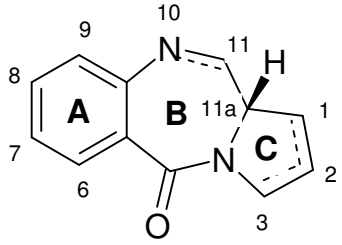
PYRROLOBENZODIAZEPINE CONJUGATES

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

Background

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

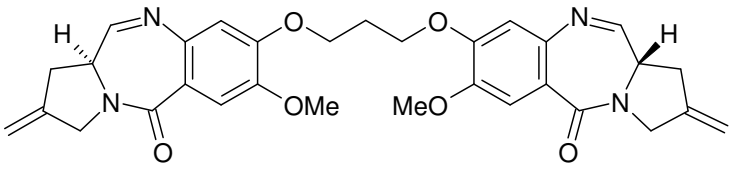


They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position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

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

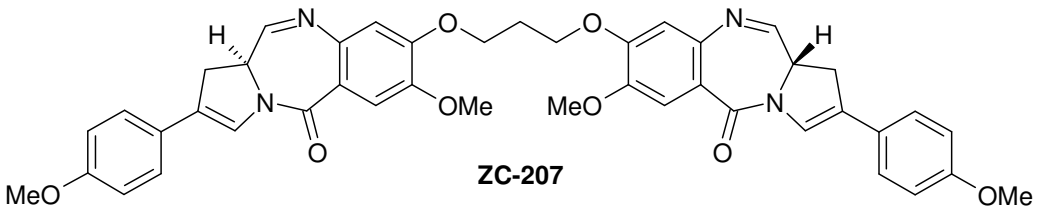
It has been previously disclosed that the biological activity of this molecules can be potentiated by joining two PBD units together through their C8/C'-hydroxyl functionalities via a flexible alkylene linker (Bose, D.S., *et al.*, *J. Am. Chem. Soc.*, **114**, 4939-4941 (1992); Thurston, D.E., *et al.*,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

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



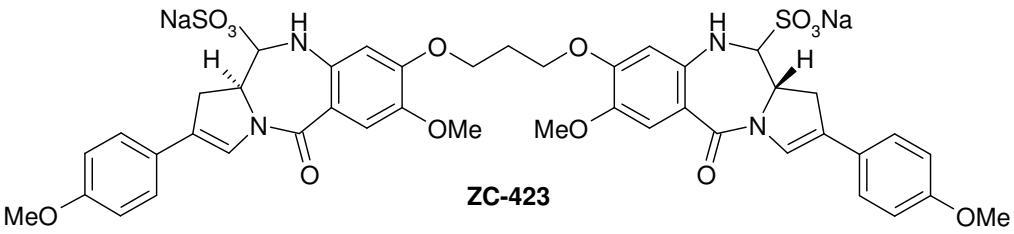
(Gregson, S., *et al.*, *J. Med. Chem.*, **44**, 737-748 (2001); Alley, M.C., *et al.*, *Cancer Research*, **64**, 6700-6706 (2004); Hartley, J.A., *et al.*, *Cancer Research*, **64**, 6693-6699 (2004)) which has been involved in clinical trials as a standalone agent, for example, NCT02034227 investigating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

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



ZC-207

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



ZC-423

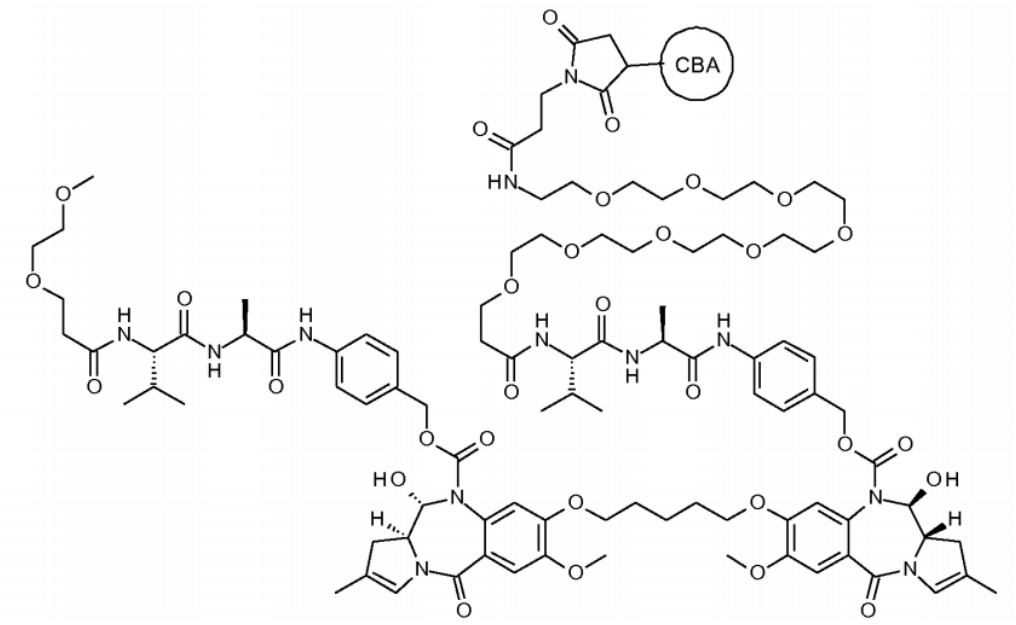
These compounds have been shown to be highly useful cytotoxic agents (Howard, P.W., *et al.*, *Bioorg. Med. Chem.* (2009), doi: 10.1016/j.bmcl.2009.09.012).

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

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

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

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



ConjC

Summary

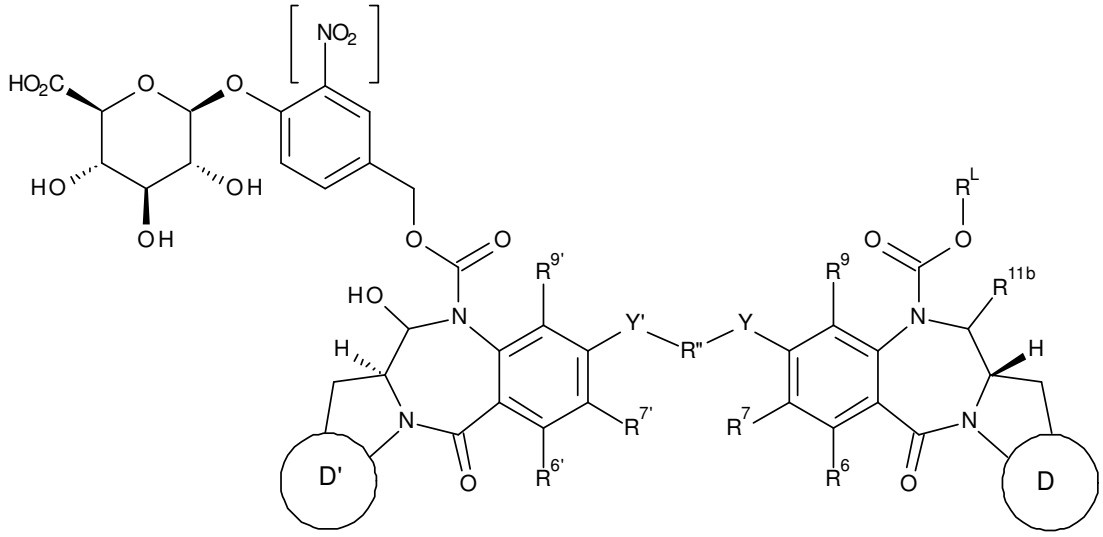
The present disclosure provides PBD and related dimer conjugates where the N10-C11 group that is not bound to the ligand unit is a β-glucuronide capping group. The cleavage trigger for the linking unit to the ligand unit is non-glucuronide cleavable.

Use of the glucuronide capping unit in these conjugates is believed to be advantageous, as if the conjugate is cleaved from the antibody outside the target cell, the released protected compound remains inactive as it is unable to permeate cell membranes due to its hydrophilicity.

The present disclosure also provided PBD and related dimer drug linkers, suitable for conjugating to a ligand unit, where the N10-C11 group that is not intended to be bound to the ligand unit is a glucuronide capping group. The cleavage trigger for the linking unit is a non-glucuronide capping unit.

Use of the glucuronide capping unit in these drug linkers is believed to be advantageous, as it increases the hydrophilicity of the generally hydrophobic drug linker, making the drug linkers easier to conjugate to a ligand unit.

In one embodiment, the present disclosure comprises a compound with the formula **I**:

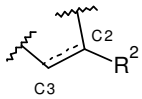


I

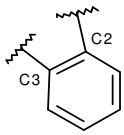
and salts and solvates thereof, wherein:

the square brackets indicate the NO_2 group is optional;

D represents either group D1 or D2:



D1



D2

;

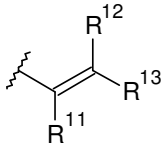
the dotted line indicates the optional presence of a double bond between C2 and C3;

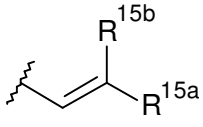
when there is a double bond present between C2 and C3, R^2 is selected from the group consisting of:

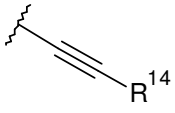
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

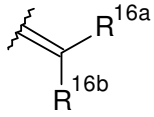
(ic) C_{3-6} saturated cycloalkyl;

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

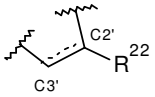
(ie)  , wherein one of R^{15a} and R^{15b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

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

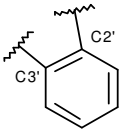
when there is a single bond present between C2 and C3,

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

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



D'1



D'2

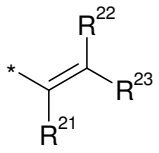
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

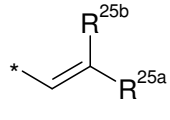
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

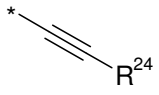
(iia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

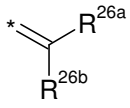
(iic) C_{3-6} saturated cycloalkyl;

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

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

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

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

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

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

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

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

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

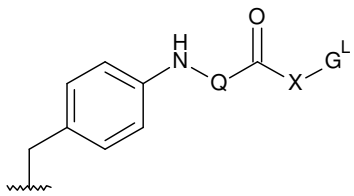
Y and Y' are selected from O, S, or NH;

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

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

R^L is a linker for connection to a cell binding agent, which is selected from:

(iiia):

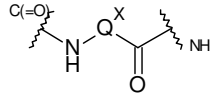


IIIa

,

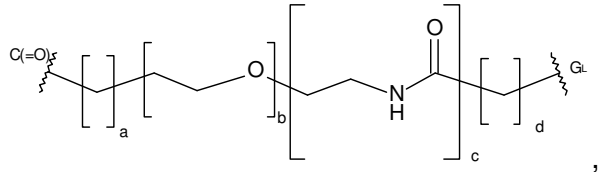
wherein

Q is:



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

X is:

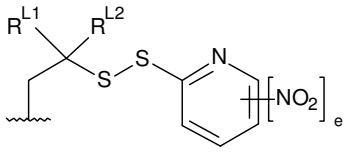


,

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

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

(iiib):

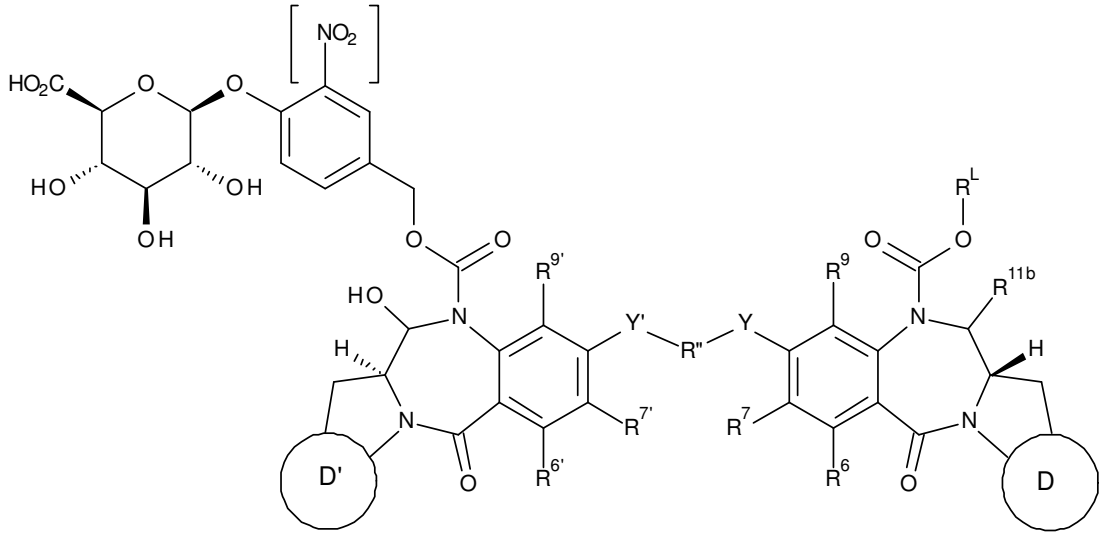


IIIb

,

where R^{L1} and R^{L2} are independently selected from H and methyl, or together with the carbon atom to which they are bound form a cyclopropylene or cyclobutylene group; and e is 0 or 1.

Disclosed herein is a compound of formula **I**:

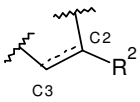


I

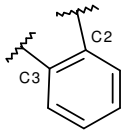
and salts and solvates thereof, wherein:

the square brackets indicate the NO_2 group is optional;

D represents either group D1 or D2:



D1



D2

;

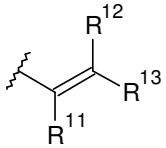
the dotted line indicates the optional presence of a double bond between C2 and C3;

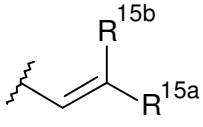
when there is a double bond present between C2 and C3, R^2 is selected from the group consisting of:

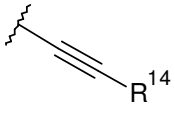
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

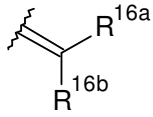
(ic) C_{3-6} saturated cycloalkyl;

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

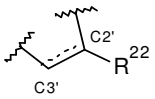
(ie)  , wherein one of R^{15a} and R^{15b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

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

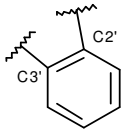
when there is a single bond present between C2 and C3,

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

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



D'1



D'2

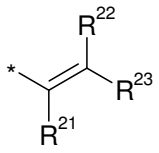
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

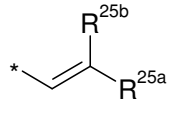
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

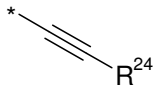
(iia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

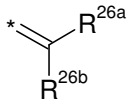
(iic) C_{3-6} saturated cycloalkyl;

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

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

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

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

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

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

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

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

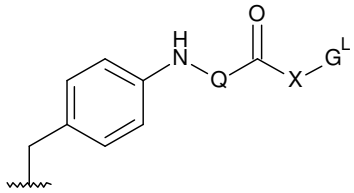
R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

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

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

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

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

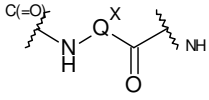


IIIa

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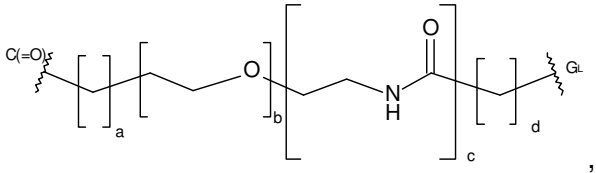
wherein

Q is:



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

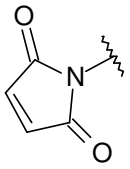
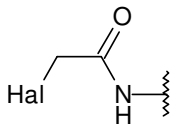
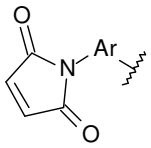
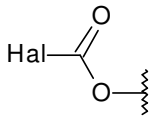
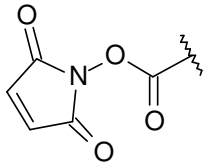
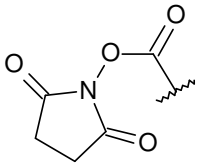
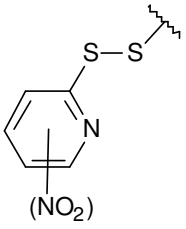
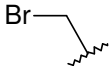
X is:

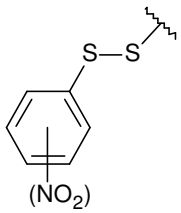
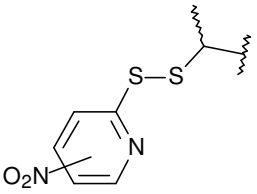
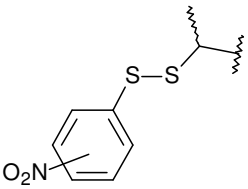


,

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

G^L is selected from:

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

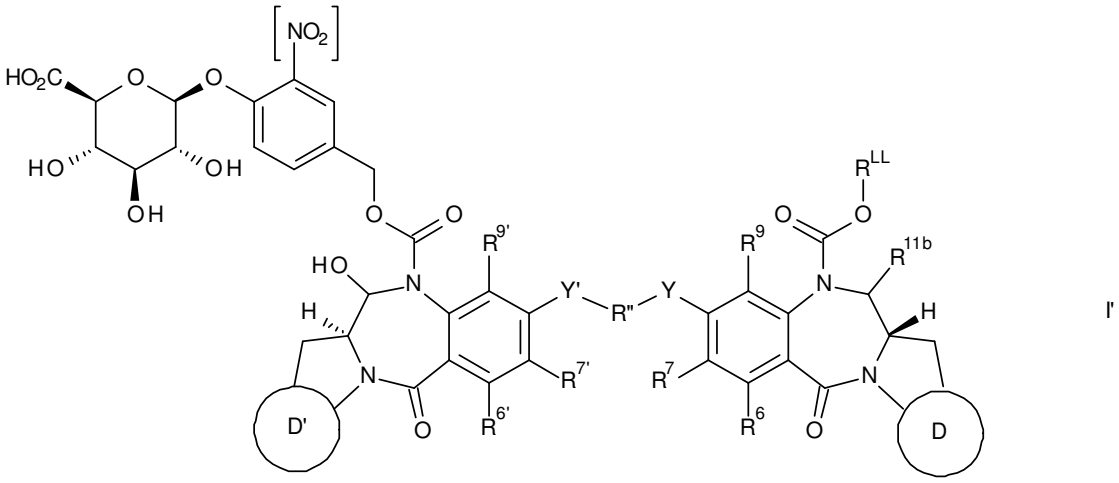
(G^{L3-2})	 where the NO_2 group is optional	(G^{L8})	
(G^{L3-3})	 where the NO_2 group is optional	(G^{L9})	
(G^{L3-4})	 where the NO_2 group is optional		

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

In one embodiment, the present disclosure provides Conjugates of formula II:

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

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

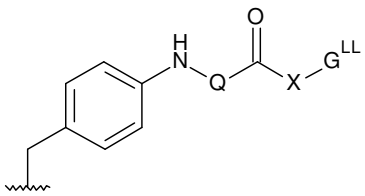


I'

wherein D, R^2, R^6, R^7, R^9, R^{11b}, Y, R", Y', D', $R^{6'}$, $R^{7'}$, $R^{9'}$ and R^{12} (including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, and the NO_2 group) are as defined herein;

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

(iiia):

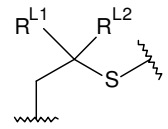


IIIa'

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

(iiib):



IIIb'

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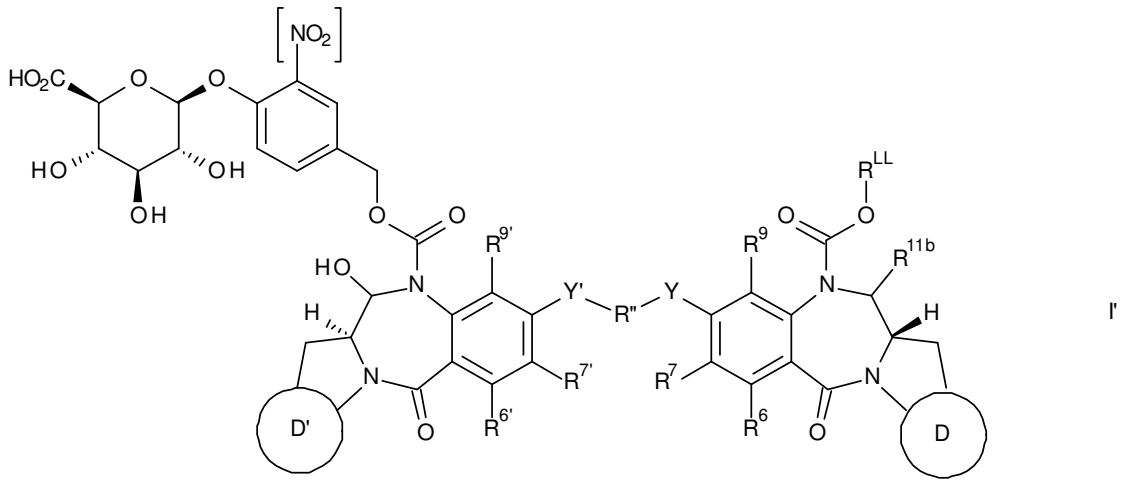
where R^{L1} and R^{L2} are as defined in the first aspect;

wherein p is an integer of from 1 to 20.

Disclosed herein is a conjugate of formula I:

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

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

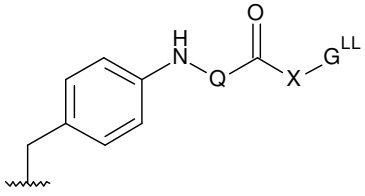


I'

wherein D, R^2, R^6, R^7, R^9, R^{11b}, Y, R", Y', D', $R^{6'}$, $R^{7'}$, $R^{9'}$ and R^{12}, including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, are as defined in any one of claims 1 to 10;

wherein p is an integer from 1 to 20;

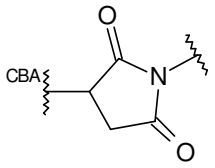
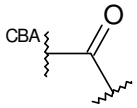
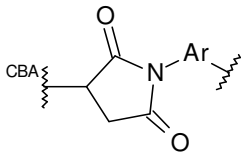
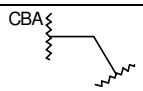
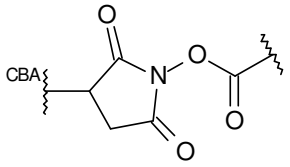
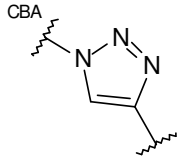
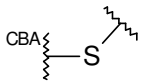
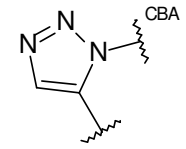
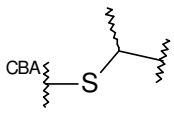
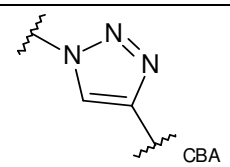
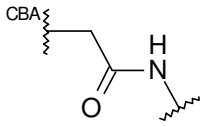
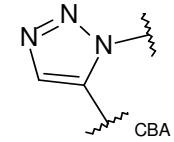
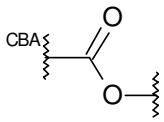
R^{LL} is a linker for connection to a cell binding agent, which is:



IIIa'

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where Q and X are as defined in any one of claims 1 and 11 to 13 and G^{LL} is selected from:

(G^{LL1-1})		(G^{LL6})	
(G^{LL1-2})		(G^{LL7})	
(G^{LL2})		(G^{LL8-1})	
(G^{LL3-1})		(G^{LL8-2})	
(G^{LL3-2})		(G^{LL9-1})	
(G^{LL4})		(G^{LL9-2})	
(G^{LL5})			

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

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

Disclosed herein is a composition comprising a mixture of conjugates as defined herein, wherein the average p in the mixture of conjugate compounds is about 1 to about 8.

Disclosed herein is the conjugate as defined herein for use in therapy.

Disclosed herein is a pharmaceutical composition comprising the conjugate as defined herein and a pharmaceutically acceptable diluent, carrier or excipient.

Disclosed herein is the conjugate as defined herein or the pharmaceutical composition as defined herein, for use in the treatment of a proliferative disease in a subject.

Disclosed herein is the use of a conjugate as defined herein in the manufacture of a medicament for the treatment of a proliferative disease. In one embodiment, there is provided a conjugate as defined herein for use in the treatment of a proliferative disease. In one embodiment, there is provided a method of treating a proliferative disease comprising administering a therapeutically effective amount of a conjugate as defined hereinto a patient in need thereof.

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

In one embodiment, the present disclosure provides the synthesis of a conjugate as defined herein comprising conjugating a compound (drug linker) as defined herein with a Ligand Unit.

Brief Description of Figures

Figure 1 shows the effect on viability of SKOV3 cells following incubation with a conjugate of the present invention;

Figure 2 shows the effect on viability of SKOV3 cells following incubation with a different conjugate of the present invention;

Figure 3 shows the effect on volume of a NCI-N87 xenograft tumour following treatment with a different conjugate of the present invention;

Figure 4 shows the effect on volume of a JIMT-1 xenograft tumour following treatment with the same conjugate of the present invention as in Figure 3.

Definitions

Substituents

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

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

Examples of substituents are described in more detail below.

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

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

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

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

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

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

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

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

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

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

saturated monocyclic hydrocarbon compounds:

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

unsaturated monocyclic hydrocarbon compounds:

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

saturated polycyclic hydrocarbon compounds:

norcarane (C_7), norpinane (C_7), norbornane (C_7).

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

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

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

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

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

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

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

O_3: trioxane (C_6);

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

N_1O_1: tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);

N_1S_1: thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);

N_2O_1: oxadiazine (C_6);

O_1S_1: oxathiole (C_5) and oxathiane (thioxane) (C_6); and,

$N_1O_1S_1$: oxathiazine (C_6).

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

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

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

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

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

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

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

N_1: pyrrole (azole) (C_5), pyridine (azine) (C_6);
O_1: furan (oxole) (C_5);
S_1: thiophene (thiole) (C_5);
N_1O_1: oxazole (C_5), isoxazole (C_5), isoxazine (C_6);
N_2O_1: oxadiazole (furazan) (C_5);
N_3O_1: oxatriazole (C_5);
N_1S_1: thiazole (C_5), isothiazole (C_5);
N_2: imidazole (1,3-diazole) (C_5), pyrazole (1,2-diazole) (C_5), pyridazine (1,2-diazine) (C_6), pyrimidine (1,3-diazine) (C_6) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (C_6);
N_3: triazole (C_5), triazine (C_6); and,
N_4: tetrazole (C_5).

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

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

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

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

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

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

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

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

Hydroxy: -OH.

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

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

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

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

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

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

Oxo (keto, -one): =O.

Thione (thioketone): =S.

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

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

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

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

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

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

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

Imidic acid: -C(=NH)OH.

Hydroxamic acid: -C(=NOH)OH.

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

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

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

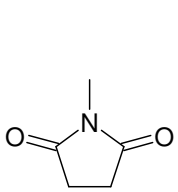
Amino: -NR^1R^2, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a

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

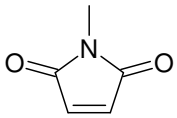
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$,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

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

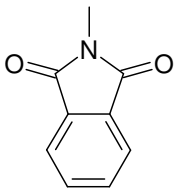
Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20}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



succinimidyl



maleimidyl



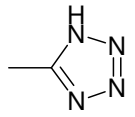
phthalimidyl

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

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

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

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



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

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

Nitro: -NO_2.

Nitroso: -NO.

Azido: -N_3.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

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

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

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

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

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

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

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$_2$CH$_3$ (ethoxysulfinyl; ethyl sulfinate).

Sulfonate (sulfonic acid ester): -S(=O)$_2$OR, wherein R is a sulfonate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonate groups include, but are not limited to, -S(=O)$_2$OCH$_3$ (methoxysulfonyl; methyl sulfonate) and -S(=O)$_2$OCH$_2$CH$_3$ (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$_2$CH$_3$.

Sulfonyloxy: -OS(=O)$_2$R, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonyloxy groups include, but are not limited to, -OS(=O)$_2$CH$_3$ (mesylate) and -OS(=O)$_2$CH$_2$CH$_3$ (esylate).

Sulfate: -OS(=O)$_2$OR; wherein R is a sulfate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfate groups include, but are not limited to, -OS(=O)$_2$OCH$_3$ and -SO(=O)$_2$OCH$_2$CH$_3$.

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): -S(=O)NR^{1}R^2, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, -S(=O)NH$_2$, -S(=O)NH(CH$_3$), -S(=O)N(CH$_3$)$_2$, -S(=O)NH(CH$_2$CH$_3$), -S(=O)N(CH$_2$CH$_3$)$_2$, and -S(=O)NHPh.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): -S(=O)$_2$NR^{1}R^2, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfonamido groups include, but are not limited to, -S(=O)$_2$NH$_2$, -S(=O)$_2$NH(CH$_3$), -S(=O)$_2$N(CH$_3$)$_2$, -S(=O)$_2$NH(CH$_2$CH$_3$), -S(=O)$_2$N(CH$_2$CH$_3$)$_2$, and -S(=O)$_2$NHPh.

Sulfamino: -$NR^1S(=O)_2OH$, 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-Bu)$_2$, and -$P(Ph)_2$.

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

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

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

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

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

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

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

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

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

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

Alkylene

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

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

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

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

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

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

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

Ligand Unit

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

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

than its affinity for binding to a non-specific molecule (e.g., BSA, casein) other than the predetermined molecule or a closely-related molecule.

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

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

Cell Binding Agent

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

Peptides

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

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

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

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, which is incorporated herein.

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 (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); 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 (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 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, 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: 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 (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, 2407-2409, 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); 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 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 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, 869-880, 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 (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) *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; 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 (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; 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 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. 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 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, 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 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.* 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) *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 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 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; 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: 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); see WO2015/159076

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)*

<u>Nucleotide</u>

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 (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; ClECT 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
- See WO2015/155345

(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 αvβ6

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 R**H**A ‘5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**B ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**C ‘5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**D ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**E ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**F ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**G ‘5
EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**K**A ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**B ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**C ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**D ‘5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**E ‘5
NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPD
RFTGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**F ‘5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**G '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, and all of which are incorporated herein by reference.

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.

Connection of Linker unit to Ligand unit

The Ligand unit is connected to the Linker unit through a disulfide bond.

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

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

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

Methods of Treatment

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

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

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

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

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

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

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

Examples of autoimmune disease include the following: rheumatoid arthritis, autoimmune demyelinative diseases (e.g., multiple sclerosis, allergic encephalomyelitis), psoriatic arthritis, endocrine ophthalmopathy, uveoretinitis, systemic lupus erythematosus, myasthenia gravis, Graves’ disease, glomerulonephritis, autoimmune hepatological disorder, inflammatory bowel disease (e.g., Crohn’s disease), anaphylaxis, allergic reaction, Sjögren’s syndrome, type I diabetes mellitus, primary biliary cirrhosis, Wegener’s granulomatosis, fibromyalgia, polymyositis, dermatomyositis, multiple endocrine failure, Schmidt’s syndrome, autoimmune uveitis, Addison’s disease, adrenalitis, thyroiditis, Hashimoto’s thyroiditis, autoimmune thyroid disease, pernicious anemia, gastric atrophy, chronic hepatitis, lupoid hepatitis, atherosclerosis, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler’s syndrome, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus,

dermatitis herpetiformis, alopecia arcata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, ulcerative colitis, mixed connective tissue disease, polyarteritis nedosa, systemic necrotizing vasculitis, atopic dermatitis, atopic rhinitis, Goodpasture's syndrome, Chagas' disease, sarcoidosis, rheumatic fever, asthma, recurrent abortion, anti-phospholipid syndrome, farmer's lung, erythema multiforme, post cardiotomy syndrome, Cushing's syndrome, autoimmune chronic active hepatitis, bird-fancier's lung, toxic epidermal necrolysis, Alport's syndrome, alveolitis, allergic alveolitis, fibrosing alveolitis, interstitial lung disease, erythema nodosum, pyoderma gangrenosum, transfusion reaction, Takayasu's arteritis, polymyalgia rheumatica, temporal arteritis, schistosomiasis, giant cell arteritis, ascariasis, aspergillosis, Sampter's syndrome, eczema, lymphomatoid granulomatosis, Behcet's disease, Caplan's syndrome, Kawasaki's disease, dengue, encephalomyelitis, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, psoriasis, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, filariasis, cyclitis, chronic cyclitis, heterochronic cyclitis, Fuch's cyclitis, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, cardiomyopathy, Eaton-Lambert syndrome, relapsing polychondritis, cryoglobulinemia, Waldenstrom's macroglobulemia, Evan's syndrome, and autoimmune gonadal failure.

