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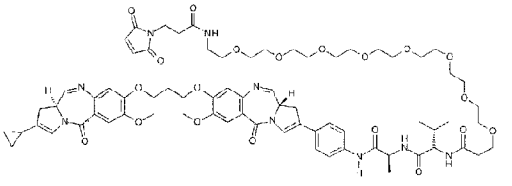
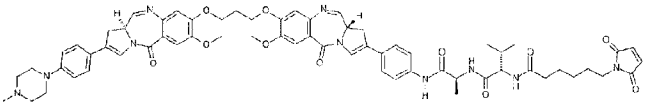
(54) **Titre : PYRROLOBENZODIAZEPINES ET LEURS CONJUGUES**
(54) **Title: PYRROLOBENZODIAZEPINES AND CONJUGATES THEREOF**



A



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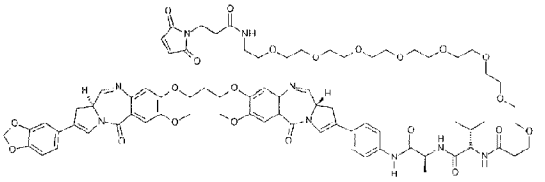

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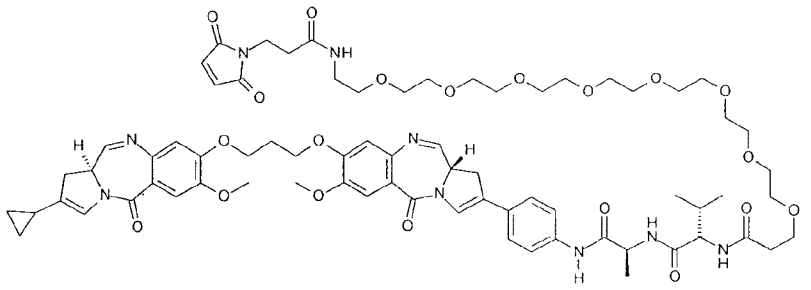
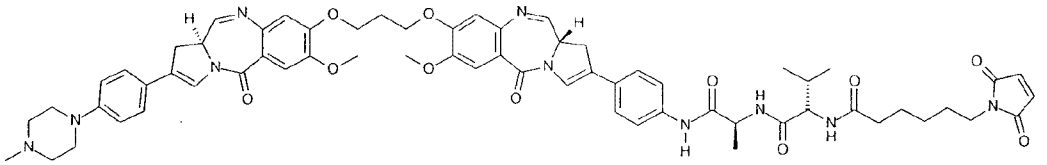
(57) **Abrégé/Abstract:**
The present application provides compounds A, B and C,
(see formula A)
(see formula B)
(see formula C)
and salts and solvates thereof, as well as conjugates thereof with cell binding agents. Also provided are methods of manufacturing the conjugates and methods of using the conjugates, alone or in combination with a chemotherapeutic agent, for the treatment of a proliferative disease in a subject.

ABSTRACT

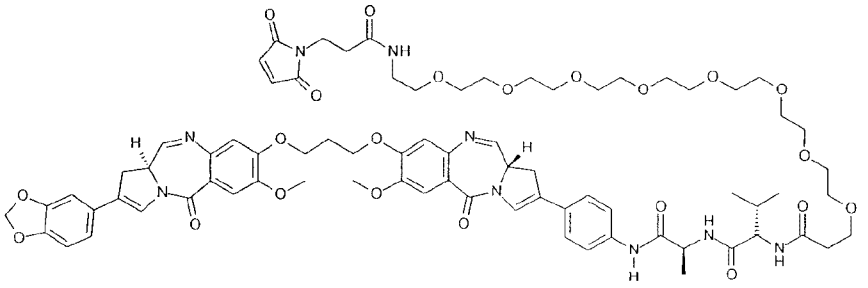
The present application provides compounds A, B and C,



A



B



C

and salts and solvates thereof, as well as conjugates thereof with cell binding agents. Also provided are methods of manufacturing the conjugates and methods of using the conjugates, alone or in combination with a chemotherapeutic agent, for the treatment of a proliferative disease in a subject.

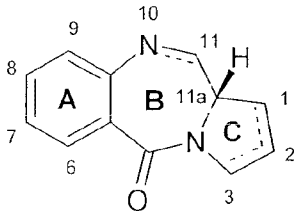
PYRROLOBENZODIAZEPINES AND CONJUGATES THEREOF

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

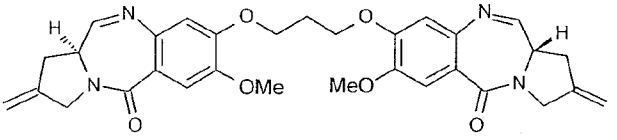
Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5793-5795 (1965); Leimgruber, *et al.*, *J. Am. Chem. Soc.*, **87**, 5791-5793 (1965)). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston, *et al.*, *Chem. Rev.* **1994**, 433-465 (1994); Antonow, D. and Thurston, D.E., *Chem. Rev.* **2011** 111 (4), 2815-2864). Family members include abbeymycin (Hochlowski, *et al.*, *J. Antibiotics*, **40**, 145-148 (1987)), chicamycin (Konishi, *et al.*, *J. Antibiotics,* **37**, 200-206 (1984)), DC-81 (Japanese Patent 58-180 487; Thurston, *et al.*, *Chem. Brit.*, **26**, 767-772 (1990); Bose, *et al.*, *Tetrahedron*, **48**, 751-758 (1992)), mazethramycin (Kuminoto, *et al.*, *J. Antibiotics*, **33**, 665-667 (1980)), neothramycins A and B (Takeuchi, *et al.*, *J. Antibiotics*, **29**, 93-96 (1976)), porothramycin (Tsunakawa, *et al.*, *J. Antibiotics*, **41**, 1366-1373 (1988)), prothracarcin (Shimizu, *et al*, *J. Antibiotics*, **29**, 2492-2503 (1982); Langley and Thurston, *J. Org. Chem.*, **52**, 91-97 (1987)), sibanomicin (DC-102)(Hara, *et al.*, *J. Antibiotics*, **41**, 702-704 (1988); Itoh, *et al.*, *J. Antibiotics*, **41**, 1281-1284 (1988)), sibiromycin (Leber, *et al.*, *J. Am. Chem. Soc.*, **110**, 2992-2993 (1988)) and tomamycin (Arima, *et al.*, *J. Antibiotics*, **25**, 437-444 (1972)). PBDs are of the general structure:



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

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

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*, 1161-1174) as compound **4a**. This compound, also known as SG2000, is shown below:



SG2000

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

Antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders (Carter, P. (2006) Nature Reviews Immunology 6:343-357). The use of antibody-drug conjugates (ADC), i.e. immunoconjugates, for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer, targets delivery of the drug moiety to tumors, and intracellular accumulation therein, whereas systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells (Xie *et al* (2006) *Expert. Opin. Biol. Ther.* 6(3):281-291; Kovtun *et al* (2006) *Cancer Res.* 66(6):3214-3121; Law *et al* (2006)

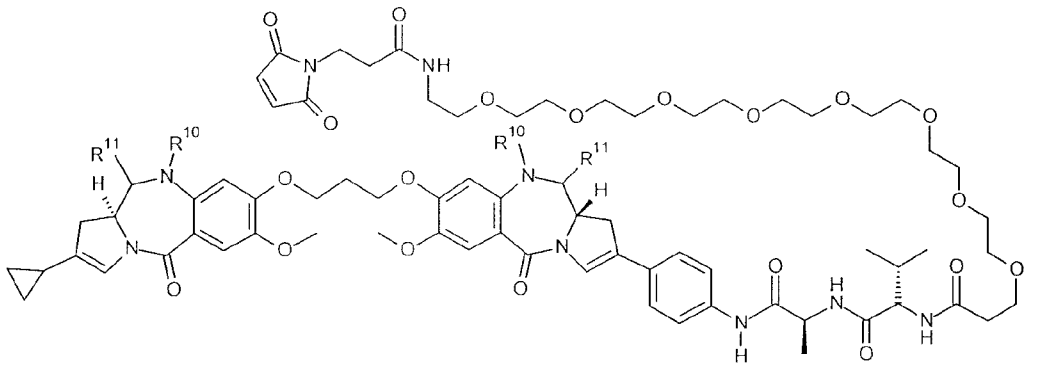
Cancer Res. 66(4):2328-2337; Wu *et al* (2005) *Nature Biotech.* 23(9):1137-1145; Lambert J. (2005) *Current Opin. in Pharmacol.* 5:543-549; Hamann P. (2005) *Expert Opin. Ther. Patents* 15(9):1087-1103; Payne, G. (2003) *Cancer Cell* 3:207-212; Trail *et al* (2003) *Cancer Immunol. Immunother.* 52:328-337; Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614).

Maximal efficacy with minimal toxicity is sought thereby. Efforts to design and refine ADC have focused on the selectivity of monoclonal antibodies (mAbs) as well as drug mechanism of action, drug-linking, drug/antibody ratio (loading), and drug-releasing properties (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; McDonagh (2006) Protein Eng. Design & Sel. 19(7): 299-307; Doronina *et al* (2006) Bioconj. Chem. 17:114-124; Erickson *et al* (2006) *Cancer Res.* 66(8):1-8; Sanderson *et al* (2005) *Clin. Cancer Res.* 11:843-852; Jeffrey *et al* (2005) *J. Med. Chem.* 48:1344-1358; Hamblett *et al* (2004) *Clin. Cancer Res.* 10:7063-7070). Drug moieties may impart their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, proteasome and/or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

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.

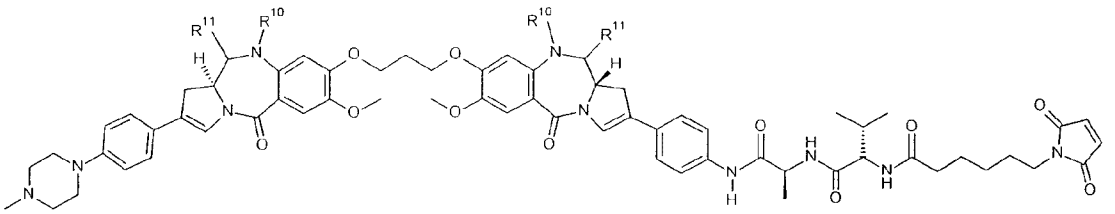
Summary of the Invention

In one aspect, the present application provides a compound, which is compound A



A ,

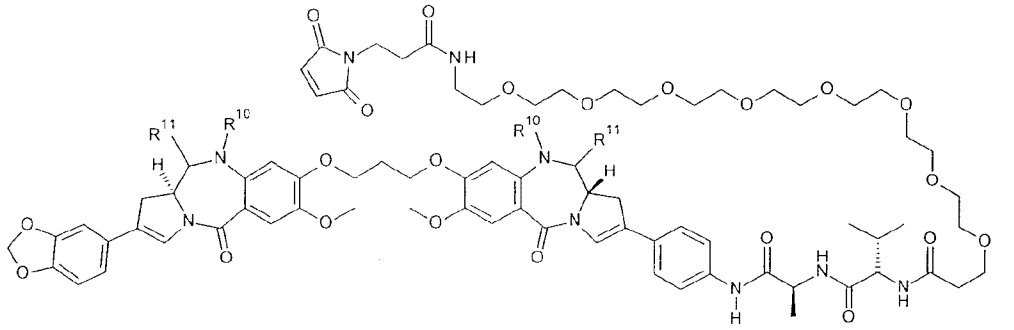
compound B



B

or

compound C



C ,

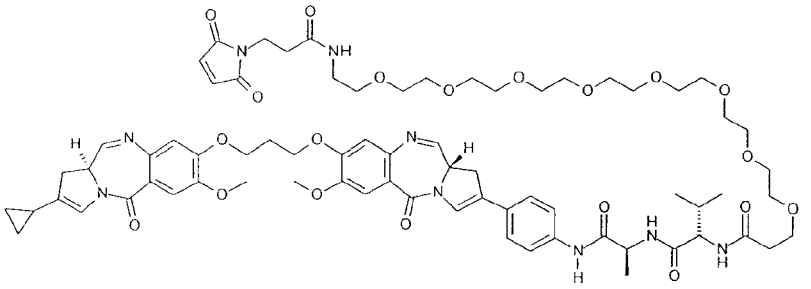
where R^{10} and R^{11} either

(a) form a double bond between the carbon and nitrogen atoms to which they are bound; or

(b) are H and OR^A respectively, where R^A is selected from H and C_{1-4} alkyl;

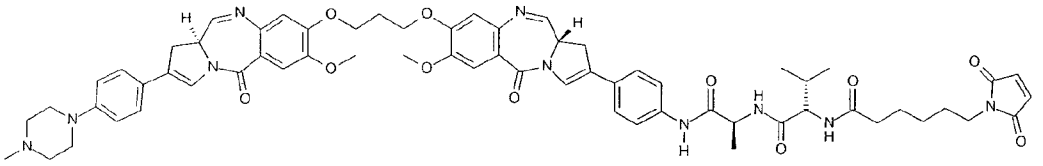
and pharmaceutically acceptable salts thereof.

In a second aspect, the present application provides a compound which is selected from A:



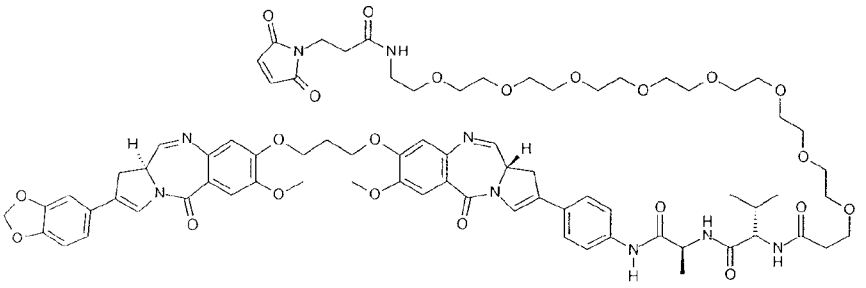
A

B:



B

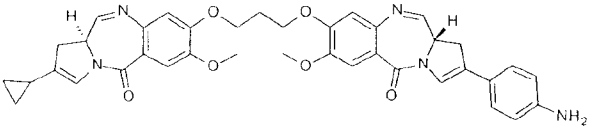
and C:



C

and salts and solvates thereof.

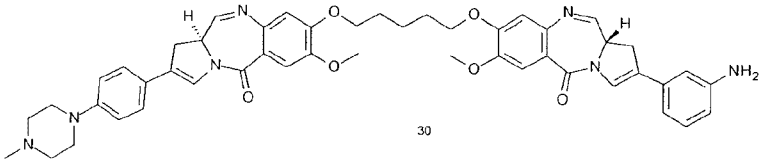
WO 2011/130615 discloses compound 26:



26

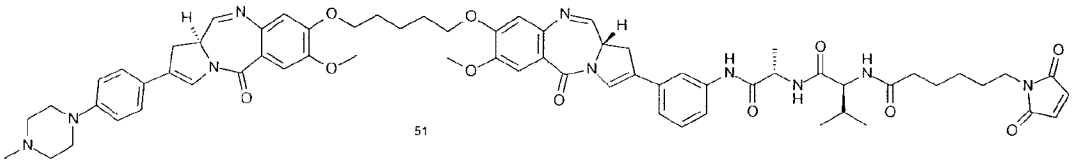
which is the parent compound of A. Compound A comprises this PBD with a linker for attachment to a cell binding agent. The cell binding agent provides a number of ethylene glycol moieties to provide solubility which is useful in the synthesis of conjugates.

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



30

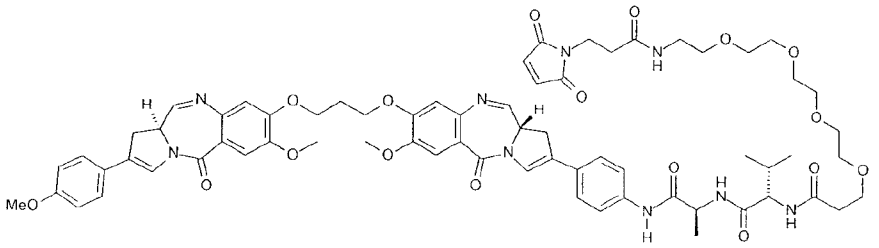
WO 2011/130613 also discloses compound 51:



51

Compound B differs from compound 30 by only having a $(CH_2)_3$ tether between the PBD moieties, instead of a $(CH_2)_5$ 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:

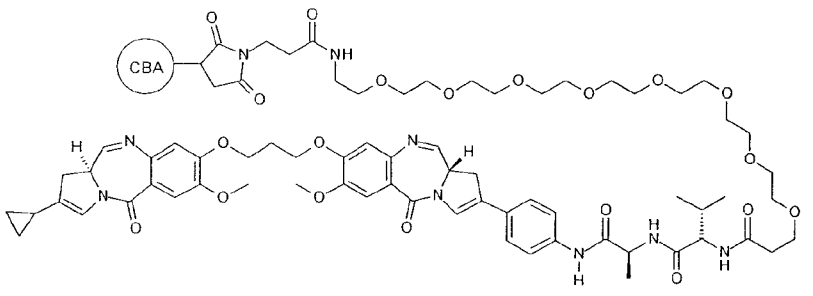


93

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 substiuent 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.

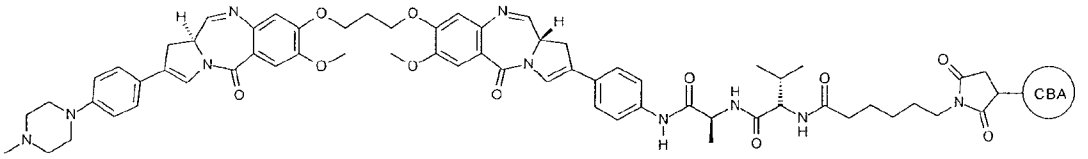
Compounds A, B and C have two sp^2 centres in each C-ring, which may allow for stronger binding in the minor groove of DNA, than for compounds with only one sp^2 centre in each C-ring.

A third aspect of the present application provides a conjugate of formula ConjA:



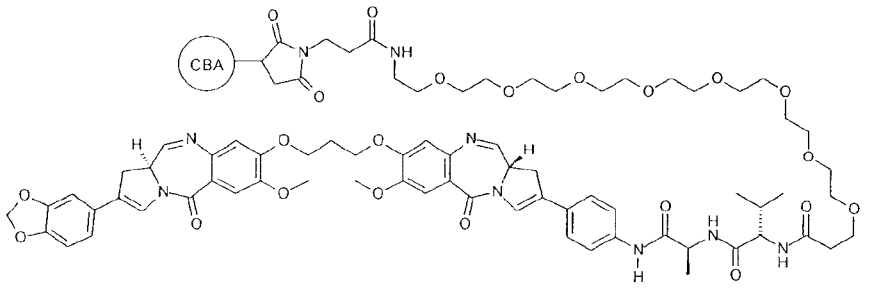
ConjA

ConjB:



ConjB

Or ConjC:



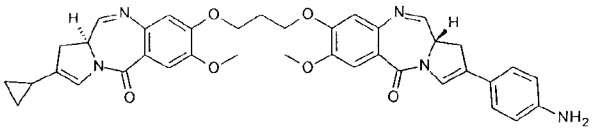
ConjC

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

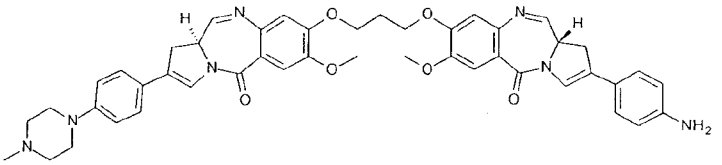
The present invention provides a PBD dimer with a linker connected through the C2 position on one of the PBD moieties suitable for forming a PBD dimer conjugated via the linker to a cell binding agent.

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:



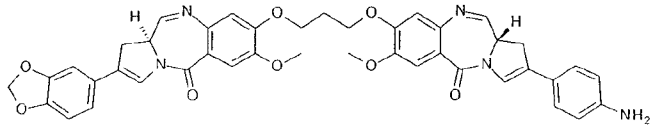
RelA

ConjB would release the compound RelB:



RelB

And ConjC would release the compound RelC:



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

Antibodies

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity

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

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

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with

the present invention may be made by the hybridoma method first described by Kohler *et al* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597 or from transgenic mice carrying a fully human immunoglobulin system (Lonberg (2008) Curr. Opinion 20(4):450-459).

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

An "intact antibody" herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

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

Humanisation

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

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

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

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

CDR grafting

In this technique, the humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as

mouse, rat, camel, bovine, goat, or rabbit having the desired properties (in effect, the non-human CDRs are 'grafted' onto the human framework). In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues (this may happen when, for example, a particular FR residue has significant effect on antigen binding).

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

Guided selection

The method consists of combining the V_H or V_L domain of a given non-human antibody specific for a particular epitope with a human V_H or V_L library and specific human V domains are selected against the antigen of interest. This selected human VH is then combined with a VL library to generate a completely human VHxVL combination. The method is described in Nature Biotechnology (N.Y.) 12, (1994) 899-903.

Composite antibodies

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

Deimmunization

This method involves the removal of human (or other second species) T-cell epitopes from the V regions of the therapeutic antibody (or other molecule). The therapeutic antibodies V-region sequence is analysed for the presence of MHC class II- binding motifs by, for example, comparison with databases of MHC-binding motifs (such as the "motifs" database

hosted at www.wehi.edu.au). Alternatively, MHC class II- binding motifs may be identified using computational threading methods such as those devised by Altuvia et al. (J. Mol. Biol. 249 244-250 (1995)); in these methods, consecutive overlapping peptides from the V-region sequences are testing for their binding energies to MHC class II proteins. This data can then be combined with information on other sequence features which relate to successfully presented peptides, such as amphipathicity, Rothbard motifs, and cleavage sites for cathepsin B and other processing enzymes.

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

All alterations can be accomplished by recombinant DNA technology, so that the final molecule may be prepared by expression from a recombinant host using well established methods such as Site Directed Mutagenesis. However, the use of protein chemistry or any other means of molecular alteration is also possible.

Resurfacing

This method involves:

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

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

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

(d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to the set of surface

exposed amino acid residues defined in step (c), wherein the heavy and light chain from the human antibody are or are not naturally paired;

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

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

(g) identifying, by comparing the three-dimensional models constructed in steps (a) and (f), any amino acid residues from the sets identified in steps (c) or (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of the non-human 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, (2007) 1986–1998).

Framework Shuffling

The CDRs of the non-human antibody are fused in-frame to cDNA pools encompassing all known heavy and light chain human germline gene frameworks. Humanised antibodies are

then selected by e.g. panning of the phage displayed antibody library. This is described in *Methods* **36**, 43-60 (2005).

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

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

TUMOR-ASSOCIATED ANTIGENS AND COGNATE ANTIBODIES

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

Nucleotide

Genbank accession no. NM_001203

Genbank version no. NM_001203.2 GI:169790809

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

Polypeptide

Genbank accession no. NP_001194

Genbank version no. NP_001194.1 GI:4502431

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

Cross-references

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

(2) *E16 (LAT1, SLC7A5)*

Nucleotide

Genbank accession no. NM_003486

Genbank version no. NM_003486.5 GI:71979931

Genbank record update date: Jun 27, 2012 12:06 PM

Polypeptide

Genbank accession no. NP_003477

Genbank version no. NP_003477.4 GI:71979932

Genbank record update date: Jun 27, 2012 12:06 PM

Cross references

Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), *Nature* 395 (6699):288-291 (1998), Gaugitsch, H.W., *et al* (1992) *J. Biol. Chem.* 267 (16):11267-11273); WO2004/048938 (Example 2); WO2004/032842 (Example IV); WO2003/042661 (Claim 12); WO2003/016475 (Claim 1); WO2002/78524 (Example 2); WO2002/99074 (Claim 19; Page 127-129); WO2002/86443 (Claim 27; Pages 222, 393); WO2003/003906 (Claim 10; Page 293); WO2002/64798 (Claim 33; Page 93-95); WO2000/14228 (Claim 5; Page 133-136); US2003/224454 (Fig 3); WO2003/025138 (Claim 12; Page 150); NP_003477 solute carrier family 7 (cationic amino acid transporter, y+system), member 5 /pid=NP_003477.3 - Homo sapiens; MIM:600182;; NM_015923.

