 15, 2012; 72(8 Supplement): 4625)

(73) *CD138 – SDC1 (syndecan 1)*

Nucleotide

Genbank accession no. AJ551176

Genbank version no. AJ551176.1 GI:29243141

Genbank record update date: Feb 01, 2011 12:09 PM

Polypeptide

Genbank accession no. CAD80245

Genbank version no. CAD80245.1 GI:29243142

Genbank record update date: Feb 01, 2011 12:09 PM

Cross-references

O'Connell FP., et al *Am J Clin Pathol.* 2004 Feb;121(2):254-63

Other information

Official Symbol: SDC1

Other Aliases: CD138, SDC, SYND1, syndecan

Other Designations: CD138 antigen; heparan sulfate proteoglycan fibroblast growth factor receptor; syndecan proteoglycan 1; syndecan-1

ANTIBODIES

Biotest: chimerized MAb (nBT062) - (Jagannath S., et al Poster *ASH* #3060, 2010; WIPO Patent Application WO/2010/128087)

For example, see US20090232810 SEQ ID NO: 1 and 2

Immunogen: B-B4 (Tassone P., et al *Blood* 104_3688-3696)

For example, see US20090175863A1 SEQ ID NO: 1 and 2

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

Nucleotide

Genbank accession no. NM_004355

Genbank version no. NM_004355.1 GI:343403784

Genbank record update date: Sep 23, 2012 02:30 PM

Polypeptide

Genbank accession no. NP_004346

Genbank version no. NP_004346.1 GI:10835071
Genbank record update date: Sep 23, 2012 02:30 PM

Cross-references

Kudo,J., et al *Nucleic Acids Res.* 13 (24), 8827-8841 (1985)

Other information

Official Symbol: CD74
Other Aliases: DHLAG, HLADG, II, Ia-GAMMA
Other Designations: CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); HLA class II histocompatibility antigen gamma chain; HLA-DR antigens-associated invariant chain; HLA-DR-gamma; Ia-associated invariant chain; MHC HLA-DR gamma chain; gamma chain of class II antigens; p33

ANTIBODIES
Immunomedics: hLL1 (Milatuzumab,) - Berkova Z., et al *Expert Opin Investig Drugs.* 2010 Jan;19(1):141-9)
For example, see US20040115193 SEQ ID NOs: 19, 20, 21, 22, 23 and 24

Genmab: HuMax-CD74 (see website)

(75) *Claudins – CLs (Claudins)*
Cross-references
Offner S., et al *Cancer Immunol Immunother.* 2005 May; 54(5):431-45, Suzuki H., et al *Ann N Y Acad Sci.* 2012 Jul;1258:65-70)

In humans, 24 members of the family have been described – see literature reference.

(76) *EGFR (Epidermal growth factor receptor)*
Nucleotide
Genbank accession no. NM_005228
Genbank version no. NM_005228.3 GI:41927737
Genbank record update date: Sep 30, 2012 01:47 PM

Polypeptide

Genbank accession no. NP_005219
Genbank version no. NP_005219.2 GI:29725609
Genbank record update date: Sep 30, 2012 01:47 PM

Cross-references
Dhomen NS., et al *Crit Rev Oncog.* 2012;17(1):31-50

Other information
Official Symbol: EGFR
Other Aliases: ERBB, ERBB1, HER1, PIG61, mENA
Other Designations: avian erythroblastic leukemia viral (v-erb-b) oncogene homolog; cell growth inhibiting protein 40; cell proliferation-inducing protein 61; proto-oncogene c-ErbB-1; receptor tyrosine-protein kinase erbB-1

ANTIBODIES
BMS: Cetuximab (Erbitux) - Broadbridge VT., et al *Expert Rev Anticancer Ther.* 2012 May;12(5):555-65.
For example, see US6217866 – ATTC deposit No. 9764.

Amgen: Panitumumab (Vectibix) - Argiles G., et al *Future Oncol.* 2012 Apr;8(4):373-89
For example, see US6235883 SEQ ID NOs: 23-38.

Genmab: Zalutumumab - Rivera F., et al *Expert Opin Biol Ther.* 2009 May;9(5):667-74.

YM Biosciences: Nimotuzumab - Ramakrishnan MS., et al *MAbs.* 2009 Jan-Feb;1(1):41-8.
For example, see US5891996 SEQ ID NOs: 27-34.

(77) *Her3 (ErbB3) – ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian))*
Nucleotide
Genbank accession no. M34309
Genbank version no. M34309.1 GI:183990
Genbank record update date: Jun 23, 2010 08:47 PM

Polypeptide

Genbank accession no. AAA35979
Genbank version no. AAA35979.1 GI:306841
Genbank record update date: Jun 23, 2010 08:47 PM

Cross-references
Plowman,G.D., et al., *Proc. Natl. Acad. Sci. U.S.A.* 87 (13), 4905-4909 (1990)

Other information
Official Symbol: ERBB3
Other Aliases: ErbB-3, HER3, LCCS2, MDA-BF-1, c-erbB-3, c-erbB3, erbB3-S, p180-ErbB3, p45-sErbB3, p85-sErbB3
Other Designations: proto-oncogene-like protein c-ErbB-3; receptor tyrosine-protein kinase erbB-3; tyrosine kinase-type cell surface receptor HER3

ANTIBODIES
Merimack Pharma : MM-121 (Schoeberl B., et al Cancer Res. 2010 Mar 15;70(6):2485-2494)
For example, see US2011028129 SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7 and 8.

(78) *RON - MST1R (macrophage stimulating 1 receptor (c-met-related tyrosine kinase))*
Nucleotide
Genbank accession no. X70040
Genbank version no. X70040.1 GI:36109
Genbank record update date: Feb 02, 2011 10:17 PM

Polypeptide
Genbank accession no. CCA49634
Genbank version no. CCA49634.1 GI:36110
Genbank record update date: Feb 02, 2011 10:17 PM

Cross-references
Ronsin C., et al *Oncogene* 8 (5), 1195-1202 (1993)

Other information
Official Symbol: MST1R

Other Aliases: CD136, CDw136, PTK8, RON
Other Designations: MSP receptor; MST1R variant RON30; MST1R variant RON62; PTK8 protein tyrosine kinase 8; RON variant E2E3; c-met-related tyrosine kinase; macrophage-stimulating protein receptor; p185-Ron; soluble RON variant 1; soluble RON variant 2; soluble RON variant 3; soluble RONvariant 4

(79) EPHA2 (EPH receptor A2)

Nucleotide

Genbank accession no. BC037166
Genbank version no. BC037166.2 GI:33879863
Genbank record update date: Mar 06, 2012 01:59 PM

Polypeptide

Genbank accession no. AAH37166
Genbank version no. AAH37166.1 GI:22713539
Genbank record update date: Mar 06, 2012 01:59 PM

Cross-references

Strausberg R.L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99 (26), 16899-16903 (2002)

Other information

Official Symbol: EPHA2
Other Aliases: ARCC2, CTPA, CTPP1, ECK
Other Designations: ephrin type-A receptor 2; epithelial cell receptor protein tyrosine kinase; soluble EPHA2 variant 1; tyrosine-protein kinase receptor ECK

ANTIBODIES

Medimmune: 1C1 (Lee JW., et al *Clin Cancer Res.* 2010 May 1;16(9):2562-2570)
For example, see US20090304721A1 Fig. 7 and 8.

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

Nucleotide

Genbank accession no. M27394
Genbank version no. M27394.1 GI:179307
Genbank record update date: Nov 30, 2009 11:16 AM

Polypeptide

Genbank accession no. AAA35581

Genbank version no. AAA35581.1 GI:179308

Genbank record update date: Nov 30, 2009 11:16 AM

Cross-references

Tedder T.F., et al *Proc. Natl. Acad. Sci. U.S.A.* 85 (1), 208-212 (1988)

Other information

Official Symbol: MS4A1

Other Aliases: B1, Bp35, CD20, CVID5, LEU-16, MS4A2, S7

Other Designations: B-lymphocyte antigen CD20; B-lymphocyte cell-surface antigen B1; CD20 antigen; CD20 receptor; leukocyte surface antigen Leu-16

ANTIBODIES

Genentech/Roche: Rituximab - Abdulla NE., et al *BioDrugs.* 2012 Apr 1;26(2):71-82.

For example, see US5736137, ATCC deposit No. HB-69119.

GSK/Genmab: Ofatumumab - Nightingale G., et al Ann Pharmacother. 2011 Oct;45(10):1248-55.

For example, see US20090169550A1 SEQ ID NOs: 2, 4 and 5.

Immunomedics: Veltuzumab - Goldenberg DM., et al *Leuk Lymphoma.* 2010 May;51(5):747-55.

For example, see US7919273B2 SEQ ID NOs: 1, 2, 3, 4, 5 and 6.

(81) *Tenascin C – TNC (Tenascin C)*

Nucleotide

Genbank accession no. NM_002160

Genbank version no. NM_002160.3 GI:340745336

Genbank record update date: Sep 23, 2012 02:33 PM

Polypeptide

Genbank accession no. NP_002151

Genbank version no. NP_002151.2 GI:153946395
Genbank record update date: Sep 23, 2012 02:33 PM

Cross-references

Nies D.E., et al J. Biol. Chem. 266 (5), 2818-2823 (1991); Siri A., et al Nucleic Acids Res. 19 (3), 525-531 (1991)

Other information

Official Symbol: TNC
Other Aliases: 150-225, GMEM, GP, HXB, JI, TN, TN-C
Other Designations: GP 150-225; cytotactin; glioma-associated-extracellular matrix antigen; hexabrachion (tenascin); myotendinous antigen; neuronectin; tenascin; tenascin-C isoform 14/AD1/16

ANTIBODIES
Philogen : G11 (von Lukowicz T., et al *J Nucl Med.* 2007 Apr;48(4):582-7) and F16 (Pedretti M., et al Lung Cancer. 2009 Apr;64(1):28-33)
For example, see US7968685 SEQ ID NOs: 29, 35, 45 and 47.

(82) *FAP (Fibroblast activation protein, alpha)*
Nucleotide
Genbank accession no. U09278
Genbank version no. U09278.1 GI:1888315
Genbank record update date: Jun 23, 2010 09:22 AM

Polypeptide
Genbank accession no. AAB49652
Genbank version no. AAB49652.1 GI:1888316
Genbank record update date: Jun 23, 2010 09:22 AM

Cross-references
Scanlan,M.J.,et al *Proc. Natl. Acad. Sci. U.S.A.* 91 (12), 5657-5661 (1994)

Other information
Official Symbol: FAP

Other Aliases: DPPIV, FAPA
Other Designations: 170 kDa melanoma membrane-bound gelatinase; integral membrane serine protease; seprase

(83) *DKK-1 (Dickkopf 1 homolog (Xenopus laevis)*
Nucleotide
Genbank accession no. NM_012242
Genbank version no. NM_012242.2 GI:61676924
Genbank record update date: Sep 30, 2012 01:48 PM

Polypeptide
Genbank accession no. NP_036374
Genbank version no. NP_036374.1 GI:7110719
Genbank record update date: Sep 30, 2012 01:48 PM

Cross-references
Fedi P. et al *J. Biol. Chem.* 274 (27), 19465-19472 (1999)

Other information
Official Symbol: DKK1
Other Aliases: UNQ492/PRO1008, DKK-1, SK
Other Designations: dickkopf related protein-1; dickkopf-1 like; dickkopf-like protein 1; dickkopf-related protein 1; hDkk-1

ANTIBODIES
Novartis: BHQ880 (Fulciniti M., et al *Blood.* 2009 Jul 9;114(2):371-379)
For example, see US20120052070A1 SEQ ID NOs: 100 and 108.

(84) *CD52 (CD52 molecule)*
Nucleotide
Genbank accession no. NM_001803
Genbank version no. NM_001803.2 GI:68342029
Genbank record update date: Sep 30, 2012 01:48 PM

Polypeptide

Genbank accession no. NP_001794
Genbank version no. NP_001794.2 GI:68342030
Genbank record update date: Sep 30, 2012 01:48 PM

Cross-references
Xia M.Q., et al *Eur. J. Immunol.* 21 (7), 1677-1684 (1991)

Other information
Official Symbol: CD52
Other Aliases: CDW52
Other Designations: CAMPATH-1 antigen; CD52 antigen (CAMPATH-1 antigen); CDW52 antigen (CAMPATH-1 antigen); cambridge pathology 1 antigen; epididymal secretory protein E5; he5; human epididymis-specific protein 5

ANTIBODIES
Alemtuzumab (Campath) - Skoetz N., et al *Cochrane Database Syst Rev.* 2012 Feb 15;2:CD008078.
For example, see Drugbank Acc. No. DB00087 (BIOD00109, BTD00109)

(85) *CS1 - SLAMF7 (SLAM family member 7)*
Nucleotide
Genbank accession no. NM_021181
Genbank version no. NM_021181.3 GI:1993571
Genbank record update date: Jun 29, 2012 11:24 AM

Polypeptide
Genbank accession no. NP_067004
Genbank version no. NP_067004.3 GI:19923572
Genbank record update date: Jun 29, 2012 11:24 AM

Cross-references
Boles K.S., et al *Immunogenetics* 52 (3-4), 302-307 (2001)

Other information
Official Symbol: SLAMF7

Other Aliases: UNQ576/PRO1138, 19A, CD319, CRACC, CS1

Other Designations: 19A24 protein; CD2 subset 1; CD2-like receptor activating cytotoxic cells; CD2-like receptor-activating cytotoxic cells; membrane protein FOAP-12; novel LY9 (lymphocyte antigen 9) like protein; protein 19A

ANTIBODIES

BMS: elotuzumab/HuLuc63 (Benson DM., et al *J Clin Oncol.* 2012 Jun 1;30(16):2013-2015)

For example, see US20110206701 SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15 and 16.

(86) *Endoglin – ENG (Endoglin)*

Nucleotide

Genbank accession no. AF035753

Genbank version no. AF035753.1 GI:3452260

Genbank record update date: Mar 10, 2010 06:36 PM

Polypeptide

Genbank accession no. AAC32802

Genbank version no. AAC32802.1 GI:3452261

Genbank record update date: Mar 10, 2010 06:36 PM

Cross-references

Rius C., et al *Blood* 92 (12), 4677-4690 (1998)

Official Symbol: ENG

Other information

Other Aliases: RP11-228B15.2, CD105, END, HHT1, ORW, ORW1

Other Designations: CD105 antigen

(87) *Annexin A1 – ANXA1 (Annexin A1)*

Nucleotide

Genbank accession no. X05908

Genbank version no. X05908.1 GI:34387

Genbank record update date: Feb 02, 2011 10:02 AM

Polypeptide

Genbank accession no. CCA29338

Genbank version no. CCA29338.1 GI:34388

Genbank record update date: Feb 02, 2011 10:02 AM

Cross-references

Wallner B.P.,et al *Nature* 320 (6057), 77-81 (1986)

Other information

Official Symbol: ANXA1

Other Aliases: RP11-71A24.1, ANX1, LPC1

Other Designations: annexin I (lipocortin I); annexin-1; calpactin II; calpactin-2; chromobindin-9; lipocortin I; p35; phospholipase A2 inhibitory protein

(88) *V-CAM (CD106) - VCAM1 (Vascular cell adhesion molecule 1)*

Nucleotide

Genbank accession no. M60335

Genbank version no. M60335.1 GI:340193

Genbank record update date: Jun 23, 2010 08:56 AM

Polypeptide

Genbank accession no. AAA61269

Genbank version no. AAA61269.1 GI:340194

Genbank record update date: Jun 23, 2010 08:56 AM

Cross-references

Hession C., et al *J. Biol. Chem.* 266 (11), 6682-6685 (1991)

Other information

Official Symbol VCAM1

Other Aliases: CD106, INCAM-100

Other Designations: CD106 antigen; vascular cell adhesion protein 1

Antibody Sequences

Anti-Integrin avβ6

RHAB6.2

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDT
EYAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTAVPNLRGDLQVLAQKVA
GPYPFDYWGQGTLVTVSS

RHCB6.2

QVQLVQSGAEVKKPGASVKVSCKASGYTFIDSYMHWVRQAPGQRLEWMGWIDPENGDT
EYAPKFQGRVTITTDTSASTAYMELSSLRSEDTAVYYCARGTPTAVPNLRGDLQVLAQKV
AGPYPFDYWGQGTLVTVSS

RHF

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGD
TEYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTGPYYFDYWGQGTLV
TVSS

RHFB6

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGD
TEYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTAVPNLRGDLQVLAQK
VAGPYYFDYWGQGTLVTVSS

RHAY100bP

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDT
EYAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTGPYPFDYWGQGTLVTV
SS

RKF

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKFL36L50

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWLQQKPGQAPRLLIYLTSNLASGIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKC
EIVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

Anti-CD33
CD33 Hum195 VH
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGT
GYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSS

CD33 Hum195 VK
DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGS
GVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK

Anti-CD19
CD19 B4 resurfaced VH
QVQLVQPGAEVVKPGASVKLSCKTSGYTFTSNWMHWVKQRPGQGLEWIGEIDPSDSYT
NYNQNFKGKAKLTVDKSTSTAYMEVSSLRSDDTAVYYCARGSNPYYYAMDYWGQGTSV
TVSS

CD19 B4 resurfaced VK
EIVLTQSPAIMSASPGERVTMTCSASSGVNYMHWYQQKPGTSPRRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEPEDAATYYCHQRGSYTFGGGTKLEIK

Anti-Her2
Herceptin VH chain
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT
VSS

Herceptin VL chain
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Anti-CD25

Simulect VK (also known as Basiliximab)

QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQQKPGTSPKRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEAEDAATYYCHQRSSYTFGGGTKLEIK

Simulect VH

QLQQSGTVLARPGASVKMSCKASGYSFTRYWMHWIKQRPGQGLEWIGAIYPGNSDTSY
NQKFEGKAKLTAVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFDFWGQGTTLTVSS

Anti-PSMA

Deimmunised VH '1

EVQLVQSGPEVKKPGATVKISCKTSGYTFTEYTIHWVKQAPGKGLEWIGNINPNNGGTTY
NQKFEDKATLTVDKSTDTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTLLTVSS

Deimmunised VK '1

DIQMTQSPSSLSTSVGDRVTLTCKASQDVGTAVDWYQQKPGPSPKLLIYWASTRHTGIPS
RFSGSGSGTDFTLTISSLQPEDFADYYCQQYNSYPLTFGPGTKVDIK

Deimmunised VH1 '5

EVKLVESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNN
FATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTGVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH2 '5

EVKLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH3 '5

EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH4 '5

EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRFTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VK1 ‘5
NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFTGSGSATDFTLTISSLQTEDLADYYCGQSYTFPYTFGQGTKLEMK

Deimmunised VK2 ‘5
NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK3 ‘5
NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK4 ‘5
NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDEADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK DI ‘5
NIVMTQFPKSMSASAGERMTLTCKASENVGTYVSWYQQKPTQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFILTISSVQAEDLVDYYCGQSYTFPYTFGGGTKLEMK

Deimmunised VH DI ‘5
EVKLEESGGGLVQPGGSMKISCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKSSVYLQMNSLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHA ‘5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHB ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHC ‘5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHD ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHE ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHF ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHG ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RKA ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKB ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKC ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKD ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKE ‘5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPD
RFTGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKF '5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKG '5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPD
RFTGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

The parent antibody may also be a fusion protein comprising an albumin-binding peptide (ABP) sequence (Dennis *et al.* (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" *J Biol Chem.* 277:35035-35043; WO 01/45746). Antibodies of the invention include fusion proteins with ABP sequences taught by: (i) Dennis *et al* (2002) *J Biol Chem.* 277:35035-35043 at Tables III and IV, page 35038; (ii) US 2004/0001827 at [0076]; and (iii) WO 01/45746 at pages 12-13.

In one embodiment, the antibody has been raised to target specific the tumour related antigen $\alpha_v\beta_6$.

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

The cell binding agent is connected to the linker. In one embodiment, the cell binding agent is connected to A, where present, of the linker.

In one embodiment, the connection between the cell binding agent and the linker is through a thioether bond.

In one embodiment, the connection between the cell binding agent and the linker is through a disulfide bond.

In one embodiment, the connection between the cell binding agent and the linker is through an amide bond.

In one embodiment, the connection between the cell binding agent and the linker is through an ester bond.

In one embodiment, the connection between the cell binding agent and the linker is formed between a thiol group of a cysteine residue of the cell binding agent and a maleimide group of the linker.

The cysteine residues of the cell binding agent may be available for reaction with the functional group of R^L to form a connection. In other embodiments, for example where the cell binding agent 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 R^L.

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

Drug loading

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

The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as UV, reverse phase HPLC, HIC, mass spectroscopy, ELISA assay, and electrophoresis. The quantitative distribution

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

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

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

Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two

reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by engineering one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues). US 7521541 teaches engineering antibodies by introduction of reactive cysteine amino acids.

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

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

Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more PBD drug

moieties and where the drug moieties may be attached to the antibody at various amino acid residues.

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

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

Use

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, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis. Cancers of particular interest include, but are not limited to, leukemias and ovarian cancers.

Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g. bowel, colon), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

In one embodiment, the treatment is of a pancreatic cancer.

In one embodiment, the treatment is of a tumour having $\alpha_v\beta_6$ integrin on the surface of the cell.

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia, haematological, and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders.

Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

Autoimmune diseases for which the ADC compounds may be used in treatment include rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g. ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and polyarteriitis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus),

hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g. Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

Methods of Treatment

The conjugates of the present invention may be used in a method of therapy. Also provided is a method of treatment, comprising administering to a subject in need of treatment a therapeutically-effective amount of a conjugate compound of the invention. 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 of the invention 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, such as chemotherapeutics); surgery; and radiation therapy.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, antibodies, photosensitizers, and kinase inhibitors. Chemotherapeutic agents include compounds used in "targeted therapy" and conventional chemotherapy.

Examples of chemotherapeutic agents include: erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTERE®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine, dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), trastuzumab (HERCEPTIN®, Genentech), temozolomide (4-methyl-5-oxo- 2,3,4,6,8-pentazabicyclo [4.3.0] nona-2,7,9-triene- 9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethylethanamine, NOLVADEX®, ISTUBAL®, VALODEX®), and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.

More examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sutent (SUNITINIB®, SU11248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (Mek inhibitor, Exelixis, WO 2007/044515), ARRY-886 (Mek inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 222584 (Novartis), fulvestrant (FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Il), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chloranmbucil, AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (TELCYTA®, Telik), thiotepa and cyclosphosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide,

mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, calicheamicin gamma1I, calicheamicin omegaI1 (*Angew Chem. Intl. Ed. Engl.* (1994) 33:183-186); dynemicin, dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, nemorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO);

retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

Also included in the definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors such as MEK inhibitors (WO 2007/044515); (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, for example, PKC-alpha, Raf and H-Ras, such as oblimersen (GENASENSE®, Genta Inc.); (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN® rIL-2; topoisomerase 1 inhibitors such as LURTOTECAN®; ABARELIX® rmRH; (ix) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and pharmaceutically acceptable salts, acids and derivatives of any of the above.

Also included in the definition of "chemotherapeutic agent" are therapeutic antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG™, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth).

Humanized monoclonal antibodies with therapeutic potential as chemotherapeutic agents in combination with the conjugates of the invention include: alemtuzumab, apolizumab, aselizumab, atlizumab, bapineuzumab, bevacizumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab,

daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pertuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a conjugate compound, 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.

Formulations

While it is possible for the conjugate compound to be used (e.g., administered) alone, it is often preferable to present it as a composition or formulation.

In one embodiment, the composition is a pharmaceutical composition (e.g., formulation, preparation, medicament) comprising a conjugate compound, as described herein, and a pharmaceutically acceptable carrier, diluent, or excipient.

In one embodiment, the composition is a pharmaceutical composition comprising at least one conjugate compound, as described herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, including, but not limited to, pharmaceutically acceptable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, colouring agents, flavouring agents, and sweetening agents.

In one embodiment, the composition further comprises other active agents, for example, other therapeutic or prophylactic agents.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, Handbook of Pharmaceutical Additives, 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse Information Resources, Inc., Endicott, New York, USA), Remington's Pharmaceutical Sciences, 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994.

Another aspect of the present invention pertains to methods of making a pharmaceutical composition comprising admixing at least one [^{11}C]-radiolabelled conjugate or conjugate-like compound, as defined herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, e.g., carriers, diluents, excipients, etc. If formulated as discrete units (e.g., tablets, etc.), each unit contains a predetermined amount (dosage) of the active compound.

The term "pharmaceutically acceptable," as used herein, pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier,

diluent, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

The formulations may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with carriers (e.g., liquid carriers, finely divided solid carrier, etc.), and then shaping the product, if necessary.

The formulation may be prepared to provide for rapid or slow release; immediate, delayed, timed, or sustained release; or a combination thereof.

Formulations suitable for parenteral administration (e.g., by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (e.g., solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (e.g., in a liposome or other microparticulate). Such liquids may additional contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active ingredient in the liquid is from about 1 ng/ml to about 10 µg/ml, for example from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

Dosage

It will be appreciated by one of skill in the art that appropriate dosages of the conjugate compound, and compositions comprising the conjugate compound, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of

therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician, veterinarian, or clinician.

In general, a suitable dose of the active compound is in the range of about 100 ng to about 25 mg (more typically about 1 µg to about 10 mg) per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, an amide, a prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 100 mg, 3 times daily.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 150 mg, 2 times daily.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 200 mg, 2 times daily.

However in one embodiment, the conjugate compound is administered to a human patient according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

In one embodiment, the conjugate compound is administered to a human patient according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

The dosage amounts described above may apply to the conjugate (including the PBD moiety and the linker to the antibody) or to the effective amount of PBD compound provided, for example the amount of compound that is releasable after cleavage of the linker.

For the prevention or treatment of disease, the appropriate dosage of an ADC of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. An exemplary dosing regimen comprises a course of administering an initial loading dose of about 4 mg/kg, followed by additional doses every week, two weeks, or three weeks of an ADC. Other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Treatment

The term "treatment," as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress,

a halt in the rate of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included.

The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

Similarly, the term "prophylactically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired prophylactic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

The Subject/Patient

The subject/patient may be an animal, mammal, a placental mammal, a marsupial (e.g., kangaroo, wombat), a monotreme (e.g., duckbilled platypus), a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), a lagomorph (e.g., a rabbit), avian (e.g., a bird), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), porcine (e.g., a pig), ovine (e.g., a sheep), bovine (e.g., a cow), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), an ape (e.g., gorilla, chimpanzee, orangutang, gibbon), or a human.

Furthermore, the subject/patient may be any of its forms of development, for example, a foetus. In one preferred embodiment, the subject/patient is a human.

In one embodiment, the patient is a population where each patient has a tumour having $\alpha_v\beta_6$ integrin on the surface of the cell.

Includes Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as

well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms.

Salts

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977).

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

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

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic,

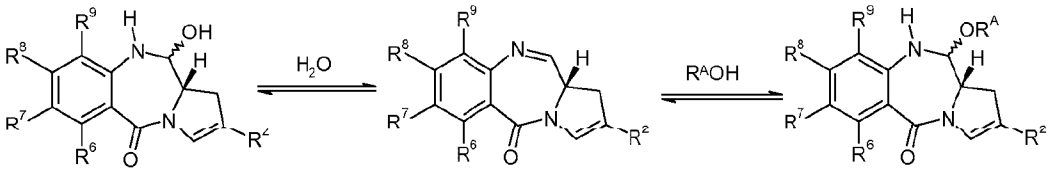
palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

Solvates

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

Carbinolamines

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



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

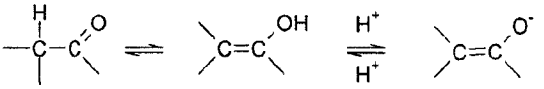
These particular compounds may be isolated in solid form, for example, by lyophilisation.

Isomers

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

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

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



keto enol enolate

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

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

General synthetic routes

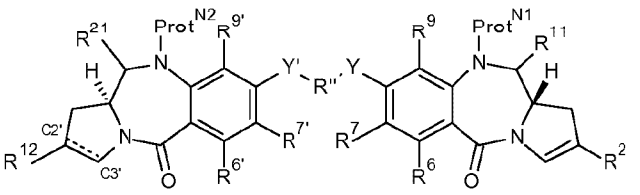
The synthesis of PBD compounds containing two imine moieties is extensively discussed in the following references:

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

b) WO 2005/023814 (pages 3 to 10);
c) WO 2004/043963 (pages 28 to 29);
d) WO 2005/085251 (pages 30 to 39);
e) WO 2010/043880 (pages 26 to 29);
f) WO 2011/130613 (pages 56 to 59); and
g) WO 2011/130616 (pages 57 to 61).

Synthesis route

The compounds of formula I, where either R^{10} and R^{11b} or R^{20} and R^{21b} 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:



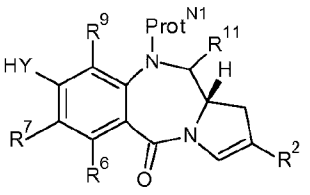
Formula 2

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, one of the pairs of R^{11} and $Prot^{N1}$ and R^{21} and $Prot^{N2}$ are $OProt^{O}$ and a carbamate nitrogen protecting group for synthesis and the other pair is selected from:
(a) =O and a hemi-aminal nitrogen protecting group for synthesis;
(b) H and a carbamate nitrogen protecting group for synthesis,
by applying the necessary conditions to remove the protecting groups.

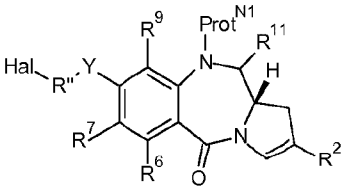
The compound of formula 2 may be used directly to make drug-linkers, and conjugates of the present invention, and thus may be a further aspect of the present invention. Part of the linking group may be added (for example, to form a protected compound of formula III), following which the deprotection discussed above can be carried out.

The group X or Q (part of R^2) in Formula 2 may be protected during the synthetic steps described below, in which case, it can be removed to give the desired compound of Formula 2.

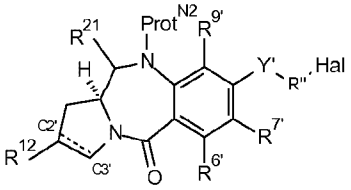
The compound of Formula 2 can be synthesised by the coupling of compounds of Formulae 3a and 4a, or compounds of Formulae 3b and 4b:



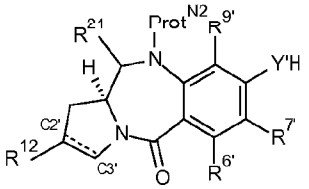
Formula 3a



Formula 3b



Formula 4a



Formula 4b

where Hal is selected from I, Cl, and Br.

The coupling can be achieved, for example, in refluxing acetone with a base, such as K_2CO_3.

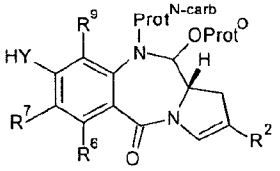
The compounds of Formulae 3b and 4a may be synthesised by reacting compounds of Formulae 3a and 4b respectively with a compound of Formula 5:

Hal-R"-Q Formula 5

where Q is selected from I, Cl, and Br, The reaction can be achieved, for example, in refluxing acetone with a base, such as K_2CO_3. An excess of the compound of Formula 5 is required to achieve the desired product.

The monomer which contains the imine or equivalent group can be synthesised in a similar manner to that described in co-pending PCT application PCT/EP2012/070232, filed 12

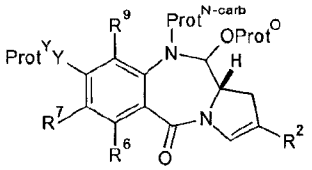
October 2012. This approach is described below in relation to the compound of Formula 3a where R^{11} and $Prot^{N1}$ are $OProt^{O}$ and a carbamate nitrogen protecting group for synthesis (Formula 3a-I), but is equally applicable to the compound of Formula 4b, where R^{21} and $Prot^{N2}$ are $OProt^{O}$ and a carbamate nitrogen protecting group for synthesis.



Formula 3a-I

$Prot^{N-carb}$ represents a carbamate nitrogen protecting group for synthesis.

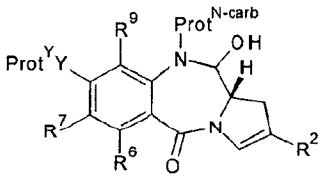
The compound of Formula 3a-I may be synthesised from a compound of Formula 6-I:



Formula 6-I

where $Prot^{Y}$ is a protecting group for Y that is orthogonal to the other protecting groups in the compound. The synthesis is achieved by deprotection of Y, under standard conditions.

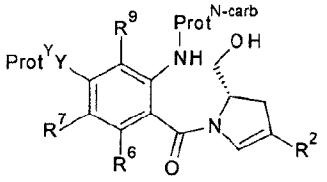
The compound of Formula 6-I may be synthesised from a compound of Formula 7-I:



Formula 7-I

by protecting the OH group with $Prot^{O}$, under non-racemising conditions.

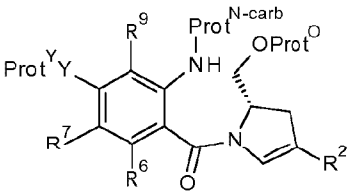
The compound of Formula 7-I may be synthesised from a compound of Formula 8-I:



Formula 8-I

by oxidation. The oxidation may be carried out, for example, with Dess-Martin periodinane (or alternatively TPAP/NMO, TFAA/DMSO, SO_3.Pyridine complex/DMSO, PDC, PCC, BAIB/TEMPO or under Swern conditions).

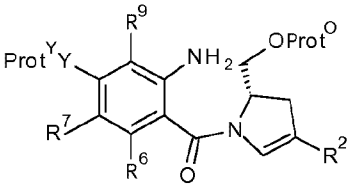
The compound of Formula 8-I may be synthesised from a compound of Formula 9-I:



Formula 9-I

by deprotection of the OH group under standard conditions.

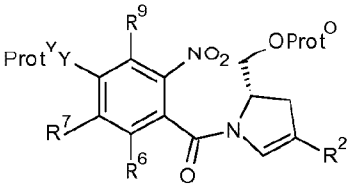
The compound of Formula 9-I may be synthesised from a compound of Formula 10-I:



Formula 10 I

by protection of the amine group by $Prot^{N\text{-}carb}$ under standard conditions.

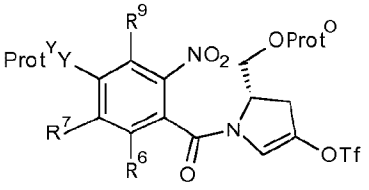
The compound of Formula 10-I may be synthesised from a compound of Formula 11-I:



Formula 11-I

by reduction of the nitro group. The reduction can be achieved by standard means, for example with Zn dust with 5% formic acid in methanol.

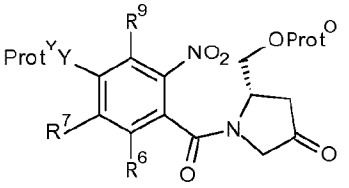
The compound of Formula 11-I may be synthesised from a compound of Formula 12-I:



Formula 12-I

by the palladium mediated coupling of the appropriate compound comprising -R^2. This coupling includes, but is not limited to: Suzuki couplings with an appropriate boron derivative; Heck coupling with alkenes, including acrylamides and acrylates; Stille couplings with organo tin reagents, such as alkyl tin reagents; Sonagishira couplings with alkynes; and hydride transfer using triethyl silanes.

The compound of Formula 12-I may be synthesised from a compound of Formula 13-I:

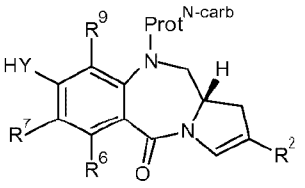


Formula 13-I

by triflation using triflic anhydride and anhydrous 2,6-lutidine or anhydrous 2,6-^{t}Bu-pyridine at a temperature of -35°C or lower in a dry organic solvent under an inert atmosphere.

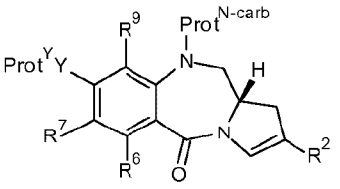
In the synthesis of compounds of Formula 4b where there is not a double bond between C2' and C3', the relevant R^{12} may be introduced at this stage.

If the other monomer contains an amine group, it can be synthesised in a similar manner to that described above. This approach is described below in relation to the compound of Formula 3a where R^{11} and $Prot^{N1}$ are H and a carbamate nitrogen protecting group for synthesis (Formula 3a-II), but is equally applicable to the compound of Formula 4b, where R^{21} and $Prot^{N2}$ are H and a carbamate nitrogen protecting group for synthesis.



Formula 3a-II

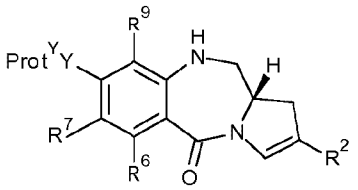
The compound of Formula 3a-II may be synthesised from a compound of Formula 6-II:



Formula 6-II

where $Prot^Y$ is a protecting group for Y that is orthogonal to the other protecting groups in the compound. The synthesis is achieved by deprotection of Y, under standard conditions.

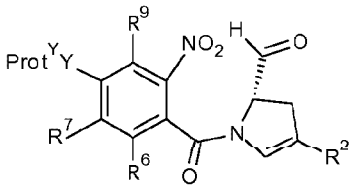
The compound of Formula 6-II may be synthesised from a compound of Formula 7-II:



Formula 7-II

by protecting the NH group with $Prot^{N\text{-}carb}$, under standard conditions.

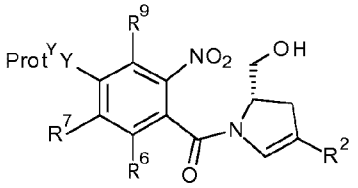
The compound of Formula 7-II may be synthesised from a compound of Formula 8-II:



Formula 8-II

by reductive amination.

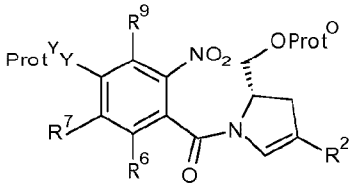
The compound of Formula 8-II may be synthesised from a compound of Formula 9-II:



Formula 9-II

by oxidation of the alcohol.

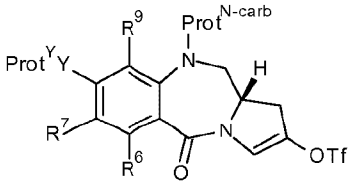
The compound of Formula 9-II may be synthesised from a compound of Formula 11-I:



Formula 11-I

by deprotection of the OH group under standard conditions.

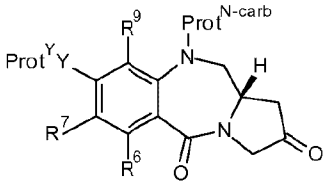
The compound of Formula 6-II may alternatively be synthesised from a compound of formula 12:



Formula 12

by the palladium mediated coupling of the appropriate compound comprising $-R^2$ (as described above).

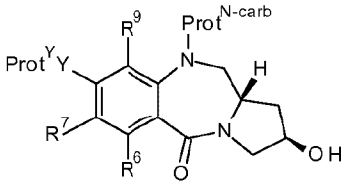
The compound of formula 12 may be synthesised from a compound of formula 13:



Formula 13

by triflation. This may be carried out with the conditions described above, or with standard conditions.

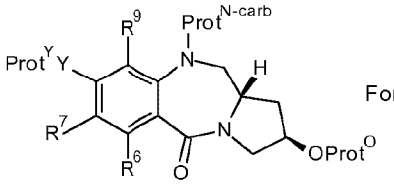
The compound of formula 13 may be synthesised from a compound of formula 14:



Formula 14

by oxidation of the alcohol group using standard conditions.

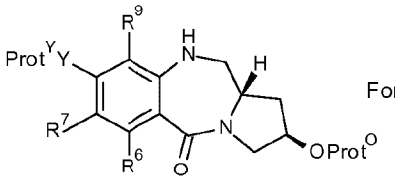
The compound of formula 14 may be synthesised from a compound of formula 15:



Formula 15

by removal of the $Prot^O$ group, which group is a alcohol protecting group orthogonal to the other protecting groups in the compound.

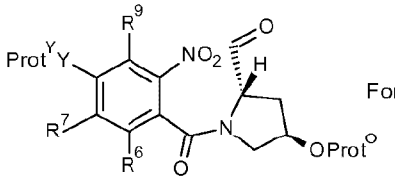
The compound of formula 15 may be synthesised from a compound of formula 16:



Formula 16

by protection of the amine with a carbamate nitrogen protecting group.

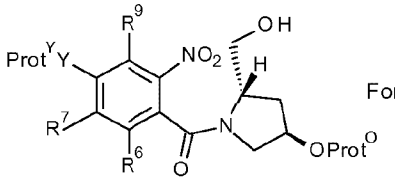
The compound of formula 16 may be synthesised from a compound of formula 17:



Formula 17

by reductive amination.

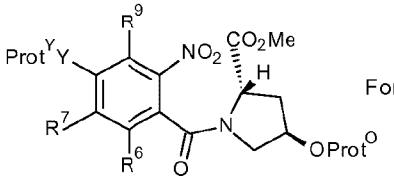
The compound of formula 17 may be synthesised from a compound of formula 18:



Formula 18

by oxidation of the unprotected alcohol group. The compound of formula 18 can be used to synthesise the compound of Formula 13-I.

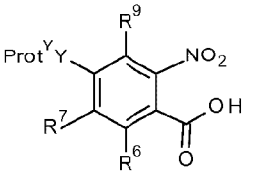
The compound of formula 18 may be synthesised from a compound of formula 19:

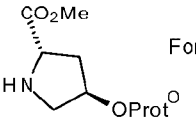


Formula 19

by reduction of the ester functionality.

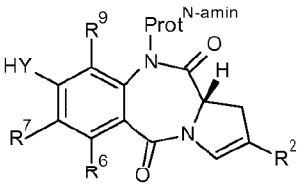
The compound of formula 19 may be synthesised from by coupling compounds of formulae 20 and 21:


Formula 20


Formula 21

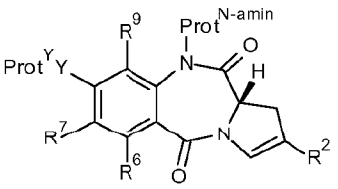
under amide coupling conditions.

If the other monomer contains an amide group, it can be synthesised in a similar manner to that described above. This approach is described below in relation to the compound of Formula 3a where R^{11} and $Prot^{N1}$ are =O and a hemi-aminal nitrogen protecting group for synthesis (Formula 3a-III), but is equally applicable to the compound of Formula 4b, where R^{21} and $Prot^{N2}$ are =O and a hemi-aminal nitrogen protecting group for synthesis.