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

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

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

Drug loading

The drug loading (p) is the average number of PBD drugs per cell binding agent, e.g. antibody. Where the compounds of the invention are bound to cysteines, drug loading may range from 1 to 8 drugs (D) per cell binding agent, i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the cell binding agent. Compositions of 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. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of Drug Linker relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

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

Cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249). The engineered cysteine thiols may react with linker reagents or the drug-linker reagents of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form ADC with cysteine engineered antibodies

and the PBD drug moieties. The location of the drug moiety can thus be designed, controlled, and known. The drug loading can be controlled since the engineered cysteine thiol groups typically react with thiol-reactive linker reagents or drug-linker reagents in high yield. Engineering an IgG antibody to introduce a cysteine amino acid by substitution at a single site on the heavy or light chain gives two new cysteines on the symmetrical antibody. A drug loading near 2 can be achieved with near homogeneity of the conjugation product ADC.

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

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

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

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

General synthetic routes

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

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

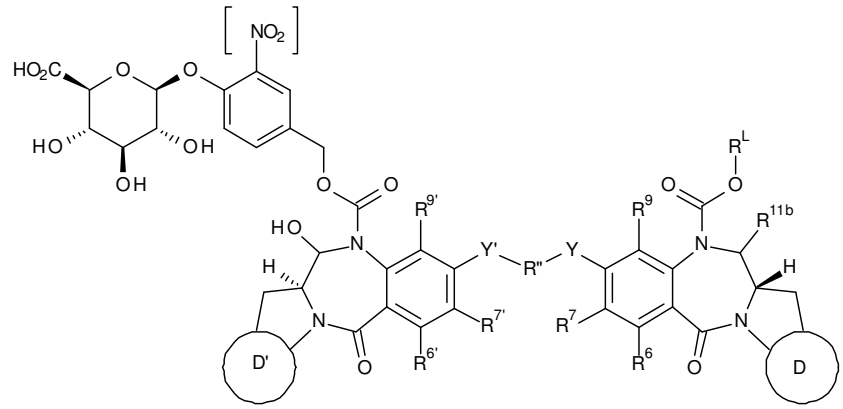
b) WO 2005/023814 (pages 3 to 10);

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

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

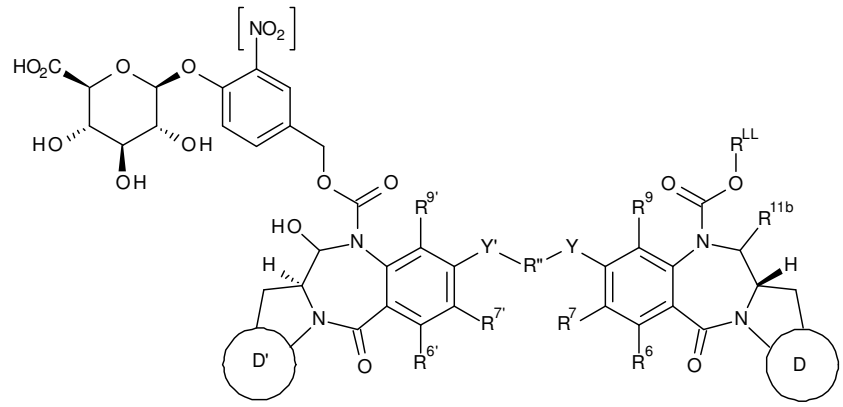
Synthesis route

Compounds of the present invention of formula I:



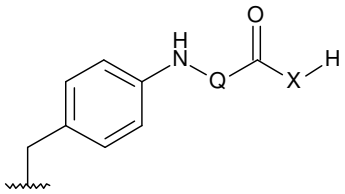
I

can be synthesised from a compound of Formula II:



Formula 2

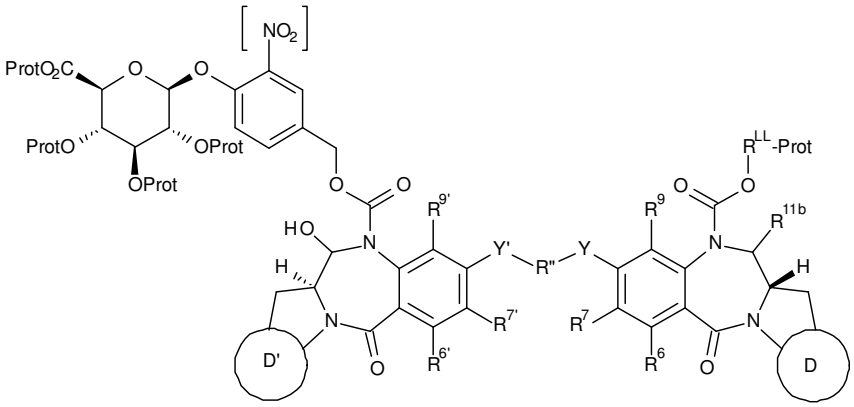
where R^2, R^6, R^7, R^9, $R^{6'}$, $R^{7'}$, $R^{9'}$, R^{11b}, R^{12}, Y, Y' and R" are as defined for compounds of formula I, and R^{LL} is a precursor of R^L – this method is particularly applicable to compounds of formula I where R^L is of formula IIIa. For these compounds, R^{LL} will typically be a portion of R^L, such as a group of formula IIIa':



IIIa'

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

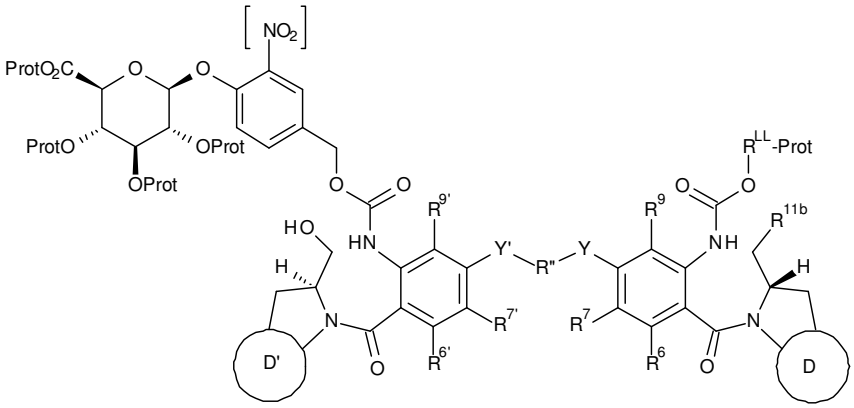
The compounds of Formula 2 may be made by deprotecting compounds of Formula 3:



Formula 3

where R^2, R^6, R^7, R^9, $R^{6'}$, $R^{7'}$, $R^{9'}$, R^{11b}, R^{12}, Y, Y' and R" are as defined for compounds of formula I, $R^{LL\text{-}Prot}$ is a protected version of R^{LL}, and the Prot represents an appropriate carboxy/hydroxy protecting group.

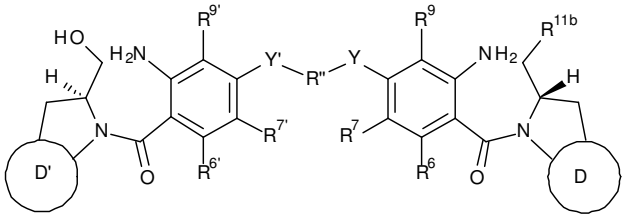
Compounds of formula 3 may be made by ring-closure of compounds of Formula 4:



Formula 4

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

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



Formula 5

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

Compounds of formula I where R^L is of formula IIIb, may be synthesised in a similar manner, although the complete R^L group may be installed starting from a compound of Formula 5, rather than with the use of a protected precursor.

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

Synthesis of Drug Conjugates

Conjugates can be prepared as previously described. Antibodies can be conjugated to the Drug Linker compound as described in Doronina et al., Nature Biotechnology, 2003, 21, 778-784). Briefly, antibodies (4-5 mg/mL) in PBS containing 50 mM sodium borate at pH 7.4 are reduced with tris(carboxyethyl)phosphine hydrochloride (TCEP) at 37 ºC. The progress of the reaction, which reduces interchain disulfides, is monitored by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) and allowed to proceed until the desired level of thiols/mAb is achieved. The reduced antibody is then cooled to 0°C and alkylated with 1.5 equivalents of maleimide drug-linker per antibody thiol. After 1 hour, the reaction is quenched by the addition of 5 equivalents of N-acetyl cysteine. Quenched drug-linker is removed by gel filtration over a PD-10 column. The ADC is then sterile-filtered through a 0.22 µm syringe filter. Protein concentration can be determined by spectral analysis at 280 nm and 329 nm, respectively, with correction for the contribution of drug absorbance at 280 nm. Size exclusion chromatography can be used to determine the extent of antibody aggregation, and RP-HPLC can be used to determine the levels of remaining NAC-quenched drug-linker.

Further Preferences

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

In some embodiments, the NO_2 group is present. In other embodiments, the NO_2 is absent.

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

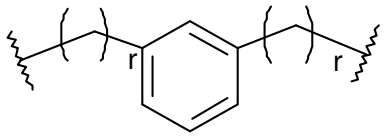
In some embodiments, R^{12} is the same as R^2.

Dimer link

In some embodiments, Y and Y' are both O.

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

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



where r is 1 or 2.

R^6 to R^9

In some embodiments, R^9 is H.

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

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

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

D and D'

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

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

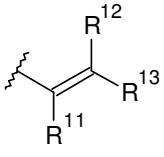
R^2

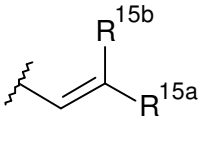
When there is a double bond present between C2 and C3, R^2 is selected from:

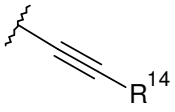
(a) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

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

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

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

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

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

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

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

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

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

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

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

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

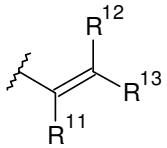
If a substituent on R^2 when R^2 is a C_{5-10} aryl group is ester, this is preferably methyl ester or ethyl ester.

Particularly preferred substituents when R^2 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^2 are dimethylaminopropyloxy and carboxy.

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

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

When R^2 is 

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

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

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

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

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

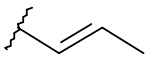
In some embodiments, R^{12} is H.

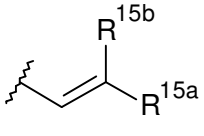
In some embodiments, R^{13} is H.

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

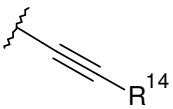
In some embodiments, R^{11} and R^{13} are H.

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

An R^2 group of particular interest is:  .

When R^2 is 

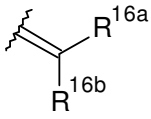
, one of R^{15a} and R^{15b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl. In some embodiments, the group which is not H is optionally substituted phenyl. If the phenyl optional substituent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

When R^2 is 

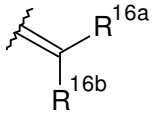
, R^{14} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment,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

In some embodiments, R^{14} is selected from H, methyl, ethyl, ethenyl and ethynyl. In some of these embodiments, R^{14} is selected from H and methyl.

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

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

In some embodiments, R^2 is H.

In some embodiments, R^2 is .

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

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

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

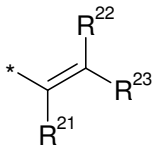
R^{12}

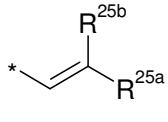
When there is a double bond present between C2' and C3', R^{12} is selected from:

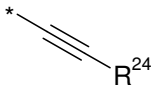
(a) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

(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.

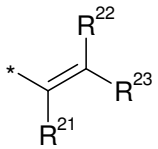
Particularly preferred substituents when R^{12} is a C_{5-10} aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-

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.

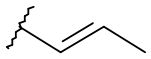
In some embodiments, R^{22} is H.

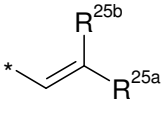
In some embodiments, R^{23} is H.

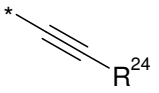
In some embodiments, R^{21} and R^{22} are H.

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

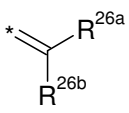
In some embodiments, R^{22} and R^{23} are H.

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

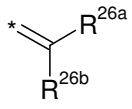
When R^{12} is , one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl. In some embodiments, the group which is not H is optionally substituted phenyl. If the phenyl optional 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.

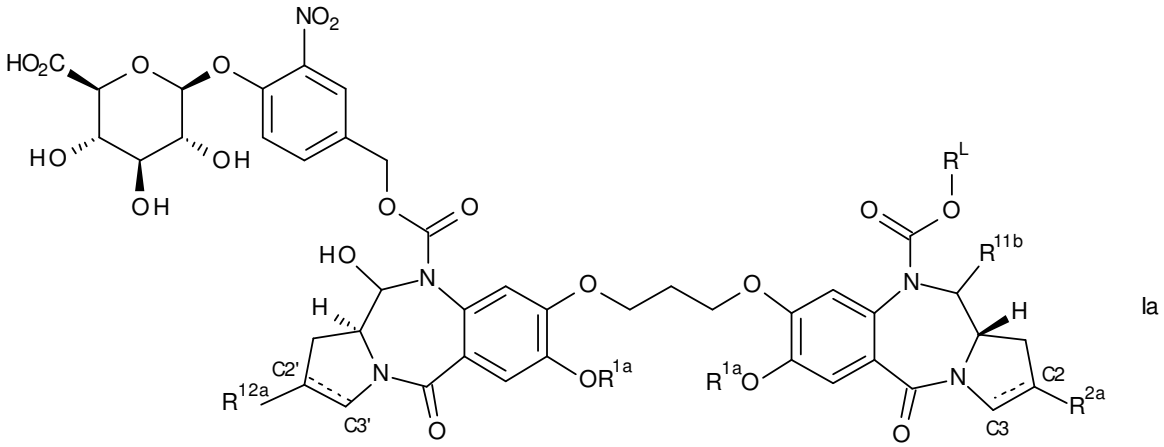
In further embodiments, it is preferred that one of R^{26a} and R^{26b} is H, and the other is selected from C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted. In these further embodiment, it may be further preferred that the group which is not H is selected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

R^{11b}

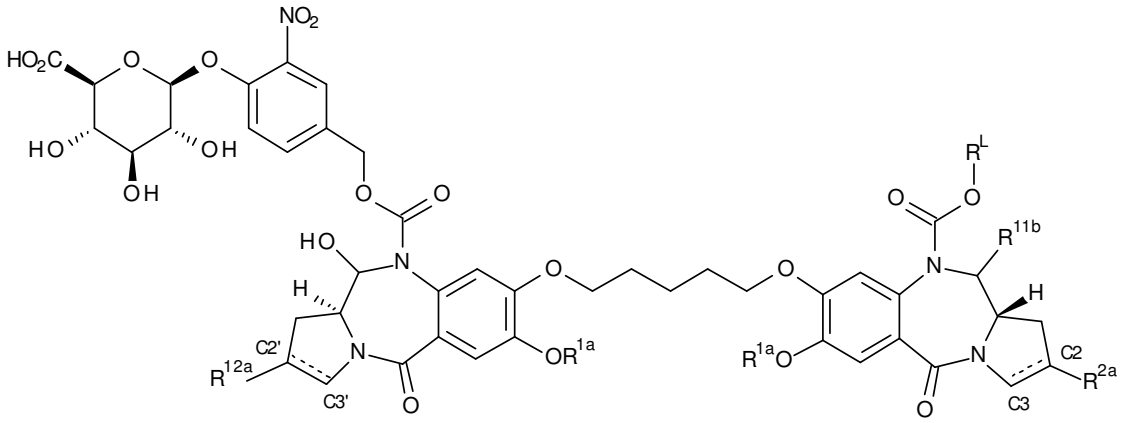
In some embodiments, R^{11b} is OH.

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

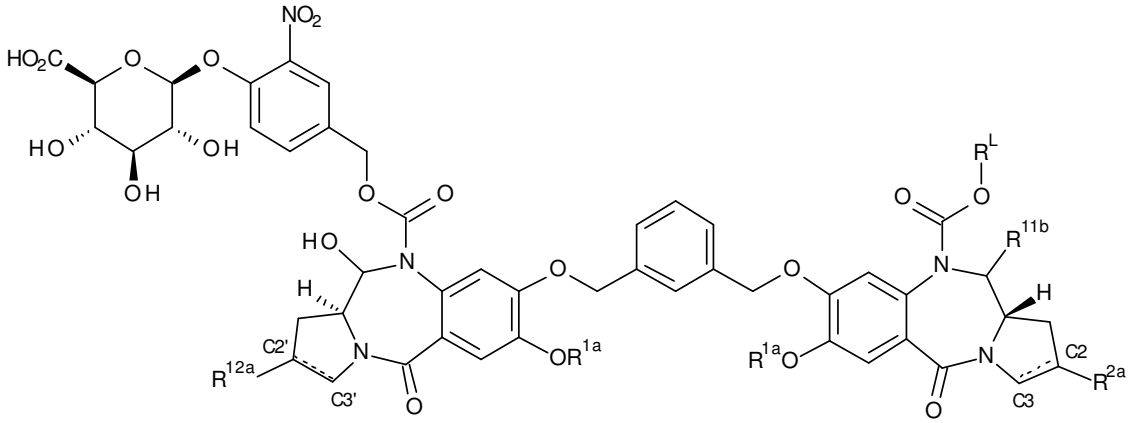
In some embodiments of the first aspect of the present invention are of formula Ia-1, Ia-2 or Ia-3:



Ia

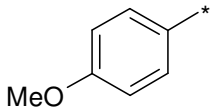


Ib

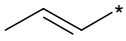


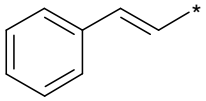
Ic

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

(a)  ;

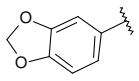
(b)  ;

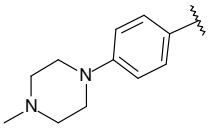
(c)  ;

(d)  ;

(e)  ;

(f)  ;

(g)  ; and

(h)  ;

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

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

In some embodiments of the present invention both R^2 and R^{12} comprise no more than 3 carbon atoms.

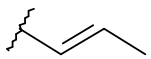
Thus in these embodiments where there is a double bond present between C2 and C3, R^2 may be selected from:

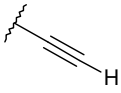
(i) Methyl;

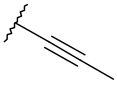
(ii) Ethyl;

(iii) Propyl;

(iv) Cyclopropyl;

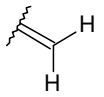
(v)  ;

(vi)  ; and

(vi) 

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

(i) H;

(ii)  ;

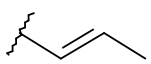
(iii)  ; and

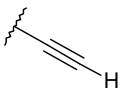
(iv)  .

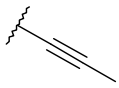
Thus in these embodiments where there is a double bond present between C2' and C3', R^{12} may be selected from:

(i) Methyl;

(ii) Ethyl;

(v)  ;

(vi)  ; and

(iii) Propyl; (vi) 

(iv) Cyclopropyl;

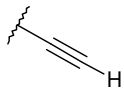
Thus in these embodiments where there is no double bond present between C2' and C3', R^{12} may be selected from:

(i) H; (iii)  ; and

(ii)  ; (iv)  .

In some of these embodiments both R^2 and R^{12} comprise no more than 2 carbon atoms.

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

(i) Methyl; (vi)  .

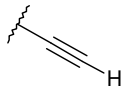
(ii) Ethyl; and

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

(i) H; (iii)  .

(ii)  ; and

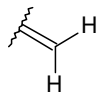
Thus in these embodiments where there is a double bond present between C2' and C3', R^{12} may be selected from:

(i) Methyl; (vi)  .

(ii) Ethyl; and

Thus in these embodiments where there is no double bond present between C2' and C3', R^{12} may be selected from:

(i) H; (iii) .

(ii)  ; and

In further of these embodiments both R^2 and R^{12} comprise no more than 1 carbon atom.

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

(i) H; and (ii) .

Thus in these embodiments where there is a double bond present between C2' and C3', R^{12} may be methyl. Thus in these embodiments where there is no double bond present between C2' and C3', R^{12} may be selected from:

(i) H; and (ii) .

Without wishing to be bound by theory, where the substituent at the C2 position of the PBD dimers are small, the use of the glucuronide capping unit in these drug linkers is believed to be particularly advantageous, as it significantly increases the hydrophilicity of the drug linker, making the drug linkers easier to conjugate to a ligand unit.

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

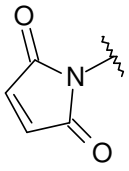
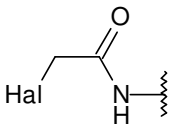
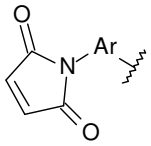
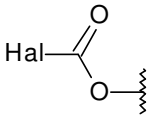
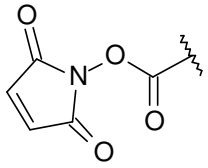
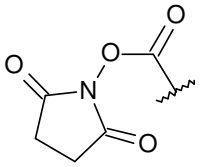
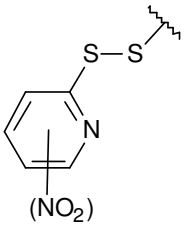
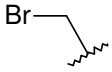
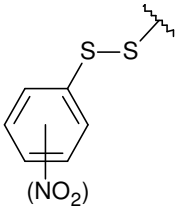
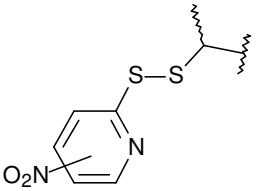
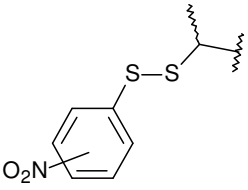
Linker (R^L)

In some embodiments, R^L is of formula IIIa.

In some embodiments, R^{LL} is of formula IIIa'.

G^L

G^L may be selected from

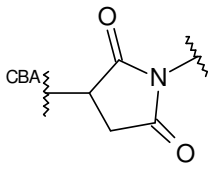
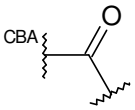
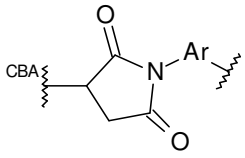
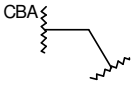
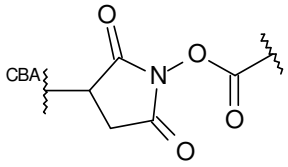
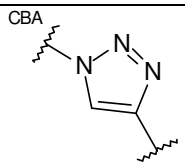
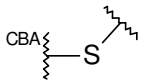
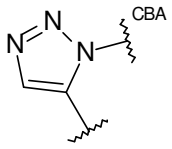
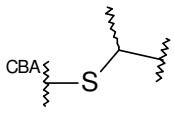
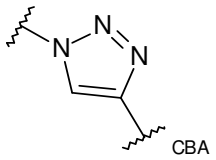
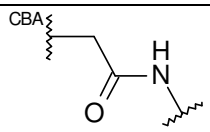
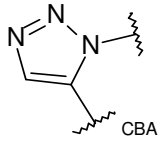
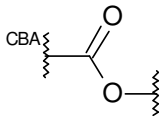
(G^{L1-1})		(G^{L4})	 Where Hal = I, Br, Cl
(G^{L1-2})		(G^{L5})	
(G^{L2})		(G^{L6})	
(G^{L3-1})	 where the NO_2 group is optional	(G^{L7})	
(G^{L3-2})	 where the NO_2 group is optional	(G^{L8})	
(G^{L3-3})	 where the NO_2 group is optional	(G^{L9})	
(G^{L3-4})	 where the NO_2 group is optional		

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

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

G^{LL}

G^{LL} may be selected from:

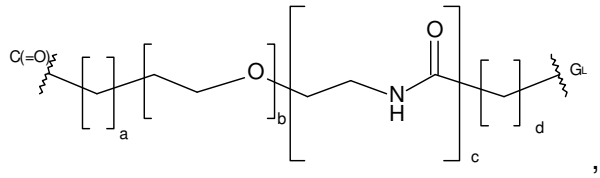
(G^{LL1-1})		(G^{LL6})	
(G^{LL1-2})		(G^{LL7})	
(G^{LL2})		(G^{LL8-1})	
(G^{LL3-1})		(G^{LL8-2})	
(G^{LL3-2})		(G^{LL9-1})	
(G^{LL4})		(G^{LL9-2})	
(G^{LL5})			

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

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

X

X is:



,

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

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

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

c may be 0 or 1.

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

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

Q^X

In one embodiment, Q^X is an amino acid residue. The amino acid may a natural amino acids or a non-natural amino acid.

In one embodiment, Q^X is selected from: Phe, Lys, Val, Ala, Cit, Leu, Ile, Arg, and Trp, where Cit is citrulline.

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

In one embodiment, Q^X is selected from:

^{CO}-Phe-Lys-^{NH},

^{CO}-Val-Ala-^{NH},

^{CO}-Val-Lys-^{NH},

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

where Cit is citrulline.

Preferably, Q^X is selected from:

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

Most preferably, Q^X is selected from ^{CO}-Phe-Lys-^{NH}, ^{CO}-Val-Cit-^{NH} and ^{CO}-Val-Ala-^{NH}.

Other dipeptide combinations of interest include:

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

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

In some embodiments, Q^X 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.

In some embodiments, R^L is of formula IIIb.
In some embodiments, R^{LL} is is formula IIIb'.

R^{L1} and R^{L2} are independently selected from H and methyl, or together with the carbon atom to which they are bound form a cyclopropylene or cyclobutylene group.

In some embodiments, both R^{L1} and R^{L2} are H.

In some embodiments, R^{L1} is H and R^{L2} is methyl.

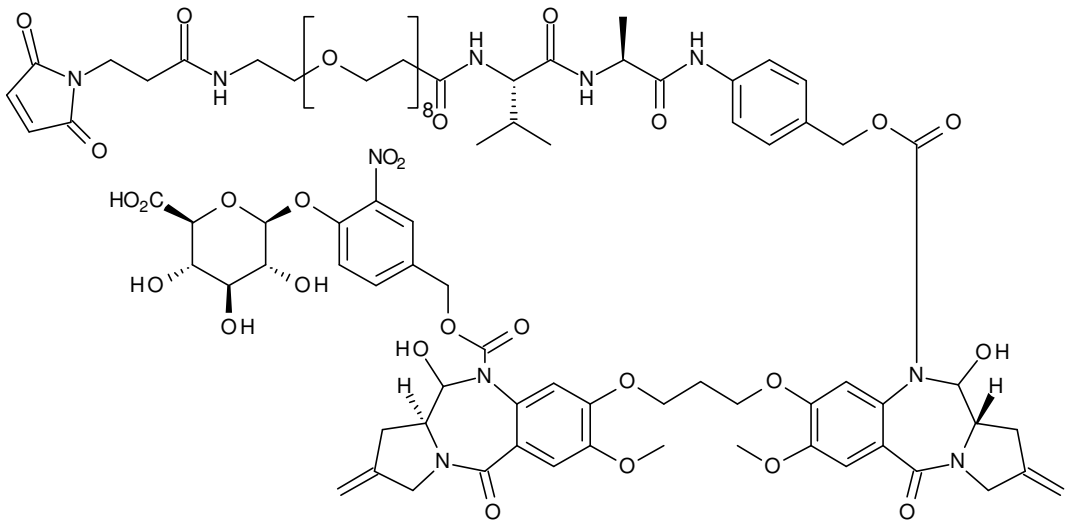
In some embodiments, both R^{L1} and R^{L2} are methyl.

In some embodiments, R^{L1} and R^{L2} together with the carbon atom to which they are bound form a cyclopropylene group.

In some embodiments, R^{L1} and R^{L2} together with the carbon atom to which they are bound form a cyclobutylene group.

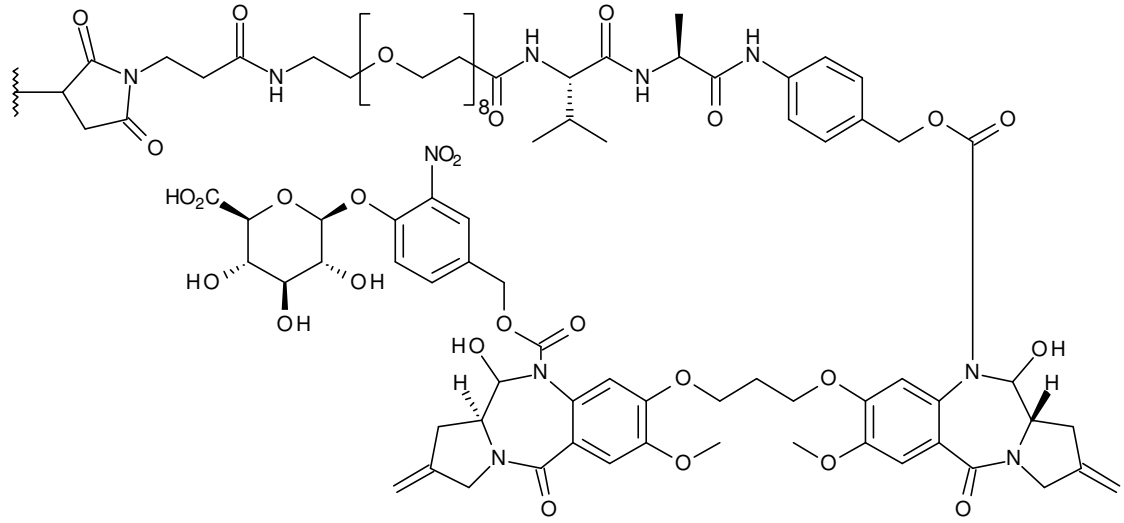
In the group IIIb, in some embodiments, e is 0. In other embodiments, e is 1 and the nitro group may be in any available position of the ring. In some of these embdoiments, it is in the ortho position. In others of these embodiments, it is in the para position.

In one particular embodiment, the first aspect of the invention comprises a compound of formula Id:



(Id)

In one particular embodiment, the second aspect of the invention, the Drug linker (D^L) is of formula (Id'):



(Id')

Additional background

In an impact study submitted to the 2014 Research Excellence Framework (REF) in the United Kingdom by University College London (available at http://impact.ref.ac.uk/casestudies2/refservice.svc/GetCaseStudyPDF/35393), it was commented that:

"The next generation of PBD dimers, which are more potent than SG2000, have been developed, including SG2057 and SG2202. They exhibit picomolar/sub-picomolar activity against a range of human tumour cell lines and demonstrate curative activity in human tumour xenograft models." making reference to:

Hartley JA, et al., *DNA interstrand cross-linking and in vivo antitumor activity of the extended pyrrolo[2,1-c][1,4]benzodiazepine dimer SG2057*. Invest New Drugs. 2012 Jun;

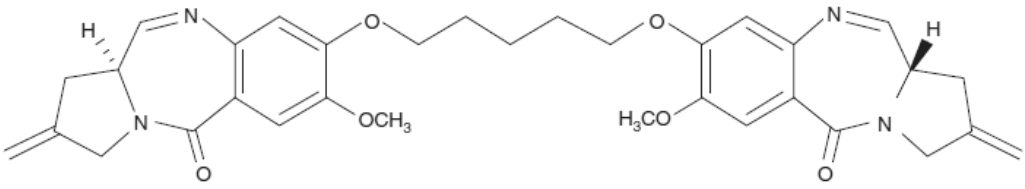
30(3):950-8. http://dx.doi.org/10.1007/s10637-011-9647-z (herein after “Hartley et al (2012)”)

and:

“The ability to generate such cytotoxic molecules that display exquisite potency suggested a potential role in strategies aimed at targeting and releasing highly cytotoxic agents directly at a tumour site. An example is as the ‘warhead’ component of an antibody drug conjugate (ADC). The fully synthetic PBD dimers are ideally suited for the role of warhead in an ADC approach.”

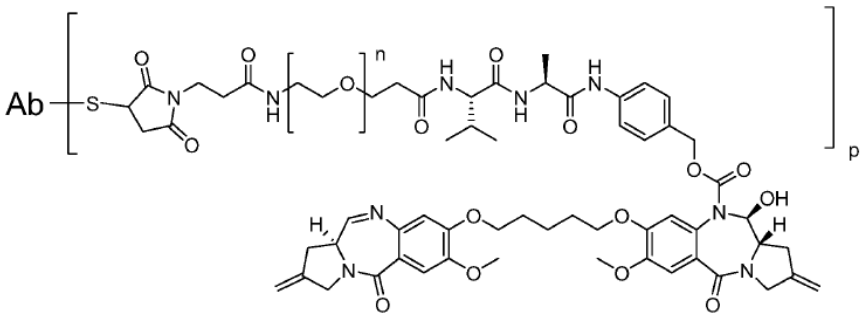
The Hartley et al (2012) paper comments in its summary that “SG2057 is therefore a highly active antiumour agent, with more potent in vitro activity and superior in vivo activity to SG2000, warranting further development”.