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

Nucleotide

Genbank accession no. NM_012449

Genbank version no. NM_012449.2 GI:22027487

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

Polypeptide

Genbank accession no. NP_036581

Genbank version no. NP_036581.1 GI:9558759

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

Cross references

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

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

(4) *0772P (CA125, MUC16)*

Nucleotide

Genbank accession no. AF361486

Genbank version no. AF361486.3 GI:34501466

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

Polypeptide

Genbank accession no. AAK74120

Genbank version no. AAK74120.3 GI:34501467

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

Cross references

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

(5) *MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin)*

Nucleotide

Genbank accession no. NM_005823

Genbank version no. NM_005823.5 GI:293651528

Genbank record update date: Sep 2, 2012 01:47 PM

Polypeptide

Genbank accession no. NP_005814

Genbank version no. NP_005814.2 GI:53988378

Genbank record update date: Sep 2, 2012 01:47 PM

Cross references

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

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

Nucleotide

Genbank accession no. NM_006424

Genbank version no. NM_006424.2 GI:110611905

Genbank record update date: Jul 22, 2012 03:39 PM

Polypeptide

Genbank accession no. NP_006415

Genbank version no. NP_006415.2 GI:110611906

Genbank record update date: Jul 22, 2012 03:39 PM

Cross references

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

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

Nucleotide

Genbank accession no. AB040878

Genbank version no. AB040878.1 GI:7959148

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

Polypeptide

Genbank accession no. BAA95969

Genbank version no. BAA95969.1 GI:7959149

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

Cross references

Nagase T., *et al* (2000) *DNA Res.* 7 (2):143-150); WO2004/000997 (Claim 1); WO2003/003984 (Claim 1); WO2002/06339 (Claim 1; Page 50); WO2001/88133 (Claim 1;

Page 41-43, 48-58); WO2003/054152 (Claim 20); WO2003/101400 (Claim 11); Accession: Q9P283; Genew; HGNC:10737

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

Nucleotide

Genbank accession no. AY358628

Genbank version no. AY358628.1 GI:37182377

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

Polypeptide

Genbank accession no. AAQ88991

Genbank version no. AAQ88991.1 GI:37182378

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

Cross references

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

(9) *ETBR (Endothelin type B receptor)*

Nucleotide

Genbank accession no. AY275463

Genbank version no. AY275463.1 GI:30526094

Genbank record update date: Mar 11, 2010 02:26 AM

Polypeptide

Genbank accession no. AAP32295

Genbank version no. AAP32295.1 GI:30526095

Genbank record update date: Mar 11, 2010 02:26 AM

Cross references

Nakamuta M., *et al Biochem. Biophys. Res. Commun.* 177, 34-39, 1991; Ogawa Y., *et al Biochem. Biophys. Res. Commun.* 178, 248-255, 1991; Arai H., *et al Jpn. Circ. J.* 56, 1303-1307, 1992; Arai H., *et al J. Biol. Chem.* 268, 3463-3470, 1993; Sakamoto A., Yanagisawa

M., *et al Biochem. Biophys. Res. Commun.* 178, 656-663, 1991; Elshourbagy N.A., *et al J. Biol. Chem.* 268, 3873-3879, 1993; Haendler B., *et al J. Cardiovasc. Pharmacol.* 20, s1-S4, 1992; Tsutsumi M., *et al Gene* 228, 43-49, 1999; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002; Bourgeois C., *et al J. Clin. Endocrinol. Metab.* 82, 3116-3123, 1997;

Okamoto Y., *et al Biol. Chem.* 272, 21589-21596, 1997; Verheij J.B., *et al Am. J. Med. Genet.* 108, 223-225, 2002; Hofstra R.M.W., *et al Eur. J. Hum. Genet.* 5, 180-185, 1997; Puffenberger E.G., *et al Cell* 79, 1257-1266, 1994; Attie T., *et al, Hum. Mol. Genet.* 4, 2407-2409, 1995; Auricchio A., *et al Hum. Mol. Genet.* 5:351-354, 1996; Amiel J., *et al Hum. Mol. Genet.* 5, 355-357, 1996; Hofstra R.M.W., *et al Nat. Genet.* 12, 445-447, 1996; Svensson P.J., *et al Hum. Genet.* 103, 145-148, 1998; Fuchs S., *et al Mol. Med.* 7, 115-124, 2001; Pingault V., *et al (2002) Hum. Genet.* 111, 198-206; WO2004/045516 (Claim 1); WO2004/048938 (Example 2); WO2004/040000 (Claim 151); WO2003/087768 (Claim 1); WO2003/016475 (Claim 1); WO2003/016475 (Claim 1); WO2002/61087 (Fig 1); WO2003/016494 (Fig 6); WO2003/025138 (Claim 12; Page 144); WO2001/98351 (Claim 1; Page 124-125); EP0522868 (Claim 8; Fig 2); WO2001/77172 (Claim 1; Page 297-299); US2003/109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004/001004.

(10) MSG783 (RNF124, hypothetical protein FLJ20315)

Nucleotide

Genbank accession no. NM_017763

Genbank version no. NM_017763.4 GI:167830482

Genbank record update date: Jul 22, 2012 12:34 AM

Polypeptide

Genbank accession no. NP_060233

Genbank version no. NP_060233.3 GI:56711322

Genbank record update date: Jul 22, 2012 12:34 AM

Cross references

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

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

Nucleotide

Genbank accession no. AF455138

Genbank version no. AF455138.1 GI:22655487

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

Polypeptide

Genbank accession no. AAN04080

Genbank version no. AAN04080.1 GI:22655488

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

Cross references

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

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

Nucleotide

Genbank accession no. NM_017636

Genbank version no. NM_017636.3 GI:304766649

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

Polypeptide

Genbank accession no. NP_060106

Genbank version no. NP_060106.2 GI:21314671

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

Cross references

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

WO2003/042661 (Claim 12); WO2002/30268 (Claim 27; Page 391); US2003/219806 (Claim 4); WO2001/62794 (Claim 10 14; Fig 1A-D); MIM:606936.

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

Nucleotide

Genbank accession no. NM_003212

Genbank version no. NM_003212.3 GI:292494881

Genbank record update date: Sep 23, 2012 02:27 PM

Polypeptide

Genbank accession no. NP_003203

Genbank version no. NP_003203.1 GI:4507425

Genbank record update date: Sep 23, 2012 02:27 PM

Cross references

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

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

Nucleotide

Genbank accession no M26004

Genbank version no. M26004.1 GI:181939

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

Polypeptide

Genbank accession no. AAA35786

Genbank version no. AAA35786.1 GI:181940

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

Cross references

Fujisaku *et al* (1989) *J. Biol. Chem.* 264 (4):2118-2125); Weis J.J., *et al J. Exp. Med.* 167, 1047-1066, 1988; Moore M., *et al Proc. Natl. Acad. Sci. U.S.A.* 84, 9194-9198, 1987; Barel M., *et al Mol. Immunol.* 35, 1025-1031, 1998; Weis J.J., *et al Proc. Natl. Acad. Sci. U.S.A.* 83, 5639-5643, 1986; Sinha S.K., *et al* (1993) *J. Immunol.* 150, 5311-5320; WO2004/045520 (Example 4); US2004/005538 (Example 1); WO2003/062401 (Claim 9); WO2004/045520 (Example 4); WO91/02536 (Fig 9.1-9.9); WO2004/020595 (Claim 1); Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

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

Nucleotide

Genbank accession no NM_000626

Genbank version no. NM_000626.2 GI:90193589

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

Polypeptide

Genbank accession no. NP_000617

Genbank version no. NP_000617.1 GI:11038674

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

Cross references

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

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

Nucleotide

Genbank accession no NM_030764

Genbank version no. NM_030764.3 GI:227430280

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

Polypeptide

Genbank accession no. NP_110391

Genbank version no. NP_110391.2 GI:19923629

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

Cross references

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

10 WO2003/089624 (Claim 25);: MIM:606509.

(17) *HER2 (ErbB2)*

Nucleotide

Genbank accession no M11730

Genbank version no. M11730.1 GI:183986

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

Polypeptide

Genbank accession no. AAA75493

Genbank version no. AAA75493.1 GI:306840

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

Cross references

Coussens L., *et al Science* (1985) 230(4730):1132-1139); Yamamoto T., *et al Nature* 319, 230-234, 1986; Semba K., *et al Proc. Natl. Acad. Sci. U.S.A.* 82, 6497-6501, 1985; Swiercz J.M., *et al J. Cell Biol.* 165, 869- 15 880, 2004; Kuhns J.J., *et al J. Biol. Chem.* 274, 36422-36427, 1999; Cho H.-S., *et al Nature* 421, 756-760, 2003; Ehsani A., *et al* (1993) *Genomics* 15, 426-429; WO2004/048938 (Example 2); WO2004/027049 (Fig 1I); WO2004/009622; WO2003/081210;

WO2003/089904 (Claim 9); WO2003/016475 (Claim 1); US2003/118592; WO2003/008537 (Claim 1); WO2003/055439 (Claim 29; Fig 1A-B); WO2003/025228 (Claim 37; Fig 5C);

20 WO2002/22636 (Example 13; Page 95-107); WO2002/12341 (Claim 68; Fig 7); WO2002/13847 (Page 71-74); WO2002/14503 (Page 114-117); WO2001/53463 (Claim 2; Page 41-46); WO2001/41787 (Page 15); WO2000/44899 (Claim 52; Fig 7); WO2000/20579

(Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004/043361 (Claim 7); WO2004/022709; WO2001/00244 25 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1

ANTIBODIES

Abbott: US20110177095

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

Biogen: US20100119511

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

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

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

Pertuzumab (Genentech)

US20110117097

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

US20090285837

US20090202546

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

US20060088523

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

US20060018899

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

US2011/0159014

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

US20090187007

Glycotope: TrasGEX antibody

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

Symphogen: US20110217305

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

(18) *NCA (CEACAM6)*

Nucleotide

Genbank accession no M18728

Genbank version no. M18728.1 GI:189084

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

Polypeptide

Genbank accession no. AAA59907

Genbank version no. AAA59907.1 GI:189085

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

Cross references

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

EMBL; M18728.

(19) *MDP (DPEP1)*

Nucleotide

Genbank accession no BC017023

Genbank version no. BC017023.1 GI:16877538

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

Polypeptide

Genbank accession no. AAH17023

Genbank version no. AAH17023.1 GI:16877539

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

Cross references

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

MIM:179780.

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

Nucleotide

Genbank accession no AF184971

Genbank version no. AF184971.1 GI:6013324

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

Polypeptide

Genbank accession no. AAF01320

Genbank version no. AAF01320.1 GI:6013325

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

Cross references

Clark H.F., *et al Genome Res.* 13, 2265-2270, 2003; Mungall A.J., *et al Nature* 425, 805-811, 2003; Blumberg H., *et al Cell* 104, 9-19, 2001; Dumoutier L., *et al J. Immunol.* 167, 3545-3549, 2001; Parrish-Novak J., *et al J. Biol. Chem.* 277, 47517-47523, 2002; Pletnev S., *et al* (2003) 10 *Biochemistry* 42:12617-12624; Sheikh F., *et al* (2004) *J. Immunol.* 172, 2006-2010; EP1394274 (Example 11); US2004/005320 (Example 5); WO2003/029262 (Page 74-75); WO2003/002717 (Claim 2; Page 63); WO2002/22153 (Page 45-47); US2002/042366 (Page 20-21); WO2001/46261 (Page 57-59); WO2001/46232 (Page 63-65); WO98/37193 (Claim 1; Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) *Brevican (BCAN, BEHAB)*

Nucleotide

Genbank accession no AF229053

Genbank version no. AF229053.1 GI:10798902

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

Polypeptide

Genbank accession no. AAG23135

Genbank version no. AAG23135.1 GI:10798903

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

Cross references

Gary S.C., *et al Gene* 256, 139-147, 2000; Clark H.F., *et al Genome Res.* 13, 2265-2270, 2003; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002; US2003/186372 (Claim 11); US2003/186373 (Claim 11); US2003/119131 (Claim 1; Fig 52); US2003/119122 (Claim 1; 20 Fig 52); US2003/119126 (Claim 1); US2003/119121 (Claim 1; Fig 52); US2003/119129 (Claim 1); US2003/119130 (Claim 1); US2003/119128 (Claim 1; Fig 52); US2003/119125 (Claim 1); WO2003/016475 (Claim 1); WO2002/02634 (Claim 1)

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

Nucleotide

Genbank accession no NM_004442

Genbank version no. NM_004442.6 GI:111118979

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

Polypeptide

Genbank accession no. NP_004433

Genbank version no. NP_004433.2 GI:21396504

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

Cross references

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

(23) *ASLG659 (B7h)*

Nucleotide

Genbank accession no. AX092328

Genbank version no. AX092328.1 GI:13444478

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

Cross references

US2004/0101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); US2003/165504 (Claim 1); US2003/124140 (Example 2); US2003/065143 (Fig 60); WO2002/102235 (Claim 13; Page 299); US2003/091580 (Example 2); WO2002/10187 (Claim 6; Fig 10); WO2001/94641 (Claim 12; Fig 7b); WO2002/02624 (Claim 13; Fig 1A-1B); US2002/034749 (Claim 54; Page 45-46); WO2002/06317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO2002/71928 (Page 468-469); WO2002/02587 (Example 1; Fig 1); WO2001/40269 (Example 3; Pages 190-192); WO2000/36107 (Example 2; Page 205-207); WO2004/053079 (Claim 12); WO2003/004989 (Claim 1); WO2002/71928 (Page 233-234, 452-453); WO 01/16318.

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

Nucleotide

Genbank accession no AJ297436

Genbank version no. AJ297436.1 GI:9367211

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

Polypeptide

Genbank accession no. CAB97347

Genbank version no. CAB97347.1 GI:9367212

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

Cross references

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

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

(25) *GEDA*

Nucleotide

Genbank accession no AY260763

Genbank version no. AY260763.1 GI:30102448

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

Polypeptide

Genbank accession no. AAP14954

Genbank version no. AAP14954.1 GI:30102449

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

Cross references

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

(26) *BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3)*

Nucleotide

Genbank accession no AF116456

Genbank version no. AF116456.1 GI:4585274

Genbank record update date: Mar 10, 2010 09:44 PM

Polypeptide

Genbank accession no. AAD25356

Genbank version no. AAD25356.1 GI:4585275

Genbank record update date: Mar 10, 2010 09:44 PM

Cross references

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

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

Nucleotide

Genbank accession no AK026467

Genbank version no. AK026467.1 GI:10439337

Genbank record update date: Sep 11, 2006 11:24 PM

Polypeptide

Genbank accession no. BAB15489

Genbank version no. BAB15489.1 GI:10439338

Genbank record update date: Sep 11, 2006 11:24 PM

Cross references

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

(27a) *CD22 (CD22 molecule)*

Nucleotide

Genbank accession no X52785

Genbank version no. X52785.1 GI:29778

Genbank record update date: Feb 2, 2011 10:09 AM

Polypeptide

Genbank accession no. CAA36988

Genbank version no. CAA36988.1 GI:29779

Genbank record update date: Feb 2, 2011 10:09 AM

Cross references

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

Other information

Official Symbol: CD22

Other Aliases: SIGLEC-2, SIGLEC2

Other Designations: B-cell receptor CD22; B-lymphocyte cell adhesion molecule; BL-CAM; CD22 antigen; T-cell surface antigen Leu-14; sialic acid binding Ig-like lectin 2; sialic acid-binding Ig-like lectin 2

ANTIBODIES

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

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

(28) *CD79a (CD79A, CD79alpha), immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M*

35 molecules, transduces a signal involved in B-cell differentiation), pI: 4.84, MW: 25028 TM: 2

[P] Gene Chromosome: 19q13.2).

Nucleotide

Genbank accession no NM_001783

Genbank version no. NM_001783.3 GI:90193587
Genbank record update date: Jun 26, 2012 01:48 PM

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

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

(29) *CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defence, plays a 10 role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia); 372 aa, pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3,*
Nucleotide
Genbank accession no NM_001716
Genbank version no. NM_001716.4 GI:342307092
Genbank record update date: Sep 30, 2012 01:49 PM

Polypeptide
Genbank accession no. NP_001707
Genbank version no. NP_001707.1 GI:4502415
Genbank record update date: Sep 30, 2012 01:49 PM

Cross references
WO2004/040000; WO2004/015426; US2003/105292 (Example 2); US6555339 (Example 2); WO2002/61087 (Fig 1); WO2001/57188 (Claim 20, page 269); WO2001/72830 (pages 12-13); WO2000/22129 (Example 1, pages 152-153, 15 Example 2, pages 254-256); WO99/28468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO94/28931 (pages

56-58); WO92/17497 (claim 7, Fig 5); Dobner *et al* (1992) *Eur. J. Immunol.* 22:2795-2799; Barella *et al* (1995) *Biochem. J.* 309:773-779

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

Nucleotide

Genbank accession no NM_002120

Genbank version no. NM_002120.3 GI:118402587

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

Polypeptide

Genbank accession no. NP_002111

Genbank version no. NP_002111.1 GI:4504403

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

Cross references

Tonnelle *et al* (1985) *EMBO J.* 4(11):2839-2847; Jonsson *et al* (1989) *Immunogenetics* 29(6):411-413; Beck *et al* (1992) *J. Mol. Biol.* 228:433-441; Strausberg *et al* (2002) *Proc. Natl. Acad. Sci USA* 99:16899- 16903; Servenius *et al* (1987) *J. Biol. Chem.* 262:8759-8766; Beck *et al* (1996) *J. Mol. Biol.* 25 255:1-13; Naruse *et al* (2002) *Tissue Antigens* 59:512-519; WO99/58658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-146); Kasahara *et al* (1989) *Immunogenetics* 30(1):66-68; Larhammar *et al* (1985) *J. Biol. Chem.* 260(26):14111-14119

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

Nucleotide

Genbank accession no NM_002561

Genbank version no. NM_002561.3 GI:325197202

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

Polypeptide

Genbank accession no. NP_002552

Genbank version no. NP_002552.2 GI:28416933
Genbank record update date: Jun 27, 2012 12:41 AM

Cross references

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

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

Nucleotide

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

Polypeptide

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

Cross references

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

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

Nucleotide

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

Polypeptide

Genbank accession no. NP_005573

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

Cross references

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

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

Nucleotide

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

Polypeptide

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

Cross references

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

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

Nucleotide

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

Polypeptide

Genbank accession no. AAK31325

Genbank version no. AAK31325.1 GI:13591710

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

Cross references

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

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

Nucleotide

Genbank accession no AF179274

Genbank version no. AF179274.2 GI:12280939

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

Polypeptide

Genbank accession no. AAD55776

Genbank version no. AAD55776.2 GI:12280940

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

Cross references

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

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

Nucleotide

Genbank accession no M99487

Genbank version no. M99487.1 GI:190663

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

Polypeptide

Genbank accession no. AAA60209

Genbank version no. AAA60209.1 GI:190664

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

Cross references

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

Other information

Official Symbol: FOLH1

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

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

ANTIBODIES

US 7,666,425:

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

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

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

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

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

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

Mouse anti-PSMA antibodies include the 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6, 4C8B9, and monoclonal antibodies. Hybridomas secreting 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6 or 4C8B9 have been publicly deposited and are described in U.S. Pat. No. 6,159,508. Relevant hybridomas have been publicly deposited and are described in U.S. Pat. No. 6,107,090. Moreover, humanized anti-PSMA antibodies, including a humanized version of J591, are described in further detail in PCT Publication WO 02/098897.

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

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

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

NW Biotherapeutics: A hybridoma cell line selected from the group consisting of 3F5.4G6 having ATCC accession number HB12060, 3D7-1.I. having ATCC accession number

HB12309, 4E10-1.14 having ATCC accession number HB12310, 3E11 (ATCC HB12488), 4D8 (ATCC HB12487), 3E6 (ATCC HB12486), 3C9 (ATCC HB12484), 2C7 (ATCC HB12490), 1G3 (ATCC HB12489), 3C4 (ATCC HB12494), 3C6 (ATCC HB12491), 4D4 (ATCC HB12493), 1G9 (ATCC HB12495), 5C8B9 (ATCC HB12492) and 3G6 (ATCC HB12485) – see US 6,150,508

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

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

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

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

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

(38.1) SSTR2 (Somatostatin receptor 2)

Nucleotide

Genbank accession no NM_001050

Genbank version no. NM_001050.2 GI:44890054

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

Polypeptide

Genbank accession no. NP_001041

Genbank version no. NP_001041.1 GI:4557859

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

Cross references

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

Other information

Official Symbol: SSTR2

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

(38.2) SSTR5 (Somatostatin receptor 5)

Nucleotide

Genbank accession no D16827

Genbank version no. D16827.1 GI:487683

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

Polypeptide

Genbank accession no. BAA04107

Genbank version no. BAA04107.1 GI:487684

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

Cross references

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

Other information

Official Symbol: SSTR5

Other Aliases: SS-5-R

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

(38.3) SSTR1

(38.4)SSTR3

(38.5) SSTR4

AvB6 – Both subunits (39+40)

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

Nucleotide

Genbank accession no M14648 J02826 M18365

Genbank version no. M14648.1 GI:340306

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

Polypeptide

Genbank accession no. AAA36808

Genbank version no. AAA36808.1 GI:340307

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

Cross references

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

Other information

Official Symbol: ITGAV

Other Aliases: CD51, MSK8, VNRA, VTNR

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

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

Nucleotide

Genbank accession no NM_000888

Genbank version no. NM_000888.3 GI:9966771

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

Polypeptide

Genbank accession no. NP_000879

Genbank version no. NP_000879.2 GI:9625002

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

Cross references

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

Other information

Official Symbol: ITGB6

Other Designations: integrin beta-6

ANTIBODIES

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

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

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

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

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

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

Nucleotide

Genbank accession no M17303

Genbank version no. M17303.1 GI:178676

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

Polypeptide

Genbank accession no. AAB59513

Genbank version no. AAB59513.1 GI:178677

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

Cross references

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

Other information

Official Symbol: CEACAM5

Other Aliases: CD66e, CEA

Other Designations: meconium antigen 100

ANTIBODIES

AstraZeneca-MedImmune:US 20100330103; US20080057063; US20020142359

- for example, an antibody having complementarity determining regions (CDRs) with the following sequences: heavy chain; CDR1 - DNYMH,

CDR2 - WIDPENGDTE YAPKFRG, CDR3 - LIYAGYLAMD Y; and light chain CDR1 - SASSSVTYMH, CDR2 - STSNLAS, CDR3 - QQRSTYPLT.

- Hybridoma 806.077 deposited as European Collection of Cell Cultures (ECACC) deposit no. 96022936.

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

Bayer Corporation: US6,013,772

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

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

Celltech Therapeutics Limited: US5,877,293

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

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

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

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

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

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

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

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

Nucleotide

Genbank accession no M35073

Genbank version no. M35073.1 GI:187553

Genbank record update date: Mar 6, 2012 11:12 AM

Polypeptide

Genbank accession no. AAA59589

Genbank version no. AAA59589.1 GI:553531

Genbank record update date: Mar 6, 2012 11:12 AM

Cross references

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

Other information

Official Symbol: MET

Other Aliases: AUTS9, HGFR, RCCP2, c-Met

Other Designations: HGF receptor; HGF/SF receptor; SF receptor; hepatocyte growth factor receptor; met proto-oncogene tyrosine kinase; proto-oncogene c-Met; scatter factor receptor; tyrosine-protein kinase Met

ANTIBODIES

Abgenix/Pfizer: US20100040629

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

Amgen/Pfizer: US20050054019

for example, an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 2 where X2 is glutamate and X4 is serine and a light chain having the amino acid sequence set forth in SEQ ID NO: 4 where X8 is alanine,

without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 6 and a light chain having the amino acid sequence set forth in SEQ ID NO: 8, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 10 and a light chain having the amino acid sequence set forth in SEQ ID NO: 12, without the signal sequences; or an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 14 and a light chain having the amino acid sequence set forth in SEQ ID NO: 16, without the signal sequences.

