Abstract:

A compound which is selected from A, B and C, and salts and solvates thereof, as well as conjugates thereof with cell binding agents.
PYRROLOBENZODIAZEPINES AND CONJUGATES THEREOF

The present invention relates to pyrrolobenzodiazepines (PBDs), in particular pyrrolobenzodiazepines having a labile C2 protecting group, in the form of a linker to a cell binding agent.

Background to the invention

Pyrrolobenzodiazepines


They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-
CH(OMe)) at the N10-C1 1 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (S)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In Antibiotics Ill. Springer-Verlag, New York, pp. 3-1 1 (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.

A particularly advantageous pyrrolobenzodiazepine compound is described by Gregson et al. (Chem. Commun. 1999, 797-798) as compound 1, and by Gregson et al. (J. Med. Chem. 2001, 44, 1161-1 174) as compound 4a. This compound, also known as SG2000, is shown below:

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SG2000
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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.

The present inventors have described dimer PBD compounds having linker groups for connection to a cell binding agent, such as an antibody, in WO 2011/130613 and WO 2011/130616. The linker in these compounds is attached to the PBD core via the C2 position, and are generally cleaved by action of an enzyme on the linker group.

*Antibody-drug conjugates*


The present inventors have developed particular PBD dimers with linking groups for the formation of PBD conjugates with cell binding agents, and in particular PBD antibody conjugates.
Summary of the Invention

In a first aspect, the present invention provides a compound which is selected from A:

![Chemical structure A](image)

B:

![Chemical structure B](image)

and C:

![Chemical structure C](image)

and salts and solvates thereof.

WO 2011/130615 discloses compound 26:

![Chemical structure 26](image)
which is the parent compound of A. Compound A comprises this PBD with a linker for attachment to a cell binding agent. The cell binding agent provides a number of ethylene glycol moieties to provide solubility which is useful in the synthesis of conjugates.

WO 2010/043380 and WO 2011/130613 disclose compound 30:

![Compound 30 diagram]

WO 2011/130613 also discloses compound 51:

![Compound 51 diagram]

Compound B differs from compound 30 by only having a \((\text{CH}_2)_3\) tether between the PBD moieties, instead of a \((\text{CH}_2)_5\) tether, which reduces the lipophilicity of the released PBD dimer. The linking group is attached to the C2-phenyl group in the para rather than meta position.

WO 2011/130613 discloses compound 93:

![Compound 93 diagram]

Compound C differs from this in two respects. The cell binding agent provides an increased number of ethylene glycol moieties to provide solubility which is useful in the synthesis of conjugates, and the phenyl substituent provide two rather than one oxygen atom, which also aids solubility. Compound C's structure may also mean it binds more strongly in the minor groove.
Compounds A, B and C have two sp² centres in each C-ring, which may allow for stronger binding in the minor groove of DNA, than for compounds with only one sp² centre in each C-ring.

A second aspect of the present invention provides a conjugate of formula ConjA:

where CBA represents a cell binding agent. The link to the moiety shown is via a free S (active thiol) on the cell binding agent.
Detailed Description of the Invention

The present invention provides a PBD dimer with a linker connected through the C2 position on one of the PBD moieties suitable for forming a PBD dimer conjugated via the linker to a cell binding agent.

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

ConjB would release the compound RelB:

And ConjC would release the compound RelC:

A further aspect of the present invention is the compounds RelB, and salts and solvates thereof.

A further aspect of the present invention is the compounds RelC, and salts and solvates thereof.

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

Delivery of the compounds of formulae RelA, RelB or RelC is achieved at the desired activation site of the conjugates of formulae ConjA, ConjB or ConjC by the action of an enzyme, such as cathepsin, on the linking group, and in particular on the valine-alanine dipeptide moiety.

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: NAVPNLRGDLQVLAAQKVRTC. Alternatively, a variant of the A20FMDV-Cys sequence may be used wherein one, two, three, four, five, six, seven, eight, nine or ten amino acid residues are substituted with another amino acid residue. Furthermore, the polypeptide may have the sequence NAVXXXXXXXXXXXXXXXRTC.

Antibodies
The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity.
(Miller et al (2003) J. Immunol. 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 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 \( \alpha \), \( \delta \), \( \epsilon \), \( \gamma \), and \( \mu \), 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 VH or VL domain of a given non-human antibody specific for a particular epitope with a human VH or VL 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 antibody 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,

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.

10 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  Gl:169790809

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

**Polypeptide**

Genbank accession no. NP_001194

Genbank version no. NP_001194.1  Gl:4502431

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

**Cross-references**

(1997); WO2004/063362 (Claim 2); WO2003/042661 (Claim 12);

US2003/134790-A1 (Page 38-39); WO2002/1 02235 (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);
15 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  Gl:71979931

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

Cross references
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);

(3) STEAP1 (six transmembrane epithelial antigen of prostate)
Nucleotide
Genbank accession no. NM_012449
Genbank version no. NM_012449.2 Gl:22027487
Genbank record update NM_015923.

Polypeptide
Genbank accession no. NP_036581
Genbank version no. NP_036581.1 Gl:9558759
Genbank record update date: Sep 9, 2012 02:57 PM

Cross references
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,
35 Example 53; Page 173, Example 2; Fig 2A); six transmembrane epithelial antigen of the prostate; MIM:604415.

(4) 0772P (CA125, MUC16)

5 Nucleotide
Genbank accession no. AF361486
Genbank version no. AF361486.3 GI:34501466
Genbank record update date: Mar 11, 2010 07:56 AM

10 Polypeptide
Genbank accession no. AAK74120
Genbank version no. AAK74120.3 GI:34501467
Genbank record update date: Mar 11, 2010 07:56 AM

15 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); Gl:34501467;

20 (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

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

30 Cross references
(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b)

**Nucleotide**

5 Genbank accession no. NM_006424
Genbank version no. NM_006424.2 GI:1 1061 1905
Genbank record update date: Jul 22, 2012 03:39 PM

**Polypeptide**

10 Genbank accession no. NP_006415
Genbank version no. NP_006415.2 GI:1 1061 1906
Genbank record update date: Jul 22, 2012 03:39 PM

**Cross references**


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

**Nucleotide**

25 Genbank accession no. AB040878
Genbank version no. AB040878.1 GI:7959148
Genbank record update date: Aug 2, 2006 05:40 PM

**Polypeptide**

30 Genbank accession no. BAA95969
Genbank version no. BAA95969.1 GI:7959149
Genbank record update date: Aug 2, 2006 05:40 PM

**Cross references**

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

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

Nucleotide
Genbank accession no. AY358628
Genbank version no. AY358628.1 GI:37182377
Genbank record update date: Dec 1, 2009 04:15 AM

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

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

(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

(10) MSG783 (RNF124, hypothetical protein FLJ20315)

Nucleotide
Genbank accession no. NM_017763
Genbank version no. NM_017763.4 GM67830482
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
WO2003/1 04275 (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  
5 Genbank accession no. AF455138  
Genbank version no. AF455138.1 GI:22655487  
Genbank record update date: Mar 11, 2010 01:54 AM  

Polypeptide  
10 Genbank accession no. AAN04080  
Genbank version no. AAN04080.1 GI:22655488  
Genbank record update date: Mar 11, 2010 01:54 AM  

Cross references  
15 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  
20 Genbank accession no. NM_017636  
Genbank version no. NM_017636.3 GI:304766649  
Genbank record update date: Jun 29, 2012 11:27 AM  

Polypeptide  
25 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  
(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
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 GM81939
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide
Genbank accession no. AAA35786
Genbank version no. AAA35786.1 GM81940
Genbank record update date: Jun 23, 2010 08:47 AM
Cross references

(15) CD79b (CD79B, Ω 79b, IgB (immunoglobulin-associated beta), B29)
Nucleotide
Genbank accession no NM_000626
Genbank version no. NM_000626.2 Gl:90193589
Genbank record update date: Jun 26, 2012 01:53 PM

Polypeptide
Genbank accession no. NP_000617
Genbank version no. NP_000617.1 Gl:1 1038674
Genbank record update date: Jun 26, 2012 01:53 PM

Cross references

(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 Gl:227430280
Genbank record update date: Jun 30, 2012 12:30 AM
Polypeptide
Genbank accession no. NP_1 10391
Genbank version no. NP_1 10391.2 Gl:1 9923629
Genbank record update date: Jun 30, 2012 12:30 AM

Cross references

(17) HER2 (ErbB2)

Nucleotide
Genbank accession no M11730
Genbank version no. M11730.1 Gl:1 83986
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide
Genbank accession no. AAA75493
Genbank version no. AAA75493.1 Gl:306840
Genbank record update date: Jun 23, 2010 08:47 AM

Cross references

35 WO2002/13847 (Page 71-74); WO2002/14503 (Page 114-1 17); WO2001/53463 (Claim 2; Page 41-46); WO2001/41787 (Page 15); WO2000/44899 (Claim 52; Fig 7); WO2000/20579
(Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61);
EP1439393 (Claim 7); WO2004/043361 (Claim 7); WO2004/022709; WO2001/00244
25 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761;
AAA35808.1

ANTIBODIES
Abbott: US201 101 77095
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: US201001 1951 1
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 BIIB71 F10 (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)
US201 101 17097
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
35 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 Shanghai Hospital Cancer Cent: HMTI-Fc Ab - Gao J., et al BMB Rep. 2009 Oct 31;42(10):636-41.

Symphogen: US2011 10217305


(18) NCA (CEACAM6)
Nucleotide
Genbank accession no M18728
Genbank version no. M18728.1 Gl:189084
Genbank record update date: Jun 23, 2010 08:48 AM
Polypeptide
Genbank accession no. AAA59907
Genbank version no. AAA59907.1 GM89085
Genbank record update date: Jun 23, 2010 08:48 AM

Cross references
EMBL; M18728.

(19) MDP (DPEP1)
Nucleotide
Genbank accession no BC017023
Genbank version no. BC017023.1 GM6877538
Genbank record update date: Mar 6, 2012 01:00 PM

Polypeptide
Genbank accession no. AAH17023
Genbank version no. AAH17023.1 Gl: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); W099/46284 (Fig 9); MIM:179780.

(20) IL20R-alpha (IL20Ra, ZCYTOR7)
Nucleotide
Genbank accession no AF184971
Genbank version no. AF184971.1 Gl:6013324
Genbank record update date: Mar 10, 2010 10:00 PM
Polypeptide
Genbank accession no. AAF01320
Genbank version no. AAF01320.1 Gl:6013325
Genbank record update date: Mar 10, 2010 10:00 PM

Cross references
EP1 394274 (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); W098/37193 (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 Gl:10798902
Genbank record update date: Mar 11, 2010 12:58 AM

Polypeptide
Genbank accession no. AAG23135
Genbank version no. AAG23135.1 Gl:10798903
Genbank record update date: Mar 11, 2010 12:58 AM

Cross references
US2003/19122 (Claim 1; 20 Fig 52); US2003/19126 (Claim 1); US2003/19121 (Claim 1; Fig 52); US2003/19129 (Claim 1); US2003/19130 (Claim 1); US2003/19128 (Claim 1; Fig 52); US2003/19125 (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 Gl:1 11118979
Genbank record update date: Sep 8, 2012 04:43 PM

Polypeptide
Genbank accession no. NP_004433
Genbank version no. NP_004433.2 Gl:21396504
Genbank record update date: Sep 8, 2012 04:43 PM

Cross references

(23) ASLG659 (B7h)

Nucleotide
Genbank accession no. AX092328
Genbank version no. AX092328.1 GM3444478
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-1 B); 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
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); W09/51824 (Claim 10; Page 94);
WO98/40403 (Claim 2; Fig 1b); Accession: 043653; 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=AAP1 4954.1 - Homo sapiens
(human); WO2003/0541 52 (Claim 20); WO2003/000842 (Claim 1); WO2003/02301 3
(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 AF1 16456
Genbank version no. AF1 16456.1 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
(5537), 2108-21 11 (2001); WO2004/058309; WO2004/01 161 1; WO2003/045422 (Example;
Page 32-33); WO2003/014294 (Claim 35; Fig 6B); WO2003/035846 (Claim 70; Page 615-
616); WO2002/94852 (Col 136-137); WO2002/38766 25 (Claim 3; Page 133);
WO2002/24909 (Example 3; Fig 3); MIM:606269; NP_443177.1; NM_052945_1; AF132600

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

Nucleotide
Genbank accession no AK026467
Genbank version no. AK026467.1 GM0439337
Genbank record update date: Sep 11, 2006 11:24 PM

Polypeptide
Genbank accession no. BAB15489
Genbank version no. BAB15489.1 GM0439338
Genbank record update date: Sep 11, 2006 11:24 PM

Cross references
Wilson et al (1991) J. Exp. Med. 173:137-146; 30 WO2003/072036 (Claim 1; Fig 1);
IM:107266; NP_001762.1; NM_001771_1.
(27a) CD22 (CD22 molecule)

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

**Polypeptide**
Genbank accession no. CAA36988
Genbank version no. CAA36988.1  Gl: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**


(28) CD79a (CD79A, CD79alpha), immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M
35 molecules, transduces a signal involved in B-cell differentiation), pi: 4.84, MW: 25028 TM: 2

[P] Gene Chromosome: 19q13.2)
(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,
(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 Gl:1 18402587
Genbank record update date: Sep 8, 2012 04:46 PM

Polypeptide
Genbank accession no. NP_002111
Genbank version no. NP_002111.1 Gl:4504403
Genbank record update date: Sep 8, 2012 04:46 PM

Cross references
W099/58658 (claim 13, Fig 15); US61 53408 (Col 35-38); US5976551 (col 168-170);

(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 Gl:325197202
Genbank record update date: Jun 27, 2012 12:41 AM

Polypeptide
Genbank accession no. NP_002552
Cross references


(32) CD72 (B-cell differentiation antigen CD72, Lyb-2); 359 aa, pi: 8.66, MW: 40225, TM: 1


Nucleotide
Genbank accession no NM_001782
Genbank version no. NM_001782.2 Gl:194018444
Genbank record update date: Jun 26, 2012 01:43 PM

Polypeptide
Genbank accession no. NP_001773
Genbank version no. NP_001773.1 Gl:4502683
Genbank record update date: Jun 26, 2012 01:43 PM

Cross references

(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 erythematosus); 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 Gl:167555126
Genbank record update date: Sep 2, 2012 01:50 PM

Polypeptide
Genbank accession no. NP_005573
**Cross references**


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

**Nucleotide**

Genbank accession no NM_052938
Genbank version no. NM_052938.4 Gl:226958543

15 Genbank record update date: Sep 2, 2012 01:43 PM

**Polypeptide**

Genbank accession no. NP_443170
Genbank version no. NP_443170.1 GM6418419

20 Genbank record update date: Sep 2, 2012 01:43 PM

**Cross references**


(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 GM3591709

Genbank record update date: Mar 11, 2010 01:16 AM

35
Polypeptide
Genbank accession no. AAK31325
Genbank version no. AAK31325.1  Gl: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_12571.1;

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

Nucleotide
Genbank accession no AF179274
Genbank version no. AF179274.2  GM2280939
Genbank record update date: Mar 11, 2010 01:05 AM

Polypeptide
Genbank accession no. AAD55776
Genbank version no. AAD55776.2  Gl: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;
Commun. 266:593-602; Liang et a/ (2000) Cancer Res. 60:4907-12; Glynne-Jones et al
(37) PSMA - FOLH1 (Folate hydrolase (prostate-specific membrane antigen) 1)

Nucleotide
Genbank accession no M99487
Genbank version no. M99487.1 GM90663

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

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 1; NAALADase 1; cell growth-inhibiting gene 27 protein; folylpolygamma-glutamate carboxypeptidase; glutamate carboxylase II; glutamate carboxypeptidase 2; glutamate carboxypeptidase II; membrane glutamate carboxypeptidase; prostate specific membrane antigen variant F; pteroyl/poly-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.


Cytogen: monoclonal antibodies 7E1 1-C5 (ATCC accession No. HB 10494) and 9H10-A4 (ATCC accession No. HB1 1430) - 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, 4E10-1.14, 3E1.1, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6, 4C8B9, and monoclonal antibodies. Hybridomas secreting 3F5.4G6, 3D7.1, 4E10-1.14, 3E1.1, 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, 8A1.1, 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, 8A1.1, 16F9, 2A1.0, 2C6, 2F5 and 1C3 are shown in SEQ ID NOs: 1-9, respectively. The V.sub.L amino acid sequences of 4A3, 7F12, 8C12, 8A1.1, 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, 3D7-1.1 having ATCC accession number
HB12309, 4E10-1 .14 having ATCC accession number HB12310, 3E1 1 (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 / Progenies / 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/F1 1 (Wolf P., et al Prostate. 2010 Apr 1;70(5):562-9).

(38) SST (Somatostatin Receptor; note that there are 5 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

Other information
Official Symbol: SSTR2
Other Designations: SRIF-1; SS2R; somatostatin receptor type 2

(38.2) SSTR5 (Somatostatin receptor 5)

**Nucleotide**

5 Genbank accession no D16827
Genbank version no. D16827.1 Gl:487683
Genbank record update date: Aug 1, 2006 12:45 PM

**Polypeptide**

10 Genbank accession no. BAA04107
Genbank version no. BAA04107.1 Gl:487684
Genbank record update date: Aug 1, 2006 12:45 PM

**Cross references**


**Other information**

Official Symbol: SSTR5
OtherAliases: SS-5-R

20 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**

30 Genbank accession no M14648 J02826 M18365
Genbank version no. M14648.1 Gl:340306
Genbank record update date: Jun 23, 2010 08:56 AM

**Polypeptide**

35 Genbank accession no. AAA36808
Genbank version no. AAA36808.1 Gl:340307
Genbank record update date: Jun 23, 2010 08:56 AM

Cross references

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

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.


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

Nucleotide

Genbank accession no M17303
Genbank version no. M17303.1 GM78676
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


Other information

Official Symbol: CEACAM5
Other Aliases: CD66e, CEA
Other Designations: meconium antigen 100

ANTIBODIES

AstraZeneca-MedImmune:ILS 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.

5

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

Bayer Corporation: US6,013,772

10 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.

15 - 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

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

US5,472,693 - for example, ATCC No. CRL-1 1215

US6,417,337 - for example, ATCC CRL-1 2208

US6,333,405 - for example, ATCC CRL-1 2208


US201 10189085

- 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);

30 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).
(42) MET (met proto-oncogene; hepatocyte growth factor receptor)

5 Nucleotide
Genbank accession no M35073
Genbank version no. M35073.1 GM87553
Genbank record update date: Mar 6, 2012 11:12 AM

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

15 Cross references

Other information
Official Symbol: MET

20 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

25 ANTIBODIES
Abgenix/Pfizer: US20100040629
for example, the antibody produced by hybridoma 13.3.2 having American Type Culture Collection (ATCC) accession number PTA-5026; the antibody produced by hybridoma 9.1.2 having ATCC accession number PTA-5027; the antibody produced by hybridoma 8.70.2 having ATCC accession number PTA-5028; or the antibody produced by hybridoma 6.90.3 having ATCC accession number PTA-5029.

Amgen/Pfizer: US20050054019
for example, an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 2 where X2 is glutamate and X4 is serine and a light chain having the amino acid sequence set forth in SEQ ID NO: 4 where X8 is alanine,
without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 6 and a light chain having the amino acid sequence set forth in SEQ ID NO: 8, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 10 and a light chain having the amino acid sequence set forth in SEQ ID NO: 12, without the signal sequences; or an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 14 and a light chain having the amino acid sequence set forth in SEQ ID NO: 16, without the signal sequences.

Agouron Pharmaceuticals (Now Pfizer): US20060035907

Eli Lilly: US20100129369

Genentech: US5,686,292; US201 00028337; US201 0001 6241; US20070129301 ;

US20070098707; US20070092520, US20060270594; US20060134104; US20060035278;
US20050233960; US20050037431

US 5,686,292 - for example, ATCC HB-1 1894 and ATCC HB-1 1895
US 20100016241 - for example, ATCC HB-1 1894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.1 1.6)


Novartis: US200901 75860

- 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 of light chain 5097 are residues 24-39, 55-61, and 94-100 of SEQ ID NO: 37.

Pharmacia Corporation: US200401 66544

Pierre Fabre: US201 10239316, US201 10097262, US201 001 15639
Sumsung: US 201 10129481 - for example a monoclonal antibody produced from a hybridoma cell having accession number KCLRF-BP-00219 or accession number of KCLRF-BP-00223.

15 Samsung: US 201 10104176 - for example an antibody produced by a hybridoma cell having Accession Number: KCLRF-BP-00220.


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

Nucleotide
Genbank accession no J05581
Genbank version no. J05581.1 GM88869
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

Other information
Official Symbol: MUC1
Other Aliases: RP1 1-263K19.2, 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.

5 AltaRex- Quest Pharma Tech: US7,147,850


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

Immunogen: US7,202,346

- for example, antibody MJ-170: hybridoma cell line MJ-170 ATCC accession no. PTA-5286
Monoclonal 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

20 Immunomedics: US 6,653,104

Ramot Tel Aviv Uni: US7,897,351


Roche GlycArt: US8,021,856


(44) CA9 (Carbonic anhydrase IX)

Nucleotide
Genbank accession no. X66839
Genbank version no. X66839.1 Gl:1000701
5 Genbank record update date: Feb 2, 2011 10:15 AM

Polypeptide
Genbank accession no. CAA47315
Genbank version no. CAA47315.1 Gl:1000702
10 Genbank record update date: Feb 2, 2011 10:15 AM

Cross references

Other information
Official Symbol: CA9
OtherAliases: CAIX, MN
OtherDesignations: 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


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 Biologie-Plasmidencollectie (LMBP) at the Universiteit Gent in Gent, Belgium, under Accession No. LMBP 6009CB.

Institute of Virology, Slovak Academy of Sciences US200801 77046; US200801 76310;

US200801 76258; US20050031 623

Novartis: US20090252738

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


Xencor: US200901 62382

(45) EGFRvll (Epidermal growth factor receptor (EGFR), transcript variant 3, Nucleotide
Genbank accession no. NM_201283

Genbank version no. NM_201283.1 Gl:41327733

Genbank record update date: Sep 30, 2012 01:47 PM

Polypeptide
Genbank accession no. NP_958440

Genbank version no. NP_958440.1 Gl:41327734

Genbank record update date: Sep 30, 2012 01:47 PM
Cross-references

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.
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).

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.

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).

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.

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

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.


(46) CD33 (CD33 molecule)

Nucleotide

15 Genbank accession no. M_23197
Genbank version no. NM_23197.1 GI:180097
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

20 Genbank accession no. AAA51948
Genbank version no. AAA51948.1 GI:188098
Genbank record update date: Jun 23, 2010 08:47 AM

Cross-references


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


(Seattle Genetics/Immunomedics)
mAb OKT9: Sutherland, D.R. et al. Proc Natl Acad Sci USA 78(7): 4515-4519 1981,


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_001 178098
Genbank version no. NM_001 178098.1 GI:296010920
Genbank record update date: Sep 10, 2012 12:43 AM

Polypeptide
Genbank accession no. NP_001 171569
Genbank version no. NP_001 171569.1 GI:296010921
Genbank record update date: Sep 10, 2012 12:43 AM

Cross-references

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


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 (RETTTVGRRYYAMDY) 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.


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.

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 Gl:269973860
Genbank record update date: Sep 09, 2012 04:59 PM

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

Cross-references

Other information
Official Symbol: IL2RA
OtherAliases: RP1 1-536K7.1, 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 having 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.