SG2057 has the structure:



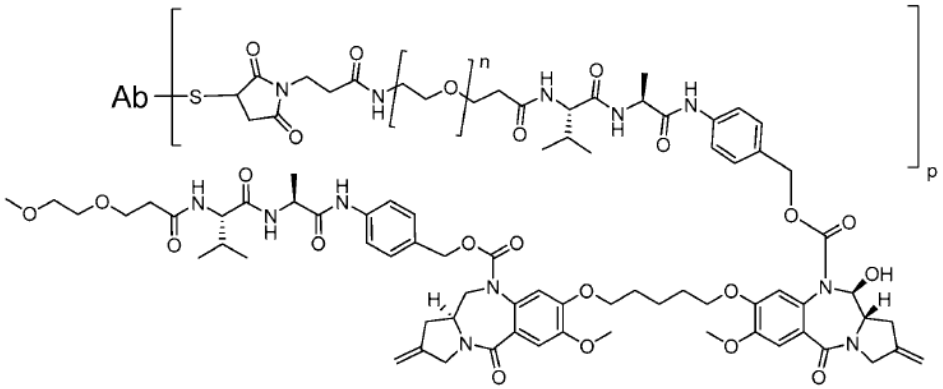
SG2057

Antibody drug conjugates using SG2057 as a warhead were first disclosed in WO 2011/130598. For example, claim 54 of this application includes the formula:



wherein n is from 1 to 24, more preferably 4 to 8. The following drug linkers were exemplified: n=4, 15c; n=8, 15d; n=24, 15e.

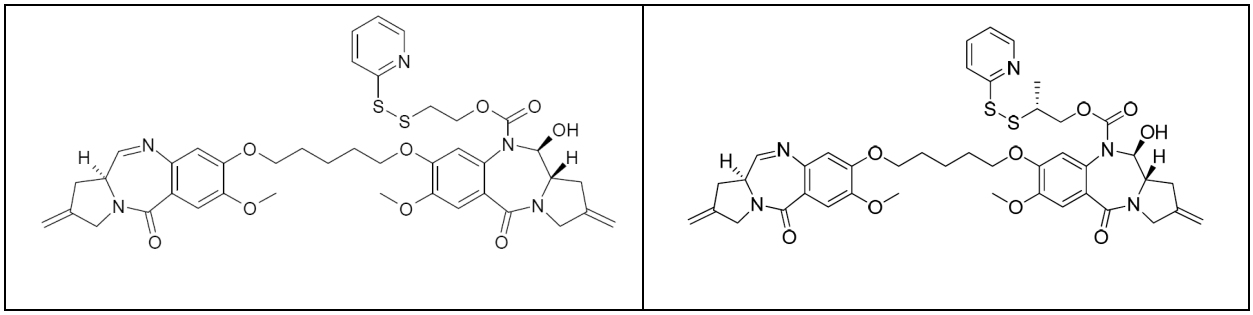
Claim 54 of this application also includes the formula:



wherein n is from 1 to 24, more preferably 4 to 8. The following drug linkers were exemplified: n=8, 58; n=24, 61.

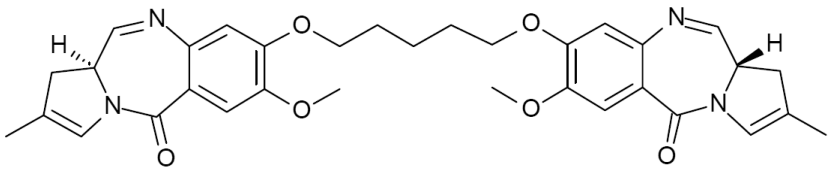
WO 2011/130598 also discloses antibody-drug conjugates including these drug linkers, for example 110 (antiSteap1-15d), example 114 (tastuzumab-15d) and example 115 (tastuzumab-58).

WO 2013/055987 discloses the drug linkers 14 and 22:

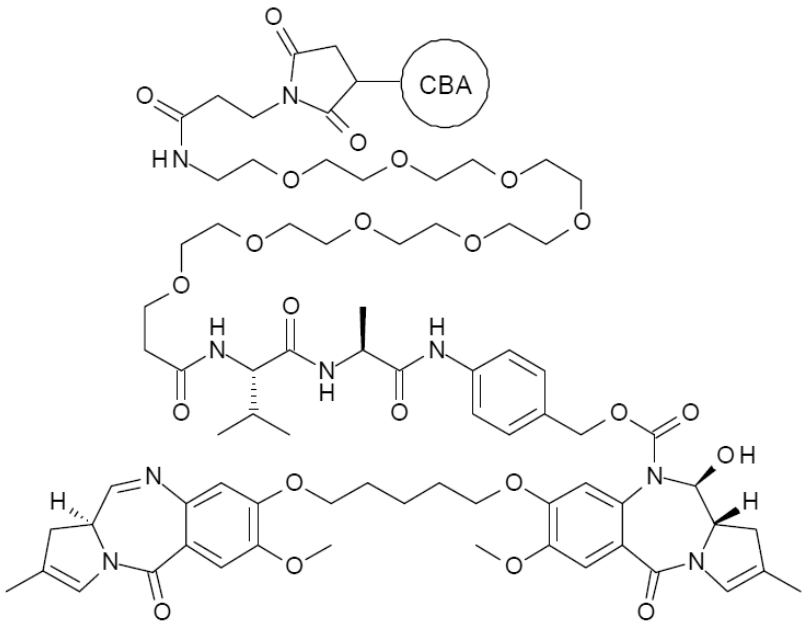


and their use in antibody-drug conjugates.

More recently, the warhead:



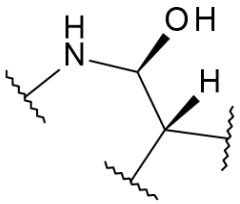
has been used in drug linkers and antibody-drug conjugates. WO 2014/057074 discloses:



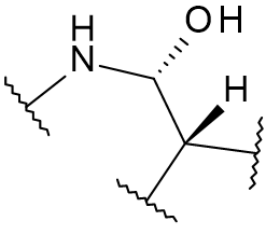
The present inventors have surprisingly found that although SG2000 is at least 10 times less cytotoxic than SG2057 (see Hartley et al 2012), particular antibody-drug conjugates, i.e. those of formula Id, appear to show at least comparable activity. These conjugates have been shown to have surprisingly well tolerated in toxicity studies in a variety of species. This leads to the conjugates exhibiting high therapeutic indices and thus are promising clinical candidates.

_____

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



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



The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Compounds of particular interest include those of the examples

Examples

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

^{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), and m (multiplet).

The analytical LC/MS conditions (for reaction monitoring and purity determination) were as follows: Positive mode electrospray mass spectrometry was performed using a Shimadzu Nexera®/Prominence® LCMS-2020. Mobile phases used were solvent A (H_2O with 0.1% formic acid) and solvent B (CH_3CN with 0.1% formic acid). Gradient for routine 3-minute run: Initial composition 5% B held over 25 seconds, then increased from 5% B to 100% B over a 1 minute 35 seconds' period. The composition was held for 50 seconds at 100% B, then returned to 5% B in 5 seconds and held there for 5 seconds. The total duration of the gradient run was 3.0 minutes. Gradient for 15-minute run: Initial composition 5% B held over 1 minute, then increased from 5% B to 100% B over a 9 minute period. The composition was held for 2 minutes at 100% B, then returned to 5% B in 10 seconds and

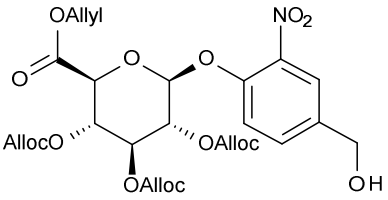
held there for 2 minutes 50 seconds. The total duration of the gradient run was 15.0 minutes. Flow rate was 0.8 mL/minute (for 3-minute run) and 0.6 mL/minute (for 15-minute run). Detection was at 254 nm. Columns: Waters Acquity UPLC® BEH Shield RP18 1.7µm 2.1 x 50 mm at 50 °C fitted with Waters Acquity UPLC® BEH Shield RP18 VanGuard Pre-column, 130A, 1.7µm, 2.1 mm x 5 mm (routine 3-minute run); and ACE Excel 2 C18-AR, 2 µ, 3.0 x 100mm fitted with Waters Acquity UPLC® BEH Shield RP18 VanGuard Pre-column, 130A, 1.7µm, 2.1 mm x 5 mm (15-minute run).

The preparative HPLC conditions were as follows: Reverse-phase ultra-fast high-performance liquid chromatography (UFLC) was carried out on a Shimazdzu Prominence® machine using a Phenomenex® Gemini NX 5µ C18 column (at 50 °C) dimensions: 150 x 21.2 mm. Eluents used were solvent A (H_2O with 0.1% formic acid) and solvent B (CH_3CN with 0.1% formic acid). All UFLC experiments were performed with gradient conditions: Initial composition 13% B increased to 60% B over a 15 minute period then increased to 100% B over 2 minutes. The composition was held for 1 minute at 100% B, then returned to 13% B in 0.1 minute and held there for 1.9 minutes. The total duration of the gradient run was 20.0 minutes. Flow rate was 20.0 mL/minute and detection was at 254 and 280 nm.

Synthesis of key intermediates

(a) Compound 5a

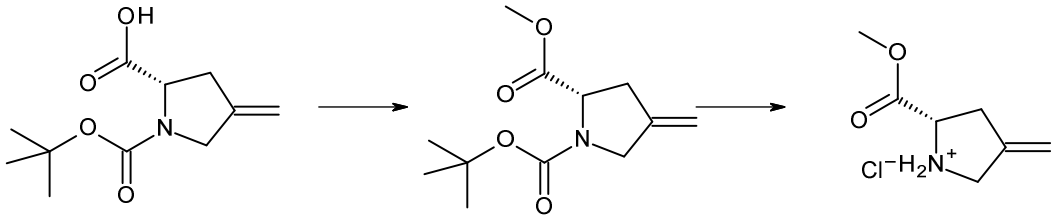
The following compound 5a:



5a

was synthesised as described (for compound 10) in Grinda, M., *et al.*, *ChemMedChem* 2011 (5), 2137-2141 (DOI: 10.1002/cmdc.201100355).

(a) (S)-2-(methoxycarbonyl)-4-methylenepyrrolidinium chloride (I3)



I1 **I2** **I3**

Commercially available proline derivative (**I1**) was obtained from Omegachem

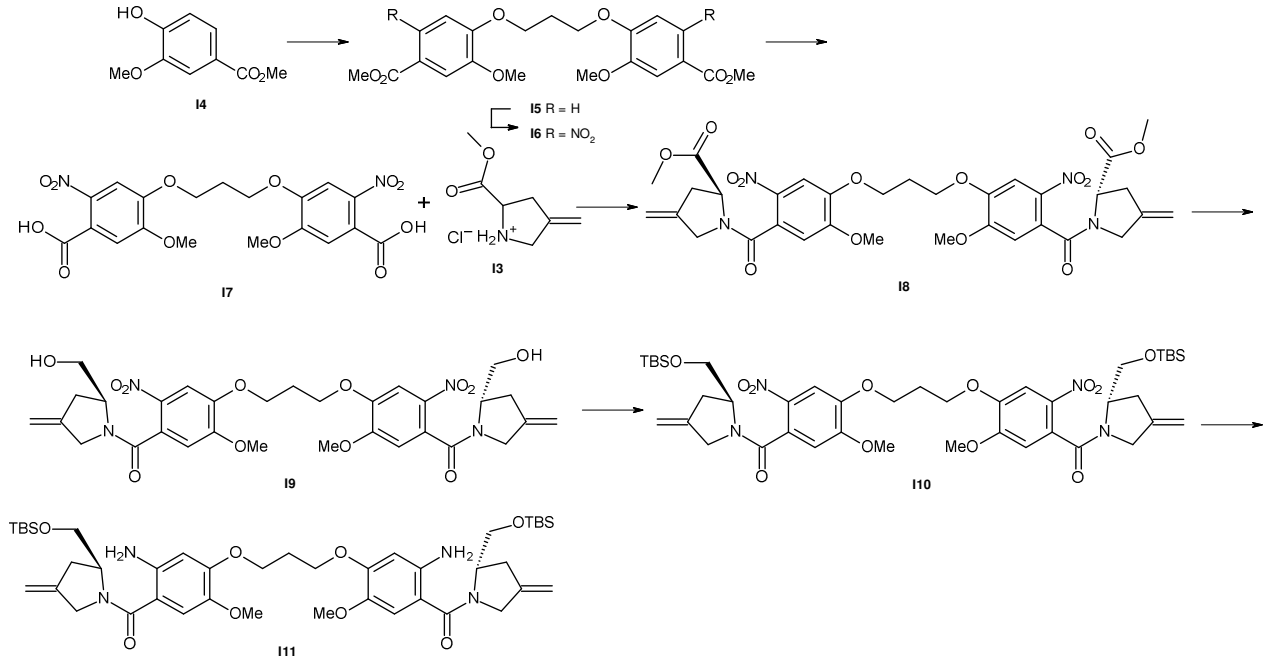
*(i) (**S**)-1-tert-butyl 2-methyl 4-methylenepyrrolidine-1,2-dicarboxylate (**I2**)*

Potassium carbonate (19.92 g, 14 mmol, 3.0 eq.) was added to a stirred solution of the carboxylic acid **I1** (10.92 g, 48 mmol, 1.0 eq.) in DMF (270 mL). The resulting white suspension was stirred at room temperature for 30 mins, at which point iodomethane (21.48 g, 9.5 mL, 151 mmol, 3.15 eq.) was added. The reaction mixture was allowed to stir at room temperature for 3 days. The DMF was removed by rotary evaporation under reduced pressure to afford a yellow residue which was partitioned between ethylacetate and water. The organic layer was separated and the aqueous phase was extracted with ethylacetate. The combined organic layers were washed with water brined and dried over magnesium sulphate. The ethylacetate was removed by rotary evaporation under reduced pressure to give the crude product as a yellow oil. The crude product was purified by flash chromatography [85% n-hexane/15% ethylacetate] to afford the product as a colourless oil (10.74 g, 93%).

*(ii) (**S**)-2-(methoxycarbonyl)-4-methylenepyrrolidinium chloride (**I3**)*

A solution of 4 M hydrochloric acid in dioxane (63 mL, 254.4 mmol, 4.5 eq.) was added to the Boc protected C-ring fragment **I2** (13.67 g, 56.6 mmol, 1.0 eq.) at room temperature. Effervescence was observed indicating liberation of CO_2 and removal of the Boc group. The product precipitated as a white solid and additional dioxane was added to facilitate stirring the reaction mixture was allowed to stir for an hour and then diluted with diethyl ether. The precipitated product was collected by vacuum filtration and washed with additional diethyl ether. Air drying afforded the desired product as a white powder (9.42 g, 94%).

(b) *((Propane-1,3-diylbis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidin-1-yl)methanone)* (**I11**)



*(i) 1',3'-Bis[2-methoxy-4-(methoxycarbonyl)phenoxy]propane **(I5)***

Diisopropyl azodicarboxylate (71.3 mL, 73.2 g, 362 mmol) was added drop-wise over a period of 60 min to an overhead stirred solution of methyl vanillate **I4** (60 g, 329 mmol) and Ph_3P (129.4 g, 494 mmol) in anhydrous THF (800 mL) at 0-5 °C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to stir at 0-5 °C for an additional 1 h after which time a solution of 1,3-propanediol (11.4 mL, 12.0 g, 158 mmol) in THF (12 mL) was added drop-wise over a period of 20 min. The reaction mixture was allowed to warm to room temperature and stirred for 5 days. The resulting white precipitate **I3** was collected by vacuum filtration, washed with THF and dried in a vacuum desiccator to constant weight. Yield = 54.68 g (84% based on 1,3-propanediol). Analytical Data: Purity satisfactory by LC/MS 3.20 min (ES+) *m/z* (relative intensity) 427 ($[M + Na]^{+\cdot}$, 10); ^{1}H NMR

(400 MHz, $CDCl_3$) δ δ7.64 (dd, 2H, *J* = 1.8, 8.3 Hz), 7.54 (d, 2H, *J* = 1.8 Hz), 6.93 (d, 2H, *J* = 8.5 Hz), 4.30 (t, 4H, *J* = 6.1 Hz), 3.90 (s, 6H), 3.89 (s, 6H), 2.40 (p, 2H, *J* = 6.0 Hz).

*(ii) 1',3'-Bis[2-methoxy-4-(methoxycarbonyl)-5-nitrophenoxy]propane (**I6**)*

Solid $Cu(NO_3)_2.3H_2O$ (81.54 g, 337.5 mmol) was added slowly to an overhead stirred slurry of the *bis*-ester **I5** (54.68 g, 135 mmol) in acetic anhydride (650 mL) at 0-5 °C (ice/acetone). The reaction mixture was allowed to stir for 1 h at 0-5 °C and then allowed to warm to room temperature. A mild exotherm (*c.* 40-50 °C), accompanied by thickening of the mixture and evolution of NO_2 was observed at this stage. Additional acetic anhydride (300 mL) was added and the reaction mixture was allowed to stir for 16 h at room temperature. The reaction mixture was poured onto ice (~ 1.5 L), stirred and allowed to return to room temperature. The resulting yellow precipitate was collected by vacuum filtration and dried in a desiccator to afford the desired *bis*-nitro compound **I6** as a yellow solid. Yield = 66.7 g (100%). Analytical Data: Purity satisfactory by LC/MS 3.25 min (ES+) *m/z* (relative intensity) 517 ([*M* + Na]$^{+\cdot}$, 40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 2H), 7.06 (s, 2H), 4.32 (t, 4H, *J* = 6.0 Hz), 3.95 (s, 6H), 3.90 (s, 6H), 2.45-2.40 (m, 2H). See ref Thurston 1996.

*(iii) 1',3'-Bis(4-carboxy-2-methoxy-5-nitrophenoxy) propane (**I7**)*

A slurry of the methyl ester **I6** (66.7 g, 135 mmol) in THF (700 mL) was treated with 1N NaOH (700 mL) and the reaction mixture was allowed to stir vigorously at room temperature. After 4 days stirring, the slurry became a dark coloured solution which was subjected to rotary evaporation under reduced pressure to remove THF. The resulting aqueous residue was acidified to pH 1 with concentrated HCl and the colourless precipitate **I7** was collected and dried thoroughly in a vacuum oven (50 °C). Yield = 54.5 g (87%). Analytical Data: Purity satisfactory by LC/MS 2.65 min (ES+) *m/z* (relative intensity) 489 ([*M* + Na]$^{+\cdot}$, 30); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (s, 2H), 7.30 (s, 2H), 4.29 (t, 4H, *J* = 6.0 Hz), 3.85 (s, 6H), 2.30-2.26 (m, 2H).

*(iv) Dimethyl 1,1'-(4,4'-(propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitrobenzoyl))(2**S**,2'**S**)-bis(4-methylenepyrrolidine-2-carboxylate) (**I8**)*

A catalytic amount of anhydrous DMF (2.4 mL) was added to a stirred suspension of oxalyl chloride (14.7 g, 9.8 mL, 115.8 mmol, 3 eq.) and dimer core **I7** (18 g, 38.6 mmol, 1 eq.) in anhydrous DCM (500 mL) at room temperature. Vigorous effervescence was observed after the addition of DMF and the reaction mixture was allowed to stir for 18 h in a round bottom flask fitted with a calcium chloride drying tube. The resulting clear solution was

evaporated under reduced pressure and the solid triturated with ether. The solid product was collected by vacuum filtration, washed with additional ether and dried *in vacuo* at 40 °C for 1.5 h. This solid was then added portion wise to a suspension of the C-ring **3** (15.1 g, 84.9 mmol, 2.2 eq.) and TEA (19.5 g, 27 ml, 119.6 mmol, 5 eq.) in dry DCM (375 mL), maintaining the temperature between -40 and -50 °C with the aid of a dry ice/acetonitrile bath. The reaction mixture was allowed to stir at -40 °C for 1h and then allowed to warm to room temperature at which point LCMS indicated the complete consumption of the starting material. The reaction mixture was diluted with additional DCM and washed sequentially with aqueous hydrochloric acid (1M, 2 x 200 mL), saturated aqueous sodium bicarbonate (2 x 250 mL), water (250 mL), brine (250 mL), dried ($MgSO_4$). DCM was removed by rotary evaporation under reduced pressure to afford the product as a yellow foam (25.72 g, 94 %). Analytical Data: RT 1.59 min; MS (ES^+) *m/z* (relative intensity) 713 ($[M + H]^{+\cdot}$, 100)

*(v) ((Propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((**S**)-2-(hydroxymethyl)-4-methylenepyrrolidin-1-yl)methanone) (**I9**)*

Solid lithium borohydride (3.18 g, 146 mmol, 3 eq.) was added in one portion to a solution of the ester **I8** (34.72 g, 48.7 mmol, 1 eq.) in dry THF (350 mL) under a nitrogen atmosphere at 0 ºC (ice bath). The reaction mixture was allowed to stir at 0 °C for 30 mins and then allowed to warm to room temperature at which point precipitation of an orange gum was observed. The reaction mixture was allowed to stir at room temperature for a futher 2 hours and then cooled in an ice bath and treated with water to give a yellow suspension. Hydrochloric acid (1M) was carefully added until effervescence ceased. The reaction mixture was extracted with ethylacetate (x 4) and the combined organic layers were washed with water (x 1), brine (x 1) and dried ($MgSO_4$). Ethylacetate was removed by rotary evaporation under reduced pressure to give a yellow foam. Purification by flash column chromatography [gradient elution DCM/MeOH 0% to 5% in 1% increments] gave the product as a pale yellow foam (23.1 g, 72%). Analytical Data: RT 1.23 min; MS (ES^+) *m/z* (relative intensity) 657 ($[M + H]^{+\cdot}$, 100)

*(vi) ((Propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidin-1-yl)methanone) (**I10**)*

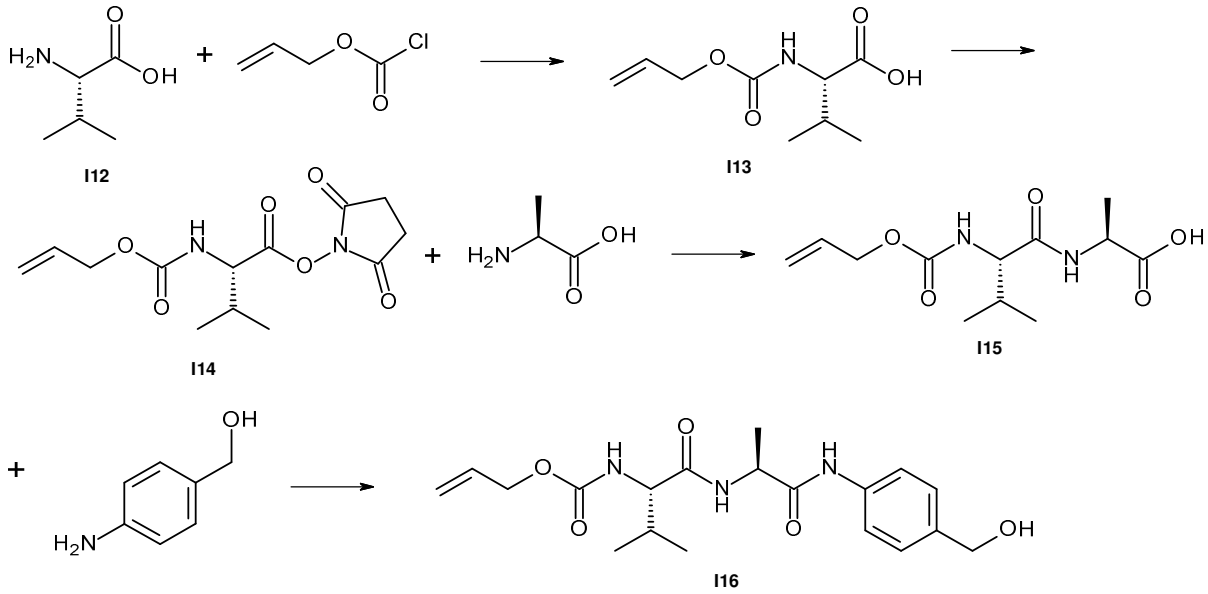
A solution of the *bis*-alcohol **I9** (10 g, 15.2 mmol, 1 eq.), *t*-butyldimethylsilylchloride (5.97 g, 39.6 mmol, 2.6 eq.) and imidazole (5.38 g, 79 mmol, 5.2 eq.) in dry DMF (80 ml) was stirred at room temperature for 3h. The reaction mixture was poured into water (500 mL) to give a yellow precipitate. The mixture was extracted with DCM (4 x 100 mL) and the combined extracts were washed with water and brine, dried ($MgSO_4$) and evaporated

under reduced pressure to give a viscous yellow oil. Purification by column chromatography [biotage isolera, gradient elution hexane 60%/EtOAc 40% to EtOAc 100%, 8 column volumes 100 g snap ultra® cartridge] gave the product as a yellow foam (11.8 g, 88%). Analytical Data: RT 2.20 min; MS (ES^+) *m/z* (relative intensity) 885 ($[M + H]^{+\cdot}$, 100), 907 ($[M + Na]^{+\cdot}$, 50)

*(vii) ((Propane-1,3-diylbis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidin-1-yl)methanone) (**I11**)*

Zinc powder (31.9 g, 488 mmol, 40 eq.) was activated by stirring/sonication with 1M HCl for 10 min. The Zinc was filtered washing with 1M HCl, water (x 3) and MeOH (x 2). The activated Zinc was added to a solution of the nitro-TBS compound **I10** (10.8 g, 12.2 mmol, 1 eq.) in MeOH (88 mL) and 5% formic acid/MeOH solution (440 mL). The temperature rose to 37°C and the reaction mixture changed from a yellow to a colourless solution. Once the exotherm had subsided (20 min.) the reaction was shown to be complete by LCMS. The reaction mixture was filtered through celite washing with EtOAc. The EtOAc portion was washed with saturated bicarbonate solution (x 4) [caution effervescence!], water (x 1), brine (x 1), dried (MgSO4) and evaporated under reduced pressure to give a yellow solid. Purification by flash column chromatography [*n*-hexane/EtOAc 50/50 v/v to EtOAc 100% in 10% increments] gave the product as a yellow foam (9.5 g, 86%). Analytical Data: RT 2.12 min; MS (ES^+) *m/z* (relative intensity) 825 ($[M + H]^{+\cdot}$, 60), 847 ($[M + Na]^{+\cdot}$, 30)

(c) Alloc-Val-Ala-PABOH (**I16**)



*(i) Alloc-Val-OH (**I13**)*

Allyl chloroformate (41 g, 36.2 mL, 0.34 mol, 1.2 eq.) was added dropwise to a stirred solution of L-valine **I12** (33.25 g, 0.28 mol, 1 eq.) and potassium carbonate (58.9 g, 0.426

mol, 1.5 eq.) in water (650 mL) and THF (650 mL). The reaction mixture was stirred at room temperature for 18h. The THF was evaporated under reduced pressure and the remaining solution was extracted with diethyl ether (or MTBE) (x 2). The aqueous portion was acidified to pH 2 with conc. HCl and extracted with DCM (x 3). The combined organic extracts were washed with brine (x 1), dried ($MgSO_4$) and evaporated under reduced pressure to give a colourless oil (57.1 g). This was used in the next step without further purification.

*(ii) Alloc-Val-OSu (**I14**)*

To a stirred solution of compound **I13** (57.1 g, 0.28 mol, 1 eq.) and N-hydroxysuccinimide (32.68 g, 0.28 mol, 1 eq.) in dry THF (800 mL) was added dicyclohexylcarbodiimide (58.6 g, 0.28 mol, 1 eq.). The reaction mixture was stirred at room temperature for 18h. The reaction mixture was filtered. The solid was washed with THF and the combined filtrate was concentrated under reduced pressure. The oil/solid residue was re-dissolved in DCM and left to stand at 0 °C for 30 min. The suspension was filtered washing with cold DCM. Evaporation of the filtrate under reduced pressure gave the succinimide ester as a white solid which was used in the next step without further purification.

*(iii) Alloc-Val-Ala-OH (**I15**)*

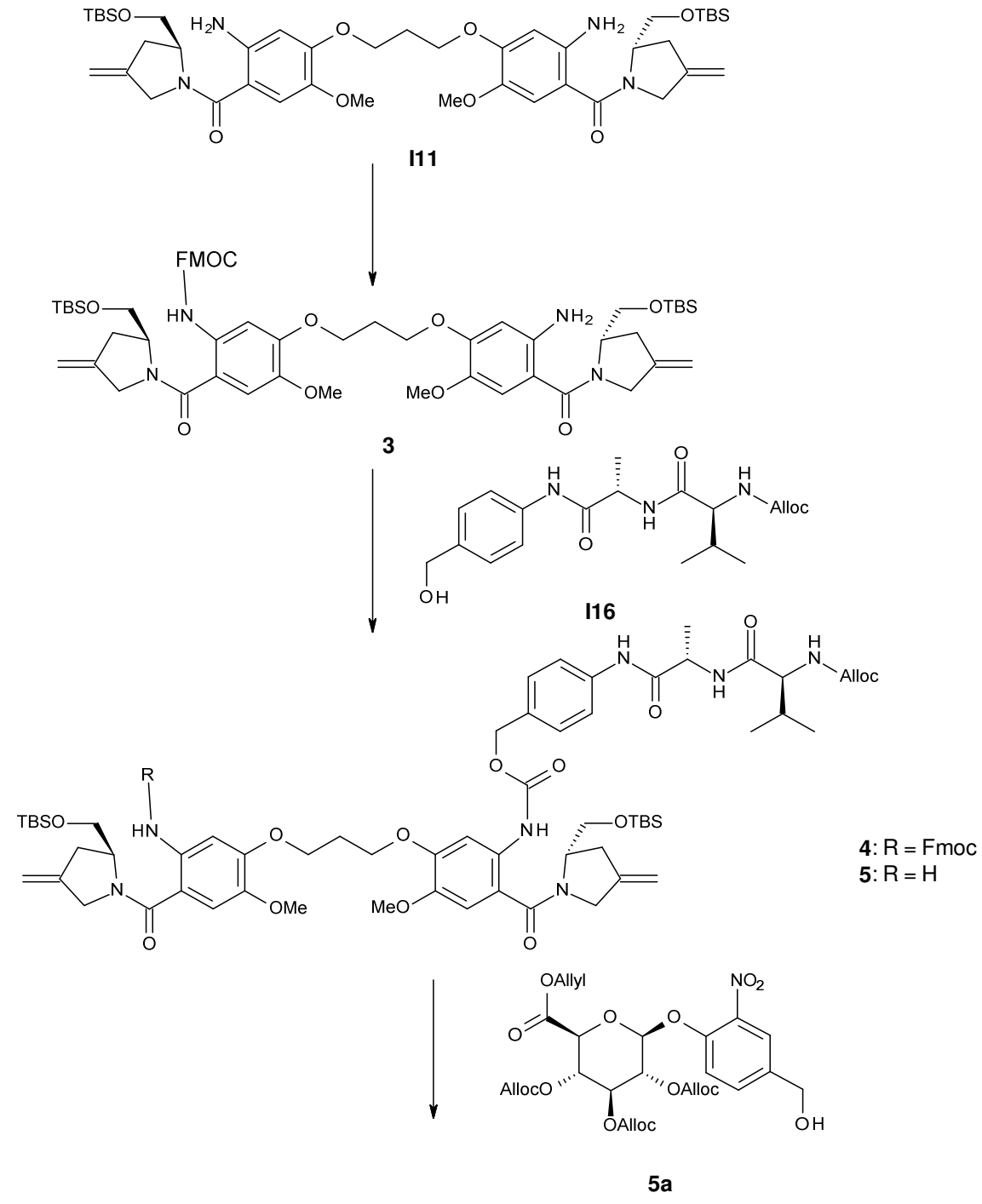
A solution of Alloc-Val-OSu **I14** (11.67 g, 39.0 mmol, 1 eq.) in THF (50 mL) was added to a solution of H-Ala-OH (3.66 g, 41.08 mmoL, 1.05 eq.) and $NaHCO_3$ (3.61 g, 43.03 mmol, 1.1 eq.) in THF (100 mL) and H_2O (100 mL). The mixture was stirred at room temperature for 72h and the THF was evaporated under reduced pressure. The pH was adjusted to 3 - 4 with citric acid to precipitate a white gum. This was extracted with ethylacetate (6 x 150 mL) and the combined extracts were washed with H_2O (200 mL), brine (200 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. Trituration with diethyl ether (xs) afforded the pure product as a white powder (7.93 g, 74%). Analytical Data: RT 2.17 min; MS (ES^+) *m/z* (relative intensity) 295 ($[M + Na]^{+\cdot}$, 63), 273 ($[M + 1]^{+\cdot}$, 60).