Agouron Pharmaceuticals (Now Pfizer): US20060035907

Eli Lilly: US20100129369

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

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

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

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

Novartis: US20090175860

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

Pharmacia Corporation: US20040166544

Pierre Fabre: US20110239316, US20110097262, US20100115639

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

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

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

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

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

Nucleotide

Genbank accession no J05581
Genbank version no. J05581.1 GI:188869
Genbank record update date: Jun 23, 2010 08:48 AM

Polypeptide

Genbank accession no. AAA59876
Genbank version no. AAA59876.1 GI:188870
Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

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

Other information

Official Symbol: MUC1
Other Aliases: RP11-263K19.2, CD227, EMA, H23AG, KL-6, MAM6, MUC-1, MUC-1/SEC, MUC-1/X, MUC1/ZD, PEM, PEMT, PUM
Other Designations: DF3 antigen; H23 antigen; breast carcinoma-associated antigen DF3; carcinoma-associated mucin; episialin; krebs von den Lungen-6; mucin 1, transmembrane; mucin-1; peanut-reactive urinary mucin; polymorphic epithelial mucin; tumor associated epithelial mucin; tumor-associated epithelial membrane antigen; tumor-associated mucin

ANTIBODIES

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

AltaRex- Quest Pharma Tech: US7,147,850

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

Glycotope GT-MAB: GT-MAB 2.5-GEX

Immunogen: US7,202,346

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

Immunomedics: US 6,653,104

Ramot Tel Aviv Uni: US7,897,351

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

Roche GlycArt: US8,021,856

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

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

(44) *CA9 (Carbonic anhydrase IX)*

Nucleotide

Genbank accession no . X66839

Genbank version no. X66839.1 GI:1000701

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

Polypeptide

Genbank accession no. CAA47315

Genbank version no. CAA47315.1 GI:1000702

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

Cross references

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

Other information

Official Symbol: CA9

Other Aliases: CAIX, MN

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

ANTIBODIES

Abgenix/Amgen: US20040018198

Affibody: Anti-CAIX Affibody molecules

Bayer: US7,462,696

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

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

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

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

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

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

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

Novartis: US20090252738

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

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

Xencor: US20090162382

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

Nucleotide

Genbank accession no. NM_201283

Genbank version no. NM_201283.1 GI:41327733

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

Polypeptide

Genbank accession no. NP_958440

Genbank version no. NP_958440.1 GI:41327734

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

Cross-references

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

ANTIBODIES:

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

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

US20100111979 (Amgen)

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

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

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

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

US20090240038 (Amgen)

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

US20090175887 (Amgen)

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

US20090156790 (Amgen)

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

US20090155282, US20050059087 and US20050053608 (Amgen)

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

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

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

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

US20090311803 (Harvard University)

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

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

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

US6,129,915 (Schering)

For example, SEQ. ID NOs: 1, 2, 3, 4, 5 and 6.

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

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

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

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

(46) *CD33 (CD33 molecule)*

Nucleotide

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

Polypeptide

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

Cross-references

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

Other information

Official Symbol: CD33
Other Aliases: SIGLEC-3, SIGLEC3, p67
Other Designations: CD33 antigen (gp67); gp67; myeloid cell surface antigen CD33; sialic acid binding Ig-like lectin 3; sialic acid-binding Ig-like lectin

ANTIBODIES

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

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

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

US6,590,088 (Human Genome Sciences)

For example, SEQ ID NOs: 1 and 2 and ATCC accession no. 97521

US7,557,189 (Immunogen)

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

(47) *CD19 (CD19 molecule)*

Nucleotide

Genbank accession no. NM_001178098

Genbank version no. NM_001178098.1 GI:296010920

Genbank record update date: Sep 10, 2012 12:43 AM

Polypeptide

Genbank accession no. NP_001171569

Genbank version no. NP_001171569.1 GI:296010921

Genbank record update date: Sep 10, 2012 12:43 AM

Cross-references

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

Other information

Official Symbol: CD19

Other Aliases: B4, CVID3

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

ANTIBODIES

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

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

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

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

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

US7,109,304 (Immunomedics)

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

US7,902,338 (Immunomedics)

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

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

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

US7,968,687 (Seattle Genetics)

An antibody or antigen-binding fragment comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:9 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 24.

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

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

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

Nucleotide

Genbank accession no. NM_000417

Genbank version no. NM_000417.2 GI:269973860

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

Polypeptide

Genbank accession no. NP_000408

Genbank version no. NP_000408.1 GI:4557667

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

Cross-references

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

Other information

Official Symbol: IL2RA

Other Aliases: RP11-536K7.1, CD25, IDDM10, IL2R, TCGFR

Other Designations: FIL-2 receptor subunit alpha; IL-2-RA; IL-2R subunit alpha; IL2-RA; TAC antigen; interleukin-2 receptor subunit alpha; p55

ANTIBODIES

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

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

For example, an antibody having an antigen binding site comprises at least one domain which comprises CDR1 having the amino acid sequence in SEQ. ID. NO: 7,

CDR2 having the amino acid sequence in SEQ. ID. NO: 8, and CDR3 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.

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

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

Nucleotide

Genbank accession no. M76125

Genbank version no. M76125.1 GI:292869

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

Polypeptide

Genbank accession no. AAA61243

Genbank version no. AAA61243.1 GI:29870

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

Cross-references

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

Other information

Official Symbol: AXL

Other Aliases: JTK11, UFO

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

ANTIBODIES

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

BergenBio: BGB324

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

Nucleotide

Genbank accession no. M83554

Genbank version no. M83554.1 GI:180095
Genbank record update date: Jun 23, 2010 08:53 AM

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

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

Other information
Official Symbol: TNFRSF8
Other Aliases: CD30, D1S166E, Ki-1
Other Designations: CD30L receptor; Ki-1 antigen; cytokine receptor CD30; lymphocyte activation antigen CD30; tumor necrosis factor receptor superfamily member 8

(51) *BCMA (B-cell maturation antigen) - TNFRSF17 (Tumor necrosis factor receptor superfamily, member 17)*
Nucleotide
Genbank accession no. Z29574
Genbank version no. Z29574.1 GI:471244
Genbank record update date: Feb 02, 2011 10:40 AM

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

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

Other information
Official Symbol: TNFRSF17
Other Aliases: BCM, BCMA, CD269

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

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

Cross-references

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

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

Nucleotide

Genbank accession no. NM000149

Genbank version no. NM000149.3 GI:148277008

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

Polypeptide

Genbank accession no. NP_000140

Genbank version no. NP_000140.1 GI:4503809

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

Cross-references

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

Other information

Official Symbol: FUT3

Other Aliases: CD174, FT3B, FucT-III, LE, Les

Other Designations: Lewis FT; alpha-(1,3/1,4)-fucosyltransferase; blood group Lewis alpha-4-fucosyltransferase; fucosyltransferase III; galactoside 3(4)-L-fucosyltransferase

(54) *CLEC14A (C-type lectin domain family 14, member A; Genbank accession no. NM175060)*

Nucleotide

Genbank accession no. NM175060

Genbank version no. NM175060.2 GI:371123930

Genbank record update date: Apr 01, 2012 03:34 PM

Polypeptide

Genbank accession no. NP_778230

Genbank version no. NP_778230.1 GI:28269707

Genbank record update date: Apr 01, 2012 03:34 PM

Other information

Official Symbol: CLEC14A

Other Aliases: UNQ236/PRO269, C14orf27, CEG1, EGFR-5

Other Designations: C-type lectin domain family 14 member A; CIECT and EGF-like domain containing protein; epidermal growth factor receptor 5

(55) *GRP78 – HSPA5 (heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)*

Nucleotide

Genbank accession no. NM005347

Genbank version no. NM005347.4 GI:305855105

Genbank record update date: Sep 30, 2012 01:42 PM

Polypeptide

Genbank accession no. NP_005338

Genbank version no. NP_005338.1 GI:16507237

Genbank record update date: Sep 30, 2012 01:42 PM

Cross-references

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

Other infromation

Official Symbol: HSPA5

Other Aliases: BIP, GRP78, MIF2

Other Designations: 78 kDa glucose-regulated protein; endoplasmic reticulum lumenal Ca(2+)-binding protein grp78; immunoglobulin heavy chain-binding protein

(56) *CD70 (CD70 molecule) L08096*

Nucleotide

Genbank accession no. L08096

Genbank version no. L08096.1 GI:307127

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

Polypeptide

Genbank accession no. AAA36175

Genbank version no. AAA36175.1 GI:307128

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

Cross-references

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

Other information

Official Symbol: CD70

Other Aliases: CD27L, CD27LG, TNFSF7

Other Designations: CD27 ligand; CD27-L; CD70 antigen; Ki-24 antigen; surface antigen CD70; tumor necrosis factor (ligand) superfamily, member 7; tumor necrosis factor ligand superfamily member 7

ANTIBODIES

MDX-1411 against CD70 (Medarex)

h1F6 (Oflazoglu, E., et al, Clin Cancer Res. 2008 Oct 1;14(19):6171-80; Seattle Genetics)

For example, see US20060083736 SEQ ID NOs: 1, 2, 11 and 12 and Fig. 1.

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

- 5T4 (see entry (63) below)
- CD25 (see entry (48) above)
- CD32
    - Polypeptide
        - Genbank accession no. ABK42161
        - Genbank version no. ABK42161.1 GI:117616286
        - Genbank record update date: Jul 25, 2007 03:00 PM
- LGR5/GPR49
    - Nucleotide
        - Genbank accession no. NM_003667
        - Genbank version no. NM_003667.2 GI:24475886
        - Genbank record update date: Jul 22, 2012 03:38 PM

- Polypeptide
  - Genbank accession no. NP_003658
  - Genbank version no. NP_003658.1 GI:4504379
  - Genbank record update date: Jul 22, 2012 03:38 PM
- Prominin/CD133
  - Nucleotide
    - Genbank accession no. NM_006017
    - Genbank version no. NM_006017.2 GI:224994187
    - Genbank record update date: Sep 30, 2012 01:47 PM
  - Polypeptide
    - Genbank accession no. NP_006008
    - Genbank version no. NP_006008.1 GI:5174387
    - Genbank record update date: Sep 30, 2012 01:47 PM

(58) *ASG-5*

Cross-references

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

ANTIBODIES

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

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

Nucleotide

Genbank accession no. AF005632

Genbank version no. AF005632.2 GI:4432589

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

Polypeptide

Genbank accession no. AAC51813

Genbank version no. AAC51813.1 GI:2465540

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

Cross-references

Jin-Hua P., et al *Genomics* 45 (2), 412-415 (1997)

Other information

Official Symbol: ENPP3

Other Aliases: RP5-988G15.3, B10, CD203c, NPP3, PD-IBETA, PDNP3

Other Designations: E-NPP 3; dJ1005H11.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); dJ914N13.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); ectonucleotide pyrophosphatase/phosphodiesterase family member 3; gp130RB13-6; phosphodiesterase I beta; phosphodiesterase I/nucleotide pyrophosphatase 3; phosphodiesterase-I beta

(60) *PRR4 (Proline rich 4 (lacrimal))*

Nucleotide

Genbank accession no. NM_007244

Genbank version no. NM_007244.2 GI:154448885

Genbank record update date: Jun 28, 2012 12:39 PM

Polypeptide

Genbank accession no. NP_009175

Genbank version no. NP_009175.2 GI:154448886

Genbank record update date: Jun 28, 2012 12:39 PM

Cross-references

Dickinson D.P., et al *Invest. Ophthalmol. Vis. Sci.* 36 (10), 2020-2031 (1995)

Other information

Official Symbol: PRR4

Other Aliases: LPRP, PROL4

Other Designations: lacrimal proline-rich protein; nasopharyngeal carcinoma-associated proline-rich protein 4; proline-rich polypeptide 4; proline-rich protein 4

(61) *GCC – GUCY2C (guanylate cyclase 2C (heat stable enterotoxin receptor)*

Nucleotide

Genbank accession no. NM_004963

Genbank version no. NM_004963.3 GI:222080082

Genbank record update date: Sep 02, 2012 01:50 PM

Polypeptide

Genbank accession no. NP_004954

Genbank version no. NP_004954.2 GI:222080083

Genbank record update date: Sep 02, 2012 01:50 PM

Cross-references

De Sauvage F.J., et al *J. Biol. Chem.* 266 (27), 17912-17918 (1991); Singh S., et al *Biochem. Biophys. Res. Commun.* 179 (3), 1455-1463 (1991)

Other information

Official Symbol: GUCY2C

Other Aliases: DIAR6, GUC2C, MUCIL, STAR

Other Designations: GC-C; STA receptor; guanylyl cyclase C; hSTAR; heat-stable enterotoxin receptor; intestinal guanylate cyclase

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

Nucleotide

Genbank accession no. U41060

Genbank version no. U41060.2 GI:12711792

Genbank record update date: Nov 30, 2009 04:35 PM

Polypeptide

Genbank accession no. AAA96258

Genbank version no. AAA96258.2 GI:12711793

Genbank record update date: Nov 30, 2009 04:35 PM

Cross-references

Taylor KM., et al *Biochim Biophys Acta.* 2003 Apr 1;1611(1-2):16-30

Other information

Official Symbol: SLC39A6

Other Aliases: LIV-1

Other Designations: LIV-1 protein, estrogen regulated; ZIP-6; estrogen-regulated protein LIV-1; solute carrier family 39 (metal ion transporter), member 6; solute carrier family 39 member 6; zinc transporter ZIP6; zrt- and Irt-like protein 6

(63) *5T4, Trophoblast glycoprotein, TPBG – TPBG (trophoblast glycoprotein)*

Nucleotide

Genbank accession no. AJ012159

Genbank version no. AJ012159.1 GI:3805946

Genbank record update date: Feb 01, 2011 10:27 AM

Polypeptide

Genbank accession no. CAA09930

Genbank version no. CAA09930.1 GI:3805947

Genbank record update date: Feb 01, 2011 10:27 AM

Cross-references

King K.W.,et al *Biochim. Biophys. Acta* 1445 (3), 257-270 (1999)

Other information

- Official Symbol: TPBG
- Other Aliases: 5T4, 5T4AG, M6P1
- Other Designations: 5T4 oncofetal antigen; 5T4 oncofetal trophoblast glycoprotein; 5T4 oncotrophoblast glycoprotein

(64) *CD56 – NCMA1 (Neural cell adhesion molecule 1)*

Nucleotide

Genbank accession no. NM_000615

Genbank version no. NM_000615.6 GI:336285433

Genbank record update date: Sep 23, 2012 02:32 PM

Polypeptide

Genbank accession no. NP_000606

Genbank version no. NP_000606.3 GI:94420689

Genbank record update date: Sep 23, 2012 02:32 PM

Cross-references

Dickson, G., et al, *Cell* 50 (7), 1119-1130 (1987)

Other information

Official Symbol: NCAM1

Other Aliases: CD56, MSK39, NCAM

Other Designations: antigen recognized by monoclonal antibody 5.1H11; neural cell adhesion molecule, NCAM

ANTIBODIES

Immunogen: HuN901 (Smith SV., et al *Curr Opin Mol Ther.* 2005 Aug;7(4):394-401)

For example, see humanized from murine N901 antibody. See Fig. 1b and 1e of Roguska, M.A., et al. Proc Natl Acad Sci USA Feb 1994;91:969-973.

(65) *CanAg (Tumor associated antigen CA242)*

Cross-references

Haglund C., et al *Br J Cancer* 60:845-851, 1989;Baeckstrom D., et al *J Biol Chem* 266:21537-21547, 1991

ANTIBODIES

huC242 (Tolcher AW et al., *J Clin Oncol.* 2003 Jan 15;21(2):211-22; Immunogen)

For example, see US20080138898A1 SEQ ID NO: 1 and 2

(66) *FOLR1 (Folate Receptor 1)*

Nucleotide

Genbank accession no. J05013

Genbank version no. J05013.1 GI:182417

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

Polypeptide

Genbank accession no. AAA35823

Genbank version no. AAA35823.1 GI:182418

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

Cross-references

Elwood P.C., et al *J. Biol. Chem.* 264 (25), 14893-14901 (1989)

Other information

Official Symbol: FOLR1

Other Aliases: FBP, FOLR

Other Designations: FR-alpha; KB cells FBP; adult folate-binding protein; folate binding protein; folate receptor alpha; folate receptor, adult; ovarian tumor-associated antigen MOv18

ANTIBODIES

M9346A - Whiteman KR., et al *Cancer Res* April 15, 2012; 72(8 Supplement): 4628 (Immunogen)

(67) *GPNMB (Glycoprotein (transmembrane) nmb)*

Nucleotide

Genbank accession no. X76534

Genbank version no. X76534.1 GI:666042

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

Polypeptide

Genbank accession no. CAA54044

Genbank version no. CAA54044.1 GI:666043

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

Cross-references

Weterman M.A., et al *Int. J. Cancer* 60 (1), 73-81 (1995)

Other information

Official Symbol: GPNMB

Other Aliases: UNQ1725/PRO9925, HGFIN, NMB

Other Designations: glycoprotein NMB; glycoprotein nmb-like protein; osteoactivin; transmembrane glycoprotein HGFIN; transmembrane glycoprotein NMB

ANTIBODIES

Celldex Therapeutics: CR011 (Tse KF., et al *Clin Cancer Res.* 2006 Feb 15;12(4):1373-82)

For example, see EP1827492B1 SEQ ID NO: 22, 24, 26, 31, 33 and 35

(68) *TIM-1 – HAVCR1 (Hepatitis A virus cellular receptor 1)*

Nucleotide

Genbank accession no. AF043724

Genbank version no. AF043724.1 GI:2827453

Genbank record update date: Mar 10, 2010 06:24 PM

Polypeptide

Genbank accession no. AAC39862

Genbank version no. AAC39862.1 GI:2827454

Genbank record update date: Mar 10, 2010 06:24 PM

Cross-references

Feigelstock D., et al *J. Virol.* 72 (8), 6621-6628 (1998)

Other information

Official Symbol: HAVCR1

Other Aliases: HAVCR, HAVCR-1, KIM-1, KIM1, TIM, TIM-1, TIM1, TIMD-1, TIMD1

Other Designations: T cell immunoglobin domain and mucin domain protein 1; T-cell membrane protein 1; kidney injury molecule 1

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

Cross-references

Parry R., et al Cancer Res. 2005 Sep 15;65(18):8397-405

(70) *B7-H4 – VTCN1 (V-set domain containing T cell activation inhibitor 1*

Nucleotide

Genbank accession no. BX648021

Genbank version no. BX648021.1 GI:34367180

Genbank record update date: Feb 02, 2011 08:40 AM

Cross-references

Sica GL., et al *Immunity.* 2003 Jun;18(6):849-61

Other information

Official Symbol: VTCN1

Other Aliases: RP11-229A19.4, B7-H4, B7H4, B7S1, B7X, B7h.5, PRO1291, VCTN1

Other Designations: B7 family member, H4; B7 superfamily member 1; T cell costimulatory molecule B7x; T-cell costimulatory molecule B7x; V-set domain-containing T-cell activation inhibitor 1; immune costimulatory protein B7-H4

(71) *PTK7 (PTK7 protein tyrosine kinase 7)*

Nucleotide

Genbank accession no. AF447176

Genbank version no. AF447176.1 GI:17432420

Genbank record update date: Nov 28, 2008 01:51 PM

Polypeptide

Genbank accession no. AAL39062

Genbank version no. AAL39062.1 GI:17432421

Genbank record update date: Nov 28, 2008 01:51 PM

Cross-references

Park S.K., et al *J. Biochem.* 119 (2), 235-239 (1996)

Other information

Official Symbol: PTK7

Other Aliases: CCK-4, CCK4

Other Designations: colon carcinoma kinase 4; inactive tyrosine-protein kinase 7; pseudo tyrosine kinase receptor 7; tyrosine-protein kinase-like 7

(72) *CD37 (CD37 molecule)*

Nucleotide

Genbank accession no. NM_001040031

Genbank version no. NM_001040031.1 GI:91807109

Genbank record update date: Jul 29, 2012 02:08 PM

Polypeptide

Genbank accession no. NP_001035120

Genbank version no. NP_001035120.1 GI:91807110

Genbank record update date: Jul 29, 2012 02:08 PM

Cross-references

Schwartz-Albiez R., et al *J. Immunol.* 140 (3), 905-914 (1988)

Other information

Official Symbol: CD37

Other Aliases: GP52-40, TSPAN26

Other Designations: CD37 antigen; cell differentiation antigen 37; leukocyte antigen CD37; leukocyte surface antigen CD37; tetraspanin-26; tspan-26

ANTIBODIES

Boehringer Ingelheim: mAb 37.1 (Heider KH., et al *Blood*. 2011 Oct 13;118(15):4159-68)

Trubion: CD37-SMIP (G28-1 scFv-Ig) ((Zhao X., et al *Blood*. 2007;110: 2569-2577)

For example, see US20110171208A1 SEQ ID NO: 253

Immunogen: K7153A (Deckert J., et al Cancer Res April 15, 2012; 72(8 Supplement): 4625)

(73) *CD138 – SDC1 (syndecan 1)*

Nucleotide

Genbank accession no. AJ551176

Genbank version no. AJ551176.1 GI:29243141

Genbank record update date: Feb 01, 2011 12:09 PM

Polypeptide

Genbank accession no. CAD80245

Genbank version no. CAD80245.1 GI:29243142

Genbank record update date: Feb 01, 2011 12:09 PM

Cross-references

O'Connell FP., et al *Am J Clin Pathol*. 2004 Feb;121(2):254-63

Other information

Official Symbol: SDC1

Other Aliases: CD138, SDC, SYND1, syndecan

Other Designations: CD138 antigen; heparan sulfate proteoglycan fibroblast growth factor receptor; syndecan proteoglycan 1; syndecan-1

ANTIBODIES

Biotest: chimerized MAb (nBT062) - (Jagannath S., et al Poster *ASH* #3060, 2010; WIPO Patent Application WO/2010/128087)

For example, see US20090232810 SEQ ID NO: 1 and 2

Immunogen: B-B4 (Tassone P., et al *Blood* 104_3688-3696)

For example, see US20090175863A1 SEQ ID NO: 1 and 2

(74) *CD74 (CD74 molecule, major histocompatibility complex, class II invariant chain)*

Nucleotide

Genbank accession no. NM_004355

Genbank version no. NM_004355.1 GI:343403784

Genbank record update date: Sep 23, 2012 02:30 PM

Polypeptide

Genbank accession no. NP_004346

Genbank version no. NP_004346.1 GI:10835071

Genbank record update date: Sep 23, 2012 02:30 PM

Cross-references

Kudo, J., et al *Nucleic Acids Res.* 13 (24), 8827-8841 (1985)

Other information

Official Symbol: CD74

Other Aliases: DHLAG, HLADG, II, Ia-GAMMA

Other Designations: CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); HLA class II histocompatibility antigen gamma chain; HLA-DR antigens-associated invariant chain; HLA-DR-gamma; Ia-associated invariant chain; MHC HLA-DR gamma chain; gamma chain of class II antigens; p33

ANTIBODIES

Immunomedics: hLL1 (Milatuzumab,) - Berkova Z., et al *Expert Opin Investig Drugs.* 2010 Jan;19(1):141-9)

For example, see US20040115193 SEQ ID NOs: 19, 20, 21, 22, 23 and 24

Genmab: HuMax-CD74 (see website)

(75) *Claudins – CLs (Claudins)*

Cross-references

Offner S., et al *Cancer Immunol Immunother.* 2005 May; 54(5):431-45, Suzuki H., et al *Ann N Y Acad Sci.* 2012 Jul;1258:65-70)

In humans, 24 members of the family have been described – see literature reference.