Formula 3a-III

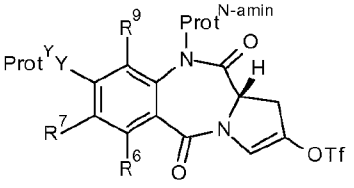
where $Prot^{N\text{-}amin}$ represents a hemi-aminal nitrogen protecting group for synthesis.

The compound of Formula 3a-III may be synthesised from a compound of Formula 22:


Formula 22

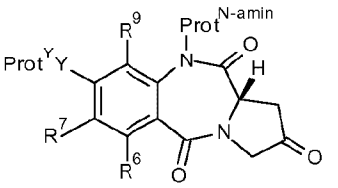
by deprotection of Y, under standard conditions.

The compound of Formula 22 may be synthesised from a compound of Formula 23:


Formula 23

by the palladium mediated coupling of the appropriate compound comprising $-R^2$ (as described above).

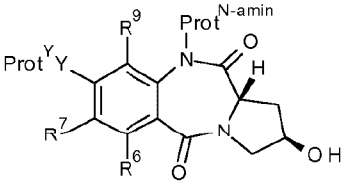
The compound of Formula 23 may be synthesised from a compound of Formula 24:



Formula 24

by triflation. This may be carried out with the conditions described above, or with standard conditions.

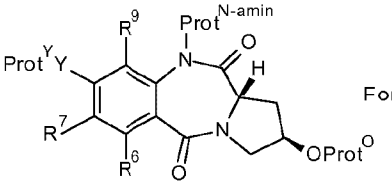
The compound of Formula 24 may be synthesised from a compound of Formula 25:



Formula 25

by oxidation of the alcohol group.

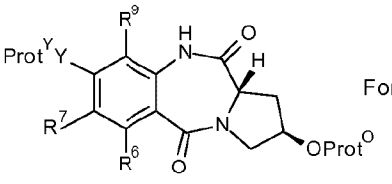
The compound of Formula 25 may be synthesised from a compound of Formula 26:



Formula 26

by removal of the $Prot^O$ group, which group is a alcohol protecting group orthogonal to the other protecting groups in the compound.

The compound of Formula 26 may be synthesised from a compound of Formula 27:



Formula 27

by protection of the amine with an hemi-aminal nitrogen protecting group.

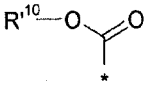
The compound of Formula 27 may be synthesised from a compound of Formula 19 by reduction of the ester functionality by hydrogen and Pd/C to achieve ring closure.

An alternative synthesis strategy is illustrated in Example 1 below.

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:



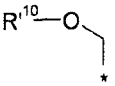
wherein R'10 is R as defined above. A large number of suitable groups are described on pages 503 to 549 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999.

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. 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.

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

Preferred oxygen protecting groups include acetates, TBS and THP.

Synthesis of Drug Conjugates

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

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

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

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

Antibodies with introduced cysteine residues can be conjugated to Linker-Drug compounds as described in International Patent Publication WO2008/070593. Antibodies containing an introduced cysteine residue in the heavy chain are fully reduced by adding 10 equivalents of TCEP and 1 mM EDTA and adjusting the pH to 7.4 with 1M Tris buffer (pH 9.0). Following a 1 hour incubation at 37°C, the reaction is cooled to 22°C and 30 equivalents of dehydroascorbic acid is added to selectively reoxidize the native disulfides, while leaving the introduced cysteine in the reduced state. The pH is adjusted to 6.5 with 1M Tris buffer (pH 3.7) and the reaction is allowed to proceed for 1 hour at 22°C. The pH of the solution is then raised again to 7.4 by addition of 1 M Tris buffer (pH 9.0). 3.5 equivalents of the PBD drug linker in DMSO is placed in a suitable container for dilution with propylene glycol prior to addition to the reaction. To maintain solubility of the PBD drug linker, the antibody itself is first diluted with propylene glycol to a final concentration of 33% (e.g., if the antibody solution was in a 60 mL reaction volume, 30 mL of propylene glycol was added). This same volume of propylene glycol (30 mL in this example) is added to the PBD drug linker as a diluent. After mixing, the solution of PBD drug linker in propylene glycol is added to the antibody solution to effect the conjugation; the final concentration of propylene glycol is 50%. The reaction is allowed to proceed for 30 minutes and then quenched by addition of 5 equivalents of N-acetyl cysteine. The ADC is purified by ultrafiltration through a 30 kD membrane. (Note that the concentration of propylene glycol used in the reaction can be reduced for any particular PBD, as its sole purpose is to maintain solubility of the drug linker in the aqueous media.)

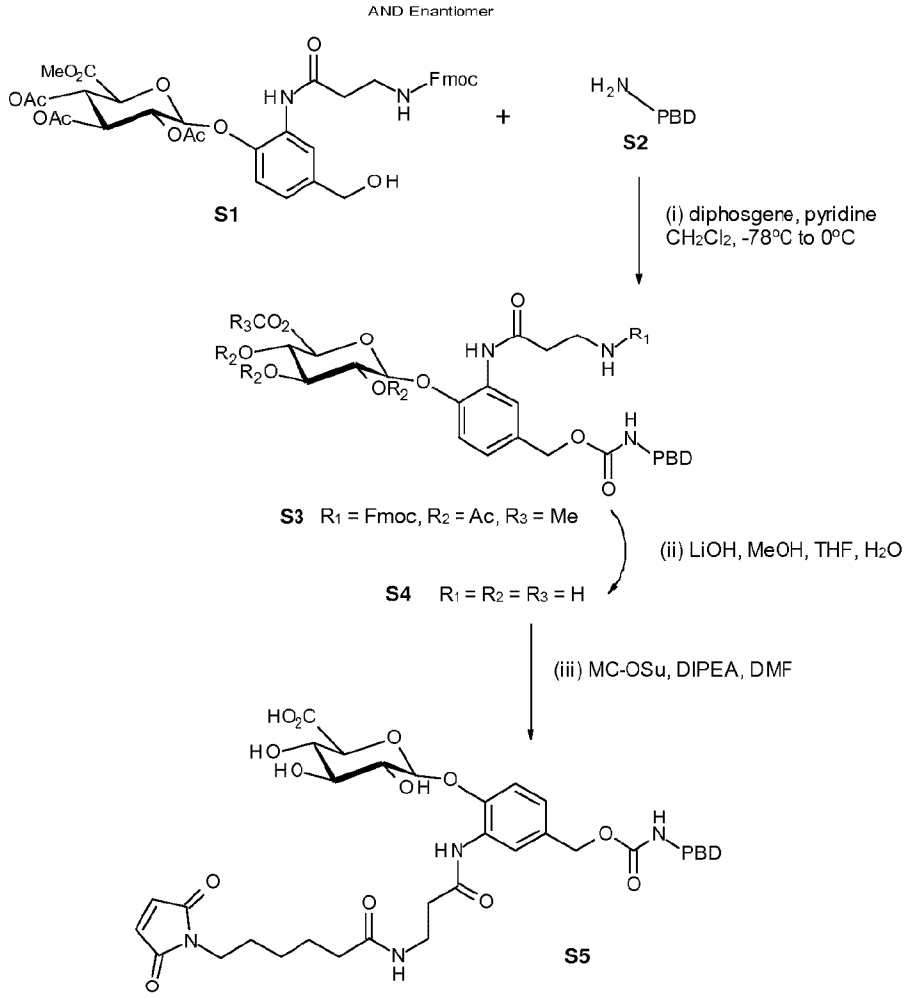
For halo-acetamide-based Linker-Drug compounds, conjugation can be performed generally as follows. To a solution of reduced and reoxidized antibodies (having introduced cysteines in the heavy chain) in 10 mM Tris (pH 7.4), 50 mM NaCl, and 2 mM DTPA is added 0.5 volumes of propylene glycol. A 10mM solution of acetamide-based

Linker-Drug compound in dimethylacetamide is prepared immediately prior to conjugation. An equivalent amount of propylene glycol as added to the antibody solution is added to a 6-fold molar excess of the Linker-Drug compound. The dilute Linker-Drug solution is added to the antibody solution and the pH is adjusted to 8-8.5 using 1 M Tris (pH 9). The conjugation reaction is allowed to proceed for 45 minutes at 37° C. The conjugation is verified by reducing and denaturing reversed phase PLRP-S chromatography. Excess Linker-Drug compound is removed with Quadrasil MP resin and the buffer is exchanged into 10 mM Tris (pH 7.4), 50 mM NaCl, and 5% propylene glycol using a PD-10 desalting column.

Illustrative synthesis schemes for Drug linkers

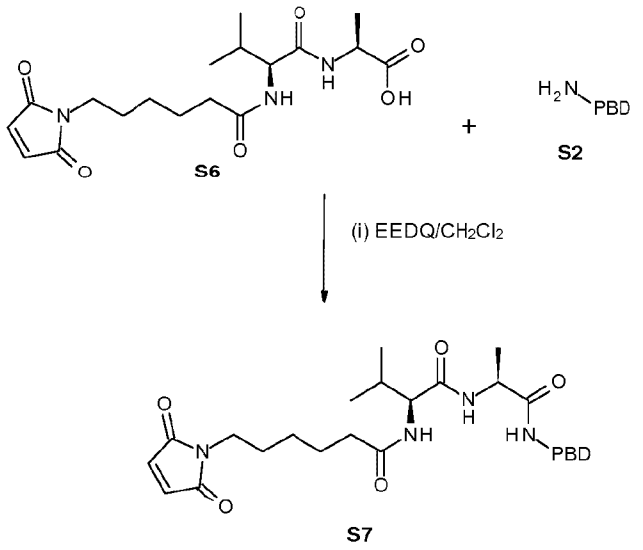
The following schemes are illustrative of routes for synthesising drug linkers, wherein PBD represents a compound of formula I of the present invention where X is NH_2, which may be varied within the scope of the present invention.

Scheme A



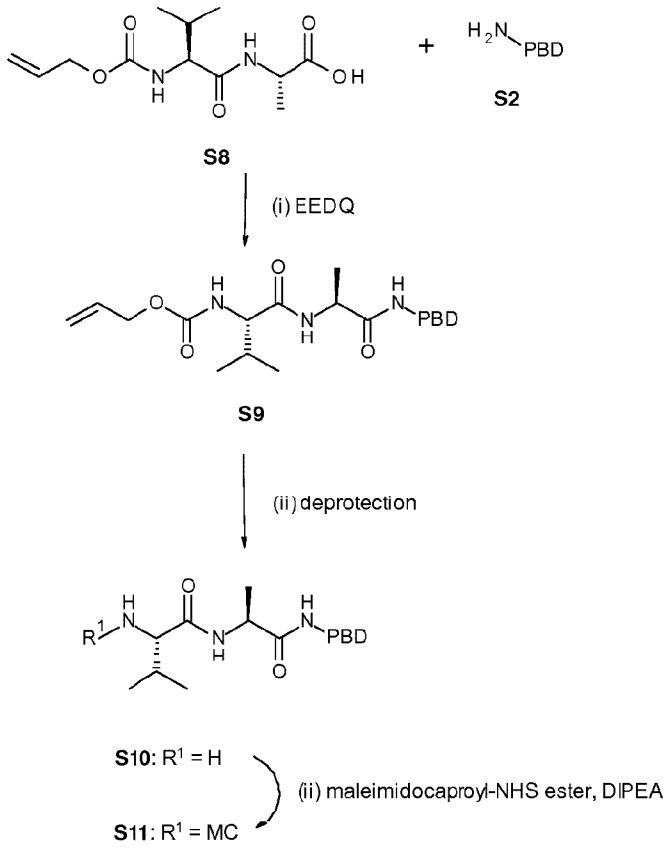
The glucuronide linker intermediate **S1** (reference: Jeffrey et al., *Bioconjugate Chemistry*, **2006**, *17*, 831-840) can be treated with diphosgene in dichlroromethane at -78°C to afford the glucuronide chloroformate, which is then reacted with the PBD dimer **S2** dissolved in CH_2Cl_2 by dropwise addition. Warming the reaction to 0°C over 2 hours followed by extraction will yield the compound **S3**. Treating a solution of **S3** in an equal solvent mixture of MeOH, tetrahydrofuran, and water (cooled to 0°C) with lithium hydroxide monohydrate for 4 hours, followed by reaction with glacial acetic acid will yield the compound **S4**. Adding maleimidocaproyl NHS ester to a solution of **S4** in DMF, followed by diisopropylethylamine and stirring at room temperature under nitrogen for 2 hours will yield the desired drug linker **S5**.

Scheme B



The maleimide linker **S6**, which can be synthesised by reacting maleimidocaproyl N-hydroxysuccinimide and H-Val-Ala-OH, can be linked to the exemplary compounds, **S2**, in the presence of EEDQ in anhydrous dichloromethane.

Scheme C



The linker **S8** can be linked to the exemplary compounds, **S2**, in the presence of EEDQ in 5% methanol/dichloromethane. The deprotection of **S9** can be carried out with the use of Ph_3P, pyrollidine and tetrakis palladium in anhydrous dichloromethane. S10 can be converted to the desired products by adding maleimidocaproyl-NHS ester, in the presence of DIPEA in DMF.

Further Preferences

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

In some embodiments, $R^{6'}$, $R^{7'}$, $R^{9'}$, and Y' are preferably the same as R^6, R^7, R^9, and Y respectively.

Dimer link

Y and Y' are preferably O.

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

R^6 to R^9

R^9 is preferably H.

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

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

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

R^2

In some embodiments, R^2 is of formula IIa.

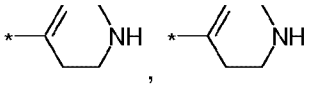
A in R^2 when it is of formula IIa may be phenyl group or a C_{5-7} heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, A is preferably phenyl.

Q^2-X may be on any of the available ring atoms of the C_{5-7} aryl group, but is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the C_{5-7} aryl group (A) is phenyl, the substituent (Q^2-X) is preferably in the meta- or para- positions, and more preferably is in the para- position.

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

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

In other embodiments, R^2 is of formula IIb. In these embodiments, R^{C1}, R^{C2} and R^{C3} are independently selected from H and unsubstituted C_{1-2} alkyl. In some preferred embodiments, R^{C1}, R^{C2} and R^{C3} are all H. In other embodiments, R^{C1}, R^{C2} and R^{C3} are all methyl. In certain embodiments, R^{C1}, R^{C2} and R^{C3} are independently selected from H and methyl.

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

In some embodiments R^2 is of formula IIc. In these embodiments, it is preferred that Q is NR^N. In other embodiments, Q is OH. In further embodiments, Q 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.

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

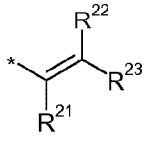
R^{12}

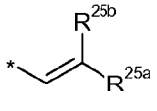
When there is a double bond present between C2' and C3', R^{12} is selected from:

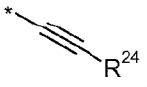
(a) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

(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-C_{1-3} alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

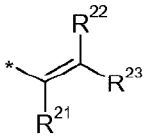
If a substituent on R^{12} when R^{12} is a C_{5-10} aryl group is ester, this is preferably methyl ester or ethyl ester.

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. Other particularly preferred substituents for R^{12} are dimethylaminopropyloxy and carboxy.

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 cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.

When R^{12} is 

, each of R^{21}, R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5. In some embodiments, the total number of carbon atoms in the R^{12} group is no more than 4 or no more than 3.

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

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

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

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

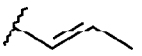
In some embodiments, R^{22} is H.

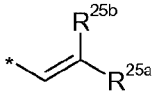
In some embodiments, R^{23} is H.

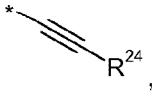
In some embodiments, R^{21} and R^{22} are H.

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

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

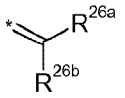
An R^{12} group of particular interest is:  .

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.

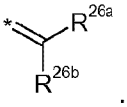
When R^{12} is  , R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl. If the phenyl optional substituent is halo, it is preferably fluoro. 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 H or  , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester.

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

In some embodiments, R^{12} is  .

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

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

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

R^{10}, R^{11a}, R^{11b}, R^{20}, R^{21a}, R^{21b}

In some embodiments, R^{20} is H and R^{21a} and R^{21b} are both H. Alternatively, R^{20} may be Me when R^{21a} and R^{21b} are both H.

In some embodiments, R^{20} is H and R^{21a} and R^{21b} are together form =O. Alternatively, R^{20} may be Me when R^{21a} and R^{21b} together form =O.

In either of these sets of embodiments, it may be preferred that R^{10} and R^{11b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are

bound and R^{11a} is H. It may be alternatively preferred that R^{10} is H, R^{11a} is H and R^{11b} is OH. It may be further alternatively preferred that R^{10} is H, R^{11a} is H and R^{11b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation.

In some embodiments, R^{10} is H and R^{11a} and R^{11b} are both H. Alternatively, R^{10} may be Me when R^{11a} and R^{11b} are both H.

In some embodiments, R^{10} is H and R^{11a} and R^{11b} together form =O. Alternatively, R^{10} may be Me when R^{11a} and R^{11b} together form =O.

In either of these sets of embodiments, it may be preferred that R^{20} and R^{21b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{21a} is H. It may be alternatively preferred that R^{20} is H, R^{21a} is H and R^{21b} is OH. It may be further alternatively preferred that R^{20} is H, R^{21a} is H and R^{21b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation.

M and z

It is preferred that M is a monovalent pharmaceutically acceptable cation, and is more preferably Na^+.

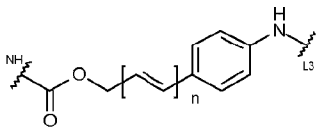
z is preferably 3.

Fourth aspect

L^4

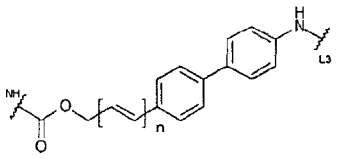
In some embodiments, L^4 is a single bond.

In some embodiments, L^4 is:



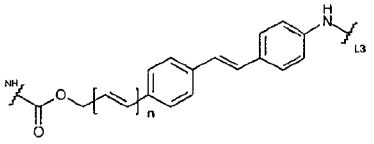
wherein n is 0 to 3. In these embodiments, n can be 0, 1, 2 or 3. n=0 and n=1 may be preferred.

In some embodiments, L^4 is:



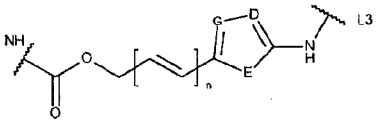
wherein n is 0 to 3. In these embodiments, n can be 0, 1, 2 or 3. n=0 and n=1 may be preferred.

In some embodiments, L^4 is:



wherein n is 0 to 3. In these embodiments, n can be 0, 1, 2 or 3. n=0 and n=1 may be preferred.

In some embodiments, L^4 is:



wherein n is 0 to 3. In these embodiments, n can be 0, 1, 2 or 3. n=0 and n=1 may be preferred. In one of these embodiments, D is N. In other of these embodiments, D is CH. In one of these embodiments, E is O or S. In one these embodiments, G is CH.

L^3

In one embodiment, L^3 is an amino acid residue. The amino acid may a natural amino acids or a non-natural amino acid.

In one embodiment, L^3 is selected from: Phe, Lys, Val, Ala, Cit, Leu, Ile, Arg, and Trp, where Cit is citrulline.

In one embodiment, L^3 comprises a dipeptide residue. The amino acids in the dipeptide may be any combination of natural amino acids and non-natural amino acids. In some embodiments, the dipeptide comprises natural amino acids. Where the linker is a cathepsin labile linker, the dipeptide is the site of action for cathepsin-mediated cleavage. The dipeptide then is a recognition site for cathepsin.

In one embodiment, L^3 is selected from:

^{Prot}-Phe-Lys-^{L4},

^{Prot}-Val-Ala-^{L4},

^{Prot}-Val-Lys-^{L4},

^{Prot}-Ala-Lys-^{L4},

^{Prot}-Val-Cit-^{L4},

^{Prot}-Phe-Cit-^{L4},

^{Prot}-Leu-Cit-^{L4},

^{Prot}-Ile-Cit-^{L4},

^{Prot}-Phe-Arg-^{L4}, and

^{Prot}-Trp-Cit-^{L4};

where Cit is citrulline.

Preferably, L^3 is selected from:

^{Prot}-Phe-Lys-^{L4},

^{Prot}-Val-Ala-^{L4},

^{Prot}-Val-Lys-^{L4},

^{Prot}-Ala-Lys-^{L4}, and

^{Prot}-Val-Cit-^{L4}.

Most preferably, L^3 is selected from ^{Prot}-Phe-Lys-^{L4}, ^{Prot}-Val-Cit-^{L4} or ^{Prot}-Val-Ala-^{L4}.

Other dipeptide combinations of interest include:

^{Prot}-Gly-Gly-^{L4},

^{Prot}-Pro-Pro-^{L4}, and

^{Prot}-Val-Glu-^{L4}.

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

In some embodiments, L^3 is a tripeptide residue. The amino acids in the tripeptide may be any combination of natural amino acids and non-natural amino acids. In some embodiments, the tripeptide comprises natural amino acids. Where the linker is a

cathepsin labile linker, the tripeptide is the site of action for cathepsin-mediated cleavage. The tripeptide then is a recognition site for cathepsin.

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, and as described above.