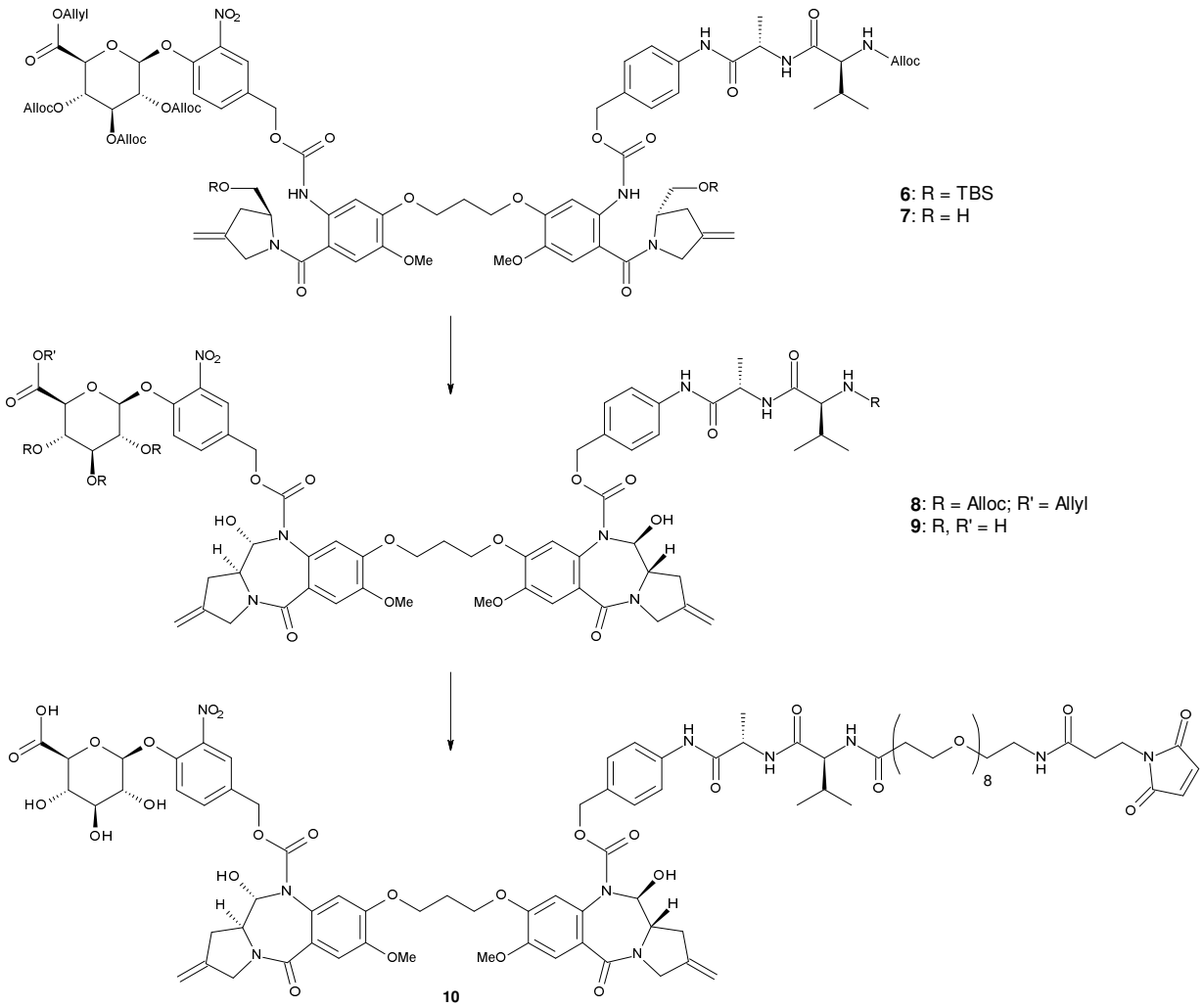
*(iv) Alloc-Val-Ala-PABOH (**I16**)*

EEDQ (4.79 g, 19.3 mmol, 1.05 eq.) was added to a solution of *p*-aminobenzyl alcohol (2.38 g, 19.3 mmol, 1.05 eq.) and Alloc-Val-Ala-OH **I15** (5.02 g, 18.4 mmol, 1.0eq) in dry THF (100 mL). The mixture was stirred at room temperature for 72h. The solvent was evaporated under reduced pressure to give a pale brown solid. The solid was triturated with diethyl ether and filtered washing with an excess of diethyl ether. This afforded the

product as a white solid (6.2 g, 89%). Analytical Data: RT 2.50 min; MS (ES^+) *m/z* (relative intensity) 400.6 ($[M + Na]^{+\cdot}$, 50), 378.6 ($[M + 1]^{+\cdot}$, 60).

Example 1


TBSO
H2N
O
O
NH2
OTBS
N
OMe
MeO
N
O
O
I11
FMOC
HN
TBSO
NH2
OTBS
OMe
MeO
3
H
N
O
H
N
Alloc
OH
I16
R
HN
TBSO
OTBS
NH
OMe
MeO
4: R = Fmoc
5: R = H
OAllyl
NO2
AllocO
OAlloc
OAlloc
OH
5a



*(a) (9H-Fluoren-9-yl)methyl (5-(3-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**3**)*

FmocCl (1.97 g, 7.63 mmol) was added to a stirred mixture of the *bis*-aniline **I11** (6.99 g, 8.48 mmol) and Na_2CO_3 (2.25 g, 21.2 mmol) in THF (65 mL) and H_2O (65 mL). The mixture was allowed to stir room temperature for 16 hours where analysis by LC/MS revealed desired mono Fmoc product **3** at retention time 2.34 minutes, I% = 40, ES+ *m*/*z* 1069 [*M*+ Na]$^{+\cdot}$, 1047 [*M*+ H]$^{+\cdot}$ along with unreacted starting material at retention time 2.15 minutes, I% = 12, and *bis*-Fmoc material at retention time 2.50 minutes, I% = 45, ES+ *m*/*z* 1291 [*M*+ Na]$^{+\cdot}$, 1269 [*M*+ H]$^{+\cdot}$. The layers were separated and the aqueous phase extracted with DCM (2 x 20 mL) and the combined organic layers washed with H_2O (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 50 g, 100 mL per minute) provided the pure mono Fmoc product **3** as an orange foam (2.67 g, 39% yield based on FmocCl, eluting at

50% Hexane/EtOAc) unreacted *bis*-aniline **2** (1.78 g, eluting at 100% EtOAc) and *bis*-Fmoc (3.28 g, eluting at 70% Hexane/EtOAc).

*(b) (9H-Fluoren-9-yl)methyl (5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**4**)*

Triphosgene (26 mg, 86 µmol) was added to a stirred solution of the mono Fmoc product **3** (250 mg, 0.24 mmol) and TEA (73 µL, 53 mg, 0.53 mmol) in dry DCM (10 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.39 minutes, ES+ *m*/*z* 1127 [*M*+ Na]$^{+\cdot}$, 1105 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional TEA (50 µL, 36 mg, 0.36 mmol) followed by the addition of linker **I16** (90 mg, 0.24 mmol). After 4 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **4** at retention time 2.34 minutes, ES+ *m*/*z* 1472 [*M*+ Na]$^{+\cdot}$, 1451 [*M*+ H]$^{+\cdot}$. The mixture was diluted with DCM (60 mL) and washed with saturated aqueous NH_4Cl (2 x 20 mL), H_2O (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 25 g, 75 mL per minute) provided the pure carbamate **4** (eluting at 28% Hexane/EtOAc) as a yellow foam (257 mg, 74% yield).

*(c) 4-((S)-2-((S)-2-(((Allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-(3-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**5**)*

Dimethylamine (16.5 mL of a 2.0M solution in THF, 33.0 mmol) was added to a stirred solution of the Fmoc protected compound **4** (2.39 g, 1.65 mmol) in THF (46 mL) at room temperature. After stirring for 1 hour at room temperature, analysis by LC/MS revealed reaction completion with desired product at retention time 2.17 minutes, ES+ *m*/*z* 1250 [*M*+ Na]$^{+\cdot}$, 1228 [*M*+ H]$^{+\cdot}$, along with Fmoc cleavage by-product at retention time 1.92 minutes and its DMA adduct at retention time 1.10 minutes. The mixture was evaporated *in vacuo* to give the crude product which was subsequently purified by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) to provide the pure aniline **5** (eluting at 100% EtOAc) as an orange foam (1.87 g, 92% yield).

*(d) Allyl (2S,3S,4S,5R,6S)-6-(4-((((5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**6**)*

Triphosgene (161 mg, 0.54 mmol) was added to a stirred solution of the aniline **5** (1.85 g, 1.51 mmol) and pyridine (269 µL, 263 mg, 3.32 mmol) in dry DCM (20 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.21 minutes, ES+ *m*/*z* 1308 $[M+ Na]^{+\cdot}$, 1286 $[M+ H]^{+\cdot}$). The mixture was treated with additional pyridine (183 µL, 179 mg, 2.27 mmol) and dibutyltin dilaurate (179 µL, 191 mg, 0.30 mmol) followed by the addition of linker **5a** (960 mg, 1.51 mmol). After 20 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **6** (retention time 2.25 minutes, ES+ *m*/*z* 1915 $[M+ Na]^{+\cdot}$, 1892 $[M+ H]^{+\cdot}$). The mixture was diluted with DCM (60 mL) and washed with saturated NH_4Cl (2 x 20 mL), H_2O (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **6** (eluting at 42% Hexane/EtOAc) as a yellow foam (2.01 g, 70% yield).

*(e) Allyl (2S,3S,4S,5R,6S)-6-(4-((((5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**7**)*

Glacial acetic acid (18 mL) was added to a stirred solution of the TBS-protected compound **6** (2.12 g, 1.12 mmol) in THF (6 mL) and H_2O (6 mL). The reaction mixture was allowed to stir for 18 hours at room temperature after which time analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.79 minutes, ES+ *m*/*z* 1663 $[M+ H]^{+\cdot}$, 1686 $[M+ Na]^{+\cdot}$. The reaction mixture was added drop-wise to a chilled (0–5 °C) saturated solution of $NaHCO_3$ (300 mL). The neutral solution was allowed to warm to room temperature and extracted with EtOAc (3 x 100 mL), the combined organic layers were washed with H_2O (50 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-alcohol **7** (eluting at 93% DCM/MeOH) as a yellowish foam (1.43 g, 77% yield).

*(f) 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl (11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine 10(5H)-carboxylate (**8**)*

45% IBX (264 mg, 0.43 mmol) was added to a stirred solution of the *bis*-OH **7** (300 mg, 0.18 mmol) in dry DMSO (5 mL). The mixture was heated to 30 °C under an argon atmosphere and reaction progress monitored by LC/MS. After 24 hours stirring, additional IBX (22 mg, 36 µmol) was added and the mixture stirred for another 24 hours. At this point analysis by LC/MS revealed predominantly a single peak corresponding to desired product at retention time 1.78 minutes, ES+ *m*/*z* 1682 $[M+ Na]^{+\cdot}$. The reaction mixture was poured onto water (40 mL), filtered and the precipitate collected, washed with H_2O (50 mL) and the filtrate kept to one side. The residue (precipitate) was washed with DCM (120 mL) and the remaining undissolved precipitate washed with saturated aqueous $NaHCO_3$ (60 mL). The combined filtrates were transferred to a separating funnel and the layers separated. The aqueous phase was extracted with DCM (2 x 20 mL) and the combined organic layers washed with $NaHCO_3$ (2 x 50 mL), brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 25 g, 75 mL per minute) gave the cyclised product **8** (eluting at 94.5% DCM/MeOH) as a white foam (261 mg, 87% yield).

*(g) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**9**)*

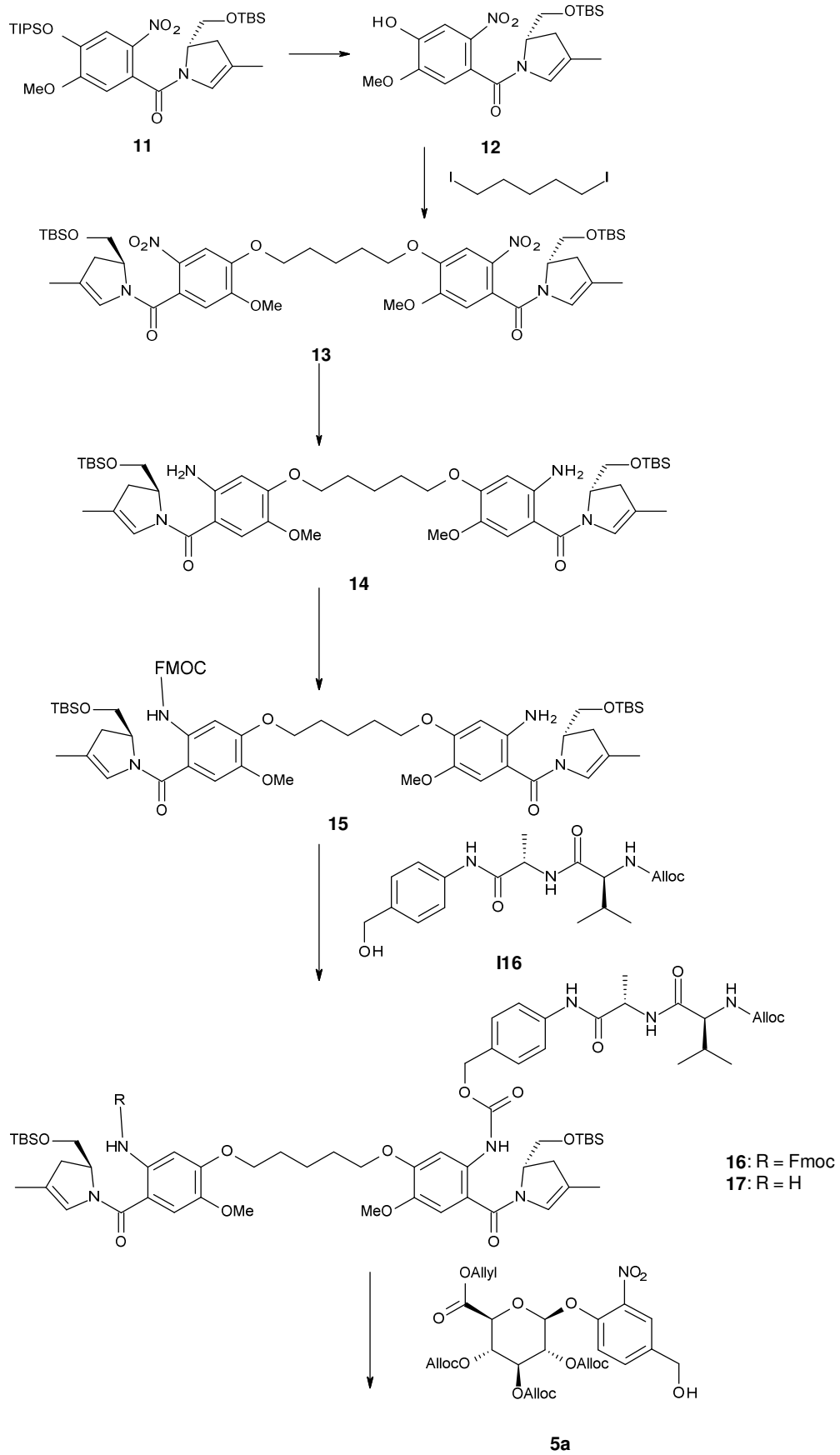
$Pd(PPh_3)_4$ (18.2 mg, 15.7 µmol) was added to a stirred solution of pyrrolidine (72 µL, 62 mg, 0.87 mmol) and the Alloc/allyl compound **8** (261 mg, 0.16 mmol) in dry DCM (3 mL). The reaction mixture was allowed to stir under an argon atmosphere where a precipitate began to form. After stirring for 30 minutes at room temperature analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.13 minutes, ES+ *m*/*z* 1284 $[M+ H]^{+\cdot}$. The solvent was removed by evaporation *in vacuo* and the resulting residue triturated with diethyl ether followed by additional evaporation *in vacuo* to

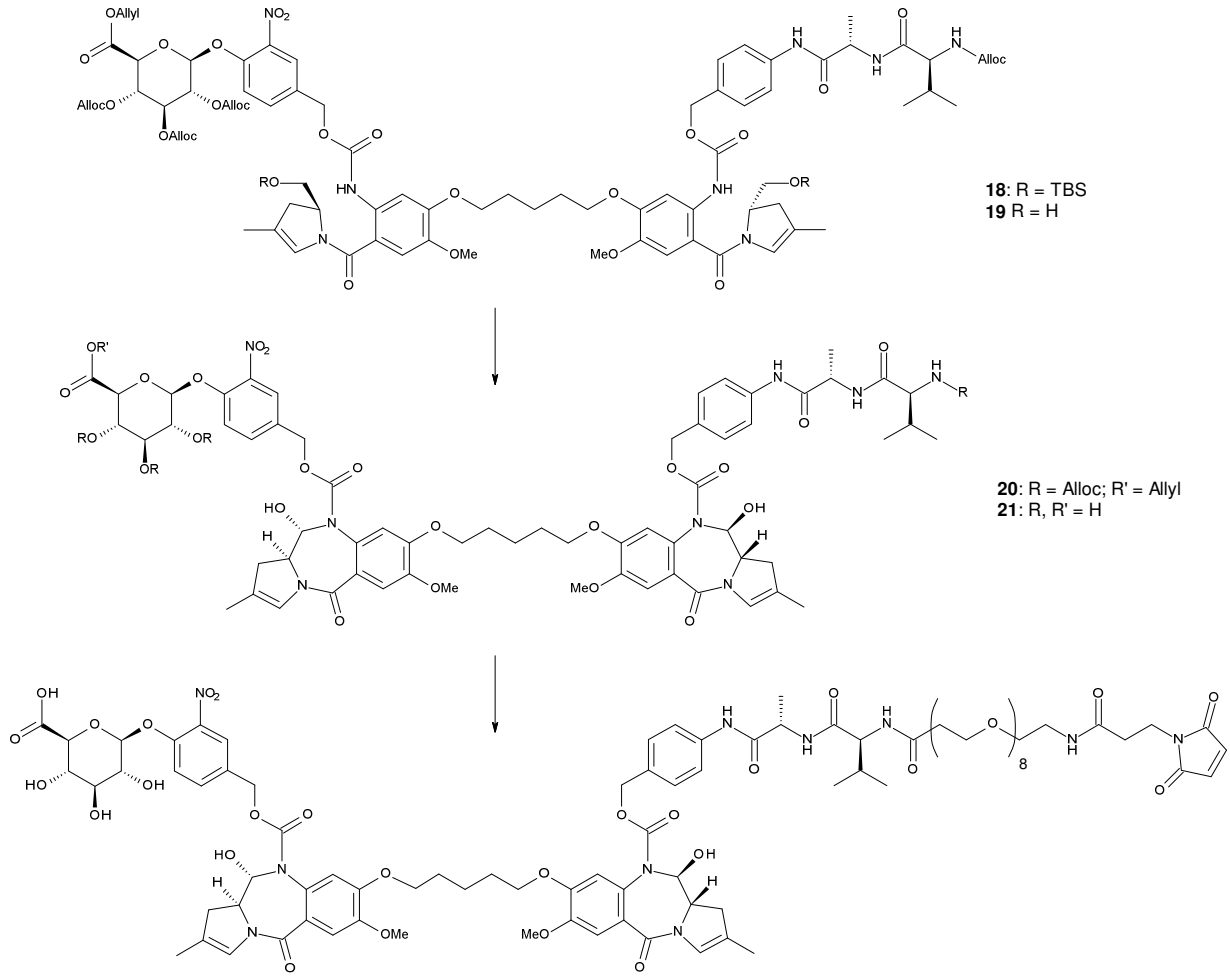
provide the crude amine **9** as a solid which was carried through to the next step without further purification or analysis.

*(h) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**10**)*

A solution of MAL-dPEG®8-TFP ester (132 mg, 0.18 mmol) in dry DCM (7 mL) was added to a stirred sample of the amine **9** (202 mg, 0.16 mmol), pyridine (24 µL, 23 mg, 0.3 mmol) and DMF (0.25 mL). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC/MS revealed formation of desired product at retention time 1.38 minutes, ES+) *m*/*z* 1857 $[M+H]^{+\cdot}$, 1880 $[M+Na]^{+}$, along with unreacted amine at retention time 1.13 minutes. Additional MAL-dPEG®8-TFP ester (66 mg, 0.09 mmol), pyridine (12 µL, 11 mg, 0.15 mmol) and DCM (3 mL) were added and the mixture allowed to stir for a further three days. At this point a satisfactory amount of desired product had formed upon analysis by LC/MS and the solvent was removed by evaporation *in vacuo*. The crude product was purified by preparative HPLC to give the maleimide **10** as a white solid (55 mg, 19%): LC/MS (15-minute run), retention time 5.93 minutes, ES+ *m*/*z* 1857 $[M+H]^{+\cdot}$, 1880 $[M+Na]^{+}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 10.0 (br s, 2H), 8.25–8.12 (m, 1H), 7.99 (t, 1H, *J* = 5.7 Hz), 7.87 (d, 1H, *J* = 8.9 Hz), 7.73 (br s, 1H), 7.62–7.48 (m, 3H), 7.40 (d, 1H, *J* = 8.7 Hz), 7.22–7.11 (m, 2H), 7.06 (s, 2H), 7.00 (s, 2H), 6.87 (s, 2H), 6.84–6.76 (m, 1H), 6.68–6.52 (m, 2H), 5.45–5.30 (m, 3H), 5.25–5.01 (m, 9H), 4.97–4.82 (m, 2H), 4.42–4.34 (m, 1H), 4.24–4.17 (m, 1H), 4.16–3.92 (m, 8H), 3.87–3.72 (m, 6H), 3.60 (t, 4H, *J* = 7.2 Hz), 3.54–3.42 (m, 30H), 3.39–3.20 (m, 5H), 3.18–3.12 (m, 2H), 2.95–2.82 (m, 2H), 2.57–2.35 (m, 4H), 2.33 (t, 2H, *J* = 7.4 Hz), 2.24–2.13 (m, 2H), 2.03–1.90 (m, 1H), 1.30 (d, 1H, *J* = 7.0 Hz), 0.87 (d, 3H, *J* = 6.7 Hz), 0.83 (d, 3H, *J* = 6.7 Hz).

Example 2


TIPSO
NO2
OTBS
MeO
HO
11
12
TBSO
O2N
OMe
13
H2N
NH2
14
FMOC
HN
15
Alloc
OH
I16
R
16: R = Fmoc
17: R = H
OAllyl
AllocO
OAlloc
5a



*(a) (S)-(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)(4-hydroxy-5-methoxy-2-nitrophenyl)methanone (**12**)*

LiOAc (2.11 g, 20.7 mmol) was added to a stirred solution of the TIPS compound **11** (12 g, 20.7 mmol) in DMF (67 mL) and H_2O (3 mL) at room temperature. A colour change from yellow to red was observed. After 2 hours stirring at room temperature analysis by LC/MS revealed complete conversion to desired product at retention time 1.83 minutes, ES+ *m*/*z* 445 $[M+ Na]^{+\cdot}$, 423 $[M+ H]^{+\cdot}$. The mixture was diluted with H_2O (300 mL) and extracted with EtOAc (3 x 150 mL). The combined organic layers were washed 10% *w/v* citric acid (2 x 200 mL), H_2O (200 mL), brine (200 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product **12** as a yellow solid (8.74 g, 100% yield). Purity satisfactory, so material carried through to next step without further purification.

*(b) ((Pentane-1,5-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone) (**13**)*

1,5-diiodopentane (1.54 mL, 3.35 g, 10.4 mmol) was added to a stirred solution of the phenol **12** (8.74 g, 20.7 mmol), TBAI (750 mg, 2.05 mmol) and K_2CO_3 (3.15 g, 22.8 mmol) in dry DMF (60 mL). The reaction mixture was heated to 70 °C and stirred under an argon atmosphere for 16 hours at which point analysis by LC/MS revealed substantial product formation at retention time 2.21 minutes, ES+ *m*/*z* 935 $[M+ Na]^{+\cdot}$, 913 $[M+ H]^{+\cdot}$. The reaction mixture was allowed to cool to room temperature and the DMF was removed by evaporation *in vacuo*. The resulting residue was redissolved in EtOAc (200 mL) and the aqueous phase was washed with water (3 x 40 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-ether **13** (eluting at 60% Hexane/EtOAc) as a yellow foam (6.75 g, 71% yield).

*(c) ((Pentane-1,5-diylbis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone) (**14**)*

Zinc dust (2.79 g, 42.8 mmol) was treated with 1N HCl (50 mL) and stirred for 10 minutes at room temperature. The mixture was then sonicated for 10 minutes and the activated Zinc collected by sinter vacuum filtration then washed with 1N HCl (25 mL), H_2O (20 mL), MeOH (20 mL) and dried *in vacuo* on the sinter filter pad. The activated zinc was added to a vigorously stirred solution of the *bis*-nitro compound **13** (974 mg, 1.07 mmol) in MeOH (15 mL) at room temperature. The reaction mixture was treated drop-wise with a solution of 5% *v/v* HCO_2H in MeOH (20 mL). A colour change from green to metallic grey and an exotherm to 37 °C were observed. The reaction mixture was allowed to cool to room temperature at which point analysis by LC/MS revealed complete conversion to desired product at retention time 2.19 minutes, ES+ *m*/*z* 875 $[M+ Na]^{+\cdot}$, 853 $[M+ H]^{+\cdot}$. The mixture was filtered through celite® and the pad washed with EtOAc (75 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (3 x 20 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude *bis*-aniline **14** as a dark orange foam (840 mg, 92% yield). Purity satisfactory, so material carried through to next step without further purification.

*(d) (9H-fluoren-9-yl)methyl(5-((5-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**15**)*

FmocCl (1.50 g, 5.93 mmol) was added to a stirred suspension of the *bis*-aniline **14** (6.74 g, 7.91 mmol) and Na_2CO_3 (2.10 g, 19.8 mmol) in THF (40 mL) and H_2O (40 mL). The

mixture was allowed to stir at room temperature for 16 hours where analysis by LC/MS revealed desired mono Fmoc product **15** at retention time 2.41 minutes, I% = 32, ES+ *m*/*z* 1098 [*M*+ Na]$^{+\cdot}$, 1076 [*M*+ H]$^{+\cdot}$ along with unreacted starting material at retention time 2.22 minutes, I% = 30, and *bis*-Fmoc material at retention time 2.60 minutes, I% = 36, ES+ *m*/*z* 1321 [*M*+ Na]$^{+\cdot}$, 1298 [*M*+ H]$^{+\cdot}$. The mixture was partitioned between H_2O (50 mL) and EtOAc (50 mL), the layers were separated and the organic layer washed with H_2O (30 mL), brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure mono Fmoc product **15** as an orange foam (2.43 g, 38% yield based on FmocCl, eluting at 58% Hexane/EtOAc) unreacted *bis*-aniline **14** (3.06 g, eluting at 40% Hexane/EtOAc) and *bis*-Fmoc (2.32 g, eluting at 74% Hexane/EtOAc).

*(e) (9H-fluoren-9-yl)methyl(5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**16**)*

Triphosgene (242 mg, 0.81 mmol) was added to a stirred solution of the mono Fmoc product **15** (2.43 g, 2.26 mmol) and TEA (692 µL, 502 mg, 4.97 mmol) in dry DCM (30 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.38 minutes, ES+ *m*/*z* 1156 [*M*+ Na]$^{+\cdot}$, 1133 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional TEA (472 µL, 342 mg, 3.39 mmol) followed by the addition of linker **I16** (852 mg, 2.26 mmol). After 3.5 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **16** at retention time 2.33 minutes, ES+ *m*/*z* 1501 [*M*+ Na]$^{+\cdot}$, 1479 [*M*+ H]$^{+\cdot}$. The mixture was diluted with DCM (50 mL) and washed with saturated aqueous NH_4Cl (2 x 20 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **16** (eluting at 46% Hexane/EtOAc) as a yellow foam (2.26 g, 68% yield).

*(f) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-((5-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**17**)*

Dimethylamine (15.0 mL of a 2.0M solution in THF, 30.6 mmol) was added to a stirred solution of the Fmoc protected compound **16** (2.26 g, 1.65 mmol) in THF (20 mL) at room temperature. After stirring for 1 hour at room temperature, analysis by LC/MS revealed reaction completion with desired product at retention time 2.17 minutes, ES+ *m*/*z* 1279 [*M*+ Na]$^{+\cdot}$, 1257 [*M*+ H]$^{+\cdot}$, along with Fmoc cleavage by-product at retention time 1.79 minutes and its DMA adduct at retention time 0.97 minutes. The mixture was evaporated *in vacuo* to give the crude product which was subsequently purified by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) to provide the pure aniline **17** (eluting at 18% Hexane/EtOAc) as an orange foam (1.94 g, 100% yield).

*(g) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**18**)*

Triphosgene (165 mg, 0.55 mmol) was added to a stirred solution of the aniline **17** (1.94 g, 1.54 mmol) and pyridine (274 µL, 268 mg, 3.39 mmol) in dry DCM (20 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.21 minutes, ES+ *m*/*z* 1337 [*M*+ Na]$^{+\cdot}$, 1315 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional pyridine (187 µL, 183 mg, 2.31 mmol) and dibutyltin dilaurate (179 µL, 191 mg, 0.30 mmol) followed by the addition of linker **5a** (984 mg, 1.54 mmol). After 18 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **18** at retention time 2.23 minutes, ES+ *m*/*z* 1942 [*M*+ Na]$^{+\cdot}$, 1920 [*M*+ H]$^{+\cdot}$. The mixture was diluted with DCM (30 mL) and washed with saturated NH_4Cl (2 x 20 mL), H_2O (20 mL), brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **18** (eluting at 43% Hexane/EtOAc) as a yellow foam (2.17 g, 74% yield).

*(h) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**19**)*

Glacial acetic acid (18 mL) was added to a stirred solution of the TBS-protected compound **18** (2.17 g, 1.13 mmol) in THF (6 mL) and H_2O (6 mL). The reaction mixture was allowed to stir for 18 hours at room temperature after which time analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.82 minutes, ES+ *m*/*z* 1691 [*M*+ H]$^{+\cdot}$, 1714 [*M*+ Na]$^{+\cdot}$. The reaction mixture was added drop-wise to a chilled (0–5 °C) saturated solution of $NaHCO_3$ (300 mL). The neutral solution was allowed to warm to room temperature and extracted with EtOAc (3 x 100 mL), the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-alcohol **19** (eluting at 96.5% DCM/MeOH) as a white foam (1.31 g, 69% yield).

*(i) 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl(11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**20**)*

45% IBX (269 mg, 0.43 mmol) was added to a stirred solution of the *bis*-OH **19** (300 mg, 0.18 mmol) in dry DMSO (5 mL). The mixture was heated to 30 °C under an argon atmosphere and reaction progress monitored by LC/MS. After 24 hours stirring, additional IBX (22 mg, 36 µmol) was added and the mixture stirred for another 24 hours. At this point analysis by LC/MS revealed formation of product at retention time 1.79 minutes, ES+ *m*/*z* 1710 [*M*+ Na]$^{+\cdot}$. The reaction mixture was added drop-wise to a saturated aqueous $NaHCO_3$ solution (100 mL) and extracted with DCM (2 x 75 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 x 20 mL), H_2O (30 mL), brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 25 g, 75 mL per minute) gave the cyclised product **11** (eluting at 97.4% DCM/MeOH) as a white foam (136 mg, 46% yield).