(76) *EGFR (Epidermal growth factor receptor)*

Nucleotide

Genbank accession no. NM_005228

Genbank version no. NM_005228.3 GI:41927737

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

Polypeptide

Genbank accession no. NP_005219

Genbank version no. NP_005219.2 GI:29725609

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

Cross-references

Dhomen NS., et al *Crit Rev Oncog.* 2012;17(1):31-50

Other information

Official Symbol: EGFR

Other Aliases: ERBB, ERBB1, HER1, PIG61, mENA

Other Designations: avian erythroblastic leukemia viral (v-erb-b) oncogene homolog; cell growth inhibiting protein 40; cell proliferation-inducing protein 61; proto-oncogene c-ErbB-1; receptor tyrosine-protein kinase erbB-1

ANTIBODIES

BMS: Cetuximab (Erbitux) - Broadbridge VT., et al *Expert Rev Anticancer Ther.* 2012 May;12(5):555-65.

For example, see US6217866 – ATTC deposit No. 9764.

Amgen: Panitumumab (Vectibix) - Argiles G., et al *Future Oncol.* 2012 Apr;8(4):373-89

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

Genmab: Zalutumumab - Rivera F., et al *Expert Opin Biol Ther.* 2009 May;9(5):667-74.

YM Biosciences: Nimotuzumab - Ramakrishnan MS., et al *MAbs.* 2009 Jan-Feb;1(1):41-8.

For example, see US5891996 SEQ ID NOs: 27-34.

(77) *Her3 (ErbB3) – ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian))*

Nucleotide

Genbank accession no. M34309

Genbank version no. M34309.1 GI:183990

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

Polypeptide

Genbank accession no. AAA35979

Genbank version no. AAA35979.1 GI:306841

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

Cross-references

Plowman, G.D., et al., *Proc. Natl. Acad. Sci. U.S.A.* 87 (13), 4905-4909 (1990)

Other information

Official Symbol: ERBB3

Other Aliases: ErbB-3, HER3, LCCS2, MDA-BF-1, c-erbB-3, c-erbB3, erbB3-S, p180-ErbB3, p45-sErbB3, p85-sErbB3

Other Designations: proto-oncogene-like protein c-ErbB-3; receptor tyrosine-protein kinase erbB-3; tyrosine kinase-type cell surface receptor HER3

ANTIBODIES

Merimack Pharma : MM-121 (Schoeberl B., et al Cancer Res. 2010 Mar 15;70(6):2485-2494)

For example, see US2011028129 SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7 and 8.

(78) *RON - MST1R (macrophage stimulating 1 receptor (c-met-related tyrosine kinase))*

Nucleotide

Genbank accession no. X70040

Genbank version no. X70040.1 GI:36109
Genbank record update date: Feb 02, 2011 10:17 PM

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

Cross-references
Ronsin C., et al *Oncogene* 8 (5), 1195-1202 (1993)

Other information
Official Symbol: MST1R
Other Aliases: CD136, CDw136, PTK8, RON
Other Designations: MSP receptor; MST1R variant RON30; MST1R variant RON62; PTK8 protein tyrosine kinase 8; RON variant E2E3; c-met-related tyrosine kinase; macrophage-stimulating protein receptor; p185-Ron; soluble RON variant 1; soluble RON variant 2; soluble RON variant 3; soluble RONvariant 4

(79) *EPHA2 (EPH receptor A2)*
Nucleotide
Genbank accession no. BC037166
Genbank version no. BC037166.2 GI:33879863
Genbank record update date: Mar 06, 2012 01:59 PM

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

Cross-references
Strausberg R.L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99 (26), 16899-16903 (2002)

Other information
Official Symbol: EPHA2
Other Aliases: ARCC2, CTPA, CTPP1, ECK

Other Designations: ephrin type-A receptor 2; epithelial cell receptor protein tyrosine kinase; soluble EPHA2 variant 1; tyrosine-protein kinase receptor ECK

ANTIBODIES

Medimmune: 1C1 (Lee JW., et al *Clin Cancer Res.* 2010 May 1;16(9):2562-2570)

For example, see US20090304721A1 Fig. 7 and 8.

(80) *CD20 – MS4A1 (membrane-spanning 4-domains, subfamily A, member 1)*

Nucleotide

Genbank accession no. M27394

Genbank version no. M27394.1 GI:179307

Genbank record update date: Nov 30, 2009 11:16 AM

Polypeptide

Genbank accession no. AAA35581

Genbank version no. AAA35581.1 GI:179308

Genbank record update date: Nov 30, 2009 11:16 AM

Cross-references

Tedder T.F., et al *Proc. Natl. Acad. Sci. U.S.A.* 85 (1), 208-212 (1988)

Other information

Official Symbol: MS4A1

Other Aliases: B1, Bp35, CD20, CVID5, LEU-16, MS4A2, S7

Other Designations: B-lymphocyte antigen CD20; B-lymphocyte cell-surface antigen B1; CD20 antigen; CD20 receptor; leukocyte surface antigen Leu-16

ANTIBODIES

Genentech/Roche: Rituximab - Abdulla NE., et al *BioDrugs.* 2012 Apr 1;26(2):71-82.

For example, see US5736137, ATCC deposit No. HB-69119.

GSK/Genmab: Ofatumumab - Nightingale G., et al Ann Pharmacother. 2011 Oct;45(10):1248-55.

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

Immunomedics: Veltuzumab - Goldenberg DM., et al *Leuk Lymphoma.* 2010 May;51(5):747-55.

For example, see US7919273B2 SEQ ID NOs: 1, 2, 3, 4, 5 and 6.

(81) *Tenascin C – TNC (Tenascin C)*

Nucleotide

Genbank accession no. NM_002160

Genbank version no. NM_002160.3 GI:340745336

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

Polypeptide

Genbank accession no. NP_002151

Genbank version no. NP_002151.2 GI:153946395

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

Cross-references

Nies D.E., et al J. Biol. Chem. 266 (5), 2818-2823 (1991); Siri A., et al Nucleic Acids Res. 19 (3), 525-531 (1991)

Other information

Official Symbol: TNC

Other Aliases: 150-225, GMEM, GP, HXB, JI, TN, TN-C

Other Designations: GP 150-225; cytotactin; glioma-associated-extracellular matrix antigen; hexabrachion (tenascin); myotendinous antigen; neuronectin; tenascin; tenascin-C isoform 14/AD1/16

ANTIBODIES

Philogen : G11 (von Lukowicz T., et al *J Nucl Med.* 2007 Apr;48(4):582-7) and F16 (Pedretti M., et al Lung Cancer. 2009 Apr;64(1):28-33)

For example, see US7968685 SEQ ID NOs: 29, 35, 45 and 47.

(82) *FAP (Fibroblast activation protein, alpha)*

Nucleotide

Genbank accession no. U09278

Genbank version no. U09278.1 GI:1888315

Genbank record update date: Jun 23, 2010 09:22 AM

Polypeptide

Genbank accession no. AAB49652

Genbank version no. AAB49652.1 GI:1888316

Genbank record update date: Jun 23, 2010 09:22 AM

Cross-references

Scanlan, M.J.,et al *Proc. Natl. Acad. Sci. U.S.A.* 91 (12), 5657-5661 (1994)

Other information

Official Symbol: FAP

Other Aliases: DPPIV, FAPA

Other Designations: 170 kDa melanoma membrane-bound gelatinase; integral membrane serine protease; seprase

(83) *DKK-1 (Dickkopf 1 homolog (Xenopus laevis)*

Nucleotide

Genbank accession no. NM_012242

Genbank version no. NM_012242.2 GI:61676924

Genbank record update date: Sep 30, 2012 01:48 PM

Polypeptide

Genbank accession no. NP_036374

Genbank version no. NP_036374.1 GI:7110719

Genbank record update date: Sep 30, 2012 01:48 PM

Cross-references

Fedi P. et al *J. Biol. Chem.* 274 (27), 19465-19472 (1999)

Other information

Official Symbol: DKK1

Other Aliases: UNQ492/PRO1008, DKK-1, SK

Other Designations: dickkopf related protein-1; dickkopf-1 like; dickkopf-like protein 1; dickkopf-related protein 1; hDkk-1

ANTIBODIES

Novartis: BHQ880 (Fulciniti M., et al *Blood.* 2009 Jul 9;114(2):371-379)

For example, see US20120052070A1 SEQ ID NOs: 100 and 108.

(84) *CD52 (CD52 molecule)*

Nucleotide

Genbank accession no. NM_001803

Genbank version no. NM_001803.2 GI:68342029

Genbank record update date: Sep 30, 2012 01:48 PM

Polypeptide

Genbank accession no. NP_001794

Genbank version no. NP_001794.2 GI:68342030

Genbank record update date: Sep 30, 2012 01:48 PM

Cross-references

Xia M.Q., et al *Eur. J. Immunol.* 21 (7), 1677-1684 (1991)

Other information

Official Symbol: CD52

Other Aliases: CDW52

Other Designations: CAMPATH-1 antigen; CD52 antigen (CAMPATH-1 antigen); CDW52 antigen (CAMPATH-1 antigen); cambridge pathology 1 antigen; epididymal secretory protein E5; he5; human epididymis-specific protein 5

ANTIBODIES

Alemtuzumab (Campath) - Skoetz N., et al *Cochrane Database Syst Rev.* 2012 Feb 15;2:CD008078.

For example, see Drugbank Acc. No. DB00087 (BIOD00109, BTD00109)

(85) *CS1 - SLAMF7 (SLAM family member 7)*

Nucleotide

Genbank accession no. NM_021181

Genbank version no. NM_021181.3 GI:1993571

Genbank record update date: Jun 29, 2012 11:24 AM

Polypeptide

Genbank accession no. NP_067004

Genbank version no. NP_067004.3 GI:19923572

Genbank record update date: Jun 29, 2012 11:24 AM

Cross-references

Boles K.S., et al *Immunogenetics* 52 (3-4), 302-307 (2001)

Other information

Official Symbol: SLAMF7

Other Aliases: UNQ576/PRO1138, 19A, CD319, CRACC, CS1

Other Designations: 19A24 protein; CD2 subset 1; CD2-like receptor activating cytotoxic cells; CD2-like receptor-activating cytotoxic cells; membrane protein FOAP-12; novel LY9 (lymphocyte antigen 9) like protein; protein 19A

ANTIBODIES

BMS: elotuzumab/HuLuc63 (Benson DM., et al *J Clin Oncol.* 2012 Jun 1;30(16):2013-2015)

For example, see US20110206701 SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15 and 16.

(86) *Endoglin – ENG (Endoglin)*

Nucleotide

Genbank accession no. AF035753

Genbank version no. AF035753.1 GI:3452260

Genbank record update date: Mar 10, 2010 06:36 PM

Polypeptide

Genbank accession no. AAC32802

Genbank version no. AAC32802.1 GI:3452261

Genbank record update date: Mar 10, 2010 06:36 PM

Cross-references

Rius C., et al *Blood* 92 (12), 4677-4690 (1998)

Official Symbol: ENG

Other information

Other Aliases: RP11-228B15.2, CD105, END, HHT1, ORW, ORW1

Other Designations: CD105 antigen

(87) *Annexin A1 – ANXA1 (Annexin A1)*

Nucleotide

Genbank accession no. X05908

Genbank version no. X05908.1 GI:34387

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

Polypeptide

Genbank accession no. CCA29338

Genbank version no. CCA29338.1 GI:34388

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

Cross-references

Wallner B.P.,et al *Nature* 320 (6057), 77-81 (1986)

Other information

Official Symbol: ANXA1

Other Aliases: RP11-71A24.1, ANX1, LPC1

Other Designations: annexin I (lipocortin I); annexin-1; calpactin II; calpactin-2; chromobindin-9; lipocortin I; p35; phospholipase A2 inhibitory protein

(88) *V-CAM (CD106) - VCAM1 (Vascular cell adhesion molecule 1)*

Nucleotide

Genbank accession no. M60335

Genbank version no. M60335.1 GI:340193

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

Polypeptide

Genbank accession no. AAA61269

Genbank version no. AAA61269.1 GI:340194

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

Cross-references

Hession C., et al *J. Biol. Chem.* 266 (11), 6682-6685 (1991)

Other information

Official Symbol VCAM1

Other Aliases: CD106, INCAM-100

Other Designations: CD106 antigen; vascular cell adhesion protein 1

Antibody Sequences

Anti-Integrin αvβ6

RHAB6.2

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDTE
YAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTAVPNLRGDLQVLAQKVAG
PYPFDYWGQGTLVTVSS

RHCB6.2

QVQLVQSGAEVKKPGASVKVSCKASGYTFIDSYMHWVRQAPGQRLEWMGWIDPENGDTE
YAPKFQGRVTITTDTSASTAYMELSSLRSEDTAVYYCARGTPTAVPNLRGDLQVLAQKVAG
PYPFDYWGQGTLVTVSS

RHF

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGDT
EYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTGPYYFDYWGQGTLVTV
SS

RHFB6

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGDT
EYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTAVPNLRGDLQVLAQKVA
GPYYFDYWGQGTLVTVSS

RHAY100bP

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDTE
YAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTGPYPFDYWGQGTLVTVSS

RKF

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKFL36L50

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWLQQKPGQAPRLLIYLTSNLASGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKC

EIVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

Anti-CD33

CD33 Hum195 VH

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTG
YNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSS

CD33 Hum195 VK

DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSG
VPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK

Anti-CD19

CD19 B4 resurfaced VH

QVQLVQPGAEVVKPGASVKLSCKTSGYTFTSNWMHWVKQRPGQGLEWIGEIDPSDSYTN
YNQNFKGKAKLTVDKSTSTAYMEVSSLRSDDTAVYYCARGSNPYYYAMDYWGQGTSVTV
SS

CD19 B4 resurfaced VK

EIVLTQSPAIMSASPGERVTMTCSASSGVNYMHWYQQKPGTSPRRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMEPEDAATYYCHQRGSYTFGGGTKLEIK

Anti-Her2

Herceptin VH chain

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
S

Herceptin VL chain

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Anti-CD25

Simulect VK (also known as Basiliximab)

QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQQKPGTSPKRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMEAEDAATYYCHQRSSYTFGGGTKLEIK

Simulect VH

QLQQSGTVLARPGASVKMSCKASGYSFTRYWMHWIKQRPGQGLEWIGAIYPGNSDTSYN
QKFEGKAKLTAVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFDFWGQGTTLTVSS

Anti-PSMA

Deimmunised VH '1

EVQLVQSGPEVKKPGATVKISCKTSGYTFTEYTIHWVKQAPGKGLEWIGNINPNNGGTTYN
QKFEDKATLTVDKSTDTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTLLTVSS

Deimmunised VK '1

DIQMTQSPSSLSTSVGDRVTLTCKASQDVGTAVDWYQQKPGPSPKLLIYWASTRHTGIPSR
FSGSGSGTDFTLTISSLQPEDFADYYCQQYNSYPLTFGPGTKVDIK

Deimmunised VH1 '5

EVKLVESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTGVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH2 '5

EVKLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNFA
THYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH3 '5

EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNFA
THYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH4 '5

EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNFA
THYAESVKGRFTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VK1 '5

NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVPD
RFTGSGSATDFTLTISSLQTEDLADYYCGQSYTFPYTFGQGTKLEMK

Deimmunised VK2 '5

NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVPD
RFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK3 '5

NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVPD
RFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK4 '5

NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVPD
RFSGSGSGTDFTLTISSLQAEDEADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK DI '5

NIVMTQFPKSMSASAGERMTLTCKASENVGTYVSWYQQKPTQSPKMLIYGASNRFTGVPD
RFSGSGSGTDFILTISSVQAEDLVDYYCGQSYTFPYTFGGGTKLEMK

Deimmunised VH DI '5

EVKLEESGGGLVQPGGSMKISCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRSQSNNFA
THYAESVKGRVIISRDDSKSSVYLQMNSLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHA '5

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNFA
THYAESVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHB '5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNFA
THYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHC '5

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNFA
THYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHD '5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNFA
THYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHE '5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNFA
THYAESVKGRFTISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHF '5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNFA
THYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RHG '5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNFA
THYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised RKA '5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPSR
FSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKB '5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPSR
FSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKC '5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKD '5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKE '5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPDR
FTGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKF '5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPSR
FSGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised RKG '5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPDR
FTGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

The parent antibody may also be a fusion protein comprising an albumin-binding peptide (ABP) sequence (Dennis *et al.* (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" *J Biol Chem.* 277:35035-35043; WO 01/45746). Antibodies of the invention include fusion proteins with ABP sequences taught by: (i) Dennis *et al* (2002) *J Biol Chem.* 277:35035-35043 at Tables III and IV, page 35038; (ii) US 2004/0001827 at [0076]; and (iii) WO 01/45746 at pages 12-13.

In one embodiment, the antibody has been raised to target specific the tumour related antigen $\alpha_v\beta_6$.

The cell binding agent may be labelled, for example to aid detection or purification of the agent either prior to incorporation as a conjugate, or as part of the conjugate. The label may be a biotin label. In another embodiment, the cell binding agent may be labelled with a radioisotope.

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 ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms.

Salts

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

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

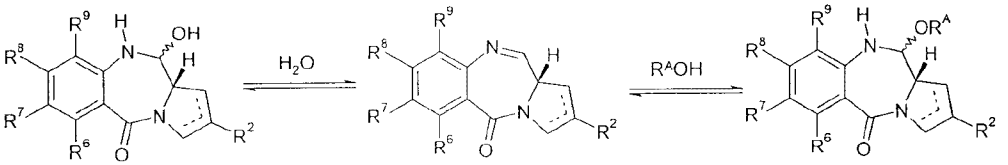
If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic,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

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, 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^AOH, where R^A 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, diasteriomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

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.

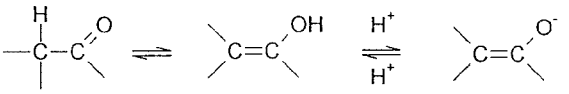
"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

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, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to

ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

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



keto enol enolate

The term "tautomer" or "tautomeric 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 ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

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 ^{2}H (deuterium, D), ^{3}H (tritium), ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}F, ^{31}P, ^{32}P, ^{35}S, ^{36}Cl, and ^{125}I. 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 (100µl 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 (20µg/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 100µl to 900µl of cell culture medium.

Four replicate wells of each ADC dilution (100µl) are dispensed in 96-well black plates, previously plated with cell suspension (100µl), resulting in a final volume of 200 µl. Control wells receive cell culture medium (100µl).

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 (20µl 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/m^2); 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 $\alpha_v\beta_6$ integrin on the surface of the cell.

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

Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

Autoimmune diseases for which the ADC compounds may be used in treatment include rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g. ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and polyarteriitis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g. Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

Methods of Treatment

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

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

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

Examples of chemotherapeutic agents include: erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTERE®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine, dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), trastuzumab (HERCEPTIN®, Genentech), temozolomide (4-methyl-5-oxo- 2,3,4,6,8-pentazabicyclo [4.3.0] nona-2,7,9-triene- 9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethylethanamine, NOLVADEX®, ISTUBAL®, VALODEX®), and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.
More examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sutent (SUNITINIB®, SU11248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (Mek inhibitor, Exelixis, WO 2007/044515), ARRY-886 (Mek inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 222584 (Novartis), fulvestrant (FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Il), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chloranmbucil,

AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (TELCYTA®, Telik), thiotepa and cyclosphosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, calicheamicin gamma1I, calicheamicin omegaI1 (*Angew Chem. Intl. Ed. Engl.* (1994) 33:183-186); dynemicin, dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, nemorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine;

pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

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

Also included in the definition of "chemotherapeutic agent" are therapeutic antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®,

Genentech/Biogen Idec), 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, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pertuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

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

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

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is

pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Formulations

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

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

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

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

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, Handbook of Pharmaceutical Additives, 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse Information Resources, Inc., Endicott, New York, USA), Remington's Pharmaceutical Sciences, 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994.

Another aspect of the present invention pertains to methods of making a pharmaceutical composition comprising admixing at least one [^{11}C]-radiolabelled conjugate or conjugate-like compound, as defined herein, together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, e.g., carriers, diluents, excipients, etc. If formulated as discrete units (e.g., tablets, etc.), each unit contains a predetermined amount (dosage) of the active compound.

The term "pharmaceutically acceptable," as used herein, pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

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

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

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

Dosage

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

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

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

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

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

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

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

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

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

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

Treatment

The term "treatment," as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate

of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included.

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

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

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, orangutan, gibbon), or a human.

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

In one embodiment, the patient is a population where each patient has a tumour having $\alpha_v\beta_6$ integrin on the surface of the cell.

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 ^{13}C NMR spectra were acquired at 300 K using a Bruker Avance™ NMR spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported relative to TMS (δ = 0.0 ppm), and signals are designated as s (singlet), d (doublet), t (triplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets) or m (multiplet), with coupling constants given in Hertz (Hz). Mass spectroscopy (MS) data were collected using a Waters Micromass™ ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA. Waters Micromass™ ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; De-solvation flow rate (L/h), 250. High-resolution mass spectroscopy (HRMS) data were recorded on a Waters Micromass™ QTOF Global in positive W-mode using metal-coated borosilicate glass tips to introduce the samples into the instrument. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F_{254}), and flash chromatography utilised silica gel (Merck 60, 230-400 mesh ASTM). Except for the HOBt (NovaBiochem) and solid-supported reagents (Argonaut), all other chemicals and solvents were purchased from Sigma-Aldrich and were used as supplied without further purification. Anhydrous solvents

were prepared by distillation under a dry nitrogen atmosphere in the presence of an appropriate drying agent, and were stored over 4Å molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

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

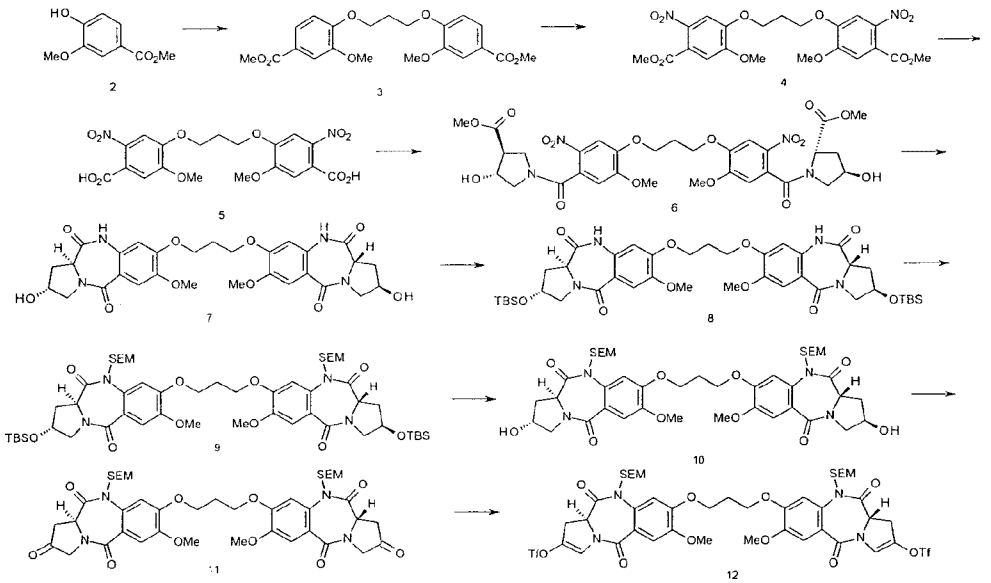
Flow rate 3.0 mL/min, 400µL was split *via* a zero dead volume tee piece which passes into the mass spectrometer. Wavelength detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex Onyx™ Monolithic C18 50 x 4.60 mm.