(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


Other information

Official Symbol: AXL

Other Aliases: JTK1, 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  Gl:180095  
Genbank record update date: Jun 23, 2010 08:53 AM

**Polypeptide**

5  Genbank accession no. AAA51947  
Genbank version no. AAA51947.1  Gl:180096  
Genbank record update date: Jun 23, 2010 08:53 AM

**Cross-references**


**Other information**

Official Symbol: TNFRSF8  
Other Aliases: CD30, D1S166E, Ki-1

15 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**

20  Genbank accession no. Z29574  
Genbank version no. Z29574.1  Gl:471244  
Genbank record update date: Feb 02, 2011 10:40 AM

**Polypeptide**

25  Genbank accession no. CAA82690  
Genbank version no. CAA82690.1  Gl:471245  
Genbank record update date: Feb 02, 2011 10:40 AM

**Cross-references**


**Other information**

Official Symbol: TNFRSF17

35 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

5 (52) CTAs - CTA (Cancer Testis Antigens)

Cross-references

10 (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

25 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

30 (54) CLEC14A (C-type lectin domain family 14, member A; Genbank accession no. NM1 75060)

Nucleotide
Genbank accession no. NM1 75060
Genbank version no. NM1 75060.2 GI:371 123930
Genbank record update date: Apr 01, 2012 03:34 PM
Polypeptide
Genbank accession no. NP_778230
Genbank version no. NP_778230.1 Gl:28269707
Genbank record update date: Apr 01, 2012 03:34 PM

Other information
Official Symbol: CLEC14A
Other Aliases: UNQ236/PR0269, C14orf27, CEG1, EGFR-5
Other Designations: C-type lectin domain family 14 member A; CIECT and EGF-like domain containing protein; epidermal growth factor receptor 5

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

Polypeptide
Genbank accession no. NP_005338
Genbank version no. NP_005338.1 GM6507237
Genbank record update date: Sep 30, 2012 01:42 PM

Cross-references

Other information
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 Gl:307127
Genbank record update date: Jun 23, 2012 08:54 AM

**Polypeptide**

Genbank accession no. AAA36175

Genbank version no. AAA361 75.1 Gl:307128

Genbank record update date: Jun 23, 2012 08:54 AM

**Cross-references**


**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 (Ofbazoglu, 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
  - 5 Polypeptide
    - Genbank accession no. ABK42161
    - Genbank version no. ABK42161.1 Gl:1 17616286
    - Genbank record update date: Jul 25, 2007 03:00 PM
  - LGR5/GPR49
    - 6 Nucleotide
      - Genbank accession no. NM_003667
      - Genbank version no. NM_003667.2 Gl:24475886
      - Genbank record update date: Jul 22, 2012 03:38 PM
o Polypeptide
  ▪ Genbank accession no. NP_003658
  ▪ Genbank version no. NP_003658.1 Gl:4504379
  ▪ Genbank record update date: Jul 22, 2012 03:38 PM

- Prominin/CD133
  o Nucleotide
    ▪ Genbank accession no. NM_006017
    ▪ Genbank version no. NM_006017.2 Gl:224994187
    ▪ Genbank record update date: Sep 30, 2012 01:47 PM

o Polypeptide
  ▪ Genbank accession no. NP_006008
  ▪ Genbank version no. NP_006008.1 Gl: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)

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

(59) ENPP3 (Ectonucleotide pyrophosphatase/phosphodiesterase 3)

25 Nucleotide
Genbank accession no. AF005632
Genbank version no. AF005632.2 Gl:4432589
Genbank record update date: Mar 10, 2010 09:41 PM

30 Polypeptide
Genbank accession no. AAC51813
Genbank version no. AAC51813.1 Gl:2465540
Genbank record update date: Mar 10, 2010 09:41 PM

35 Cross-references
Other information
Official Symbol: ENPP3
Other Aliases: RP5-988G15.3, B10, CD203c, NPP3, PD-IBETA, PDNP3
Other Designations: E-NPP 3; dJ1005H1 1.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); dJ914N13.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); ectonucleotide pyrophosphatase/phosphodiesterase family member 3; gp130RB13-6; phosphodiesterase l beta; phosphodiesterase I/nucleotide pyrophosphatase 3; phosphodiesterase-l beta

(60) PRR4 (Proline rich 4 (lacral))
Nucleotide
Genbank accession no. NM_007244
Genbank version no. NM_007244.2 Gl:154448885
Genbank record update date: Jun 28, 2012 12:39 PM

Polypeptide
Genbank accession no. NP_009175
Genbank version no. NP_009175.2 Gl:154448886
Genbank record update date: Jun 28, 2012 12:39 PM

Cross-references

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 Gl:222080082
Genbank record update date: Sep 02, 2012 01:50 PM
Polypeptide
Genbank accession no. NP_004954
Genbank version no. NP_004954.2 Gl:222080083
Genbank record update date: Sep 02, 2012 01:50 PM

Cross-references

Other information
Official Symbol: GUCY2C
OtherAliases: DIAR6, GUC2C, MUCIL, STAR
OtherDesignations: GC-C; STA receptor; guanylyl cyclase C; hSTAR; heat-stable enterotoxin receptor; intestinal guanylate cyclase

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

Nucleotide
Genbank accession no. U41060
Genbank version no. U41060.2 Gl:1271 1792
Genbank record update date: Nov 30, 2009 04:35 PM

Polypeptide
Genbank accession no. AAA96258
Genbank version no. AAA96258.2 Gl:1271 1793
Genbank record update date: Nov 30, 2009 04:35 PM

Cross-references

Other information
Official Symbol: SLC39A6
OtherAliases: LIV-1
OtherDesignations: 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) 574, Trophoblast glycoprotein, TPBG - TPBG (trophoblast glycoprotein)

Nucleotide
Genbank accession no. AJ012159
Genbank version no. AJ012159.1  Gl:3805946
5 Genbank record update date: Feb 01, 2011 10:27 AM

Polypeptide
Genbank accession no. CAA09930
Genbank version no. CAA09930.1  Gl:3805947
10 Genbank record update date: Feb 01, 2011 10:27 AM

Cross-references

(64) CD56 - NCMA1 (Neural cell adhesion molecule 1)

Nucleotide
Genbank accession no. NM_000615
Genbank version no. NM_000615.6  Gl:336285433
25 Genbank record update date: Sep 23, 2012 02:32 PM

Polypeptide
Genbank accession no. NP_000606
Genbank version no. NP_000606.3  Gl:94420689
30 Genbank record update date: Sep 23, 2012 02:32 PM

Cross-references

Other information
• Official Symbol: NCAM1
Other Aliases: CD56, MSK39, NCAM
Other Designations: antigen recognized by monoclonal antibody 5.1 H11; neural cell adhesion molecule, NCAM

5 ANTIbODIES
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.

10 (65) CanAg (Tumor associated antigen CA242)
Cross-references

15 ANTIbODIES
huC242 (Tolcher AW et al., J Clin Oncol. 2003 Jan 15;21 (2):21 1-22; Immunogen)
For example, see US20080138898A1 SEQ ID NO: 1 and 2

20 (66) FOLR1 (Folate Receptor 1)
Nucleotide
Genbank accession no. J05013
Genbank version no. J05013.1 Gl:182417
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide
Genbank accession no. AAA35823
Genbank version no. AAA35823.1 Gl:182418
Genbank record update date: Jun 23, 2010 08:47 AM

30 Cross-references

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

5 ANTIBODIES

(67) GPNMB (Glycoprotein (transmembrane) nmb)
10 Nucleotide
Genbank accession no. X76534
Genbank version no. X76534.1 Gl:666042
Genbank record update date: Feb 02, 2011 10:10 AM

15 Polypeptide
Genbank accession no. CAA54044
Genbank version no. CAA54044.1 Gl:666043
Genbank record update date: Feb 02, 2011 10:10 AM

20 Cross-references

Other information
Official Symbol: GPNMB

25 Other Aliases: UNQ1725/PR09925, HGFIN, NMB
Other Designations: glycoprotein NMB; glycoprotein nmb-like protein; osteoactivin; transmembrane glycoprotein HGFIN; transmembrane glycoprotein NMB

ANTIBODIES
   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
35 Genbank accession no. AF043724
Genbank version no. AF043724.1 Gl:2827453
Polypeptide
Genbank accession no. AAC39862
Genbank version no. AAC39862.1 GI:2827454
Genbank record update date: Mar 10, 2010 06:24 PM

Cross-references

Other information
Official Symbol: HAVCR1
OtherAliases: HAVCR, HAVCR-1, KIM-1, KIM1, TIM, TIM-1, TIM1, TIMD-1, TIMD1
OtherDesignations: T cell immunoglobulin 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

(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

Other information
Official Symbol: VTCN1
OtherAliases: RP1 1-229A19.4, B7-H4, B7H4, B7S1, B7X, B7h.5, PR01291, VCTN1
OtherDesignations: 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
5 Genbank version no. AF447176.1 GI:17432420
Genbank record update date: Nov 28, 2008 01:51 PM

**Polypeptide**
Genbank accession no. AAL39062
10 Genbank version no. AAL39062.1 GM7432421
Genbank record update date: Nov 28, 2008 01:51 PM

**Cross-references**

**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
25 Genbank version no. NM_001040031.1 GI:91807109
Genbank record update date: Jul 29, 2012 02:08 PM

**Polypeptide**
Genbank accession no. NP_001035120
30 Genbank version no. NP_001035120.1 GI:91807110
Genbank record update date: Jul 29, 2012 02:08 PM

**Cross-references**
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


For example, see US201 10171208A1 SEQ ID NO: 253


(73) CD138 - SDC1 (syndecan 1)

Nucleotide
Genbank accession no. AJ551 176
Genbank version no. AJ551 176.1 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

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
**ANTIBODES**


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**


**Other information**

Official Symbol: CD74

Other Aliases: DHLAG, HLADG, II, la-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; la-associated invariant chain; MHC HLA-DR gamma chain; gamma chain of class II antigens; p33

**ANTIBODES**


For example, see US200401 15193 SEQ ID NOs: 19, 20, 21, 22, 23 and 24

Genmab: HuMax-CD74 (see website)
(75) Claudins - CLs (Claudins)

Cross-references

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 Gl:41927737
Genbank record update date: Sep 30, 2012 01:47 PM

Polypeptide
Genbank accession no. NP_005219
Genbank version no. NP_005219.2 Gl:29725609
Genbank record update date: Sep 30, 2012 01:47 PM

Cross-references

Other information
Official Symbol: EGFR

OtherAliases: ERBB, ERBB1, HER1, PIG61, mENA

OtherDesignations: 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
For example, see US6217866 - ATTC deposit No. 9764.

For example, see US6235883 SEQ ID NOs: 23-38.


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


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


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  Gl:36109
Genbank record update date: Feb 02, 2011 10:17 PM

Polypeptide

Genbank accession no. CCA49634
Genbank version no. CCA49634.1  Gl:36110
Genbank record update date: Feb 02, 2011 10:17 PM

Cross-references


Other information

Official Symbol: MST1R
Other Aliases: CD136, CDw136, PTK8, RON

Other Designations: MSP receptor; MST1 R variant RON30; MST1 R 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 RON variant 4

(79) EPHA2 (EPH receptor A2)

Nucleotide

Genbank accession no. BC037166
Genbank version no. BC037166.2  Gl:33879863
Genbank record update date: Mar 06, 2012 01:59 PM

Polypeptide

Genbank accession no. AAH37166
Genbank version no. AAH37166.1  Gl:22713539
Genbank record update date: Mar 06, 2012 01:59 PM

Cross-references


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
5 Medimmune: 1C1 (Lee JW., et al Clin Cancer Res. 2010 May 1;16(9):2562-2570)
For example, see US20090304721 A1 Fig. 7 and 8.

(80) CD20 - MS4A1 (membrane-spanning 4-domains, subfamily A, member 1)
Nucleotide
10 Genbank accession no. M27394
Genbank version no. M27394.1 GM79307
Genbank record update date: Nov 30, 2009 11:16 AM

Polypeptide
15 Genbank accession no. AAA35581
Genbank version no. AAA35581.1 GM79308
Genbank record update date: Nov 30, 2009 11:16 AM

Cross-references

Other information
Official Symbol: MS4A1
Other Aliases: B1, Bp35, CD20, CVID5, LEU-16, MS4A2, S7
25 Other Designations: B-lymphocyte antigen CD20; B-lymphocyte cell-surface antigen B1; CD20 antigen; CD20 receptor; leukocyte surface antigen Leu-16

ANTIBODIES
For example, see US5736137, ATCC deposit No. HB-691 19.

For example, see US20090169550A1 SEQ ID NOs: 2, 4 and 5.

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 Gl:340745336
Genbank record update date: Sep 23, 2012 02:33 PM

Polypeptide
Genbank accession no. NP_002151
Genbank version no. NP_002151.2 Gl:153946395
Genbank record update date: Sep 23, 2012 02:33 PM

Cross-references

Other information
Official Symbol: TNC
OtherAliases: 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

ANTIBODIES

(82) FAP (Fibroblast activation protein, alpha)
Nucleotide
Genbank accession no. U09278
Genbank version no. U09278.1 Gl:1888315
Genbank record update date: Jun 23, 2010 09:22 AM

For example, see US7968685 SEQ ID NOs: 29, 35, 45 and 47.
Polypeptide
Genbank accession no. AAB49652
Genbank version no. AAB49652.1 GM888316
Genbank record update date: Jun 23, 2010 09:22 AM

Cross-references

Other information
Official Symbol: FAP
Other Aliases: DPPIV, FAPA
Other Designations: 170 kDa melanoma membrane-bound gelatinase; integral membrane serine protease; seprase

(83) DKK1 (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

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


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

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


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 GM993571
Genbank record update date: Jun 29, 2012 11:24 AM
**Polypeptide**
Genbank accession no. NP_067004
Genbank version no. NP_067004.3  GM9923572
Genbank record update date: Jun 29, 2012 11:24 AM

**Cross-references**

**Other information**
Official Symbol: SLAMF7
Other Aliases: UNQ576/PR01 138, 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**
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**
Official Symbol: ENG

**Other information**
Other Aliases: RP1 1-228B15.2, CD105, END, HHT1, ORW, ORW1
Other Designations: CD105 antigen

(87) Annexin A 1 - ANXA 1 (Annexin A1)

Nucleotide
5 Genbank accession no. X05908
Genbank version no. X05908.1 GI:34387
Genbank record update date: Feb 02, 2011 10:02 AM

Polypeptide
10 Genbank accession no. CCA29338
Genbank version no. CCA29338.1 GI:34388
Genbank record update date: Feb 02, 2011 10:02 AM

Cross-references

Other information
Official Symbol: ANXA1
OtherAliases: RP1 1-71A24.1, ANX1, LPC1
20 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
25 Genbank accession no. M60335
Genbank version no. M60335.1 GI:340193
Genbank record update date: Jun 23, 2010 08:56 AM

Polypeptide
30 Genbank accession no. AAA61269
Genbank version no. AAA61269.1 GI:340194
Genbank record update date: Jun 23, 2010 08:56 AM

Cross-references
Other information
Official Symbol VCAM1
Other Aliases: CD106, INCAM-100
Other Designations: CD106 antigen; vascular cell adhesion protein 1

Antibody Sequences
Anti-Integrin αββ

RHAB6.2
QVQLVQSGSEKLKPGASVKISCKASGFAFTDSYMHWRQAPGQGLEWMGWIDPENGDT
YAPKFQGRFVSLDTSVSTAYLQISLKAEDTAVYYCTRGPTAVPNLRGDLQVLAQKVAG
PYPFDYWGQGTLVTVSS

RHCB6.2
QVQLVQSGAEVKPGASVKSCASKASYTFIDSYMHWRQAPGQRLEWMGWIDPENGDT
YAPKFQGRVTITTDTSASTAYMELSSLLRSEDATAVYYCARGTPTAVPNLRGDLQVLAQKVAG
PYPFDYWGQGTLVTVSS

RHF
QVQLVQSGAEVKPGASVKSCASKASGNYFSYMHWRQAPGQIRLEWMGWIDPENGDT
EYAPKFQGRVTITTDTSASTAYMELSSLLRSEDATAVYYCNEGTPTGYPYFDYWGQGTLVTVSS

RHFB6
QVQLVQSGAEVKPGASVKSCASKASGNGFIDSYMHWRQAPGQRLEWMGWIDPENGDT
EYAPKFQGRVTITTDTSASTAYMELSSLLRSEDATAVYYCNEGTPTAVPNLRGDLQVLAQKVAG
GPYYFDYWGQGTLVTVSS

RHAYIOObP
QVQLVQSGSEKLKPGASVKISCKASGFAFTDSYMHWRQAPGQGLEWMGWIDPENGDT
EYAPKFQGRFVSLDTSVSTAYLQISLKAEDTAVYYCTRGPTGYPYFDYWGQGTLVTVSS

RKF
ENVLTQSPGTLSPGERATLCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASSGIPDRF
SGGSGTDFLTISRLEPEDFAVYYCQRSSYPLTFGGGTKVEIK
RKFL36L50
ENVTQSPGTLSPGERATLSASSSASASSASVSYMHWLQQKPGQAPRLLIYLSNLASHGIPDRFS
SGSGSTDFTLITISRLEPEDFAVYCYCQRSSYPLTFGGGTKEIK

5 RKC
EIVLTQSPGTLSPGERATLSASSSASASSASVSYMHWQKPGQAPRLLIYLYSNLASHGIPDRFS
SGSGSTDFTLITISRLEPEDFAVYCYCQRSSYPLTFGGGTKEIK

Anti-CD33
10 CD33 Hum195 VH
QVQLVQSGAEVKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPNYNGGTG
YNQKFKSKATITADESTNTAYMELSSLRSEDTHAVYYCARGRPAMDYWGQGLTVSS

CD33 Hum195 VK
15 DIQMTQSPSSLSASVGRVTICRASESVVDNYGISFMNWFQKPGKAPKLLIYAAASNQGSG
VPSRFSGSGSTDFTLITISSLPDDFATYYCQQSKETSVPWTFQGQGTKKEIK

Anti-CD19
19 B4 resurfaced VH
20 QVQLVQPGAEVKPGASVKLSCKTSGYTFTSNWMHWKVQRPGQGLEWIEIDPSYTN
YNQNFKGKAKLTVDKSTAYMEVSSLRSDDTAVYYCARGSNYYAMDYWGQGTAVTVSS

CD19 B4 resurfaced VK
25 EIVLTQPAIMSASGGERVMTCSASSGVNYMHWQKPGTSPRRWYDTKLSANVAPAR
FSGSGSTYSSTLISMEPDAATYYCHQRTYGSTFGGGTKLEIK

Anti-Her2
Herceptin VH chain
30 EVQLVESGGGLVQPGGSLRLSCAASSGFIKDITYIHWVRQAPGKLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLMNLSRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVS

Herceptin VL chain
35 DIQMTQSPSSLSASVGRVTICRASQDVTAVAWYQQKPGKAPKLLIYASFLYSGVPSR
FSGSGSTDFTLITISSLQPDDFATYYCQQHYTTPTFGQGTKEIK
Anti-CD25
Simulect VK (also known as Basiliximab)
QIVSTQSPAIMSASPGEKTMTCSASSSSRSYMQWYQQPKPGTSPKRWYDTSKLASGVPAR
FSGSGGTYSYSLTISSEMAEADAATYYCHQRSSYTFGGGTKLEIK

Simulect VH
QLQQSGTVLRAPGASVKMSCKASGYSFTRYWMIHKQRPQGQGLEWIGAIYPGNSDTSYN
QKFEGKAKLTAVTSASTAYMEMLSELLTHEDAVYYCSRDGYFYDFWGQGGTTLTVSS

Anti-PSMA
Deimmunised VH 1
EVQLVQSGPEKPKPAGTVKISCKTSGYTFETYIHWVKQAPKGLEWIGNINPNNGATTYN
QKFEDKATLTVDKSTDATAYMEMLSELLRSDTAHYCAAGWNFDYWQGQTTLTVSS

Deimmunised VK 1
DIQMTSPSSLSTSVGDRVTLTCKASQDVGTAHDWYQQPKPGPGLLYWASRHTGIPSR
FSGSGSTFDTLTISSLPEDFADYYCQQYNSYPPLTFPGGTKVDIK

Deimmunised VH 1 '5
EVKLVEGGLVQPPGSKLSCVASGFTSYWWMNWVRQAPKGLEWVAEIRSQSNF
ATHYAESVKGRVTISRDSSIVLYQMNNLAEDTVYYCTRRWNFWGQGTTTVSS

Deimmunised VH 2 '5
EVKLVEGGLVQPPGSLKLSCVASGFTSYWWMNWVRQAPKGLEWVAEIRSQSNFA
THYAESVKGRVTISRDSSIVLYQMNNLRAEDTVYYCCTRRWNFWGQGTTTVSS

Deimmunised VH 3 '5
EVQLVESGGGLVQPPGSLKLSCVASGFTSYWWMNWVRQAPKGLEWVAEIRSQSNFA
THYAESVKGRVTISRDSSIVLYQMNNLRAEDTVYYCTRRWNFWGQGTTTVSS

Deimmunised VH 4 '5
EVQLVESGGGLVQPPGSLKLSCVASGFTSYWWMNWVRQAPKGLEWVAEIRSQSNFA
THYAESVKGRFTISRDSSIVLYQMNNLRAEDTVYYCCTRRWNFWGQGTTTVSS
Deimmunised VK1 '5
NIVMTQFPSSMASVGDRVTITCKASENVGTYVSWyQQKPDQSPKMLIYGASNRF TGVPD
RFTGSGSATDFTLTISSLQTEDLADYYCGQSYTFPYPGQGKLEMK

5 Deimmunised VK2 '5
NIVMTQFPSSMASVGDRVTITCKASENVGTYVSWyQQKPDQSPKMLIYGASNRF TGVPD
RFSGSGSTGDTFTLTISSLQAEELADYYCGQSYTFPYPGQGKLEIK

Deimmunised VK3 '5
NIQMTQFPSSMASVGDRVTITCKASENVGTYVSWyQQKPDQSPKMLIYGASNRF TGVPD
RFSGSGSTGDTFTLTISSLQAEELADYYCGQSYTFPYPGQGKLEIK

Deimmunised VK4 '5
NIQMTQFPSSMASVGDRVTITCKASENVGTYVSWyQQKPDQSPKMLIYGASNRF TGVPD
RFSGSGSTGDTFTLTISSLQAEELADYYCGQSYTFPYPGQGKLEIK

Deimmunised VK D1 '5
NIVMTQFPKSMASASAGERTTLTCKASENVGTYVSWyQQKPTQSPKMLIYGASNRF TGVPD
RFSGSGSTGDTFTLTISSLQAEELADYYCGQSYTFPYPGQGKLEIK

20 Deimmunised VH D1 '5
EVKLEESGGGLVQPGGSMKISCVASGFTFSNYWMNWVRQSEQKLEWVAGEIRSQSN
THYAESVKGRVIISRPPSKSSYVLQMNSLRAEPTAVYCTRRWNNFQGQGTVTSS

25 Humanised RHA '5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKLEWVGEIRSQSN
THYAESVKGRFTISRPPKNTAYLQMNLKEPTAVYCTRRWNNFQGQGTVTSS

Humanised RHB '5
EVKLEESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKLEWVGEIRSQSN
THYAESVKGRVIISRPPKNTAYLQMNLKEPTAVYCTRRWNNFQGQGTVTSS

Humanised RHC '5
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKLEWVGEIRSQSN
THYAESVKGRVIISRPPKNTAYLQMNLKEPTAVYCTRRWNNFQGQGTVTSS
Humanised RHP '5
EVKLVESGGGLVQPGGLKLSAASGFTFSNYWMNWVRQASGKGEWVGEIRSQSNNFA
THYAESVKGRVIISRDDSNTYVQLMNSLRTEDAVYYCTRWRWNFWGQGTTTVSS

5 Humanised RHE '5
EVKLVESGGGLVQPGGLKLSAASGFTFSNYWMNWVRQASGKGEWVGEIRSQSNNFA
THYAESVKGRFTISRDDSNTYVQLMNSLRTEDAVYYCTRWRWNFWGQGTTTVSS

Humanised RHF '5
EVKLVESGGGLVQPGGLKLSAASGFTFSNYWMNWVRQASGKGEWVGEIRSQSNNFA
THYAESVKGRVIISRDDSNTAYLQMNSLRTEDAVYYCTRWRWNFWGQGTTTVSS

Humanised RHG '5
EVKLVESGGGLVQPGGLKLSAASGFTFSNYWMNWVRQASGKGEWVGEIRSQSNNFA
THYAESVKGRVIISRDDSNTAYLQMNSLRTEDAVYYCTRWRWNFWGQGTTTVSS

Humanised RKA '5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSQWYQQKPGTAPKLLIYGASNRFTGVPSR
FSGSGSATDFLTINNLQPEFATYYCGQSYTFPGQGTKVEIK

20 Humanised RKB '5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSQWYQQKPGTAPKLLIYGASNRFTGVPSR
FSGSGSATDFLTINNLQPEFATYYCGQSYTFPGQGTKVEIK

Humanised RKC '5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSQWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFLTINNLQPEFATYYCGQSYTFPGQGTKVEIK

Humanised RKD '5
DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSQWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFLTINNLQPEFATYYCGQSYTFPGQGTKVEIK

30 Humanised RKE '5
NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSQWYQQKPGTAPKLLIYGASNRFTGPDR
FTGSGSATDFLTINNLQPEFATYYCGQSYTFPGQGTKVEIK
Humanised RKF '5
NIVMTQSPSSVSASVGDRVTITCKASENGTYVSWYQQKPGTAPKMLIYGASNRFTGVPSR
FSGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

5 Humanised RKG '5
NIVMTQSPSSVSASVGDRVTITCKASENGTYVSWYQQKPGTAPKMLIYGASNRFTGVVPDR
FTGSGSATDFLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

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 $\alpha_\beta_6$.