(j) Alternate synthesis of 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl(11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-

*hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**20**)*

TEMPO (6.6 mg, 42 µmol) and DAIB (125 mg, 0.39 mmol) were added to a stirred solution of the *bis*-OH **19** (300 mg, 0.18 mmol) in dry DCM (15 mL) at room temperature. The mixture was stirred under an argon atmosphere and reaction progress monitored by LC/MS. After 24 hours stirring, additional TEMPO (6.6 mg, 42 µmol) and DAIB (25 mg, 78 µmol) were added and the mixture stirred for another 24 hours. At this point analysis by LC/MS revealed formation of product at retention time 1.79 minutes, ES+ *m*/*z* 1710 $[M+Na]^{+\cdot}$. The mixture was diluted with DCM (50 mL) and washed with saturated aqueous sodium thiosulfate (40 mL), saturated aqueous $NaHCO_3$ (30 mL), brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera ™ (DCM/MeOH, SNAP Ultra 25 g, 75 mL per minute) gave the cyclised product **21** (eluting at 96.5% DCM/MeOH) as a white foam (231 mg, 78% yield).

*(k) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**21**)*

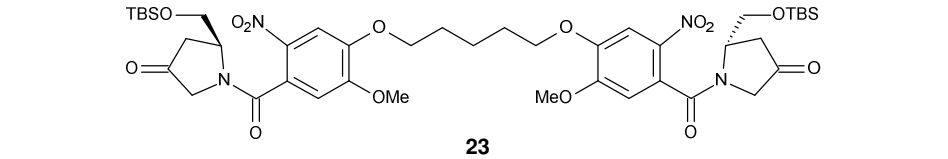
$Pd(PPh_3)_4$ (15.8 mg, 13.7 µmol) was added to a stirred solution of pyrrolidine (63 µL, 54 mg, 0.75 mmol) and the Alloc/allyl compound **20** (231 mg, 0.14 mmol) in dry DCM (4 mL). The reaction mixture was allowed to stir under an argon atmosphere where a precipitate began to form. After stirring for 30 minutes at room temperature analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.18 minutes, ES+ *m*/*z* 1312 $[M+H]^{+\cdot}$. The solvent was removed by evaporation *in vacuo* and the resulting residue triturated with diethyl ether followed by additional evaporation *in vacuo* to provide the crude amine **12** as a solid which was carried through to the next step without further purification or analysis.

(l) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-

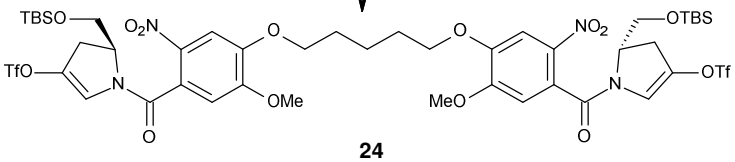
*pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**22**)*

A solution of MAL-dPEG®8-TFP ester (122 mg, 0.16 mmol) in dry DCM (5 mL) was added to a stirred sample of the amine **21** (180 mg, 0.16 mmol), pyridine (28 µL, 27 mg, 0.34 mmol) and DMF (0.25 mL). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC/MS revealed formation of desired product at retention time 1.43 minutes, ES- *m*/*z* 1884 $[M- H]^{+\cdot}$, along with unreacted amine at retention time 1.18 minutes. Additional MAL-dPEG®8-TFP ester (66 mg, 0.09 mmol), pyridine (12 µL, 11 mg, 0.15 mmol) and DCM (3 mL) were added and the mixture allowed to stir for a further three days. At this point a satisfactory amount of desired product had formed upon analysis by LC/MS and the solvent was removed by evaporation *in vacuo*. The crude product was purified by preparative HPLC to give the maleimide **22** as a white solid (55 mg, 19%): LC/MS (15-minute run), retention time 5.82 minutes, ES+ *m*/*z* 1886 $[M+ H]^{+\cdot}$, 1908 $[M+ Na]^{+\cdot}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 9.98 (br s, 2H), 8.25–8.15 (m, 1H), 7.99 (t, 1H, *J* = 5.7 Hz), 7.87 (d, 1H, *J* = 8.7 Hz), 7.69 (br s, 1H), 7.62–7.48 (m, 3H), 7.37 (d, 1H, *J* = 8.9 Hz), 7.21–7.09 (m, 2H), 7.04 (s, 1H), 7.03 (s, 1H), 6.98 (s, 2H), 6.79 (s, 1H), 6.74 (s, 1H), 6.72–6.65 (m, 2H), 6.62–6.58 (m, 2H), 5.63–5.50 (m, 2H), 5.41–5.32 (m, 2H), 5.25–5.05 (m, 4H), 4.93–4.80 (m, 2H), 4.41–4.32 (m, 1H), 4.23–4.16 (m, 1H), 4.00–3.90 (m, 4H), 3.87–3.72 (m, 6H), 3.70–3.60 (m, 2H), 3.58 (t, 4H, *J* = 7.2 Hz), 3.52–3.43 (m, 28H), 3.39–3.20 (m, 5H), 3.18–3.10 (m, 2H), 2.95–2.82 (m, 2H), 2.53–2.34 (m, 4H), 2.31 (t, 2H, *J* = 7.3 Hz), 1.99–1.90 (m, 1H), 1.82–1.68 (m, 4H), 1.73 (s, 6H), 1.60–1.47 (m, 2H), 1.28 (d, 1H, *J* = 7.0 Hz), 0.85 (d, 3H, *J* = 6.8 Hz), 0.81 (d, 3H, *J* = 6.8 Hz).

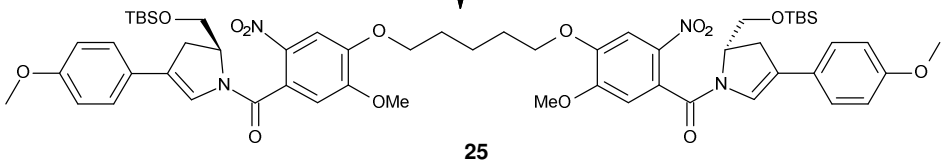
Example 3



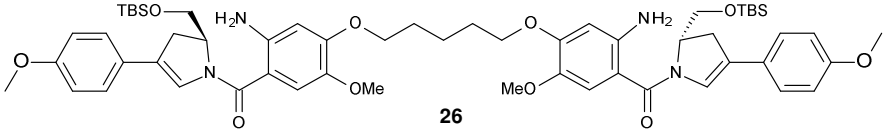
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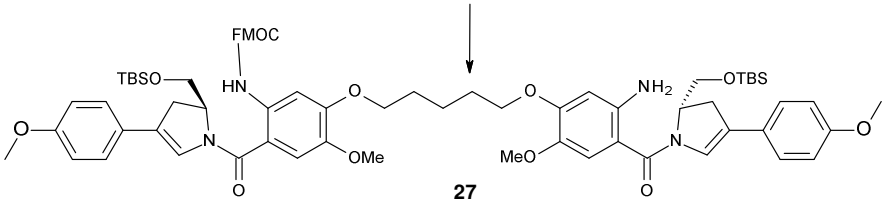
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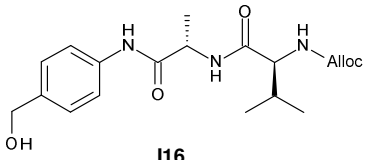
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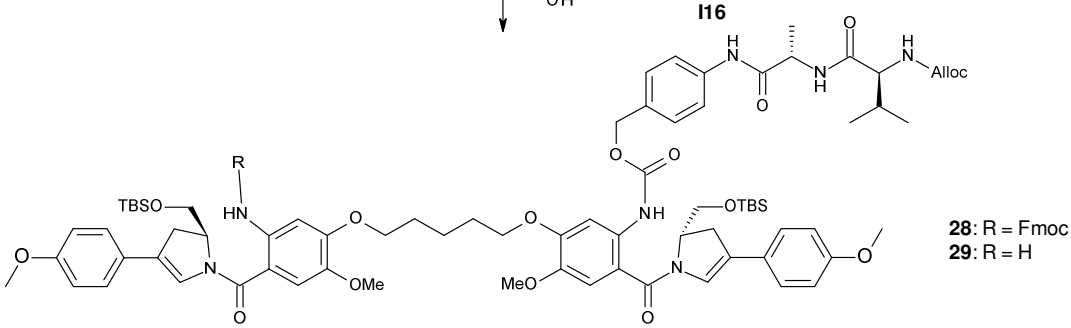
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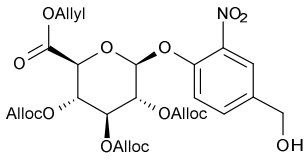
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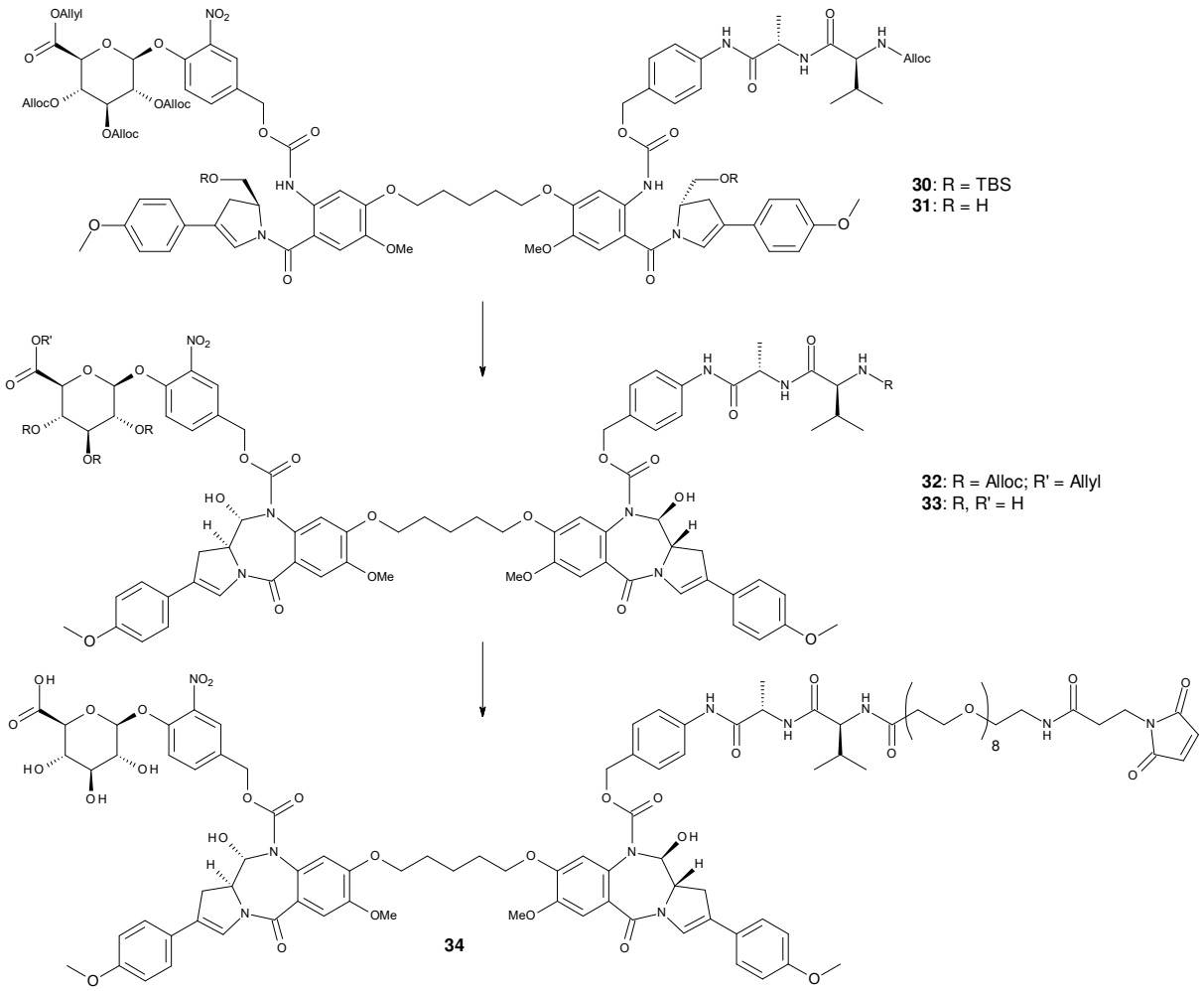
I16



28: R = Fmoc
29: R = H



5a



Compound 23 is compound 46 in WO2014/057074.

*(a) (5S,5'S)-(4,4'-(Pentane-1,5-diylbis(oxy))bis(5-methoxy-2-nitrobenzoyl))bis(5-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydro-1H-pyrrole-1,3-diyl)bis(trifluoromethanesulfonate) (**24**)*

Anhydrous 2,6-lutidine (8.90 mL, 8.22 g, 76.7 mmol) was added via syringe to a stirred solution of *bis*-ketone **23** (5.41 g, 5.90 mmol) in dry DCM (200 mL) at -45 °C (dry ice/acetonitrile) under an argon atmosphere. Anhydrous trifluoromethanesulfonic anhydride (5.96 mL, 10.0 g, 35.5 mmol), taken from a freshly opened ampoule, was then added via syringe, while maintaining the temperature at -40 °C or below. The reaction mixture was allowed to stir at -45 °C for 1 hour at which point analysis by LC/MS revealed conversion to product at retention time retention time 2.23 minutes, ES+ *m/z* 1203 $[M+Na]^{+\cdot}$, 1181 $[M+H]^{+\cdot}$. The cold reaction mixture was immediately diluted with DCM (200 mL) and washed with water (1 x 200 mL), 5% citric acid solution (2 x 200 mL), saturated aqueous $NaHCO_3$ (200 mL), brine (300 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per

minute) afforded the *bis*-enol triflate **3** (eluting at 78% Hexane/EtOAc) as a yellow foam (5.02 g, 72% yield).

*(b) ((Pentane-1,5-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrol-1-yl)methanone) (**25**)*

$Pd(PPh_3)_4$ (196 mg, 0.17 mmol) was added to a stirred mixture of the *bis*-enol triflate **24** (5.02 g, 4.25 mmol), 4-methoxyphenylboronic acid (1.68 g, 11.1 mmol), Na_2CO_3 (1.44 g, 13.6 mmol), EtOH (20 mL), toluene (40 mL) and water (20 mL). The reaction mixture was heated at 30 °C and stirred under an argon atmosphere for 3 hours after which time complete conversion to desired product was observed by LC/MS, retention time 2.23 minutes, ES+ *m*/*z* 1120 $[M+ Na]^{+\cdot}$, 1098 $[M+ H]^{+\cdot}$. The reaction mixture was partitioned between EtOAc (200 mL) and H_2O (100 mL) and the aqueous layer extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with H_2O (2 x 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) afforded bis-C2-aryl compound **25** (eluting at 63% Hexane/EtOAc) as a yellowish foam (3.11 g, 67% yield).

*(c) ((Pentane-1,5-diylbis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrol-1-yl)methanone) (**26**)*

Non-activated zinc (4.13 g, 63.1 mmol) was added to a vigorously stirred solution of the *bis*-nitro compound **25** (1.73 g, 1.58 mmol) in EtOH (15 mL) and EtOAc (15 mL) at room temperature. The reaction mixture was treated with water (1 mL) then drop-wise with a solution of 5% *v/v* HCO_2H in MeOH (19 mL). A colour change from green to metallic grey and an exotherm to 40 °C were observed. The reaction mixture was allowed to cool to room temperature at which point analysis by LC/MS revealed complete conversion to desired product at retention time 2.19 minutes, ES+ *m*/*z* 1060 $[M+ Na]^{+\cdot}$, 1038 $[M+ H]^{+\cdot}$. The mixture was filtered through celite® and the pad washed with EtOAc (100 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (3 x 50 mL) [care: effervescence!], brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude *bis*-aniline **26** as a dark orange foam (1.56 g, 95% yield). Purity satisfactory, so material carried through to next step without further purification.

*(d) (9H-Fluoren-9-yl)methyl(5-((5-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**27**)*

FmocCl (1.22 g, 4.70 mmol) was added to a stirred suspension of the *bis*-aniline **26** (6.50 g, 6.27 mmol) and Na_2CO_3 (1.66 g, 15.7 mmol) in THF (32 mL) and H_2O (32 mL). The mixture was allowed to stir at room temperature for 3 hours where analysis by LC/MS revealed desired mono Fmoc product **27** at retention time 2.33 minutes, I% = 51, ES+ *m*/*z* 1282 $[M+ Na]^{+\cdot}$, 1260 $[M+ H]^{+\cdot}$ along with unreacted starting material at retention time 2.19 minutes, I% = 24, and *bis*-Fmoc material at retention time 2.47 minutes, I% = 24, ES+ *m*/*z* 1504 $[M+ Na]^{+\cdot}$, 1482 $[M+ H]^{+\cdot}$. The mixture was partitioned between H_2O (50 mL) and EtOAc (75 mL), the layers were separated and the aqueous phase extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with H_2O (30 mL), brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure mono Fmoc product **27** as an orange foam (3.31 g, 56% yield based on FmocCl, eluting at 53% Hexane/EtOAc) unreacted *bis*-aniline **26** (2.09 g, eluting at 40% Hexane/EtOAc) and *bis*-Fmoc (1.39 g, eluting at 68% Hexane/EtOAc).

*(e) (9H-Fluoren-9-yl)methyl(5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**28**)*

Triphosgene (281 mg, 0.81 mmol) was added to a stirred solution of the mono Fmoc product **27** (3.31 g, 2.63 mmol) and TEA (805 µL, 584 mg, 5.79 mmol) in dry DCM (35 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.35 minutes, ES+ *m*/*z* 1340 $[M+ Na]^{+\cdot}$, 1318 $[M+ H]^{+\cdot}$). The mixture was treated with additional TEA (549 µL, 398 mg, 3.95 mmol) followed by the addition of linker **I16** (992 mg, 2.63 mmol). After 20 hours stirring under argon, LC/MS revealed conversion to desired carbamate **28** at retention time 2.33 minutes, I% = 40, ES+ *m*/*z* 1685 $[M+ Na]^{+\cdot}$, along with Fmoc cleaved material **29** at retention time 2.19 minutes, I% = 28, ES+ *m*/*z* 1463 $[M+ Na]^{+\cdot}$, 1441 $[M+ H]^{+\cdot}$. The mixture was diluted with DCM (40 mL) and washed with saturated aqueous NH_4Cl (2 x 30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100

g, 100 mL per minute) provided the pure carbamate **28** as a yellow foam (1.64 g, 38% yield, eluting at 40% Hexane/EtOAc) along with Fmoc cleaved product **29** (1.31 g, eluting at 20% Hexane/EtOAc).

*(f) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-((5-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamate (**29**)*

Dimethylamine (9.9 mL of a 2.0M solution in THF, 19.7 mmol) was added to a stirred solution of the Fmoc protected compound **28** (1.64 g, 0.99 mmol) in THF (15 mL) at room temperature. After stirring for 1.5 hours at room temperature, analysis by LC/MS revealed reaction completion with desired product at retention time 2.19 minutes, ES+ *m*/*z* 1463 $[M+Na]^{+\cdot}$, 1441 $[M+H]^{+\cdot}$, along with Fmoc cleavage by product DMA adduct at retention time 0.97 minutes. The mixture was evaporated *in vacuo* to give the crude product which was subsequently purified by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) to provide the pure aniline **29** (eluting at 20% Hexane/EtOAc) as an orange foam (1.36 g, 96% yield).

*(g) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**30**)*

Triphosgene (198 mg, 0.67 mmol) was added to a stirred solution of the aniline **29** (2.67 g, 1.85 mmol) and pyridine (329 µL, 322 mg, 4.07 mmol) in dry DCM (25 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.22 minutes, ES+ *m*/*z* 1521 $[M+Na]^{+\cdot}$, 1499 $[M+H]^{+\cdot}$). The mixture was treated with additional pyridine (224 µL, 220 mg, 2.78 mmol) and dibutyltin dilaurate (219 µL, 234 mg, 0.37 mmol) followed by the addition of linker **5a** (1.18 g, 1.85 mmol). After 16 hours stirring under argon, LC/MS revealed consumption of isocyanate and decrease in signal intensity for linker **5a** but no M^{2+} for desired product at retention time 2.23 minutes. The mixture was diluted with DCM (30 mL) and washed with saturated NH_4Cl (2 x 30 mL), brine (40 mL),

dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (Hexane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **30** (eluting at 39% Hexane/EtOAc) as a yellow foam (2.40 g, 62% yield).

*(h) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-((5-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-2-methoxyphenoxy)pentyl)oxy)-2-((S)-2-(hydroxymethyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**31**)*

Glacial acetic acid (21 mL) was added to a stirred solution of the TBS-protected compound **30** (2.40 g, 1.14 mmol) in THF (7 mL) and H_2O (7 mL). The reaction mixture was allowed to stir for 16 hours at room temperature after which time analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.88 minutes, ES+ *m*/*z* 1876 $[M+ H]^{+\cdot}$, 1898 $[M+ Na]^{+\cdot}$. The reaction mixture was added drop-wise to a chilled (0–5 °C) saturated solution of $NaHCO_3$ (350 mL). The neutral solution was allowed to warm to room temperature and partitioned with EtOAc (200 mL). The aqueous phase was extracted with EtOAc (2 x 50 mL), the combined organic layers were washed with $NaHCO_3$ (2 x 60 mL), brine (70 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-alcohol **31** (eluting at 95.6% DCM/MeOH) as a yellowish foam (2.07 g, 97% yield).

*(i) 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl(11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-11,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**32**)*

$Cu(NCCH_3)_4 \cdot CF_3SO_3$ (2 mg, 5.3 µmmol) was added to a stirred solution of the bis-OH **31** (100 mg, 53 µmmol) and the Stahl aerobic oxidation TEMPO solution (27 µL of a 0.2M solution in CH_3CN, 5.3 µmmol) in CH_3CN (1 mL). The reaction mixture was allowed to stir under an air atmosphere (balloon) for 3 hours at which point analysis by LC/MS revealed no apparent change in retention time or ES+. Additional $Cu(NCCH_3)_4 \cdot CF_3SO_3$ (2 mg, 5.3 µmmol) and Stahl aerobic oxidation TEMPO solution (27 µL of a 0.2M solution in CH_3CN, 5.3 µmmol) were added and the reaction mixture allowed to stir for a further 16 hours. Analysis by LC/MS again revealed no change in retention time, however, ES^+ showed

absence of starting material *m/z* and strong presence of desired product *m/z* 1894 [*M*+Na]$^{+\cdot}$. The solvent was removed by evaporation *in vacuo* and the resulting residue purified by Isolera™ (DCM/MeOH, SNAP Ultra 10 g, 36 mL per minute) to give the cyclised product **32** (eluting at 96.2% DCM/MeOH) as a white foam (91 mg, 92% yield).

*(j) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**33**)*

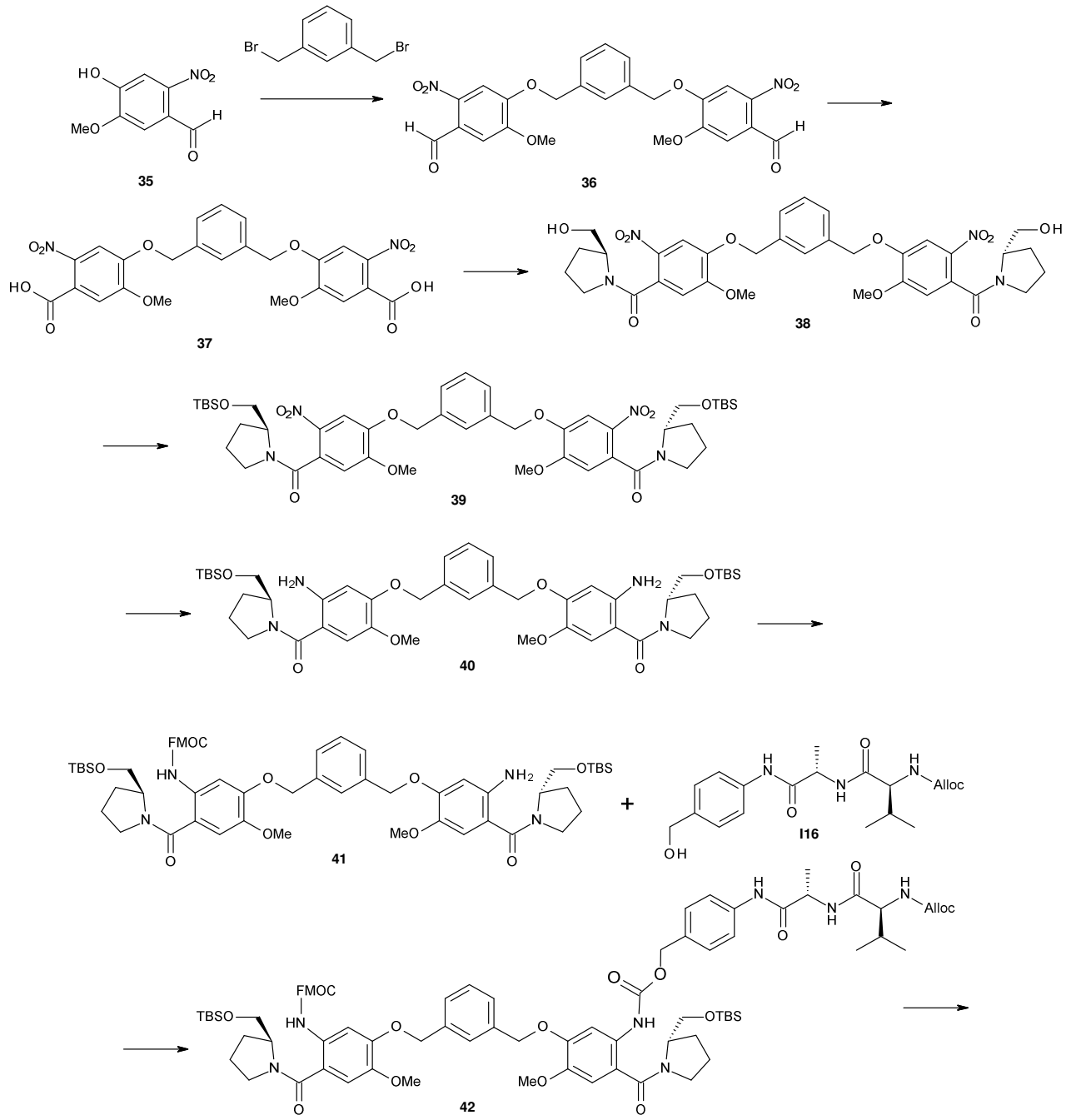
$Pd(PPh_3)_4$ (15.0 mg, 12.8 µmol) was added to a stirred solution of pyrrolidine (59 µL, 50 mg, 0.70 mmol) and the Alloc/allyl compound **32** (240 mg, 0.13 mmol) in dry DCM (5 mL). The reaction mixture was allowed to stir under an argon atmosphere, after stirring for 30 minutes at room temperature analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.31 minutes, ES+ *m/z* 1496 [*M*+H]$^{+\cdot}$. The solvent was removed by evaporation *in vacuo* and the resulting residue triturated with diethyl ether, sonicated, followed by additional evaporation *in vacuo* to provide the crude amine **33** as a yellow solid which was carried through to the next step without further purification or analysis.

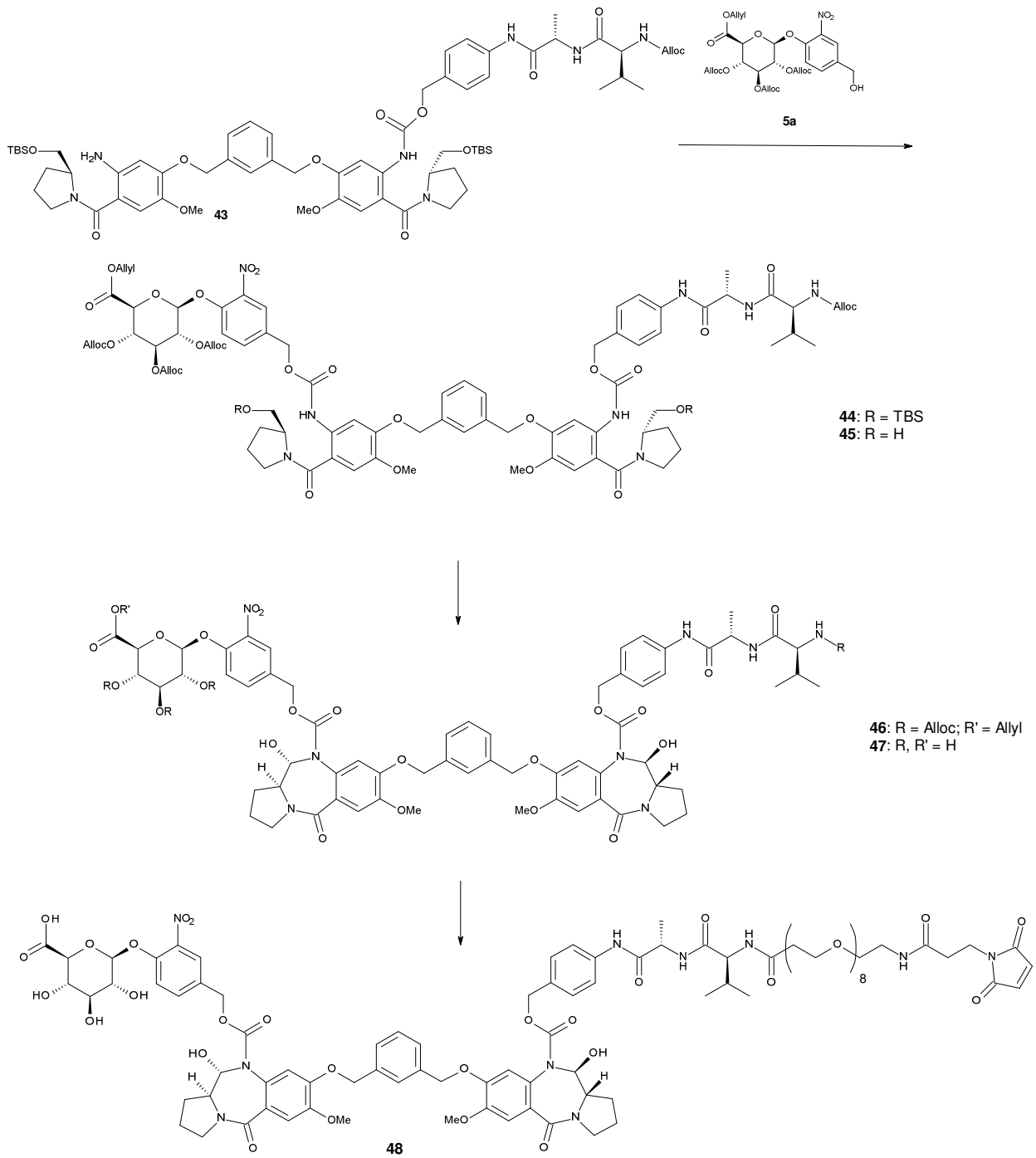
*(k) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4] benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**34**)*

A solution of MAL-dPEG®8-TFP ester (114 mg, 0.15 mmol) in dry DCM (5 mL) was added to a stirred sample of the amine **33** (191 mg, 0.13 mmol) and pyridine (26 µL, 25 mg, 0.32 mmol). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC/MS revealed formation of desired product at retention time 1.59 minutes (desired *m/z* for M^{2+} not detected during reaction monitoring) along with unreacted amine at retention time 1.31 minutes. Additional MAL-dPEG®8-TFP ester (114 mg, 0.15 mmol), pyridine (26 µL, 25 mg, 0.32 mmol) and DCM (3 mL) were added and the mixture allowed to stir for a further six days. At this point a satisfactory

amount of desired product had formed upon analysis by LC/MS and the solvent was removed by evaporation *in vacuo*. The crude product was purified by Isolera™ (DCM/MeOH, SNAP Ultra 10 g, 36 mL per minute, eluting at 82% DCM/MeOH) followed by preparative HPLC to give the maleimide **34** as a slightly yellow solid (46 mg, 13%): LC/MS (15-minute run), retention time 6.71 minutes, ES+ *m*/*z* 1036 [$M^{2+\cdot}$ + H], 1058 [M^{2+} + Na], 1046 [*M* + Na] $^{2+}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 10.02 (br s, 2H), 8.27–8.16 (m, 1H), 8.01 (t, 1H, *J* = 5.6 Hz), 7.90 (d, 1H, *J* = 8.6 Hz), 7.72 (br s, 1H), 7.60–7.51 (m, 3H), 7.46–7.38 (m, 5H), 7.23–7.15 (m, 2H), 7.11 (s, 1H), 7.09 (s, 1H), 6.99 (s, 2H), 6.92 (d, 2H, *J* = 8.9 Hz), 6.91 (d, 2H, *J* = 8.9 Hz), 6.88–6.77 (m, 6H), 5.70–5.60 (m, 2H), 5.39–5.33 (m, 2H), 5.24–5.09 (m, 4H), 4.96–4.81 (m, 2H), 4.42–4.34 (m, 1H), 4.24–4.17 (m, 1H), 4.04–3.93 (m, 4H), 3.87–3.79 (m, 8H), 3.77 (s, 6H), 3.59 (t, 4H, *J* = 7.3 Hz), 3.52–3.46 (m, 28H), 3.40–3.26 (m, 7H, obscured by H_2O), 3.19–3.11 (m, 2H), 2.95–2.82 (m, 2H), 2.53–2.31 (m, 4H), 2.02–1.92 (m, 1H), 1.86–1.73 (m, 4H), 1.62–1.52 (m, 2H), 1.30 (d, 1H, *J* = 7.0 Hz), 0.87 (d, 3H, *J* = 6.7 Hz), 0.83 (d, 3H, *J* = 6.8 Hz).

