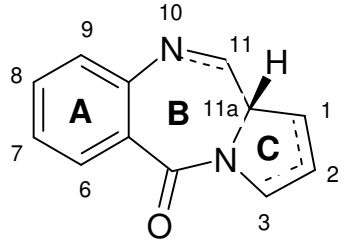
PYRROLOBENZODIAZEPINE CONJUGATES

The present invention relates to conjugates comprising a specific pyrrolobenzodiazepine (PBD), and the precursor drug linker used to make such conjugates.

Background to the invention

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

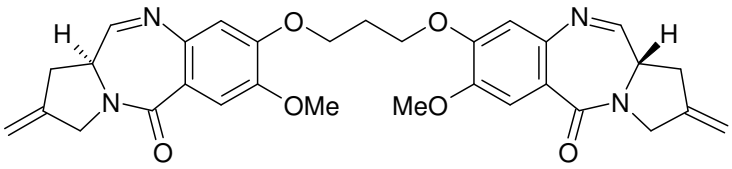


They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position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

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

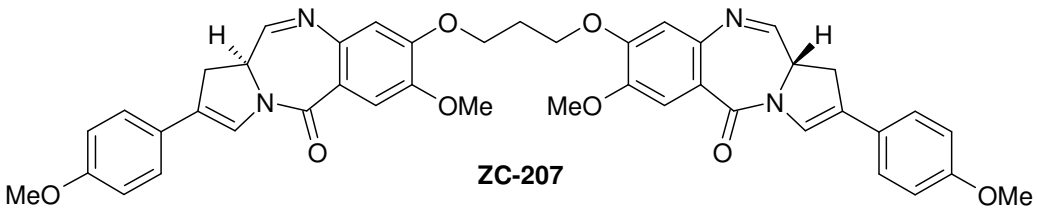
It has been previously disclosed that the biological activity of this molecules can be potentiated by joining two PBD units together through their C8/C'-hydroxyl functionalities via a flexible alkylene linker (Bose, D.S., *et al.*, *J. Am. Chem. Soc.*, **114**, 4939-4941 (1992); Thurston, D.E., *et al.*,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

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



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

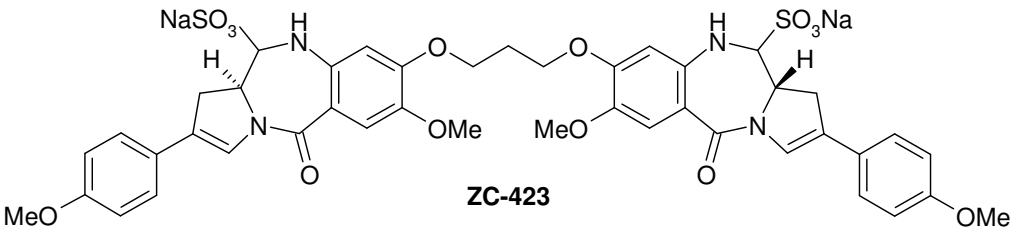
Dimeric PBD compounds bearing C2 aryl substituents, such as SG2202 (ZC-207), are disclosed in WO 2005/085251:



ZC-207

,

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



ZC-423

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

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

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

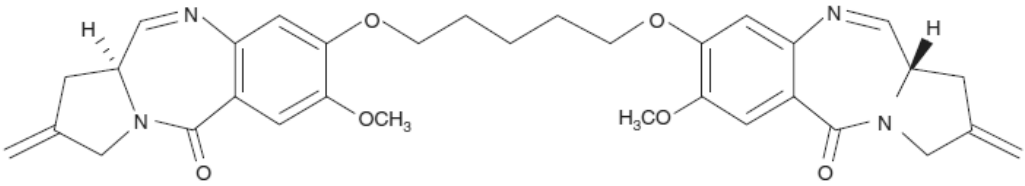
Hartley JA, et al., *DNA interstrand cross-linking and in vivo antitumor activity of the extended pyrrolo[2,1-c][1,4]benzodiazepine dimer SG2057*. Invest New Drugs. 2012 Jun; 30(3):950-8. http://dx.doi.org/10.1007/s10637-011-9647-z (herein after "Hartley et al (2012)")

and:

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

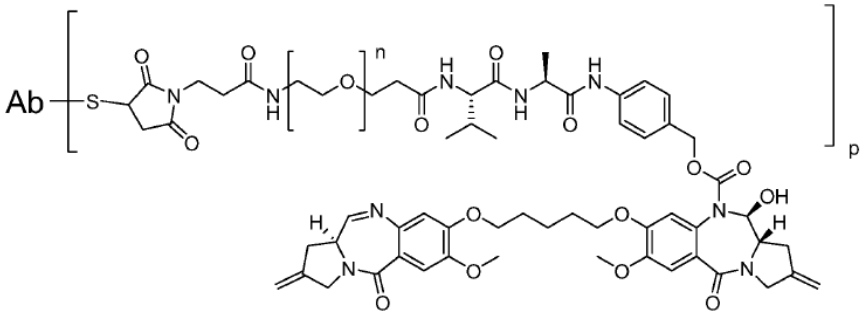
The Hartley et al (2012) paper comments in its summary that "SG2057 is therefore a highly active antiumour agent, with more potent in vitro activity and superior in vivo activity to SG2000, warranting further development".

SG2057 has the structure:



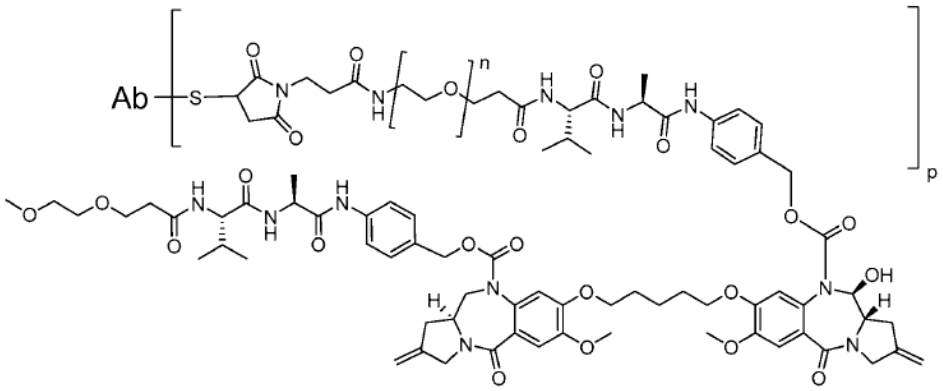
SG2057

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



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

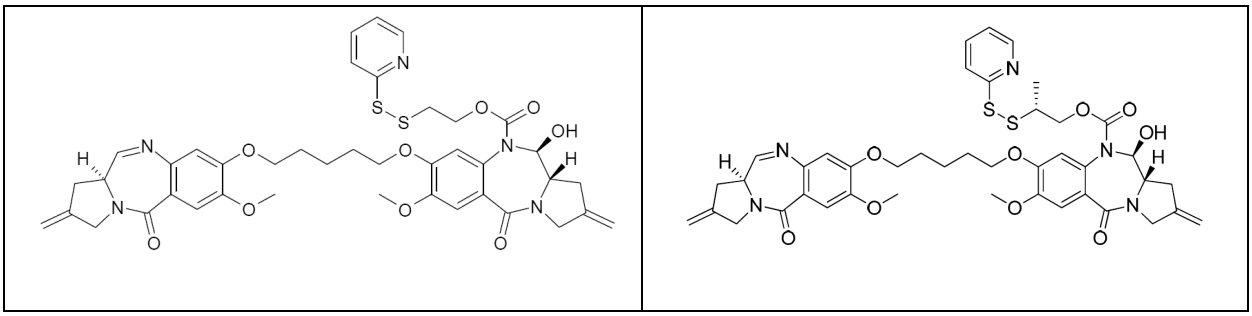
Claim 54 of this application also includes the formula:



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

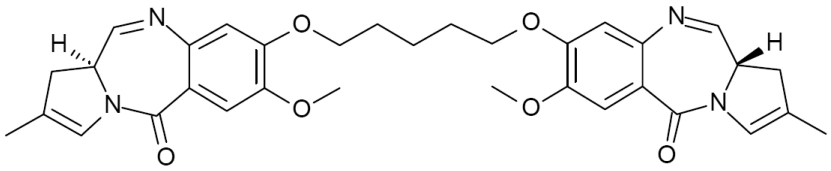
WO 2011/130598 also discloses antibody-drug conjugates including these drug linkers, for example 110 (antiSteap1-15d), example 114 (tastuzumab-15d) and example 115 (tastuzumab-58).