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.

Embodiments of the present invention include ConjA wherein the cell binding agent is selected from an antibody to any of the antigens discussed above.

Embodiments of the present invention include ConjB wherein the cell binding agent is selected from an antibody to any of the antigens discussed above.

Embodiments of the present invention include ConjA wherein the cell binding agent is selected from any of the antibodies discussed above.

Embodiments of the present invention include ConjB wherein the cell binding agent is selected from any of the antibodies discussed above.
The present invention may also relate to conjugates where the cell binding agent is selected from an antibody to any of the antigens discussed above and any of the antibodies discussed above linked to different drugs.

**Drug loading**

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

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

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. Higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.
Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the drug-linker intermediate (D-L) or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of drug-linker intermediate (D-L) or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

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

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

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

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

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

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

Includes Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form (-COO⁻), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form (-N⁺HR'R²), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form (-O⁻), a salt or solvate thereof, as well as conventional protected forms.
Salts

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, et al., J. Pharm. Sci., 66, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be -COO⁻), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na⁺ and K⁺, alkaline earth cations such as Ca²⁺ and Mg²⁺, and other cations such as Al³⁺. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH₄⁺) and substituted ammonium ions (e.g. NH₃R⁺, NH₂R₂⁺, NHR₃⁺, NR₄⁺). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is N(CH₃)₄⁺.

If the compound is cationic, or has a functional group which may be cationic (e.g. -NH₂ may be -NH₃⁺), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

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

Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.
**Solvates**

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

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

These forms can be called the carbinolamine and carbinolamine ether forms of the PBD (as described in the section relating to $R^{10}$ above). The balance of these equilibria depend on the conditions in which the compounds are found, as well as the nature of the moiety itself.

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

**Isomers**

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

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.
"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., "Stereochemistry of Organic Compounds", John Wiley & Sons, Inc., New York, 1994. The compounds of the invention may contain asymmetric or chiral centers, and therefore exist in different stereoisomeric forms. It is intended that all stereoisomeric forms of the compounds of the invention, including but not limited to, diastereomers, enantiomers and atropisomers, as well as mixtures thereof such as racemic mixtures, form part of the present invention. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH₃, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH₂OH. Similarly, a reference to
ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C₆H₇alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

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

\[
\begin{align*}
\text{keto} & \quad \leftrightarrow \quad \text{enol} & \quad \leftrightarrow \quad \text{enolate} \\
\end{align*}
\]

The term "tautomer" or "tautomer form" refers to structural isomers of different energies which are interconvertible via a low energy barrier. For example, proton tautomers (also known as prototropic tautomers) include interconversions via migration of a proton, such as keto-enol and imine-enamine isomerizations. Valence tautomers include interconversions by reorganization of some of the bonding electrons.

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

Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, and chlorine, such as, but not limited to ²H (deuterium, D), ³H (tritium), ¹¹C, ¹³C, ¹⁴C, ¹⁵N, ¹⁸F, ³¹P, ³²P, ³⁵S, ³⁶Cl, and ¹²⁵I. Various isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ³H, ¹³C, and ¹⁴C are incorporated. Such isotopically labelled compounds may be useful in metabolic studies, reaction kinetic studies, detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. Deuterium labelled or substituted therapeutic compounds of the invention may have improved DMPK (drug metabolism and
pharmacokinetics) properties, relating to distribution, metabolism, and excretion (ADME). Substitution with heavier isotopes such as deuterium may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements. An 18F labeled compound may be useful for PET or SPECT studies. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples and preparations described below by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent. Further, substitution with heavier isotopes, particularly deuterium (i.e., 2H or D) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements or an improvement in therapeutic index. It is understood that deuterium in this context is regarded as a substituent. The concentration of such a heavier isotope, specifically deuterium, may be defined by an isotopic enrichment factor. In the compounds of this invention any atom not specifically designated as a particular isotope is meant to represent any stable isotope of that atom.

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

**Biological Activity**

In vitro cell proliferation assays

Generally, the cytotoxic or cytostatic activity of an antibody-drug conjugate (ADC) is measured by: exposing mammalian cells having receptor proteins, e.g. HER2, to the antibody of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays are used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of an ADC of the invention.

The in vitro potency of antibody-drug conjugates can be measured by a cell proliferation assay. The CellTiter-Glo® Luminescent Cell Viability Assay is a commercially available (Promega Corp., Madison, WI), homogeneous assay method based on the recombinant expression of *Coleoptera* luciferase (US Patent Nos. 5583024; 5674713 and 5700670). This cell proliferation assay determines the number of viable cells in culture based on quantitation
of the ATP present, an indicator of metabolically active cells (Crouch et al (1993) J. Immunol. Meth. 160:81-88; US 6602677). The CellTiter-Glo® Assay is conducted in 96 well format, making it amenable to automated high-throughput screening (HTS) (Cree et al (1995) Anticancer Drugs 6:398-404). The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent and mixing. The cells may be treated continuously with ADC, or they may be treated and separated from ADC. Generally, cells treated briefly, i.e. 3 hours, showed the same potency effects as continuously treated cells.

The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiter-Glo® Assay generates a "glow-type" luminescent signal, produced by the luciferase reaction, which has a half-life generally greater than five hours, depending on cell type and medium used. Viable cells are reflected in relative luminescence units (RLU). The substrate, Beetle Luciferin, is oxidatively decarboxylated by recombinant firefly luciferase with concomitant conversion of ATP to AMP and generation of photons.

The in vitro potency of antibody-drug conjugates can also be measured by a cytotoxicity assay. Cultured adherent cells are washed with PBS, detached with trypsin, diluted in complete medium, containing 10% FCS, centrifuged, re-suspended in fresh medium and counted with a haemocytometer. Suspension cultures are counted directly. Monodisperse cell suspensions suitable for counting may require agitation of the suspension by repeated aspiration to break up cell clumps.

The cell suspension is diluted to the desired seeding density and dispensed (100 µl per well) into black 96 well plates. Plates of adherent cell lines are incubated overnight to allow adherence. Suspension cell cultures can be used on the day of seeding.

A stock solution (1ml) of ADC (20pg/ml) is made in the appropriate cell culture medium. Serial 10-fold dilutions of stock ADC are made in 15ml centrifuge tubes by serially transferring 100 µl to 900 µl of cell culture medium.
Four replicate wells of each ADC dilution (100 µl) are dispensed in 96-well black plates, previously plated with cell suspension (100 µl), resulting in a final volume of 200 µl. Control wells receive cell culture medium (100 µl).

If the doubling time of the cell line is greater than 30 hours, ADC incubation is for 5 days, otherwise a four day incubation is done.

At the end of the incubation period, cell viability is assessed with the Alamar blue assay. AlamarBlue (Invitrogen) is dispensed over the whole plate (20 µl per well) and incubated for 4 hours. Alamar blue fluorescence is measured at excitation 570nm, emission 585nm on the Varioskan flash plate reader. Percentage cell survival is calculated from the mean fluorescence in the ADC treated wells compared to the mean fluorescence in the control wells.

**In vivo efficacy**

The in vivo efficacy of antibody-drug conjugates (ADC) of the invention can be measured by tumor xenograft studies in mice. For example, the in vivo efficacy of an anti-HER2 ADC of the invention can be measured by a high expressing HER2 transgenic explant mouse model. An allograft is propagated from the Fo5 mmtv transgenic mouse which does not respond to, or responds poorly to, HERCEPTIN® therapy. Subjects are treated once with ADC at certain dose levels (mg/kg) and PBD drug exposure (µg/m²); and placebo buffer control (Vehicle) and monitored over two weeks or more to measure the time to tumor doubling, log cell kill, and tumor shrinkage.

**Use**

The conjugates of the invention may be used to provide a PBD compound at a target location.

The target location is preferably a proliferative cell population. The antibody is an antibody for an antigen present on a proliferative cell population.

In one embodiment the antigen is absent or present at a reduced level in a non-proliferative cell population compared to the amount of antigen present in the proliferative cell population, for example a tumour cell population.
At the target location the linker may be cleaved so as to release a compound RelA or RelB. Thus, the conjugate may be used to selectively provide a compound RelA or RelB to the target location.

The linker may be cleaved by an enzyme present at the target location.

The target location may be in vitro, in vivo or ex vivo.

The antibody-drug conjugate (ADC) compounds of the invention include those with utility for anticancer activity. In particular, the compounds include an antibody conjugated, i.e. covalently attached by a linker, to a PBD drug moiety, i.e. toxin. When the drug is not conjugated to an antibody, the PBD drug has a cytotoxic effect. The biological activity of the PBD drug moiety is thus modulated by conjugation to an antibody. The antibody-drug conjugates (ADC) of the invention selectively deliver an effective dose of a cytotoxic agent to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved.

Thus, in one aspect, the present invention provides a conjugate compound as described herein for use in therapy.

In a further aspect there is also provides a conjugate compound as described herein for use in the treatment of a proliferative disease. A second aspect of the present invention provides the use of a conjugate compound in the manufacture of a medicament for treating a proliferative disease.

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

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 carcinoma, ovarian carcinoma, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), lymphomas, leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis. Cancers of particular interest include, but are not limited to, leukemias and ovarian cancers.

Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g. bowel, colon), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

In one embodiment, the treatment is of a pancreatic cancer.

In one embodiment, the treatment is of a tumour having $\alpha_\beta_6$ integrin on the surface of the cell.

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia, haematological, and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders.

Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.
Autoimmune diseases for which the ADC compounds may be used in treatment include rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjogren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g. ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and polyarteritis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neutromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g. Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjogren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

Methods of Treatment

The conjugates of the present invention may be used in a method of therapy. Also provided is a method of treatment, comprising administering to a subject in need of treatment a therapeutically-effective amount of a conjugate compound of the invention. The term "therapeutically effective amount" is an amount sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.
A compound of the invention may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g. drugs, such as chemotherapeutics); surgery; and radiation therapy.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, antibodies, photosensitizers, and kinase inhibitors. Chemotherapeutic agents include compounds used in "targeted therapy" and conventional chemotherapy.

Examples of chemotherapeutic agents include: erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTERE®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine, dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), trastuzumab (HERCEPTIN®, Genentech), temozolomide (4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo[4.3.0]nona-2,7,9-triene-9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-V./V-dimethylethanamine, NOLVADEX®, ISTUBAL®, VALODEX®, and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.

More examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sutent (SUNITINIB®, SU1 1248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (Mek inhibitor, Exelixis, WO 2007/044515), ARRY-886 (Mek inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1 126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 22584 (Novartis), fulvestrant (FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lopatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, IL), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chlorambucil,
AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (TELCYTA®, Telik), thiotapec and cyclophosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, imposulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamemelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystemon; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherochalin; panaxadiol; a sarcodecicin; spongistatin; nitrogen mustards such as chlorambucil, clonaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorothamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin,\n\ncalicheamicin gamma 1, calicheamicin omega-H (Angew Chem. Ed. Engl. (1994)\n33:183-186); dynemicin, dynemicin A; bisphosphonates, such as clodronate; an\nesperamycin; as well as neocarzinostatin chromophore and related chromoprotein enediyne\nantibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins,\ncactinomycin, carubicin, carminomycin, carzinophilin, chromomycinis, dactinomycin,\ndaunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin,\ncyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin,\nesorubicin, idarubicin, nemorubicin, marcellomycin, mitomycins such as mitomycin C,\nmypcophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin,\nquelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin,\nzorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs\nsuch as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as\nfludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as\nancticline, azacitidine, 6-azauridine, carbomof, cytarabine, dideoxyuridine, doxifluridine,\nenocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate,\nepitostanol, meptiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane,\ntrilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide\nglycoside; aminolevulinic acid; eniluracil; amsacrine; bestrubucil; bisantrene; edatraxate;\ndefopamine; demecolcline; diaziquone; elfornithine; elliptinium acetate; an epothilone;\netogulcid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as\nmaytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine;
pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichotheccenes (especially T-2 toxin, verrucarin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

Also included in the definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminogluthethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestane, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacinabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors such as MEK inhibitors (WO 2007/044515); (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, for example, PKC-alpha, Raf and H-Ras, such as oblimersen (GENASENSE®, Genta Inc.); (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN® rlL-2; topoisomerase 1 inhibitors such as LURTOTECAN®; ABARELIX® rmRH; (ix) antiangiogenic agents such as bevacizumab (AVASTIN®, Genentech); and pharmaceutically acceptable salts, acids and derivatives of any of the above.

Also included in the definition of "chemotherapeutic agent" are therapeutic antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®,
Genentech/Biogen Idee), ofatumumab (ARZERRA®, GSK), pertuzumab (PERJETA™, OMNITARG™, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth).

Humanized monoclonal antibodies with therapeutic potential as chemotherapeutic agents in combination with the conjugates of the invention include: alemtuzumab, apolizumab, aselizumab, atlizumab, bapineuzumab, bevacizumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidxtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pefusituzumab, pectuzumab, pertuzumab, pexelizumab, ralizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sonotuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

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

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

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

**Formulations**

While it is possible for the conjugate compound to be used (e.g., administered) alone, it is often preferable to present it as a composition or formulation.

In one embodiment, the composition is a pharmaceutical composition (e.g., formulation, preparation, medicament) comprising a conjugate compound, as described herein, and a pharmaceutically acceptable carrier, diluent, or excipient.

In one embodiment, the composition is a pharmaceutical composition comprising at least one conjugate compound, as described herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, including, but not limited to, pharmaceutically acceptable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, colouring agents, flavouring agents, and sweetening agents.

In one embodiment, the composition further comprises other active agents, for example, other therapeutic or prophylactic agents.


Another aspect of the present invention pertains to methods of making a pharmaceutical composition comprising admixing at least one $^{11}C$-radiolabelled conjugate or conjugate-like compound, as defined herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, e.g., carriers, diluents, excipients, etc. If formulated as discrete units (e.g., tablets, etc.), each unit contains a predetermined amount (dosage) of the active compound.
The term "pharmaceutically acceptable," as used herein, pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

The formulations may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with carriers (e.g., liquid carriers, finely divided solid carrier, etc.), and then shaping the product, if necessary.

The formulation may be prepared to provide for rapid or slow release; immediate, delayed, timed, or sustained release; or a combination thereof.

Formulations suitable for parenteral administration (e.g., by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (e.g., solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (e.g., in a liposome or other microparticulate). Such liquids may additional contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active ingredient in the liquid is from about 1 ng/ml to about 10 µg/ml, for example from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.
Dosage

It will be appreciated by one of skill in the art that appropriate dosages of the conjugate compound, and compositions comprising the conjugate compound, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician, veterinarian, or clinician.

In general, a suitable dose of the active compound is in the range of about 100 ng to about 25 mg (more typically about 1 μg to about 10 mg) per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, an amide, a prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 100 mg, 3 times daily.

In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 150 mg, 2 times daily.
In one embodiment, the active compound is administered to a human patient according to the following dosage regime: about 200 mg, 2 times daily.

However in one embodiment, the conjugate compound is administered to a human patient according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

In one embodiment, the conjugate compound is administered to a human patient according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

The dosage amounts described above may apply to the conjugate (including the PBD moiety and the linker to the antibody) or to the effective amount of PBD compound provided, for example the amount of compound that is releasable after cleavage of the linker.

For the prevention or treatment of disease, the appropriate dosage of an ADC of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. An exemplary dosing regimen comprises a course of administering an initial loading dose of about 4 mg/kg, followed by additional doses every week, two weeks, or three weeks of an ADC. Other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

**Treatment**

The term “treatment,” as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate
of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included.

The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

Similarly, the term "prophylactically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired prophylactic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

**Preparation of Drug conjugates**

Antibody drug conjugates, as well as conjugates with other cell binding agents, may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including reaction of a nucleophilic group of an antibody or cell binding agent with a drug-linker reagent. This method may be employed with a variety of antibodies and cell binding agents to prepare the antibody-drug conjugates of the invention.

Nucleophilic groups on antibodies include, but are not limited to side chain thiol groups, e.g. cysteine. Thiol groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties such as those of the present invention. 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 (Cleland's reagent, dithiothreitol) or TCEP (tris(2-carboxyethyl)phosphine hydrochloride; Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA). Each cysteine disulfide 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.
The Subject/Patient

The subject/patient may be an animal, mammal, a placental mammal, a marsupial (e.g., kangaroo, wombat), a monotreme (e.g., duckbilled platypus), a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), a lagomorph (e.g., a rabbit), avian (e.g., a bird), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), porcine (e.g., a pig), ovine (e.g., a sheep), bovine (e.g., a cow), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), an ape (e.g., gorilla, chimpanzee, orangutang, gibbon), or a human.

Furthermore, the subject/patient may be any of its forms of development, for example, a foetus. In one preferred embodiment, the subject/patient is a human.

In one embodiment, the patient is a population where each patient has a tumour having α6β₄ integrin on the surface of the cell.

Examples

General Experimental Methods

Optical rotations were measured on an ADP 220 polarimeter (Bellingham Stanley Ltd.) and concentrations (c) are given in g/100mL. Melting points were measured using a digital melting point apparatus (Electrothermal). IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. ¹H and ¹³C NMR spectra were acquired at 300 K using a Bruker Avance NMR spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported relative to TMS (δ = 0.0 ppm), and signals are designated as s (singlet), d (doublet), t (triplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets) or m (multiplet), with coupling constants given in Hertz (Hz). Mass spectroscopy (MS) data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; De-solvation flow rate (L/h), 250. High-resolution mass spectroscopy (HRMS) data were recorded on a Waters Micromass QTOF Global in positive W-mode using metal-coated borosilicate glass tips to introduce the samples into the instrument. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F₂₅₄). and flash chromatography utilised silica gel (Merck 60, 230-400 mesh ASTM). Except for the HOBT (NovaBiochem) and solid-supported reagents (Argonaut), all other chemicals and solvents were purchased from Sigma-Aldrich and were used as supplied without further purification. Anhydrous solvents
were prepared by distillation under a dry nitrogen atmosphere in the presence of an appropriate drying agent, and were stored over 4A molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

5 General LC/MS conditions:

Method 1 (default method, used unless stated otherwise)
The HPLC (Waters Alliance 2695) was run using a mobile phase of water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B held over 1.0 min, then increase from 5% B to 95% B over a 3 min period. The composition was held for 0.1 min at 95% B, then returned to 5% B in 0.03 minutes and hold there for 0.87 min. Total gradient run time equals 5 minutes.

Method 2
The HPLC (Waters Alliance 2695) was run using a mobile phase of water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%). Gradient: initial composition 5% B held over 1.0 minute, then increase from 5% B to 95% B over a 2.5 minute period. The composition was held for 0.5 minutes at 95% B, then returned to 5% B in 0.1 minutes and hold there for 0.9 min. Total gradient run time equals 5 minutes.

For both methods
Flow rate 3.0 mL/min, 400μL was split via a zero dead volume tee piece which passes into the mass spectrometer. Wavelength detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex Onyx Monolithic C18 50 x 4.60 mm.

The reverse phase flash purification conditions were as follows: The Flash purification system (Varian 971-Fp) was run using a mobile phase of water (A) and acetonitrile (B). Gradient: initial composition 5% B over 20 C.V. (Column Volume) then 5% B to 70% B within 60 C.V. The composition was held for 15 C.V. at 95% B, and then returned to 5% B in 5 C.V. and held at 5%B for 10 C.V. Total gradient run time equals 120 C.V. Flow rate 6.0 mL/min. Wavelength detection range: 254 nm. Column: Agilent AX1 372-1 SF10-5.5gC8.

Preparative HPLC: Reverse-phase ultra-high-performance liquid chromatography (UPLC) was carried out on Phenomenex Gemini NX 5μ C-18 columns of the following dimensions: 150 x 4.6 mm for analysis, and 150 x 21.20 mm for preparative work. All UPLC experiments were performed with gradient conditions. Eluents used were solvent A (H₂O with 0.1% Formic acid) and solvent B (CH₃CN with 0.1% Formic acid). Flow rates used were 1.0
ml/min for analytical, and 20.0 ml/min for preparative HPLC. Detection was at 254 and 280 nm.

**Synthesis of Intermediate 12**

(a) \(1',3'-\text{Bis[2-methoxy-4-\{(methoxycarbonyl)phenoxy\}propane (3)}\)

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 2 (60.0 g, 329 mmol) and \(\text{Ph}_3\text{P}\) (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 hour 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 3 was collected by vacuum filtration, washed with THF and dried in a vacuum desiccator to constant weight. Yield = 54.7 g (84% based on 1,3-propanediol). Purity satisfactory by LC/MS (3.20 min (ES+) m/z (relative intensity) 427 ([M + Na], 10); \(^1\text{H NMR (400 MHz, CDCl}_3\) δ 7.64 (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).

(b) \(1',3'-\text{Bis[2-methoxy-4-\{(methoxycarbonyl)\}-5-nitrophenoxy\}propane (4)}\)

Solid \(\text{Cu(NO}_3\text{)}_2\cdot3\text{H}_2\text{O\) (81.5 g, 337.5 mmol) was added slowly to an overhead stirred slurry of the bis-ester 3 (54.7 g, 135 mmol) in acetic anhydride (650 mL) at 0-5°C (ice/aceton). The
reaction mixture was allowed to stir for 1 hour at 0-5°C and then allowed to warm to room temperature. A mild exotherm (ca. 40-50°C), accompanied by thickening of the mixture and evolution of NO₂ was observed at this stage. Additional acetic anhydride (300 mL) was added and the reaction mixture was allowed to stir for 16 hours at room temperature. The reaction mixture was poured on to 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 6'-nitro compound 4 as a yellow solid. Yield = 66.7 g (100%). Purity satisfactory by LC/MS (3.25 min (ES+) m/z (relative intensity) 517 \([M + Na]^+, 40\); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 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).