Example 4





*(a) 4,4'-((1,3-phenylenebis(methylene))bis(oxy))bis(5-methoxy-2-nitrobenzaldehyde) (**36**)*

1,3-bis(bromomethyl)benzene (5.0 g, 19.1 mmol) was added to a stirred solution of the phenol **35** (7.5 g, 38.2 mmol), TBAI (705 mg, 1.91 mmol) and K_2CO_3 (5.81 g, 42.0 mmol) in dry DMF (60 mL). The reaction mixture was heated to 70 °C and stirred under an argon atmosphere for 2 hours at which point analysis by LC/MS revealed substantial product formation at retention time 1.67 minutes, ES+ *m*/*z* 519 $[M+ Na]^{+\cdot}$, 497 $[M+ H]^{+\cdot}$. The reaction mixture was allowed to cool to room temperature and the DMF was removed by

evaporation *in vacuo*. The resulting residue was diluted with water (300 mL) and the orange precipitate **36** collected by vacuum filtration then washed with H_2O, diethyl ether and dried in a vacuum oven to constant weight (9.18 g, 97% yield).

*(b) 4,4'-((1,3-phenylenebis(methylene))bis(oxy))bis(5-methoxy-2-nitrobenzoic acid) (**37**)*
A solution of sodium chlorite (8.44 g, 92.7 mmol, 80 % technical grade) and sodium dihydrogenphosphate monobasic (6.2 g, 51.8 mmol) in water (140 mL) was added to a solution of the aldehyde **36** (9.18 g, 18.5 mmol) in tetrahydrofuran (100 mL) at room temperature. Hydrogen peroxide (30 % w/w, 53 mL, 518 mmol) was immediately added drop-wise to the vigorously stirred biphasic mixture. Effervescence and an exotherm to 30 °C were observed along with a colour change from orange to pale yellow and dissolution of the suspension. The reaction mixture was cooled in an ice bath and acidified to pH2 with concentrated hydrochloric acid. The reaction mixture was then extracted with EtOAc (4 x 150 mL) and the combined organic phase washed with brine (2 x 100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude acid **37**. The solid was triturated with diethyl ether and dried in a vacuum oven to constant weight (9.90 g, >100% yield). Purity satisfactory by LC/MS (retention time 1.56 minutes, ES- *m*/*z* 527 $[M+ H]^{+\cdot}$).

*(c) (((1,3-phenylenebis(methylene))bis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(hydroxymethyl)pyrrolidin-1-yl)methanone) (**38**)*
DMF (12 drops) was added to a stirred suspension of the *bis*-nitrobenzoic acid **37** (9.90 g, 18.7 mmol) and oxalyl chloride (4.9 mL, 7.1 g, 56.3 mmol) in anhydrous DCM (130 mL). Following initial effervescence the reaction suspension became a solution and the mixture was allowed to stir at room temperature for 16 hours. The majority of solvent was removed by evaporation *in vacuo* and the concentrated solution was triturated with diethyl ether. The resulting yellow precipitate (LC/MS sampled in MeOH gave methyl ester at retention time 1.69 minutes, ES+ *m*/*z* 579 $[M+ Na]^{+\cdot}$) was collected by vacuum filtration, washed with cold diethyl ether and dried for 1 hour in a vacuum oven at 40 °C. The solid acid chloride was added portion-wise to a stirred suspension of (*S*)-(+)-2-pyrrolidinemethanol (4.35 g, 4.25 mL, 43.0 mmol) and TEA (13.0 mL, 9.46 g, 93.5 mmol) in DCM (100mL) at -40 °C (dry ice/CH_3CN). After 1 hour stirring, the reaction was complete as judged by LC/MS with exclusively desired product at retention time 1.44 minutes, ES+ *m/z* 717 $[M+ Na]^{+\cdot}$, 695 $[M + H]^{+\cdot}$. The mixture was diluted with DCM (100 mL) and washed with 1N HCl (3 x 50 mL), saturated $NaHCO_3$ (3 x 40 mL), brine (70 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the pure product **38** as a yellow solid (12.7 g, 100% yield).

*(d) (((1,3-phenylenebis(methylene))bis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)methanone) (**39**)*

TBSCl (8.27 g, 54.9 mmol) was added to a stirred solution of the *bis*-alcohol **38** (12.7 g, 18.3 mmol) and imidazole (7.47 g, 110 mmol) in dry DCM (100 mL). After stirring at room temperature for 1.5 hours analysis by LC/MS revealed complete conversion to desired product at retention time 2.11 minutes, ES+ *m*/*z* 946 $[M+ Na]^{+\cdot}$, 924 $[M+ H]^{+\cdot}$. The mixture was diluted with DCM (100 mL) and washed with water (2 x 75 mL), brine (80 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the crude product **39** as a yellow foam (16.7 g, 99% yield). Material sufficiently pure to carry through to next step without further purification

*(e) (((1,3-phenylenebis(methylene))bis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)methanone) (**40**)*

Non-activated zinc (18.5 g, 282 mmol) was added to a vigorously stirred solution of the *bis*-nitro compound **39** (6.50 g, 7.10 mmol) in EtOH (65 mL) and EtOAc (65 mL) at room temperature. The reaction mixture was treated with water (6.5 mL) then drop-wise with a solution of 5% *v/v* HCO_2H in EtOH (130 mL). A colour change from green to metallic grey and an exotherm to 37 °C were observed. The reaction mixture was allowed to cool to room temperature at which point analysis by LC/MS revealed complete conversion to desired product at retention time 2.04 minutes, ES+ *m*/*z* 885 $[M+ Na]^{+\cdot}$, 863 $[M+ H]^{+\cdot}$. The mixture was filtered through celite® and the pad washed with EtOAc (200 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (3 x 100 mL) [care: effervescence!], brine (150 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the crude *bis*-aniline **40** as a dark orange foam (5.41 g, 89% yield). Purity satisfactory, so material carried through to next step without further purification.

*(f) (9H-fluoren-9-yl)methyl (5-((3-((5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)methyl)benzyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**41**)*

FmocCl (1.43 g, 5.50 mmol) was added to a stirred suspension of the *bis*-aniline **40** (6.34 g, 7.35 mmol) and Na_2CO_3 (1.95 g, 18.4 mmol) in THF (32 mL) and H_2O (32 mL). The mixture was allowed to stir at room temperature for 2 hours where analysis by LC/MS revealed desired mono Fmoc product **41** at retention time 2.24 minutes, I% = 41, ES+ *m*/*z* 1108 $[M+ Na]^{+\cdot}$, 1086 $[M+ H]^{+\cdot}$ along with unreacted starting material at retention time 2.03 minutes, I% = 19, and *bis*-Fmoc material at retention time 2.39 minutes, I% = 40, ES+ *m*/*z*

1330 [*M*+ Na]$^{+\cdot}$, 1308 [*M*+ H]$^{+\cdot}$. The mixture was partitioned between H_2O (50 mL) and EtOAc (50 mL), the layers were separated and the organic layer washed with H_2O (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure mono Fmoc product **41** as an orange foam (2.40 g, 40% yield based on FmocCl, eluting at 30% heptane/EtOAc) unreacted *bis*-aniline **40** (1.72 g, eluting at 100% EtOAc) and *bis*-Fmoc (1.95 g, eluting at 69% heptane/EtOAc).

(g) (9*H*-fluoren-9-yl)methyl(5-((3-((5-((((4-((*S*)-2-((*S*)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((*S*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)methyl)benzyl)oxy)-2-((*S*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**42**)
Triphosgene (236 mg, 0.80 mmol) was added to a stirred solution of the mono Fmoc product **41** (2.40 g, 2.21 mmol) and TEA (676 µL, 491 mg, 4.86 mmol) in dry DCM (30 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.27 minutes, ES+ *m*/*z* 1166 [*M*+ Na]$^{+\cdot}$, 1144 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional TEA (461 µL, 335 mg, 3.32 mmol) followed by the addition of linker **I16** (835 mg, 2.21 mmol). After 2.5 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **9** at retention time 2.24 minutes, ES+ *m*/*z* 1511 [*M*+ Na]$^{+\cdot}$, 1489 [*M*+ H]$^{+\cdot}$. The mixture was diluted with DCM (50 mL) and washed with saturated aqueous NH_4Cl (2 x 40 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **42** (eluting at 38% heptane/EtOAc) as a yellow foam (2.40 g, 73% yield).

*(h) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-((3-((5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)methyl)benzyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**43**)*
Dimethylamine (16.1 mL of a 2.0M solution in THF, 32.2 mmol) was added to a stirred solution of the Fmoc protected compound **42** (2.40 g, 1.61 mmol) in THF (20 mL) at room temperature. After stirring for 1 hour at room temperature, analysis by LC/MS revealed reaction completion with desired product at retention time 2.07 minutes, ES+ *m*/*z* 1289 [*M*+ Na]$^{+\cdot}$, 1267 [*M*+ H]$^{+\cdot}$, along with Fmoc cleavage by-product at retention time 1.74 minutes

and its DMA adduct at retention time 0.95 minutes. The mixture was evaporated *in vacuo* to give the crude product which was subsequently purified by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) to provide the pure aniline **43**(eluting at 100% EtOAc) as a white foam (1.77 g, 87% yield).

*(i) Allyl(2S,3S,4S,5R,6S)-6-(4-(((((5-((3-((5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)methyl)benzyl)oxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**44**)*

Triphosgene (150 mg, 0.50 mmol) was added to a stirred solution of the aniline **43** (1.77 g, 1.40 mmol) and pyridine (249 µL, 243 mg, 3.08 mmol) in dry DCM (20 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.11 minutes, ES+ *m*/*z* 1347 $[M+ Na]^{+\cdot}$, 1325 $[M+ H]^{+\cdot}$). The mixture was treated with additional pyridine (170 µL, 166 mg, 2.10 mmol) and dibutyltin dilaurate (165 µL, 177 mg, 0.28 mmol) followed by the addition of linker **5a** (891 mg, 1.40 mmol). After 24 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **44** at retention time 2.16 minutes, ES+ *m*/*z* 1952 $[M+ Na]^{+\cdot}$, 1930 $[M+ H]^{+\cdot}$. The mixture was diluted with DCM (40 mL) and washed with saturated NH_4Cl (2 x 35 mL), brine (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **44** (eluting at 32% heptane/EtOAc) as a white solid (2.16 g, 80% yield).

*(j) Allyl(2S,3S,4S,5R,6S)-6-(4-(((((5-((3-((5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)methyl)benzyl)oxy)-2-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**45**)*

Glacial acetic acid (18 mL) was added to a stirred solution of the TBS-protected compound **44** (2.16 g, 1.12 mmol) in THF (6 mL) and H_2O (6 mL). The reaction mixture was allowed to stir for 5 hours at room temperature after which time analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.77 minutes, ES+ *m*/*z* 1702 $[M+ H]^{+\cdot}$, 1724 $[M+ Na]^{+\cdot}$. The reaction mixture was added drop-wise to a chilled (0–5 °C)

saturated solution of $NaHCO_3$ (300 mL). The neutral solution was allowed to warm to room temperature and extracted with EtOAc (3 x 50 mL), the combined organic layers were washed with brine (60 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-alcohol **45** (eluting at 94.8% DCM/MeOH) as a white foam (1.86 g, 98% yield).

*(k) 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl(11S,11aS)-8-((3-((((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)methyl)benzyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**46**)*

45% IBX (263 mg, 0.42 mmol) was added to a stirred solution of the *bis*-OH **45** (300 mg, 0.18 mmol) in dry DMSO (4 mL). The mixture was heated to 30 °C under an argon atmosphere and reaction progress monitored by LC/MS. After 24 hours stirring, additional IBX (2 x 22 mg, 2 x 36 µmol) was added and the mixture stirred for another 28 hours. At this point analysis by LC/MS revealed formation of resolved product at retention time 1.72 minutes, ES+ *m*/*z* 1698 $[M+H]^{+\cdot}$,1720 $[M+Na]^{+\cdot}$. The reaction mixture was added drop-wise to a saturated aqueous $NaHCO_3$ solution (100 mL) and extracted with DCM (2 x 40 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 25 g, 75 mL per minute) gave the cyclised product **46** (eluting at 96.5% DCM/MeOH) as a white foam (219 mg, 73% yield).

*(l) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((3-((((11S,11aS)-10-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)methyl)benzyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**47**)*

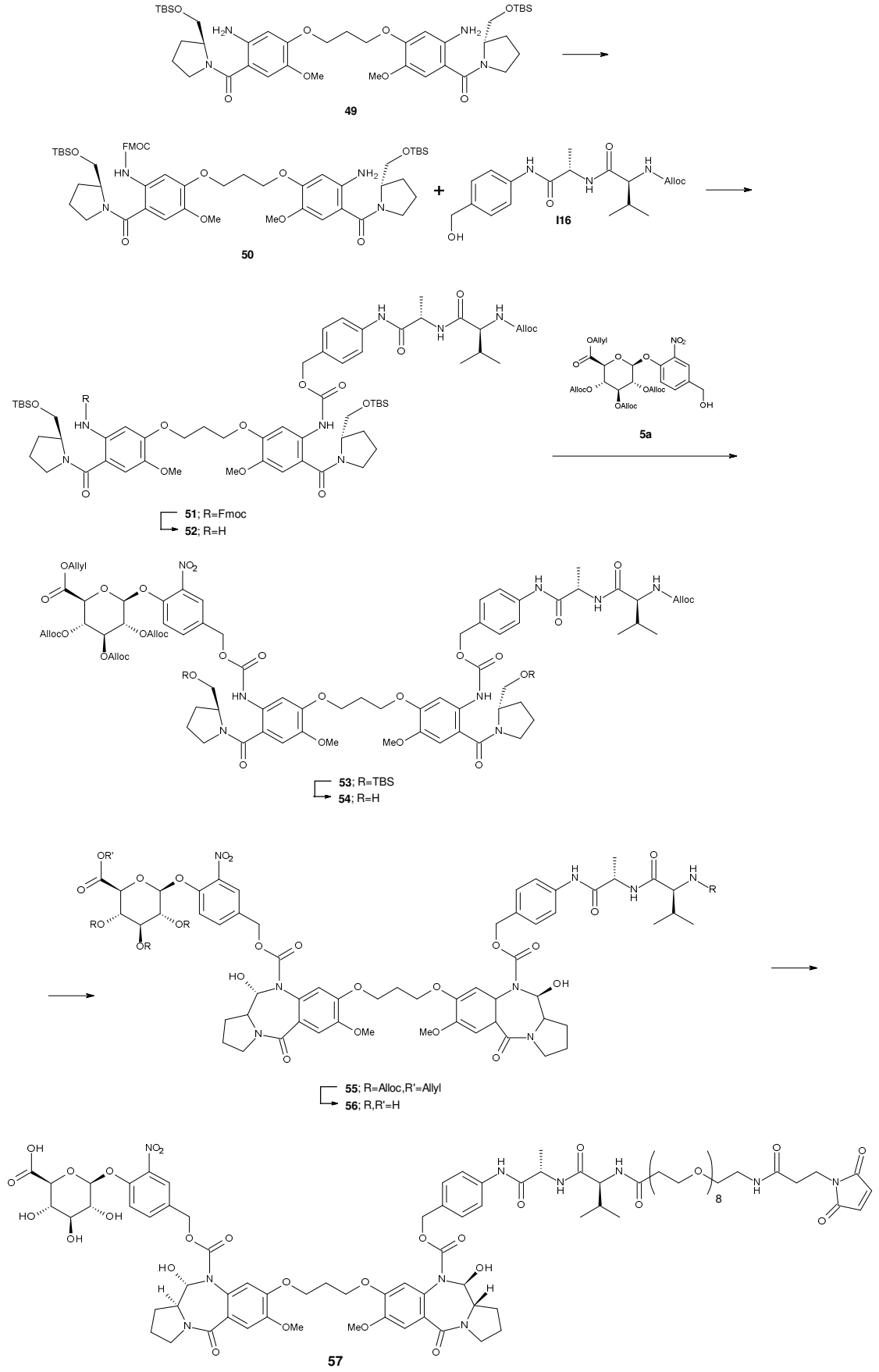
$Pd(PPh_3)_4$ (13.0 mg, 11.1 µmol) was added to a stirred solution of pyrrolidine (51 µL, 43 mg, 0.61 mmol) and the Alloc/allyl compound **46** (189 mg, 0.11 mmol) in dry DCM (2 mL). The reaction mixture was allowed to stir under an argon atmosphere for 30 minutes at room temperature where analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.13 minutes, ES+ *m*/*z* 1322 $[M+H]^{+\cdot}$. The solvent was

removed by evaporation *in vacuo* and the resulting residue triturated with diethyl ether followed by additional evaporation *in vacuo* to provide the crude amine **47** as a solid which was carried through to the next step without further purification or analysis.

*(m) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((3-(((((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)methyl)benzyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**48**)*

A solution of MAL-dPEG®8-TFP ester (123 mg, 0.16 mmol) in dry DCM (2 mL) was added to a stirred sample of the amine **47** (147 mg, 0.11 mmol) and pyridine (22 µL, 22 mg, 0.28 mmol). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC/MS revealed formation of desired product at retention time 1.37 minutes, ES+ *m*/*z* 1896 $[M+H]^{+\cdot}$, 1918 $[M+Na]^{+\cdot}$ along with trace unreacted amine at retention time 1.13 minutes. Substantial decomposition of product and the solvent was removed by evaporation *in vacuo*. The crude product was purified by preparative HPLC to give the maleimide **48** as a white solid (75 mg, 36%): LC/MS (15-minute run), retention time 5.48 minutes, ES+ *m*/*z* 1896 $[M+H]^{+\cdot}$, 1918 $[M+Na]^{+\cdot}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 9.92 (br s, 2H), 8.20–8.14 (m, 1H), 8.01 (t, 1H, *J* = 5.6 Hz), 7.87 (d, 1H, *J* = 8.7 Hz), 7.73 (br s, 1H), 7.62–7.36 (m, 8H), 7.23–7.15 (m, 2H), 7.11 (s, 1H), 7.09 (s, 1H), 7.00 (s, 2H), 6.99 (s, 1H), 6.94 (s, 1H), 6.62–6.51 (m, 2H), 5.53–5.41 (m, 3H), 5.35–5.85 (m, 9H), 4.41–4.32 (m, 1H), 4.23–4.16 (m, 1H), 4.00–3.90 (m, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 3.61–3.58 (m, 4H), 3.52–3.45 (m, 28H), 3.42–3.23 (m, 9H), 3.18–3.12 (m, 2H), 2.50–2.35 (m, 2H), 2.34–2.31 (m, 2H), 2.10–1.8 (m, 9H), 1.28 (d, 1H, *J* = 7.1 Hz), 0.87 (d, 3H, *J* = 6.7 Hz), 0.83 (d, 3H, *J* = 6.8 Hz).

Example 5


49
50
I16
5a
51; R=Fmoc
52; R=H
53; R=TBS
54; R=H
55; R=Alloc,R'=Allyl
56; R,R'=H
57

*(a) (9H-fluoren-9-yl)methyl(5-(3-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**50**)*

FmocCl (1.58 g, 6.09 mmol) was added to a stirred mixture of the *bis*-aniline **49** (6.50 g, 8.12 mmol) and Na_2CO_3 (2.15 g, 20.3 mmol) in THF (35 mL) and H_2O (35 mL). The mixture was allowed to stir room temperature for 2.5 hours where analysis by LC/MS revealed desired mono Fmoc product **50** at retention time 2.19 minutes, I% = 49, ES+ *m*/*z* 1046 [*M*+ Na]$^{+\cdot}$, 1024 [*M*+ H]$^{+\cdot}$ along with unreacted starting material at retention time 1.96 minutes, I% = 19, and *bis*-Fmoc material at retention time 2.34 minutes, I% = 32, ES+ *m*/*z* 1270 [*M*+ Na]$^{+\cdot}$, 1246 [*M*+ H]$^{+\cdot}$. The mixture was partitioned between H_2O (50 mL) and EtOAc (50 mL), the layers were separated and the aqueous phase was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with H_2O (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure mono Fmoc product **50** as an orange foam (3.61 g, 58% yield based on FmocCl, eluting at 61% heptane/EtOAc) unreacted *bis*-aniline **2** (2.04 g, eluting at 100% EtOAc) and *bis*-Fmoc (1.71 g, eluting at 30% heptane/EtOAc).

*(b) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-(3-(5-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**51**)*

Triphosgene (377 mg, 1.27 mmol) was added to a stirred solution of the mono Fmoc product **50** (3.61g, 3.53 mmol) and TEA (1.08 mL, 784 mg, 7.70 mmol) in dry DCM (40 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.23 minutes, ES+ *m*/*z* 1104 [*M*+ Na]$^{+\cdot}$, 1082 [*M*+ H]$^{+\cdot}$). The mixture was treated with additional TEA (737 µL, 535 mg, 5.30 mmol) followed by the addition of linker **I16** (1.33 g, 3.53 mmol). After 2.5 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **51** at retention time 2.21 minutes, ES+ *m*/*z* 1449 [*M*+ Na]$^{+\cdot}$, 1427 [*M*+ H]$^{+\cdot}$. The mixture was diluted with DCM (50 mL) and washed with saturated aqueous NH_4Cl (2 x 30 mL), H_2O (30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra `100 g, 100 mL

per minute) provided the pure carbamate **51** (eluting at 40% Hexane/EtOAc) as a yellow foam (3.34 g, 66% yield).

*(c) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-(3-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**52**)*

Dimethylamine (23.4 mL of a 2.0M solution in THF, 46.8 mmol) was added to a stirred solution of the Fmoc protected compound **51** (3.34 g, 2.34 mmol) in THF (15 mL) at room temperature. After stirring under argon for 1 hour at room temperature, analysis by LC/MS revealed reaction completion with desired product at retention time 2.02 minutes, ES+ *m*/*z* 1227 $[M+ Na]^{+\cdot}$, 1205 $[M+ H]^{+\cdot}$, along with Fmoc cleavage by-product at retention time 1.72 minutes and its DMA adduct at retention time 0.93 minutes. The mixture was evaporated *in vacuo* to give the crude product which was subsequently purified by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) to provide the pure aniline **52** (eluting at 100% EtOAc) as an orange foam (2.51 g, 89% yield).

*(d) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**53**)*

Triphosgene (222 mg, 0.75 mmol) was added to a stirred solution of the aniline **52** (2.51 g, 2.08 mmol) and pyridine (370 µL, 362 mg, 4.58 mmol) in dry DCM (30 mL) at room temperature. After stirring for 10 minutes under argon, analysis by LC/MS revealed complete conversion to isocyanate (sampled in MeOH to give methyl carbamate, retention time 2.06 minutes, ES+ *m*/*z* 1285 $[M+ Na]^{+\cdot}$, 1263 $[M+ H]^{+\cdot}$). The mixture was treated with additional pyridine (252 µL, 247 mg, 3.12 mmol) and dibutyltin dilaurate (247 µL, 263 mg, 0.42 mmol) followed by the addition of linker **5a** (1.33 g, 2.08 mmol). After 28 hours stirring under argon, LC/MS revealed satisfactory conversion to carbamate **53** (retention time 2.12 minutes, ES+ *m*/*z* 1890 $[M+ Na]^{+\cdot}$, 1868 $[M+ H]^{+\cdot}$). The mixture was diluted with DCM (40 mL) and washed with saturated NH_4Cl (3 x 30 mL), brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (heptane/EtOAc, SNAP Ultra 100 g, 100 mL per minute) provided the pure carbamate **53** (eluting at 37% heptane/EtOAc) as a yellow foam (3.12 g, 80% yield).

*(e) Allyl(2S,3S,4S,5R,6S)-6-(4-((((5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-carboxylate (**54**)*

Glacial acetic acid (24 mL) was added to a stirred solution of the TBS-protected compound **53** (3.12 g, 1.67 mmol) in THF (8 mL) and H_2O (8 mL). The reaction mixture was allowed to stir for 18 hours at room temperature after which time analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.72 minutes, ES+ *m*/*z* 1640 $[M+H]^{+\cdot}$, 1662 $[M+Na]^{+\cdot}$. The reaction mixture was added drop-wise to a chilled (0–5 °C) saturated solution of $NaHCO_3$ (450 mL). The neutral solution was allowed to warm to room temperature and extracted with EtOAc (3 x 80 mL), the combined organic layers were washed with brine (80 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 100 g, 100 mL per minute) gave the *bis*-alcohol **54** (eluting at 96.6% DCM/MeOH) as a white foam (2.05 g, 75% yield).

*(f) 4-(((2S,3R,4S,5S,6S)-6-((allyloxy)carbonyl)-3,4,5-tris(((allyloxy)carbonyl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-nitrobenzyl(11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**55**)*

45% IBX (273 mg, 0.44 mmol) was added to a stirred solution of the *bis*-OH **54** (300 mg, 0.18 mmol) in dry DMSO (4 mL). The mixture was heated to 30 °C under an argon atmosphere and reaction progress monitored by LC/MS. After 24 hours stirring, additional IBX (26 mg, 42 µmol) was added and the mixture stirred for another 24 hours. At this point analysis by LC/MS revealed predominantly a single peak corresponding to desired product at retention time 1.68 minutes, ES+ *m*/*z* 1658 $[M+Na]^{+\cdot}$. The reaction mixture was added drop-wise to a chilled saturated aqueous $NaHCO_3$ solution (100 mL) and extracted with DCM (2 x 40 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product. Purification by Isolera™ (DCM/MeOH, SNAP Ultra 25 g, 75 mL per minute) gave the cyclised product **55** (eluting at 97% DCM/MeOH) as a white foam (256 mg, 87% yield).

*(g) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**56**)*

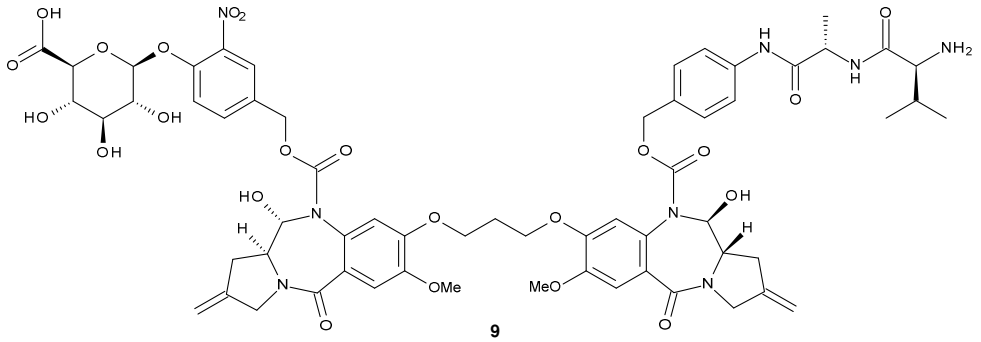
$Pd(PPh_3)_4$ (18.1 mg, 15.7 µmol) was added to a stirred solution of pyrrolidine (72 µL, 62 mg, 0.86 mmol) and the Alloc/allyl compound **55** (256 mg, 0.16 mmol) in dry DCM (2 mL). The reaction mixture was allowed to stir under an argon atmosphere where a precipitate began to form. After stirring for 30 minutes at room temperature analysis by LC/MS revealed reaction completion with desired product observed at retention time 1.07 minutes, ES+ *m*/*z* 1260 $[M+H]^{+\cdot}$. The solvent was removed by evaporation *in vacuo* and the resulting residue triturated with diethyl ether followed by additional evaporation *in vacuo* to provide the crude amine **56** as a solid which was carried through to the next step without further purification or analysis.

*(h) (2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**57**)*

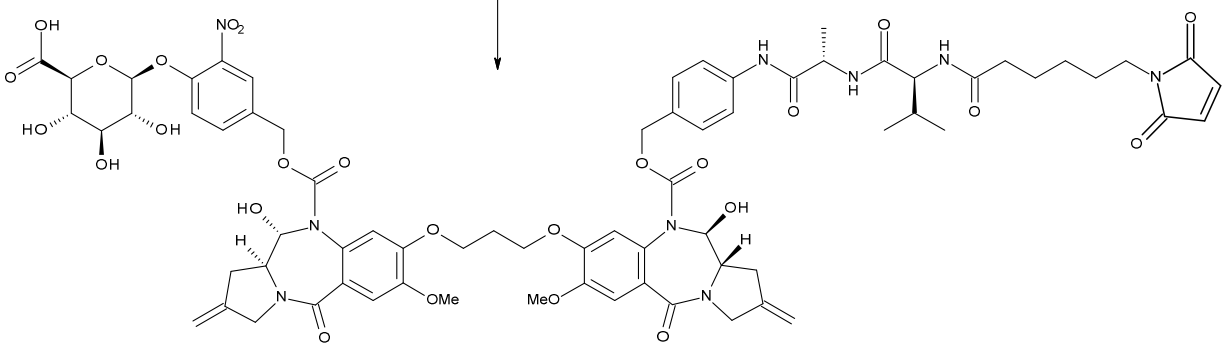
A solution of MAL-dPEG®8-TFP ester (140 mg, 0.19 mmol) in dry DCM (2 mL) was added to a stirred sample of the amine **56** (198 mg, 0.16 mmol), pyridine (32 µL, 31 mg, 0.39 mmol) and DMF (0.25 mL). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC/MS revealed formation of desired product at retention time 1.31 minutes, ES+ *m*/*z* 1834 $[M+H]^{+\cdot}$, 1857 $[M+Na]^{+}$, along with unreacted amine at retention time 1.07 minutes. Additional MAL-dPEG®8-TFP ester (70 mg, 0.09 mmol), pyridine (16 µL, 16 mg, 0.2 mmol) and DCM (2 mL) were added and the mixture allowed to stir for a further 24 hours. The solvent volume was then reduced by 50% by evaporation *in vacuo*, at this point a satisfactory amount of desired product had formed upon analysis by LC/MS and the solvent was completely removed by evaporation *in vacuo*. The crude product was purified by preparative HPLC to give the maleimide **56** as a white solid (48 mg, 17%): LC/MS (15-minute run), retention time 5.22 minutes, ES+ *m*/*z* 1834 $[M+H]^{+\cdot}$, 1856 $[M+Na]^{+}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 9.99 (br s, 2H), 8.24–8.17

(m, 1H), 8.01 (t, 1H, *J* = 5.6 Hz), 7.88 (d, 1H, *J* = 8.7 Hz), 7.74 (br s, 1H), 7.64–7.50 (m, 3H), 7.44–7.34 (m, 1H), 7.23–7.13 (m, 2H), 7.06 (s, 2H), 7.00 (s, 2H), 6.86 (s, 1H), 6.79 (s, 1H), 6.59–6.47 (m, 2H), 5.52–5.37 (m, 3H), 5.32–5.02 (m, 4H), 4.98–4.84 (m, 2H), 4.44–4.34 (m, 1H), 4.24–4.19 (m, 1H), 4.17–3.96 (m, 5H), 3.92–3.84 (m, 1H), 3.77 (s, 6H), 3.60 (t, 4H, *J* = 7.3 Hz), 3.54–3.45 (m, 29H), 3.40–3.24 (m, 8H), 3.19–3.12 (m, 2H), 2.57–2.35 (m, 2H), 2.34–2.30 (m, 2H), 2.25–2.12 (m, 2H), 2.10–1.78 (m, 9H), 1.30 (d, 1H, *J* = 6.9 Hz), 0.87 (d, 3H, *J* = 6.7 Hz), 0.83 (d, 3H, *J* = 6.7 Hz).