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

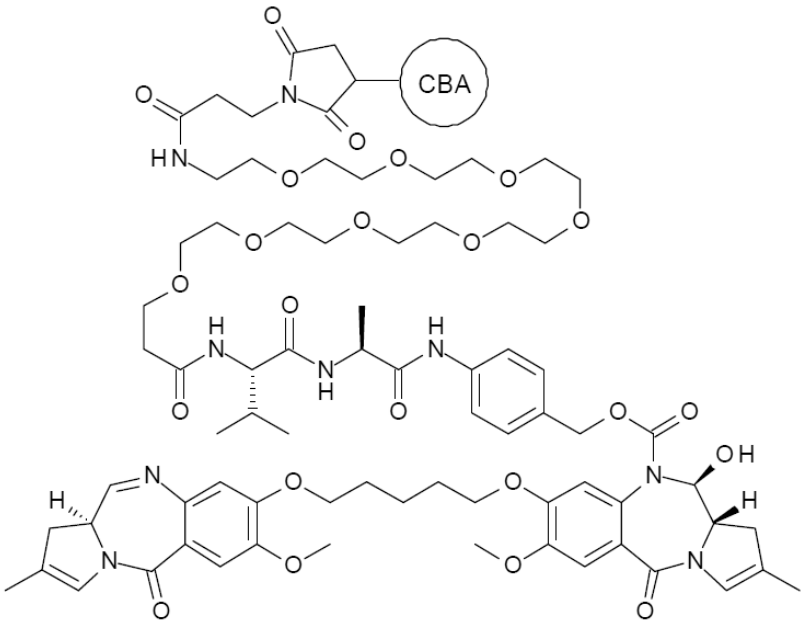


and their use in antibody-drug conjugates.

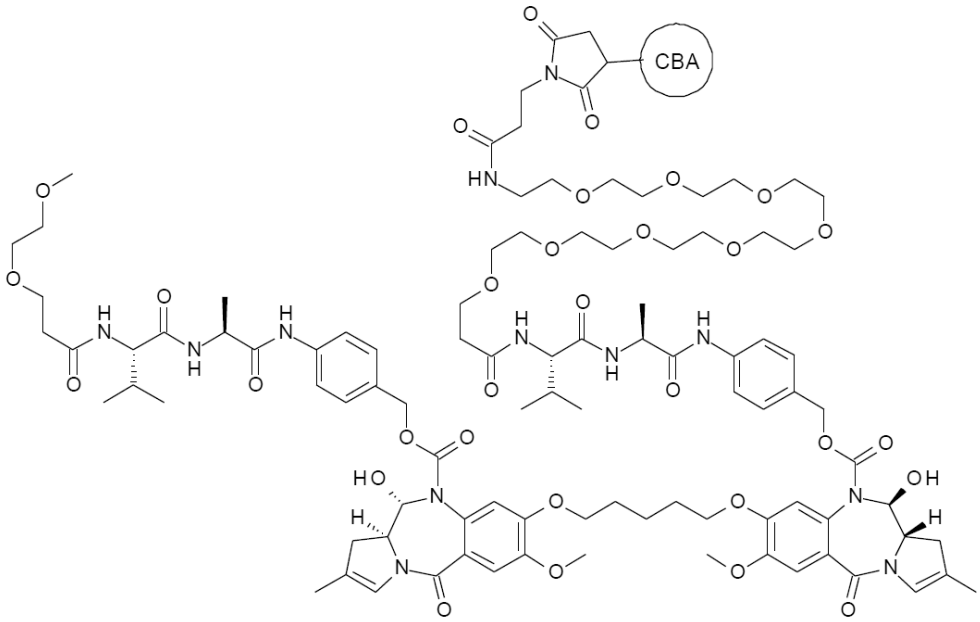
More recently, the warhead:



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



WO2015/052322 discloses:



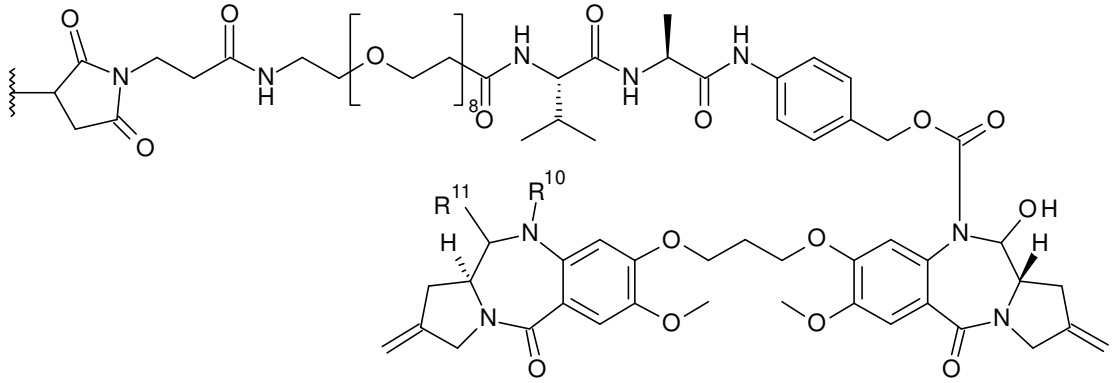
Disclosure of the invention

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

In a first aspect, the present invention provides Conjugates of formula I:

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

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



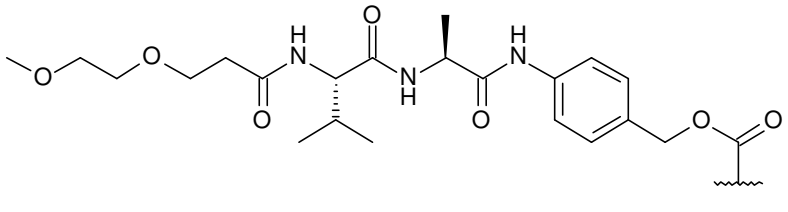
(II)

wherein

either:

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

(b) R^{11} is OH, and R^{10} is:

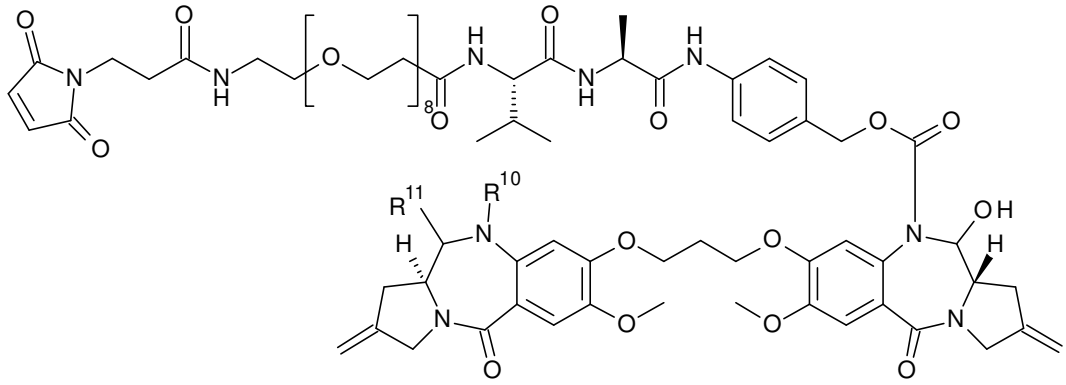


;

p is an integer of from 1 to 20.