(c) 1',3'-Bis(4-carboxy-2-methoxy-5-nitrophenoxy) propane (5)

A slurry of the methyl ester 4 (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 5 was collected and dried thoroughly in a vacuum oven (50 °C). Yield = 54.5 g (87%). Purity satisfactory by LC/MS (2.65 min (ES+) m/z (relative intensity) 489 \([M + Na]^+, 30\)); \(^1\)H NMR (400 MHz, DMSO-de) \(\delta\) 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).

(d) 1,1'-[[((Propane-1,3-diyl)dioxy)bis[[5-methoxy-2-nitro-1,4-phenylene]carbonyl]]bis[(2S,4R)-methyl-4-hydroxyprrolidine-2-carboxylate] (6)

Oxalyl chloride (24.5 mL, 35.6 g, 281 mmol) was added to a stirred suspension of the nitrobenzoic acid 5 (43 g, 92.3 mmol) and DMF (6 mL) in anhydrous DCM (600mL). Following initial effervescence the reaction suspension became a solution and the mixture was allowed to stir at room temperature for 16 hours. Conversion to the acid chloride was confirmed by treating a sample of the reaction mixture with MeOH and the resulting bis-methyl ester was observed by LC/MS. The majority of solvent was removed by evaporation under reduced pressure; the resulting concentrated solution was re-dissolved in a minimum amount of dry DCM and triturated with diethyl ether. The resulting yellow precipitate was collected by filtration, washed with cold diethyl ether and dried for 1 hour in a vacuum oven at 40°C. The solid acid chloride was added portionwise over a period of 25 min to a stirred suspension of (2S,4R)-methyl-4-hydroxyprrolidine-2-carboxylate hydrochloride (38.1 g, 210 mmol) and TEA (64.5 mL, g, 463 mmol) in DCM (400mL) at -40°C (dry ice/CH₃CN).
Immediately, the reaction was complete as judged by LC/MS (2.47 min (ES+) m/z (relative intensity) 721 ([M + H]+, 100). The mixture was diluted with DCM (200 mL) and washed with 1N HCl (300 mL), saturated NaHCO₃ (300 mL), brine (400 mL), dried (MgSO₄), filtered and the solvent evaporated in vacuo to give the pure product 6 as an orange solid (66.7 g, 100%). [a]²⁰_D = -46.1° (c = 0.47, CHCl₃); ¹H NMR (400 MHz, CDCl₃) (rotamers) δ 7.63 (s, 2H), 6.82 (s, 2H), 4.79-4.72 (m, 2H), 4.49-4.28 (m, 6H), 3.96 (s, 6H), 3.79 (s, 6H), 3.46-3.38 (m, 2H), 3.02 (d, 2H, J = 11.1 Hz), 2.48-2.30 (m, 4H), 2.29-2.04 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) (rotamers) δ 172.4, 166.7, 154.6, 148.4, 137.2, 127.0, 109.7, 108.2, 69.7, 65.1, 57.4, 57.0, 56.7, 52.4, 37.8, 29.0; IR (ATR, CHCl₃) 3410 (br), 3010, 2953, 1741, 1622, 1577, 1519, 1455, 1429, 1334, 1274, 1211, 1177, 1072, 1050, 1008, 871 cm⁻¹; MS (ES+) m/z (relative intensity) 721 ([M + H]+, 47), 388 (80); HRMS [M + H]+ theoretical C₂₉H₃₂N₄O₁₀ m/z 597.2191, found (ES+) m/z 597.2205.

(e) 1.1'-[[Propane-1,3-diyldioxy]bis(1 1aS,2R)-2-(hydroxy)-7-methoxy-1,2,3,10, 11.11a-hexahydro-5H-pyrrolo[2, 1-c][1,4]benzodiazepin-5,11-dione] (7)

**Method A**: A solution of the nitro-ester 6 (44 g, 61.1 mmol) in MeOH (2.8 L) was added to freshly purchased Raney® nickel (~ 50 g of a ~ 50% slurry in H₂O) and anti-bumping granules in a 5L 3-neck round bottomed flask. The mixture was heated at reflux and then treated dropwise with a solution of hydrazine hydrate (21.6 mL, 22.2 g, 693 mmol) in MeOH (200 mL) at which point vigorous effervescence was observed. When the addition was complete (~ 45 min) additional Raney® nickel was added carefully until effervescence had ceased and the initial yellow colour of the reaction mixture was discharged. The mixture was heated at reflux for a further 5 min at which point the reaction was deemed complete by TLC (90:10 v/v CHCl₃/MeOH) and LC/MS (2.12 min (ES+) m/z (relative intensity) 597 ([M + H]+, 100)). The reaction mixture was filtered hot immediately through a sinter funnel containing celite with vacuum suction. The filtrate was reduced in volume by evaporation in vacuo at which point a colourless precipitate formed which was collected by filtration and dried in a vacuum desiccator to provide 7 (31 g, 85%). [a]²⁰_D = +404° (c = 0.10, DMF); ¹H NMR (400 MHz, DMSO-d₆) δ 10.2 (s, 2H, NH), 7.26 (s, 2H), 6.73 (s, 2H), 5.11 (d, 2H, J = 3.98 Hz, OH), 4.32-4.27 (m, 2H), 4.19-4.07 (m, 6H), 3.78 (s, 6H), 3.62 (dd, 2H, J = 12.1, 3.60 Hz), 3.43 (dd, 2H, J = 12.0, 4.72 Hz), 2.67-2.57 (m, 2H), 2.26 (p, 2H, J = 5.90 Hz), 1.99-1.89 (m, 2H); ¹³C NMR (100 MHz, DMSO-d₆) δ 169.1, 164.0, 149.9, 144.5, 129.8, 117.1, 111.3, 104.5, 54.8, 54.4, 53.1, 33.5, 27.5; IR (ATR, neat) 3438, 1680, 1654, 1610, 1051, 1490, 1434, 1379, 1263, 1234, 1216, 1177, 1156, 1115, 1089, 1038, 981, 870 cm⁻¹; MS (ES+) m/z (relative intensity) 619 ([M + Na]+, 10), 597 ([M + H]+, 52), 445 (12), 326 (11); HRMS [M + H]+ theoretical C₂₉H₃₂N₄O₁₀ m/z 597.2191, found (ES+) m/z 597.2205.
**Method B**: A suspension of 10% Pd/C (7.5 g, 10% w/w) in DMF (40 mL) was added to a solution of the nitro-ester 6 (75 g, 104 mmol) in DMF (360 mL). The suspension was hydrogenated in a Parr hydrogenation apparatus over 8 hours. Progress of the reaction was monitored by LC/MS after the hydrogen uptake had stopped. Solid Pd/C was removed by filtration and the filtrate was concentrated by rotary evaporation under vacuum (below 10 mbar) at 40°C to afford a dark oil containing traces of DMF and residual charcoal. The residue was digested in EtOH (500 mL) at 40°C on a water bath (rotary evaporator bath) and the resulting suspension was filtered through celite and washed with ethanol (500 mL) to give a clear filtrate. Hydrazine hydrate (10 mL, 321 mmol) was added to the solution and the reaction mixture was heated at reflux. After 20 minutes the formation of a white precipitate was observed and reflux was allowed to continue for a further 30 minutes. The mixture was allowed to cool down to room temperature and the precipitate was retrieved by filtration, washed with diethyl ether (2:1 volume of precipitate) and dried in a vacuum desiccator to provide 7 (50 g, 81%). Analytical data for method B: Identical to those obtained for Method A (optical rotation, $^1$H NMR, LC/MS and TLC).

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.65 (s, 2H, NH), 7.44 (s, 2H), 6.54 (s, 2H), 4.50 (p, 2H, $J = 5.38$ Hz), 4.21-4.10 (m, 6H), 3.87 (s, 6H), 3.73-3.63 (m, 4H), 2.85-2.79 (m, 2H), 2.36-2.29 (m, 2H), 2.07-1.99 (m, 2H), 0.86 (s, 18H), 0.08 (s, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.4, 165.7, 151.4, 146.6, 129.7, 118.9, 112.8, 105.3, 69.2, 65.4, 56.3, 55.7, 54.2, 35.2, 28.7, 25.7, 18.0, -4.82 and -4.86; IR (ATR, CHCl$_3$) 3235, 2955, 2926, 2855, 1698, 1695, 1603, 1518, 1491, 1446, 1380, 1356, 1251, 1220, 1120, 1099, 1033 cm$^{-1}$; MS (ES$^+$) m/z (relative intensity) 825 ([M + H]$^+$, 100). The reaction mixture was poured onto ice (~ 1.75 L) and allowed to warm to room temperature with stirring. The resulting white precipitate was collected by vacuum filtration, washed with H$_2$O, diethyl ether and dried in the vacuum desiccator to provide pure 8 (30.1 g, 99%). $[\alpha]^2$D = $+234^\circ$ (c = 0.41, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.65 (s, 2H, NH), 7.44 (s, 2H), 6.54 (s, 2H), 4.50 (p, 2H, $J = 5.38$ Hz), 4.21-4.10 (m, 6H), 3.87 (s, 6H), 3.73-3.63 (m, 4H), 2.85-2.79 (m, 2H), 2.36-2.29 (m, 2H), 2.07-1.99 (m, 2H), 0.86 (s, 18H), 0.08 (s, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.4, 165.7, 151.4, 146.6, 129.7, 118.9, 112.8, 105.3, 69.2, 65.4, 56.3, 55.7, 54.2, 35.2, 28.7, 25.7, 18.0, -4.82 and -4.86; IR (ATR, CHCl$_3$) 3235, 2955, 2926, 2855, 1698, 1695, 1603, 1518, 1491, 1446, 1380, 1356, 1251, 1220, 1120, 1099, 1033 cm$^{-1}$; MS (ES$^+$) m/z (relative intensity) 825 ([M + H]$^+$, 62), 721 (14), 440 (38); HRMS [M + H]$^+$ theoretical C$_{41}$H$_{57}$O$_{14}$Si$_2$ m/z 825.3921, found (ES$^+$) m/z 825.3948.
A solution of n-BuLi (68.3 mL of a 1.6 M solution in hexane, 109 mmol) was added dropwise to a stirred suspension of the tetralactam 8 (30.08 g, 36.4 mmol) in anhydrous THF (600 mL) at -30°C (dry ice/ethylene glycol) under a nitrogen atmosphere. The reaction mixture was allowed to stir at this temperature for 1 hour (now a reddish orange colour) at which point a solution of SEMCl (19.3 mL, 18.2 g, 109 mmol) in anhydrous THF (120 mL) was added dropwise. The reaction mixture was allowed to slowly warm to room temperature and was stirred for 16 hours under a nitrogen atmosphere. The reaction was deemed complete as judged by TLC (EtOAc) and LC/MS (4.77 min (ES+) m/z (relative intensity) 1085 [{M + H}]^+, 100). The THF was removed by evaporation in vacuo and the resulting residue dissolved in EtOAc (750 mL), washed with H_2O (250 mL), brine (250 mL), dried (MgSO_4) filtered and evaporated in vacuo to provide the crude N10-SEM-protected tetralactam 9 as an oil (max^m 39.5 g, 100%). Product carried through to next step without purification. [a]_D^23 = +163° (c = 0.41, CHCl_3); ^1H NMR (400 MHz, CDCl_3) 7.33 (s, 2H), 7.22 (s, 2H), 5.47 (d, 2H, J = 9.98 Hz), 4.68 (d, 2H, J = 9.99 Hz), 4.57 (p, 2H, J = 5.77 Hz), 4.29-4.19 (m, 6H), 3.89 (s, 6H), 3.79-3.51 (m, 8H), 2.87-2.81 (m, 2H), 2.41 (p, 2H, J = 5.81 Hz), 2.03-1.90 (m, 2H), 1.02-0.81 (m, 22H), 0.09 (s, 12H), 0.01 (s, 18H); ^13C NMR (100 MHz, CDCl_3) 170.0, 165.7, 151.2, 147.5, 133.8, 121.8, 111.6, 106.9, 78.1, 69.6, 67.1, 65.5, 56.6, 56.3, 53.7, 35.6, 30.0, 25.8, 18.4, 18.1, -1.24, -4.73; IR (ATR, CHCl_3) 2951, 1685, 1604, 1606, 1517, 1462, 1433, 1360, 1247, 1127, 1065 cm^-1; MS (ES+) m/z (relative intensity) 1113 [{M + Na}]^+, 808 [{M + H}]^+, 100), 1009 (5), 813 (6); HRMS [M + H]^+ theoretical C_{53}H_{99}N_{12}O_{12}Si_{12} m/z 1085.5548, found (ES+) m/z 1085.5542.

A solution of TBAF (150 mL of a 1.0 M solution in THF, 150 mmol) was added to a stirred solution of the crude bis-silyl ether 9 [84.0 g (max^m 56.8 g), 52.4 mmol] in THF (800 mL) at room temperature. After stirring for 1 hour, analysis of the reaction mixture by TLC (95:5 v/v CHCl_3/MeOH) revealed completion of reaction. The THF was removed by evaporation under reduced pressure at room temperature and the resulting residue dissolved in EtOAc (500 mL) and washed with NH_4Cl (300 mL). The combined organic layers were washed with brine (60 mL), dried (MgSO_4), filtered and evaporated under reduced pressure to provide the crude product. Purification by flash chromatography (gradient elution: 100% CHCl_3 to 96:4
v/v CHCl₃/MeOH) gave the pure tetralactam 10 as a white foam (36.0 g, 79%). LC/MS 3.33 min (ES+) m/z (relative intensity) 879 ([M + Na]⁺, 100), 857 ([M + H]⁺, 40); [α]²⁰D = +202° (c = 0.34, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.28 (s, 2H), 7.20 (s, 2H), 5.44 (d, 2H, J = 10.0 Hz), 4.72 (d, 2H, J = 10.0 Hz), 4.61-4.58 (m, 2H), 4.25 (t, 4H, J = 5.83 Hz), 4.20-4.16 (m, 2H), 3.91-3.85 (m, 8H), 3.77-3.54 (m, 6H), 3.01 (br s, 2H, OH), 2.96-2.90 (m, 2H), 2.38 (p, 2H, J = 5.77 Hz), 2.1 1-2.05 (m, 2H), 1.00-0.91 (m, 4H), 0.00 (s, 18H); ¹³C NMR (100 MHz, CDCl₃) δ 169.5, 165.9, 151.3, 147.4, 133.7, 121.5, 111.6, 106.9, 79.4, 69.3, 67.2, 65.2, 56.5, 56.2, 54.1, 35.2, 29.1, 18.4, -1.23; IR (ATR, CHCl₃) 2956, 1684, 1625, 1564, 1418, 1464, 1434, 1361, 1238, 1058, 1021 cm⁻¹; MS (ES⁺) m/z (relative intensity) 885 ([M + 29]⁺, 70), 857 ([M + H]⁺, 100), 711 (8), 448 (17); HRMS [M + H]⁺ theoretical C₄₁H₆₀N₄O₁₂Si₂ m/z 854.2104, found (ES⁺) m/z 854.2103.

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

Diol 10 (25.6 g, 30 mmol, 1 eq.), NaOAc (6.9 g, 84 mmol, 2.8 eq.) and TEMPO (188 mg, 1.2 mmol, 0.04 eq.) were dissolved in DCM (326 mL) under Ar. This was cooled to -8°C (internal temperature) and TCCA (9.7 g, 42 mmol, 1.4 eq.) was added portionwise over 15 minutes. TLC (EtOAc) and LC/MS [3.60 min. (ES⁺) m/z (relative intensity) 854.21 ([M + H]⁺, 40), (ES⁻) m/z (relative intensity) 887.07 ([M - H + Cl]⁻, 10)] after 30 minutes indicated that reaction was complete. Cold DCM (200 mL) was added and the mixture was filtered through a pad of Celite before washing with a solution of saturated sodium hydrogen carbonate/ sodium thiosulfate (1:1 v/v; 200 mL x 2). The organic layer was dried with MgSO₄, filtered and the solvent removed in vacuo to yield a yellow/orange sponge (25.4 g, 99%). LC/MS [3.60 min. (ES⁺) m/z (relative intensity) 854.21 ([M + H]⁺, 40); [α]²⁰D = +291° (c = 0.26, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.32 (s, 2H), 7.25 (s, 2H), 5.50 (d, 2H, J = 10.1 Hz), 4.75 (d, 2H, J = 10.1 Hz), 4.60 (dd, 2H, J = 9.85, 3.07 Hz), 4.31-4.18 (m, 6H), 3.89-3.84 (m, 8H), 3.78-3.62 (m, 4H), 3.55 (dd, 2H, J = 19.2, 2.85 Hz), 2.76 (dd, 2H, J = 19.2, 9.90 Hz), 2.42 (p, 2H, J = 5.77 Hz), 0.98-0.91 (m, 4H), 0.00 (s, 18H); ¹³C NMR (100 MHz, CDCl₃) δ 206.8, 168.8, 165.9, 151.8, 148.0, 133.9, 120.9, 112.0, 107.2, 78.2, 67.3, 65.6, 56.3, 54.9, 52.4, 37.4, 29.0, 18.4, -1.24; IR (ATR, CHCl₃) 2957, 1763, 1685, 1644, 1606, 1516, 1457, 1434, 1360, 1247, 1209, 1098, 1066, 1023 cm⁻¹; MS (ES⁺) m/z (relative intensity) 881 ([M + 29]⁺, 38), 853 ([M + H]⁺, 100), 707 (8), 542 (12); HRMS [M + H]⁺ theoretical C₄₁H₆₀N₄O₁₂Si₂ m/z 853.3506, found (ES⁺) m/z 853.3502.
Anhydrous 2,6-lutidine (5.15 mL, 4.74 g, 44.2 mmol) was injected in one portion to a vigorously stirred solution of bis-ketone 11 (6.08 g, 7.1 mmol) in dry DCM (180 mL) at -45°C (dry ice/acetone) under a nitrogen atmosphere. Anhydrous triflic anhydride, taken from a freshly opened ampoule (7.2 mL, 12.08 g, 42.8 mmol), was injected rapidly dropwise, while maintaining the temperature at -40°C or below. The reaction mixture was allowed to stir at -45°C for 1 hour at which point TLC (50/50 v/v n-hexane/EtOAc) revealed the complete consumption of starting material. The cold reaction mixture was immediately diluted with DCM (200 mL) and, with vigorous shaking, washed with water (1 x 100 mL), 5% citric acid solution (1 x 200 mL) saturated NaHCO₃ (200 mL), brine (100 mL) and dried (MgSO₄). Filtration and evaporation of the solvent under reduced pressure afforded the crude product which was purified by flash column chromatography (gradient elution: 90:10 v/v n-hexane/EtOAc to 70:30 v/v n-hexane/EtOAc) to afford bis-enol triflate 12 as a yellow foam (5.5 g, 70%). LC/MS 4.32 min (ES+) m/z (relative intensity) 1139 ([M + Na]⁺, 20); [α]²⁴_D = +271° (c = 0.18, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.33 (s, 2H), 7.26 (s, 2H), 7.14 (t, 2H, J = 1.97 Hz), 5.51 (d, 2H, J = 10.1 Hz), 4.76 (d, 2H, J = 10.1 Hz), 4.62 (dd, 2H, J = 11.0, 3.69 Hz), 4.32-4.23 (m, 4H), 3.94-3.90 (m, 8H), 3.81-3.64 (m, 4H), 3.16 (ddd, 2H, J = 16.3, 11.0, 2.36 Hz), 2.43 (p, 2H, J = 5.85 Hz), 1.23-0.92 (m, 4H), 0.02 (s, 18H); ¹³C NMR (100 MHz, CDCl₃) δ 167.1, 162.7, 151.9, 148.0, 138.4, 133.6, 120.2, 118.8, 111.9, 107.4, 78.6, 67.5, 65.6, 56.7, 56.3, 30.8, 29.0, 18.4, -1.25; IR (ATR, CHCl₃) 2958, 1690, 1646, 1605, 1517, 1456, 1428, 1360, 1327, 1207, 1136, 1096, 1060, 1022, 938, 913 cm⁻¹; MS (ES⁺) m/z (relative intensity) 1144 ([M + 28]⁺, 100), 1117 ([M + H]⁺, 48), 1041 (40), 578 (8); HRMS [M + H]⁺ theoretical C₄₃H₄₄N₄O₁₀Si₂S₂F₆ m/z 1117.2491, found (ES⁺) m/z 1117.2465.
Example 1

\[(a) \ (S)-8-(3-(((S)-2-(4-aminophenyl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,1-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,1-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl trifluoromethanesulfonate \ (13)\]

\[\text{Pd(PPh}_3\text{)}_4 \ (116.9 \text{ mg, 0.101 mmol)} \text{ was added to a stirred mixture of the bis-enol triflate} \ 12 \ \text{(5.65 g, 5.06 mmol), 4-Aminophenylboronic acid pinacol ester (1 g, 4.56 mmol), Na}_2\text{CO}_3 \ (2.46 g, 23.2 mmol), MeOH (37 mL), toluene (74 mL) and water (37 mL). The reaction mixture was allowed to stir at 30\textdegreeC under a nitrogen atmosphere for 24 hours after which time all the boronic ester has consumed. The reaction mixture was then evaporated to dryness before the residue was taken up in EtOAc (150 mL) and washed with H}_2\text{O (2 x 100 mL), brine (150 mL), dried (MgSO}_4\text{), filtered and evaporated under reduced pressure to provide the crude product. Purification by flash chromatography (gradient elution: 80:20 v/v Hexane/EtOAc to 60:40 v/v Hexane/EtOAc) afforded product 13 as a yellowish foam (2.4 g, 45%).} \]

\[\text{LC/MS 4.02 min (ES+)} \ m/z \ \text{(relative intensity)} \ 1060.21 \ \{(M + H)\}^+ \ (100); \ 1^\text{H-NMR: (CDCl}_3\text{, 400 MHz))} \ \delta 7.40 \text{ (s, 1H), 7.33 (s, 1H), 7.27 (bs, 3H), 7.24 (d, 2H, J} = 8.5 \text{ Hz), 7.15 (t, 1H, J} = 2.0 \text{ Hz), 6.66 (d, 2H, J} = 8.5 \text{ Hz), 5.52 (d, 2H, J} = 10.0 \text{ Hz), 4.77 (d, 1H, J} = 10.0 \text{ Hz),}\]
4.76 (d, 1H, J = 10.0 Hz), 4.62 (dd, 1H, J = 3.7, 11.0 Hz), 4.58 (dd, 1H, J = 3.4, 10.6 Hz),
4.29 (t, 4H, J = 3.61, 7.30 Hz), 3.80 - 3.60 (m, 4H), 3.16 (ddd, 1H, J = 2.4, 11.0, 16.3 Hz), 3.11 (dd, 1H, J = 2.2, 10.5, 16.1 Hz), 2.43 (p, 2H, J = 5.9 Hz), 1.1-0.9 (m, 4H), 0.2 (s, 18H).

13C-NMR: (CDCl₃, 100 MHz) δ 169.8, 168.3, 164.0, 162.7, 153.3, 152.6, 149.28,
149.0, 147.6, 139.6, 134.8, 127.9, 127.5, 125.1, 121.5, 120.5, 120.1, 116.4, 113.2, 108.7, 79.8, 79.6, 68.7, 68.5, 67.0, 66.8, 58.8, 58.0, 57.6, 32.8, 32.0, 30.3, 19.7, 0.25.