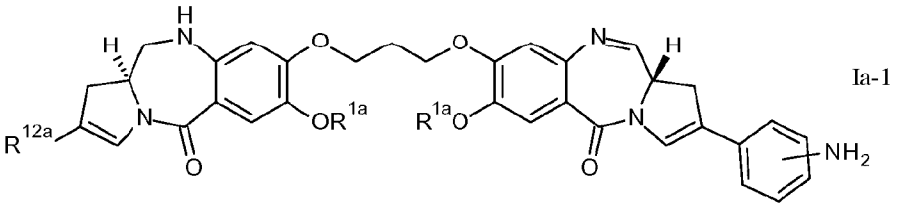
Prot

Prot is selected from Fmoc (fluorenylmethyloxycarbonyl), Teoc (2-(trimethylsilyl)ethoxycarbonyl) , Boc (t-butoxycarbonyl) and Alloc (alylocycarbonyl). In some embodiments, Prot is selected from Fmoc and Teoc.

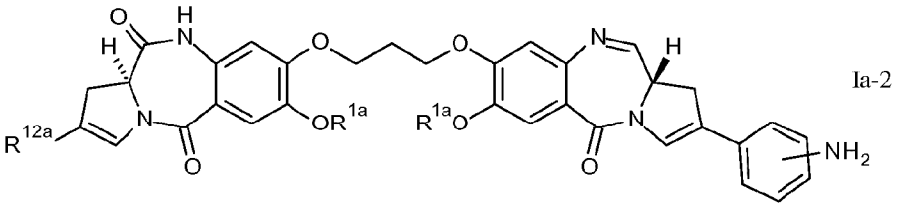
In some embodiments, Prot is Fmoc.

In some embodiments, Prot is Teoc.

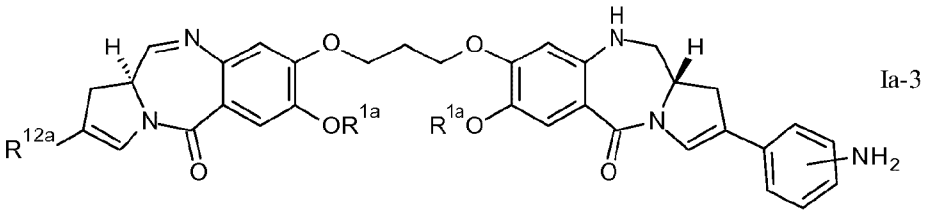
Particularly preferred compounds of the first aspect of the present invention are of formula Ia-1, Ia-2, Ia-3 or Ia-4:



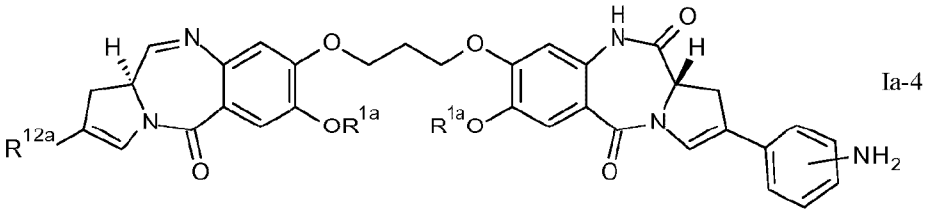
Ia-1



Ia-2

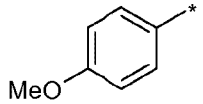


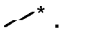
Ia-3

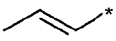


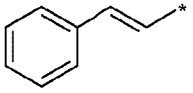
Ia-4

where R^{12a} is selected from:

(a)  ;

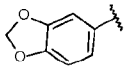
(b)  ;

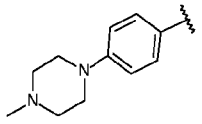
(c)  ;

(d)  ;

(e)  ;

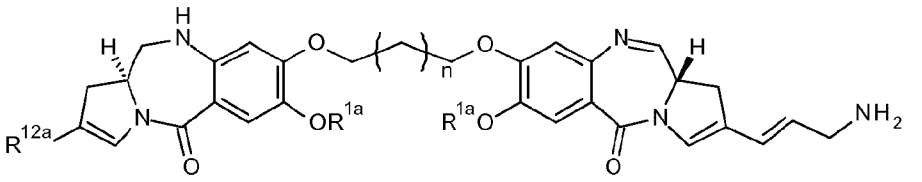
(f)  ;

(g)  ; and

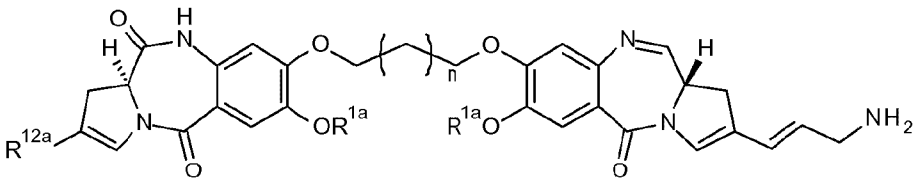
(h)  .

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

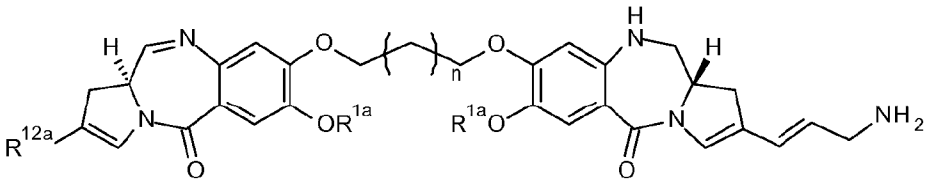
Further particularly preferred compounds of the first aspect of the present invention are of formula Ib-1, Ib-2, Ib-3 or Ib-4:



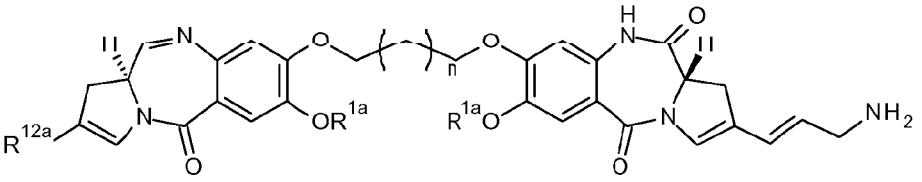
Ib-1



Ib-2



Ib-3



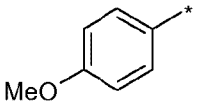
Ib-4

where

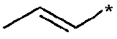
n is 1 or 3;

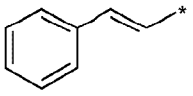
R^{1a} is methyl or phenyl;

R^{12a} is selected from:

(a)  ;

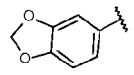
(b)  ;

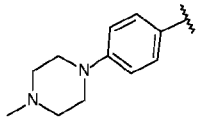
(c)  ;

(d)  ;

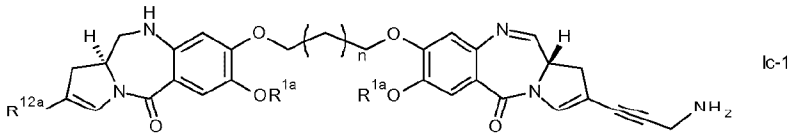
(e)  ;

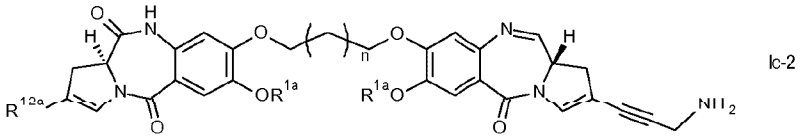
(f)  ;

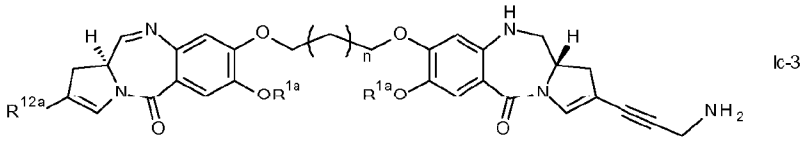
(g)  ; and

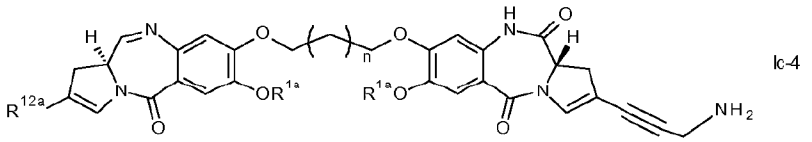
(h)  .

Further particularly preferred compounds of the first aspect of the present invention are of formula Ic-1, Ic-2, Ic-3 or Ic-4:


Ic-1


Ic-2


Ic-3

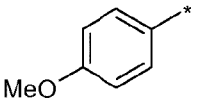

Ic-4

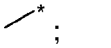
where

n is 1 or 3;

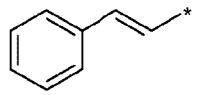
R^{1a} is methyl or phenyl;

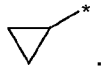
R^{12a} is selected from:

(a)  ;

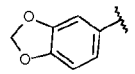
(b)  ;

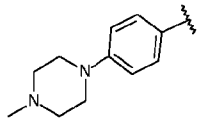
(c)  ;

(d)  ;

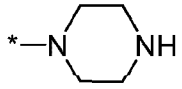
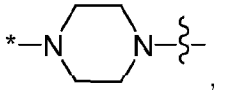
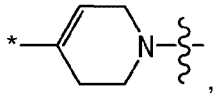
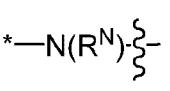
(e)  ;

(f)  ;

(g)  ; and

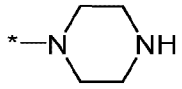
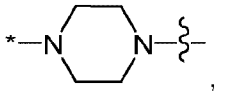
(h)  .

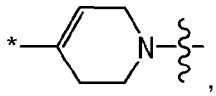
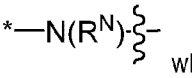
Fifth aspect

The preferences for compounds of formula I apply as appropriate to D in the fifth aspect of the invention. . For example, in the fifth aspect, the PBD dimer is any of the compounds of formula I, or a pharmaceutically acceptable salt or solvate thereof, described herein expect that,  is replaced with  , is replaced with  , and  is replaced with  where the wavy line indicates the point of attachment to the Linker Unit.

Accordingly, the Conjugates of the present invention include those having the following formula (V)

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

or a pharmaceutically acceptable salt or solvate thereof, wherein L is a Ligand unit (i.e., a targeting agent), LU is a Linker unit and the PBD dimer D. D is any of the compounds of formula I, or a pharmaceutically acceptable salt or solvate thereof, described herein expect that,  is replaced with  , is replaced with

 , and *—NHR^N is replaced with  where the wavy line indicates the point of attachment to the Linker Unit.

(a) Conjugates of the present invention include, for example, those of the formula:

CBA – A^1 – L^1– *

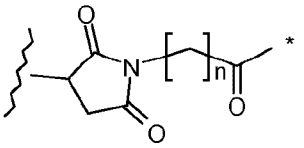
where the asterisk indicates the point of attachment to the PBD dimer (D), CBA is the Cell Binding Agent, L^1 is a Specificity unit that is cleavable by the action of an enzyme, and A^1 is a Stretcher unit connecting L^1 to the Cell Binding Agent.

(b) Conjugates of the present invention include, for example, those of the formula:

CBA – A^1 – L^1– *

where the asterisk indicates the point of attachment to the PBD dimer (D), CBA is the Cell Binding Agent, A^1 is a Stretcher unit connecting L^1 to the Cell Binding Agent and L^1 is a Specificity unit that is cleavable by the action of cathepsin, L^1 is a dipeptide, L^1 is a dipeptide that is cleavable by the action of cathepsin or L^1 is a dipeptide selected from -Phe-Lys-, -Val-Ala-, -Val-Lys-, -Ala-Lys-, and -Val-Cit-.

Preferred conjugates of the present invention include any of those described in (a) and (b) wherein A^1 is



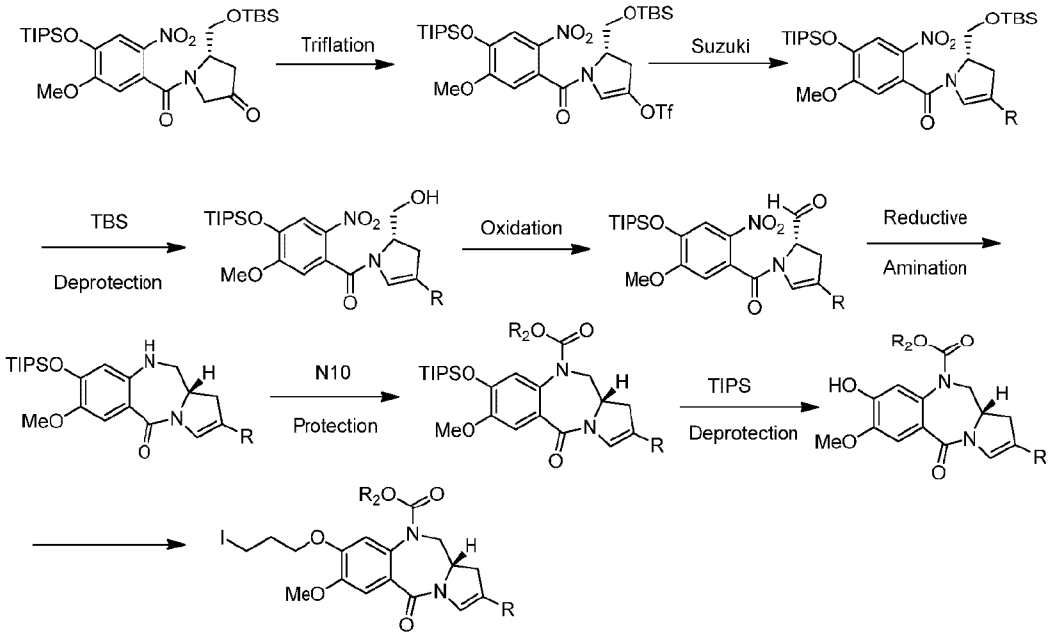
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to CBA, and n is 0 to 6 (preferably n is 5).

Illustrative Synthetic Schemes

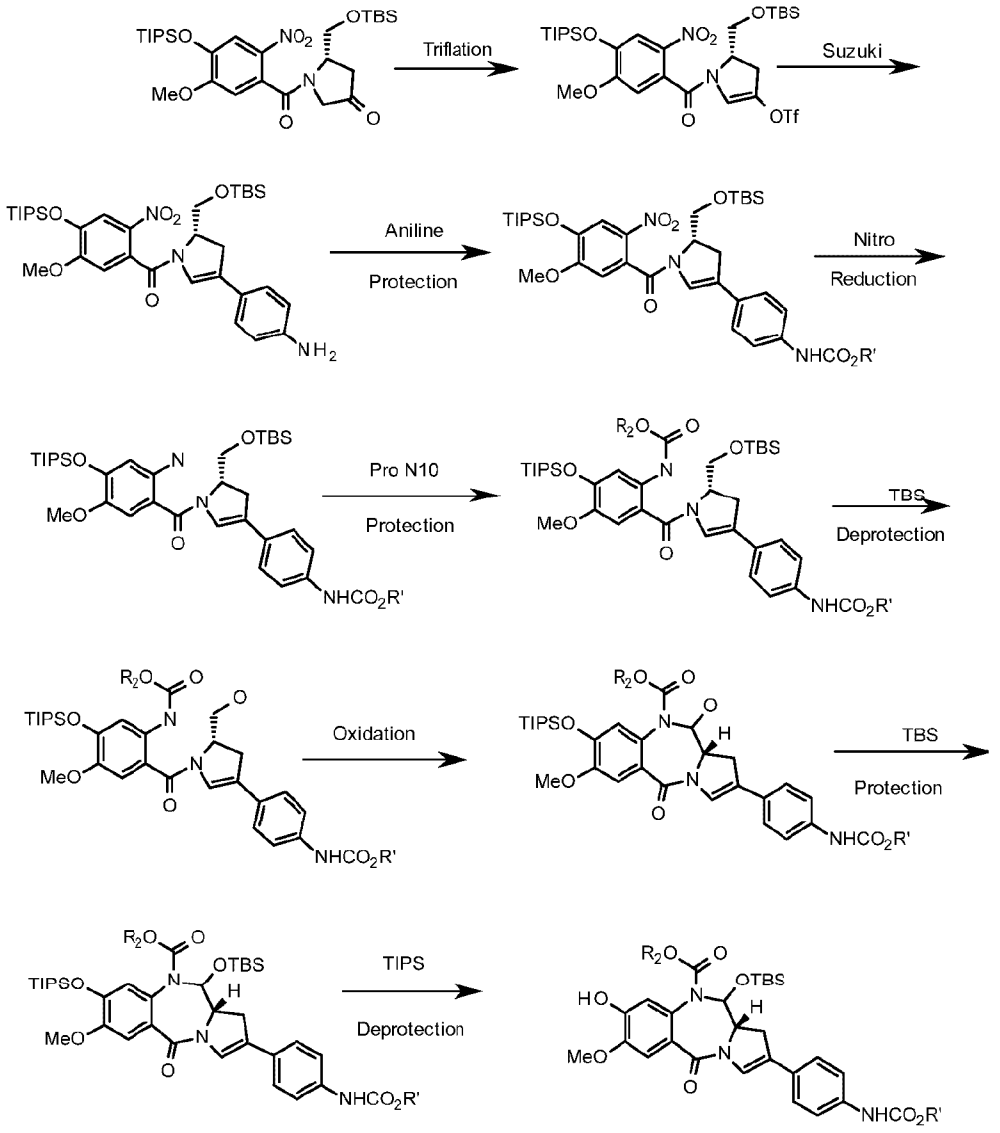
The following schemes illustrate a way of making certain compounds of the present invention, in which certain groups are illustrated generically as R, R' and R_2. The groups of which these form a part should be interpreted in accordance with the disclosure of the

invention. In schemes where protecting groups are explicitly described, these may also be varied within the scope of the present invention.

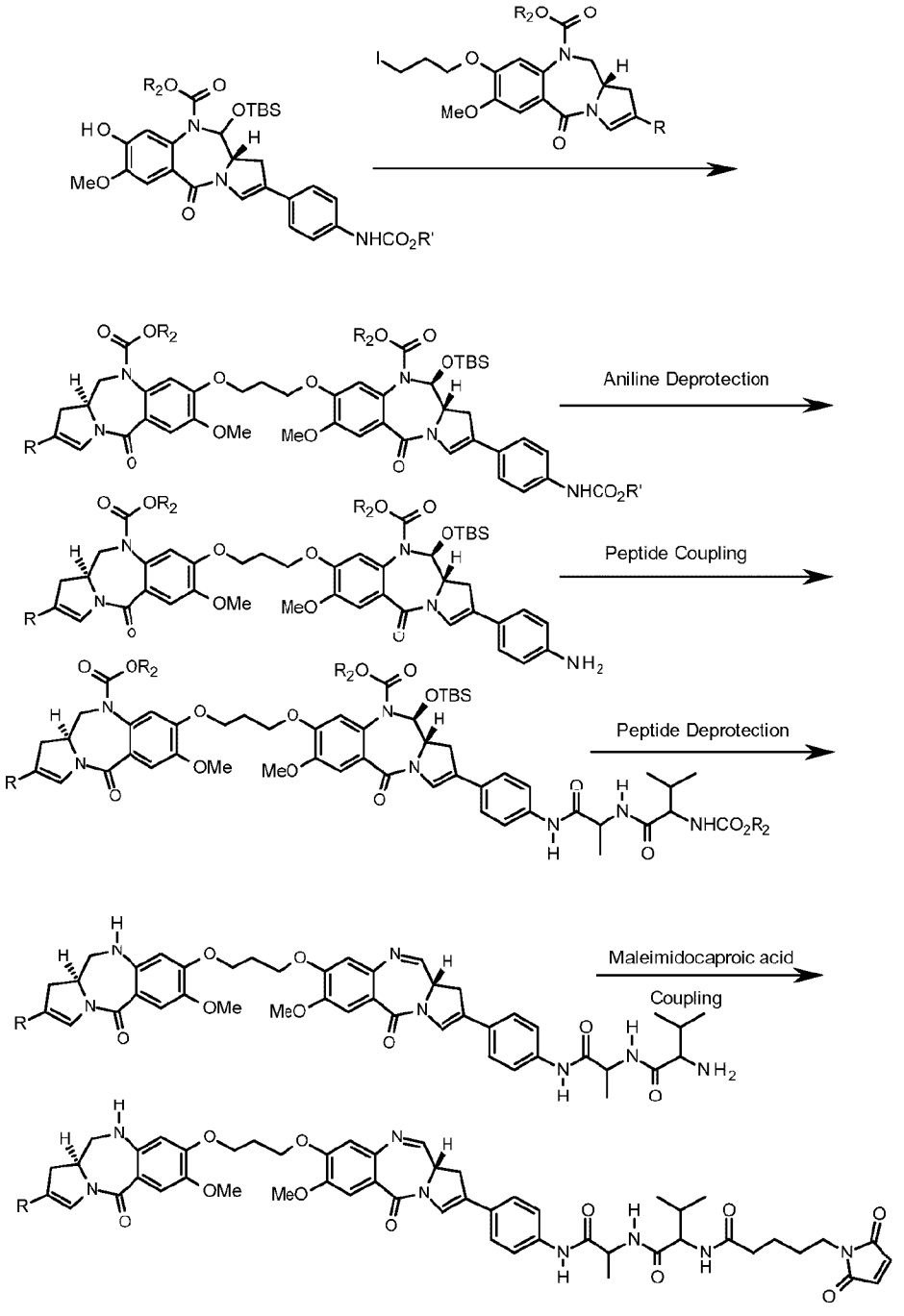
Scheme 1a – synthesis of amine building block