Example 6



9



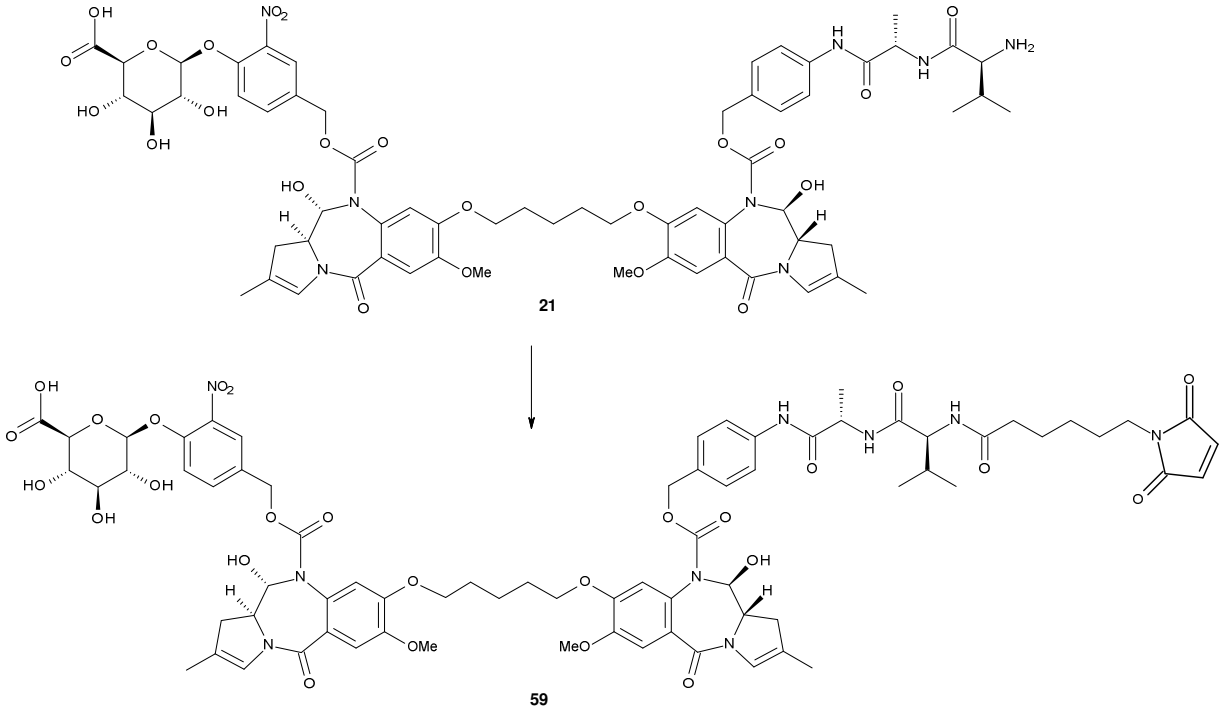
58

*(2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**58**)*

A solution of 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (51 mg, 0.17 mmol) in dry DCM (2 mL) was added to a stirred sample of the amine **9** (141 mg, 0.11 mmol), pyridine (22 µL, 21 mg, 0.33 mmol) and DMF (0.25 mL). The reaction mixture was allowed to stir under argon for 20 hours and began to darken in colour over time. Analysis by LC-MS revealed formation of desired product at retention time 1.42 minutes, ES- *m*/*z* 1474 [*M*-

H]$^{-\cdot}$, along with trace unreacted amine at retention time 1.12 minutes. The solvent was removed by evaporation *in vacuo* and the resulting residue purified by Isolera™ (DCM/MeOH, SNAP Ultra 10 g, 36 mL per minute, eluting at 80% DCM/MeOH) to give 90 mg of enriched **58**. Further purification by preparative HPLC gave the maleimide **58**, as a white solid (26 mg, 16%): LC-MS (15-minute run), retention time 5.85 minutes, ES+ *m*/*z* 1477 [*M*+ H]$^{+\cdot}$, 1499 [*M*+ Na]$^{+\cdot}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ10.0 (br s, 2H), 8.16 (d, 1H, *J* = 7.0 Hz), 7.80 (d, 1H, *J* = 8.6 Hz), 7.74 (br s, 1H), 7.62–7.50 (m, 3H), 7.39 (d, 1H, *J* = 8.7 Hz), 7.22–7.11 (m, 2H), 7.06 (s, 2H), 7.00 (s, 2H), 6.89 (s, 2H), 6.84–6.78 (m, 1H), 6.68–6.56 (m, 2H), 5.45–5.30 (m, 3H), 5.29–5.01 (m, 9H), 4.98–4.85 (m, 2H), 4.42–4.33 (m, 1H), 4.24–3.92 (m, 9H), 3.77 (s, 6H), 3.44 (t, 2H, *J* = 9.5 Hz), 3.39–3.30 (m, 2H), 2.93–2.80 (m, 2H), 2.57–2.35 (m, 2H), 2.23–2.06 (m, 4H) 2.03–1.90 (m, 1H), 1.48 (p, 4H, *J* = 7.3 Hz), 1.30 (d, 3H, *J* = 7.0 Hz), 1.18 (p, 2H, *J* = 7.6 Hz), 0.86 (d, 3H, *J* = 6.7 Hz), 0.83 (d, 3H, *J* = 6.7 Hz).

Example 7



*(2S,3S,4S,5R,6S)-6-(4-((((11S,11aS)-8-((5-(((11S,11aS)-10-(((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10-carbonyl)oxy)methyl)-2-nitrophenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**59**)*

A solution of 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (42 mg, 0.14 mmol) in dry DCM (2 mL) was added to a stirred sample of the amine **21** (118 mg, 0.09 mmol) and pyridine (18 µL, 18 mg, 0.23 mmol). The reaction mixture was allowed to stir under argon for 24 hours and began to darken in colour over time. Analysis by LC-MS revealed formation of desired product at retention time 1.45 minutes, ES- *m*/*z* 1502 $[M\text{-H}]^{-\cdot}$, along with trace unreacted amine at retention time 1.15 minutes. The solvent was removed by evaporation *in vacuo* and the resulting residue purified by Isolera™ (DCM/MeOH, SNAP Ultra 10 g, 36 mL per minute, eluting at 66% DCM/MeOH) to give 76 mg of enriched **59**. Further purification by preparative HPLC gave the maleimide **59** as a white solid (27 mg, 20%): LC-MS (15-minute run), retention time 6.13 minutes, ES+ *m*/*z* 1505 $[M\text{+ H}]^{+\cdot}$, 1527 $[M\text{+ Na}]^{+\cdot}$; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 9.95 (br s, 2H), 8.15 (d, 1H, *J* = 6.9 Hz), 7.80 (d, 1H, *J* = 8.7 Hz), 7.87 (d, 1H, *J* = 8.7 Hz), 7.71 (br s, 1H), 7.62–7.48 (m, 3H), 7.39 (d, 1H, *J* = 8.6 Hz), 7.21–7.12 (m, 2H), 7.06 (s, 1H), 7.05 (s, 1H), 6.99 (s, 2H), 6.82 (s, 1H), 6.76 (s, 1H), 6.72–6.65 (m, 2H), 6.62 (s, 2H), 5.63–5.50 (m, 2H), 5.48–5.40 (m, 2H), 5.31–5.08 (m, 6H), 4.98–4.82 (m, 3H), 4.43–4.34 (m, 1H), 4.21–4.14 (m, 1H), 4.03–3.86 (m, 4H), 3.79 (s x 2, 6H), 3.72–3.61 (m, 2H), 3.38–3.35 (m, 2H), 2.98–2.85 (m, 2H), 2.59–2.42 (m, 2H), 2.23–2.06 (m, 2H), 2.00–1.88 (m, 1H), 1.82–1.68 (m, 4H), 1.74 (s, 6H), 1.61–1.41 (m, 8H), 1.22–1.14 (m, 2H), 1.28 (d, 1H, *J* = 7.0 Hz), 0.86 (d, 3H, *J* = 6.7 Hz), 0.82 (d, 3H, *J* = 6.8 Hz).

Example 8 - Conjugation

Conjugate Tratuzumab-10 (ConjA)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 16 micromoles, 320 µL) to a 12.0 mL solution of antibody, Trastuzumab (30 mg, 200 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.5 mg/mL. The reduction mixture was allowed to react at room temperature for 4-5 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (50 rpm) shaking. The reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 4 micromoles, 80 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (50 rpm) shaking at an antibody concentration of 1.5 mg/mL (or more DHAA added and reaction left for longer until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1

mM EDTA for a final antibody concentration of 1.0 mg/mL. Compound **10** was added as a DMSO solution (10 molar equivalent/antibody, 1 micromole, in 1.5 mL DMSO) to 13.5 mL of this reoxidised antibody solution (15 mg, 100 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (15 micromoles, 150 µL at 100 mM), then purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjA at 280 nm and 330 nm (Compound **10** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of compound **10**, consistent with a drug-per-antibody ratio (DAR) of 1.89 molecules of compound **10** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjA at 280 nm shows a monomer purity of 99%. UHPLC SEC analysis gives a concentration of final ConjA at 1.45 mg/mL in 8.5 mL, obtained mass of ConjA is 12.3 mg (82% yield).

Conjugate Tratuzumab-22 (ConjB)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 42.7 micromoles, 853 µL) to a 20.0 mL solution of antibody, Trastuzumab (80 mg, 533 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4.0 mg/mL. The reduction mixture was allowed to react at room temperature for 4-5 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. The reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 10.7 micromoles, 213 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (50 rpm) shaking at an antibody concentration of 1.5 mg/mL (or more DHAA added and reaction left for longer until full reoxidation of the

cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 1.0 mg/mL. Compound **22** was added as a DMSO solution (10 molar equivalent/antibody, 1.33 micromoles, in 2.0 mL DMSO) to 18.0 mL of this reoxidised antibody solution (20 mg, 133 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (20 micromoles, 200 µL at 100 mM), then purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjB at 280 nm and 330 nm (Compound **22** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **22**, consistent with a drug-per-antibody ratio (DAR) of 1.71 molecules of Compound **22** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjB at 280 nm shows a monomer purity of 99%. UHPLC SEC analysis gives a concentration of final ConjB at 1.39 mg/mL in 10.3 mL, obtained mass of ConjB is 14.3 mg (72% yield).

Conjugate Tratuzumab-34 (ConjC)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 42.7 micromoles, 853 µL) to a 20.0 mL solution of antibody, Trastuzumab (80 mg, 533 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4.0 mg/mL. The reduction mixture was allowed to react at room temperature for 4-5 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. The reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 10.7 micromoles, 213 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours

at room temperature with gentle (50 rpm) shaking at an antibody concentration of 1.5 mg/mL (or more DHAA added and reaction left for longer until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 1.0 mg/mL. Compound **34** was added as a DMSO solution (10 molar equivalent/antibody, 1.33 micromoles, in 2.0 mL DMSO) to 18.0 mL of this reoxidised antibody solution (20 mg, 133 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (20 micromoles, 200 µL at 100 mM), then purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, and further purified on an AKTA™ Start FPLC using a GE Healthcare HiLoad™ 26/600 column packed with Superdex 200 PG, eluting with 2.5 mL/min PBS. Fractions corresponding to ConjC monomer peak were pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjC at 280 nm and 330 nm (Compound **34** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **34**, consistent with a drug-per-antibody ratio (DAR) of 1.66 molecules of Compound **34** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjC at 280 nm shows a monomer purity of 99%. UHPLC SEC analysis gives a concentration of final ConjC at 1.36 mg/mL in 10.4 mL, obtained mass of ConjC is 14.1 mg (71% yield).

Conjugate Tratuzumab-10 (ConjD)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (150 molar equivalent/antibody, 40 micromoles, 800 µL) to a 16.0 mL solution of antibody, Tratuzumab (40 mg, 267 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.5 mg/mL. The reduction mixture was allowed to react at room temperature for 4 hours (or

until full reduction is observed by UHPLC) in an orbital shaker with gentle (50 rpm) shaking. The reduced antibody solution was buffer exchanged (to remove all the excess reducing agent), *via* spin filter centrifugation, into a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 2.5 mg/mL. Compound **10** was added as a DMSO solution (25 molar equivalent/antibody, 1.67 micromoles, in 0.4 mL DMSO) to 3.6 mL of this reduced antibody solution (10 mg, 67 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (8.33 micromoles, 83.3 µL at 100 mM), then purified on an AKTA™ Start FPLC using a GE Healthcare HiLoad™ 26/600 column packed with Superdex 200 PG, eluting with 2.5 mL/min PBS. Fractions corresponding to ConjD monomer peak were pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjD at 280 nm and 330 nm (Compound **10** specific) shows a mixture of unconjugated light chains, light chains attached to a single molecule of Compound **10**, unconjugated heavy chains and heavy chains attached to up to three molecules of Compound **10**, consistent with a drug-per-antibody ratio (DAR) of 7.47 molecules of Compound **10** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjD at 280 nm shows a monomer purity of 99%. UHPLC SEC analysis gives a concentration of final ConjD at 1.90 mg/mL in 4.4 mL, obtained mass of ConjA is 8.4 mg (84% yield).

Conjugate Tratuzumab-22 (ConjE)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (150 molar equivalent/antibody, 40 micromoles, 800 mL) to a 16.0 mL solution of antibody, Tratuzumab (40 mg, 267 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.5 mg/mL. The reduction mixture was allowed to react at room temperature for 4 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (50 rpm) shaking.

The reduced antibody solution was buffer exchanged (to remove all the excess reducing agent), *via* spin filter centrifugation, into a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 2.5 mg/mL. Compound **22** was added as a DMSO solution (25 molar equivalent/antibody, 1.67 micromoles, in 0.4 mL DMSO) to 3.6 mL of this reduced antibody solution (10 mg, 67 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (8.33 micromoles, 83.3 mL at 100 mM), then purified on an AKTA™ Start FPLC using a GE Healthcare HiLoad™ 26/600 column packed with Superdex 200 PG, eluting with 2.5 mL/min PBS. Fractions corresponding to ConjE monomer peak were pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjE at 280 nm and 330 nm (Compound **22** specific) shows a mixture of unconjugated light chains, light chains attached to a single molecule of Compound **22**, unconjugated heavy chains and heavy chains attached to up to three molecules of Compound **22**, consistent with a drug-per-antibody ratio (DAR) of 7.39 molecules of Compound **22** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjE at 280 nm shows a monomer purity of 96%. UHPLC SEC analysis gives a concentration of final ConjE at 1.93 mg/mL in 4.6 mL, obtained mass of ConjE is 8.9 mg (89% yield).

Conjugate Tratuzumab-34 (ConjF)

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (150 molar equivalent/antibody, 10 micromoles, 200 µL) to a 4.0 mL solution of antibody (10 mg, 66.7 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.5 mg/mL. The reduction mixture was allowed to react at room temperature for 4 hours (or until full reduction is observed by UHPLC) in an orbital shaker with gentle (50 rpm) shaking. The reduced antibody solution was buffer exchanged (to remove all the excess reducing agent),

via spin filter centrifugation, into a conjugation buffer containing PBS and 1 mM EDTA for a final antibody concentration of 2.5 mg/mL. Compound **34** was added as a DMSO solution (25 molar equivalent/antibody, 1.67 micromoles, in 0.4 mL DMSO) to 3.6 mL of this reduced antibody solution (10 mg, 67 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 0.75 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (8.33 micromoles, 83.3 µL at 100 mM), then purified on an AKTA™ Start FPLC using a GE Healthcare HiLoad™ 26/600 column packed with Superdex 200 PG, eluting with 2.5 mL/min PBS. Fractions corresponding to ConjF peak were pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, re-purified on an AKTA™ Start FPLC using a GE Healthcare HiLoad™ 26/600 column packed with Superdex 200 PG, eluting with 2.5 mL/min PBS. Fractions corresponding to ConjF peak were pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ConjF at 280 nm and 330 nm (Compound **34** specific) shows a mixture of unconjugated light chains, light chains attached to a single molecule of Compound **34**, unconjugated heavy chains and heavy chains attached to up to three molecules of Compound **34**, consistent with a drug-per-antibody ratio (DAR) of 7.41 molecules of Compound **34** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjF at 280 nm shows a monomer purity of 0% (100% dimer-trimer). UHPLC SEC analysis gives a concentration of final ConjF at 1.09 mg/mL in 4.4 mL, obtained mass of ConjF is 4.8 mg (48% yield).

Conjugate Tratuzumab-58 (ConjG)

A 50 mM solution of Dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (120 molar equivalent/antibody, 32 micromoles, 0.64 mL at 50 mM) to a 3.7 mL solution of antibody (40 mg, 0.267 micromoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.0 mg/mL. The reduction mixture was heated at +25°C for 4 hours (or until full reduction

observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter using 50 KDa MWCO vivaspin, into a reoxidation buffer containing PBS pH 7.4 and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 5.3 micromoles, 0.1 mL at 50 mM) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (60 rpm) shaking at an antibody concentration of ~ 2.0 mg/mL (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was centrifuged for 3 min at 4000 rpm and then sterile-filtered using 0.22 µm membrane filter. Compound **58** was added as a DMSO solution (11 molar equivalent/antibody, 1.46 micromoles, in 0.9 mL DMSO) to 9.1 mL of this reoxidised antibody solution (20 mg, 0.13 micromoles) for a 10% (*v/v*) final DMSO concentration. The solution was shaken for 3 hours at +25°C and then the conjugation was quenched with *N*-acetyl cysteine (7.3 micromoles, 0.07 mL at 100 mM).

Excess free drug was removed *via* spin filter using 50 kDa MWCO vivaspin into buffer containing PBS pH 7.4. Extent of free drug removal was monitored by UHPLC-RP using neat conjugate. After complete removal of free drug, ConjG was filtered using 0.22 µm, Mustang filter under sterile atmosphere and then stored at +4°C.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm and 330 nm (Compound **58** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **58**, consistent with a drug-per-antibody ratio (DAR) of 1.83 molecules of Compound **58** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjG at 280 nm shows a monomer purity of greater than 99%. UHPLC SEC analysis gives a concentration of final ConjG at 1.34 mg/mL in 8.9 mL, obtained mass of ConjG is 11.9 mg (60% yield).

Conjugate Tratuzumab-59 (ConjH)

Antibody (30 mg) was loaded onto solid support and reduced, reoxidised, conjugated to Compound **59**, purified, released from the resin and formulated onto 25 mM Histidine, 200 mM Sucrose, Tween-20 0.02%, pH 6.0 according to the method described in US2014-0037961A1, which is incorporated herein by reference.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm and 330 nm (Compound **59** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **59**, consistent with a drug-per-antibody ratio (DAR) of 1.9 molecules of Compound **59** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjH at 280 nm shows a monomer purity of greater than 97%. UHPLC SEC analysis gives a concentration of final ConjH at 1.49 mg/mL in 7.5 mL, obtained mass of ConjH is 11.2 mg (37% yield).

Conjugate Tratuzumab-48 (ConjI)

A 50 mM solution of Dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 21.4 micromoles, 0.43 mL at 50 mM) to a 3.7 mL solution of antibody (40 mg, 0.267 micromoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.0 mg/mL. The reduction mixture was heated at +25°C for 4 hours (or until full reduction observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter using 50 KDa MWCO vivaspin, into a reoxidation buffer containing PBS pH 7.4 and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 20 molar equivalent/antibody, 5.3 micromoles, 0.1 mL at 50 mM) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (60 rpm) shaking at an antibody concentration of ~ 2.0 mg/mL (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was centrifuged for 3 min at 4000 rpm and then sterile-filtered using

0.22 µm membrane filter. Compound **48** was added as a DMSO solution (11 molar equivalent/antibody, 1.46 micromoles, in 1.7 mL DMSO) to 16.8 mL of this reoxidised antibody solution (20 mg, 0.13 micromoles) for a 10% (*v/v*) final DMSO concentration. The solution was shaken for 3 hours at +25°C and then the conjugation was quenched with *N*-acetyl cysteine (7.3 micromoles, 0.07 mL at 100 mM).

Excess free drug was removed *via* spin filter using 50 kDa MWCO vivaspin into buffer containing PBS pH 7.4. Extent of free drug removal was monitored by UHPLC-RP using neat conjugate. After complete removal of free drug, ADC was filtered using 0.22 µm, Mustang filter under sterile atmosphere and then stored at +4°C.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm and 330 nm (Compound **48** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **48**, consistent with a drug-per-antibody ratio (DAR) of 1.86 molecules of Compound **48** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjI at 280 nm shows a monomer purity of greater than 99%. UHPLC SEC analysis gives a concentration of final ConjI at 1.7 mg/mL in 7.3 mL, obtained mass of ConjI is 12.4 mg (62 % yield).

Conjugate Tratuzumab-57 (ConjJ)

A 50 mM solution of Dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 21.4 micromoles, 0.43 mL at 50 mM) to a 3.7 mL solution of antibody (40 mg, 0.267 micromoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 2.0 mg/mL. The reduction mixture was heated at +25°C for 4 hours (or until full reduction observed by UHPLC) in an orbital shaker with gentle (60 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter using 50 KDa MWCO vivaspin, into a reoxidation buffer containing PBS pH 7.4 and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA,

20 molar equivalent/antibody, 5.3 micromoles, 0.1 mL at 50 mM) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (60 rpm) shaking at an antibody concentration of ~ 2.0 mg/mL (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was centrifuged for 3 min at 4000 rpm and then sterile-filtered using 0.22 µm membrane filter. Compound **57** was added as a DMSO solution (11 molar equivalent/antibody, 1.46 micromoles, in 1.7 mL DMSO) to 16.8 mL of this reoxidised antibody solution (20 mg, 0.13 micromoles) for a 10% (*v/v*) final DMSO concentration. The solution was shaken for 3 hours at +25°C and then the conjugation was quenched with *N*-acetyl cysteine (7.3 micromoles, 0.07 mL at 100 mM).

Excess free drug was removed *via* spin filter using 50 kDa MWCO vivaspin into buffer containing PBS pH 7.4. Extent of free drug removal was monitored by UHPLC-RP using neat conjugate. After complete removal of free drug, ADC was filtered using 0.22 µm, Mustang filter under sterile atmosphere and then stored at +4°C.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm and 330 nm (Compound **57** specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound **57**, consistent with a drug-per-antibody ratio (DAR) of 1.87 molecules of Compound **57** per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ConjJ at 280 nm shows a monomer purity of greater than 100%. UHPLC SEC analysis gives a concentration of final ConjJ at 0.86 mg/mL in 10.3 mL, obtained mass of ConjJ is 8.9 mg (45 % yield).

Example 9

In vitro testing of Compound 10

DU145 (2000/well, 72 hour assay) and MDA-MB-361 (5000/well, 144 hour assay) cells were plated in culture medium to tissue culture-treated 96-well plates at a volume of 80 µL per well and allowed to adhere overnight. For warhead dilutions, first a 60X concentration

of each dose to be tested was prepared by diluting the test articles in DMSO. The 60X dilutions of warhead were then further diluted to a 5x concentration in either cell culture medium alone or cell culture medium containing 5x β-glucuronidase enzyme (Sigma Cat # G0799-25KU). 20 µL of cell culture medium containing test article +/- enzyme was added to cells in triplicate wells, and the final dose curve ranged from 6.4 nM to 61 pM in a stepwise 1:4 serial dilution series. DMSO alone diluted to the culture medium was used as the controls. The treated cells were cultured at 37°C / 5% CO_2 for 72 to 144 hours (depending on established growth kinetics of each particular cell line). At the end of the assay, CellTiter-Glo® (CTG) Luminescent Viability Assay (Promega) was used to determine relative cytotoxicity. 100 µL of CTG reagent was added to each well, and plates were incubated for 10 minutes at room temperature with gentle shaking, absorbance of each sample was read at 560nM using an EnVision luminometer (Perkin Elmer). The percent cell viability was calculated by the following formula:
(average luminescence of treated samples/average luminescence of control samples) x 100.

IC_{50} values were determined using logistic non-linear regression analysis with GraphPad Prism software.

Compound **10**	No glucuronidase	With glucuronidase
DU145 - IC_{50}	6.84 µM	201 nM
MDA-MB-361 - IC_{50}	778 nM	32 nM

In vitro testing of ConjA

A a similar method was used as described above with MDA-MB-361 (HER2 high, 5000 cells/well, 144hr assay) and MCF-7 cells (HER2 low, 1500 cells/well, 144hr assay). Cell plating density and duration of the assay were determined based on established growth kinetics of each particular cell line. A 5x concentration of each dose of ADC to be tested was prepared by diluting the test articles in culture medium. Twenty µL of ADC was added to the cells in triplicate wells with a final dose curve ranging from 3 µg/mL to 11 pg/mL in a 1:4 serial dilution series. The treated cells were cultured at 37°C / 5% CO_2 for 144 hours and the CellTiter-Glo® (CTG) Luminescent Viability Assay (Promega) was used to determine relative cytotoxicity as described above.

IC_{50}	MDA-MB-361	MCF-7
ConjA	14.5 ng/mL	~ 3µg/mL

Testing of ConjB and ConjC in cells with siRNA knock down of β-glucuronidase

HER2-overexpressing SKOV3 cells were subjected to reverse transfection to knock down GUSB expression levels. First, a 4x stock of RNAiMAX (final concentration of 0.125 ul/well, Life Technologies) and a 4x stock of siRNA oligos (Non-targeting/Negative siRNA, GAPDH siRNA and GUSB siRNA final concentration of 100 nM/well, Life Technologies) were prepared in OptiMEM medium. An RNAiMAX/OptiMEM only mixture was included as the lipid control. The 4x RNAiMAX solution and 4x siRNA solutions were brought to 2x by mixing in a one to one ratio, and the RNAiMAX/siRNA mixture was left to incubate at room temperature for 20 minutes. After incubation, 40 µl of the RNAiMAX/siRNA mixtures and lipid control were added per well to tissue culture-treated 96-well plates and 40 µl of cell solution was added onto the lipid/siRNA mixture, with a final plating density of 2000 cells/well. Plates were cultured at 37°C / 5% CO_2 for 72 hours at which point 20 µl of a 5X concentration of each dose of ConjB/ConjC was added to the cells in duplicate wells with a final dose curve ranging from 10 µg/mL to 0.15 ng/mL in a 1:4 serial dilution series. The ADC treated cells were cultured at 37°C / 5% CO_2 for an additional 72 hours and the CellTiter-Glo® (CTG) Luminescent Viability Assay (Promega) was used to determine relative cytotoxicity as described above. Efficiency of the siRNA knock down was determined by RNA expression levels and measurement of β-glucuronidase enzyme activity.

The results are shown in Figure 1 (ConjB) and Figure 2 (ConjC), wherein the followed symbols are used:

	Untrasfected
	Lipid Only
	Ne siRNA oligo2
	GUSB oligo3
	GAPDH oligo2
	Neg2 siRNA tox
	GUSB siRNA tox
	GAPDH siRNA tox

IC_{50} (ng/mL)	Untransfected	Neg siRNA oligo2	Lipid only	GUSB oligo3	GAPDH oligo2
ConjB	12.66	18.19	13.1	26.05	27.84
ConjC	4.871	13.07	5.818	31.59	23.00

Also noticeable is the much higher maximum kill for ConjC.

Example 10 – *in vitro* testing

Medium from sub-confluent (80-90% confluency) cell culture in a T75 flask was aspirated and the flask rinsed with PBS (about 20ml) and emptied. Trypsin-EDTA (5ml) was added, the flask returned to the 37°C gassed incubator for up to about 5 minutes, then rapped sharply to dislodge and dissociate cells from the plastic. The cell suspension was transferred to a sterile 50ml screw-top centrifuge tube, diluted with growth medium to a final volume of 15ml, then centrifuged (400g for 5 min). The supernatant was aspirated and the pellet re-suspended in 10ml culture medium. Repeated pipetting may be necessary to produce monodisperse cell suspensions. The cell concentration and viability are measured of trypan blue cell stained cells, and counted using the LUNA-II™ Automated Cell Counter. Cells were diluted to $2x10^5$/ml, dispensed (50µl /well) into 96 well flat bottom plates and incubated overnight before use.

A stock solution (1ml) of antibody drug conjugate (ADC) (20µg/ml) was made by dilution of filter-sterilised ADC into cell culture medium. A set of 8x 10-fold dilutions of stock ADC were made in a 24 well plate by serial transfer of 100µl onto 900µl of cell culture medium.

ADC dilution was dispensed (50µl/ well) into 4 replicate wells of the 96-well plate, containing 50µl cell suspension seeded the previous day. Control wells received 50µl cell culture medium. The 96-well plate containing cells and ADCs was incubated at 37°C in a CO_2-gassed incubator for the exposure time.

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

Cell Line	Description	ADC Exposure	Cell growth medium
MDA MB 468	HER2 negative, Breast carcinoma	4 days	RPMI 1640 with glutamax, 10% (v/v) HyClone™ Fetal Bovine Serum
NCIN87	Gastric carcinoma	7 days	RPMI 1640 with glutamax, 10% (v/v) HyClone™ Fetal Bovine Serum

ADC	**IC_{50} (nM) in:**	
	MDMA MB 468	**NCI-N87**
ConjA	>100	0.263
ConjB	>100	0.0222
ConjC	68	0.0013
ConjD	>100	0.185
ConjE	>100	0.0023
ConjF	81	0.0003

ADC	**EC_{50} (µg/ml)**
ConjA	0.0133
ConjB	0.00141
ConjC	0.00000187
ConjD	0.00271
ConjE	0.00000203
ConjF	3×10^{-16}
ConjG	0.00369
ConjH	0.000496
ConjI	0.00844
ConjJ	0.0829

Example 11 – Xenograft testing

NCI-N87 Xenografted Mice

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

Tumour Cell Culture

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

In Vivo Implantation and Tumour Growth

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

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

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

where w = width and l = length, in mm, of the tumour. Tumour weight may be estimated

with the assumption that 1 mg is equivalent to 1 mm^3 of tumour volume.

Treatment

Treatment began on Day 1 in groups of 10 mice (n=10) with established subcutaneous NCI-N87 tumours (108–144 mm^3). Trastuzumab-**10** (ConjA) was administered intravenously once on Day 1 (qd x 1). A vehicle-treated group served as the control group for efficacy analysis. Tumours were measured twice per week until the study was ended on Day 79. Each mouse was euthanized when its tumour reached the endpoint volume of 800 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

Treatment outcome was determined from percent tumour growth delay (%TGD), defined as the percent increase in median TTE for treated versus control mice, with differences between groups deemed statistically significant at $P \leq 0.05$ using logrank survival analysis. Mice were monitored for complete regression (CR) and partial regression (PR) responses.

Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. All regimens were well tolerated. The median TTE for vehicle-treated controls was 50.7 days, establishing a maximum possible TGD of 28.3 days (56%) for the 79-day study.

The minimum effective dose was defined as the minimum dosage required to produce tumour stasis for twenty-eight days after test article administration. Based on visual inspection of the mean and median tumour volume plots and percent change of tumour volume from Day 1, trastuzumab-**10** (ConjA) at 1.0 mg/kg, appeared to be the dosage that achieved a response consistent with the minimum effective dose

Two of the 0.3 mg/kg group animals were end of study survivors with an MTV of 708 mm^3. Eight of the 1.0 mg/kg group animals were end of study survivors with an MTV of 650 mm^3. The results are illustrated in Figure 3.

<u>JIMT-1 Xenografted Mice</u>

Mice

Female severe combined immunodeficient mice (Fox Chase SCID®, CB17/Icr-Prkdcscid/IcrIcoCrl, Charles River) were ten weeks old with a body weight (BW) range of 15.1 to 22.2 g on Day 1 of the study.

Tumour Cell Culture

JIMT-1 human breast carcinoma cells were grown in DMEM containing 10% fetal bovine serum, 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulphate, 25 µg/mL gentamicin, and 2 mM glutamine. Cell cultures were maintained in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO_2 and 95% air.

In Vivo Implantation and Tumour Growth

The JIMT-1 cells used for implantation were harvested during exponential growth and re-suspended in 50% Matrigel (BD Biosciences): 50% phosphate buffered saline at a concentration of 5 x 10^7 cells/mL. On the day of tumour implant, each test mouse was injected subcutaneously in the right flank with 1 x 10^7 cells (0.2 mL cell suspension), and tumour growth was monitored as the average size approached the target range of 100 to 150 mm^3. Tumours were measured in two dimensions using calipers, and volume was calculated using the formula:

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

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

Treatment

Treatment began on Day 1 in groups of 10 mice (n=10) with established subcutaneous JIMT-1 tumours with volume range (88-196 mm^3) and group mean timour volume of 120 to 122 mm^3. Trastuzumab-**10** (ConjA) was administered intravenously once on Day 1 (qd x 1). A vehicle-treated group served as the control group for efficacy analysis. Tumours were measured twice per week until the study was ended on Day 59. Each mouse was euthanized when its tumour reached the endpoint volume of 1000 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

Treatment outcome was determined from percent tumour growth delay (%TGD), defined as the percent increase in median TTE for treated versus control mice, with differences between groups deemed statistically significant at $P \leq 0.05$ using logrank survival analysis. Mice were monitored for complete regression (CR) and partial regression (PR) responses.

Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. All regimens were well tolerated. The median TTE for vehicle-treated controls was 48.4 days, establishing a maximum possible TGD of 10.6 days (22%) for the 59-day study. All ADC regimens tested produced significant survival benefit compared to vehicle-treated controls ($P < 0.01$).

The minimum effective dose was defined as the minimum dosage required to produce tumour stasis for twenty-eight days after test article administration. Based on visual inspection of the mean and median tumour volume plots and percent change of tumour volume from Day 1, trastuzumab-**10** (ConjA) at 1.0 mg/kg, appeared to be the dosage that achieved a response consistent with the minimum effective dose.

A dose-dependent effect was observed where the 0.3 mg/kg group median tumour volume slowly increased through Day 34 then progressed thereafter, while the 1.0 mg/kg group showed static median tumour volume through Day 38 which progressed thereafter. The median TTE for the 0.3 mg/kg group was 56.9 days corresponding to a significant TGD of 8.5 days (18%) versus the control ($P < 0.05$). The 0.3 mg/kg group had no objective regression responses. Five of the 0.3 mg/kg group animals were end of study survivors with an MTV of 486 mm^3.

The median TTE for the 1.0 mg/kg group was a maximal 59.0 days corresponding to a significant TGD of 10.6 days (22%) versus the control ($P < 0.001$). The 1.0 mg/kg group had no objective regression responses. Nine of the 1.0 mg/kg group animals were 'end of study' survivors with an MTV of 446 mm3. The 0.3 and 1.0 mg/kg groups were significantly different from one another ($P < 0.05$).

The results are illustrated in Figure 4.

Example 12 – Toxicity studies/Therapeutic Index

Rat study:

A single dose toxicity study was used to determine the maximum tolerated dose (MTD) and safety profile of Trastuzumab-**10** (ConjA). Male Sprague Dawley rats (ENVIGO) were dosed once by slow bolus intravenous injection via the tail vein with vehicle control (25mM Histidine, 200mM Sucrose, pH 6.0) or test material (ConjA). Parameters evaluated during the study included mortality, physical examinations, cageside observations, body weights, body weight changes, clinical pathology (clinical chemistry, hematology, and coagulation),

and gross pathology findings. All animals in groups 1-6 were terminated on Study Day (SD) 29, except one animal in Group 6 who was terminated on Study Day (SD) 25.

Group	Treatment	Dose Route	Dose (mg/kg)	Frequency	Male Rats Main Study N
1	Control	IV	0	Single	5
2	ConjA	IV	4	Single	5
3	ConjA	IV	6	Single	5
4	ConjA	IV	8	Single	5
5	ConjA	IV	12	Single	5
6	ConjA	IV	14	Single	5

Control = 25 mM Histidine-HCl, 200mM sucrose, pH 6.0

Tolerability was determined based on toxicity end points, including decreased absolute reticulocytes, white blood cells and cellular components, as well as macroscopic and microscopic lesions in the lungs. The major finding at dose levels of ≥ 4 mg/kg were histopathological observations of lung lesions. At the 4 mg/kg dose, findings were mild and considered reversible. As the dose increased, the lung lesions were of increasing severity and were not considered reversible. Based on this finding, the maximum tolerated dose (MTD) in the rat after a single dose of ConjA was determined to be 6mg/kg.

Therapeutic Index

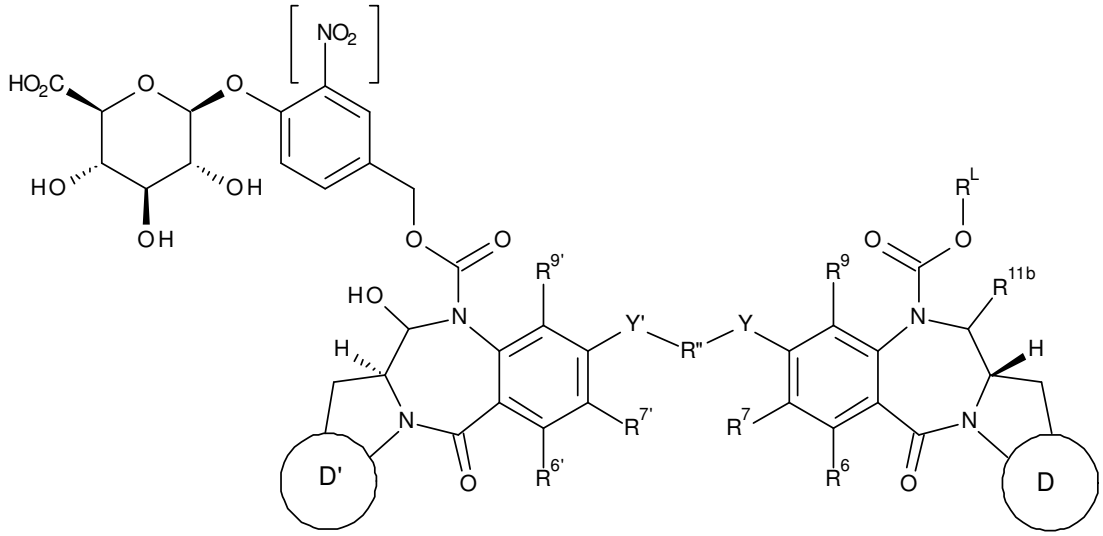
The Therapeutic Index can be calculated by dividing the maximum tolerated single dose (MTD) of non-targeted ADC in rat, by the minimal effective single dose (MED) of the a targeted ADC. The MED is the single dose necessary to achieve tumour stasis in an *in vivo* model at 28 days (for NCI-N87 xenograft).

Thus for conjugates of compound **10**, the therapeutic index is the MTD of 6 mg/kg divided by the MED which is 1.0 mg/kg (see above), giving a Therapeutic Index of 6.

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

CLAIMS

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

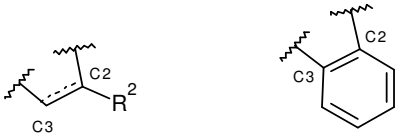


I

and salts and solvates thereof, wherein:

the square brackets indicate the NO_2 group is optional;

D represents either group D1 or D2:



D1 D2

;

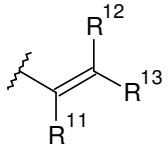
the dotted line indicates the optional presence of a double bond between C2 and C3;

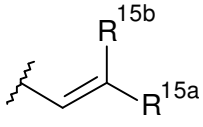
when there is a double bond present between C2 and C3, R^2 is selected from the group consisting of:

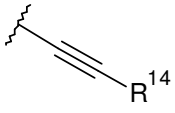
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

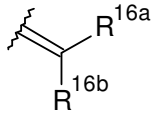
(ic) C_{3-6} saturated cycloalkyl;

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

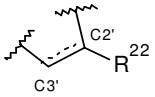
(ie)  , wherein one of R^{15a} and R^{15b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

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

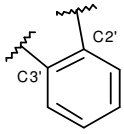
when there is a single bond present between C2 and C3,

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

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



D'1



D'2

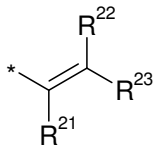
wherein the dotted line indicates the optional presence of a double bond between C2' and C3';

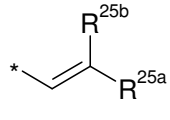
when there is a double bond present between C2' and C3', R^{12} is selected from the group consisting of:

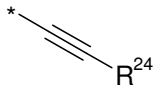
(iia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene;

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

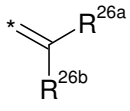
(iic) C_{3-6} saturated cycloalkyl;

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

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

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

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

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

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

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

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

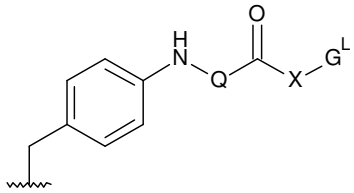
R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

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

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

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

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

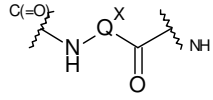


IIIa

,

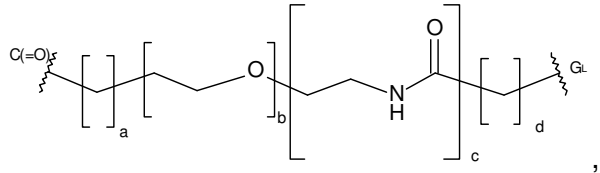
wherein

Q is:



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

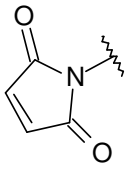
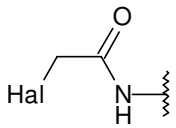
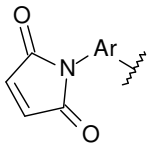
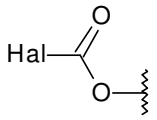
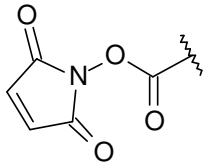
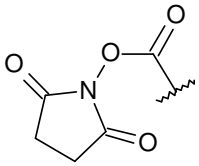
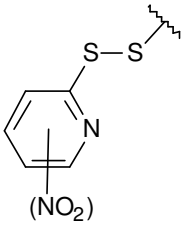
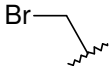
X is:

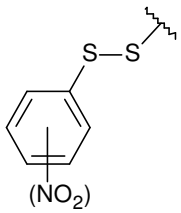
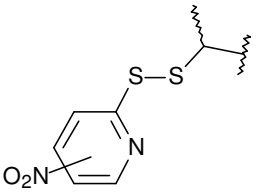
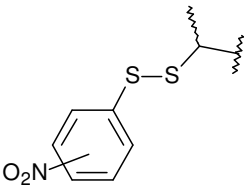


,

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

G^L is selected from:

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

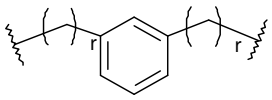
(G^{L3-2})	 where the NO_2 group is optional	(G^{L8})	
(G^{L3-3})	 where the NO_2 group is optional	(G^{L9})	
(G^{L3-4})	 where the NO_2 group is optional		

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

2. A compound according to claim 1, wherein:

a) both Y and Y' are O; and/or

b) R'' is C_{3-7} alkylene or a group of formula:



where r is 1 or 2; and/or

c) R^9 is H; and /or

d) R^6 is H; and/or

e) R^7 is selected from H, OH and OR or a C_{1-4} alkyloxy group.

3. A compound according to claim 1 or claim 2, wherein:

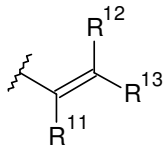
a) D is D1, there is a double bond between C2 and C3, and R^2 is a C_{5-7} aryl group, wherein R^2 optionally bears one to three substituent groups selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl; or

b) D is D1, there is a double bond between C2 and C3, and R^2 is a C_{8-10} aryl group, wherein R^2 optionally bears one to three substituent groups selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl; or

c) D is D1, there is a double bond between C2 and C3, and R^2 is methyl, ethyl or propyl; or

d) there is a double bond between C2 and C3, and R^2 is cyclopropyl; or

e) wherein D is D1, there is a double bond between C2 and C3, and R^2 is a group of formula:



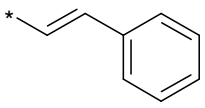
, wherein:

i) the total number of carbon atoms in the R^2 group is no more than 4; and /or

ii) one of R^{11}, R^{12} and R^{13} is H, with the other two groups being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl; or

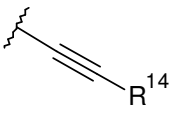
iii) two of R^{11}, R^{12} and R^{13} are H, with the other group being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl; or

f) D is D1, there is a double bond between C2 and C3, and R^2 is the group:



; or

g) D is D1, there is a double bond between C2 and C3, and R^2 is a group of formula:

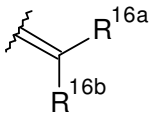


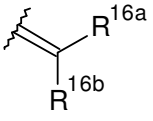
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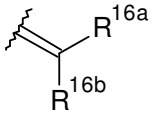
wherein R^{14} is selected from H, methyl, ethyl, ethenyl and ethynyl.

4. A compound according to claim 1 or claim 2, wherein

a) D is D1, there is a single bond between C2 and C3, and R^2 is H; or

b) D is D1, there is a single bond between C2 and C3, R^2 is  and R^{16a} and R^{16b} are both H; or

c) D is D1, there is a single bond between C2 and C3, R^2 is , and R^{16a} and R^{16b} are both methyl; or

d) D is D1, there is a single bond between C2 and C3, R^2 is , one of R^{16a} and R^{16b} is H, and the other is selected from C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted.

5. A compound according to any one of claims 1 to 4, wherein:

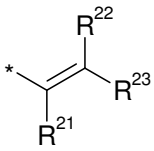
a) D' is D'1, there is a double bond between C2' and C3', and R^{12} is a C_{5-7} aryl group, wherein R^{12} optionally bears one to three substituent groups selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl; or

b) D' is D'1,there is a double bond between C2' and C3', and R^{12} is a C_{8-10} aryl group, wherein R^{12} optionally bears one to three substituent groups selected from methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl; or

c) D' is D'1, there is a double bond between C2' and C3', and R^{12} is methyl, ethyl or propyl; or

d) D' is D'1, there is a double bond between C2' and C3', and R^{12} is cyclopropyl; or

e) D' is D'1, there is a double bond between C2' and C3', and R^{12} is a group of formula:



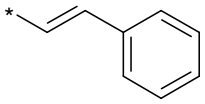
wherein:

i) the total number of carbon atoms in the R^{12} group is no more than 3;

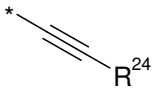
ii) one of R^{21}, R^{22} and R^{23} is H, with the other two groups being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl; or

iii) 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; or

f) D' is D'1, there is a double bond between C2' and C3', and R^{12} is the group:


; or

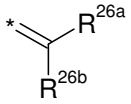
g) D' is D'1, there is a double bond between C2' and C3', and R^{12} is a group of formula:

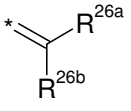


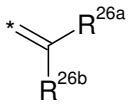
wherein R^{24} is selected from H, methyl, ethyl, ethenyl and ethynyl.

6. A compound according to any one of claims 1 to 4, wherein

a) D' is D'1, there is a single bond between C2' and C3', and R^{12} is H; or

b) D' is D'1, there is a single bond between C2' and C3', R^{12} is  and R^{26a} and R^{26b} are both H; or

c) D' is D'1, there is a single bond between C2' and C3', R^{12} is , and R^{26a} and R^{26b} are both methyl; or

d) D' is D'1, there is a single bond between C2' and C3', R^{12} is  , one of R^{26a} and R^{26b} is H, and the other is selected from C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted.

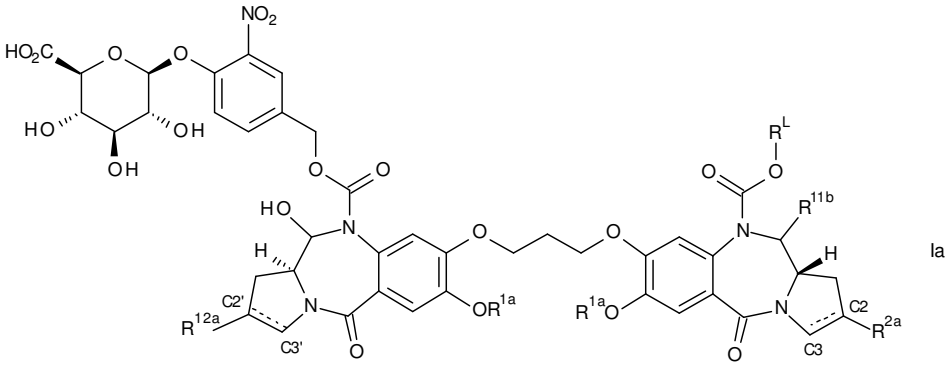
7. A compound according to any one of claims 1 to 6, wherein

a) $R^{6'}$ is selected from the same groups as R^6, $R^{7'}$ is selected from the same groups as R^7, $R^{9'}$ is selected from the same groups as R^9 and Y' is selected from the same groups as Y; and/or

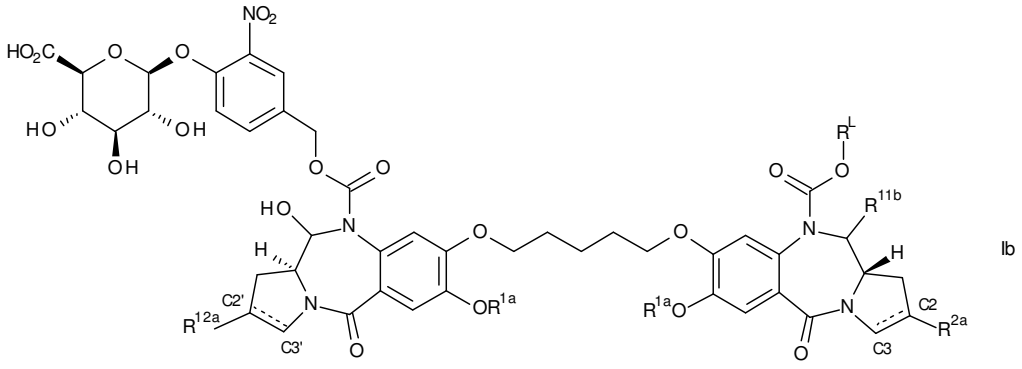
b) $R^{6'}$ is the same groups as R^6, $R^{7'}$ is the same groups as R^7, $R^{9'}$ is the same groups as R^9 and Y' is the same groups as Y; and/or

c) R^{12} is the same group as R^2.

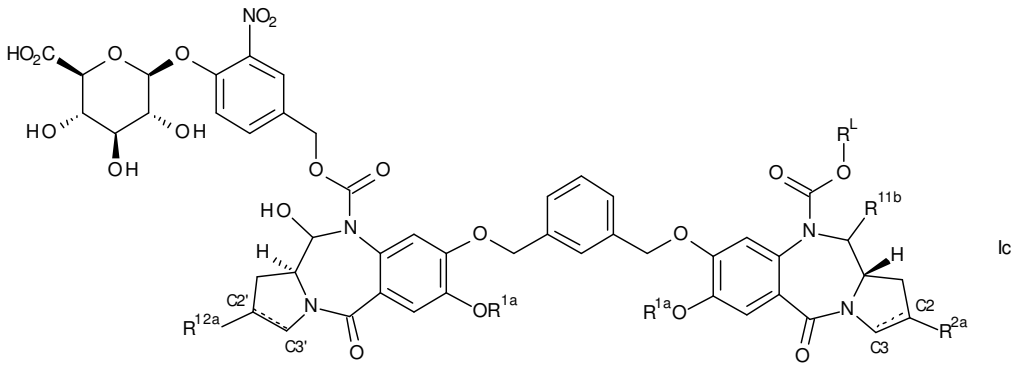
8. A compound according to claim 1, which is of formula Ia-1, Ia-2 or Ia-3:



Ia

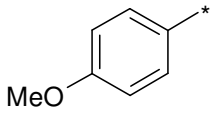


Ib

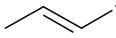


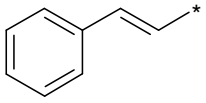
Ic

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

(a)  ;

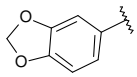
(b)  ;

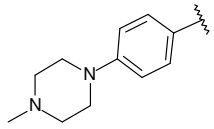
(c)  ;

(d)  ;

(e)  ;

(f)  ;

(g)  ; and

(h)  ;

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

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

9. A compound according to claim 1 or claim 2, wherein both R^2 and R^{12} comprise no more than 3 carbon atoms.

10. A compound according to any one of claims 1 to 9, wherein R^{11b} is OH or OR^A, where R^A is C_{1-4} alkyl, optionally wherein R^A is methyl.

11. A compound according to any one of claims 1 to 10, wherein Q^x is:

a) an amino acid residue selected from Phe, Lys, Val, Ala, Cit, Leu, Ile, Arg, and Trp; or

b) a dipeptide residue selected from:

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

c) a tripeptide residue.

12. A compound according to any one of claims 1 to 11, wherein

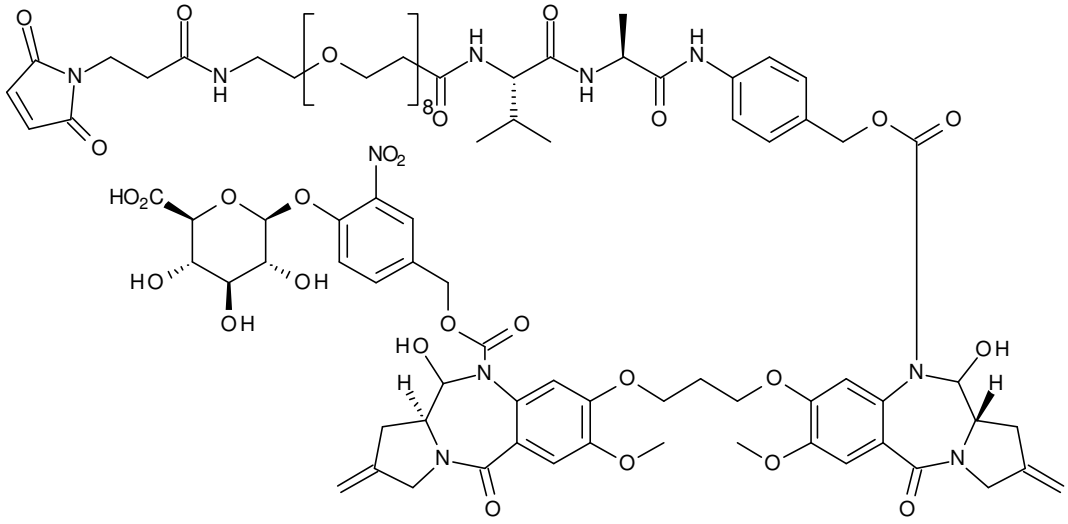
a) a is 0 to 3; and/or

b) b is 0 to 12; and/or

c) d is 0 to 3.

13. A compound according to any one of claims 1 to 11, wherein a is 0, c is 1 and d is 2, and b is from 0 to 8.

14. A compound according to claim 1, wherein the compound is of formula Id:



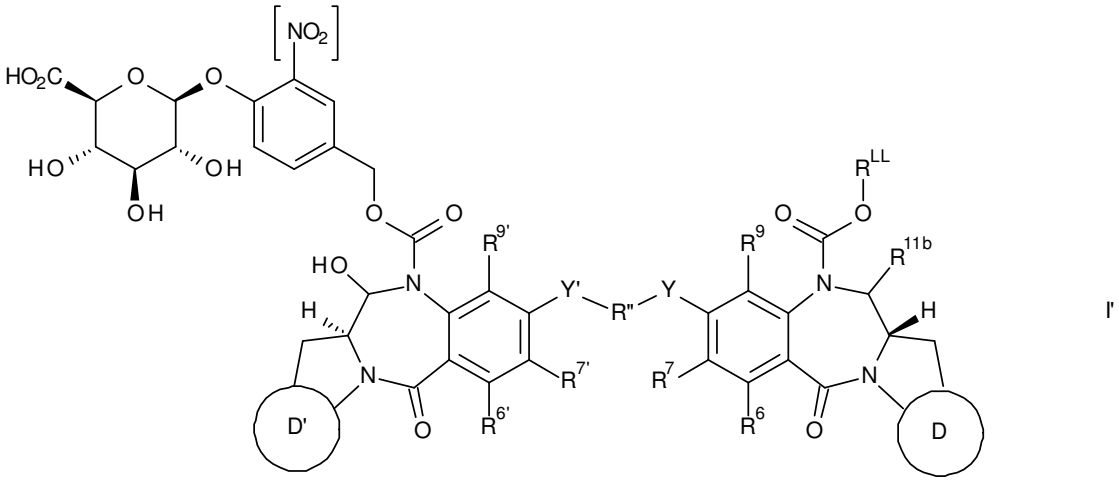
(Id)

.

15. A conjugate of formula I:

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

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

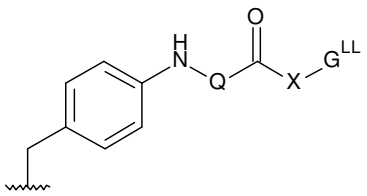


I'

wherein D, R^2, R^6, R^7, R^9, R^{11b}, Y, R", Y', D', $R^{6'}$, $R^{7'}$, $R^{9'}$ and R^{12}, including the presence or absence of double bonds between C2 and C3 and C2' and C3' respectively, are as defined in any one of claims 1 to 10;

wherein p is an integer from 1 to 20;

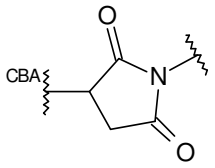
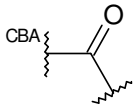
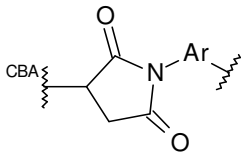
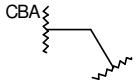
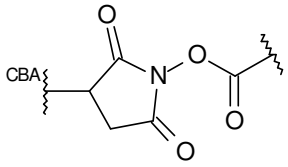
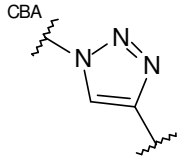
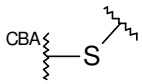
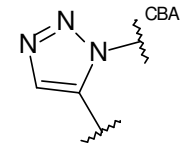
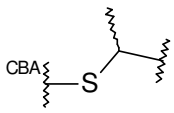
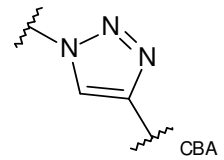
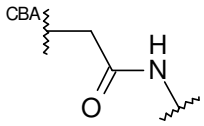
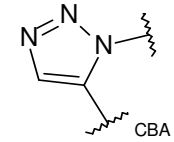
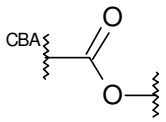
R^{LL} is a linker for connection to a cell binding agent, which is:



IIIa'

,

where Q and X are as defined in any one of claims 1 and 11 to 13 and G^{LL} is selected from:

(G^{LL1-1})		(G^{LL6})	
(G^{LL1-2})		(G^{LL7})	
(G^{LL2})		(G^{LL8-1})	
(G^{LL3-1})		(G^{LL8-2})	
(G^{LL3-2})		(G^{LL9-1})	
(G^{LL4})		(G^{LL9-2})	
(G^{LL5})			

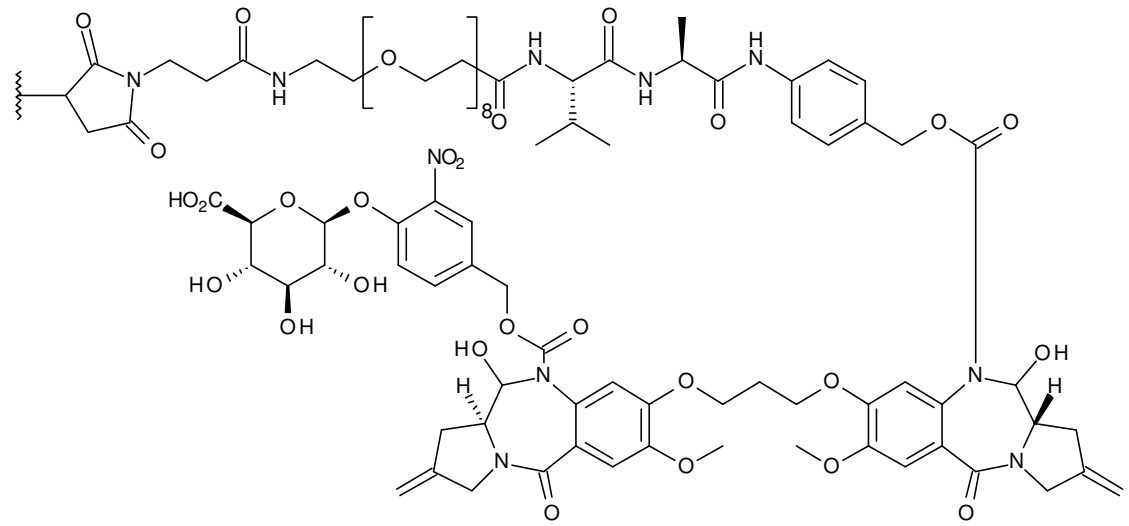
where Ar represents a C_{5-6} arylene group.

16. A compound according to any one of claims 1 to 14 or a conjugate according to claim 15, wherein

a) Ar is a phenylene group; and/or

b) G^{LL} is selected from G^{LL1-1} and G^{LL1-2}.

17. A conjugate according to claim 15, wherein D^{L} is of formula (Id'):



(Id')

.

18. A conjugate according to any one of claims 15 to 17, wherein the Ligand Unit is an antibody or an active fragment thereof.

19. The conjugate according to claim 18, wherein the antibody or antibody fragment is an antibody or antibody fragment for a tumour-associated antigen, optionally wherein the antibody or antibody fragment is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(88):

(1) BMPR1B;
(2) E16;
(3) STEAP1;
(4) 0772P;
(5) MPF;
(6) Napi3b;
(7) Sema 5b;
(8) PSCA hlg;
(9) ETBR;
(10) MSG783;
(11) STEAP2;
(12) TrpM4;
(13) CRIPTO;
(14) CD21;
(15) CD79b;
(16) FcRH2;

(17) HER2;

(18) NCA;

(19) MDP;

(20) IL20R-alpha;

(21) Brevican;

(22) EphB2R;

(23) ASLG659;

(24) PSCA;

(25) GEDA;

(26) BAFF-R;

(27) CD22;

(28) CD79a;

(29) CXCR5;

(30) HLA-DOB;

(31) P2X5;

(32) CD72;

(33) LY64;

(34) FcRH1;

(35) IRTA2;

(36) TENB2;

(37) PSMA – FOLH1;

(38) SST;

(38.1) SSTR2;

(38.2) SSTR5;

(38.3) SSTR1;

(38.4)SSTR3;

(38.5) SSTR4;

(39) ITGAV;

(40) ITGB6;

(41) CEACAM5;

(42) MET;

(43) MUC1;

(44) CA9;

(45) EGFRvIII;

(46) CD33;

(47) CD19;

(48) IL2RA;

(49) AXL;

(50) CD30 - TNFRSF8;

(51) BCMA - TNFRSF17;

(52) CT Ags – CTA;

(53) CD174 (Lewis Y) - FUT3;

(54) CLEC14A;

(55) GRP78 – HSPA5;

(56) CD70;

(57) Stem Cell specific antigens;

(58) ASG-5;

(59) ENPP3;

(60) PRR4;

(61) GCC – GUCY2C;

(62) Liv-1 – SLC39A6;

(63) 5T4;

(64) CD56 – NCMA1;

(65) CanAg;

(66) FOLR1;

(67) GPNMB;

(68) TIM-1 – HAVCR1;

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

(70) B7-H4 – VTCN1;

(71) PTK7;

(72) CD37;

(73) CD138 – SDC1;

(74) CD74;

(75) Claudins – CLs;

(76) EGFR;

(77) Her3;

(78) RON - MST1R;

(79) EPHA2;

(80) CD20 – MS4A1;

(81) Tenascin C – TNC;

(82) FAP;

(83) DKK-1;

(84) CD52;

(85) CS1 - SLAMF7;
(86) Endoglin – ENG;
(87) Annexin A1 – ANXA1;
(88) V-CAM (CD106) - VCAM1.

20. The conjugate of claim 18 or claim 19 wherein the antibody or antibody fragment is a cysteine-engineered antibody.

21. The conjugate according to any one of claims 15 to 20 wherein p is an integer from 1 to 8.

22. A composition comprising a mixture of conjugates according to any one of claims 15 to 21, wherein the average p in the mixture of conjugate compounds is about 1 to about 8.

23. The conjugate according to any one of claims 15 to 21, for use in therapy.

24. A pharmaceutical composition comprising the conjugate of any one of claims 15 to 21 and a pharmaceutically acceptable diluent, carrier or excipient.

25. The conjugate according to any one of claims 15 to 21 or the pharmaceutical composition according to claim 24, for use in the treatment of a proliferative disease in a subject.

26. Use of a conjugate according to any one of claims 15 to 21 in a method of manufacture of a medicament for the treatment of a proliferative disease.

27. The conjugate according to clam 25 or the use according to claim 26, wherein the proliferative disease is a cancer.