(b) (S)-2-(4-Aminophenyl)-8-(3-(((S)-2-cyclopropyl-7-methoxy-5, 11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)propoxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl) 1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5, 11(10H, 11aH)-dione (14)
Triphenylarsine (0.24 g, 0.8 mmol), silver (I) oxide (1.02 g, 4.4 mmol), cyclopropylboronic acid (0.47 g, 5.5 mmol) and starting material 13 (1.15 g, 1.1 mmol) were dissolved in dioxane (30 mL) under an argon atmosphere. Potassium phosphate tribasic (2.8 g, 13.2 mmol) was ground-up with a pestle and mortar and quickly added to the reaction mixture. The reaction mixture was evacuated and flushed with argon 3 times and heated to 71°C.
Palladium (II) bis (benzonitrile chloride) (84 mg, 0.22 mmol) was added and the reaction vessel was evacuated and flushed with argon 3 times. After 10 minutes a small sample was taken for analysis by TLC (80:20 v/v ethyl acetate/hexane) and LC/MS. After 30 minutes the reaction had gone to completion (LC/MS analysis indicated complete consumption of starting material) and the reaction was filtered through celite and the filter pad washed with ethyl acetate (400 mL). The filtrate was washed with water (2x 200 mL) and brine (2x 200 mL). The organic layer was dried with MgSO₄, filtered and the solvent removed in vacuo.

Purification by silica gel column chromatography (30:70 v/v Hexane/ Ethyl acetate) afforded the product 14 as an orange/yellow solid (0.66 g, 63%). Method 1, LC/MS (3.85 min (ES⁺) m/z (relative intensity) 952.17 ([M + H]+, 100). 
1H NMR (400 MHz, CDCl₃) δ 7.36 (d, 2H, J = 8.4 Hz), 7.30 (s, 1H), 7.25 - 7.19 (m, 4H), 6.68 (s, 1H), 6.62 (d, 2H, J = 8.4 Hz), 5.49 (dd, 2H, J = 5.6, 10.0 Hz), 4.73 (app. t, 2H, J = 10.8 Hz), 4.54 (dd, 1H, J = 3.2, 10.4 Hz), 4.40 (dd, 1H, J = 3.2, 10.4 Hz), 4.29 - 4.23 (m, 4H), 3.91 - 3.85 (m, 7H), 3.80 - 3.71 (m, 2H), 3.70 - 3.61 (m, 2H), 3.38 - 3.32 (m, 1H), 3.12 - 3.01 (m, 1H), 2.50 - 2.69 (m, 1H), 2.40 (q, 2H, J = 5.6 Hz), 1.50 - 1.43 (m, 1H), 0.99 - 0.71 (m, 6H), 0.54 - 0.59 (m, 2H), 0.00 (s, 18H) ppm.
(c) (S)-2-(4-Aminophenyl)-8-((3-((S)-2-cyclopropyl-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-1 H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(1 1aH)-one (15)

SEM dilactam 14 (0.66 g, 0.69 mmol) was dissolved in THF (23 mL) and cooled to -78°C under an argon atmosphere. Super-Hydride® solution (1.7 mL, 1 M in THF) was added dropwise over 5 minutes while monitoring the temperature. After 20 minutes a small sample was taken and washed with water for LC/MS analysis. Water (50 mL) was added and the cold bath was removed. The organic layer was extracted and washed with brine (60 mL). The combined aqueous layers were washed with CH₂Cl₂/MeOH (90/1 0 v/v) (2 x 50 mL). The combined organic layers were dried with MgSO₄, filtered and the solvent removed in vacuo. The crude product was dissolved in MeOH (48 mL), CH₂Cl₂ (18 mL) and water (6 mL) and sufficient silica gel was added to afford a thick suspension. After 5 days stirring, the suspension was filtered through a sintered funnel and washed with CH₂Cl₂/MeOH (9:1) (~200 mL) until product ceased to be eluted. The organic layer was washed with brine (2 x 70 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by silica gel column chromatography (100% CHCl₃ to 96/4 v/v CHCl₃/MeOH) afforded the product 15 as a yellow solid (302 mg, 66%). Method 1, LC/MS (2.42 min (ES+)) m/z (relative intensity) 660.74 ([M + H]+, 30). ¹H NMR (400 MHz, CDCl₃) δ 7.86 (d, 1H, J = 3.6 Hz), 7.78 (d, 1H, J = 3.6 Hz), 7.58 - 7.44 (m, 3H), 7.34 - 7.20 (m, 3H), 6.88 - 6.66 (m, 4H), 4.35 - 4.15 (m, 6H), 3.95 - 3.75 (m, 7H), 3.39 - 3.22 (m, 1H), 3.14 - 3.04 (m, 1H), 2.93 - 2.85 (m, 1H), 2.46 - 2.36 (m, 2H), 1.49 - 1.41 (m, 1H), 0.80 - 0.72 (m, 2H), 0.58 - 0.51 (app. s, 2H) ppm.

(d) Allyl ((2S)-1-(((2S)-1-(((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (16)

In a degassed round bottom flask filled with argon, HO-Ala-Val-alloc (149.6 mg, 0.549 mmol) and EEDQ (135.8 mg, 0.549 mmol) were dissolved in a 9:1 mixture of dry CH₂Cl₂/MeOH (5 mL). The flask was wrapped in aluminium foil and the reaction mixture was allowed to stir at room temperature for 1 hour before starting material 15 (302 mg, 0.457 mmol) was added. The reaction mixture was left to stir for a further 40 hours at room temperature before the volatiles were removed by rotary evaporation under reduced pressure (the reaction was followed by LC/MS, RT starting material 2.32 min, (ES+ 660.29 ([M+H]+, 100)). The crude product was directly purified by silica gel chromatography column (100% CHCl₃ to 90/1 0 v/v CHCl₃/MeOH) to afford the pure product (16) in 42% yield (174 mg). Method 2 LC/MS (2.70 min (ES+) m/z (relative intensity) 914.73 ([M+H]+, 60), 660.43 (60), 184.31 (100)).
(e) (2S)-2-amino-N-((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (17)

The starting material 16 (170 mg, 0.185 mmol) was dissolved in dry CH$_2$Cl$_2$ (5 mL) in a round bottom flask filled with argon, before pyrrolidine (41 µL, 0.21 mmol) was added. The flask was purged/refilled three times with argon before Pd(PPh)$_3$ (14 mg, 0.084 mmol) was added and the flushing operation repeated. After 1 hour, complete consumption of starting material was observed (the reaction was followed by LC/MS) and Et$_2$O (50 mL) was added to the reaction mixture which was allowed to stir until all the product had crashed out of solution. The solid was filtered through a sintered funnel and washed twice with Et$_2$O (2 x 25 mL). The collecting flask was replaced and the isolated solid was dissolved in CHCl$_3$ (100 mL or until all the product had passed through the sintered funnel). The volatiles were then removed by rotary evaporation under reduced pressure to afford the crude product 17 which was used directly in the next step (168 mg). LC/MS method 2 (2.70 min (ES+) m/z (relative intensity) 830.27 ([M+H]$^+$, 50), 660.13 (80), 171.15 (100)).

(l) N-((R)-$^-$-I-(("S;+"-3-("S;+"-2-cyclopropyl-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutano-2-yl)-1-((3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (18)

Starting material 17 (154 mg, 0.185 mmol) and EDCI.HCl (110 mg, 0.185 mmol) were solubilised in dry CH$_2$Cl$_2$ (5 mL) in a round bottom flask purged and filled with argon. The mixture was left to stir at room temperature for 1 hour before PEG$_8$-maleimide (35.6 mg, 0.185 mmol) was added and the reaction mixture stirred for a further 16 hours (or until the reaction is complete, monitored by LC/MS). The reaction solution was diluted with CH$_2$Cl$_2$ (50 mL) and the organics were washed with H$_2$O (50 mL) and brine (50 mL) before being dried with MgSO$_4$, filtered and the solvent removed by rotary evaporation under reduced pressure to afford the crude product. Purification on silica gel column chromatography (100% CHCl$_3$ to 85/15 v/v CHCl$_3$/MeOH) gave the desired product (135mg), however remaining traces of unreacted PEG$_8$-maleimide were observed (by LC/MS, 2.21 min, method 2). Automated reverse phase silica gel chromatography (H$_2$O/CH$_3$CN) (see general information for conditions) successfully removed the impurity affording pure final product (18, 37mg of pure product starting from 110mg, 33%). Overall yield = 17%. Method 2 LC/MS
(2.58 min (ES+) m/z (relative intensity) 1404.03 ([M+H]^+ 20), 702.63 (100)). ¹H NMR (400 MHz, CDCl₃) δ 7.91 (t, J = 3.5 Hz, 1H), 7.80 (d, J = 4.0 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.54 - 7.50 (m, 2H), 7.45 (s, 1H), 7.39 - 7.31 (m, 2H), 6.87 (d, J = 10.5 Hz, 2H), 6.76 (s, 1H), 6.72 - 6.68 (m, 2H), 4.74 - 4.62 (m, 1H), 4.45 - 4.17 (m, 7H), 3.95 (s, 3H), 3.94 (s, 3H), 3.67 - 3.58 (m, 3H), 3.54 (m, 2H), 3.42 (dd, J = 10.2, 5.2 Hz, 2H), 3.16 - 3.07 (m, 1H), 2.92 (dd, J = 16.1, 4.1 Hz, 1H), 2.62 - 2.49 (m, 4H), 2.48 - 2.39 (m, 2H), 2.37 - 2.25 (m, 1H), 1.92 (s, 1H), 1.52 - 1.44 (m, 3H), 1.10 - 0.93 (m, 6H), 0.79 (dd, J = 9.2, 5.3 Hz, 2H), 0.57 (dd, J = 9.2, 5.3 Hz, 2H), NH were not observed.
Example 2

Chemical structures and reactions are depicted with various chemical entities and transformations indicated. The images show a series of molecular structures and reactions, with labels 20a, 20b, 20, 12, 21, 22, 23, 17, 18, and 19. The reactions and structures are connected to illustrate the chemical processes described in the example.
(a) (R)-2-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanoic acid (20b)

HO-Ala-Val-H 20a (350 mg, 1.86 mmol) and Na₂CΟ₃ (493 mg, 4.65 mmol) were dissolved in distilled H₂O (15 ml.) and the mixture was cooled to 0°C before dioxane (15 ml.) was added (partial precipitation of the amino acid salt occurred). A solution of Fmoc-Cl (504 mg, 1.95 mmol) in dioxane (15 ml.) was added dropwise with vigorous stirring over 10 minutes. The resulting mixture was stirred at 0°C for 2 hours before the ice bath was removed and stirring was maintained for 16 hours. The solvent was removed by rotary evaporation under reduced pressure and the residue dissolved in water (150 ml). The pH was adjusted from 9 to 2 with 1N HCl and the aqueous layer was subsequently extracted with EtOAc (3x100 ml). The combined organics were washed with brine (100 ml.), dried with MgSO₄, filtered and the volatiles removed by rotary evaporation under reduced pressure to afford pure HO-Ala-Val-Fmoc 20b (746 mg, 97% yield). LC/MS 2.85 min (ES+) m/z (relative intensity) 410.60 ; 1H-NMR (400 MHz, CDCl₃) δ 7.79 (d, J=7.77 Hz, 2H), 7.60 (d, J=7.77 Hz, 2H), 7.43 (d, J=7.5 Hz, 2H), 7.34 (d, J=7.5 Hz, 2H), 6.30 (bs, 1H), 5.30 (bs, 1H), 4.71-7.56 (m, 1H), 4.54-4.36 (m, 2H), 4.08-3.91 (m, 1H), 2.21-2.07 (m, 1H), 1.50 (d, J=7 Hz, 3H), 1.06-0.90 (m, 6H).

(b) (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-oxo-1-(((S)-1-oxo-1-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)amino)propan-2-yl)amino)butan-2-yl)carbamate (20)

4-Aminophenylboronic acid pinacol ester was added (146.9 mg, 0.67 mmol) was added to a solution of HO-Ala-Val-Fmoc 20b (330mg, 0.8 mmol), DCC (166 mg, 0.8 mmol) and DMAP (5 mg, cat.) in dry DCM (8 ml.) previously stirred for 30 minutes at room temperature in a flask flushed with argon. The reaction mixture was then allowed to stir at room temperature overnight. The reaction was followed by LCMS and TLC. The reaction mixture was diluted with CH₂Cl₂ and the organics were washed with H₂O and brine before being dried with MgSO₄, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dryloaded on a silicagel chromatography column (Hexane/EtOAc, 6:4) and pure product 20 was isolated as a white solid in 88% yield (360 mg).

(c) 8-(3-(((2-(4-(4-(4,5-tetramethyl-1,3,2-dioxaborolan-2-yl)oxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-7-methoxy-5, 11-dioxo-10-(((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5, 11-dioxo-10-(((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl trifluoromethanesulfonate (21)

Bis-triflate 12 (2.03g, 1.81 mmol), boronic pinacol ester (1g, 1.63 mmol) and Na₂CΟ₃ (881 mg, 8.31 mmol) were dissolved in a mixture of toluene/MeOH/H₂O, 2:1:1 (40 ml). The
reaction flask was purged and filled with argon three times before Addition of (triphenylphosphine) palladium(0) (41 mg, 0.035 mmol) was added and the reaction mixture was heated to 30°C overnight. The solvents were removed under reduced pressure and the residue was taken up in H₂O (100 ml) and extracted with EtOAc (3 x 100 ml). The combined organics were washed with brine (100 ml), dried with MgSO₄, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel chromatography column (Hexane/ EtOAc, 8:2 to 25:75) to afford pure 21 in 33% yield (885 mg). LC/MS 3.85 min (ES+) m/z (relative intensity) 1452.90; ¹H NMR (400 MHz, CDCl₃) δ 7.78 - 7.16 (m, 17H), 7.13 (s, 1H), 6.51 - 6.24 (m, 1H), 5.51 (dd, J = 10.0, 5.1 Hz, 2H), 5.36 - 5.11 (m, 1H), 4.74 (dd, J = 10.1, 4.4 Hz, 2H), 4.70 - 4.53 (m, 2H), 4.47 (d, J = 6.4 Hz, 1H), 4.37 (d, J = 7.2 Hz, 1H), 4.27 (m, 4H), 4.20 - 4.14 (m, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.77 (ddd, J = 16.7, 9.0, 6.4 Hz, 3H), 3.71 - 3.61 (m, 2H), 3.24 - 2.91 (m, 3H), 2.55 - 2.33 (m, 2H), 2.22 - 2.07 (m, 1H), 1.52 - 1.37 (m, 3H), 1.04 - 0.86 (m, 10H), 0.00 (s, 18H).

(d) (9H-fluoren-9-yl)methyl[(2S)-1-1((2S)-1-((4-(8-((3-(4H)-1,4-diazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-(2-(trimethylsilyl)ethoxy)methyl)-5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy]-7-methoxy-5,11-dioxo-10-(2-(trimethylsilyl)ethoxy)methyl]-5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)(phenyl)amino)-1-oxopropan-2-yl)carbamate (22)

Triphenylarsine (42 mg, 0.137 mmol) was added to a mixture of PBD-triflate 21 (250 mg, 0.172 mmol), cyclopropylboronic acid (73.9 mg, 0.86 mmol), silver oxide (159 mg, 0.688 mmol) and potassium phosphate tribasic (438 mg, 2.06 mmol) in dry dioxane (10 ml) under an argon atmosphere. The reaction was flushed with argon 3 times and subsequently heated to 75°C and stirred for 10 minutes. The reaction mixture was filtered through a pad of celite which was subsequently rinsed with ethyl acetate. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 1% methanol/chloroform). Pure fractions were collected and combined, and excess eluent was removed by rotary evaporation under reduced pressure to afford the desired product 22 (132 mg, 50 % yield). LC/MS 3.83 min (ES+) m/z (relative intensity) 1345.91; ¹H NMR (400 MHz, CDCl₃) δ 7.88 - 7.14 (m, 17H), 6.69 (s, 1H), 6.45 - 6.25 (m, 1H), 5.57 - 5.41 (m, 2H), 5.34 - 5.14 (m, 1H), 4.78 - 4.67 (m, 2H), 4.62 - 4.55 (m, 1H), 4.50 - 4.45 (m, 2H), 4.51 - 4.44 (m, 1H), 4.31 - 4.21 (m, 4H), 4.16 (m, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 - 3.71 (m, 2H), 3.66 (m, 3H), 3.40 - 3.28 (m, 1H), 3.07 (m, 1H), 2.70 - 2.57 (m, 1H), 2.47 - 2.36 (m, 2H), 2.15
(m, 1H), 1.51 - 1.40 (m, 3H), 1.03 - 0.87 (m, 11H), 0.77 - 0.71 (m, 2H), 0.60 - 0.54 (m, 2H),
0.00 (t, J = 3.0 Hz, 18H).

(e) (9H-fluoren-9-yl)methyl[(2S)-1-((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-
dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-
dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino]-
3-methyl-1-oxobutan-2-yl)carbamate (23)
A solution of Super-Hydride® (0.5 mL, 1M in THF) was added dropwise to a solution of SEM
dilactam 22 (265 mg, 0.19 mmol) in THF (10 mL) at -78°C under an argon atmosphere.

The addition was completed over 5 minutes in order to maintain the internal temperature of
the reaction mixture constant. After 20 minutes, an aliquot was quenched with water for
LC/MS analysis, which revealed that the reaction was complete. Water (20 mL) was added
to the reaction mixture and the cold bath was removed. The organic layer was extracted with
EtOAc (3 x 30 mL) and the combined organics were washed with brine (50 mL), dried with
MgSO₄, filtered and the solvent removed by rotary evaporation under reduced pressure. The
crude product was dissolved in MeOH (12 mL), CH₂Cl₂ (6 mL), water (2 mL) and enough
silica gel to form a thick stirring suspension. After 5 days, the suspension was filtered
through a sintered funnel and washed with CH₂Cl₂/MeOH (9:1) (200 mL) until the elution
of the product was complete. The organic layer was washed with brine (2 x 70 mL), dried with
MgSO₄, filtered and the solvent removed by rotary evaporation under reduced pressure.
Purification by silica gel column chromatography (100% CHCl₃ to 96% CHCl₃/ 4% MeOH)
afforded the product 23 as a yellow solid (162 mg, 78%). LC/MS 3.02 min (ES⁺) m/z
(relative intensity) 1052.37.

(f) (2S)-2-amino-N-((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-1H-
benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-
benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-
methybutanamide (17)
Excess piperidine was added (0.2 mL, 2 mmol) to a solution of SEM-dilactam 23 (76 mg,
0.073 mmol) in DMF (1 mL). The mixture was allowed to stir at room temperature for 20 min,
at which point the reaction had gone to completion (as monitored by LC/MS). The reaction
mixture was diluted with CH₂Cl₂ (75 mL) and the organic phase was washed with H₂O (3x75
mL) until complete piperidine removal. The organic phase was dried over MgSO₄, filtered
and excess solvent removed by rotary evaporation under reduced pressure to afford crude
product 17 which was used as such in the next step. LC/MS 2.32 min (ES⁺) m/z (relative
intensity) 830.00.
(g) N-((2S)-1-(((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,1,1a-dihydro-1H-
benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,1,1a-dihydro-1H-
benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-
oxobutan-2-yl)-1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (18)

EDCI hydrochloride (14 mg, 0.0732 mmol) was added to a suspension of Maleimide-PEG₈ acid (43.4 mg, 0.0732 mmol) in dry CH₂Cl₂ (5 ml) under argon atmosphere. The mixture was stirred for 1 hour at room temperature before PBD 17 (60.7 mg, 0.0732 mmol) was added. Stirring was maintained until the reaction was complete (usually 5 hours). The reaction was diluted with CH₂Cl₂ and the organic phase was washed with H₂O and brine before being dried over MgSO₄, filtered and excess solvent removed by rotary evaporation under reduced pressure. The product was purified by careful silica gel chromatography (slow elution starting with 100% CHCl₃ up to 9:1 CHCl₃/MeOH) followed by reverse phase chromatography to remove unreacted maleimide-PEG₈-acid. The product 18 was isolated in 17.6% (21.8 mg). LC/MS 2.57 min (ES+) m/z (relative intensity) 1405.30; ¹H NMR (400 MHz, CDCl₃) δ 7.91 (t, J = 3.5 Hz, 1H), 7.80 (d, J = 4.0 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.54 - 7.50 (m, 2H), 7.45 (s, 1H), 7.39 - 7.31 (m, 2H), 6.87 (d, J = 10.5 Hz, 2H), 6.76 (s, 1H), 6.72 - 6.68 (m, 2H), 4.74 - 4.62 (m, 1H), 4.45 - 4.17 (m, 7H), 3.95 (s, 3H), 3.94 (s, 3H), 3.67 - 3.58 (m, 34H), 3.54 (m, 2H), 3.42 (dd, J = 10.2, 5.2 Hz, 2H), 3.16 - 3.07 (m, 1H), 2.92 (dd, J = 16.1, 4.1 Hz, 1H), 2.62 - 2.49 (m, 4H), 2.48 - 2.39 (m, 2H), 2.37 - 2.25 (m, 1H), 1.92 (s, 1H), 1.52 - 1.44 (m, 3H), 1.10 - 0.93 (m, 6H), 0.79 (dd, J = 9.2, 5.3 Hz, 2H), 0.57 (dd, J = 9.2, 5.3 Hz, 2H), NH were not observed.
Example 3

(a) (S)-7-methoxy-8-(3-(((S)-7-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)ph enyl)-5, 11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5, 11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)trifluoromethanesulfonate (24)

Pd(PPh₃)₄ (20.6 mg, 0.018 mmol) was added to a stirred mixture of the bis-enol triflate 12 (500 mg, 0.44 mmol), N-methyl piperazine boronic ester (100 mg, 0.4 mmol), Na₂CO₃ (218 mg, 2.05 mmol), MeOH (2.5 ml), toluene (5 ml) and water (2.5 ml). The reaction mixture was allowed to stir at 30°C under a nitrogen atmosphere for 24 hours after which time all the boronic ester has consumed. The reaction mixture was then evaporated to dryness before the residue was taken up in EtOAc (100 ml) and washed with H₂O (2 x 50 ml), brine (50 ml), dried (MgSO₄), filtered and evaporated under reduced pressure to provide the crude product. Purification by flash chromatography (gradient elution: 80:20 v/v Hexane/EtOAc to 60:40 v/v Hexane/EtOAc) afforded product 24 as a yellowish foam (122.6 mg, 25%).
LC/MS 3.15 min (ES+) m/z (relative intensity) 1144 \([M + H]^+\), 20%.

(b) \((9H\text{-fluoren-9-yl})\text{methyl}\) \((\text{CH}_3\text{-i-})^+(\text{CH}_3\text{-i-})^+\)(4-\((\text{CH}_3\text{-i-})^+\)(7-methyl/\text{oxy}-8-\((\text{CH}_3\text{-i-})^+\)(7-methyl/\text{oxy}-2-(4-(\text{CH}_3\text{-i-})^+\)(7-methyl/\text{oxy}-1-yl)phenyl)-5, 11-dioxa-10-(2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1 H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5, 11-dioxa-10-(2-(trimethylsilyl)ethoxy)methyl)-5, 10, 11, 11a-tetrahydro-1 H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl][amino]-1-oxopropan-2-yl]amino)-3-methyl-1-oxobutan-2-yl]carbamate \(25\)

PBD-triflate \(24\) (359 mg, 0.314 mmol), boronic pinacol ester \(20\) (250 mg, 0.408 mmol) and triethylamine (0.35 mL, 2.51 mmol) were dissolved in a mixture of toluene/MeOH/H$_2$O, 2:1:1 (3 mL). The microwave vessel was purged and filled with argon three times before tetra/i/s(triphenylphosphine)/palladium(0) (21.7 mg, 0.018 mmol) was added and the reaction mixture placed in the microwave at 80°C for 10 minutes. Subsequently, CH$_2$Cl$_2$ (100 mL) was added and the organics were washed with water (2 x 50 mL) and brine (50 mL) before being dried with MgSO$_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel chromatography column (CHCl$_3$/MeOH, 100% to 9:1) to afford pure \(25\) (200 mg, 43% yield). LC/MS 3.27 min (ES+) m/z (relative intensity) 1478 \([M + H]^+\), 100%.