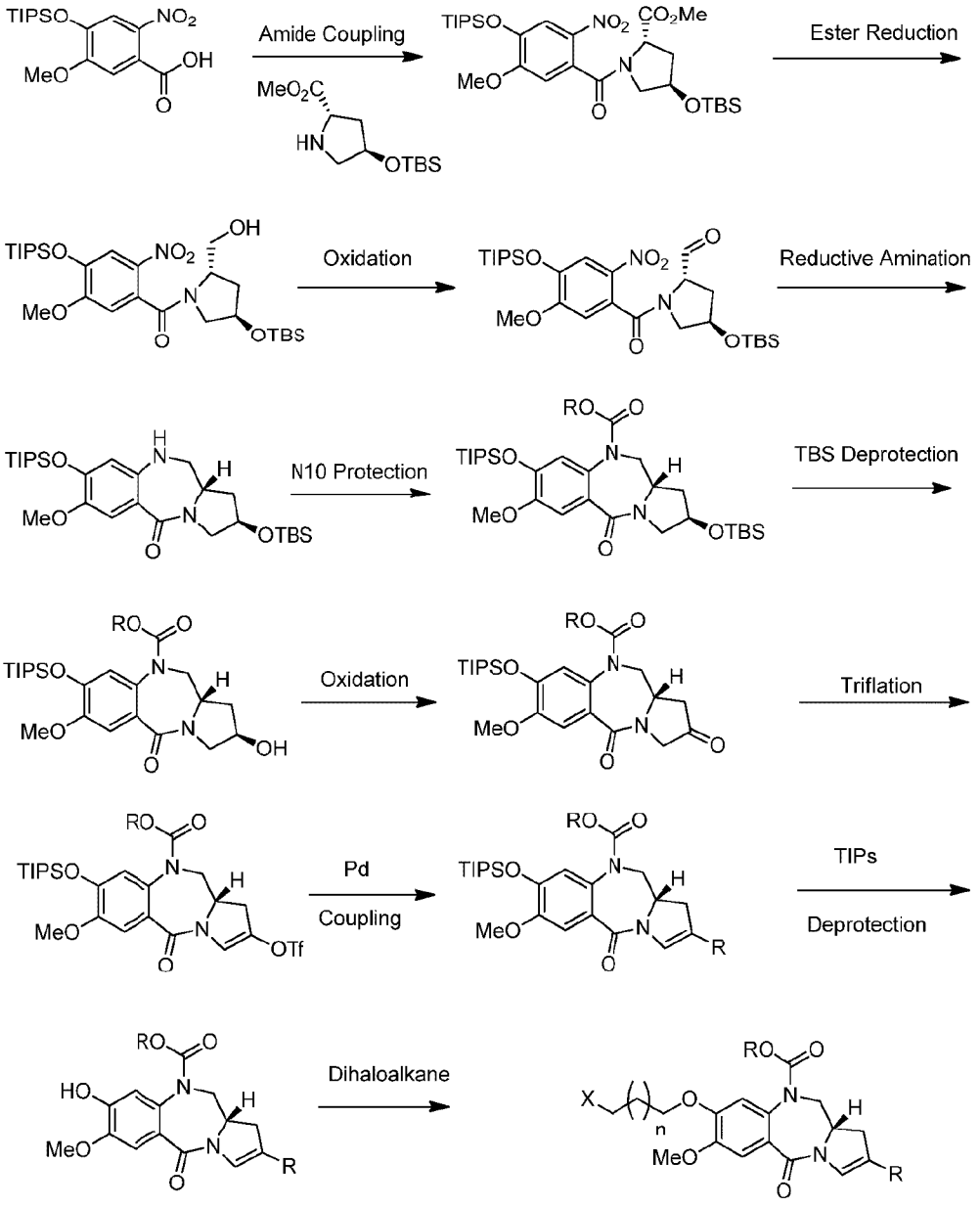
Scheme 1b – synthesis of protected carbinolamine building block



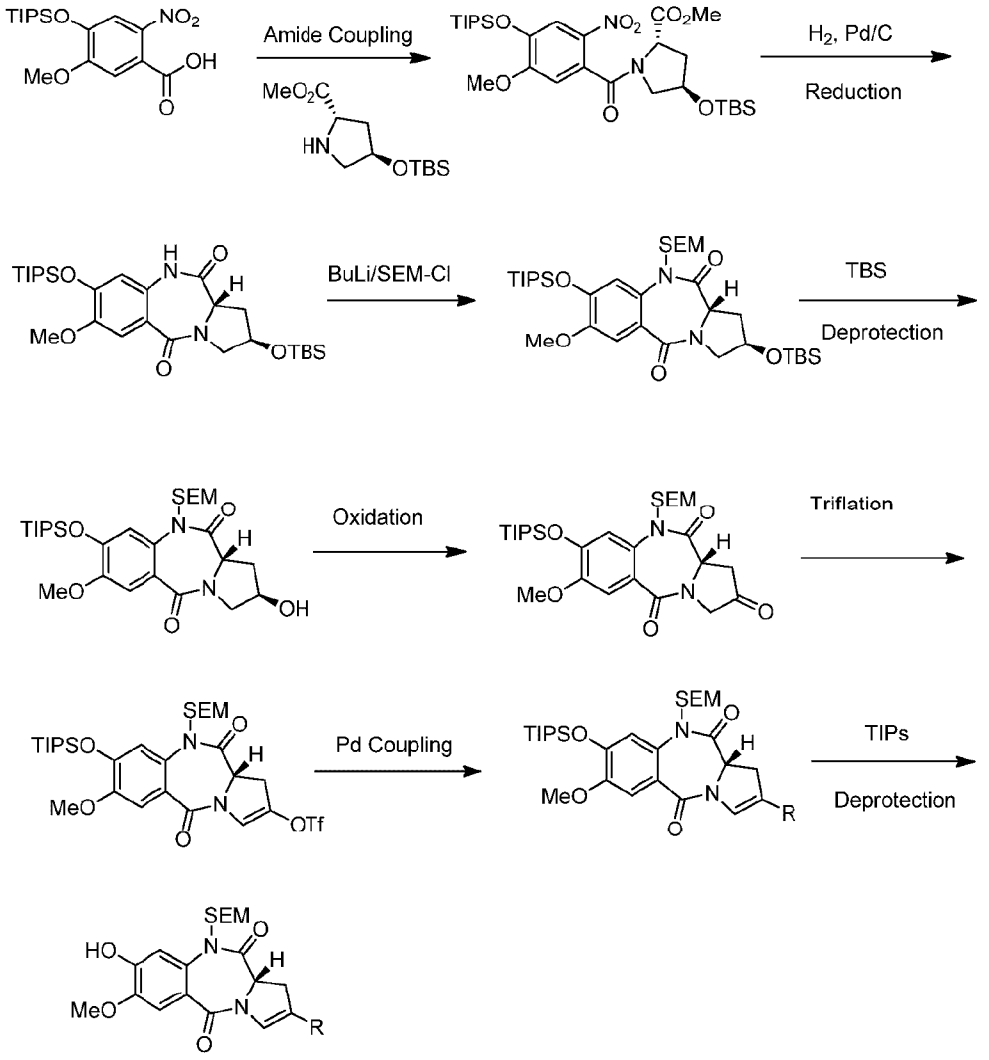
Scheme 1c – synthesis of dimer with linker attached (drug-linker)



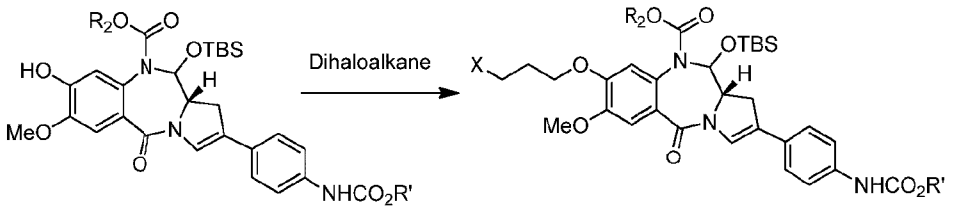
Scheme 2 – alternative synthesis of secondary amine building block



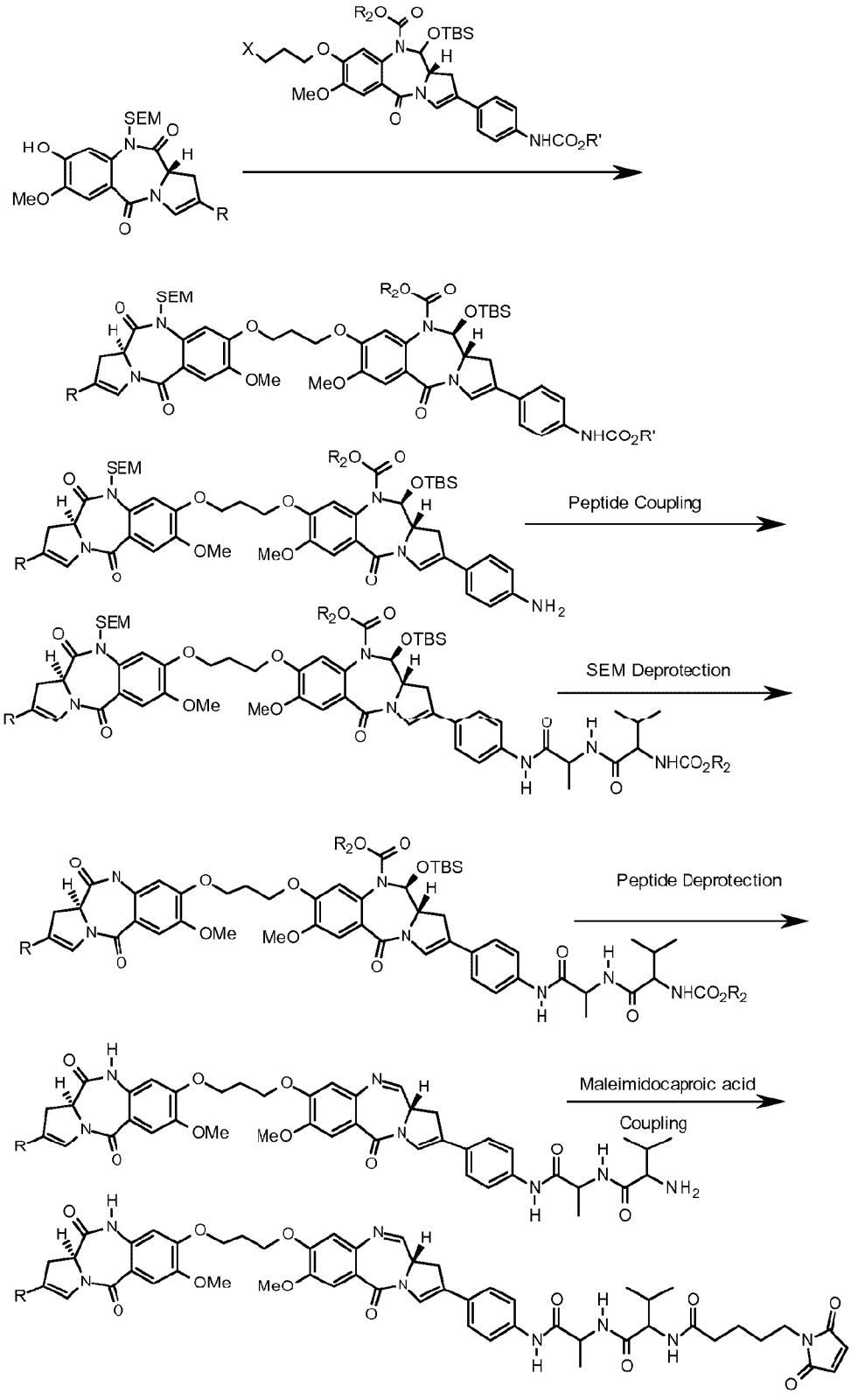
Scheme 3a – synthesis of dilactam building block



Scheme 3b - synthesis of protected carbinolamine building block with tether



Scheme 3c – synthesis of dimer with linker attached (drug-linker)



In the above schemes, the protecting groups may be orthogonal to one another to provide for synthetic flexibility.

Examples

General Experimental Methods

Reaction progress was monitored by thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel, with fluorescent indicator on aluminium plates. Visualisation of TLC was achieved with UV light or iodine vapour unless otherwise stated. Flash chromatography was performed using Merck Kieselgel 60 F254 silica gel. Extraction and chromatography solvents were bought and used without further purification from Fisher Scientific, U.K. All chemicals were purchased from Aldrich, Lancaster or BDH.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer. Coupling constants are quoted in hertz (Hz). Chemical shifts are recorded in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentuplet) and m (multiplet). IR spectra were recorded on a Perkin-Elmer FT/IR paragon 1000 spectrophotometer by application of the sample in a solution of chloroform using the ATR "golden gate" system. Optical rotations were measured at ambient temperature using a Bellingham and Stanley ADP 220 polarimeter. Mass spectrometry was performed on a ThermoQuest Navigator from Thermo Electron, Electrospray (ES) spectra were obtained at 20 to 30 V. Accurate mass measurements were performed using Micromass Q-TOF global tandem. All samples were run under electrospray ionization mode using 50% acetonitrile in water and 0.1% formic acid as a solvent. Samples were run on W mode which gives a typical resolution of 19000 at FWHH. The instrument was calibrated with [Glu]-Fibrinopeptide B immediately prior to measurement.

General LC/MS conditions:

Method 1 (default method, used unless stated otherwise)

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 held over 1.0 min, then increase from 5% B to 95% B over a 3 min period. The composition was held for 0.1 min at 95% B, then returned to 5% B in 0.03 minutes and hold there for 0.87 min. Total gradient run time equals 5 minutes.

Flow rate 3.0 mL/min, 400µL was split *via* a zero dead volume tee piece which passes into the mass spectrometer. Wavelength detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex Onyx Monolithic C18 50 x 4.60 mm.

The reverse phase flash purification conditions were as follows: The Flash purification system (Varian 971-Fp) was run using a mobile phase of water (A) and acetonitrile (B). Gradient: initial composition 5% B over 20 C.V. (Column Volume) then 5% B to 70% B within 60 C.V. The composition was held for 15 C.V. at 95% B, and then returned to 5% B in 5 C.V. and held at 5%B for 10 C.V. Total gradient run time equals 120 C.V. Flow rate 6.0 mL/min. Wavelength detection range: 254 nm. Column: Agilent AX1372-1 SF10-5.5gC8.

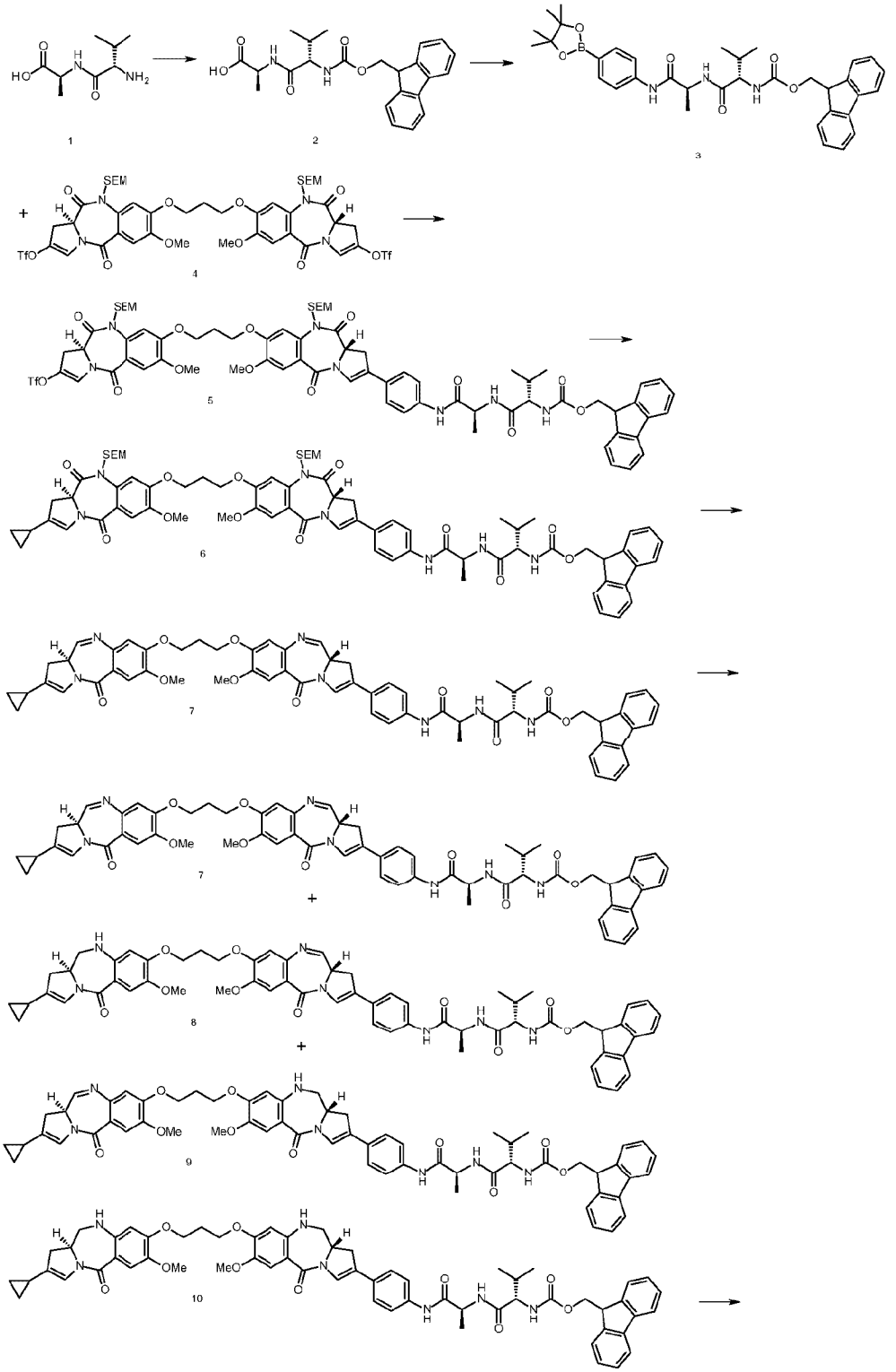
Fast Formic:
Positive mode electrospray mass spectrometry (ESI-MS) was performed using a Shimazu LCMS-2020 (single quadrupole mass spectrometer). Mobile phase used were water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B held over 0.25 min, then increase from 5% B to 100% B over a 2 min period. The composition was held for 0.50 min at 100% B, then returned to 5% B in 0.05 minutes and hold there for 0.05 min. Total gradient run time equals 3 min. Flow rate 0.8 mL/min. Wavelength detection range: 220 to 400 nm. Column: Waters Acquity UPLC BEH Shield RP18 1.7µm 2.1x50mm.

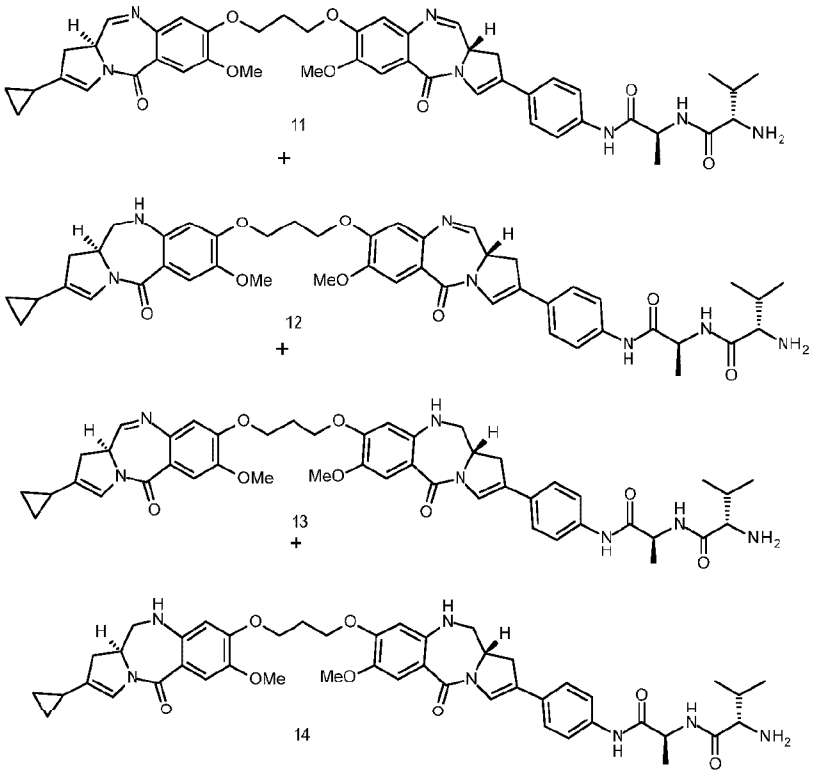
C18 15min formic:
Positive mode electrospray mass spectrometry (ESI-MS) was performed using a Shimazu LCMS-2020 (single quadrupole mass spectrometer). Oven temperature 50°C. Mobile phase used were water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B held over 1 min, then increase from 5% B to 100% B over a 9 min period. The composition was held for 2 min at 100% B, then returned to 5% B in 0.10 minutes and hold there for 2.90 min. Total gradient run time equals 15 min. Flow rate 0.6 mL/min. Wavelength detection range: 220 to 400 nm. Column: Gemini-NX UPLC C18 3µm 2x100mm.