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

A second aspect of the present invention provides a compound of formula III:



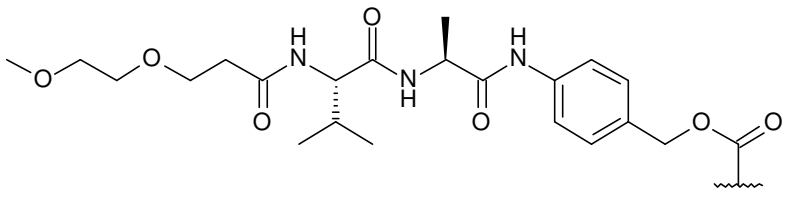
(III)

wherein

either:

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

(b) R^{11} is OH, and R^{10} is:



.

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

treatment of a proliferative disease. The third aspect also provides a method of treating a proliferative disease comprising administering a therapeutically effective amount of a conjugate of the first aspect of the invention to a patient in need thereof.

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

A fourth aspect of the present invention provides the synthesis of a conjugate of the first aspect of the invention comprising conjugating a compound (drug linker) of the second aspect of the invention with a Ligand Unit.

Brief Description of Figures

Figure 1 shows the effect on volume of a BT474 tumour following treatment with a conjugate of the present invention;

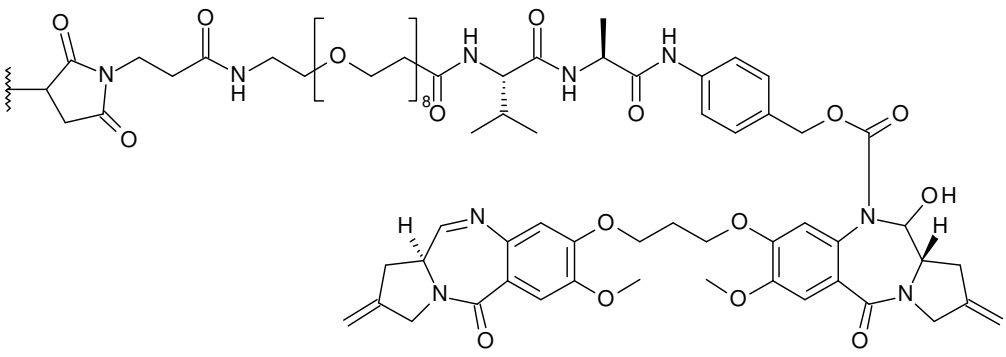
Figure 2 shows the effect on volume of a BT474 tumour following treatment with a different conjugate of the present invention;

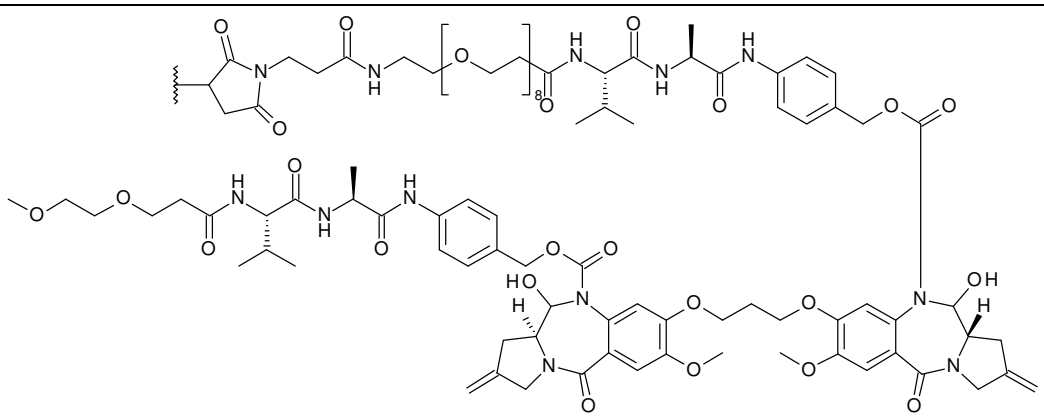
Figure 3 shows the effect on volume of a NCI-N87 tumour following treatment with a conjugate of the present invention;

Figure 4 shows the effect on volume of a NCI-N87 tumour following treatment with a different conjugate of the present invention.

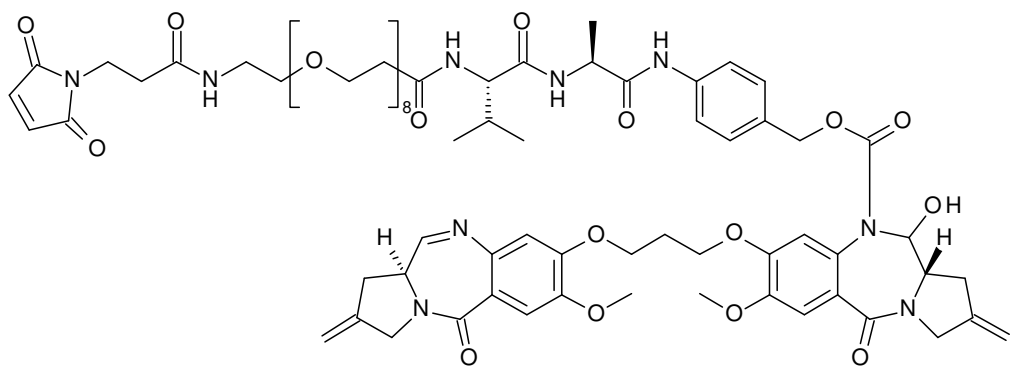
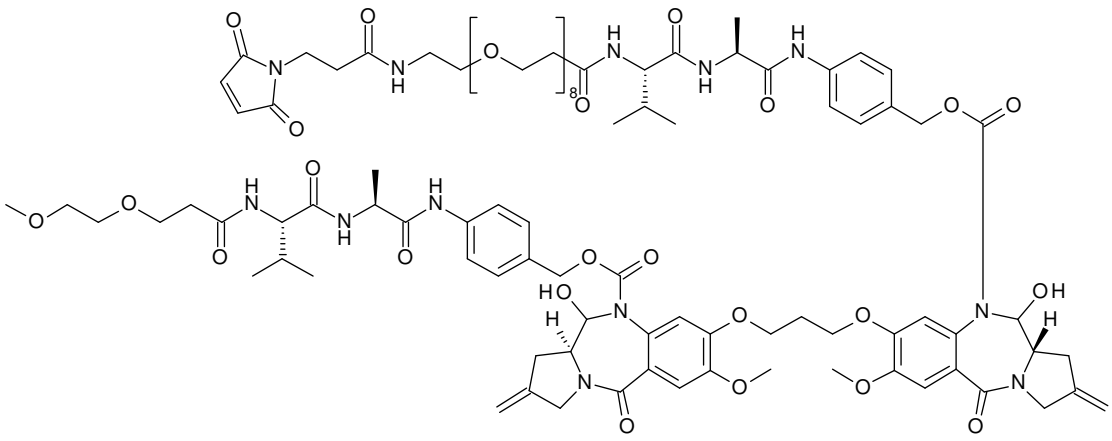
D^L

In the first aspect D^L is selected from D^L-A and D^L-B:

D^L-A	

D^L-B	

In the second aspect, the compound is selected from A and B:

A (23)	
B (40)	

Ligand Unit

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

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

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

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

Cell Binding Agent

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

Peptides

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

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

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

Antibodies

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

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and scFv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining

region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

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

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

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

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

Humanisation

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

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

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

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

CDR grafting

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

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

Guided selection

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

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

Composite antibodies

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

Deimmunization

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

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

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

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

Resurfacing

This method involves:

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

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

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

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

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

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

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

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

Superhumanization

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

Human String Content Optimization

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

Framework Shuffling

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

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

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

TUMOR-ASSOCIATED ANTIGENS AND COGNATE ANTIBODIES

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

Nucleotide

Genbank accession no. NM_001203

Genbank version no. NM_001203.2 GI:169790809

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

Polypeptide

Genbank accession no. NP_001194

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

Cross-references

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

(2) *E16 (LAT1, SLC7A5)*

Nucleotide

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

Polypeptide

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

Cross references

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

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

Nucleotide

Genbank accession no. NM_012449

Genbank version no. NM_012449.2 GI:22027487

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

Polypeptide

Genbank accession no. NP_036581

Genbank version no. NP_036581.1 GI:9558759

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

Cross references

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

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

Nucleotide

Genbank accession no. AF361486

Genbank version no. AF361486.3 GI:34501466

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

Polypeptide

Genbank accession no. AAK74120

Genbank version no. AAK74120.3 GI:34501467

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

Cross references

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

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

Nucleotide

Genbank accession no. NM_005823

Genbank version no. NM_005823.5 GI:293651528

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

Polypeptide

Genbank accession no. NP_005814

Genbank version no. NP_005814.2 GI:53988378

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

Cross references

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

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

Nucleotide

Genbank accession no. NM_006424

Genbank version no. NM_006424.2 GI:110611905

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

Polypeptide

Genbank accession no. NP_006415

Genbank version no. NP_006415.2 GI:110611906

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

Cross references

J. Biol. Chem. 277 (22):19665-19672 (2002), *Genomics* 62 (2):281-284 (1999), Feild, J.A., *et al* (1999) *Biochem. Biophys. Res. Commun.* 258 (3):578-582); WO2004/022778 (Claim 2); EP1394274 (Example 11); WO2002/102235 (Claim 13; Page 326); EP0875569 (Claim

1; Page 17-19); WO2001/57188 (Claim 20; Page 329); WO2004/032842 (Example IV); WO2001/75177 (Claim 24; Page 139-140); MIM:604217.

(7) *Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, 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 (Claim 11); US2003/096961 (Claim 11); US2003/232056 (Example 5); WO2003/105758 16 (Claim 12); US2003/206918 (Example 5); EP1347046 (Claim 1); WO2003/025148 (Claim 20); GI:37182378.

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

Nucleotide

Genbank accession no. AY275463

Genbank version no. AY275463.1 GI:30526094

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

Polypeptide

Genbank accession no. AAP32295

Genbank version no. AAP32295.1 GI:30526095

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

Cross references

Nakamuta M., *et al Biochem. Biophys. Res. Commun.* 177, 34-39, 1991; Ogawa Y., *et al Biochem. Biophys. Res. Commun.* 178, 248-255, 1991; Arai H., *et al Jpn. Circ. J.* 56, 1303-1307, 1992; Arai H., *et al J. Biol. Chem.* 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., *et al Biochem. Biophys. Res. Commun.* 178, 656-663, 1991; Elshourbagy N.A., *et al J. Biol. Chem.* 268, 3873-3879, 1993; Haendler B., *et al J. Cardiovasc. Pharmacol.* 20, s1-S4, 1992; Tsutsumi M., *et al Gene* 228, 43-49, 1999; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002; Bourgeois C., *et al J. Clin. Endocrinol. Metab.* 82, 3116-3123, 1997; Okamoto Y., *et al Biol. Chem.* 272, 21589-21596, 1997; Verheij J.B., *et al Am. J. Med. Genet.* 108, 223-225, 2002; Hofstra R.M.W., *et al Eur. J. Hum. Genet.* 5, 180-185, 1997; Puffenberger E.G., *et al Cell* 79, 1257-1266, 1994; Attie T., *et al, Hum. Mol. Genet.* 4, 2407-2409, 1995; Auricchio A., *et al Hum. Mol. Genet.* 5:351-354, 1996; Amiel J., *et al Hum. Mol. Genet.* 5, 355-357, 1996; Hofstra R.M.W., *et al Nat. Genet.* 12, 445-447, 1996; Svensson P.J., *et al Hum. Genet.* 103, 145-148, 1998; Fuchs S., *et al Mol. Med.* 7, 115-124, 2001; Pingault V., *et al (2002) Hum. Genet.* 111, 198-206; WO2004/045516 (Claim 1); WO2004/048938 (Example 2); WO2004/040000 (Claim 151); WO2003/087768 (Claim 1); WO2003/016475 (Claim 1); WO2003/016475 (Claim 1); WO2002/61087 (Fig 1); WO2003/016494 (Fig 6); WO2003/025138 (Claim 12; Page 144); WO2001/98351 (Claim 1;

Page 124-125); EP0522868 (Claim 8; Fig 2); WO2001/77172 (Claim 1; Page 297-299); US2003/109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004/001004.

(10) MSG783 (RNF124, hypothetical protein FLJ20315)

Nucleotide

Genbank accession no. NM_017763

Genbank version no. NM_017763.4 GI:167830482

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

Polypeptide

Genbank accession no. NP_060233

Genbank version no. NP_060233.3 GI:56711322

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

Cross references

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

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

Nucleotide

Genbank accession no. AF455138

Genbank version no. AF455138.1 GI:22655487

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

Polypeptide

Genbank accession no. AAN04080

Genbank version no. AAN04080.1 GI:22655488

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

Cross references

Lab. Invest. 82 (11):1573-1582 (2002)); WO2003/087306; US2003/064397 (Claim 1; Fig 1); WO2002/72596 (Claim 13; Page 54-55); WO2001/72962 (Claim 1; Fig 4B);

WO2003/104270 (Claim 11); WO2003/104270 (Claim 16); US2004/005598 (Claim 22); WO2003/042661 (Claim 12); US2003/060612 (Claim 12; Fig 10); WO2002/26822 (Claim 23; Fig 2); WO2002/16429 (Claim 12; Fig 10); GI:22655488.

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

Nucleotide

Genbank accession no. NM_017636

Genbank version no. NM_017636.3 GI:304766649

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

Polypeptide

Genbank accession no. NP_060106

Genbank version no. NP_060106.2 GI:21314671

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

Cross references

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

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

Nucleotide

Genbank accession no. NM_003212

Genbank version no. NM_003212.3 GI:292494881

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

Polypeptide

Genbank accession no. NP_003203

Genbank version no. NP_003203.1 GI:4507425

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

Cross references

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

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

Nucleotide

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

Polypeptide

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

Cross references

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

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

Nucleotide

Genbank accession no NM_000626
Genbank version no. NM_000626.2 GI:90193589
Genbank record update date: Jun 26, 2012 01:53 PM

Polypeptide

Genbank accession no. NP_000617
Genbank version no. NP_000617.1 GI:11038674

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

Cross references

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

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

Nucleotide

Genbank accession no NM_030764
Genbank version no. NM_030764.3 GI:227430280
Genbank record update date: Jun 30, 2012 12:30 AM

Polypeptide

Genbank accession no. NP_110391
Genbank version no. NP_110391.2 GI:19923629
Genbank record update date: Jun 30, 2012 12:30 AM

Cross references

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

(17) *HER2 (ErbB2)*

Nucleotide

Genbank accession no M11730

Genbank version no. M11730.1 GI:183986
Genbank record update date: Jun 23, 2010 08:47 AM

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

Cross references
Coussens L., *et al Science* (1985) 230(4730):1132-1139); Yamamoto T., *et al Nature* 319, 230-234, 1986; Semba K., *et al Proc. Natl. Acad. Sci. U.S.A.* 82, 6497-6501, 1985; Swiercz J.M., *et al J. Cell Biol.* 165, 869-880, 2004; Kuhns J.J., *et al J. Biol. Chem.* 274, 36422-36427, 1999; Cho H.-S., *et al Nature* 421, 756-760, 2003; Ehsani A., *et al* (1993) *Genomics* 15, 426-429; WO2004/048938 (Example 2); WO2004/027049 (Fig 1I); WO2004/009622; WO2003/081210; WO2003/089904 (Claim 9); WO2003/016475 (Claim 1); US2003/118592; WO2003/008537 (Claim 1); WO2003/055439 (Claim 29; Fig 1A-B); WO2003/025228 (Claim 37; Fig 5C); WO2002/22636 (Example 13; Page 95-107); WO2002/12341 (Claim 68; Fig 7); WO2002/13847 (Page 71-74); WO2002/14503 (Page 114-117); WO2001/53463 (Claim 2; Page 41-46); WO2001/41787 (Page 15); WO2000/44899 (Claim 52; Fig 7); WO2000/20579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004/043361 (Claim 7); WO2004/022709; WO2001/00244 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1

ANTIBODIES

Abbott: US20110177095

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

Biogen: US20100119511

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

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

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

Pertuzumab (Genentech)

US20110117097

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

US20090285837

US20090202546

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

US20060088523

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

US20060018899

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

US2011/0159014

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

US20090187007

Glycotope: TrasGEX antibody http://www.glycotope.com/pipeline
For example, see International Joint Cancer Institute and Changhai Hospital Cancer Cent: HMTI-Fc Ab - Gao J., et al *BMB Rep*. 2009 Oct 31;42(10):636-41.