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 AX1372-1 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 (H_2O with 0.1% Formic acid) and solvent B (CH_3CN with 0.1% Formic acid). Flow rates used were 1.0

ml/min for analytical, and 20.0 ml/min for preparative HPLC. Detection was at 254 and 280 nm.

Synthesis of Intermediate 12



(a) 1',3'-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 Ph_3P (129.4 g, 494 mmol) in anhydrous THF (800 mL) at 0-5°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to stir at 0-5°C for an additional 1 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); ^1H 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'-Bis[2-methoxy-4-(methoxycarbonyl)-5-nitrophenoxy]propane ***(4)***

Solid $Cu(NO_3)_2.3H_2O$ (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/acetone). 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 NO_2 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 *bis*-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]$^{+\cdot}$, 40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 2H), 7.06 (s, 2H), 4.32 (t, 4H, *J* = 6.0 Hz), 3.95 (s, 6H), 3.90 (s, 6H), 2.45–2.40 (m, 2H).

*(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]$^{+\cdot}$, 30)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (s, 2H), 7.30 (s, 2H), 4.29 (t, 4H, *J* = 6.0 Hz), 3.85 (s, 6H), 2.30–2.26 (m, 2H).

*(d) 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(5-methoxy-2-nitro-1,4-phenylene)carbonyl]]bis[(2S,4R)-methyl-4-hydroxypyrrolidine-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 (2*S*,4*R*)-methyl-4-hydroxypyrrolidine-2-carboxylate hydrochloride (38.1 g, 210 mmol) and TEA (64.5 mL, g, 463 mmol) in DCM (400mL) at -40°C (dry ice/CH_3CN).

Immediately, the reaction was complete as judged by LC/MS (2.47 min (ES+) *m/z* (relative intensity) 721 ([*M* + H]$^+$, 100). The mixture was diluted with DCM (200 mL) and washed with 1N HCl (300 mL), saturated $NaHCO_3$ (300 mL), brine (400 mL), dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to give the pure product **6** as an orange solid (66.7 g, 100%). $[\alpha]^{22}_D$ = -46.1° (*c* = 0.47, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) (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); ^{13}C NMR (100 MHz, $CDCl_3$) (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, $CHCl_3$) 3410 (br), 3010, 2953, 1741, 1622, 1577, 1519, 1455, 1429, 1334, 1274, 1211, 1177, 1072, 1050, 1008, 871 cm^{-1}; MS (ES$^+$) *m/z* (relative intensity) 721 ([*M* + H]$^+$, 47), 388 (80); HRMS [*M* + H]$^+$ theoretical $C_{31}H_{36}N_4O_{16}$ *m/z* 721.2199, found (ES$^+$) *m/z* 721.2227.

(e) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(hydroxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] ***(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 H_2O) 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 $CHCl_3$/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%). $[\alpha]^{27}_D$ = +404° (*c* = 0.10, DMF); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.2 (s, 2H, N*H*), 7.26 (s, 2H), 6.73 (s, 2H), 5.11 (d, 2H, *J* = 3.98 Hz, O*H*), 4.32–4.27 (m, 2H), 4.19–4.07 (m, 6H), 3.78 (s, 6H), 3.62 (dd, 2H, *J* = 12.1, 3.60 Hz), 3.43 (dd, 2H, *J* = 12.0, 4.72 Hz), 2.67–2.57 (m, 2H), 2.26 (p, 2H, *J* = 5.90 Hz), 1.99–1.89 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 164.0, 149.9, 144.5, 129.8, 117.1, 111.3, 104.5, 54.8, 54.4, 53.1, 33.5, 27.5; IR (ATR, neat) 3438, 1680, 1654, 1610, 1605, 1516, 1490, 1434, 1379, 1263, 1234, 1216, 1177, 1156, 1115, 1089, 1038, 1018, 952, 870 cm^{-1}; MS (ES$^+$) *m/z* (relative intensity) 619 ([*M* + Na]$^+$, 10), 597 ([*M* + H]$^+$, 52), 445 (12), 326 (11); HRMS [*M* + H]$^+$ theoretical $C_{29}H_{32}N_4O_{10}$ *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 10mbar) 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).

(f) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] ***(8)***

TBSCl (27.6 g, 182.9 mmol) and imidazole (29.9 g, 438.8 mmol) were added to a cloudy solution of the tetralactam **7** (21.8 g, 36.6 mmol) in anhydrous DMF (400 mL) at 0°C (ice/acetone). The mixture was allowed to stir under a nitrogen atmosphere for 3 hours after which time the reaction was deemed complete as judged by LC/MS (3.90 min (ES+) *m/z* (relative intensity) 825 ([*M* + H]$^+$, 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_2O, diethyl ether and dried in the vacuum desiccator to provide pure **8** (30.1 g, 99%). $[\alpha]^{23}_D$ = +234° (*c* = 0.41, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 2H, N*H*), 7.44 (s, 2H), 6.54 (s, 2H), 4.50 (p, 2H, *J* = 5.38 Hz), 4.21–4.10 (m, 6H), 3.87 (s, 6H), 3.73–3.63 (m, 4H), 2.85–2.79 (m, 2H), 2.36–2.29 (m, 2H), 2.07-1.99 (m, 2H), 0.86 (s, 18H), 0.08 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 165.7, 151.4, 146.6, 129.7, 118.9, 112.8, 105.3, 69.2, 65.4, 56.3, 55.7, 54.2, 35.2, 28.7, 25.7, 18.0, -4.82 and -4.86; IR (ATR, $CHCl_3$) 3235, 2955, 2926, 2855, 1698, 1695, 1603, 1518, 1491, 1446, 1380, 1356, 1251, 1220, 1120, 1099, 1033 cm^{-1}; MS (ES$^+$) *m/z* (relative intensity) 825 ([*M* + H]$^+$, 62), 721 (14), 440 (38); HRMS [*M* + H]$^+$ theoretical $C_{41}H_{60}N_4O_{10}Si_2$ *m/z* 825.3921, found (ES$^+$) *m/z* 825.3948.

(g) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS,2R)-2-(tert-butyldimethylsilyloxy)-7-methoxy-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] ***(9)***

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. $[\alpha]^{23}_D$ = +163° (*c* = 0.41, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 2H), 7.22 (s, 2H), 5.47 (d, 2H, *J* = 9.98 Hz), 4.68 (d, 2H, *J* = 9.99 Hz), 4.57 (p, 2H, *J* = 5.77 Hz), 4.29–4.19 (m, 6H), 3.89 (s, 6H), 3.79–3.51 (m, 8H), 2.87–2.81 (m, 2H), 2.41 (p, 2H, *J* = 5.81 Hz), 2.03–1.90 (m, 2H), 1.02–0.81 (m, 22H), 0.09 (s, 12H), 0.01 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 165.7, 151.2, 147.5, 133.8, 121.8, 111.6, 106.9, 78.1, 69.6, 67.1, 65.5, 56.6, 56.3, 53.7, 35.6, 30.0, 25.8, 18.4, 18.1, -1.24, -4.73; IR (ATR, $CHCl_3$) 2951, 1685, 1640, 1606, 1517, 1462, 1433, 1360, 1247, 1127, 1065 cm^{-1}; MS (ES^+) *m/z* (relative intensity) 1113 ($[M + Na]^+$, 48), 1085 ($[M + H]^+$, 100), 1009 (5), 813 (6); HRMS $[M + H]^+$ theoretical $C_{53}H_{88}N_4O_{12}Si_4$ *m/z* 1085.5548, found (ES^+) *m/z* 1085.5542.

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

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 $CHCl_3$/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); $[\alpha]^{23}_D$ = +202° (*c* = 0.34, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 2H), 7.20 (s, 2H), 5.44 (d, 2H, *J* = 10.0 Hz), 4.72 (d, 2H, *J* = 10.0 Hz), 4.61–4.58 (m, 2H), 4.25 (t, 4H, *J* = 5.83 Hz), 4.20–4.16 (m, 2H), 3.91–3.85 (m, 8H), 3.77–3.54 (m, 6H), 3.01 (br s, 2H, O*H*), 2.96–2.90 (m, 2H), 2.38 (p, 2H, *J* = 5.77 Hz), 2.11–2.05 (m, 2H), 1.00–0.91 (m, 4H), 0.00 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 165.9, 151.3, 147.4, 133.7, 121.5, 111.6, 106.9, 79.4, 69.3, 67.2, 65.2, 56.5, 56.2, 54.1, 35.2, 29.1, 18.4, -1.23; IR (ATR, $CHCl_3$) 2956, 1684, 1625, 1604, 1518, 1464, 1434, 1361, 1238, 1058, 1021 cm^{-1}; MS (ES$^+$) *m/z* (relative intensity) 885 ([*M* + 29]$^+$, 70), 857 ([*M* + H]$^+$, 100), 711 (8), 448 (17); HRMS [*M* + H]$^+$ theoretical $C_{41}H_{60}N_4O_{12}Si_2$ *m/z* 857.3819, found (ES$^+$) *m/z* 857.3826.

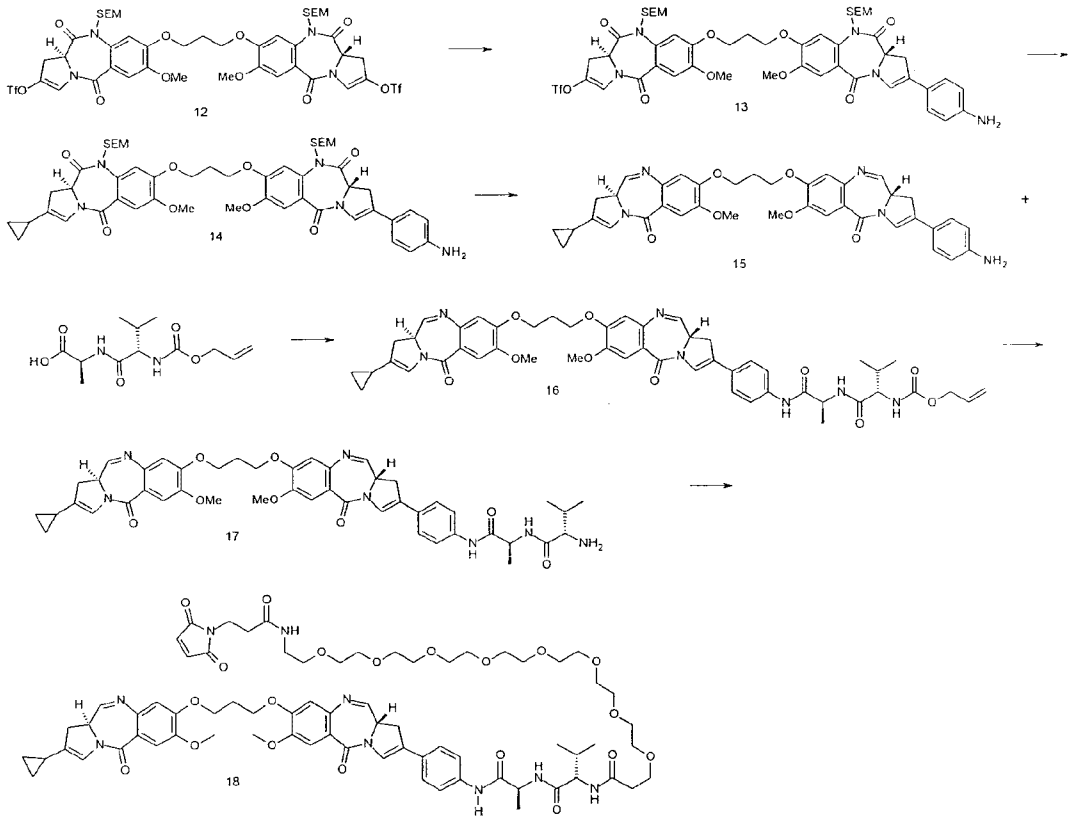
(i) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS)-7-methoxy-2-oxo-10-((2-(trimethylsilyl)ethoxy)methyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] ***(11)***

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 $MgSO_4$, 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); $[\alpha]^{20}_D$ = +291° (*c* = 0.26, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 2H), 7.25 (s, 2H), 5.50 (d, 2H, *J* = 10.1 Hz), 4.75 (d, 2H, *J* = 10.1 Hz), 4.60 (dd, 2H, *J* = 9.85, 3.07 Hz), 4.31–4.18 (m, 6H), 3.89–3.84 (m, 8H), 3.78–3.62 (m, 4H), 3.55 (dd, 2H, *J* = 19.2, 2.85 Hz), 2.76 (dd, 2H, *J* = 19.2, 9.90 Hz), 2.42 (p, 2H, *J* = 5.77 Hz), 0.98–0.91 (m, 4H), 0.00 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.8, 168.8, 165.9, 151.8, 148.0, 133.9, 120.9, 111.6, 107.2, 78.2, 67.3, 65.6, 56.3, 54.9, 52.4, 37.4, 29.0, 18.4, -1.24; IR (ATR, $CHCl_3$) 2957, 1763, 1685, 1644, 1606, 1516, 1457, 1434, 1360, 1247, 1209, 1098, 1066, 1023 cm^{-1}; MS (ES$^+$) *m/z* (relative intensity) 881 ([*M* + 29]$^+$, 38), 853 ([*M* + H]$^+$, 100), 707 (8), 542 (12); HRMS [*M* + H]$^+$ theoretical $C_{41}H_{56}N_4O_{12}Si_2$ *m/z* 853.3506, found (ES$^+$) *m/z* 853.3502.

(j) 1,1'-[[(Propane-1,3-diyl)dioxy]bis(11aS)-7-methoxy-2-[[(trifluoromethyl)sulfonyl]oxy]-10-((2-(trimethylsilyl)ethoxy)methyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5,11-dione] ***(12)***

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/acetonitrile) under a nitrogen atmosphere. Anhydrous triflic anhydride, taken from a freshly opened ampoule (7.2 mL, 12.08 g, 42.8 mmol), was injected rapidly dropwise, while maintaining the temperature at -40°C or below. The reaction mixture was allowed to stir at -45°C for 1 hour at which point TLC (50/50 v/v *n*-hexane/EtOAc) revealed the complete consumption of starting material. The cold reaction mixture was immediately diluted with DCM (200 mL) and, with vigorous shaking, washed with water (1 x 100 mL), 5% citric acid solution (1 x 200 mL) saturated $NaHCO_3$ (200 mL), brine (100 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent 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 ($[M + Na]^{+\cdot}$, 20); $[\alpha]^{24}_D$ = +271° (*c* = 0.18, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 2H), 7.26 (s, 2H), 7.14 (t, 2H, *J* = 1.97 Hz), 5.51 (d, 2H, *J* = 10.1 Hz), 4.76 (d, 2H, *J* = 10.1 Hz), 4.62 (dd, 2H, *J* = 11.0, 3.69 Hz), 4.32–4.23 (m, 4H), 3.94–3.90 (m, 8H), 3.81–3.64 (m, 4H), 3.16 (ddd, 2H, *J* = 16.3, 11.0, 2.36 Hz), 2.43 (p, 2H, *J* = 5.85 Hz), 1.23–0.92 (m, 4H), 0.02 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 162.7, 151.9, 148.0, 138.4, 133.6, 120.2, 118.8, 111.9, 107.4, 78.6, 67.5, 65.6, 56.7, 56.3, 30.8, 29.0, 18.4, -1.25; IR (ATR, $CHCl_3$) 2958, 1690, 1646, 1605, 1517, 1456, 1428, 1360, 1327, 1207, 1136, 1096, 1060, 1022, 938, 913 cm^{-1}; MS (ES^+) *m/z* (relative intensity) 1144 ($[M + 28]^{+\cdot}$, 100), 1117 ($[M + H]^{+\cdot}$, 48), 1041 (40), 578 (8); HRMS [*M* + H]$^{+\cdot}$ theoretical $C_{43}H_{54}N_4O_{16}Si_2S_2F_6$ *m/z* 1117.2491, found (ES^+) *m/z* 1117.2465.

Example 1



*(a) (S)-8-(3-(((S)-2-(4-aminophenyl)-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 (**13**)*

$Pd(PPh_3)_4$ (116.9 mg, 0.101 mmol) was added to a stirred mixture of the bis-enol triflate **12** (5.65 g, 5.06 mmol), 4-Aminophenylboronic acid pinacol ester (1 g, 4.56 mmol), Na_2CO_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°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 (150 mL) and washed with H_2O (2 x 100 mL), brine (150 mL), dried ($MgSO_4$), 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%). LC/MS 4.02 min (ES+) *m/z* (relative intensity) 1060.21 ($[M + H]^+$, 100); ^{1}H–NMR: ($CDCl_3$, 400 MHz) δ 7.40 (s, 1H), 7.33 (s, 1H), 7.27 (bs, 3H), 7.24 (d, 2H, J = 8.5 Hz), 7.15 (t, 1H, J = 2.0 Hz), 6.66 (d, 2H, J = 8.5 Hz), 5.52 (d, 2H, J = 10.0 Hz), 4.77 (d, 1H, J = 10.0 Hz),

4.76 (d, 1H, *J* = 10.0 Hz), 4.62 (dd, 1H, *J* = 3.7, 11.0 Hz), 4.58 (dd, 1H, *J* = 3.4, 10.6 Hz), 4.29 (t, 4H, *J* = 5.6 Hz), 4.00-3.85 (m, 8H), 3.80 – 3.60 (m, 4H), 3.16 (ddd, 1H, *J* = 2.4, 11.0, 16.3 Hz), 3.11 (ddd, 1H, *J* = 2.2, 10.5, 16.1 Hz), 2.43 (p, 2H, *J* = 5.9 Hz), 1.1-0.9 (m, 4H), 0.2 (s, 18H). ^{13}C-NMR: ($CDCl_3$, 100 MHz) δ 169.8, 168.3, 164.0, 162.7, 153.3, 152.6, 149.28, 149.0, 147.6, 139.6, 134.8, 134.5, 127.9, 127.5, 125.1, 123.21, 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-yl)oxy)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 (2 x 200 mL) and brine (2 x 200 mL). The organic layer was dried with $MgSO_4$, 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 orangey/yellow solid (0.66 g, 63%). Method 1, LC/MS (3.85 min (ES^+) *m/z* (relative intensity) 952.17 ($[M + H]^+$, 100). ^{1}H NMR (400 MHz, $CDCl_3$) 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-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-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 drop wise 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 CH_2Cl_2/MeOH (90/10 v/v) (2 x 50 mL). The combined organic layers were dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was dissolved in MeOH (48 mL), CH_2Cl_2 (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 CH_2Cl_2/MeOH (9:1) (~ 200 mL) until product ceased to be eluted. The organic layer was washed with brine (2 x 70 mL), dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography (100% $CHCl_3$ to 96/4 v/v $CHCl_3$/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, $CDCl_3$) 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-2-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 CH_2Cl_2/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% $CHCl_3$ to 90/10 v/v $CHCl_3$/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**)*

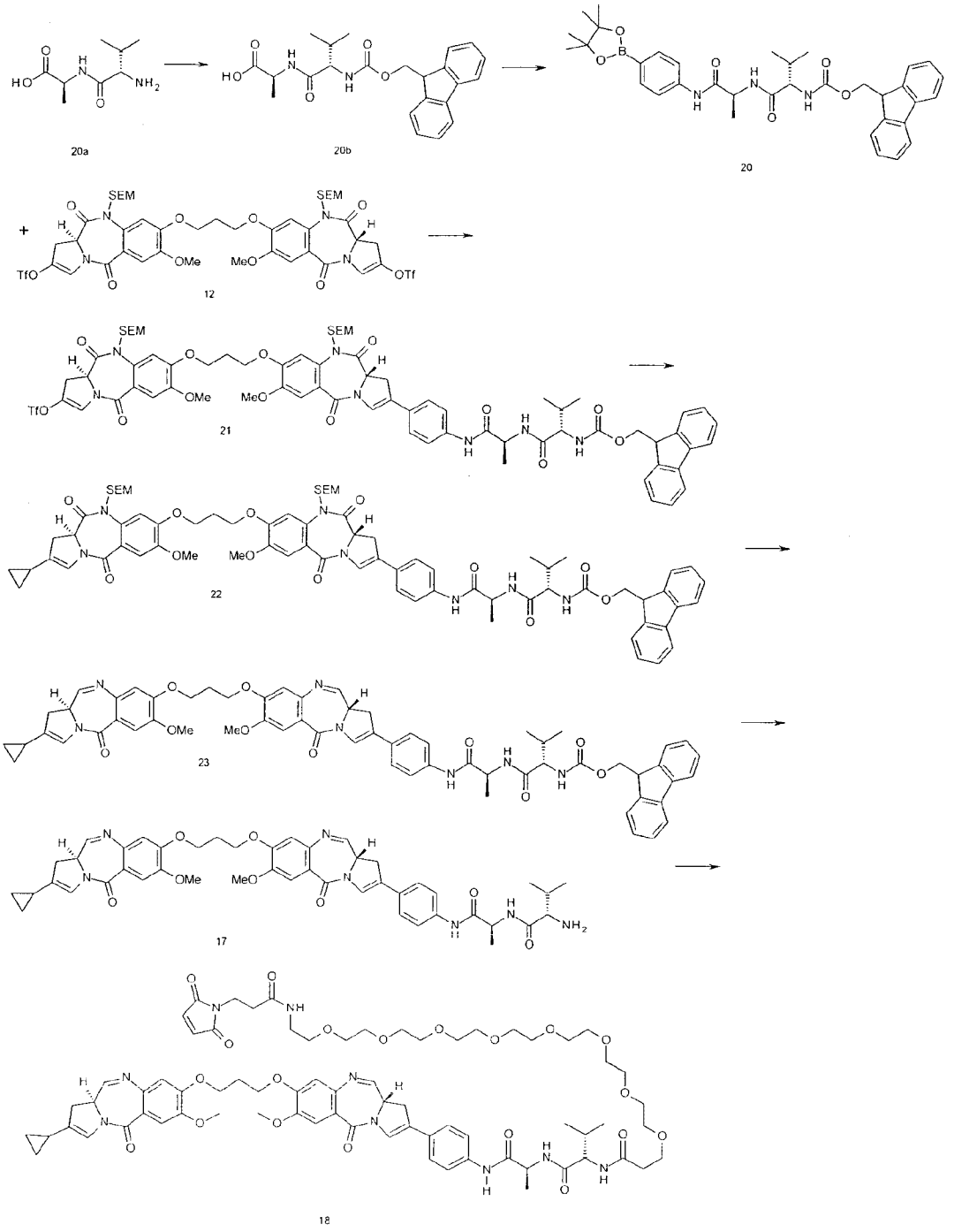
The starting material **16** (170 mg, 0.185 mmol) was dissolved in dry CH_2Cl_2 (5 mL) in a round bottom flask filled with argon, before pyrrolidine (41 µL, 0.21 mmol) was added. The flask was purged/refilled three times with argon before $Pd(PPh_3)_4$ (14 mg, 0.084 mmol) was added and the flushing operation repeated. After 1 hour, complete consumption of starting material was observed (the reaction was followed by LC/MS) and Et_2O (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_2O (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)).