(c) \((9H\text{-fluoren-9-yl})\text{methyl}\) \(((S)-1-f)((T)-1-f)(4-\((\text{CH}_3\text{-i-})^+\)(7-methyl/\text{oxy}-8-\((\text{CH}_3\text{-i-})^+\)(7-methoxy-2-(4-(\text{CH}_3\text{-i-})^+\)(7-methyl/\text{oxy}-1-yl)phenyl)-5-oxo-5, 11a-dihydro-1 H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5, 11a-dihydro-1 H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl][amino]-1-oxopropan-2-yl]amino)-3-methyl-1-oxobutan-2-yl]carbamate \(26\)

A solution of Super-Hydride® (0.34 mL, 1M in THF) was added dropwise to a solution of SEM-dilactam \(25\) (200 mg, 0.135 mmol) in THF (5 mL) at -78°C under an argon atmosphere. The addition was completed over 5 minutes in order to maintain the internal temperature of the reaction mixture constant. After 20 minutes, an aliquot was quenched with water for LC/MS analysis, which revealed that the reaction was complete. Water (20 mL) was added to the reaction mixture and the cold bath was removed. The organic layer was extracted with EtOAc (3 x 30 mL) and the combined organics were washed with brine (50 mL), dried with MgSO$_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dissolved in MeOH (6 mL), CH$_2$Cl$_2$ (3 mL), water (1 mL) and enough silica gel to form a thick stirring suspension. After 5 days, the suspension was filtered through a sintered funnel and washed with CH$_2$Cl$_2$/MeOH (9:1) (100 mL) until the elution of the product was complete. The organic layer was washed with brine (2 x 50 mL), dried with MgSO$_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. Purification by silica gel column chromatography (100% CHCl$_3$ to 96% CHCl$_3$/4% MeOH)
afforded the product 26 as a yellow solid (100 mg, 63%). LC/MS 2.67 min (ES+) m/z (relative intensity) 1186 ([M + H]+, 5%).

(d) (S)-2-amino-N-(((S)-1-(4-((R)-7-methoxy-8-(3-(((R)-7-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-5-oxo-5,11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (27)

Excess piperidine was added (0.1 ml, 1 mmol) to a solution of PBD 26 (36.4 mg, 0.03 mmol) in DMF (0.9 ml). The mixture was allowed to stir at room temperature for 20 min, at which point the reaction had gone to completion (as monitored by LC/MS). The reaction mixture was diluted with CH$_2$Cl$_2$ (50 ml) and the organic phase was washed with H$_2$O (3 x 50 ml) until complete piperidine removal. The organic phase was dried over MgSO$_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure to afford crude product 27 which was used as such in the next step. LC/MS 2.20 min (ES+) m/z (relative intensity) 964 ([M + H]+, 5%).

(e) 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(((S)-1-(((S)-1-(((S)-7-methoxy-8-(3-(((S)-7-methoxy-2-(4-methylpiperazin-1-yl)phenyl)-5-oxo-5,11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)hexanamide (28)

EDCI hydrochloride (4.7 mg, 0.03 mmol) was added to a suspension of 6-maleimidohexanoic acid (6.5 mg, 0.03 mmol) in dry CH$_2$Cl$_2$ (3 mL) under argon atmosphere. The mixture was stirred for 1 hour at room temperature before PBD 27 (34 mg, crude) was added. Stirring was maintained until the reaction was complete (6 hours). The reaction was diluted with CH$_2$Cl$_2$ and the organic phase was washed with H$_2$O and brine before being dried over MgSO$_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure by rotary evaporation under reduced pressure. The product was purified by careful silica gel chromatography (slow elution starting with 100% CHCl$_3$ up to 9:1 CHCl$_3$/MeOH) followed by reverse phase chromatography to remove unreacted maleimide-PEG$_6$-acid. The product 28 was isolated in 41% over two steps (14.6 mg). LC/MS 2.40 min (ES+) m/z (relative intensity) 1157 ([M + H]+, 5%).
Example 4 - alternative synthesis of compound 25

PBD-triflate 21 (469 mg, 0.323 mmol), boronic pinacol ester (146.5 mg, 0.484 mmol) and Na₂CO₃ (157 mg, 1.48 mmol) were dissolved in a mixture of toluene/MeOH/H₂O, 2:1:1 (10 mL). The reaction flask was purged with argon three times before [tris(triphenylphosphine)palladium(0)] (7.41 mg, 0.0064 mmol) was added and the reaction mixture heated to 30°C overnight. The solvents were removed under reduced pressure and the residue was taken up in H₂O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organics were washed with brine (100 mL), dried with MgSO₄, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel column chromatography (CHCl₃ 100% to CHCl₃/MeOH 95%/5%) to afford pure 25 in 33% yield (885 mg). LC/MS 3.27 min (ES⁺) m/z (relative intensity) 1478 ([M + H]⁺, 100%).
Example 5

(a) (S)-2-(4-Aminophenyl)-8-(3-(((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxa-10-
(2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5, 11(10H, 11aH)-dione (29)

3,4-(Methylenedioxy)phenyl boronic acid (356 mg, 2.1 mmol, 1.3 equiv.), TEA (1.8 mL, 12.9 mmol, 8 equiv.) and triflate/aniline 13 (1.75 g, 1.7 mmol, 1 equiv.) were dissolved in a mixture of ethanol (7 mL), toluene (13 mL) and water (2 mL) under an Ar atmosphere. The reaction mixture was evacuated and flushed with Ar 3 times, before addition of tetrakis(triphenylphosphine)palladium(0) (114 mg, 0.1 mmol, 0.06 equiv.). The flask was
again evacuated and flushed with Ar 3 times and heated in a microwave at 80°C for 8 minutes with 30 seconds pre-stirring time. Analysis by TLC (80:20 v/v ethyl acetate/hexane) indicated complete consumption of starting material. The reaction mixture was diluted with dichloromethane (50 mL) and washed with water (50 mL). The organic layer was dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by silica gel column chromatography (60:40 to 20:80 v/v hexane/ethyl acetate) afforded the product 29 as a yellow solid (1.21 g, 71%). LC/MS (3.92 min (ES⁺) m/z (relative intensity) 1032.44 \([M + H]^+\), 100).

(b) \((S)-2-(4-Aminophenyl)-8-(3-(((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)(oxy)propoxy)-7-methoxy-1H-pyrrolo[2,1-c][1,4]benzodiazepin-5(1H)-one\) (30)

SEM dilactam 29 (0.25 g, 0.24 mmol, 1 equiv.) was dissolved in THF (8 mL) and cooled to -78°C under an Ar atmosphere. Super-Hydride® (0.6 mL, 1 M in THF, 2.5 equiv.) was added dropwise over 5 minutes while monitoring the temperature. After 20 minutes a small sample was taken and worked-up for LCMS analysis. Water (50 mL) was added, the cold bath was removed and the solution washed with ethyl acetate (50 mL). The organic layer was extracted and washed with brine (60 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. The crude product was dissolved in EtOH (15 mL), \(\text{CH}_2\text{Cl}_2\) (7.5 mL) and water (2.5 mL) and enough silica gel was added until it was a thick suspension. After 5 days stirring, it was filtered through a sintered funnel and washed with \(\text{CH}_2\text{Cl}_2\)/MeOH (9:1) (100 mL) until product ceased to be eluted. The organic layer was washed with brine (2 x 50 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by silica gel column chromatography (CHCl₃ with 1% to 4% MeOH gradient) afforded the product 30 as a yellow solid (94 mg, 53%). LC/MS (2.53 min (ES⁺) m/z (relative intensity) 739.64 \([M]^+\), 70).

(c) Allyl \(\((S)-1-((\text{S})-1-((\text{S})-8-(3-(((\text{S})-2-(benzo[d][1,3]dioxo}^\text{̲})-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)(oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)(phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate\) (31)

Under an Ar atmosphere, Alanine-Valine-Alloc (180 mg, 0.66 mmol, 1.2 equiv.) was stirred with EEDQ (163 mg, 0.66 mmol, 1.2 equiv.) in anhydrous \(\text{CH}_2\text{Cl}_2\) (21 mL) and methanol (1 mL) for 1 hour. The PBD 30 (407 mg, 0.55 mmol, 1 equiv.) was dissolved in anhydrous \(\text{CH}_2\text{Cl}_2\) (21 mL) and methanol (1 mL) and added to the reaction. LC/MS after 5 days stirring at room temperature showed majority product formation. The solvent was removed in vacuo before purification by column chromatography (\(\text{CH}_2\text{Cl}_2\) with 1% to 6% MeOH gradient) to
yield the product 31 as a yellow solid (184 mg, 34%). LC/MS (2.95 min (ES+) m/z (relative intensity) 994.95 ([M + H]+, 60).


The imine 31 (100 mg, 0.1 mmol, 1 equiv.) was dissolved in anhydrous DCM (10 ml) (with the aid of one drop of methanol to aid dissolution) under an Ar atmosphere. Pyrrolidine (30 µL, 0.15 mmol, 1.5 equiv.) was added drop wise before the flask was evacuated and flushed with Ar three times. Pd(PPh3)4 (7 mg, 6 µmol, 0.06 equiv.) was added and the flask was evacuated and flushed with Ar three times. LC/MS analysis after 1 hour indicated product formation and complete loss of starting material. Et2O (60 ml) was added to the reaction mixture and it was left to stir until all the product had crashed out of solution. The precipitate was filtered through a sintered funnel and washed twice with Et2O (2 x 20 ml). The collection flask was replaced and the isolated solid was dissolved and washed through the sinter with CHCl3 (100 ml). The solvent was removed in vacuo to afford the crude product 32 as a yellow solid which was used directly in the next step. LC/MS (1.14 min (ES+) m/z (relative intensity) 910.40 ([M + H]+, 67).

(e) N-((S)-1-((((S)-8-(3-(((S)-2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo-5, 11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-1-(3-(2,5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)propanamido)-3,6,9, 12, 15, 18,21,24-octaoxaheptacosan-27-amide (33)

The imine 32 (92 mg, 0.1 mmol, 1.1 equiv.) was dissolved in CHCl3 (6 ml) with one drop of anhydrous MeOH to aid dissolution. Maleimide-PEG-8-acid (53 mg, 0.09 mmol, 1 equiv.) was added followed by EEDQ (33 mg, 0.14 mmol, 1.5 equiv.). This was left to stir vigorously at room temperature under Ar for 4 days until LC/MS analysis showed majority product formation. The solvent was removed in vacuo and the crude product was partially purified by silica gel column chromatography (CHCl3 with 1% to 10% MeOH gradient) yielding 33 (81 mg). The material was purified further by preparative HPLC to give 33 as a yellow solid (26.3 mg, 18%). Fast Formic run: LC/MS (1.39 min (ES+) m/z (relative intensity) 1485.00 ([M + H]+, 64).
Example 6

(a) 9H-Fluoren-9-yl)methyl (S)-1-(((S)-1-(((S)-8-(((S)-2-((benzo[d][1,3]dioxol-5-yl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (34)

The triflate (0.5 g, 0.35 mmol, 1 equiv.), 3,4-(methylenedioxy)phenyl boronic acid (75 mg, 0.45 mmol, 1.3 equiv.) and Na₂CO₃ (0.17 g, 1.6 mmol, 4.5 equiv.) were dissolved in toluene (11 mL), EtOH (5.5 mL) and water (5.5 mL) under an Ar atmosphere. The flask was evacuated and flushed with Ar three times. Pd(PPh₃)₄ (24 mg, 0.02 mmol, 0.06 equiv.) was added and again the flask was evacuated and flushed with Ar three times. This was heated to 30°C and left stirring overnight. Analysis by LC/MS showed complete loss of starting material. The solvent was removed in vacuo and the residue dissolved in water (60 mL) before washing with ethyl acetate (60 mL x 3). The combined organic layers were washed with brine (50 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by column chromatography (50:50 to 25:75 v/v hexane/ethyl acetate) afforded the product 34 as a yellow solid (310 mg, 64%). LC/MS (1.44 min (ES⁻) m/z (relative intensity) 1423.35 ([M - H]⁻, 79).
(b) (9H-Fluoren-9-yl)methyl (S)-1-(((S)-1-((4-((S)-8-((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo-5, 11a-dihydro-1H-pyrrolo[2, 1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5, 11a-dihydro-1'H-pyrrolo[2, 1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)(amo)-3-methyl-1-oxobutan-2-yl)carbamate (35)

SEM dilactam 34 (0.31 g, 0.22 mmol, 1 equiv.) was dissolved in THF (10 mL) and cooled to -78°C under an Ar atmosphere. Super-Hydride® (0.5 mL, 1 M in THF, 2.5 equiv.) was added drop wise over 5 minutes while monitoring the temperature. After 30 minutes a small sample was taken and worked-up for LC/MS analysis. Water (50 mL) was added, the cold bath was removed and the solution washed with ethyl acetate (50 mL). The organic layer was extracted and washed with brine (60 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. The crude product was dissolved in EtOH (13.2 mL), CH₂Cl₂ (6.6 mL) and water (2.2 mL) and enough silica gel was added until it was a thick suspension. After 5 days stirring, it was filtered through a sintered funnel and washed with CH₂Cl₂/MeOH (9:1) (100 mL) until product ceased to be eluted. The organic layer was washed with brine (2 x 50 mL), dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by silica gel column chromatography (CHCl₃ with 1% to 4% MeOH gradient) afforded the pure product 35 as a yellow solid (185 mg, 75%). LC/MS (1.70 min (ES⁺) m/z (relative intensity) 1132.85 [M + H]+, 60).


The imine 35 (82 mg, 0.07 mmol, 1 equiv.) was dissolved in DMF (1 mL) before piperidine (0.2 mL, 2 mmol, excess) was added slowly. This solution was left to stir at room temperature for 20 minutes until LC/MS analysis showed complete consumption of starting material. The reaction mixture was diluted with CH₂Cl₂ (50 mL), washed with water (50 mL x 4), dried with MgSO₄, filtered and the solvent removed in vacuo. The product 33 was used without further purification in the next step. LC/MS (1.15 min (ES⁺) m/z (relative intensity) 910.60 [M + H]+, 58).

**General Experimental Methods for Example 7**

Reaction progress was monitored by thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel, with fluorescent indicator on aluminium plates. Visualisation of TLC was achieved with UV light or iodine vapour unless otherwise stated. Flash chromatography was performed using Merck Kieselgel 60 F254 silica gel. Extraction and chromatography solvents
were bought and used without further purification from Fisher Scientific, U.K. All chemicals were purchased from Aldrich, Lancaster or BDH.

\(^1\)H and \(^{13}\)C NMR spectra were obtained on a Bruker Avance 400 spectrometer. Coupling constants are quoted in hertz (Hz). Chemical shifts are recorded in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentuplet) and m (multiplet). IR spectra were recorded on a Perkin-Elmer FT/IR paragon 1000 spectrophotometer by application of the sample in a solution of chloroform using the ATR "golden gate" system. Optical rotations were measured at ambient temperature using a Bellingham and Stanley ADP 220 polarimeter. Mass spectrometry was performed on a ThermoQuest Navigator from Thermo Electron, Electrospray (ES) spectra were obtained at 20 to 30 V. Accurate mass measurements were performed using Micromass Q-TOF global tandem. All samples were run under electrospray ionization mode using 50% acetonitrile in water and 0.1% formic acid as a solvent. Samples were run on W mode which gives a typical resolution of 19000 at FWHH. The instrument was calibrated with [Glu]-Fibrinopeptide B immediately prior to measurement.

**LCMS**

LC/MS (Shimazu LCMS-2020) using a mobile phase of water (A) (formic acid 0.1%) and acetonitrile (B) (formic acid 0.1%).

Gradient: initial composition 5% B held over 0.25 min, then increase from 5% B to 100% B over a 2 min period. The composition was held for 0.50 min at 100% B, then returned to 5% B in 0.05 minutes and hold there for 0.05 min. Total gradient run time equals 3 min. Flow rate 0.8 mL/min. Wavelength detection range: 190 to 800 nm. Oven temperature: 50°C.

Column: Waters Acquity UPLC BEH Shield RP18 1.7 µm 2.1x50mm.

**Preparative HPLC**

The conditions for the preparative HPLC were as follow: the HPLC (Shimadzu UFLC) was run using a mobile phase of water (0.1% formic acid) A and acetonitrile (0.1% formic acid) B. Wavelength detection range: 254 nm.

Column: Phenomenex Gemini 5µ C18 150x21-20mm.
Gradient:

B

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Total gradient run time is 20 min; flow rate 20.00 mL/min.

Example 7

(a) (S)-5-(((terf-butyl(dimethyl)silyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((tri/sopropylsilyl)oxy)benzoyl)-4,5-dihydro-1H-pyrrol-3-yl trifluoromethanesulfonate (37)

Anhydrous 2,6-lutidine (16.06 mL, 0.137 mol) was injected in one portion to a vigorously stirred solution of ketone 36 (20 g, 0.034 mol) in dry CH₂Cl₂ (350 mL) at -45°C (dry ice/acetonitrile) under an argon atmosphere. Anhydrous triflic anhydride, taken from a freshly opened bottle (17.37 mL, 0.1 mol), was injected rapidly, while maintaining the temperature at -40 °C or below. The reaction mixture was allowed to stir at -45°C for 1 hour at which point TLC (Hexane/EtOAc ; 95/5) revealed complete consumption of starting material. The cold reaction mixture was immediately diluted with CH₂Cl₂ (400 mL) and, with vigorous shaking, washed with ice cold water (1 x 200 mL), ice cold 5% citric acid solution (1 x 300 mL), saturated NaHCO₃ (300 mL), brine (200 mL). The organics were dried over MgSO₄, filtered and the solvent evaporated under reduced pressure. The crude material was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 90:10) to afford enol-triflate 37 as a yellow foam (22.06 g, 89%). ¹H-NMR (400 MHz, CDCl₃) δ 7.72 (s, 1H), 7.26 (s, 1H), 6.75 (s, 1H), 60.6 (bm, 1H), 5.75 (d, J = 5.7 Hz, 0.5H), 4.78 (m, 1H), 4.59 (d, J = 8.2 Hz, 0.5H), 3.92 (s, 3H), 3.18 (dd, J = 15.2, 3.2 Hz, 4H), 2.99 (dd, J = 15.7, 3.2 Hz, 4H), 1.36 - 1.22 (m, 3H), 1.11 (d, J = 7.3 Hz, 18H), 0.92 (s, 9H), 0.12 (s, 6H); ES⁺ = 2.39 min, m/z 1447.05 [2M + Na]⁺.

(b) tert-butyl (1S)-8-(3-bromopropoxy)-1-(((terf-butyl(dimethyl)silyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-1,1a-dihydro-1H-benzooelpyrrole,2-1diazepine-1(5H)-carboxylate (45)
(i) (S)-(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-cyclopropyl-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone (38)

Triphenylarsine (0.343 g, 1.12 mmol), silver (I) oxide (1.3 g, 5.6 mmol), cyclopropylboronic acid (0.6 g, 7.01 mmol) and triflate 37 (1 g, 1.4 mmol) were dissolved in dioxane (20 ml) under an argon atmosphere. Potassium phosphate tribasic (3.6 g, 16.8 mmol) was ground-up with a pestle and mortar and quickly added to the reaction mixture. The reaction mixture was evacuated and flushed with argon three times and heated to 71°C.

Bis(benzonitrile)palladium(II) chloride (107 mg, 0.28 mmol) was added and the reaction vessel was evacuated and flushed with argon three times. After 10 minutes, a small sample
was taken for analysis by TLC (Hexane/EtOAc ; 80:20) revealing the reaction to be complete. The reaction mixture was filtered through celite and the filter pad washed with EtOAc (200 ml). The filtrate was washed with water (200 ml.) and brine (200 ml.). The organic layer was dried with MgSO₄, filtered and the solvent removed in vacuo. Purification by silica gel chromatography (Hexane/EtOAc ; 100% to 95:5) afforded product 38 as a yellow solid (0.663 g, 78%). ¹H-NMR (400 MHz, CDCl₃) δ 7.70 (s, 1H), 7.33 (s, 1H), 6.77 (s, 1H), 4.64 (m, 1H), 3.90 (s, 3H), 3.70 (s, 2H), 2.64 (dd, J = 16.2, 2.42 Hz, 1H), 2.42 (dd, J = 16.2, 2.4 Hz, 1H), 1.35 - 1.22 (m, 3H), 1.19 (m, 1H), 1.10 (d, J = 7.3 Hz, 18H), 0.91 (s, 9H), 0.61 (m, 2H), 0.40 (dd, J = 7.2, 3.4 Hz, 2H), 0.10 (d, J = 1.9 Hz, 6H). ES⁺ = 2.39 min, m/z 605.30 [M + H]+.

(ii) (S)-(2-amino-5-methoxy-4-((trisopropylsilyl)oxy)phenyl)(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-cyclopropyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (39)

In a dry two-neck round bottom flask previously flushed with argon and fitted with a thermometer, nitrophenyl 38 (3.03g 5 mmol) was solubilised in a solution of 5% formic acid in methanol (25 ml). Zinc (1.64g, 25 mmol) was rapidly poured into the solution. The temperature instantaneously rose to 45°C and slowly cooled down back to room temperature at which point the reaction is complete (=15 min, reaction monitored by LCMS). The reaction mixture was then filtered through celite and the pad further washed with EtOAc (2 x 100 ml). The combined organics were subsequently washed with saturated NaHCO₃ (100 ml), H₂O (100 ml) and brine (100 ml), before being dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified silica gel chromatography (Hexane/EtOAc ; 100% to 80:20) and pure product 39 was isolated as a pale colourless oil (1.35 g, 47% yield). ¹H-NMR (400 MHz, CDCl₃) δ 7.26 (s, 2H), 6.71 (s, 1H), 4.61 (bs, 1H), 4.22 (s, 2H), 3.88 (s, 1H), 3.77 (s, 1H), 3.71 (s, 3H), 2.60 (dd, J = 16.5, 3.7 Hz, 1H), 2.43 (dd, J = 16.5, 3.7 Hz, 1H), 1.35 (m, 1H), 1.22 (m, 3H), 1.09 (d, J = 7.2 Hz, 18H), 0.89 (s, 9H), 0.68 - 0.58 (m, 2H), 0.48 - 0.36 (m, 2H), 0.05 (d, J = 5.8 Hz, 6H). ES⁺ = 2.40 min, m/z 575.30 [M + H]+.

(hi) tert-butyl (S)-(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-cyclopentyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((trisopropylsilyl)oxy)phenyl)carbamate (40)

Amine 39 (770 mg, 1.34 mmol) and Boc₂O (350 mg, 1.6 mmol) were heated together at 70°C in a round bottom flask. To help with solubility, CHCl₃ (3 ml.) was added and the mixture left to stir until the reaction was complete (followed by LCMS). The thick crude solution was left to cool down to room temperature before being directly loaded on a silica gel chromatography column (Hexane/EtOAc ; 100% to 95:5). Product 40 was isolated as a
colourless foam (741 mg, 82% yield). \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.73 (s, 1H), 7.26 (s, 2H), 6.73 (s, 1H), 4.64 (s, 1H), 3.91 (s, 1H), 3.78 (s, 1H), 3.74 (s, 3H), 2.61 (dd, \(J = 16.2, 3.0\) Hz, 1H), 2.45 (dd, \(J = 16.2, 3.0\) Hz, 1H), 1.47 (s, 9H), 1.36 (m, 1H), 1.33 - 1.23 (m, 3H), 1.11 (d, \(J = 7.3\) Hz, 18H), 0.89 (s, 9H), 0.64 (m, 2H), 0.43 (m, 2H), 0.05 (d, \(J = 7.2\) Hz, 6H) ; ES\(^+\) = 2.56 min, m/z 675.30 [M+H]\(^+\).