Symphogen: US20110217305

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

(18) *NCA (CEACAM6)*

Nucleotide

Genbank accession no M18728
Genbank version no. M18728.1 GI:189084
Genbank record update date: Jun 23, 2010 08:48 AM

Polypeptide

Genbank accession no. AAA59907
Genbank version no. AAA59907.1 GI:189085
Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

Barnett T., *et al Genomics* 3, 59-66, 1988; Tawaragi Y., *et al Biochem. Biophys. Res. Commun.* 150, 89-96, 1988; Strausberg R.L., *et al Proc. Natl. Acad. Sci. U.S.A.* 99:16899-16903, 2002; WO2004/063709; EP1439393 (Claim 7); WO2004/044178 (Example 4); WO2004/031238; WO2003/042661 (Claim 12); WO2002/78524 (Example 2); WO2002/86443 (Claim 27; Page 427); WO2002/60317 (Claim 2); Accession: P40199; Q14920; EMBL; M29541; AAA59915.1.
EMBL; M18728.

(19) *MDP (DPEP1)*

Nucleotide

Genbank accession no BC017023

Genbank version no. BC017023.1 GI:16877538

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

Polypeptide

Genbank accession no. AAH17023

Genbank version no. AAH17023.1 GI:16877539

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

Cross references

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

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

Nucleotide

Genbank accession no AF184971

Genbank version no. AF184971.1 GI:6013324

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

Polypeptide

Genbank accession no. AAF01320

Genbank version no. AAF01320.1 GI:6013325

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

Cross references

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

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

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; Fig 52); US2003/119126 (Claim 1); US2003/119121 (Claim 1; Fig 52); US2003/119129 (Claim 1); US2003/119130 (Claim 1); US2003/119128 (Claim 1; Fig 52); US2003/119125 (Claim 1); WO2003/016475 (Claim 1); WO2002/02634 (Claim 1)

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

Nucleotide

Genbank accession no NM_004442
Genbank version no. NM_004442.6 GI:111118979
Genbank record update date: Sep 8, 2012 04:43 PM

Polypeptide

Genbank accession no. NP_004433
Genbank version no. NP_004433.2 GI:21396504

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

Cross references

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

(23) *ASLG659 (B7h)*

Nucleotide

Genbank accession no. AX092328

Genbank version no. AX092328.1 GI:13444478

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

Cross references

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

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

Nucleotide

Genbank accession no AJ297436

Genbank version no. AJ297436.1 GI:9367211

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

Polypeptide

Genbank accession no. CAB97347

Genbank version no. CAB97347.1 GI:9367212

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

Cross references

Reiter R.E., *et al Proc. Natl. Acad. Sci. U.S.A.* 95, 1735-1740, 1998; Gu Z., *et al Oncogene* 19,
1288-1296, 2000; *Biochem. Biophys. Res. Commun.* (2000) 275(3):783-788; WO2004/022709; EP1394274 (Example 11); US2004/018553 (Claim 17); WO2003/008537 (Claim 1); WO2002/81646 (Claim 1; Page 164); WO2003/003906 (Claim 10; Page 288); WO2001/40309 (Example 1; Fig 17); US2001/055751 (Example 1; Fig 1b); WO2000/32752 (Claim 18; Fig 1); WO98/51805 (Claim 17; Page 97); WO98/51824 (Claim 10; Page 94); WO98/40403 (Claim 2; Fig 1B); Accession: O43653; EMBL; AF043498; AAC39607.1

(25) *GEDA*

Nucleotide

Genbank accession no AY260763
Genbank version no. AY260763.1 GI:30102448
Genbank record update date: Mar 11, 2010 02:24 AM

Polypeptide

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

Cross references

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

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

Nucleotide

Genbank accession no AF116456
Genbank version no. AF116456.1 GI:4585274
Genbank record update date: Mar 10, 2010 09:44 PM

Polypeptide

Genbank accession no. AAD25356
Genbank version no. AAD25356.1 GI:4585275

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

Cross references

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

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

Nucleotide

Genbank accession no AK026467

Genbank version no. AK026467.1 GI:10439337

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

Polypeptide

Genbank accession no. BAB15489

Genbank version no. BAB15489.1 GI:10439338

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

Cross references

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

(27a) *CD22 (CD22 molecule)*

Nucleotide

Genbank accession no X52785

Genbank version no. X52785.1 GI:29778

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

Polypeptide

Genbank accession no. CAA36988

Genbank version no. CAA36988.1 GI:29779

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

Cross references

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

Other information

Official Symbol: CD22

Other Aliases: SIGLEC-2, SIGLEC2

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

ANTIBODIES

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

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

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

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

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

Nucleotide

Genbank accession no NM_001783

Genbank version no. NM_001783.3 GI:90193587

Genbank record update date: Jun 26, 2012 01:48 PM

Polypeptide

Genbank accession no. NP_001774

Genbank version no. NP_001774.1 GI:4502685

Genbank record update date: Jun 26, 2012 01:48 PM

Cross references

WO2003/088808, US2003/0228319; WO2003/062401 (claim 9); US2002/150573 (claim 4, pages 13-14); WO99/58658 (claim 13, Fig 16); WO92/07574 (Fig 1); US5644033; Ha *et al* (1992) *J. Immunol.* 148(5):1526-1531; Müller *et al* (1992) *Eur. J. Immunol..* 22:1621-1625;

Hashimoto *et al* (1994) *Immunogenetics* 40(4):287-295; Preud'homme *et al* (1992) *Clin. Exp.*
5 *Immunol.* 90(1):141-146; Yu *et al* (1992) *J. Immunol.* 148(2) 633-637; Sakaguchi *et al* (1988)
EMBO J. 7(11):3457-3464

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

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

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

(30) *HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and 20 presents them to CD4+ T lymphocytes); 273 aa, pI: 6.56, MW: 30820.TM: 1 [P] Gene Chromosome: 6p21.3)*
Nucleotide
Genbank accession no NM_002120
Genbank version no. NM_002120.3 GI:118402587
Genbank record update date: Sep 8, 2012 04:46 PM

Polypeptide

Genbank accession no. NP_002111

Genbank version no. NP_002111.1 GI:4504403

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

Cross references

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

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

Nucleotide

Genbank accession no NM_002561

Genbank version no. NM_002561.3 GI:325197202

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

Polypeptide

Genbank accession no. NP_002552

Genbank version no. NP_002552.2 GI:28416933

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

Cross references

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

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

Nucleotide

Genbank accession no NM_001782

Genbank version no. NM_001782.2 GI:194018444

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

Polypeptide

Genbank accession no. NP_001773

Genbank version no. NP_001773.1 GI:4502683

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

Cross references

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

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

Nucleotide

Genbank accession no NM_005582

Genbank version no. NM_005582.2 GI:167555126

Genbank record update date: Sep 2, 2012 01:50 PM

Polypeptide

Genbank accession no. NP_005573

Genbank version no. NP_005573.2 GI:167555127

Genbank record update date: Sep 2, 2012 01:50 PM

Cross references

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

(34) *FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain*
that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte 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; US2004/005563; US2003/124579; Horie *et al* (2000) *Genomics* 67:146-152; Uchida *et al* (1999) *Biochem. Biophys. Res. Commun.* 266:593-602; Liang *et al* (2000) *Cancer Res.* 60:4907-12; Glynne-Jones *et al* (2001) *Int J Cancer.* Oct 15; 94(2):178-84.