*(f) N-((R)-1-(((S)-1-((4-((S)-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-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)-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_2Cl_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, monitered by LC/MS). The reaction solution was diluted with CH_2Cl_2 (50 mL) and the organics were washed with H_2O (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_2O/CH_3CN) (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]$^{+\cdot}$, 20), 702.63 (100)). ^{1}H NMR (400 MHz, $CDCl_3$) 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 2



*(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 Na_2CO_3 (493 mg, 4.65 mmol) were dissolved in distilled H_2O (15 mL) and the mixture was cooled to 0°C before dioxane (15 mL) was added (partial precipitation of the amino acid salt occurred). A solution of Fmoc-Cl (504 mg, 1.95 mmol) in dioxane (15 mL) was added dropwise with vigorous stirring over 10 minutes. The resulting mixture was stirred at 0°C for 2 hours before the ice bath was removed and stirring was maintained for 16 hours. The solvent was removed by rotary evaporation under reduced pressure and the residue dissolved in water (150 mL). The pH was adjusted from 9 to 2 with 1N HCl and the aqueous layer was subsequently extracted with EtOAc (3x100 mL). The combined organics were washed with brine (100 mL), dried with $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure to afford pure HO-Ala-Val-Fmoc **20b** (746 mg, 97% yield). LC/MS 2.85 min (ES+) *m/z* (relative intensity) 410.60 ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J*=7.77 Hz, 2H), 7.60(d, *J*=7.77 Hz, 2H), 7.43(d, *J*=7.5 Hz, 2H), 7.34 (d, *J*=7.5 Hz, 2H), 6.30 (bs, 1H), 5.30 (bs, 1H), 4.71-7.56 (m, 1H), 4.54-4.36 (m, 2H), 4.08-3.91 (m, 1H), 2.21-2.07 (m, 1H), 1.50 (d, *J*=7.1 Hz, 3H), 1.06-0.90 (m, 6H).

*(b) (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-oxo-1-(((S)-1-oxo-1-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)amino)propan-2-yl)amino)butan-2-yl)carbamate (**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 CH_2Cl_2 and the organics were washed with H_2O and brine before being dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dryloaded on a silicagel chromatography column (Hexane/EtOAc, 6:4) and pure product **20** was isolated as a white solid in 88% yield (360 mg).

*(c) 8-(3-((2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl trifluoromethanesulfonate (**21**)*

Bis-triflate **12** (2.03g, 1.81 mmol), boronic pinacol ester (1g, 1.63 mmol) and Na_2CO_3 (881 mg, 8.31 mmol) were dissolved in a mixture of toluene/MeOH/H_2O, 2:1:1 (40 mL). The

reaction flask was purged and filled with argon three times before *tetrakis*(triphenylphosphine)palladium(0) (41 mg, 0.035 mmol) was added and the reaction mixture heated to 30°C overnight. The solvents were removed under reduced pressure and the residue was taken up in H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organics were washed with brine (100 mL), dried with $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel chromatography column (Hexane/EtOAc, 8:2 to 25:75) to afford pure **21** in 33% yield (885 mg). LC/MS 3.85 min (ES+) *m/z* (relative intensity) 1452.90 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.16 (m, 17H), 7.13 (s, 1H), 6.51 – 6.24 (m, 1H), 5.51 (dd, *J* = 10.0, 5.1 Hz, 2H), 5.36 – 5.11 (m, 1H), 4.74 (dd, *J* = 10.1, 4.4 Hz, 2H), 4.70 – 4.53 (m, 2H), 4.47 (d, *J* = 6.4 Hz, 1H), 4.37 (d, *J* = 7.2 Hz, 1H), 4.27 (m, 4H), 4.20 – 4.14 (m, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.77 (ddd, *J* = 16.7, 9.0, 6.4 Hz, 3H), 3.71 – 3.61 (m, 2H), 3.24 – 2.91 (m, 3H), 2.55 – 2.33 (m, 2H), 2.22 – 2.07 (m, 1H), 1.52 – 1.37 (m, 3H), 1.04 – 0.86 (m, 10H), 0.00 (s, 18H).

*(d) (9H-fluoren-9-yl)methyl((2S)-1-(((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**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 *bis*(benzonitrile)palladium(II) chloride (13.2 mg, 0.034 mmol) was added. The reaction was flushed with Argon 3 more times before being warmed to 75°C and stirred for 10 minutes. The reaction mixture was filtered through a pad of celite which was subsequently rinsed with ethyl acetate. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 1 % methanol/chloroform). Pure fractions were collected and combined, and excess eluent was removed by rotary evaporation under reduced pressure to afford the desired product **22** (132 mg, 50 % yield). LC/MS 3.83 min (ES+) *m/z* (relative intensity) 1345.91 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.14 (m, 17H), 6.69 (s, 1H), 6.45 – 6.25 (m, 1H), 5.57 – 5.41 (m, 2H), 5.34 – 5.14 (m, 1H), 4.78 – 4.67 (m, 2H), 4.62 – 4.55 (m, 1H), 4.50 – 4.45 (m, 2H), 4.51 – 4.44 (m, 1H), 4.31 – 4.21 (m, 4H), 4.16 (m, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 – 3.71 (m, 2H), 3.66 (m, 3H), 3.40 – 3.28 (m, 1H), 3.07 (m, 1H), 2.70 – 2.57 (m, 1H), 2.47 – 2.36 (m, 2H), 2.15

(m, 1H), 1.51 – 1.40 (m, 3H), 1.03 – 0.87 (m, 11H), 0.77 – 0.71 (m, 2H), 0.60 – 0.54 (m, 2H), 0.00 (t, *J* = 3.0 Hz, 18H).

*(e) (9H-fluoren-9-yl)methyl((2S)-1-(((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**23**)*

A solution of Super-Hydride® (0.5 mL, 1M in THF) was added dropwise to a solution of SEM dilactam **22** (265 mg g, 0.19 mmol) in THF (10 mL) at -78°C under an argon atmosphere. The addition was completed over 5 minutes in order to maintain the internal temperature of the reaction mixture constant. After 20 minutes, an aliquot was quenched with water for LC/MS analysis, which revealed that the reaction was complete. Water (20 mL) was added to the reaction mixture and the cold bath was removed. The organic layer was extracted with EtOAc (3 x 30 mL) and the combined organics were washed with brine (50 mL), dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was dissolved in MeOH (12 mL), CH_2Cl_2 (6 mL), water (2 mL) and enough silica gel to form a thick stirring suspension. After 5 days, the suspension was filtered through a sintered funnel and washed with CH_2Cl_2/MeOH (9:1) (200 mL) until the elution of the product was complete. The organic layer was washed with brine (2 x 70 mL), dried with $MgSO_4$, filtered and the solvent removed by rotary evaporation under reduced pressure. Purification by silica gel column chromatography (100% $CHCl_3$ to 96% $CHCl_3$/ 4% MeOH) afforded the product **23** as a yellow solid (162 mg, 78%). LC/MS 3.02 min (ES+) *m/z* (relative intensity) 1052.37.

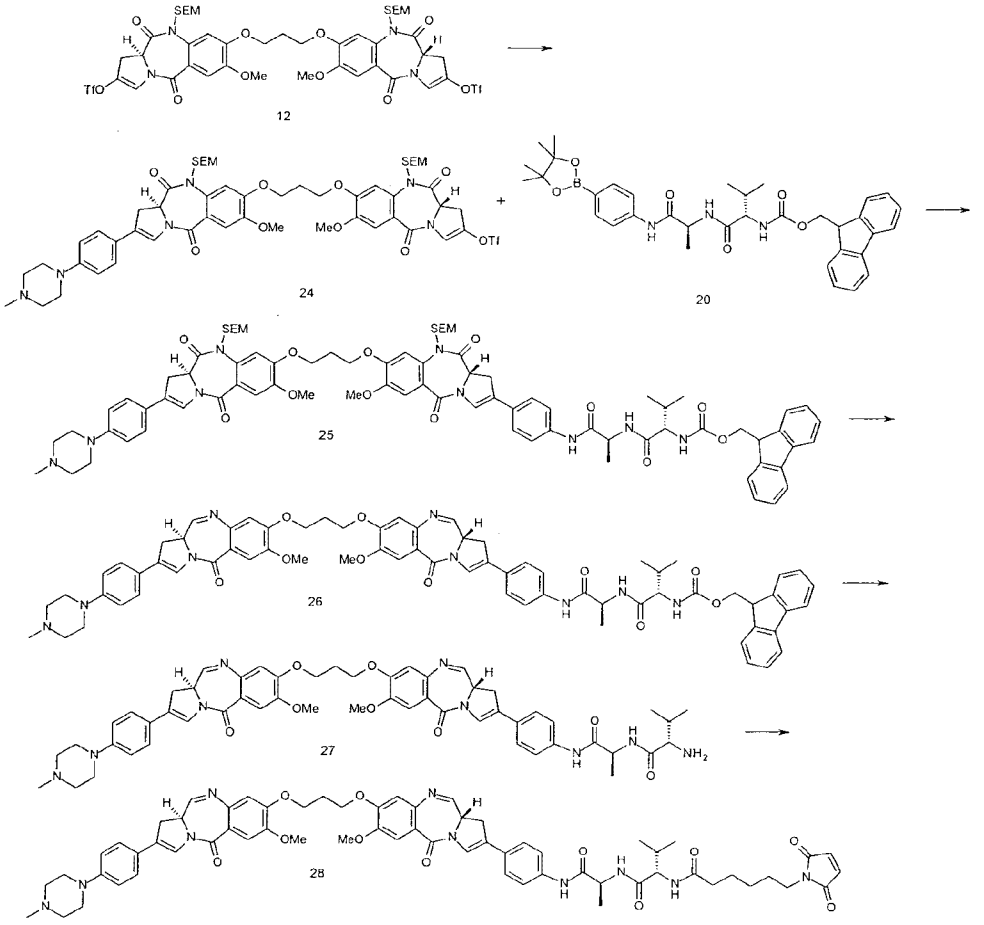
*(f) (2S)-2-amino-N-((2S)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (**17**)*

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 CH_2Cl_2 (75 mL) and the organic phase was washed with H_2O (3x75 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 **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-5,11a-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)-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-PEG_8-acid (43.4 mg, 0.0732 mmol) in dry CH_2Cl_2 (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 CH_2Cl_2 and the organic phase was washed with H_2O and brine before being dried over $MgSO_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure 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_8-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, $CDCl_3$) 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)-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)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl trifluoromethanesulfonate (**24**)*

$Pd(PPh_3)_4$ (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), Na_2CO_3 (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 H_2O (2 x 50 mL), brine (50 mL), dried ($MgSO_4$), 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]$^{+\cdot}$, 20%).

*(b) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-7-methoxy-8-(3-(((S)-7-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-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 (**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_2O, 2:1:1 (3 mL). The microwave vessel was purged and filled with argon three times before *tetrakis*(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_2Cl_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]$^{+\cdot}$, 100%).

*(c) (9H-fluoren-9-yl)methyl ((**S**)-1-(((**S**)-1-((4-((**S**)-7-methoxy-8-(3-(((**S**)-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)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_2Cl_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_2Cl_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]$^{+\cdot}$, 5%).

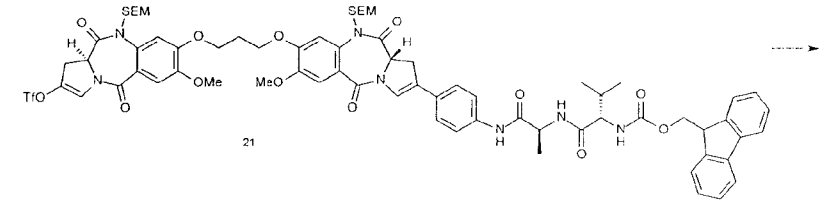
*(d) (**S**)-2-amino-N-((**S**)-1-((4-((**R**)-7-methoxy-8-(3-(((**R**)-7-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (**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_2Cl_2 (50 mL) and the organic phase was washed with H_2O (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]$^{+\cdot}$, 5%).

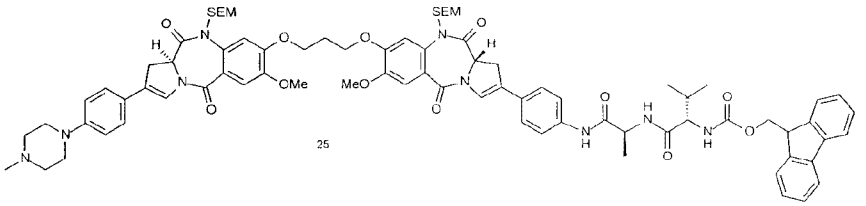
*(e) 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((**S**)-1-(((**S**)-1-((4-((**S**)-7-methoxy-8-(3-(((S)-7-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)hexanamide (**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_2Cl_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_2Cl_2 and the organic phase was washed with H_2O and brine before being dried over $MgSO_4$, filtered and excess solvent removed by rotary evaporation under reduced pressure 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_8-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]$^{+\cdot}$, 5%)

Example 4 – alternative synthesis of compound 25



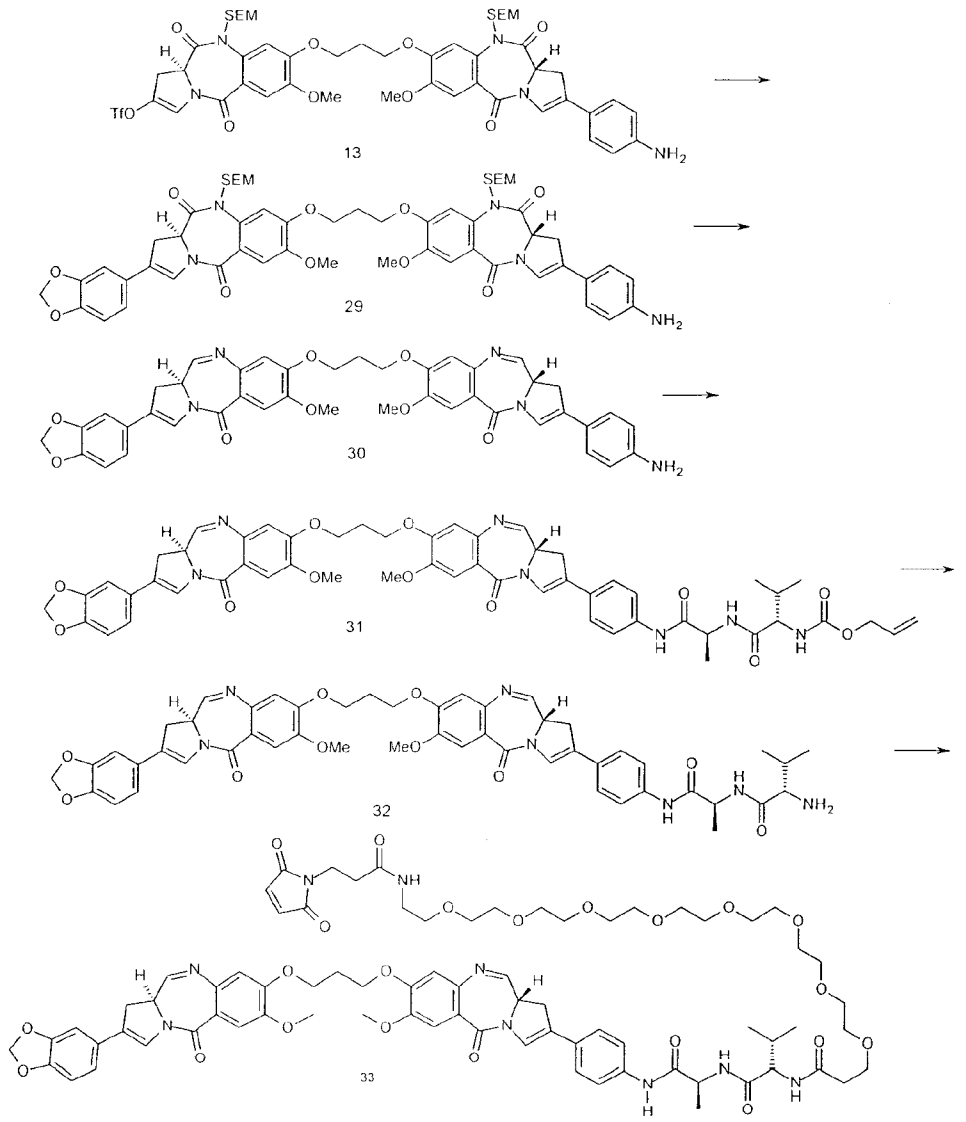
21



25

PBD-triflate **21** (469 mg, 0.323 mmol), boronic pinacol ester (146.5 mg, 0.484 mmol) and Na_2CO_3 (157 mg, 1.48 mmol) were dissolved in a mixture of toluene/MeOH/H_2O, 2:1:1 (10 mL). The reaction flask was purged with argon three times before *tetrakis*(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 H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organics were washed with brine (100 mL), dried with $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$ 100% to $CHCl_3$/MeOH 95%:5%) to afford pure **25** in 33% yield (885 mg). LC/MS 3.27 min (ES+) *m/z* (relative intensity) 1478 ($[M + H]^{+\cdot}$, 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-10-((2-(trimethylsilyl)ethoxy)methyl)-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 $MgSO_4$, 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(11aH)-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 drop wise 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 $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was dissolved in EtOH (15 mL), CH_2Cl_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 CH_2Cl_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 $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography ($CHCl_3$ 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-(((S)-1-((4-((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)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 CH_2Cl_2 (21 mL) and methanol (1 mL) for 1 hour. The PBD **30** (407 mg, 0.55 mmol, 1 equiv.) was dissolved in anhydrous CH_2Cl_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 (CH_2Cl_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 ($[M + H]^{+\cdot}$, 60).

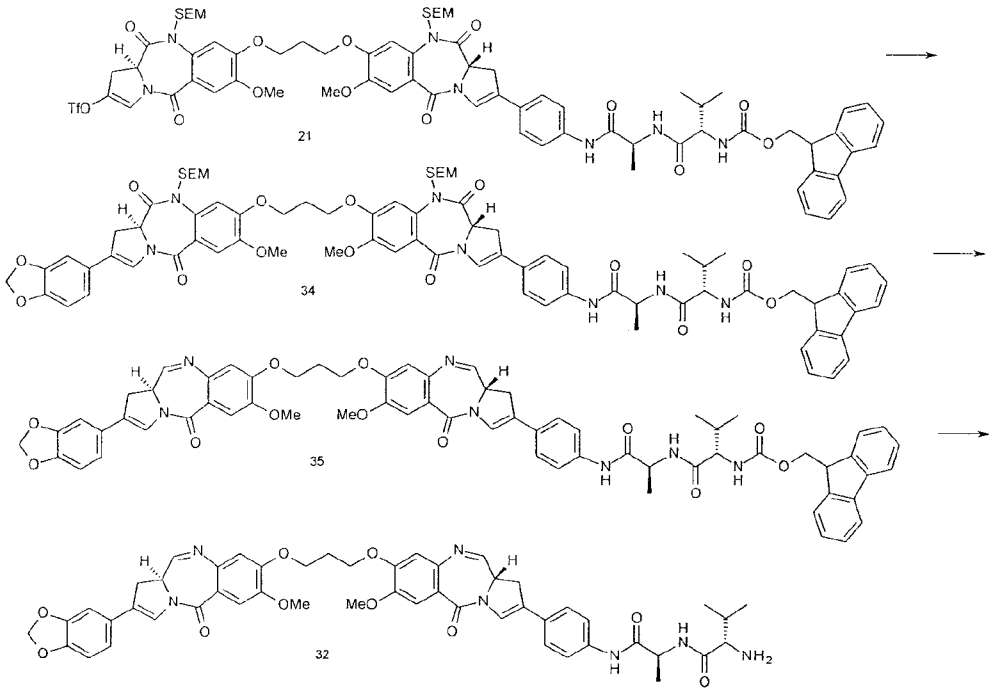
*(d) (S)-2-Amino-N-((S)-1-((4-((S)-8-(3-(((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11a-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-2-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (**32**)*

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. $Pd(PPh_3)_4$ (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. Et_2O (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 Et_2O (2 x 20 mL). The collection flask was replaced and the isolated solid was dissolved and washed through the sinter with $CHCl_3$ (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 ($[M + H]^{+\cdot}$, 67).

*(e) N-((S)-1-(((S)-1-((4-((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)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 (**33**)*

The imine **32** (92 mg, 0.1 mmol, 1.1 equiv.) was dissolved in $CHCl_3$ (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** (81mg). 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-((4-((S)-8-(3-(((S)-2-(benzo[d][1,3]dioxol-5-yl)-7-methoxy-5,11-dioxo-10-((2-(trimethylsilyl)ethoxy)methyl)-5,10,11,11a-tetrahydro-1H-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 **21** (0.5 g, 0.35 mmol, 1 equiv.), 3, 4-(methylenedioxy)phenyl boronic acid (75 mg, 0.45 mmol, 1.3 equiv.) and Na_2CO_3 (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. $Pd(PPh_3)_4$ (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 $MgSO_4$, 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-(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)amino)-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 $MgSO_4$, filtered and the solvent removed *in vacuo*. The crude product was dissolved in EtOH (13.2 mL), CH_2Cl_2 (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 CH_2Cl_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 $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography ($CHCl_3$ 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).

*(c) (S)-2-Amino-N-((S)-1-((4-((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)-3-methylbutanamide (**32**)*

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 CH_2Cl_2 (50 mL), washed with water (50 mL x 4), dried with $MgSO_4$, 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 1.7µm 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.

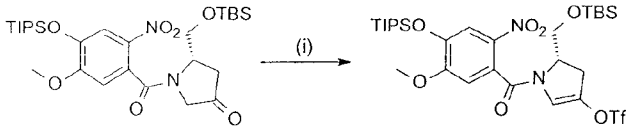
Gradient:

	B
t=0	13%
t=15.00	95%
t=17.00	95%
t=17.10	13%
t=20.00	13%

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

Example 7

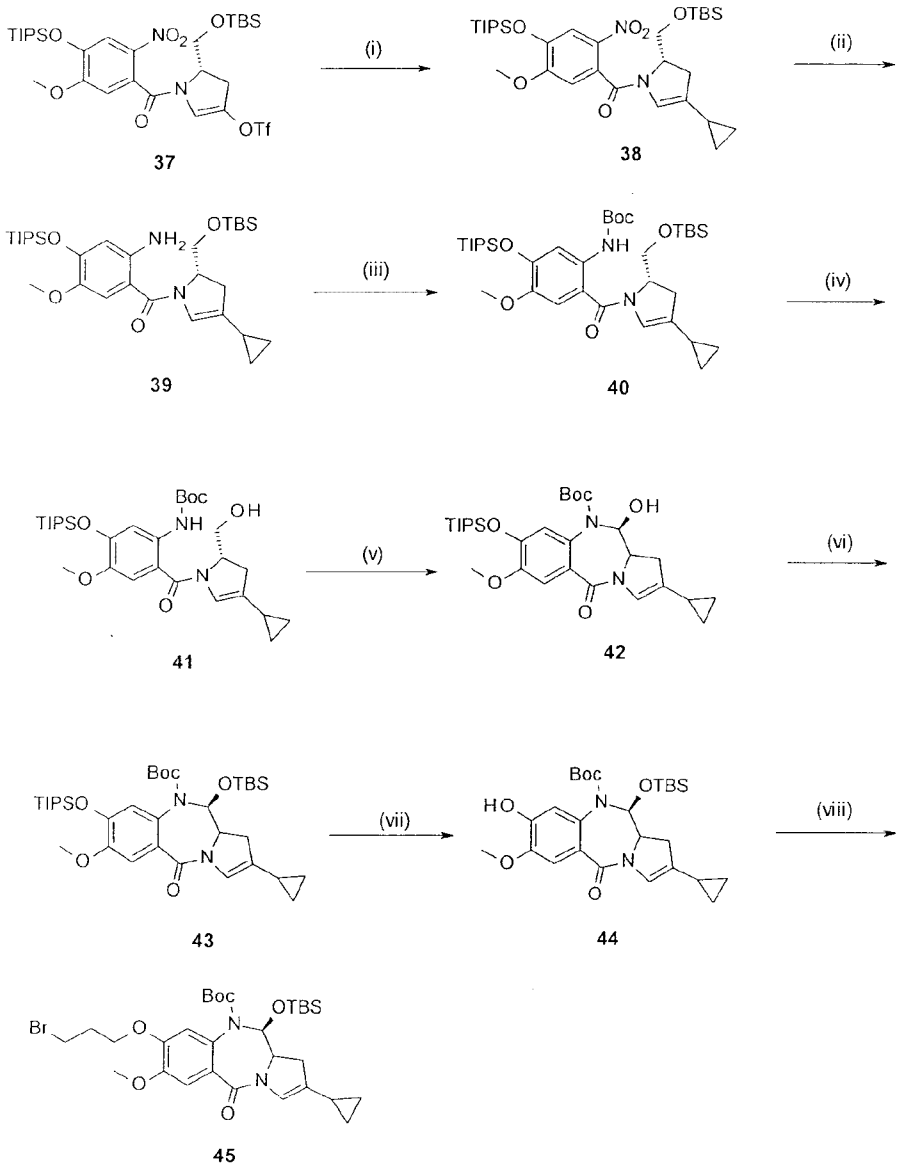
(a) (*S*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((tri*iso*propylsilyl)oxy)benzoyl)-4,5-dihydro-1*H*-pyrrol-3-yl trifluoromethanesulfonate **(37)**



36 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 CH_2Cl_2 (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 CH_2Cl_2 (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 $NaHCO_3$ (300 mL), brine (200 mL). The organics were dried over $MgSO_4$, 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%). ^{1}H-NMR (400 MHz, $CDCl_3$) 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 (11S)-8-(3-bromopropoxy)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(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 $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel chromatography (Hexane/EtOAc ; 100% to 80:20) afforded product **38** as a yellow solid (0.663 g, 78%). ^{1}H-NMR (400 MHz, $CDCl_3$) 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-((triisopropylsilyl)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 $NaHCO_{3(aq)}$ (100 mL), H_2O (100 mL) and brine (100 mL), before being dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was purified silica gel chromatography (Hexane/EtOAc ; 100% to 80:20) and pure product **39** was isolated as a pale colourless oil (1.35 g, 47% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 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 $[M + H]^+$.