(iv) tert-butyl (S)-(2-(4-cyclopropyl-2-(hydroxymethyl)-2,3-dihydro-1H-pyrrole-1'-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (41)

Silyl ether 40 (741 mg, 1.1 mmol) was solubilised in a 7:2:1:1 mixture of AcOH/H\(_2\)O/MeOH/THF (11 mL) and the mixture was stirred at room temperature until the reaction was complete (~3 hours). The volatiles were removed in vacuo and the residue was taken up in EtOAc (50 mL). The organic phase was washed with saturated NaHCO\(_3\)(aq) (50 mL), H\(_2\)O (50 mL) and brine (50 mL), before being dried over MgSO\(_4\), filtered and concentrated in vacuo. The crude material was purified by silica gel chromatography

(11S)-2-cyclopropyl-1 1-hydroxy-7-methoxy-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (42)

DMSO (163 \(\mu\)L, 2.29 mmol) was added to a cooled solution of oxalyl chloride (93 \(\mu\)L, 1.1 mmol) in CH\(_2\)Cl\(_2\) (2 mL) at -78°C. After 15 minutes, a solution of alcohol 41 (515 mg, 0.91 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added dropwise to the oxidising mixture. The reaction was left to stir at -78°C for 1 hour before NEt\(_3\) (640 \(\mu\)L, 4.59 mmol) was added and the mixture allowed to warm to room temperature. Upon completion, the reaction mixture was diluted with CH\(_2\)Cl\(_2\) (40 mL) and the solution was washed with 0.1M HCl(aq) (50 mL), H\(_2\)O (50 mL), saturated NaHCO\(_3\)(aq.) (50 mL) and brine (50 mL). The organics were dried with MgSO\(_4\), filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 60:40) to provide pure 42 as a white foam (350 mg, 68%).

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.17 (s, 1H), 6.74 (s, 1H), 6.67 (s, 1H), 5.65 (dd, \(J = 8.6, 2.3\) Hz, 1H), 3.85 (s, 3H), 3.77 (dt, \(J = 13.2, 6.7\) Hz, 1H), 3.38 (s, 1H), 2.88 (dd, \(J = 17.7, 9.2\) Hz, 1H), 2.52 (d, \(J = 14.5\) Hz, 1H), 1.46 (m, 1H), 1.39 (s, 9H), 1.31 - 1.19 (m, 3H), 1.10 (dd, \(J = 8.3, 1.5\) Hz, 18H), 0.89 (s, 9H), 0.64 (m, 2H), 0.43 (m, 2H), 0.05 (d, \(J = 7.2\) Hz, 6H) ; ES\(^+\) = 2.56 min, m/z 675.30 [M+H]\(^+\).
7.4, 2.1 Hz, 18H), 0.77 - 0.70 (m, 2H), 0.56 - 0.48 (m, 2H) ; ES⁺ = 1.89 min, m/z 559.45 [M+H]⁺.

(vi) tert-butyl (11S)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-8-
((trisopropylsilyl)oxy)-11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-
carboxylate (43)

(43) Alcohol 42 (350 mg, 0.62 mmol) was solubilised in dry CH₂Cl₂ (5 mL) in a sealed round bottom flask previously flushed three times with argon. The solution was cooled to 0°C before lutidine (0.3 mL, 2.5 mmol) and TBS-OTf (0.43 mL, 1.8 mmol) were subsequently added. The reaction mixture was left to warm to room temperature and stirred until complete (monitored by LCMS). Upon completion, the solution was diluted with CH₂Cl₂ (50 mL), washed with saturated NH₄Cl (aq) (50 mL), H₂O (50 mL), saturated NaHCO₃ (aq) (50 mL) and brine (50 mL). The organics were dried with MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 80:20) to provide pure 43 as a colourless oil (397.3 mg, 94%). ¹H-NMR (400 MHz, CDCl₃) δ 7.16 (s, 1H), 6.69 (s, 1H), 6.63 (s, 1H), 5.78 (d, J = 9.0 Hz, 1H), 3.84 (s, 3H), 3.65 (td, J = 10.1, 3.7 Hz, 1H), 2.82 (ddd, J = 16.8, 10.3, 2.6 Hz, 1H), 2.30 (dd, J = 16.8, 2.6 Hz, 1H), 1.45 - 1.37 (m, 1H), 1.32 (s, 9H), 1.25 (dd, J = 14.1, 8.0 Hz, 3H), 1.09 (dd, J = 7.4, 4.1 Hz, 18H), 0.85 (s, 9H), 0.74 - 0.67 (m, 2H), 0.55 - 0.49 (m, 1H), 0.47 - 0.40 (m, 1H), 0.25 (s, 3H), 0.20 (s, 3H) ; ES⁺ = 2.39 min, m/z 695.55 [M+Na]⁺.

(vii) tert-butyl (11S)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-8-hydroxy-7-methoxy-5-
oxo-11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (44)

Monomer 43 (518.8 mg, 0.77 mmol) was solubilised in wet DMF (5 mL +0.1 mL H₂O ) before LiOAc (78.5 mg, 0.77 mmol) was added and the mixture left to stir at room temperature until complete (followed by LCMS). The mixture was subsequently diluted with EtOAc (50 mL), quenched with citric acid (aq.) (pH=3, 40 mL), then washed with H₂O (50 mL) and brine (50 mL). The organic layer was dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude product was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 60:40) and pure product 44 was isolated as a white solid (351 mg, 88% yield). ¹H-NMR (400 MHz, CDCl₃) δ 7.20 (s, 1H), 6.68 (s, 1H), 6.68 (s, 1H), 5.79 (d, J = 8.9 Hz, 1H), 3.94 (s, 3H), 3.70 (td, J = 10.1, 3.7 Hz, 1H), 2.82 (ddd, J = 16.9, 10.3, 2.0 Hz, 1H), 2.31 (dd, J = 16.9, 2.0 Hz, 1H), 1.44 - 1.37 (m, 1H), 1.32 (s, 9H), 0.96 (s, 9H), 0.75 - 0.68 (m, 1H), 0.57 - 0.49 (m, 1H), 0.46 (m, 1H), 0.23 (d, J = 6.9 Hz, 6H) ; ES⁺ = 1.82 min, m/z 517.35 [M+Na]⁺.
(viii) tert-butyl (11S)-8-(3-bromopropoxy)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-1,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (45)

In a dry round bottom flask previously flushed three times with argon, alcohol 44 (300 mg, 0.58 mmol) was solubilised in dry DMF (5 mL). K₂CO₃ (123 mg, 0.88 mmol) and 1,3-dibromopropane (0.3 mL, 2.9 mmol) were subsequently added. The reaction mixture was heated to 70°C and left to stir until complete (=1 hour, followed by LCMS). The reaction was diluted with EtOAc (50 mL), washed with H₂O (75 mL) and brine (50 mL) before being dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (Hexane/EtOAc; 100% to 70:30) and pure product 45 was isolated as a colourless foam (311 mg, 84% yield). ¹H-NMR (400 MHz, CDCl₃) δ 7.21 (s, 1H), 6.69 (s, 1H), 6.63 (s, 1H), 5.82 (d, J = 8.8 Hz, 1H), 4.14 (t, J = 5.9 Hz, 2H), 3.90 (s, 3H), 3.69 (ddd, J = 10.2, 9.0, 3.7 Hz, 1H), 3.63 (t, J = 6.3 Hz, 2H), 2.84 (ddd, J = 16.7, 10.4, 1.9 Hz, 1H), 2.38 (p, J = 6.1 Hz, 2H), 2.31 (dd, J = 16.5, 2.1 Hz, 1H), 1.45 - 1.37 (m, 1H), 1.33 (s, 9H), 0.87 (s, 9H), 0.77 - 0.69 (m, 2H), 0.57 - 0.49 (m, 1H), 0.49 - 0.42 (m, 1H), 0.24 (d, J = 5.4 Hz, 6H); ES⁺ = 2.16 min, m/z 638.95 [M+Na]⁺.
(c) tert-butyl (11SV2-(4-((S)-2-((SV2-((((9H-fluoren-9-vnmethoxy \_carbonvnaminoV3-methylbutanamido)propanamido)phenyl)-1-((ferf-butyldimethylsilyl)oxy)-8-hvdroxy-7-methoxy-5-oxo-1.1\_1-a-dihvdro-1/\_/-benzoelpyrrolori,2-alM,4Idiazepine-10(5/-/)-carboxylate

(Pd(PPh)\textsubscript{3})\textsubscript{4} (609 mg, 0.52 mmol) was added to a stirred mixture of triflate 37 (18.8 g, 26.3 mmol), 4-aminophenylboronic acid pinacol ester (8.64 g, 39.4 mmol), Na\textsubscript{2}CO\textsubscript{3} (12.78 g, 120 mmol), MeOH (80 ml\textsubscript{1}), toluene (160 ml\textsubscript{1}) and water (80 ml\textsubscript{1}). The reaction mixture was allowed to stir at 30°C under a nitrogen atmosphere for 24 hours after which time all the boronic ester has consumed. The reaction mixture was then evaporated to dryness before
the residue was taken up in EtOAc (100 mL) and washed with H$_2$O (100 mL), brine (100 mL), dried (MgSO$_4$), filtered and evaporated under reduced pressure to provide the crude product. Purification by silica gel chromatography (Hexane/EtOAc ; 100% to 70:30) afforded product 46 as a yellowish foam (11.06 g, 64%). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.74 (s, 1H), 7.00 (d, $J$ = 8.3 Hz, 2H), 6.81 (s, 1H), 6.58 (d, $J$ = 8.3 Hz, 2H), 6.06 (s, 1H), 4.77 (bm, 1H), 3.91 (d, $J$ = 6.7 Hz, 3H), 3.68 (bs, 2H), 3.13 (bm, 1H), 2.97 (d, $J$ = 14.5 Hz, 1H), 1.36 - 1.21 (m, 3H), 1.12 (d, $J$ = 7.3 Hz, 18H), 0.89 (s, 10H), 0.10 (s, 6H). $^1$ES$^+$ = 2.27 min, m/z 698 [M + CH$_3$CN]$^+$. 

To a dry round bottom flask previously flushed with argon was added aniline 46 (10.05 g, 15.3 mmol), the dipeptide (6.3 g, 15.3 mmol) and dry CH$_2$Cl$_2$ (500 mL). The flask was then purged three times with argon before EEDQ (3.79 g, 15.3 mmol) was added and the mixture left to stir at room temperature. The reaction was followed by LCMS and after 3.5 hours the reaction was complete. The reaction was quenched with H$_2$O (200 mL) and extracted twice with CH$_2$Cl$_2$ (250 mL). The combined organics were washed with brine (150 mL), dried over MgSO$_4$, filtered and the solvent removed in vacuo. The crude product was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 55:45) to afford pure product 47 (13.821 g, 86%). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 8.26 (s, 1H), 7.64 (s + d, $J$ = 4.9 Hz, 3H), 7.43 (t, $J$ = 7.3 Hz, 1H), 7.36 (d, $J$ = 7.3 Hz, 1H), 7.28 (t, $J$ = 7.3 Hz, 1H), 7.19 (d, $J$ = 7.7 Hz, 1H), 6.99 (d, $J$ = 7.9 Hz, 1H), 6.71 (s, 1H), 6.27 (d, $J$ = 6.3 Hz, 1H), 6.08 (s, 1H), 5.11 (d, $J$ = 6.6 Hz, 1H), 4.69 (bs, 1H), 4.52 (bm, 1H), 4.36 (d, $J$ = 6.5 Hz, 2H), 4.08 (t, $J$ = 5.9 Hz, 1H), 3.89 (m, 1H), 3.80 (s, 3H), 3.11 - 2.97 (bm, 1H), 2.88 (bd, $J$ = 15.2 Hz, 1H), 2.03 (bs, 1H), 1.33 (d, $J$ = 6.9 Hz, 3H), 1.24 - 1.11 (m, 3H), 1.01 (d, $J$ = 7.4 Hz, 18H), 0.86 - 0.79 (m, 6H), 0.77 (s, 9H), 0.00 (s, 6H); $^1$ES$^+$ = 2.37 min, no mass.

In a dry two-neck round bottom flask previously flushed with argon and fitted with a thermometer, nitrophenyl 47 (2.97g, 2.8 mmol) was solubilised in a solution of 5% formic acid in methanol (50 mL). Zinc (1.85g, 28 mmol) was rapidly poured into the solution. The temperature instantaneously rose to 40°C and slowly cooled down back to room temperature at which point the reaction is complete (=15 minutes, reaction monitored by
LCMS). The reaction mixture was then filtered through celite and the pad further washed with EtOAc (2 x 150 mL). The combined organics were subsequently washed with saturated NaHCO₃ (100 mL), H₂O (100 mL) and brine (100 mL), before being dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified silica gel chromatography (Hexane/EtOAc 75:25 to 50:50) and pure product 48 was isolated as a pale yellow oil (2.291 g, 79% yield).  1H-NMR (400 MHz, CDCl₃) δ 8.37 (s, 1H), 7.74 (s+d, J = 4.9 Hz, 3H), 7.53 (t, J = 7.4 Hz, 2H), 7.46 (d, J = 11.3 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.28 (t, J = 11.3 Hz, 2H), 7.09 (d, J = 7.9 Hz, 2H), 6.38 (d, J = 6.3 Hz, 1H), 6.18 (s, 1H), 5.21 (d, J = 2.9 Hz, 1H), 4.81 (bs, 1H), 4.72 - 4.57 (m, 1H), 4.47 (d, J = 6.5 Hz, 2H), 4.19 (t, J = 5.0 Hz, 1H), 4.00 - 3.94 (m, 1H), 3.91 (s, 3H), 3.23 - 3.07 (m, 1H), 2.98 (d, J = 16.8 Hz, 1H), 2.15 (s, 1H), 1.43 (d, J = 6.9 Hz, 3H), 1.36 - 1.18 (m, 3H), 1.12 (d, J = 7.4 Hz, 18H), 0.97 - 0.89 (m, 6H), 0.88 (s, 9H), 0.10 (s, 6H). ES+ = 2.37 min, m/z no mass.

(iv) (9H-floumn-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-^rt-butoxycarbonyl)amino)-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-((tert-butyl(dimethyl)silyl)oxy)methyl)-4,5-dihydro-1H-pyrrol-3-yl)(phenyl)amino)-1-oxopropan-2-yl)(amino)-3-methyl-1-oxobutan-2-yl)carbamate (49)

Amine 48 (14.913 g, 14.6 mmol) and Boc₂O (3.83 g, 17.5 mmol) were heated together at 70°C in a round bottom flask. To help with solubility, CHCl₃ (25 mL) was added and the mixture left to stir until the reaction was complete (followed by LCMS). The thick crude solution was left to cool down to room temperature before being directly loaded on a silica gel chromatography column (Hexane/EtOAc ; 100% to 65:35). Product 49 was isolated as a cream foam (13.2 g, 80% yield).

1H-NMR (400 MHz, CDCl₃) δ 8.40 (s, 1H), 8.21 (s, 1H), 7.74 (d, J = 7.8 Hz, 3H), 7.54 (t, J = 7.0 Hz, 2H), 7.48 (d, J = 7.7 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.31 - 7.25 (m, 3H), 7.14 (d, J = 6.7 Hz, 2H), 6.84 (bs, 1H), 6.80 (s, 1H), 6.50 (d, J = 6.4 Hz, 1H), 5.28 (d, J = 6.0 Hz, 1H), 4.77 (d, J = 2.6 Hz, 1H), 4.70 - 4.58 (m, 1H), 4.47 (t, J = 5.7 Hz, 2H), 4.19 (t, J = 6.1 Hz, 1H), 4.00 (m, 2H), 3.88 (bs, 1H), 3.73 (s, 3H), 3.05 (m, 1H), 2.98 (dd, J = 15.4, 3.3 Hz, 1H), 2.15 (bm, 1H), 1.46 (s, 9H), 1.43 (d, J = 1.7 Hz, 3H), 1.36 - 1.22 (m, 3H), 1.12 (d, J = 7.4 Hz, 18H), 1.00 - 0.89 (m, 6H), 0.84 (s, 9H), 0.05 (d, J = 6.0 Hz, 6H) ; ES+ = 2.53 min, m/z no mass.

(v) (9H-flouren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-^rt-butoxycarbonyl)amino)-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(hydroxymethyl)-4,5-dihydro-1H-pyrrol-3-yl)(phenyl)amino)-1-oxopropan-2-yl)(amino)-3-methyl-1-oxobutan-2-yl)carbamate (50)

Silyl ether 49 (13.2 g, 11.8 mmol) was solubilised in a 7:2:1 : mixture of AcOH/H₂O/MeOH/THF (220 mL) and the mixture was stirred at room temperature until the
reaction was complete (left overnight). The volatiles were removed in vacuo and the residue
was taken up in EtOAc (400 mL). The organic phase was washed with saturated NaHCO₃ (200 mL), H₂O (200 mL) and brine (10 mL) before being dried over MgSO₄, filtered and
concentrated in vacuo. The crude material was purified by silica gel chromatography

(vi) tert-butyl (1S)-2-((4-((S)-2-(((S)-2-((((9H-fluoren-9-yl)methoxy)propanamido)phenyl)-1 1-((tert-butyldimethylsilyl)oxy)

15 methylbutanamido)propanamido)phenyl)-1 1-hydroxy-7-methoxy-5-oxo-8-(triisopropylsilyl)oxy)-1, 11a-dihydro-1H-benzo[elpyrropoi [1,2-a][1,4]diazepine- 10(5H)-carboxylate (51)

DMSO (1.55 L, 21.9 mmol) was added to a cooled solution of oxalyl chloride (0.89 mL, 10.5 mmol) in CH₂Cl₂ (50 mL) at -78°C. After 15 minutes, a solution of alcohol 50 (8.8 mg, 8.76 mmol) in CH₂Cl₂ (100 mL) was added dropwise to the oxidising mixture. The reaction was left to stir at -78°C for 1 hour before NEt₃ (6.11 mL, 43.8 mmol) was added and the mixture allowed to warm to room temperature. Upon completion, the reaction mixture was diluted with CH₂Cl₂ (100 mL) and the solution was washed with 0.1M HCl(aq.) (250 mL), H₂O (250 mL), saturated NaHCO₃ (aq) (250 mL) and brine (200 mL). The organics were dried with

MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (CH₂Cl₂/EtOAc ; 100% to 50:50) to provide pure 51 as a yellow oil (8.8 mg, 100%).

(vii) tert-butyl (1S)-2-((4-((S)-2-(((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-1 1-((tert-butyl(dimethylsilyl)oxy)-7-methoxy-5-oxo-8-
((trisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (52)

Alcohol 51 (8.8 g, 8.78 mmol) was solubilised in dry CH₂Cl₂ (150 mL) in a sealed round bottom flask previously flushed three times with argon. The solution was cooled to 0°C before lutidine (4 mL, 35.1 mmol) and TBS-OTf (6 mL, 26.3 mmol) were subsequently added. The reaction mixture was left to warm to room temperature and stirred until complete (monitored by LCMS). Upon completion, the solution was diluted with CH₂Cl₂ (100 mL), washed with saturated NH₄Cl(aq.) (150 mL), H₂O (100 mL), saturated NaHCO₃(aq.) (100 mL) and brine (100 mL). The organics were dried with MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (Hexane/EtOAc: 100% to 80:20) to provide pure 52 as a colourless oil (6.18 mg, 70%). ¹H-NMR (400 MHz, CDCl₃) δ 8.40 (s, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.55 (dd, J = 13.0, 6.7 Hz, 4H), 7.40 (t, J = 7.3 Hz, 4H), 7.33 - 7.27 (m, 3H), 7.21 (s, 1H), 6.67 (s, 1H), 6.49 (s, 1H), 5.87 (d, J = 8.8 Hz, 1H), 5.30 (d, J = 5.7 Hz, 1H), 4.71 - 4.59 (m, 1H), 4.48 (d, J = 6.8 Hz, 2H), 4.20 (t, J = 6.7 Hz, 1H), 4.04 - 3.96 (m, 1H), 3.86 (s, 3H), 3.84 - 3.77 (m, 1H), 3.25 (m, 1H), 2.79 (d, J = 1.5 Hz, 1H), 2.26 - 2.11 (m, 1H), 1.46 (d, J = 6.9 Hz, 3H), 1.33 (s, 9H), 1.27 (dd, J = 17.1, 9.7 Hz, 3H), 1.11 (dd, J = 7.4, 4.0 Hz, 18H), 0.93 (s, 6H), 0.89 (s, 9H), 0.27 (s, 3H), 0.22 (s, 3H); ES⁺ = 2.55 min, m/z 116.30 [M+H]⁺.

(viii) tert-butyl (11S)-2-(4-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbon yl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (53)

Monomer 52 (1 g, 0.89 mmol) was solubilised in wet DMF (5 mL +0.5 mL H₂O) before LiOAc (91 mg, 0.89 mmol) was added and the mixture left to stir at room temperature until complete (=3h, followed by LCMS). The mixture was subsequently diluted with EtOAc (50 mL), quenched with citric acid(aq.) (pH=3, 40 mL), then washed with H₂O (50 mL) and brine (50 mL). The organic layer was dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude product was purified by silica gel chromatography (Hexane/EtOAc/MeOH; 63.0:10 to 60:30:10) and pure product 53 was isolated as a cream solid (675 mg, 78% yield).

¹H-NMR (400 MHz, CDCl₃) δ 8.36 (s, 1H), 7.76 (d, J = 7.6 Hz, 2H), 7.55 (dd, J = 16.0, 7.5 Hz, 4H), 7.40 (t, J = 7.4 Hz, 4H), 7.30 (ddd, J = 14.7, 7.4, 1.1 Hz, 3H), 7.24 (s, 1H), 6.72 (s, 1H), 6.38 (d, J = 5.3 Hz, 1H), 5.87 (s, 1H), 5.23 (d, J = 6.2 Hz, 1H), 4.69 - 4.57 (m, 1H), 4.49 (d, J = 6.6 Hz, 2H), 4.20 (t, J = 5.3 Hz, 1H), 4.04 - 3.96 (m, 1H), 3.96 (s, 3H), 3.87 (dd, J = 10.1, 3.5 Hz, 1H), 3.29 (dd, J = 18.0, 8.5 Hz, 1H), 2.80 (d, J = 19.4 Hz, 1H), 2.24 - 2.08 (m,
1H), 1.46 (d, J = 10.5 Hz, 3H), 1.33 (s, 9H), 1.00 - 0.91 (m, 6H), 0.90 (s, 9H), 0.25 (d, J = 8.6 Hz, 6H). ; ES⁺ = 2.08 min, m/z 960.35 [M+H]⁺.

(d) /V-((2SV1-((2SV1-((4-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-1H-

benzolepyrroliri,2-al[1,4)diazepin-2-yl]phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-

oxobutan-2-yl)-1 -(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-ylpropanamido)-

(i) tert-butyl (11S)-2-(4-((S)-2-amino-3-methylbutanamido)propanamido)phenyl-8-(3-(((11S)-10-(tert-butoxycarbonyl)-11-((tert-butylimidemethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-5, 10, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1-((tert-butylimidemethylsilyl)oxy)-7-methoxy-5-oxo-11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (54)

In a dry round bottom flask previously flushed three times with argon, monomer 45 (310 mg, 0.48 mmol), monomer 53 (513 mg, 0.53 mmol), K₂CO₃ (103 mg, 0.48 mmol) and TBAI (18 mg, 0.048 mmol) were solubilised in dry DMF (5 mL) and the mixture was heated to 60°C. The reaction was left to stir until complete (followed by LCMS), before being diluted with EtOAc (50 mL), washed with H₂O (75 mL) and brine (50 mL). The organics were dried over MgSO₄, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (CHCl₃/MeOH; 100% to 98:2) and pure product 54 was isolated as a white solid (280.3 mg, 46% yield). ¹H-NMR (400 MHz, CDCl₃) δ 8.93 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.40 (s, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.19 (s, 2H), 6.69 (s, 1H), 6.63 (s, 1H), 6.61 (s, 1H), 5.90 (d, J = 9.3 Hz, 1H), 5.81 (d, J = 5.5 Hz, 1H), 4.60 (p, J = 7.1 Hz, 1H), 4.20 (dd, J = 15.9, 11.1 Hz, 4H), 3.88 (s, 3H), 3.87 (s, 3H), 3.84 (dd, J = 6.3, 4.5 Hz, 1H), 3.68 (td, J = 10.2, 3.7 Hz, 1H), 3.39 - 3.22 (m, 2H), 2.89 - 2.73 (m, 2H), 2.48 - 2.26 (m, 4H), 1.47 (d, J = 7.0 Hz, 3H), 1.42 (m, 1H), 1.30 (s, 18H), 1.02 (d, J = 7.0 Hz, 3H), 0.89 (s, 9H), 0.86 (s, 10H), 0.84 (s, 6H), 0.72 (dd, J = 8.1, 3.3 Hz, 2H), 0.57 - 0.50 (m, 1H), 0.45 (m, 1H), 0.28 - 0.20 (m, 12H); ES⁺ = 2.16 min, m/z 1297.55 [M+Na]⁺.