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

Nucleotide

Genbank accession no M99487

Genbank version no. M99487.1 GI:190663

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

Polypeptide

Genbank accession no. AAA60209

Genbank version no. AAA60209.1 GI:190664
Genbank record update date: Jun 23, 2010 08:48 AM

Cross references

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

Other information

Official Symbol: FOLH1
Other Aliases: GIG27, FGCP, FOLH, GCP2, GCPII, NAALAD1, NAALAdase, PSM, PSMA, mGCP
Other Designations: N-acetylated alpha-linked acidic dipeptidase 1; N-acetylated-alpha-linked acidic dipeptidase I; NAALADase I; cell growth-inhibiting gene 27 protein; folylpoly-gamma-glutamate carboxypeptidase; glutamate carboxylase II; glutamate carboxypeptidase 2; glutamate carboxypeptidase II; membrane glutamate carboxypeptidase; prostate specific membrane antigen variant F; pteroylpoly-gamma-glutamate carboxypeptidase

ANTIBODIES

US 7,666,425:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(38.1) SSTR2 (Somatostatin receptor 2)

Nucleotide

Genbank accession no NM_001050

Genbank version no. NM_001050.2 GI:44890054

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

Polypeptide

Genbank accession no. NP_001041

Genbank version no. NP_001041.1 GI:4557859

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

Cross references

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

Other information

Official Symbol: SSTR2

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

(38.2) SSTR5 (Somatostatin receptor 5)

Nucleotide

Genbank accession no D16827

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

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

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

Other information
Official Symbol: SSTR5
Other Aliases: SS-5-R
Other Designations: Somatostatin receptor subtype 5; somatostatin receptor type 5

(38.3) SSTR1
(38.4)SSTR3
(38.5) SSTR4

AvB6 – Both subunits (39+40)
(39) *ITGAV (Integrin, alpha V;*
Nucleotide
Genbank accession no M14648 J02826 M18365
Genbank version no. M14648.1 GI:340306
Genbank record update date: Jun 23, 2010 08:56 AM

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

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

Other information

Official Symbol: ITGAV

Other Aliases: CD51, MSK8, VNRA, VTNR

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

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

Nucleotide

Genbank accession no NM_000888

Genbank version no. NM_000888.3 GI:9966771

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

Polypeptide

Genbank accession no. NP_000879

Genbank version no. NP_000879.2 GI:9625002

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

Cross references

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

Other information

Official Symbol: ITGB6

Other Designations: integrin beta-6

ANTIBODIES

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

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

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

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

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

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

Nucleotide

Genbank accession no M17303
Genbank version no. M17303.1 GI:178676
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

Genbank accession no. AAB59513
Genbank version no. AAB59513.1 GI:178677
Genbank record update date: Jun 23, 2010 08:47 AM

Cross references

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

Other information

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

ANTIBODIES

AstraZeneca-MedImmune:US 20100330103; US20080057063; US20020142359

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

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

Bayer Corporation: US6,013,772

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

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

Celltech Therapeutics Limited: US5,877,293

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

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

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

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

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

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

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

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

<u>Nucleotide</u>

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

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

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

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

ANTIBODIES

Abgenix/Pfizer: US20100040629

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

Amgen/Pfizer: US20050054019

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

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

Agouron Pharmaceuticals (Now Pfizer): US20060035907

Eli Lilly: US20100129369

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

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

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

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

Novartis: US20090175860

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

Pharmacia Corporation: US20040166544

Pierre Fabre: US20110239316, US20110097262, US20100115639

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

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

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

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

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

Nucleotide

Genbank accession no J05581

Genbank version no. J05581.1 GI:188869

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

Polypeptide

Genbank accession no. AAA59876

Genbank version no. AAA59876.1 GI:188870

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

Cross references

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

Other information

Official Symbol: MUC1

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

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

ANTIBODIES

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

AltaRex- Quest Pharma Tech: US7,147,850

CRT: 5E5 - Sørensen AL., et al *Glycobiology* vol. 16 no. 2 pp. 96–107, 2006; HMFG2 – Burchell J., et al *Cancer Res.*, 47, 5476–5482 (1987); see WO2015/159076

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

Immunogen: US7,202,346

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

Immunomedics: US 6,653,104

Ramot Tel Aviv Uni: US7,897,351

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

Roche GlycArt: US8,021,856

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

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

(44) *CA9 (Carbonic anhydrase IX)*

<u>Nucleotide</u>

Genbank accession no . X66839

Genbank version no. X66839.1 GI:1000701

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

<u>Polypeptide</u>

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

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

Other information
Official Symbol: CA9
Other Aliases: CAIX, MN
Other Designations: CA-IX; P54/58N; RCC-associated antigen G250; RCC-associated protein G250; carbonate dehydratase IX; carbonic anhydrase 9; carbonic dehydratase; membrane antigen MN; pMW1; renal cell carcinoma-associated antigen G250

ANTIBODIES
Abgenix/Amgen: US20040018198

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

Bayer: US7,462,696

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

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

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

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

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

- for example the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128; or the V/10 monoclonal antibody secreted from the hybridoma V/10-VU, which was deposited at the

International Depository Authority of the Belgian Coordinated Collection of Microorganisms (BCCM) at the Laboratorium voor Moleculaire Bioloqie-Plasmidencollectie (LMBP) at the Universeit Gent in Gent, Belgium, under Accession No. LMBP 6009CB.

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

Novartis: US20090252738

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

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

Xencor: US20090162382

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

Nucleotide

Genbank accession no. NM_201283

Genbank version no. NM_201283.1 GI:41327733

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

Polypeptide

Genbank accession no. NP_958440

Genbank version no. NP_958440.1 GI:41327734

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

Cross-references

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

ANTIBODIES:

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

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

amino acid sequence selected from the group consisting of: SEQ ID NO: 144 and variants.

US20100111979 (Amgen)

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

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

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

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

US20090240038 (Amgen)

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

US20090175887 (Amgen)

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

US20090156790 (Amgen)

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

US20090155282, US20050059087 and US20050053608 (Amgen)

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

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

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

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

US20090311803 (Harvard University)

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

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

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

US6,129,915 (Schering)

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

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

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

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

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

(46) *CD33 (CD33 molecule)*

Nucleotide

Genbank accession no. M_23197

Genbank version no. NM_23197.1 GI:180097

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

Polypeptide

Genbank accession no. AAA51948

Genbank version no. AAA51948.1 GI:188098

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

Cross-references

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

Other information

Official Symbol: CD33

Other Aliases: SIGLEC-3, SIGLEC3, p67

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

ANTIBODIES

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

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

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

US6,590,088 (Human Genome Sciences)

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

US7,557,189 (Immunogen)

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

(47) *CD19 (CD19 molecule)*

Nucleotide

Genbank accession no. NM_001178098

Genbank version no. NM_001178098.1 GI:296010920

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

Polypeptide

Genbank accession no. NP_001171569

Genbank version no. NP_001171569.1 GI:296010921

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

Cross-references

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

Other information

Official Symbol: CD19

Other Aliases: B4, CVID3

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

ANTIBODIES

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

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

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

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

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

US7,109,304 (Immunomedics)

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

US7,902,338 (Immunomedics)

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

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

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

US7,968,687 (Seattle Genetics)

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

4G7 chim - Lang P., et al Blood. 2004 May 15;103(10):3982-5 (University of Tübingen)