*(iii) tert-butyl (S)-(2-(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-cyclopropyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (**40**)*

Amine **39** (770 mg, 1.34 mmol) and Boc_2O (350 mg, 1.6 mmol) were heated together at 70°C in a round bottom flask. To help with solubility, $CHCl_3$ (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, $CDCl_3$) δ 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_2O/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_2O (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 (Hex/EtOAc ; 100% to 60:40) and pure product **41** was isolated as a colourless foam (521 mg, 84% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.65 (s, 1H), 6.75 (s, 1H), 6.17 (s, 1H), 4.71 (s, 1H), 4.60 (s, 1H), 3.82 (t, *J* = 8.7 Hz, 1H), 3.76 (s, 3H), 3.72 (d, *J* = 8.7 Hz, 1H), 2.76 (ddd, *J* = 16.4, 10.2, 1.6 Hz, 1H), 2.08 (dd, *J* = 16.3, 4.4 Hz, 1H), 1.48 (s, 9H), 1.42 – 1.33 (m, 1H), 1.33 – 1.23 (m, 3H), 1.11 (d, *J* = 7.3 Hz, 18H), 0.67 (dd, *J* = 5.0, 3.1 Hz, 2H), 0.46 – 0.39 (m, 2H) ; ES^+ = 2.25 min, *m/z* 561.45 $[M+H]^+$.

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

DMSO (163 µL, 2.29 mmol) was added to a cooled solution of oxallyl chloride (93 µL, 1.1 mmol) in CH_2Cl_2 (2 mL) at -78°C. After 15 minutes, a solution of alcohol **41** (515 mg, 0.91 mmol) in CH_2Cl_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 µL, 4.59 mmol) was added and the mixture allowed to warm to room temperature. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and the solution was washed with 0.1M $HCl_{(aq.)}$ (50 mL), H_2O (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, $CDCl_3$) δ 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* =

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-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate ***(43)***

Alcohol **42** (350 mg, 0.62 mmol) was solubilised in dry CH_2Cl_2 (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 CH_2Cl_2 (50 mL), washed with saturated $NH_4Cl_{(aq.)}$ (50 mL), H_2O (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 80:20) to provide pure **43** as a colourless oil (397.3 mg, 94%). ^{1}H-NMR (400 MHz, $CDCl_3$) 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 $[M+Na]^+$.

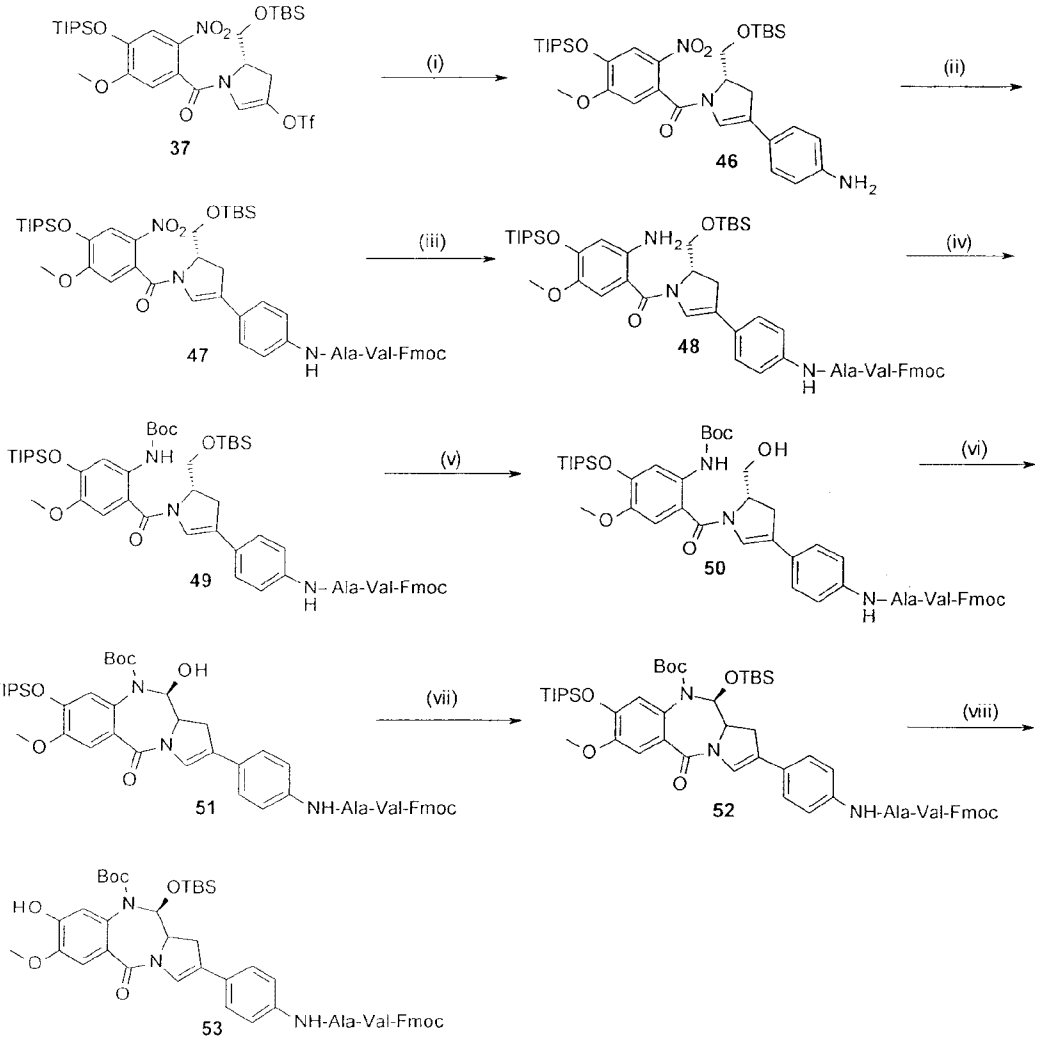
(vii) tert-butyl (11S)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-8-hydroxy-7-methoxy-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate ***(44)***

Monomer **43** (518.8 mg, 0.77 mmol) was solubilised in wet DMF (5 mL +0.1 mL H_2O) 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 H_2O (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude product was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 60:40) and pure product **44** was isolated as a white solid (351 mg, 88% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 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), 0.86 (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-11,11a-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). K_2CO_3 (123 mg, 0.58 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 H_2O (75 mL) and brine (50 mL) before being dried over $MgSO_4$, 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). ^{1}H-NMR (400 MHz, $CDCl_3$) 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 (11*S*)-2-(4-((*S*)-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((*tert*-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-5-oxo-11,11*a*-dihydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate **(53)**



*(i) (S)-(4-(4-aminophenyl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone **(46)***

$Pd(PPh_3)_4$ (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_2CO_3 (12.78 g, 120 mmol), MeOH (80 mL), toluene (160 mL) and water (80 mL). The reaction mixture was allowed to stir at 30°C under a nitrogen atmosphere for 24 hours after which time all the boronic ester has consumed. The reaction mixture was then evaporated to dryness before

the residue was taken up in EtOAc (100 mL) and washed with H_2O (100 mL), brine (100 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure to provide the crude product. Purification by silica gel chromatography (Hexane/EtOAc ; 100% to 70:30) afforded product **46** as a yellowish foam (11.06 g, 64%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.81 (s, 1H), 6.58 (d, *J* = 8.3 Hz, 2H), 6.06 (s, 1H), 4.77 (bm, 1H), 3.91 (d, *J* = 6.7 Hz, 3H), 3.68 (bs, 2H), 3.13 (bm, 1H), 2.97 (d, *J* = 14.5 Hz, 1H), 1.36 – 1.21 (m, 3H), 1.12 (d, *J* = 7.3 Hz, 18H), 0.89 (s, 10H), 0.10 (s, 6H).) ; ES^+ = 2.27 min, *m/z* 698 [M + CH_3CN]$^+$.

(ii) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate ***(47)***

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_2Cl_2 (500 mL). The flask was then purged three times with argon before EEDQ (3.79 g, 15.3 mmol) was added and the mixture left to stir at room temperature. The reaction was followed by LCMS and after 3.5 hours the reaction was complete. The reaction was quenched with H_2O (200 mL) and extracted twice with CH_2Cl_2 (250 mL). The combined organics were washed with brine (150 mL), dried over $MgSO_4$, filtered and the solvent removed in *vacuo*. The crude product was purified by silica gel chromatography (Hexane/EtOAc ; 100% to 55:45) to afford pure product **47** (13.821 g, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.64 (s + d, *J* = 4.9 Hz, 3H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.36 (d, *J* = 7.3 Hz, 1H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 6.71 (s, 1H), 6.27 (d, *J* = 6.3 Hz, 1H), 6.08 (s, 1H), 5.11 (d, *J* = 6.6 Hz, 1H), 4.69 (bs, 1H), 4.52 (bm, 1H), 4.36 (d, *J* = 6.5 Hz, 2H), 4.08 (t, *J* = 5.9 Hz, 1H), 3.89 (m, 1H), 3.80 (s, 3H), 3.11 – 2.97 (bm, 1H), 2.88 (bd, *J* = 15.2 Hz, 1H), 2.03 (bs, 1H), 1.33 (d, *J* = 6.9 Hz, 3H), 1.24 – 1.11 (m, 3H), 1.01 (d, *J* = 7.4 Hz, 18H), 0.86 – 0.79 (m, 6H), 0.77 (s, 9H), 0.00 (s, 6H) ; ES^+ = 2.37 min, no mass.

(iii) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-amino-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate ***(48)***

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 $NaHCO_{3(aq)}$ (100 mL), H_2O (100 mL) and brine (100 mL), before being dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was purified silica gel chromatography (Hexane/EtOAC 75:25 to 50:50) and pure product **48** was isolated as a pale yellow oil (2.291 g, 79% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.74 (s+d, *J* = 4.9 Hz, 3H), 7.53 (t, *J* = 7.4 Hz, 2H), 7.46 (d, *J* = 11.3 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.28 (t, *J* = 11.3 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H), 6.38 (d, *J* = 6.3 Hz, 1H), 6.18 (s, 1H), 5.21 (d, *J* = 2.9 Hz, 1H), 4.81 (bs, 1H), 4.72 – 4.57 (m, 1H), 4.47 (d, *J* = 6.5 Hz, 2H), 4.19 (t, *J* = 5.0 Hz, 1H), 4.00 – 3.94 (m, 1H), 3.91 (s, 3H), 3.23 – 3.07 (m, 1H), 2.98 (d, *J* = 16.8 Hz, 1H), 2.15 (s, 1H), 1.43 (d, *J* = 6.9 Hz, 3H), 1.36 – 1.18 (m, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 0.97 – 0.89 (m, 6H), 0.88 (s, 9H), 0.10 (s, 6H). ES^+ = 2.37 min, *m/z* no mass.

(iv) (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((S)-1-(2-((tert-butoxycarbonyl)amino)-5-methoxy-4-((triisopropylsilyl)oxy)benzoyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydro-1H-pyrrol-3-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate ***(49)***

Amine **48** (14.913 g, 14.6 mmol) and Boc_2O (3.83 g, 17.5 mmol) were heated together at 70°C in a round bottom flask. To help with solubility, $CHCl_3$ (25 mL) was added and the mixture left to stir until the reaction was complete (followed by LCMS). The thick crude solution was left to cool down to room temperature before being directly loaded on a silica gel chromatography column (Hexane/EtOAc ; 100% to 65:35). Product **49** was isolated as a cream foam (13.2 g, 80% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.21 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 3H), 7.54 (t, *J* = 7.0 Hz, 2H), 7.48 (d, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.31 – 7.25 (m, 3H), 7.14 (d, *J* = 6.7 Hz, 2H), 6.84 (bs, 1H), 6.80 (s, 1H), 6.50 (d, *J* = 6.4 Hz, 1H), 5.28 (d, *J* = 6.0 Hz, 1H), 4.77 (d, *J* = 2.6 Hz, 1H), 4.70 – 4.58 (m, 1H), 4.47 (t, *J* = 5.7 Hz, 2H), 4.19 (t, *J* = 6.1 Hz, 1H), 4.00 (m, 2H), 3.88 (bs, 1H), 3.73 (s, 3H), 3.05 (m, 1H), 2.98 (dd, *J* = 15.4, 3.3 Hz, 1H), 2.15 (bm, 1H), 1.46 (s, 9H), 1.43 (d, *J*=11.7 Hz, 3H), 1.36 – 1.22 (m, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 1.00 – 0.89 (m, 6H), 0.84 (s, 9H), 0.05 (d, *J* = 6.0 Hz, 6H)) ; ES^+ = 2.53 min, no mass.

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

Silyl ether **49** (13.2 g, 11.8 mmol) was solubilised in a 7:2:1:1 mixture of AcOH/H_2O/MeOH/THF (220 mL) and the mixture was stirred at room temperature until the

reaction was complete (left overnight). The volatiles were removed in vacuo and the residue was taken up in EtOAc (400 mL). The organic phase was washed with saturated $NaHCO_{3(aq)}$ (200 mL), H_2O (200 mL) and brine (10 mL) before being dried over $MgSO_4$, filtered and concentrated in *vacuo*. The crude material was purified by silica gel chromatography (Hex/EtOAc ; 50:50 to 0:100) and pure product **50** was isolated as a light yellow foam (11.168 g, 94% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.93 (s, 1H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.64 (s, 1H), 7.52 (dd, *J* = 17.9, 8.9 Hz, 4H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.26 (m, 3H), 7.13 (d, *J* = 7.4 Hz, 2H), 6.81 (s, 1H), 6.45 (s, 1H), 5.26 (s, 1H), 4.84 (s, 1H), 4.69 – 4.58 (m, 1H), 4.47 (d, *J* = 6.2 Hz, 2H), 4.43 (s, 1H), 4.17 (d, *J* = 14.2 Hz, 1H), 3.99 (s, 1H), 3.89 (s, 2H), 3.74 (s, 3H), 3.30 – 3.17 (m, 1H), 2.64 (d, *J* = 16.9 Hz, 1H), 2.23 – 2.09 (m, 1H), 1.44 (s, 9H), 1.44 (d, *J* = 10.9 Hz, 2H), 1.29 (ddd, *J* = 14.3, 13.0, 7.4 Hz, 3H), 1.12 (d, *J* = 7.4 Hz, 18H), 0.92 (m, 6H) ; ES^+ = 2.23 min, no mass

(vi) tert-butyl (11S)-2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-hydroxy-7-methoxy-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate ***(51)***

DMSO (1.55 L, 21.9 mmol) was added to a cooled solution of oxallyl chloride (0.89 mL, 10.5 mmol) in CH_2Cl_2 (50 mL) at -78°C. After 15 minutes, a solution of alcohol **50** (8.8 mg, 8.76 mmol) in CH_2Cl_2 (100 mL) was added dropwise to the oxidising mixture. The reaction was left to stir at -78°C for 1 hour before NEt_3 (6.11 mL, 43.8 mmol) was added and the mixture allowed to warm to room temperature. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and the solution was washed with 0.1M HCl(aq.) (250 mL), H_2O (250 mL), saturated $NaHCO_{3(aq.)}$ (250 mL) and brine (200 mL). The organics were dried with $MgSO_4$, filtered and the volatiles removed in vacuo. The crude material was purified by silica gel chromatography (CH_2Cl_2/EtOAc ; 100% to 50:50) to provide pure **51** as a yellow oil (8.8 mg, 100%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.74 (t, *J* = 8.4 Hz, 3H), 7.52 (d, *J* = 7.4 Hz, 5H), 7.43 – 7.33 (m, 4H), 7.23 – 7.17 (m, 2H), 6.69 (s, 1H), 6.42 (d, *J* = 7.9 Hz, 1H), 5.78 (d, *J* = 7.8 Hz, 1H), 5.62 (s, 1H), 5.23 (d, *J* = 7.7 Hz, 1H), 4.84 – 4.69 (m, 1H), 4.65 (d, *J* = 22.5 Hz, 1H), 4.45 – 4.29 (m, 2H), 3.91 (dd, *J* = 11.3, 8.1 Hz, 1H), 3.86 (s, 3H), 3.28 (q, *J* = 11.9 Hz, 1H), 2.98 (t, *J* = 12.6 Hz, 1H), 2.14 (dd, *J* = 12.9, 10.0 Hz, 1H), 1.52 – 1.42 (m, 3H), 1.38 (s, 9H), 1.26 (m, 3H), 1.16 – 1.05 (m, 18H), 0.93 (d, *J* = 6.0 Hz, 6H) ; ES^+ = 2.19 min, no mass.

(vii) tert-butyl (11S)-2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)phenyl)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-8-

((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate ***(52)***

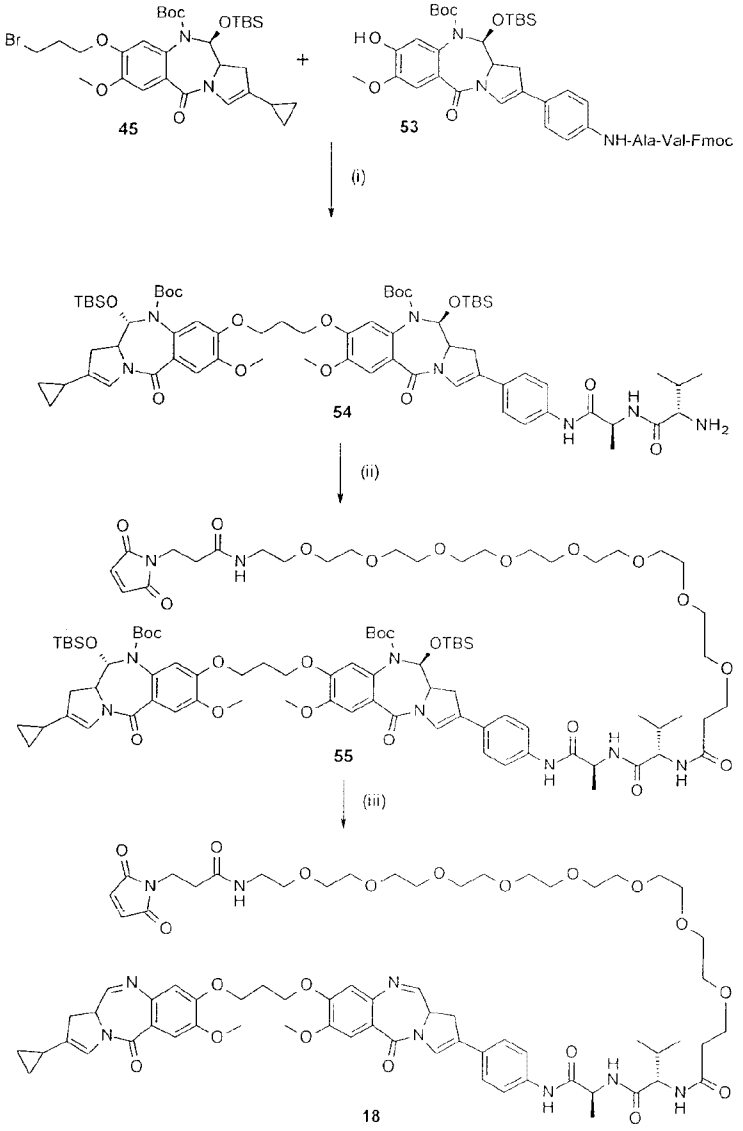
Alcohol **51** (8.8 g, 8.78 mmol) was solubilised in dry CH_2Cl_2 (150 mL) in a sealed round bottom flask previously flushed three times with argon. The solution was cooled to 0°C before lutidine (4 mL, 35.1 mmol) and TBS-OTf (6 mL, 26.3 mmol) were subsequently added. The reaction mixture was left to warm to room temperature and stirred until complete (monitored by LCMS). Upon completion, the solution was diluted with CH_2Cl_2 (100 mL), washed with saturated $NH_4Cl_{(aq.)}$ (150 mL), H_2O (100 mL), saturated $NaHCO_3$(aq.) (100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was purified by silica gel chromatography (Hexane/EtOAc : 100% to 80:20) to provide pure **52** as a colourless oil (6.18 mg, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.55 (dd, *J* = 13.0, 6.7 Hz, 4H), 7.40 (t, *J* = 7.3 Hz, 4H), 7.33 – 7.27 (m, 3H), 7.21 (s, 1H), 6.67 (s, 1H), 6.49 (s, 1H), 5.87 (d, *J* = 8.8 Hz, 1H), 5.30 (d, *J* = 5.7 Hz, 1H), 4.71 – 4.59 (m, 1H), 4.48 (d, *J* = 6.8 Hz, 2H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.04 – 3.96 (m, 1H), 3.86 (s, 3H), 3.84 – 3.77 (m, 1H), 3.25 (m, 1H), 2.79 (d, *J* = 1.5 Hz, 1H), 2.26 – 2.11 (m, 1H), 1.46 (d, *J* = 6.9 Hz, 3H), 1.33 (s, 9H), 1.27 (dd, *J* = 17.1, 9.7 Hz, 3H), 1.11 (dd, *J* = 7.4, 4.0 Hz, 18H), 0.93 (s, 6H), 0.89 (s, 9H), 0.27 (s, 3H), 0.22 (s, 3H); ES^+ = 2.55 min, *m/z* 116.30 $[M+H]^+$.

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

Monomer **52** (1 g, 0.89 mmol) was solubilised in wet DMF (5 mL +0.5 mL H_2O) before LiOAc (91 mg, 0.89 mmol) was added and the mixture left to stir at room temperature until complete (≈3h, followed by LCMS). The mixture was subsequently diluted with EtOAc (50 mL), quenched with citric acid(aq.) (pH=3, 40 mL), then washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude product was purified by silica gel chromatography (Hexane/EtOAc/MeOH ; 60:40:0 to 60:30:10) and pure product **53** was isolated as a cream solid (675 mg, 78% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.55 (dd, *J* = 16.0, 7.5 Hz, 4H), 7.40 (t, *J* = 7.4 Hz, 4H), 7.30 (ddd, *J* = 14.7, 7.4, 1.1 Hz, 3H), 7.24 (s, 1H), 6.72 (s, 1H), 6.38 (d, *J* = 5.3 Hz, 1H), 5.87 (s, 1H), 5.23 (d, *J* = 6.2 Hz, 1H), 4.69 – 4.57 (m, 1H), 4.49 (d, *J* = 6.6 Hz, 2H), 4.20 (t, *J* = 5.3 Hz, 1H), 4.04 – 3.96 (m, 1H), 3.96 (s, 3H), 3.87 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.29 (dd, *J* = 18.0, 8.5 Hz, 1H), 2.80 (d, *J* = 19.4 Hz, 1H), 2.24 – 2.08 (m,

1H), 1.46 (d, *J* = 10.5 Hz, 3H), 1.33 (s, 9H), 1.00 – 0.91 (m, 6H), 0.90 (s, 9H), 0.25 (d, *J* = 8.6 Hz, 6H). ; ES$^+$ = 2.08 min, *m/z* 960.35 $[M+H]^+$.