Preparative HPLC: Reverse-phase ultra-high-performance liquid chromatography (UPLC) was carried out on Phenomenex Gemini NX 5µ C-18 columns of the following dimensions: 150 x 4.6 mm for analysis, and 150 x 21.20 mm for preparative work. All UPLC

experiments were performed with gradient conditions: initial fixed composition 13% B to 75% B over 15 min, held for 2.0 min at 75% B, then 75% B to 13% B within 0.10 min held at 13% for 2.90 min. Total duration of gradient run was 20.00 min. Eluents used were solvent A (H_2O with 0.1% Formic acid) and solvent B (CH_3CN with 0.1% Formic acid). Flow rates used were 1.0 ml/min for analytical, and 20.0 ml/min for preparative HPLC. Detection was at 254 and 280 nm.

Example 1





*(a) (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido) propanoic acid (**2**)*

HO-Ala-Val-H **1** (350 mg, 1.86 mmol) and Na_2CO_3 (493 mg, 4.65 mmol) were dissolved in distilled H_2O (15 mL) and the mixture was cooled to 0°C before dioxane (15 mL) was added (partial precipitation of the amino acid salt occurred). A solution of Fmoc-Cl (504 mg, 1.95 mmol) in dioxane (15 mL) was added dropwise with vigorous stirring over 10 minutes. The resulting mixture was stirred at 0°C for 2 hours before the ice bath was removed and stirring was maintained for 16 hours. The solvent was removed by rotary evaporation under reduced pressure and the residue dissolved in water (150 mL). The pH was adjusted from 9 to 2 with 1N HCl and the aqueous layer was subsequently extracted with EtOAc (3x100 mL). The combined organics were washed with brine (100 mL), dried with $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure to afford pure HO-Ala-Val-Fmoc **2** (746 mg, 97% yield). LC/MS 2.85 min (ES+) *m/z* (relative intensity) 410.60 ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J*=7.77 Hz, 2H), 7.60(d, *J*=7.77 Hz, 2H), 7.43(d, *J*=7.5 Hz, 2H), 7.34 (d, *J*=7.5 Hz, 2H), 6.30 (bs, 1H), 5.30 (bs, 1H), 4.71-7.56 (m, 1H), 4.54-4.36 (m, 2H), 4.08-3.91 (m, 1H), 2.21-2.07 (m, 1H), 1.50 (d, *J*=7.1 Hz, 3H), 1.06-0.90 (m, 6H).

*(b) (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-oxo-1-(((S)-1-oxo-1-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)amino)propan-2-yl)amino)butan-2-yl)carbamate (**3**)*

4-Aminophenylboronic acid pinacol ester was added (146.9 mg, 0.67 mmol) was added to a solution of HO-Ala-Val-Fmoc **2** (330mg, 0.8 mmol), DCC (166 mg, 0.8 mmol) and DMAP (5 mg, cat.) in dry DCM (8 mL) previously stirred for 30 minutes at room temperature in a flask flushed with argon. The reaction mixture was then allowed to stir at room temperature overnight. The reaction was followed by LCMS and TLC. The reaction mixture was diluted with CH_2Cl_2 and the organics were washed with H_2O and brine before being dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dryloaded on a silicagel chromatography column (Hexane/EtOAc, 6:4) and pure product **3** was isolated as a white solid in 88% yield (360 mg).

*(c) 8-(3-((2-(4-((S)-2-((S)-2-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-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-yl)oxy)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 (**5**)*

1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS)-7-methoxy-2-[[(trifluoromethyl)sulfonyl]oxy]-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] **4** (2.03g, 1.81 mmol), boronic pinacol ester (1g, 1.63 mmol) and Na_2CO_3 (881 mg, 8.31 mmol) were dissolved in a mixture of toluene/MeOH/H_2O, 2:1:1 (40 mL). The reaction flask was purged and filled with argon three times before *tetrakis*(triphenylphosphine)palladium(0) (41 mg, 0.035 mmol) was added and the reaction mixture heated to 30°C overnight. The solvents were removed under reduce pressure and the residue was taken up in H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organics were washed with brine (100 mL), dried with $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel chromatography column (Hexane/EtOAc, 8:2 to 25:75) to afford pure **5** in 33% yield (885 mg). LC/MS 3.85 min (ES+) *m/z* (relative intensity) 1452.90 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.16 (m, 17H), 7.13 (s, 1H), 6.51 – 6.24 (m, 1H), 5.51 (dd, *J* = 10.0, 5.1 Hz, 2H), 5.36 – 5.11 (m, 1H), 4.74 (dd, *J* = 10.1, 4.4 Hz, 2H), 4.70 – 4.53 (m, 2H), 4.47 (d, *J* = 6.4 Hz, 1H), 4.37 (d, *J* = 7.2 Hz, 1H), 4.27 (m, 4H), 4.20 – 4.14 (m, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.77 (ddd, *J* = 16.7, 9.0, 6.4 Hz, 3H), 3.71 – 3.61 (m, 2H), 3.24 – 2.91

(m, 3H), 2.55 – 2.33 (m, 2H), 2.22 – 2.07 (m, 1H), 1.52 – 1.37 (m, 3H), 1.04 – 0.86 (m, 10H), 0.00 (s, 18H).

*(d) (9H-fluoren-9-yl)methyl((2S)-1-(((2S)-1-((4-(8-(3-((2-cyclopropyl-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-yl)oxy)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)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**6**)*

Triphenylarsine (42 mg, 0.137 mmol) was added to a mixture of PBD-triflate **5** (250 mg, 0.172 mmol), cyclopropylboronic acid (73.9 mg, 0.86 mmol), silver oxide (159 mg, 0.688 mmol) and potassium phosphate tribasic (438 mg, 2.06 mmol) in dry dioxane (10 mL) under an argon atmosphere. The reaction was flushed with argon 3 times and *bis*(benzonitrile)palladium(II) chloride (13.2 mg, 0.034 mmol) was added. The reaction was flushed with Argon 3 more times before being warmed to 75°C and stirred for 10 minutes. The reaction mixture was filtered through a pad of Celite™ which was subsequently rinsed with ethyl acetate. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 1 % methanol/chloroform). Pure fractions were collected and combined, and excess eluent was removed by rotary evaporation under reduced pressure to afford the desired product **22** (132 mg, 50 % yield). LC/MS 3.83 min (ES+) *m/z* (relative intensity) 1345.91 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.14 (m, 17H), 6.69 (s, 1H), 6.45 – 6.25 (m, 1H), 5.57 – 5.41 (m, 2H), 5.34 – 5.14 (m, 1H), 4.78 – 4.67 (m, 2H), 4.62 – 4.55 (m, 1H), 4.50 – 4.45 (m, 2H), 4.51 – 4.44 (m, 1H), 4.31 – 4.21 (m, 4H), 4.16 (m, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 – 3.71 (m, 2H), 3.66 (m, 3H), 3.40 – 3.28 (m, 1H), 3.07 (m, 1H), 2.70 – 2.57 (m, 1H), 2.47 – 2.36 (m, 2H), 2.15 (m, 1H), 1.51 – 1.40 (m, 3H), 1.03 – 0.87 (m, 11H), 0.77 – 0.71 (m, 2H), 0.60 – 0.54 (m, 2H), 0.00 (t, *J* = 3.0 Hz, 18H).

*(e) (9H-fluoren-9-yl)methyl((2S)-1-(((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**7**)*

A solution of Super-Hydride® (0.5 mL, 1M in THF) was added dropwise to a solution of SEM dilactam 6 (265 mg g, 0.19 mmol) in THF (10 mL) at -78°C under an argon atmosphere. The addition was completed over 5 minutes in order to maintain the internal

temperature of the reaction mixture constant. After 20 minutes, an aliquot was quenched with water for LC/MS analysis, which revealed that the reaction was complete. Water (20 mL) was added to the reaction mixture and the cold bath was removed. The organic layer was extracted with EtOAc (3 x 30 mL) and the combined organics were washed with brine (50 mL), dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dissolved in MeOH (12 mL), CH_2Cl_2 (6 mL), water (2 mL) and enough silica gel to form a thick stirring suspension. After 5 days, the suspension was filtered through a sintered funnel and washed with CH_2Cl_2/MeOH (9:1) (200 mL) until the elution of the product was complete. The organic layer was washed with brine (2 x 70 mL), dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. Purification by silica gel column chromatography (100% $CHCl_3$ to 96% $CHCl_3$/ 4% MeOH) afforded the product 23 as a yellow solid (162 mg, 78%). LC/MS 3.02 min (ES+) *m/z* (relative intensity) 1052.37.

(f) Imine reduction

A solution of Super-Hydride® (95 µL, 1 eq, 1M in THF) was added dropwise to a solution of bis imine **7** (100 mg, 0.095 mmol) in THF (10 mL) at -78°C under an argon atmosphere. After 20 minutes, an aliquot was quenched with water for LC/MS analysis, which revealed that the reaction was complete, with some over-reduction towards the bis amine **10**. (Observed LC: Bis-imine **7** 19%, Imine-Amine **8** + **9** 36%, bis-amine **10** 45%; theoretical target for 1 eq of reducing agent is **7** 25%, **8+9** 50%, **10** 25%). Water (20 mL) was added to the reaction mixture and the cold bath was removed. The organic layer was extracted with chloroform (40 mL) and the combined organics were washed with water (1 x 40 mL), brine (50 mL), dried with Na_2SO_4, filtered and the solvent removed by rotary evaporation under reduced pressure. Purification by silica gel column chromatography (100% $CHCl_3$ to 96% $CHCl_3$/ 4% MeOH) improved the ratio of **8+9** to: **7** 25%, **8+9** 50%, **10** 25% (20 mg, 25%, as a mixture).

LC/MS (Fast Formic, 2.5 min system) Bis-Imine **7** 1.66 min (ES+) *m/z* (relative intensity) 1052.15 ; Hybrids Amine-Imine **8+9** (no separation on the 2.5 min system) 1.71 min (ES+) *m/z* (relative intensity) 1054.45; Bis-Amine **10** 1.66 min (ES+) *m/z* (relative intensity) 1056.95, in a 1/2/1 ratio.

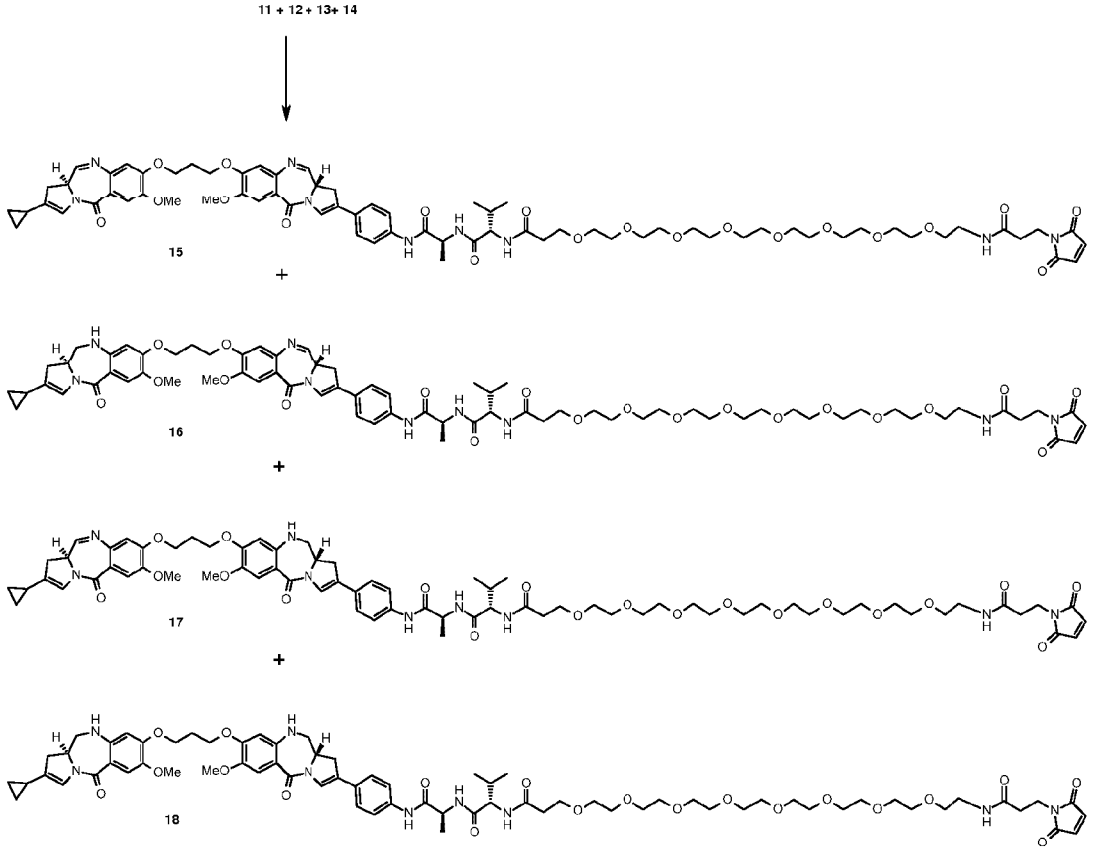
(g) Fmoc deprotection

Excess piperidine was added (0.1 mL, 1 mmol) to a 1/2/1 mixture of Fmoc protected **7, 8+9** and **10** (20 mg, 0.019 mmol) in DMF (1 mL). The mixture was allowed to stir at room

temperature for 20 minutes, at which point the reaction had gone to completion (as monitored by LC/MS). The reaction mixture was diluted with CH_2Cl_2 (30 mL) and the organic phase was washed with H_2O (2x30 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 products **11**, **12 + 13** and **14** (1/2/1 ratio), which were used as such in the next step.

LC/MS (Fast Formic, 2.5 min system) Bis-Imine **11** 1.12 min (ES+) *m/z* (relative intensity) 830.45 ; Hybrids Amine-Imine **12+13** (no separation) 1.15 min (ES+) *m/z* (relative intensity) 832.35; Bis-Amine **14** 1.19 min (ES+) *m/z* (relative intensity) 834.35, in a 1/2/1 ratio.

Example 2



PEG maleimide coupling

EDCI hydrochloride (5.46 mg, 0.028 mmol, 1.5 eq) was added to a suspension of Maleimide-PEG_8-acid (11.3 mg, 0.019 mmol, 1 eq) in chloroform (5 mL) under argon atmosphere. The mixture was stirred for 1h at room temperature before crude PBD mixture **11+12+13+14** (20 mg, 0.019 mmol, 1eq) was added. Stirring was maintained until the reaction was complete (usually 2 hours). The reaction was diluted with CH_2Cl_2 and the

organic phase was washed with H_2O and brine before being dried over $MgSO_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure. The four products were purified and separated individually by reverse phase chromatography (see method below). The bis-imine **15** was isolated (2.0 mg, 7.5%), followed by the two separable amine-imine hybrids **16** (3.8 mg, 14.2%) and **17** (2.7 mg, 10.1%), and the bis-amine **18** (2.2 mg, 8.2%). ^{1}H NMR analysis unambiguously identified **16** from **17**. One key feature is the imine proton (d, J = 4.0 Hz, 1H), which is at 7.78 ppm on the cyclopropyl side, and 7.88 ppm on the aromatic side of the molecule.

LC/MS C18 15 min formic:

Bis-Imine **15** 5.23 min (ES+) *m/z* (relative intensity) 703.20 (100, (M+2H)/2), 1404.55 (10, M+H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.89 (d, J = 4.0 Hz, 1H), 7.78 (d, J = 4.0 Hz, 1H), 7.77 – 7.64 (m, 2H), 7.53 – 7.47 (m, 2H), 7.44 (s, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.12 (s, 1H), 6.89 – 6.81 (m, 2H), 6.74 (s, 1H), 6.69 (d, J = 1.6 Hz, 2H), 6.50 (s, 1H), 4.72 – 4.57 (m, 1H), 4.43 – 4.14 (m, 7H), 4.11 – 4.03 (m, 1H), 3.93 (d, J = 4.6 Hz, 6H), 3.83 (t, J = 7.2 Hz, 2H), 3.80 – 3.71 (m, 2H), 3.68 – 3.56 (m, 28H), 3.55 – 3.48 (m, 3H), 3.44 – 3.34 (m, 3H), 3.16 – 3.04 (m, 1H), 2.95 – 2.84 (m, 1H), 2.58 – 2.47 (m, 4H), 2.47 – 2.37 (m, 2H), 2.33 – 2.16 (m, 1H), 1.51 – 1.41 (m, 4H), 1.05 – 0.94 (m, 6H), 0.77 (dt, J = 5.5, 4.8 Hz, 2H), 0.55 (dd, J = 9.3, 4.9 Hz, 2H).

Hybrid Amine-Imine **16** 5.48 min (ES+) *m/z* (relative intensity) 704.20 (100, (M+2H)/2), 1406.70 (5, M+H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.88 (d, J = 3.9 Hz, 1H), 7.78 – 7.64 (m, 2H), 7.55 – 7.48 (m, 2H), 7.43 (s, 1H), 7.36 – 7.29 (m, 2H), 7.12 (s, 1H), 6.93 – 6.80 (m, 3H), 6.69 (d, J = 1.7 Hz, 2H), 6.49 (s, 1H), 6.10 (s, 1H), 4.72 – 4.58 (m, 1H), 4.41 – 4.32 (m, 1H), 4.32 – 4.24 (m, 2H), 4.24 – 4.16 (m, 2H), 4.15 – 4.02 (m, 2H), 3.94 (s, 3H), 3.87 – 3.80 (m, 5H), 3.79 – 3.71 (m, 2H), 3.69 – 3.55 (m, 28H), 3.55 – 3.49 (m, 2H), 3.46 – 3.34 (m, 4H), 2.93 – 2.81 (m, 2H), 2.60 – 2.46 (m, 4H), 2.42 – 2.34 (m, 2H), 2.24 (dd, J = 14.0, 6.5 Hz, 2H), 1.52 – 1.38 (m, 4H), 1.07 – 0.92 (m, 6H), 0.75 – 0.66 (m, 2H), 0.53 – 0.44 (m, 2H).

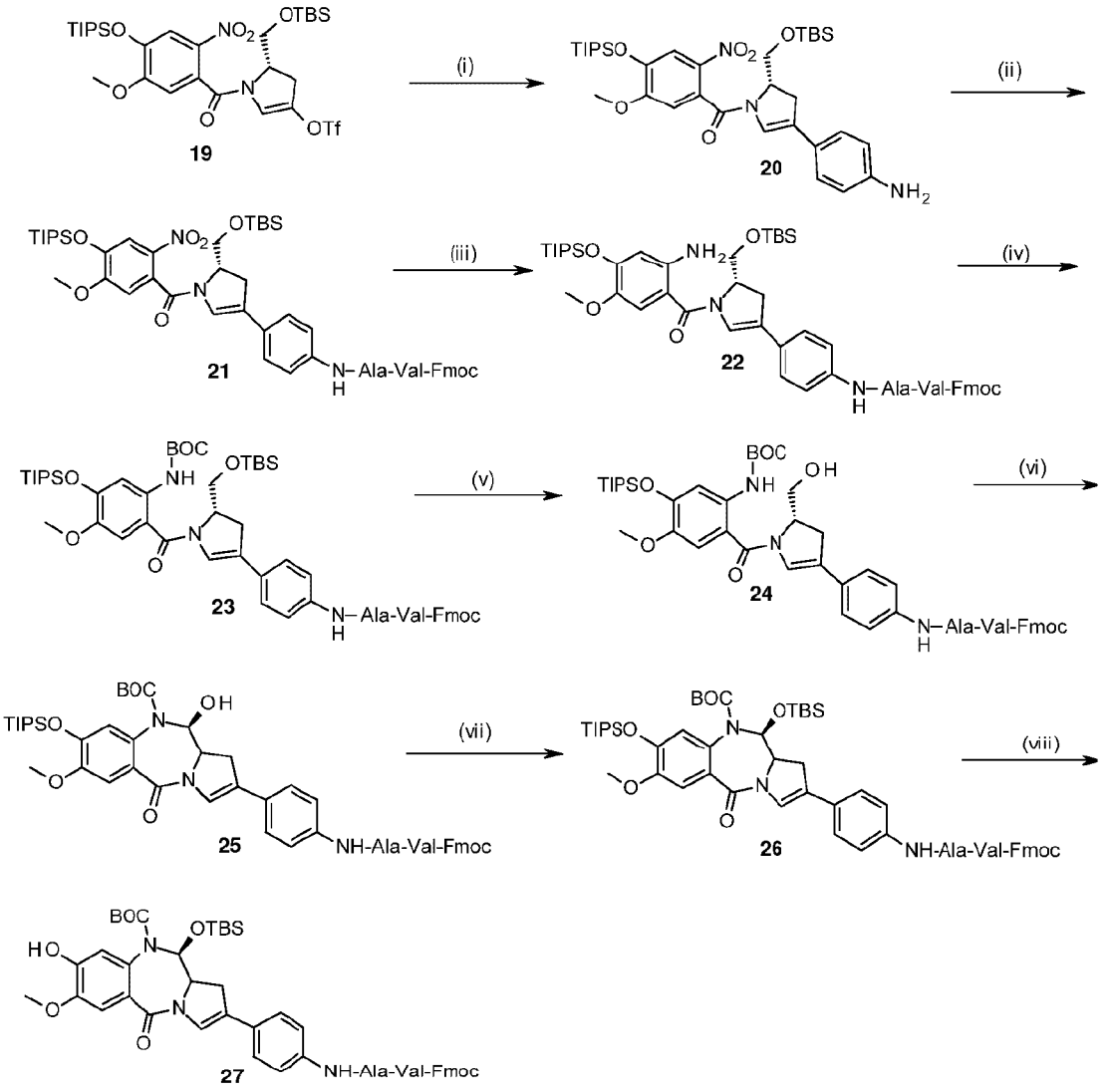
Hybrid Imine-Amine **17** 5.41 min (ES+) *m/z* (relative intensity) 704.25 (100, (M+2H)/2), 1406.45 (3, M+H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.78 (d, J = 4.0 Hz, 1H), 7.74 – 7.58 (m, 2H), 7.56 (s, 1H), 7.53 – 7.49 (m, 2H), 7.36 – 7.27 (m, 2H), 7.17 – 7.04 (m, 1H), 6.95 – 6.86 (m, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 6.71 – 6.65 (m, 2H), 6.53 (s, 1H), 6.12 (s, 1H), 4.74 – 4.57 (m, 2H), 4.40 – 4.14 (m, 7H), 4.13 – 4.05 (m, 1H), 3.93 (s, 3H), 3.88 – 3.79 (m, 5H), 3.79 – 3.68 (m, 2H), 3.69 – 3.55 (m, 28H), 3.55 – 3.48 (m, 3H), 3.46 – 3.29 (m, 3H), 3.16 – 3.03 (m, 1H), 2.91 (s, 1H), 2.71 (s, 1H), 2.60 – 2.45 (m, 4H), 2.42 – 2.33

(m, 2H), 2.29 – 2.19 (m, 1H), 1.51 – 1.39 (m, 4H), 1.08 – 0.92 (m, 6H), 0.81 – 0.74 (m, 2H), 0.55 (dd, *J* = 9.3, 5.4 Hz, 2H).