For example, fig. 6 and SEQ ID No: 80 of US20120082664

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

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

Nucleotide

Genbank accession no. NM_000417

Genbank version no. NM_000417.2 GI:269973860

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

Polypeptide

Genbank accession no. NP_000408

Genbank version no. NP_000408.1 GI:4557667

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

Cross-references

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

Other information

Official Symbol: IL2RA

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

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

ANTIBODIES

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

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

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

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

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

Nucleotide

Genbank accession no. M76125

Genbank version no. M76125.1 GI:292869

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

Polypeptide

Genbank accession no. AAA61243

Genbank version no. AAA61243.1 GI:29870

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

Cross-references

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

Other information

Official Symbol: AXL

Other Aliases: JTK11, UFO

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

ANTIBODIES

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

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

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

Nucleotide

Genbank accession no. M83554

Genbank version no. M83554.1 GI:180095

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

Polypeptide

Genbank accession no. AAA51947

Genbank version no. AAA51947.1 GI:180096

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

Cross-references

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

Other information

Official Symbol: TNFRSF8

Other Aliases: CD30, D1S166E, Ki-1

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

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

Nucleotide

Genbank accession no. Z29574

Genbank version no. Z29574.1 GI:471244

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

Polypeptide

Genbank accession no. CAA82690

Genbank version no. CAA82690.1 GI:471245

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

Cross-references

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

Other information

Official Symbol: TNFRSF17

Other Aliases: BCM, BCMA, CD269

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

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

Cross-references

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

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

Nucleotide

Genbank accession no. NM000149

Genbank version no. NM000149.3 GI:148277008

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

Polypeptide

Genbank accession no. NP_000140

Genbank version no. NP_000140.1 GI:4503809

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

Cross-references

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

Other information

Official Symbol: FUT3

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

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

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

Nucleotide

Genbank accession no. NM175060

Genbank version no. NM175060.2 GI:371123930

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

Polypeptide

Genbank accession no. NP_778230

Genbank version no. NP_778230.1 GI:28269707

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

Other information

Official Symbol: CLEC14A

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

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

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

Nucleotide

Genbank accession no. NM005347

Genbank version no. NM005347.4 GI:305855105

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

Polypeptide

Genbank accession no. NP_005338

Genbank version no. NP_005338.1 GI:16507237

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

Cross-references

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

Other infromation

Official Symbol: HSPA5

Other Aliases: BIP, GRP78, MIF2

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

(56) *CD70 (CD70 molecule) L08096*

Nucleotide

Genbank accession no. L08096

Genbank version no. L08096.1 GI:307127

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

Polypeptide

Genbank accession no. AAA36175

Genbank version no. AAA36175.1 GI:307128

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

Cross-references

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

Other information

Official Symbol: CD70

Other Aliases: CD27L, CD27LG, TNFSF7

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

ANTIBODIES

MDX-1411 against CD70 (Medarex)

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

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

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

- Genbank accession no. NM_006017
- Genbank version no. NM_006017.2 GI:224994187
- Genbank record update date: Sep 30, 2012 01:47 PM

o Polypeptide

- Genbank accession no. NP_006008
- Genbank version no. NP_006008.1 GI:5174387
- Genbank record update date: Sep 30, 2012 01:47 PM

(58) *ASG-5*

Cross-references

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

ANTIBODIES

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

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

Nucleotide

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

Polypeptide

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

Cross-references

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

Other information

Official Symbol: ENPP3
Other Aliases: RP5-988G15.3, B10, CD203c, NPP3, PD-IBETA, PDNP3
Other Designations: E-NPP 3; dJ1005H11.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); dJ914N13.3 (phosphodiesterase I/nucleotide pyrophosphatase 3);

ectonucleotide pyrophosphatase/phosphodiesterase family member 3; gp130RB13-6; phosphodiesterase I beta; phosphodiesterase I/nucleotide pyrophosphatase 3; phosphodiesterase-I beta

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

Nucleotide

Genbank accession no. NM_007244

Genbank version no. NM_007244.2 GI:154448885

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

Polypeptide

Genbank accession no. NP_009175

Genbank version no. NP_009175.2 GI:154448886

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

Cross-references

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

Other information

Official Symbol: PRR4

Other Aliases: LPRP, PROL4

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

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

Nucleotide

Genbank accession no. NM_004963

Genbank version no. NM_004963.3 GI:222080082

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

Polypeptide

Genbank accession no. NP_004954

Genbank version no. NP_004954.2 GI:222080083

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

Cross-references

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

Other information

Official Symbol: GUCY2C

Other Aliases: DIAR6, GUC2C, MUCIL, STAR

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

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

Nucleotide

Genbank accession no. U41060

Genbank version no. U41060.2 GI:12711792

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

Polypeptide

Genbank accession no. AAA96258

Genbank version no. AAA96258.2 GI:12711793

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

Cross-references

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

Other information

Official Symbol: SLC39A6

Other Aliases: LIV-1

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

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

Nucleotide

Genbank accession no. AJ012159

Genbank version no. AJ012159.1 GI:3805946

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

Polypeptide

Genbank accession no. CAA09930

Genbank version no. CAA09930.1 GI:3805947

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

Cross-references

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

Other information

- Official Symbol: TPBG
- Other Aliases: 5T4, 5T4AG, M6P1
- Other Designations: 5T4 oncofetal antigen; 5T4 oncofetal trophoblast glycoprotein; 5T4 oncotrophoblast glycoprotein
- See WO2015/155345

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

Nucleotide

Genbank accession no. NM_000615

Genbank version no. NM_000615.6 GI:336285433

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

Polypeptide

Genbank accession no. NP_000606

Genbank version no. NP_000606.3 GI:94420689

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

Cross-references

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

Other information

Official Symbol: NCAM1

Other Aliases: CD56, MSK39, NCAM

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

ANTIBODIES

Immunogen: HuN901 (Smith SV., et al *Curr Opin Mol Ther.* 2005 Aug;7(4):394-401)
For example, see humanized from murine N901 antibody. See Fig. 1b and 1e of Roguska, M.A., et al. Proc Natl Acad Sci USA Feb 1994;91:969-973.

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

Cross-references

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

ANTIBODIES

huC242 (Tolcher AW et al., *J Clin Oncol.* 2003 Jan 15;21(2):211-22; Immunogen)
For example, see US20080138898A1 SEQ ID NO: 1 and 2

(66) *FOLR1 (Folate Receptor 1)*

Nucleotide

Genbank accession no. J05013
Genbank version no. J05013.1 GI:182417
Genbank record update date: Jun 23, 2010 08:47 AM

Polypeptide

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

Cross-references

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

Other information

Official Symbol: FOLR1
Other Aliases: FBP, FOLR
Other Designations: FR-alpha; KB cells FBP; adult folate-binding protein; folate binding protein; folate receptor alpha; folate receptor, adult; ovarian tumor-associated antigen MOv18

ANTIBODIES

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

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

Nucleotide

Genbank accession no. X76534

Genbank version no. X76534.1 GI:666042

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

Polypeptide

Genbank accession no. CAA54044

Genbank version no. CAA54044.1 GI:666043

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

Cross-references

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

Other information

Official Symbol: GPNMB

Other Aliases: UNQ1725/PRO9925, HGFIN, NMB

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

ANTIBODIES

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

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

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

Nucleotide

Genbank accession no. AF043724

Genbank version no. AF043724.1 GI:2827453

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

Polypeptide

Genbank accession no. AAC39862

Genbank version no. AAC39862.1 GI:2827454

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

Cross-references

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

Other information

Official Symbol: HAVCR1

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

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

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

Cross-references

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

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

Nucleotide

Genbank accession no. BX648021

Genbank version no. BX648021.1 GI:34367180

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

Cross-references

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

Other information

Official Symbol: VTCN1

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

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

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

Nucleotide

Genbank accession no. AF447176

Genbank version no. AF447176.1 GI:17432420

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

Polypeptide

Genbank accession no. AAL39062

Genbank version no. AAL39062.1 GI:17432421

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

Cross-references

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

Other information

Official Symbol: PTK7

Other Aliases: CCK-4, CCK4

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

(72) *CD37 (CD37 molecule)*

Nucleotide

Genbank accession no. NM_001040031

Genbank version no. NM_001040031.1 GI:91807109

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

Polypeptide

Genbank accession no. NP_001035120

Genbank version no. NP_001035120.1 GI:91807110

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

Cross-references

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

Other information

Official Symbol: CD37

Other Aliases: GP52-40, TSPAN26

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

ANTIBODIES

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

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

For example, see US20110171208A1 SEQ ID NO: 253

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

(73) *CD138 – SDC1 (syndecan 1)*

Nucleotide

Genbank accession no. AJ551176

Genbank version no. AJ551176.1 GI:29243141

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

Polypeptide

Genbank accession no. CAD80245

Genbank version no. CAD80245.1 GI:29243142

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

Cross-references

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

Other information

Official Symbol: SDC1

Other Aliases: CD138, SDC, SYND1, syndecan

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

ANTIBODIES

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

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

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

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

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

Nucleotide

Genbank accession no. NM_004355

Genbank version no. NM_004355.1 GI:343403784

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

Polypeptide

Genbank accession no. NP_004346

Genbank version no. NP_004346.1 GI:10835071

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

Cross-references

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

Other information

Official Symbol: CD74

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

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

ANTIBODIES

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

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

Genmab: HuMax-CD74 (see website)