(d) *N*-((2*S*)-1-(((2*S*)-1-((4-(8-(3-((2-cyclopropyl-7-methoxy-5-oxo-5,11*a*-dihydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-5-oxo-5,11*a*-dihydro-1*H*-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-1*H*-pyrrol-1-yl)propanamido)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide **(18)**



(i) tert-butyl (11S)-2-(4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)phenyl)-8-(3-(((11S)-10-(tert-butoxycarbonyl)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-11,11a-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), K_2CO_3 (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 H_2O (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 ($CHCl_3$/MeOH ; 100% to 98:2) and pure product **54** was isolated as a white solid (280.3 mg, 46% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 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), 3.38 – 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-(3-(((11S)-10-(tert-butoxycarbonyl)-11-((tert-butyldimethylsilyl)oxy)-2-(4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)phenyl)-7-methoxy-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-((tert-butyldimethylsilyl)oxy)-2-cyclopropyl-7-methoxy-5-oxo-11,11a-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 CH_2Cl_2 (6 mL). EDCI hydrochloride (40 mg, 0.021 mmol) and maleimide-PEG_8-OH (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 CH_2Cl_2 (50 mL) and the organic phase was washed with H_2O (50 mL) and brine (50 mL) before being dried over $MgSO_4$, filtered and the volatiles removed by rotary evaporation under reduced pressure. The crude material was purified by silica gel chromatography ($CHCl_3$/MeOH 100% to 97:3) and pure product **55** was isolated as a light yellow foam (318.8 mg, 82% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 8.64 (s, 1H), 7.69 (d,

J = 15.0 Hz, 2H), 7.39 (s, 1H), 7.28 (d, J = 15.0 Hz, 2H), 7.26, (s, 1H), 7.22 (s, 1H), 7.20 (s, 1H),7.03 (d, J = 4.5 Hz, 1H), 6.92 (d, J = 7.5 Hz, 1H), 6.69 (s, 2H), 6.65 (s, 1H), 6.63 (s, 1H), 6.37 (t, J = 4.7 Hz, 1H), 5.89 (d, J = 6.8 Hz, 1H), 5.81 (d, J = 8.4 Hz, 1H), 4.67 (p, J = 7.2 Hz, 1H), 4.27 – 4.12 (m, 5H), 3.88 (s, 3H), 3.87 (s, 3H), 3.84 (t, J = 5.6 Hz, 1H), 3.73 – 3.56 (m, 46H), 3.53 (t, J = 5.0 Hz, 1H), 3.41 (dd, J = 10.3, 5.2 Hz, 1H), 3.35 – 3.22 (m, 1H), 2.90 – 2.74 (m, 1H), 2.67 (ddd, J = 13.6, 9.2, 4.1 Hz, 1H), 2.52 (t, J = 7.2 Hz, 1H), 2.48 – 2.45 (m, 1H), 2.45 – 2.37 (m, 1H), 2.37 – 2.22 (m, 1H), 2.02 (t, J = 9.0 Hz, 1H), 1.45 (d, J = 7.1 Hz, 3H), 1.42 – 1.37 (m, 1H), 1.30 (s, 9H), 0.99 (s, 6H), 0.89 (s, 9H), 0.86 (s, 9H), 0.78 – 0.67 (m, 2H), 0.58 – 0.49 (m, 1H), 0.48 – 0.42 (m, 1H), 0.28 – 0.20 (m, 12H) ; ES^+ = 2.15 min, *m/z* 1891.60 $[M+Na]^+$.

(iii) N-((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)-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)***

Dimer **55** (318 mg, 0.017 mmol) was solubilised in dry H_2O (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 CH_2Cl_2 (50 mL) and the organic phase was washed with ice cold $NaHCO_3$ (2 x 50 mL), H_2O (50 mL) and brine (50 mL) before being dried over $MgSO_4$, filtered and the volatiles removed in *vacuo*. The crude material was directly purified by reverse phase preparative HPLC (H_2O/CH_3CN, see conditions below) and pure product **18** was isolated as a yellow solid (61 mg, 26% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 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% CO_2 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 (10^4 cells per well, 8 wells per sample). Plates were then kept in the dark at 37°C in a humidified atmosphere containing 5% CO_2. The assay is based on the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-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 IC_{50} value was read as the dose required to reduce the final optical density to 50% of the control value.

Compound RelC has an IC_{50} 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

Antibody1 (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, 100μL 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 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 **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

Antibody1 (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 1.0 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 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 **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). 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 **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 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 **ADC1B** at 280 nm shows a monomer purity of 96.6% with 3.4% aggregates.

ADC1C

Antibody1 (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 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 **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 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 **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 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 **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, 100µL at 30 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 **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 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 **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 cytoxicity 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 mm^3 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 mm^3 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 **ADC1B** at 0.3 (grey) or 1.0 mg/kg (purple) compared to vehicle (black) or naked Ig (blue) controls.

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*H*-fluoren-9-ylmethoxycarbonyl
imp	*N*-10 imine protecting group: 3-(2-methoxyethoxy)propanoate-Val-Ala-PAB
MC-OSu	maleimidocaproyl-O-*N*-succinimide
Moc	methoxycarbonyl
MP	maleimidopropanamide
Mtr	4-methoxy-2,3,6-trimethtylbenzenesulfonyl
PAB	para-aminobenzyloxycarbonyl
PEG	ethyleneoxy
PNZ	*p*-nitrobenzyl carbamate
Psec	2-(phenylsulfonyl)ethoxycarbonyl
TBDMS	tert-butyldimethylsilyl
TBDPS	tert-butyldiphenylsilyl
Teoc	2-(trimethylsilyl)ethoxycarbonyl
Tos	tosyl
Troc	2,2,2-trichlorethoxycarbonyl chloride
Trt	trityl
Xan	xanthyl

SEQUENCES

SEQ ID NO. 1 (Her VH):
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
S

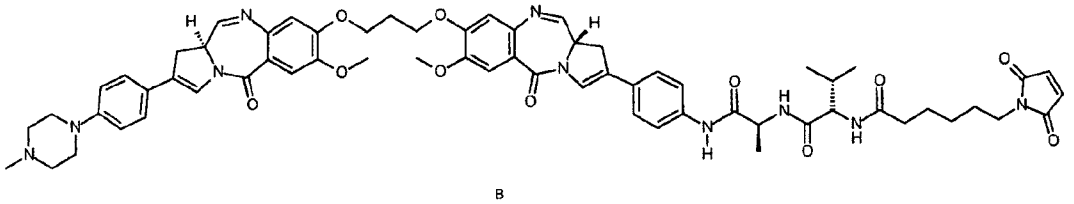
SEQ ID NO. 2 (Her VL):
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

SEQ ID NO. 3 (Simulect VH):
QLQQSGTVLARPGASVKMSCKASGYSFTRYWMHWIKQRPGQGLEWIGAIYPGNSDTSYN
QKFEGKAKLTAVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFDFWGQGTTLTVS

SEQ ID NO. 4 (Simulect VL):
QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQQKPGTSPKRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMEAEDAATYYCHQRSSYTFGGGTKLEIK

Claims

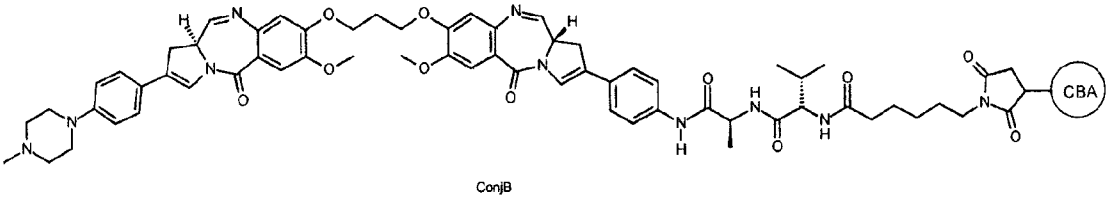
1. A compound which is B:



B

and salts and solvates thereof.

2. A conjugate of formula ConjB:



ConjB

where CBA represents a cell binding agent.

3. The conjugate according to claim 2, wherein the cell binding agent is an antibody or an antigen-binding 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) BMPR1B;

(2) E16;

(3) STEAP1;

(4) 0772P;

(5) MPF;

(6) Napi3b;

(7) Sema 5b;
(8) PSCA hlg;
(9) ETBR;
(10) MSG783;
(11) STEAP2;
(12) TrpM4;
(13) CRIPTO;
(14) CD21;
(15) CD79b;
(16) FcRH2;
(17) HER2;
(18) NCA;
(19) MDP;
(20) IL20R-alpha;
(21) Brevican;
(22) EphB2R;
(23) ASLG659;
(24) PSCA;
(25) GEDA;
(26) BAFF-R;
(27) CD22;
(28) CD79a;
(29) CXCR5;
(30) HLA-DOB;
(31) P2X5;
(32) CD72;
(33) LY64;
(34) FcRH1;
(35) IRTA2;
(36) TENB2;
(37) PSMA – FOLH1;
(38) SST;
(38.1) SSTR2;
(38.2) SSTR5;
(38.3) SSTR1;
(38.4)SSTR3;

(38.5) SSTR4;
(39) ITGAV;
(40) ITGB6;
(41) CEACAM5;
(42) MET;
(43) MUC1;
(44) CA9;
(45) EGFRvIII;
(46) CD33;
(47) CD19;
(48) IL2RA;
(49) AXL;
(50) CD30 - TNFRSF8;
(51) BCMA - TNFRSF17;
(52) CT Ags – CTA;
(53) CD174 (Lewis Y) - FUT3;
(54) CLEC14A;
(55) GRP78 – HSPA5;
(56) CD70;
(57) Stem Cell specific antigens;
(58) ASG-5;
(59) ENPP3;
(60) PRR4;
(61) GCC – GUCY2C;
(62) Liv-1 – SLC39A6;
(63) 5T4;
(64) CD56 – NCMA1;
(65) CanAg;
(66) FOLR1;
(67) GPNMB;
(68) TIM-1 – HAVCR1;
(69) RG-1/Prostate tumor target Mindin – Mindin/RG-1;
(70) B7-H4 – VTCN1;
(71) PTK7;
(72) CD37;
(73) CD138 – SDC1;

(74) CD74;

(75) Claudins – CLs;

(76) EGFR;

(77) Her3;

(78) RON - MST1R;

(79) EPHA2;

(80) CD20 – MS4A1;

(81) Tenascin C – TNC;

(82) FAP;

(83) DKK-1;

(84) CD52;

(85) CS1 - SLAMF7;

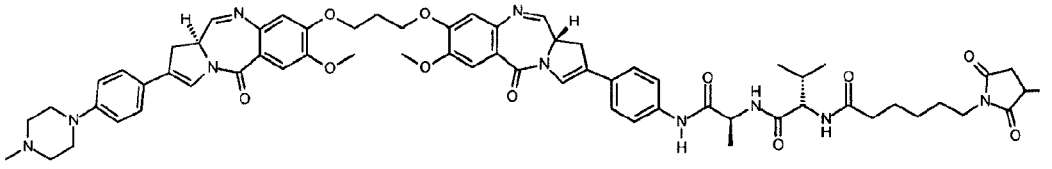
(86) Endoglin – ENG;

(87) Annexin A1 – ANXA1; and

(88) V-CAM (CD106) - VCAM1.

6. The conjugate of any one of claims 3 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 conjugate has a drug loading that is an integer from 1 to about 8, where the drug loading is the average number of drug moieties D per CBA



D

8. The conjugate according to claim 7, wherein the drug loading is 1, 2, 3, or 4.

9. A composition comprising a mixture of conjugates according to claim 7, wherein the average drug loading of the conjugates is from 1 to about 8.

10. 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.

11. The pharmaceutical composition of claim 10 further comprising a therapeutically effective amount of a chemotherapeutic agent.

12. 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.

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

14. The conjugate or composition according to claim 13, wherein the cancer is chronic myeloid leukemia.

15. Use of the conjugate according to any one of claims 2 to 8 or the composition according to claim 9 for the treatment of cancer in a subject.

16. The use according to claim 15, wherein the cancer is chronic myeloid leukemia.

17. The use according to claim 15 or 16, which comprises the use of a chemotherapeutic agent in combination with the conjugate or composition.

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 B as defined in claim 1.

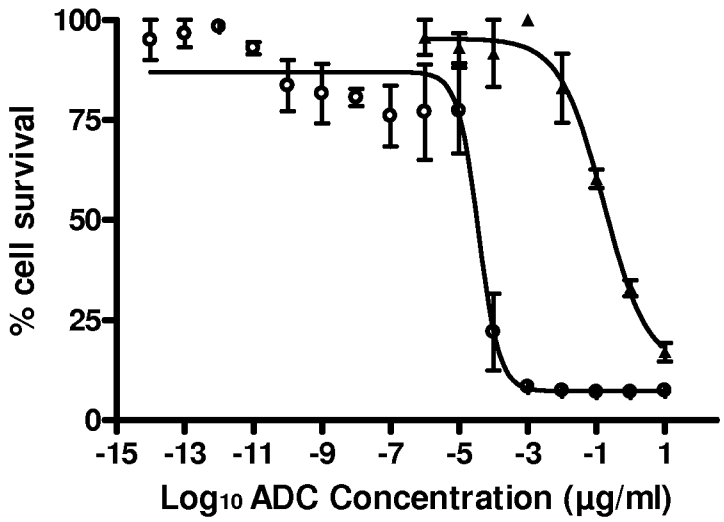


Figure 1

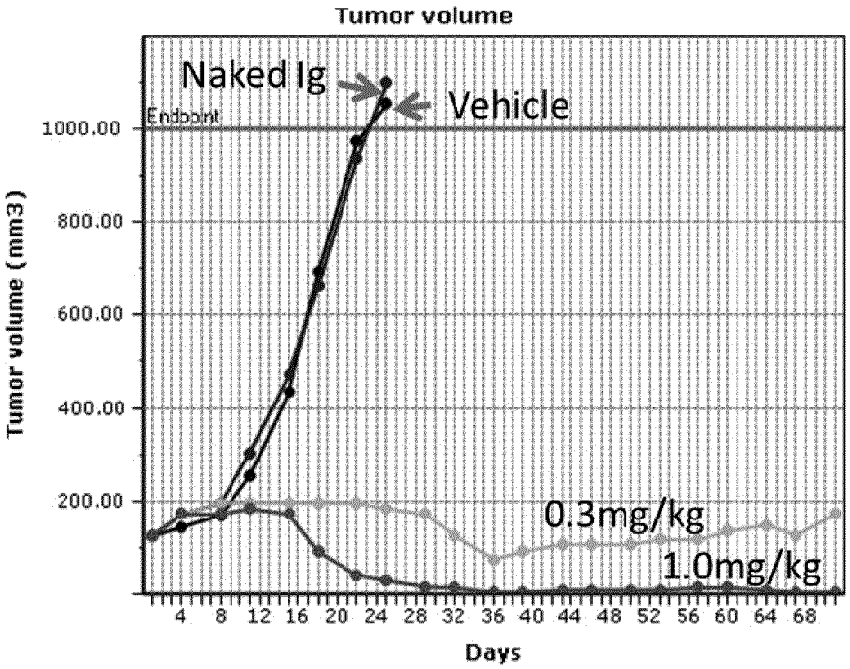


Figure 2

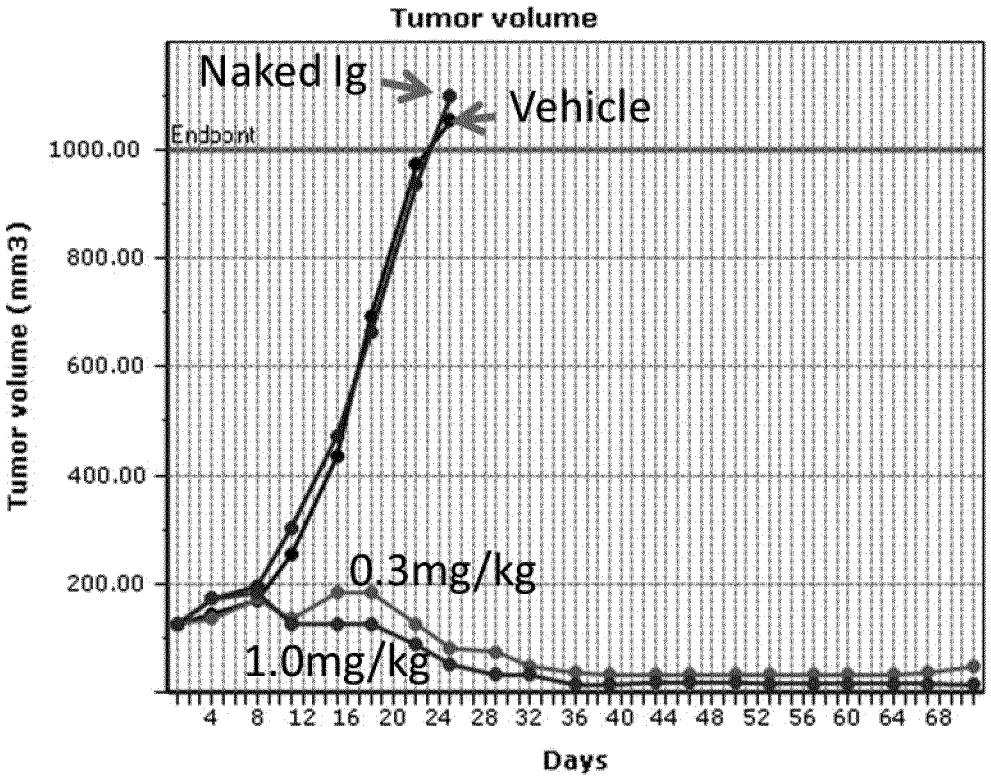
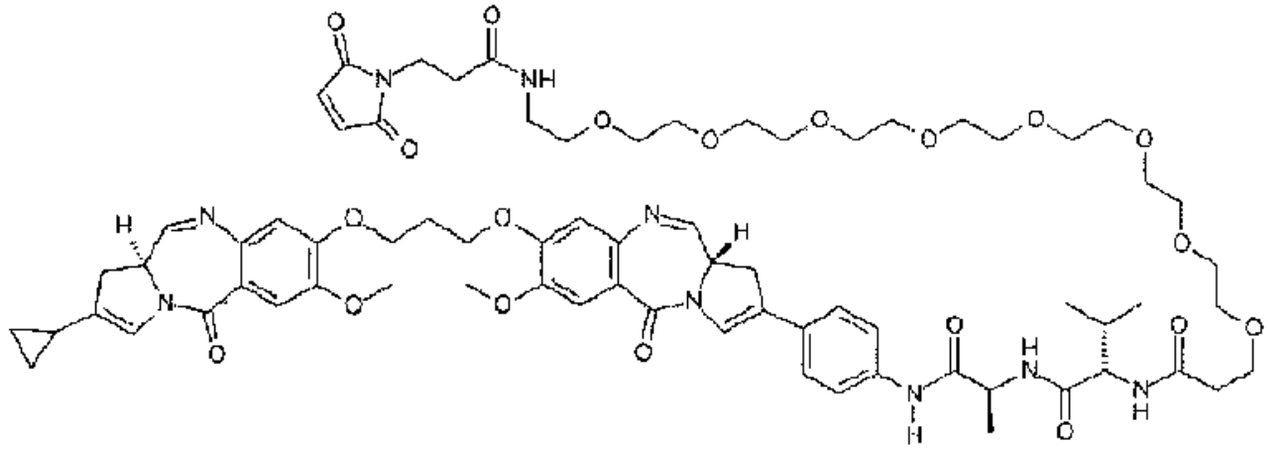
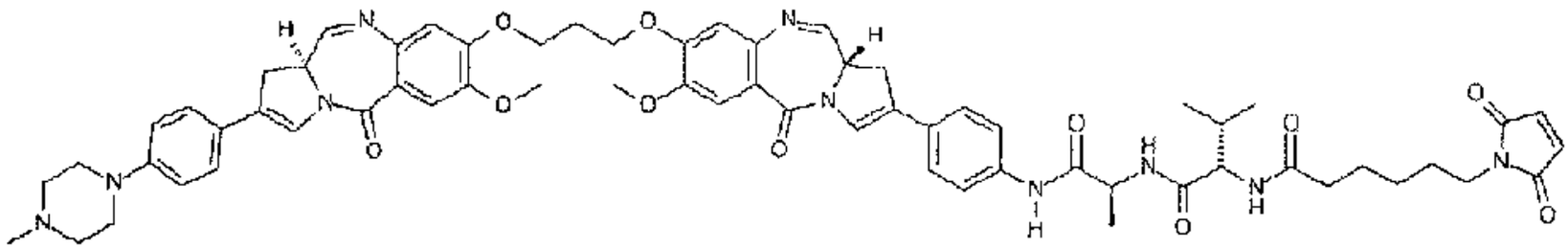

Tumor volume
Naked Ig
Vehicle
Endpoint
1000.00
800.00
600.00
400.00
200.00
Tumor volume (mm3)
0.3mg/kg
1.0mg/kg
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68
Days

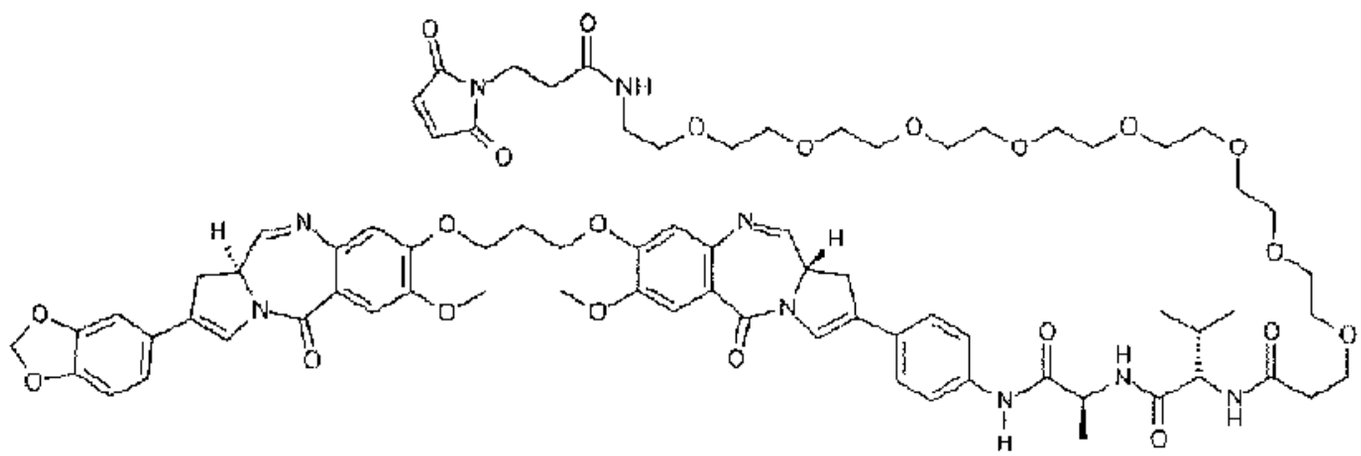
Figure 3



A



B



C