Bis-amine **18** 5.72 min (ES+) *m/z* (relative intensity) 705.15 (100, (M+2H)/2), 1408.45 (3, M+H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.73 – 7.59 (m, 2H), 7.57 – 7.49 (m, 3H), 7.29 (d, *J* = 2.5 Hz, 2H), 7.13 (s, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 2H), 6.49 (s, 1H), 6.08 (d, *J* = 3.9 Hz, 3H), 4.72 – 4.57 (m, 2H), 4.19 (dd, *J* = 10.6, 4.3 Hz, 5H), 4.09 (dd, *J* = 12.6, 5.8 Hz, 2H), 3.88 – 3.80 (m, 8H), 3.79 – 3.69 (m, 2H), 3.68 – 3.56 (m, 28H), 3.54 – 3.46 (m, 2H), 3.44 – 3.26 (m, 4H), 2.85 (dd, *J* = 15.9, 10.4 Hz, 2H), 2.73 (dd, *J* = 16.2, 4.7 Hz, 2H), 2.61 – 2.43 (m, 4H), 2.38 – 2.16 (m, 5H), 1.51 – 1.38 (m, 4H), 1.05 – 0.93 (m, 6H), 0.69 (dt, *J* = 4.9, 4.3 Hz, 2H), 0.49 (dd, *J* = 5.1, 3.3 Hz, 2H).

Example 3

(a) tert-butyl (11*S*)-2-(4-((*S*)-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((*tert*-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-5-oxo-11,11*a*-dihydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate **(27)**



*(i) (S)-(4-(4-aminophenyl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone **(20)***

$Pd(PPh_3)_4$ (609 mg, 0.52 mmol) was added to a stirred mixture of triflate **19** (18.8 g, 26.3 mmol), 4-aminophenylboronic acid pinacol ester (8.64 g, 39.4 mmol), Na_2CO_3 (12.78 g, 120 mmol), MeOH (80 mL), toluene (160 mL) and water (80 mL). The reaction mixture was

allowed to stir at 30°C under a nitrogen atmosphere for 24 hours after which time all the boronic ester has consumed. The reaction mixture was then evaporated to dryness before the residue was taken up in EtOAc (100 mL) and washed with H_2O (100 mL), brine (100 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure to provide the crude product. Purification by silica gel chromatography (Hexane/EtOAc ; 100% to 70:30) afforded product **20** as a yellowish foam (11.06 g, 64%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.81 (s, 1H), 6.58 (d, *J* = 8.3 Hz, 2H), 6.06 (s, 1H), 4.77 (bm, 1H), 3.91 (d, *J* = 6.7 Hz, 3H), 3.68 (bs, 2H), 3.13 (bm, 1H), 2.97 (d, *J* = 14.5 Hz, 1H), 1.36 – 1.21 (m, 3H), 1.12 (d, *J* = 7.3 Hz, 18H), 0.89 (s, 10H), 0.10 (s, 6H).) ; ES^+ = 2.27 min, *m/z* 698 $[M + CH_3CN]^+$.

*(ii) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **(21)***

To a dry round bottom flask previously flushed with argon was added aniline **20** (10.05 g, 15.3 mmol), the dipeptide (6.3 g, 15.3 mmol) and dry CH_2Cl_2 (500 mL). The flask was then purged three times with argon before EEDQ (3.79 g, 15.3 mmol) was added and the mixture left to stir at room temperature. The reaction was followed by LCMS and after 3.5 hours the reaction was complete. The reaction was quenched with H_2O (200 mL) and extracted twice with CH_2Cl_2 (250 mL). The combined organics were washed with brine (150 mL), dried over $MgSO_4$, filtered and the solvent removed in *vacuo*. The crude product was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 55:45) to afford pure product **21** (13.821 g, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.64 (s + d, *J* = 4.9 Hz, 3H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.36 (d, *J* = 7.3 Hz, 1H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 6.71 (s, 1H), 6.27 (d, *J* = 6.3 Hz, 1H), 6.08 (s, 1H), 5.11 (d, *J* = 6.6 Hz, 1H), 4.69 (bs, 1H), 4.52 (bm, 1H), 4.36 (d, *J* = 6.5 Hz, 2H), 4.08 (t, *J* = 5.9 Hz, 1H), 3.89 (m, 1H), 3.80 (s, 3H), 3.11 – 2.97 (bm, 1H), 2.88 (bd, *J* = 15.2 Hz, 1H), 2.03 (bs, 1H), 1.33 (d, *J* = 6.9 Hz, 3H), 1.24 – 1.11 (m, 3H), 1.01 (d, *J* = 7.4 Hz, 18H), 0.86 – 0.79 (m, 6H), 0.77 (s, 9H), 0.00 (s, 6H) ; ES^+ = 2.37 min, no mass.

*(iii) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-amino-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**22**)*

In a dry two-neck round bottom flask previously flushed with argon and fitted with a thermometer, nitrophenyl **21** (2.97g, 2.8 mmol) was solubilised in a solution of 5% formic

acid in methanol (50 mL). Zinc (1.85g, 28 mmol) was rapidly poured into the solution. The temperature instantaneously rose to 40°C and slowly cooled down back to room temperature at which point the reaction is complete (≈15 minutes, reaction monitored by LCMS). The reaction mixture was then filtered through celite and the pad further washed with EtOAc (2 x 150 mL). The combined organics were subsequently washed with saturated $NaHCO_{3(aq)}$ (100 mL), H_2O (100 mL) and brine (100 mL), before being dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was purified silica gel chromatography (Hexane/EtOAC 75:25 to 50:50) and pure product **22** was isolated as a pale yellow oil (2.291 g, 79% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.74 (s+d, *J* = 4.9 Hz, 3H), 7.53 (t, *J* = 7.4 Hz, 2H), 7.46 (d, *J* = 11.3 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.28 (t, *J* = 11.3 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H), 6.38 (d, *J* = 6.3 Hz, 1H), 6.18 (s, 1H), 5.21 (d, *J* = 2.9 Hz, 1H), 4.81 (bs, 1H), 4.72 – 4.57 (m, 1H), 4.47 (d, *J* = 6.5 Hz, 2H), 4.19 (t, *J* = 5.0 Hz, 1H), 4.00 – 3.94 (m, 1H), 3.91 (s, 3H), 3.23 – 3.07 (m, 1H), 2.98 (d, *J* = 16.8 Hz, 1H), 2.15 (s, 1H), 1.43 (d, *J* = 6.9 Hz, 3H), 1.36 – 1.18 (m, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 0.97 – 0.89 (m, 6H), 0.88 (s, 9H), 0.10 (s, 6H). ES^+ = 2.37 min, *m/z* no mass.

*(iv) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-((tert-butoxycarbonyl)amino)-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **(23)***

Amine **22** (14.913 g, 14.6 mmol) and Boc_2O (3.83 g, 17.5 mmol) were heated together at 70°C in a round bottom flask. To help with solubility, $CHCl_3$ (25 mL) was added and the mixture left to stir until the reaction was complete (followed by LCMS). The thick crude solution was left to cool down to room temperature before being directly loaded on a silica gel chromatography column (Hexane/EtOAc ; 100% to 65:35). Product **23** was isolated as a cream foam (13.2 g, 80% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.21 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 3H), 7.54 (t, *J* = 7.0 Hz, 2H), 7.48 (d, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.31 – 7.25 (m, 3H), 7.14 (d, *J* = 6.7 Hz, 2H), 6.84 (bs, 1H), 6.80 (s, 1H), 6.50 (d, *J* = 6.4 Hz, 1H), 5.28 (d, *J* = 6.0 Hz, 1H), 4.77 (d, *J* = 2.6 Hz, 1H), 4.70 – 4.58 (m, 1H), 4.47 (t, *J* = 5.7 Hz, 2H), 4.19 (t, *J* = 6.1 Hz, 1H), 4.00 (m, 2H), 3.88 (bs, 1H), 3.73 (s, 3H), 3.05 (m, 1H), 2.98 (dd, *J* = 15.4, 3.3 Hz, 1H), 2.15 (bm, 1H), 1.46 (s, 9H), 1.43 (d, *J*=11.7 Hz, 3H), 1.36 – 1.22 (m, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 1.00 – 0.89 (m, 6H), 0.84 (s, 9H), 0.05 (d, *J* = 6.0 Hz, 6H)) ; ES^+ = 2.53 min, no mass.

*(v) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-((tert-butoxycarbonyl)amino)-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(hydroxymethyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **(24)***

Silyl ether **23** (13.2 g, 11.8 mmol) was solubilised in a 7:2:1:1 mixture of AcOH/H_2O/MeOH/THF (220 mL) and the mixture was stirred at room temperature until the reaction was complete (left overnight). The volatiles were removed in vacuo and the residue was taken up in EtOAc (400 mL). The organic phase was washed with saturated $NaHCO_{3(aq)}$ (200 mL), H_2O (200 mL) and brine (10 mL) before being dried over $MgSO_4$, filtered and concentrated in *vacuo*. The crude material was purified by silica gel chromatography (Hex/EtOAc ; 50:50 to 0:100) and pure product **24** was isolated as a light yellow foam (11.168 g, 94% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.93 (s, 1H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.64 (s, 1H), 7.52 (dd, *J* = 17.9, 8.9 Hz, 4H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.26 (m, 3H), 7.13 (d, *J* = 7.4 Hz, 2H), 6.81 (s, 1H), 6.45 (s, 1H), 5.26 (s, 1H), 4.84 (s, 1H), 4.69 – 4.58 (m, 1H), 4.47 (d, *J* = 6.2 Hz, 2H), 4.43 (s, 1H), 4.17 (d, *J* = 14.2 Hz, 1H), 3.99 (s, 1H), 3.89 (s, 2H), 3.74 (s, 3H), 3.30 – 3.17 (m, 1H), 2.64 (d, *J* = 16.9 Hz, 1H), 2.23 – 2.09 (m, 1H), 1.44 (s, 9H), 1.44 (d, *J* = 10.9 Hz, 2H), 1.29 (ddd, *J* = 14.3, 13.0, 7.4 Hz, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 0.92 (m, 6H) ; ES^+ = 2.23 min, no mass.

*(vi) tert-butyl (11S)-2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-hydroxy-7-methoxy-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **(25)***

DMSO (1.55 L, 21.9 mmol) was added to a cooled solution of oxallyl chloride (0.89 mL, 10.5 mmol) in CH_2Cl_2 (50 mL) at -78°C. After 15 minutes, a solution of alcohol **24** (8.8 mg, 8.76 mmol) in CH_2Cl_2 (100 mL) was added dropwise to the oxidising mixture. The reaction was left to stir at -78°C for 1 hour before NEt_3 (6.11 mL, 43.8 mmol) was added and the mixture allowed to warm to room temperature. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and the solution was washed with 0.1M HCl(aq.) (250 mL), H_2O (250 mL), saturated $NaHCO_{3(aq.)}$ (250 mL) and brine (200 mL). The organics were dried with $MgSO_4$, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (CH_2Cl_2/EtOAc ; 100% to 50:50) to provide pure **25** as a yellow oil (8.8 mg, 100%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.74 (t, *J* = 8.4 Hz, 3H), 7.52 (d, *J* = 7.4 Hz, 5H), 7.43 – 7.33 (m, 4H), 7.23 – 7.17 (m, 2H), 6.69 (s, 1H), 6.42 (d, *J* = 7.9 Hz, 1H), 5.78 (d, *J* = 7.8 Hz, 1H), 5.62 (s, 1H), 5.23 (d, *J* = 7.7 Hz, 1H), 4.84 – 4.69 (m, 1H), 4.65 (d, *J* = 22.5 Hz, 1H), 4.45 – 4.29 (m, 2H), 3.91 (dd, *J* = 11.3, 8.1

Hz, 1H), 3.86 (s, 3H), 3.28 (q, *J* = 11.9 Hz, 1H), 2.98 (t, *J* = 12.6 Hz, 1H), 2.14 (dd, *J* = 12.9, 10.0 Hz, 1H), 1.52 – 1.42 (m, 3H), 1.38 (s, 9H), 1.26 (m, 3H), 1.16 – 1.05 (m, 18H), 0.93 (d, *J* = 6.0 Hz, 6H) ; ES^+ = 2.19 min, no mass.

*(vii) tert-butyl (11S)-2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **(26)***

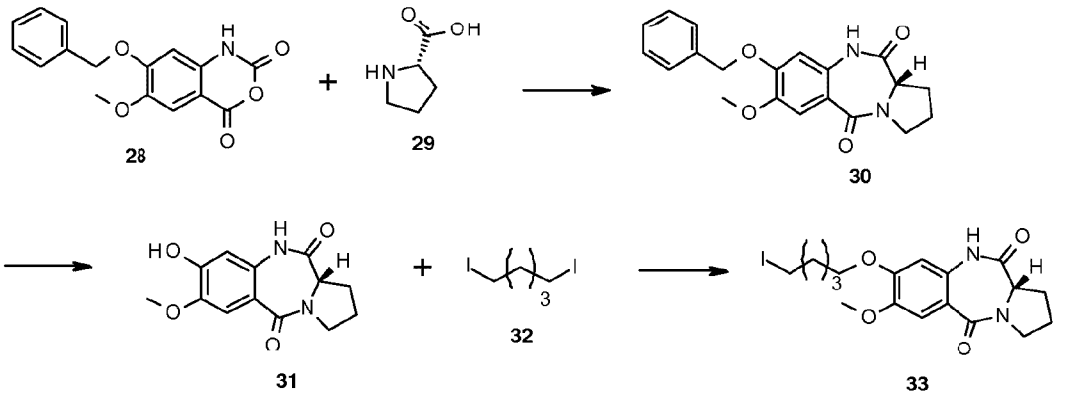
Alcohol **25** (8.8 g, 8.78 mmol) was solubilised in dry CH_2Cl_2 (150 mL) in a sealed round bottom flask previously flushed three times with argon. The solution was cooled to 0°C before lutidine (4 mL, 35.1 mmol) and TBS-OTf (6 mL, 26.3 mmol) were subsequently added. The reaction mixture was left to warm to room temperature and stirred until complete (monitored by LCMS). Upon completion, the solution was diluted with CH_2Cl_2 (100 mL), washed with saturated $NH_4Cl_{(aq.)}$ (150 mL), H_2O (100 mL), saturated $NaHCO_3$(aq.) (100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was purified by silica gel chromatography (Hexane/EtOAc : 100% to 80:20) to provide pure **26** as a colourless oil (6.18 mg, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.55 (dd, *J* = 13.0, 6.7 Hz, 4H), 7.40 (t, *J* = 7.3 Hz, 4H), 7.33 – 7.27 (m, 3H), 7.21 (s, 1H), 6.67 (s, 1H), 6.49 (s, 1H), 5.87 (d, *J* = 8.8 Hz, 1H), 5.30 (d, *J* = 5.7 Hz, 1H), 4.71 – 4.59 (m, 1H), 4.48 (d, *J* = 6.8 Hz, 2H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.04 – 3.96 (m, 1H), 3.86 (s, 3H), 3.84 – 3.77 (m, 1H), 3.25 (m, 1H), 2.79 (d, *J* = 1.5 Hz, 1H), 2.26 – 2.11 (m, 1H), 1.46 (d, *J* = 6.9 Hz, 3H), 1.33 (s, 9H), 1.27 (dd, *J* = 17.1, 9.7 Hz, 3H), 1.11 (dd, *J* = 7.4, 4.0 Hz, 18H), 0.93 (s, 6H), 0.89 (s, 9H), 0.27 (s, 3H), 0.22 (s, 3H); ES^+ = 2.55 min, *m/z* 116.30 $[M+H]^+$.

*(viii) tert-butyl (11S)-2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **(27)***

Monomer **26** (1 g, 0.89 mmol) was solubilised in wet DMF (5 mL +0.5 mL H_2O) before LiOAc (91 mg, 0.89 mmol) was added and the mixture left to stir at room temperature until complete (≈3h, followed by LCMS). The mixture was subsequently diluted with EtOAc (50 mL), quenched with citric acid(aq.) (pH=3, 40 mL), then washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude product was purified by silica gel chromatography

(Hexane/EtOAc/MeOH ; 60:40:0 to 60:30:10) and pure product **27** was isolated as a cream solid (675 mg, 78% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.55 (dd, *J* = 16.0, 7.5 Hz, 4H), 7.40 (t, *J* = 7.4 Hz, 4H), 7.30 (ddd, *J* = 14.7, 7.4, 1.1 Hz, 3H), 7.24 (s, 1H), 6.72 (s, 1H), 6.38 (d, *J* = 5.3 Hz, 1H), 5.87 (s, 1H), 5.23 (d, *J* = 6.2 Hz, 1H), 4.69 – 4.57 (m, 1H), 4.49 (d, *J* = 6.6 Hz, 2H), 4.20 (t, *J* = 5.3 Hz, 1H), 4.04 – 3.96 (m, 1H), 3.96 (s, 3H), 3.87 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.29 (dd, *J* = 18.0, 8.5 Hz, 1H), 2.80 (d, *J* = 19.4 Hz, 1H), 2.24 – 2.08 (m, 1H), 1.46 (d, *J* = 10.5 Hz, 3H), 1.33 (s, 9H), 1.00 – 0.91 (m, 6H), 0.90 (s, 9H), 0.25 (d, *J* = 8.6 Hz, 6H). ; ES^+ = 2.08 min, *m/z* 960.35 $[M+H]^+$.

(b) (***S***)-8-((5-iodopentyl)oxy)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (**33**)



*(i) (**S**)-8-(benzyloxy)-7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H)-dione (**30**)*

A suspension of benzyl isatoic anhydride **28** (1.34 g, 4.48 mmol, 1.0 eq.)and L-proline **29** (0.705 g, 6.12 mmol, 1.36 eq.) in anhydrous DMSO (20 mL), in a sealed vial, was heated under microwave irradiation at 150°C with stirring for 12 minutes. The resultant yellow solution was allowed to cool to room temperature and poured onto ice. The precipitated product was collected by filtration, dissolved in DCM (200 mL) and the solution was washed with saturated NaCl solution (200 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give the product **30** as a yellow solid (1.35 g, 85%). Analytical Data: RT 1.39 min; MS (ES^+) *m/z* (relative intensity) 353 ($[M + H]^+$,100).

*(ii) (**S**)-8-hydroxy-7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H)-dione (**31**)*

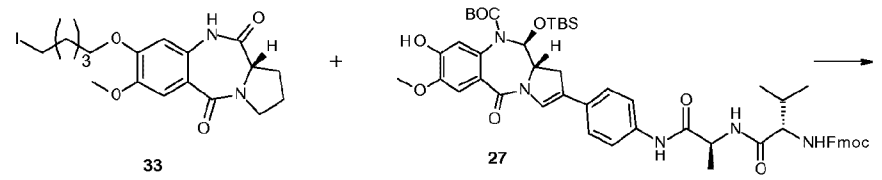
A slurry of 10% Palladium on carbon (0.27 g, 20 wt.%) in ethylacetate (10 mL) was added to a suspension of benzyl dilactam **30** (1.35 g, 3.8 mmol) in a mixture of ethanol (60 mL),

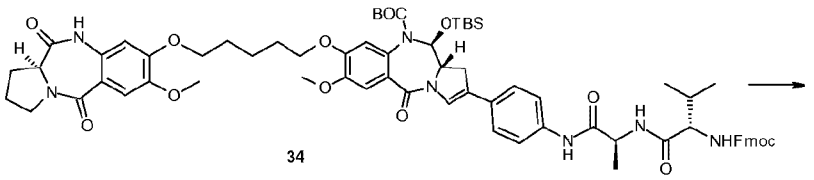
ethylacetate (40 mL) and DMF (5 mL). The mixture was hydrogenated at 45 psi for 2 hours. The reaction mixture was filtered through celite and the solvent evaporated under reduced pressure to give a viscous gum. The gum was sonicated with diethyl ether (50 mL) and the resultant product collected by filtration. This gave the desired product **31** as an off-white powder (0.86 g, 85%). Analytical Data: RT 1.02 min; MS (ES^+) *m/z* (relative intensity) 263 ($[M + H]^+$,100).