(ii) tert-butyl (11S)-8-((1S)-10-(tert-butoxycarbonyl)-1-((tert-butylimidemethylsilyl)oxy)-2-(4-((2S,5S)-3-7-(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-octaaxo-3,6,34-triazatehtacontamido)phenyl)-7-methoxy-5-oxo-5, 10, 11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1-((tert-butylimidemethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (55)

In a dry round bottom flask previously flushed three times with argon, dimer 54 (270 mg, 0.021 mmol) was solubilised in dry CH₂Cl₂ (6 mL). EDCI hydrochloride (40 mg, 0.021 mmol) and maleimide-PEG₆OH (123 mg, 0.021 mmol) were subsequently added to the solution which was left to stir at room temperature until complete (=1 hour, followed by LCMS). Upon completion, the reaction was diluted with CH₂Cl₂ (50 mL) and the organic phase was washed with H₂O (50 mL) and brine (50 mL) before being dried over MgSO₄, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude material was purified by silica gel chromatography (CHCl₃/MeOH 100% to 97:3) and pure product 55 was isolated as a light yellow foam (318.8 mg, 82% yield). ¹H-NMR (400 MHz, CDCl₃) 8.64 (s, 1H), 7.69 (d,
The product was isolated directly from the mixture being solubilised in dry 
H₂O (160 µL) and the slurry was cooled to 0°C before TFA (4 mL) was added and
the mixture left to stir until complete (=20 minutes, followed by LCMS). Upon completion, the reaction was diluted with CH₂Cl₂ (50 mL) and
the organic phase was washed with ice cold NaHCO₃ (2 x 50 mL), H₂O (50 mL) and
brine (50 mL) before being dried over MgSO₄, filtered and the volatiles removed in vacuo.
The crude material was directly purified by reverse phase preparative HPLC (H₂O/CH₃CN,
see conditions below) and pure product 18 was isolated as a yellow solid (61 mg, 26% yield). ¹H-NMR (400 MHz, CDCl₃) δ 8.76 (s, 1H), 7.88 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 4.0 Hz, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.51 - 7.48 (m, 2H), 7.43 (s, 1H), 7.33 (d, J = 8.6 Hz, 2H), 7.20 (s, 1H), 7.15 (s, 1H), 6.86 (s, 1H), 6.84 (s, 1H), 6.74 (s, 1H), 6.68 (s, 2H), 6.62 (s, 1H), 4.69 (p, J = 7.1 Hz, 1H), 4.41 - 4.24 (m, 5H), 4.24 - 4.16 (m, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.83 (t, J = 7.2 Hz, 4H), 3.67 - 3.56 (m, 33H), 3.55 - 3.49 (m, 1H), 3.39 (dt, J = 14.0, 7.0 Hz, 1H), 3.10 (dd, J = 15.0, 11.6 Hz, 1H), 2.89 (dd, J = 16.9, 3.6 Hz, 1H), 2.75 - 2.64 (m, 1H), 2.51 (t, J = 7.2 Hz, 2H), 2.48 - 2.44 (m, 1H), 2.44 - 2.38 (m, 1H), 2.28 (dt, J = 13.3, 6.8 Hz, 1H), 1.47 (s, 1H), 1.46 (d, J = 7.1 Hz, 3H), 1.02 (dd, J = 10.7, 6.9 Hz, 6H), 0.82 - 0.72 (m, 2H), 0.55 (q, J = 5.2 Hz, 2H). ES⁺ = 1.39 min, m/z 1404.45 [M+H]⁺
Example 8: Activity of released compounds

K562 assay

K562 human chronic myeloid leukaemia cells were maintained in RPM1 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37°C in a humidified atmosphere containing 5% CO₂ and were incubated with a specified dose of drug for 1 hour or 96 hours at 37°C in the dark. The incubation was terminated by centrifugation (5 min, 300 g) and the cells were washed once with drug-free medium. Following the appropriate drug treatment, the cells were transferred to 96-well microtiter plates (10⁴ cells per well, 8 wells per sample). Plates were then kept in the dark at 37°C in a humidified atmosphere containing 5% CO₂. The assay is based on the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Aldrich-Sigma), to an insoluble purple formazan precipitate. Following incubation of the plates for 4 days (to allow control cells to increase in number by approximately 10 fold), 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and the plates further incubated for 5 h. The plates were then centrifuged for 5 min at 300 g and the bulk of the medium pipetted from the cell pellet leaving 10-20 μL per well. DMSO (200 μL) was added to each well and the samples agitated to ensure complete mixing. The optical density was then read at a wavelength of 550 nm on a Titertek Multiscan ELISA plate reader, and a dose-response curve was constructed. For each curve, an IC₅₀ value was read as the dose required to reduce the final optical density to 50% of the control value.

Compound ReIC has an IC₅₀ of less than 0.1 pM in this assay.

Example 9: Formation of conjugates

General antibody conjugation procedure

Antibodies are diluted to 1-5 mg/mL in a reduction buffer (examples: phosphate buffered saline PBS, histidine buffer, sodium borate buffer, TRIS buffer). A freshly prepared solution of TCEP (tris(2-carboxyethyl)phosphine hydrochloride) is added to selectively reduce cysteine disulfide bridges. The amount of TCEP is proportional to the target level of reduction, within 1 to 4 molar equivalents per antibody, generating 2 to 8 reactive thiols. After reduction for several hours at 37°C, the mixture is cooled down to room temperature and excess drug-linker (A, B, C) added as a diluted DMSO solution (final DMSO content of up to 10% volume/volume of reaction mixture). The mixture was gently shaken at either 4°C or room temperature for the appropriate time, generally 1-3 hours. Excess reactive thiols can be reacted with a 'thiol capping reagent' like N-ethyl maleimide (NEM) at the end of the conjugation. Antibody-drug conjugates are concentrated using centrifugal spin-filters with a
molecular weight cut-off of 10 kDa or higher, then purified by tangential flow filtration (TFF) or Fast Protein Liquid Chromatography (FPLC). Corresponding antibody-drug conjugates can be determined by analysis by High-Performance Liquid Chromatography (HPLC) or Ultra-High-Performance Liquid Chromatography (UHPLC) to assess drug-per-antibody ratio (DAR) using reverse-phase chromatography (RP) or Hydrophobic-Interaction Chromatography (HIC), coupled with UV-Visible, Fluorescence or Mass-Spectrometer detection; aggregate level and monomer purity can be analysed by HPLC or UHPLC using size-exclusion chromatography coupled with UV-Visible, Fluorescence or Mass-Spectrometer detection. Final conjugate concentration is determined by a combination of spectroscopic (absorbance at 280, 214 and 330 nm) and biochemical assay (bicinchonic acid assay BCA; Smith, P.K., et al. (1985) Anal. Biochem. 150 (1): 76-85; using a known-concentration IgG antibody as reference). Antibody-drug conjugates are generally sterile filtered using 0.2 μm filters under aseptic conditions, and stored at +4°C, -20°C or -80°C.

Examples of particular conjugations are described below.

**ADC1A**

Antibody (15 mg, 100 nanomoles) is diluted into 13.5 mL of a reduction buffer containing 10 mM sodium borate pH 8.4, 2.5 mM EDTA and a final antibody concentration of 1.1 mg/mL. A 20 mM solution of TCEP is added (2 molar equivalent/antibody, 200 nanomoles, 10 μL) and the reduction mixture heated at 37°C for one hour in an orbital incubator. After cooling down to room temperature, A is added as a DMSO solution (5 molar equivalent/antibody, 510 nanomoles, in 1.2 mL DMSO). The solution is mixed 3 hour at room temperature, and then quenched by addition of N-ethylmaleimide (NEM, 10 molar equivalent, 1000 nanomoles, 100 μL at 10 mM), then transferred into a 15mL Amicon Ultracell 50KDa MWCO spin filter, concentrated to ca. 2.0 mL and injected into a AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with 1.5 mL/min of sterile-filtered Phosphate-buffered saline (PBS). Fractions corresponding to ADC1A monomer peak are pooled, analysed and sterile-filtered. BCA assay gives a concentration of final ADC1A at 1.25 mg/mL in 10.0 mL, obtained mass is 12.5 mg (83% yield). UHPLC analysis on a Shimadzu Prominence system using an Agilent PLRP-S 1000 A 8um 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ADC1A at 280 nm and 330 nm (drug-linker specific) shows a mixture of light and heavy chains attached to several molecules of A, consistent with a drug-per-antibody ratio (DAR) of 2.5 molecules of A per antibody. SEC analysis on a AKTA™FPLC using a GE Healthcare XK16/70 column
packed with Superdex 200 PG, eluting with sterile-filtered Phosphate-buffered saline (PBS) on a sample of ADC1A at 280 nm shows a monomer purity of 99.4% with 0.6% aggregates.

ADC1B

Antibody (15 mg, 100 nanomoles) is diluted into 13.5 mL of a reduction buffer containing 10 mM sodium borate pH 8.4, 2.5 mM EDTA and a final antibody concentration of 1.1 mg/mL. A 10 mM solution of TCEP is added (3 molar equivalent/antibody, 300 nanomoles, 30 μL) and the reduction mixture was heated at 37°C for two hours in an orbital incubator. After cooling down to room temperature, B is added as a DMSO solution (7 molar equivalent/antibody, 700 nanomoles, in 1 mL DMSO). The solution is mixed 3 hour at room temperature, then transferred into a 15mL Amicon Ultracell 50KDa MWCO spin filter, concentrated to ca. 2.0 mL and injected into an AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with 1.5 mL/min of sterile-filtered Phosphate-buffered saline (PBS). Fractions corresponding to ADC1B monomer peak are pooled, analysed and sterile-filtered. BCA assay gives a concentration of final ADC1B at 1.57 mg/mL in 6.3 mL, obtained mass is 9.9 mg (66% yield). HPLC analysis on a Shimadzu Prominence system using an Agilent PLRP-S 1000 A 8um 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ADC1B at 280 nm and 330 nm (drug-linker specific) shows a mixture of light and heavy chains attached to several molecules of B, consistent with a drug-per-antibody ratio (DAR) of 2.8 molecules of B per antibody. SEC analysis on an AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with sterile-filtered Phosphate-buffered saline (PBS) on a sample of ADC1B at 280 nm shows a monomer purity of 96.6% with 3.4% aggregates.

ADC1C

Antibody (1.0 mg, 6.7 nanomoles) is diluted into 0.9 mL of a reduction buffer containing 10 mM sodium borate pH 8.4, 2.5 mM EDTA and a final antibody concentration of 1.1 mg/mL. A 1 mM solution of TCEP is added (3 molar equivalent/antibody, 300 nanomoles, 30 μL) and the reduction mixture is heated at 37°C for 1.5 hours in an orbital incubator. After cooling down to room temperature, C is added as a DMSO solution (10 molar equivalent/antibody, 67 nanomoles, in 0.1 mL DMSO). The solution is mixed for 3 hours at room temperature, then quenched by addition of N-ethylmaleimide (NEM, 37 molar equivalent, 250 nanomoles, 10μL at 25 mM), then injected into an AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with 1.5 mL/min of sterile-filtered Phosphate-buffered saline (PBS). Fractions corresponding to ADC1C monomer peak are pooled, transferred into
a 15ml Amicon Ultracell 50KDa MWCO spin filter, concentrated to ca. 1.0 mL, analysed and sterile-filtered. BCA assay gives a concentration of final ADC1C at 0.63 mg/mL in 1.0 mL, obtained mass is 0.63 mg (63% yield). UHPLC analysis on a Shimadzu Prominence system using a Agilent PLRP-S 1000 A 8um 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ADC1C at 280 nm and 330 nm (drug-linker specific) shows a mixture of light and heavy chains attached to several molecules of C, consistent with a drug-per-antibody ratio (DAR) of 2.9 molecules of C per antibody. SEC analysis on an AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with sterile-filtered Phosphate-buffered saline (PBS) on a sample of ADC1C at 280 nm shows a monomer purity of 99.0% with 1.0% aggregates.

ADC2A

Antibody2 (15 mg, 100 nanomoles) is diluted into 13.5 mL of a reduction buffer containing 10 mM sodium borate pH 8.4, 2.5 mM EDTA and a final antibody concentration of 1.1 mg/mL. A 40 mM solution of TCEP is added (3 molar equivalent/antibody, 300 nanomoles, 7.5 µL) and the reduction mixture heated at 37°C for one hour in an orbital incubator. After cooling down to room temperature, A is added as a DMSO solution (7 molar equivalent/antibody, 700 nanomoles, in 1.0 mL DMSO). The solution is mixed 2.5 hour at room temperature, then is quenched by addition of N-ethylmaleimide (NEM, 30 molar equivalent, 3000 nanomoles, 100 µL at 30 mM), then transferred into a 15mL Amicon Ultracell 50KDa MWCO spin filter, concentrated to ca. 2.0 mL and injected into an AKTA™FPLC using a GE Healthcare XK16/70 column packed with Superdex 200 PG, eluting with 1.5 mL/min of sterile-filtered Phosphate-buffered saline (PBS). Fractions corresponding to ADC2A monomer peak are pooled, concentrated using a 15mL Amicon Ultracell 50KDa MWCO spin filter, analysed and sterile-filtered. BCA assay gives a concentration of final ADC2A at 3.94 mg/mL in 2.7 mL, obtained mass is 10.6 mg (71% yield). UHPLC analysis on a Shimadzu Prominence system using an Agilent PLRP-S 1000 A 8um 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of ADC2A at 280 nm and 330 nm (drug-linker specific) shows a mixture of light and heavy chains attached to several molecules of A, consistent with a drug-per-antibody ratio (DAR) of 2.4 molecules of A per antibody. UHPLC analysis on a Shimadzu Prominence system using a Waters Acquity UPLC BEH200 SEC 1.7 um 4.6 x 150 mm column eluting with sterile-filtered Phosphate-buffered saline (PBS) on a sample of ADC2A at 280 nm shows a monomer purity of 97.5% with 1.9% aggregates.
As used herein, "Antibody 1" is an anti-Her2 antibody comprising a VH domain having the sequence according to SEQ ID NO. 1 and a VL domain having the sequence according to SEQ ID NO. 2.

As used herein, "Antibody 2" is an anti-CD25 antibody ("Simulect") comprising a VH domain having the sequence according to SEQ ID NO. 3 and a VL domain having the sequence according to SEQ ID NO. 4.

**Example 10: In vitro ADC efficacy studies**

The cytotoxicity of ADC2A was assessed in a cytotoxicity assay as described above, and the results are shown in Figure 1. • represents the antigen expressing cell line (SU-DHL-1 cells from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.), and ▲ represents the antigen non-expressing cell line (Daudi cells from the American Type Culture Collection), and the error bars indicate ± standard deviation.

**Example 11: In vivo ADC efficacy studies**

CB.17 SCID mice, aged 8-12 weeks, are injected with 1 mm³ tumour fragments derived from the BT-474 cell line sub cutaneously in the flank. When tumours reach an average size of 100-150 mg, treatment is begun. Mice are weighed twice a week. Tumour size is measured twice a week. Animals are monitored individually. The endpoint of the experiment is a tumour volume of 1000 mm³ or 60 days, whichever comes first. Responders can be followed longer.

Groups of 10 xenografted mice are injected i.v. with 0.2ml of antibody drug conjugate (ADC), or naked antibody, in phosphate buffered saline (vehicle) or with 0.2ml of vehicle alone. The concentration of ADC is adjusted to give, for example, 0.3 or 1.0 mg ADC/ kg body weight in a single dose. Three identical doses may be given to each mouse at intervals of, for example, 1 week.

Figure 2 shows the effect on mean tumour volume in groups of 10 miced dosed with ADC1A at 0.3 (yellow) or 1.0 mg/kg (purple) compared to vehicle (black) or naked antibody (blue) controls.

Figure 3 shows the effect on mean tumour volume in groups of 10 miced dosed with ADC1 B at 0.3 (grey) or 1.0 mg/kg (purple) compared to vehicle (black) or naked Ig (blue) controls.
All documents and other references mentioned above are herein incorporated by reference.

Abbreviations

Ac acetyl
Acm acetamidomethyl
Alloc allyloxycarbonyl
Boc di-tert-butyl dicarbonate
t-Bu tert-butyl
Bzl benzyl, where Bzl-OMe is methoxybenzyl and Bzl-Me is methylbenzene
Cbz or Z benzyloxy-carbonyl, where Z-Cl and Z-Br are chloro- and bromobenzyloxy carbonyl respectively
DMF N,N-dimethylformamide
Dnp dinitrophenyl
DTT dithiothreitol
Fmoc 9/-fluoren-9-ylmethoxycarbonyl
imp \( \Lambda \)-10 imine protecting group: 3-(2-methoxyethoxy)propanoate-Val-Ala-PAB
MC-OSu maleimidocaproyl-0/-succinimide
Moc methoxycarbonyl
MP maleimidopropanamide
Mtr 4-methoxy-2,3,6-trimethylbenzenesulfonyl
PAB para-aminobenzyloxy carbonyl
PEG ethyleneoxy
PNZ p-nitrobenzyl carbamate
Psec 2-(phenylsulfonyl)ethoxycarbonyl
TBDMS tert-butyldimethylsilyl
TBDPS tert-butyldiphenylsilyl
Teoc 2-(trimethylsilyl)ethoxycarbonyl
tosyl
troclorothoxycarbonyl chloride
Trt trityl
Xan xanthyl
SEQUENCES

SEQ ID NO. 1 (Her VH):
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMYDYGWGQTLTVS

SEQ ID NO. 2 (Her VL):
DIQMTQSPSSLSASVGVDRVTITCRASQDVNTAVAAYQKPGKAPKLLIYSASFLYSGVPERS
FSGSRSGTDFTLTISSLQPEFDAYCQQHYTTTPFGQGTKVEIK

SEQ ID NO. 3 (Simulect VH):
QLQQSGTVLARPGASKASGYSFTRYWMHVIKQRPGGLEWIAIPGNSDTSYN
QKFGKAKLTAVTSASTAYMELSSLTHEDSAYVYCSRDGYFDFWGGQTTLTVS

SEQ ID NO. 4 (Simulect VL):
QIVSTQSPAAMSASPGEKVTMCASASSSRYMQWYQQKPGRWYDTKLASGVPAR
FSGSGSTSYLTISMEEADAATYYCHQRSSYTFGGGTLEIK
Claims

1. A compound which is selected from A:

\[ \text{A} \]

B:

\[ \text{B} \]

and C:

\[ \text{C} \]

and salts and solvates thereof.
2. A conjugate of formula ConjA:

![ConjA](image)

Conjugate ConjA:

3. The conjugate according to claim 2, wherein the cell binding agent is an antibody or an active fragment thereof.

4. The conjugate according to claim 3, wherein the antibody or antibody fragment is an antibody or antibody fragment for a tumour-associated antigen.

5. The conjugate of claim 3 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) BMPR1 B;
(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;
6. The conjugate of any one of claims 2 to 5 wherein the antibody or antibody fragment is a cysteine-engineered antibody.

7. The conjugate according to any one of claims 2 to 6 wherein the drug loading \( p \) of drugs \( D \) to antibody \( Ab \) is an integer from 1 to about 8.

8. The conjugate according to claim 7, wherein \( p \) is 1, 2, 3, or 4.

9. A composition comprising a mixture of drug conjugate compounds according to any one of claims 2 to 8, wherein the average drug loading per antibody in the mixture of antibody-drug conjugate compounds is about 1 to about 8.

10. The conjugate according to any one of claims 2 to 8 or the composition according to claim 9, for use in therapy.
11. The conjugate according to any one of claims 2 to 8 or the composition according to claim 9, for use in the treatment of a proliferative disease in a subject.

12. The conjugate or composition according to claim 11, wherein the disease is cancer.

13. A pharmaceutical composition comprising the conjugate according to any one of claims 2 to 8 or the composition according to claim 9, and a pharmaceutically acceptable diluent, carrier or excipient.

14. The pharmaceutical composition of claim 13 further comprising a therapeutically effective amount of a chemotherapeutic agent.

15. Use of the conjugate according to any one of claims 2 to 8 or the composition according to claim 9 in the preparation of a medicament for use in the treatment of a proliferative disease in a subject.


17. The method of claim 16 wherein the patient is administered a chemotherapeutic agent, in combination with the conjugate.

18. A method of preparing a conjugate according to any one of claims 2 to 8, the method comprising the step of reacting a cell binding agent with compound A, B or C as defined in claim 1.
Figure 3
INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/071235

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 A61P39/00 A61P35/00 C07D487/04 C07D519/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Relevant to claim No.</th>
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<td>WO 2011/130613 AI (SEATTLE GENETICS INC [US]; SPI ROGEN LTD [GB]; HOWARD PHILI P W LSON [GB] 20 October 2011 (2011-10-20) cited in the application on page 45, line 10 - line 14; compounds 51,93</td>
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<td>WO 2011/130616 AI (SPI ROGEN LTD [GB]; SEATTLE GENETICS INC [US]; HOWARD PHILI P W LSON [GB] 20 October 2011 (2011-10-20) cited in the application on compounds 24,30 page 45, line 5 - line 9 page 37, line 29</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:
  *"A" document defining the general state of the art which is not considered to be of particular relevance
  *"E" earlier application or patent but published on or after the international filing date
  *"L" document which may throw doubts on priority claim(s) or one which is cited to establish the publication date of another citation or other special reason (as specified)
  *"O" document referring to an oral disclosure, use, exhibition or other means
  *"P" document published prior to the international filing date but later than the priority date claimed
  *"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  *"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  *"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  *"A" document member of the same patent family

Date of the actual completion of the international search
22 January 2014

Date of mailing of the international search report
29/01/2014

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV RIJSWIJK
Tel. (+31-70) 340-2040
Fax. (+31-70) 340-3016

Authorized officer
SCHILDENBAUM, A
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<td>Y</td>
<td>WO 2005/085251 Al (SPIRGEN LTD [GB]; HOWARD PHILIP WILSON [GB]; GREGSON STEPHEN JOHN [GB]) 15 September 2005 (2005-09-15) compound 30n</td>
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### Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claims Nos.:**
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. **Claims Nos.:**
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. **Claims Nos.:**
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see additional sheet

1. **Claims Nos.:**
   - As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **Claims Nos.:**
   - As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. **Claims Nos.:**
   - As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. **Claims Nos.:**
   - No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest
- The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. **Claims**: 1-18 (partially)
   - Compound A (cyclopropyl) and conjugates.

2. **Claims**: 1-18 (partially)
   - Compound B (perazinyl) and conjugates.

3. **Claims**: 1-18 (partially)
   - Compound C (benzodioxol) and conjugates.
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