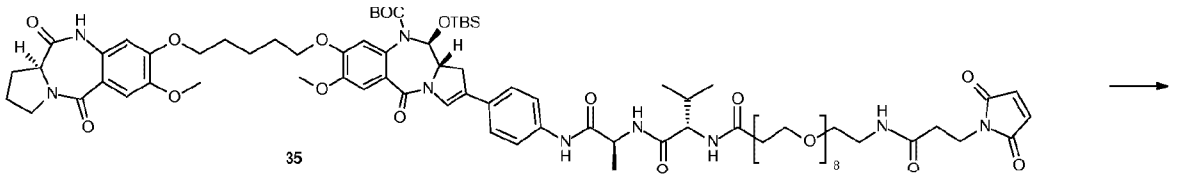
*(iii) (**S**)-8-((5-iodopentyl)oxy)-7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H)-dione (**33**)*

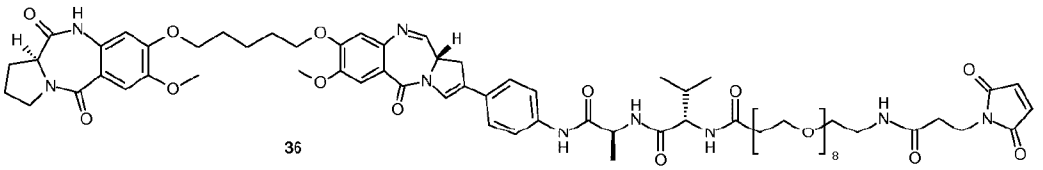
To a solution of **31** (400mg, 1.5 mmol) in anhydrous DMF (4 mL), in a flask purged with argon, was added K_2CO_3 (320 mg, 1.5 mmol) and 1,5-diiodopentane **32** (1.1 mL, 7.6 mmol). The reaction mixture was heated to 60°C until complete (30 minutes). The solution was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL) and brine (50 mL) before the organics were dried with $MgSO_4$, filtered and the volatiles remove in *vacuo*. The crude material was purified by silica gel column chromatography (Hexane/EtOAc ; 100% to 3:7) to afford pure product **33** as a light brown foamy gum (611 mg, 87% yield). Analytical Data: RT 1.51 min; MS (ES^+) *m/z* (relative intensity) 458.95 ($[M + H]^+$,100).

(c) 1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-N-((***S***)-1-(((***S***)-1-((4-((***S***)-7-methoxy-8-((5-(((***S***)-7-methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-5-oxo-5,11a-dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12,15,18,21-heptaoxatetracosan-24-amide (**36**)









*(i) tert-butyl (11**S**,11a**S**)-2-(4-((**S**)-2-((**S**)-2-amino-3-methylbutanamido)propanamido)phenyl)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-8-((5-(((**S**)-7-methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**34**)*

To a solution of **33** (250mg, 0.545 mmol) and **27** (570 mg, 0.6mmol) in anhydrous DMF (4 mL), in a flask purged with argon, was added K_2CO_3 (115 mg, 0.545 mmol) and the mixture was heated to 60°C until reaction was complete (45 minutes). The solution was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL) and brine (50 mL) before the organics were dried with $MgSO_4$, filtered and the volatiles remove in *vacuo*. The crude material was purified by silica gel column chromatography ($CHCl_3$/MeOH ; 100% to 95:5) to afford pure

product **34** as a white foam (337 mg, 58% yield). Analytical Data: RT 1.42 min; MS (ES^+) *m/z* (relative intensity) 1069.05 ($[M + H]^+$,80).

(ii) tert-butyl (11***S***)-11-((*tert*-butyldimethylsilyl)oxy)-2-(4-((2***S***,5***S***)-37-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-5-*iso*propyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)phenyl)-7-methoxy-8-((5-(((***S***)-7-methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-11,11a-dihydro-1*H*-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5*H*)-carboxylate (**35**)

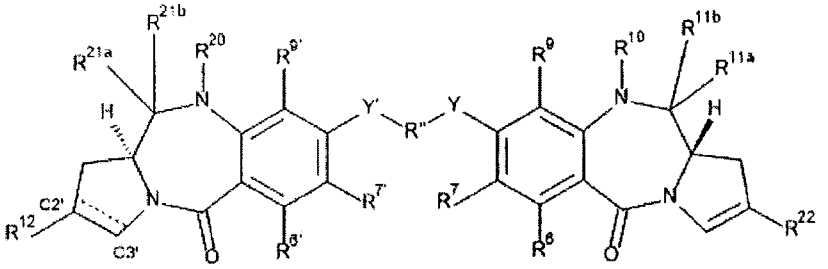
To a solution of **34** (337mg, 0.31 mmol) in dry CH_2Cl_2 (5 mL) was added the PEG moiety (186 mg, 0.31 mmol) and EDCI.HCl (60 mg, 0.31 mmol). The mixture was stirred at room temperature under an atmosphere of argon until completion. The mixture was subsequently diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL) and brine (50 mL) before removing the volatiles in *vacuo*. . The crude material was purified by silica gel column chromatography ($CHCl_3$/MeOH ; 100% to 95:5) to afford pure product **35** as a light yellow foam (408.8 mg, 58% yield). Analytical Data: RT 1.75 min; MS (ES^+) *m/z* (relative Intensity) 1643.15 ($[M + H]^+$,10) 822.25 ($[M + 2H]^{2+}$, 100).

*(iii) 1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-N-((**S**)-1-(((**S**)-1-((4-((**S**)-7-methoxy-8-((5-(((**S**)-7-methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12,15,18,21-heptaoxatetracosan-24-amide (**36**)*

To a flask containing **35** (400 mg, 0.24 mmol) cooled to 0°C were subsequently added H_2O (160 µL) and TFA (3.5 mL). The mixture was left to stir until complete before quenching with ice cold $NaHCO_3$ (50 mL) and extracting with CH_2Cl_2 (50 mL + 25 mL). The organics were then washed with brine (25 mL), dried over $MgSO_4$, filtered and the volatiles were removed in *vacuo* to give crude product **36** without further purification. Analytical Data: RT 1.40 min; MS (ES^+) *m/z* (relative intensity) 1410.60 ($[M + H]^+$,5) 706.10 ($[M + 2H]^{2+}$, 100).

CLAIMS

1. A compound of formula III:



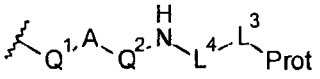
III

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

R^{22} is selected from the group consisting of:

(a) formula IVa:



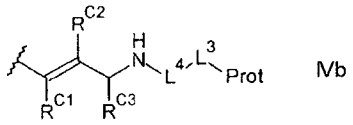
IVa

wherein A is a C_{5-7} aryl group, and either

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

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

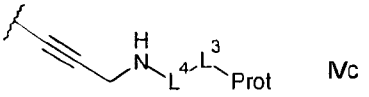
(b) formula IVb:



IVb

wherein R^{C1}, R^{C2} and R^{C3} are independently selected from the group consisting of H and unsubstituted C_{1-2} alkyl; and

(c) formula IVc:

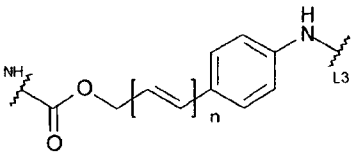


IVc

;

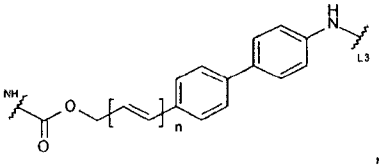
L^4 is selected from the group consisting of a single bond and a group of:

(a):



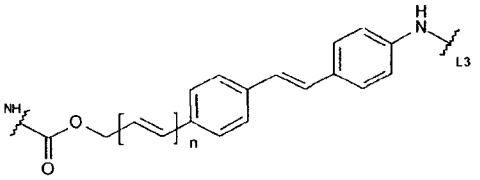
wherein n is 0 to 3;

(b)



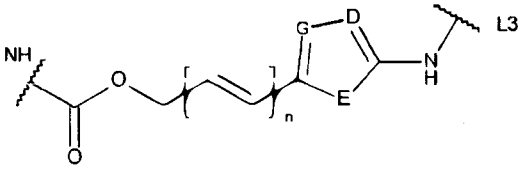
, wherein n is 0 to 3;

(c)



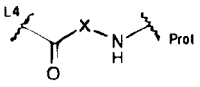
, wherein n is 0 to 3; and

(d)



, wherein n is 0 to 3, E is O, S or NR, D is N, CH, or CR, and G is N, CH, or CR;

L^3 is:



, where X is such that L^3 is an amino-acid residue, a dipeptide residue or a tripeptide residue;

Prot is selected from the group consisting of Fmoc (fluorenylmethyloxycarbonyl), Teoc (2-(trimethylsilyl)ethoxycarbonyl) and Boc (t-butoxycarbonyl);

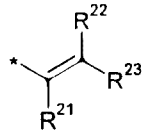
and either:

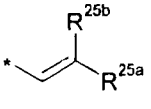
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

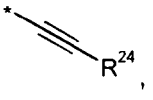
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group consiting of: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

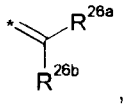
(ic) C_{3-6} saturated cycloalkyl;

(id)  , wherein each of R^{21}, R^{22} and R^{23} are independently selected from the group consisting of 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;

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

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

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

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

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

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

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

R'' is a C_{3-12} alkylene group, which chain is interrupted by one or more aromatic rings, selected from the group consisting of benzene and pyridine;

Y and Y' are selected from the group consisting of O, S, and NH;

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

either:

(A) R^{20} is H or Me and R^{21a} and R^{21b} are both H or together form =O and either:

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

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

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

(B) R^{10} is H or Me and R^{11a} and R^{11b} are both H or together form =O and either:

(i) R^{20} is H, R^{21a} is H and R^{21b} is OH or OR^A, where R^A is C_{1-4} alkyl; or

(ii) R^{20} and R^{21b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{11a} is H; or

(iii) R^{20} is H, R^{21a} is H and R^{21b} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation.

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

3. A compound according to either claim 1 or claim 2, wherein Y is O and R" is C_{3-7} alkylene.

4. A compound according to any one of claims 1 to 3, wherein R^6 and R^9 are H.

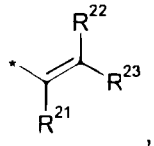
5. A compound according to any one of claims 1 to 4, wherein there is a double bond between C2' and C3', and R^{12} is:

(a) a C_{5-7} aryl group, which may bear one to three substituent groups selected from the group consisting of methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl; or

(b) methyl, ethyl or propyl; or

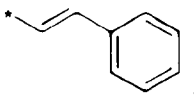
(c) cyclopropyl; or

(d) a group of formula:



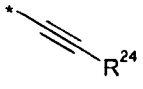
, wherein the total number of carbon atoms in the R^{12} group is no more than 4; or

(e) the group:

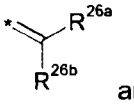


; or

(f) a group of formula:



, wherein R^{24} is selected from the group consisting of H and methyl.

6. A compound according to any one of claims 1 to 4, wherein there is a single bond between C2' and C3', R^{12} is  and:

(a) R^{26a} and R^{26b} are both H; or

(b) R^{26a} and R^{26b} are both methyl; or

(c) one of R^{26a} and R^{26b} is H, and the other is selected from the group consisting of C_{1-4} saturated alkyl and C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted.

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

8. A compound according to any one of claims 1 to 7, wherein R^{20} is H or Me and R^{21a} and R^{21b} are both H.

9. A compound according to any one of claims 1 to 7, wherein R^{20} is H or Me and R^{21a} and R^{21b} together form =O.

10. A compound according to either claim 8 or claim 9, wherein R^{10} and R^{11b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{11a} is H.

11. A compound according to any one of claims 1 to 7, wherein R^{10} is H or Me and R^{11a} and R^{11b} are both H.

12. A compound according to any one of claims 1 to 7, wherein R^{10} is H or Me and R^{11a} and R^{11b} together form =O.

13. A compound according to either claim 11 or claim 12, wherein R^{20} and R^{21b} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound and R^{11a} is H.

14. Use of a compound according to any one of claims 1 to 13 in the manufacture of a medicament for treating a proliferative disease.

15. A compound according to any one of claims 1 to 13 for use in the treatment of a proliferative disease.

16. A conjugate comprising a compound of formula I according to any one of claims 1 to 13, or a pharmaceutically acceptable salt or solvate thereof, linked to a targeting agent.

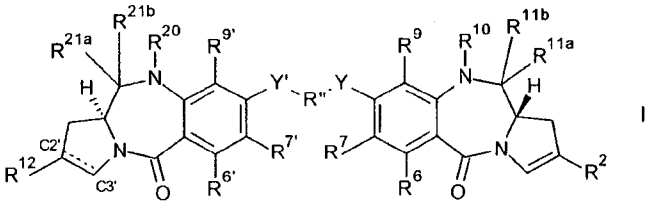
17. A Conjugate having formula V:

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

wherein L is an antibody or antibody fragment,

p is 1 to 20; and

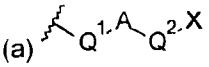
D is a Drug unit according to formula I:



I

wherein R^6, $R^{6'}$, R^7, $R^{7'}$, R^9, $R^{9'}$, R^{10}, R^{11a}, R^{11b}, R^{12}, R^{20}, R^{21a}, R^{21b}, Y, Y' and R'' are as defined in any one of claims 1 to 13; and

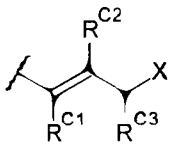
R^2 is of formula IIa, formula IIb or formula IIc:

(a)  IIa

where A is a C_{5-7} aryl group, and either

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

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

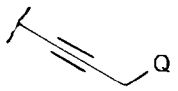


IIb

(b)

where;

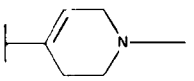
R^{C1}, R^{C2} and R^{C3} are independently selected from the group consisting of H and unsubstituted C_{1-2} alkyl;



IIc

(c)

where Q is selected from the group consisting of -O-, -S- and $-N(R^N)-$, and R^N is selected from the group consisting of H, methyl and ethyl

X is selected from the group consisting of: *-O-, *-S-, *-CO_2-, *-CO-, *-NH(C=O)-, *-NHNH-, *-CONHNH-,  and $-N(R^N)-$, wherein R^N is selected from the group consisting of H and C_{1-4} alkyl; wherein the asterix or wavy line indicates the point of attachment to the remainder of the drug unit;

wherein LU is connected to D via the X or Q substituent of R^2, wherein LU has the formula (Va):

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

wherein:

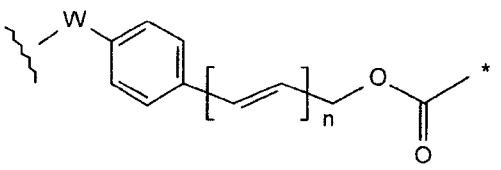
a is 1 or 2,

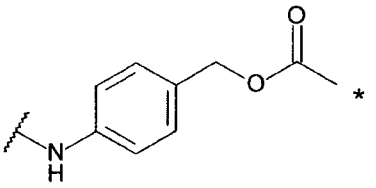
s is an integer ranging from 0 to 12,

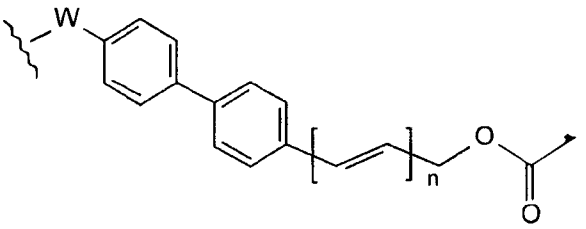
y is 0, 1 or 2, and

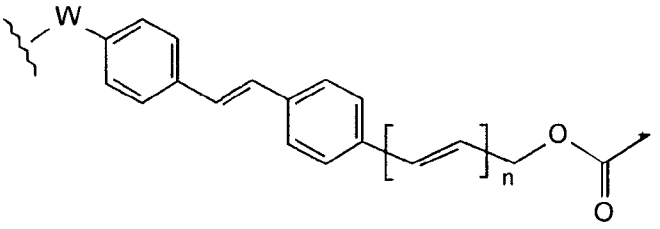
L^1 comprises an amino acid sequence;

L^2 is selected from the groups consisting of

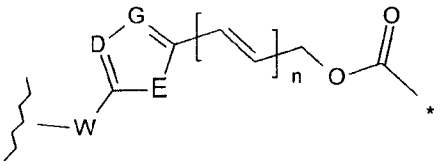








and



wherein the asterisk indicates the point of attachment to the Drug unit, and the wavy line indicates the point of attachment to L^1,

W is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-,

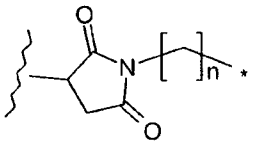
n is 0 to 3

E is O, S or NH,

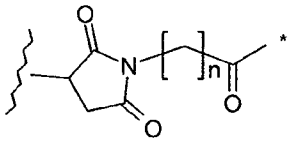
D is N or CH,

G is N or CH;

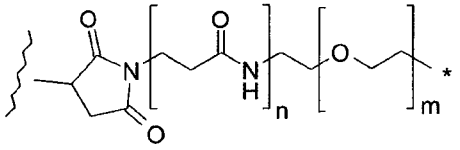
-A^1- is selected from the group consisting of:



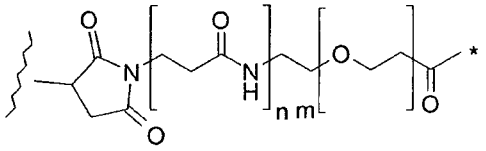
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to L, and n is 0 to 6;



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



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

18. A conjugate according to claim 17, wherein R^2 is of formula IIa, and A is phenyl, Q^1 is a single bond, and Q^2 is a single bond.

19. A conjugate according to claim 17, wherein R^2 is of formula IIb, and R^{C1}, R^{C2} and R^{C3} are all H.

20. A conjugate according to claim 17, wherein X is NH.

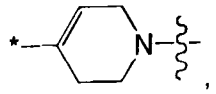
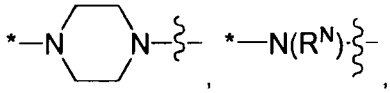
21. A conjugate according to claim 17, wherein R^2 is of formula IIc, and Q is NR^N, wherein R^N is H or methyl.

22. Use of a conjugate according to any one of claims 16 to 21 to treat a proliferative disease or an autoimmune disease.

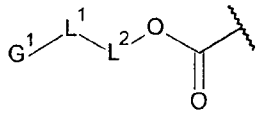
23. Use of an effective amount of the conjugate of any one of claims 16 to 21 to treat a mammal having a proliferative disease or an autoimmune disease.

24. A drug linker of formula VI:

LU-D (VI)

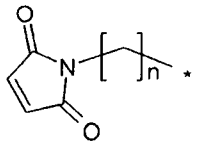
or a pharmaceutically acceptable salt or solvate thereof, wherein D is a Drug unit according to formula I as defined in claim 17, but where X is selected from the group consisting of *-O-^{q}, *-S-^{q}, *-CO_2-^{q}, *-CO-^{q}, *-NH(C=O)-^{q}, *-NHNH-^{q}, *-CONHNH-^{q},  ,  , wherein R^N is selected from the group consisting of H and C_{1-4} alkyl, and the asterix indicates the point of attachment to the remainder of the Drug unit and the wavy line or ^{q} indicates the point of attachment to LU;

wherein LU is selected from the group consisting of: G^1-L^1-L^2- and

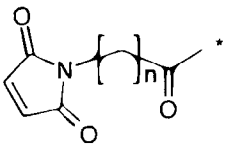
 ;

wherein L^1 and L^2 are as defined in claim 17;

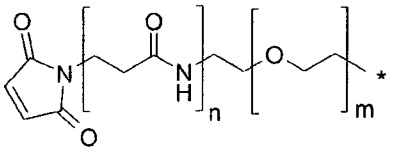
and -G^1- is selected from the group consisting of:



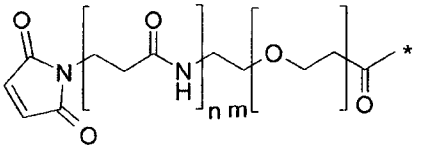
where the asterisk indicates the point of attachment to L^1 and n is 0 to 6;



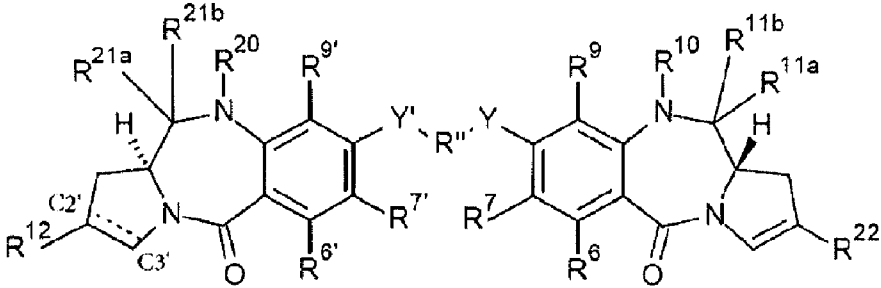
where the asterisk indicates the point of attachment to L^1 and n is 0 to 6;



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



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


R21b
R21a
R20
R9'
H
N
Y'
R''
Y
R9
R10
R11b
R11a
H
C2'
N
R7'
R7
R12
C3'
O
R6'
R6
O
N
R22

III