(75) *Claudins – CLs (Claudins)*

Cross-references

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

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

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

Nucleotide

Genbank accession no. NM_005228

Genbank version no. NM_005228.3 GI:41927737

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

Polypeptide

Genbank accession no. NP_005219

Genbank version no. NP_005219.2 GI:29725609

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

Cross-references

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

Other information

Official Symbol: EGFR

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

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

ANTIBODIES

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

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

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

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

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

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

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

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

Nucleotide

Genbank accession no. M34309

Genbank version no. M34309.1 GI:183990

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

Polypeptide

Genbank accession no. AAA35979

Genbank version no. AAA35979.1 GI:306841

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

Cross-references

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

Other information

Official Symbol: ERBB3

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

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

ANTIBODIES

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

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

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

Nucleotide

Genbank accession no. X70040

Genbank version no. X70040.1 GI:36109

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

Polypeptide

Genbank accession no. CCA49634

Genbank version no. CCA49634.1 GI:36110

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

Cross-references

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

Other information

Official Symbol: MST1R

Other Aliases: CD136, CDw136, PTK8, RON

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

(79) *EPHA2 (EPH receptor A2)*

Nucleotide

Genbank accession no. BC037166

Genbank version no. BC037166.2 GI:33879863

Genbank record update date: Mar 06, 2012 01:59 PM

Polypeptide

Genbank accession no. AAH37166

Genbank version no. AAH37166.1 GI:22713539

Genbank record update date: Mar 06, 2012 01:59 PM

Cross-references

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

Other information

Official Symbol: EPHA2

Other Aliases: ARCC2, CTPA, CTPP1, ECK

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

ANTIBODIES

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

For example, see US20090304721A1 Fig. 7 and 8.

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

Nucleotide

Genbank accession no. M27394

Genbank version no. M27394.1 GI:179307

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

Polypeptide

Genbank accession no. AAA35581

Genbank version no. AAA35581.1 GI:179308

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

Cross-references

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

Other information

Official Symbol: MS4A1

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

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

ANTIBODIES

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

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

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

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

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

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

(81) *Tenascin C – TNC (Tenascin C)*

Nucleotide

Genbank accession no. NM_002160

Genbank version no. NM_002160.3 GI:340745336
Genbank record update date: Sep 23, 2012 02:33 PM

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

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

Other information
Official Symbol: TNC
Other Aliases: 150-225, GMEM, GP, HXB, JI, TN, TN-C
Other Designations: GP 150-225; cytotactin; glioma-associated-extracellular matrix antigen; hexabrachion (tenascin); myotendinous antigen; neuronectin; tenascin; tenascin-C isoform 14/AD1/16

ANTIBODIES
Philogen : G11 (von Lukowicz T., et al *J Nucl Med.* 2007 Apr;48(4):582-7) and F16 (Pedretti M., et al Lung Cancer. 2009 Apr;64(1):28-33)
For example, see US7968685 SEQ ID NOs: 29, 35, 45 and 47.

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

Polypeptide
Genbank accession no. AAB49652
Genbank version no. AAB49652.1 GI:1888316
Genbank record update date: Jun 23, 2010 09:22 AM

Cross-references

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

Other information

Official Symbol: FAP

Other Aliases: DPPIV, FAPA

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

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

Nucleotide

Genbank accession no. NM_012242

Genbank version no. NM_012242.2 GI:61676924

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

Polypeptide

Genbank accession no. NP_036374

Genbank version no. NP_036374.1 GI:7110719

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

Cross-references

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

Other information

Official Symbol: DKK1

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

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

ANTIBODIES

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

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

(84) *CD52 (CD52 molecule)*

Nucleotide

Genbank accession no. NM_001803

Genbank version no. NM_001803.2 GI:68342029

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

Polypeptide

Genbank accession no. NP_001794

Genbank version no. NP_001794.2 GI:68342030

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

Cross-references

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

Other information

Official Symbol: CD52

Other Aliases: CDW52

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

ANTIBODIES

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

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

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

Nucleotide

Genbank accession no. NM_021181

Genbank version no. NM_021181.3 GI:1993571

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

Polypeptide

Genbank accession no. NP_067004

Genbank version no. NP_067004.3 GI:19923572

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

Cross-references

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

Other information

Official Symbol: SLAMF7

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

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

ANTIBODIES

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

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

(86) *Endoglin – ENG (Endoglin)*

Nucleotide

Genbank accession no. AF035753

Genbank version no. AF035753.1 GI:3452260

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

Polypeptide

Genbank accession no. AAC32802

Genbank version no. AAC32802.1 GI:3452261

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

Cross-references

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

Official Symbol: ENG

Other information

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

Other Designations: CD105 antigen

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

Nucleotide

Genbank accession no. X05908

Genbank version no. X05908.1 GI:34387

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

Polypeptide

Genbank accession no. CCA29338

Genbank version no. CCA29338.1 GI:34388

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

Cross-references

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

Other information

Official Symbol: ANXA1

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

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

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

Nucleotide

Genbank accession no. M60335

Genbank version no. M60335.1 GI:340193

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

Polypeptide

Genbank accession no. AAA61269

Genbank version no. AAA61269.1 GI:340194

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

Cross-references

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

Other information

Official Symbol VCAM1

Other Aliases: CD106, INCAM-100

Other Designations: CD106 antigen; vascular cell adhesion protein 1

Antibody Sequences

Anti-Integrin αvβ6

RHAB6.2

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDT
EYAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTAVPNLRGDLQVLAQKVA
GPYPFDYWGQGTLVTVSS

RHCB6.2

QVQLVQSGAEVKKPGASVKVSCKASGYTFIDSYMHWVRQAPGQRLEWMGWIDPENGDT
EYAPKFQGRVTITTDTSASTAYMELSSLRSEDTAVYYCARGTPTAVPNLRGDLQVLAQKV
AGPYPFDYWGQGTLVTVSS

RHF

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGD
TEYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTGPYYFDYWGQGTLV
TVSS

RHFB6

QVQLVQSGAEVKKPGASVKVSCKASGFNFIDSYMHWVRQAPGQRLEWMGWIDPENGD
TEYAPKFQGRVTFTTDTSASTAYMELSSLRSEDTAVYYCNEGTPTAVPNLRGDLQVLAQK
VAGPYYFDYWGQGTLVTVSS

RHAY100bP

QVQLVQSGSELKKPGASVKISCKASGFAFTDSYMHWVRQAPGQGLEWMGWIDPENGDT
EYAPKFQGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCTRGTPTGPYPFDYWGQGTLVTV
SS

RKF

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKFL36L50

ENVLTQSPGTLSLSPGERATLSCSASSSVSYMHWLQQKPGQAPRLLIYLTSNLASGIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

RKC

EIVLTQSPGTLSLSPGERATLSCSASSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQRSSYPLTFGGGTKVEIK

Anti-CD33

CD33 Hum195 VH

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGT
GYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSS

CD33 Hum195 VK

DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGS
GVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK

Anti-CD19

CD19 B4 resurfaced VH

QVQLVQPGAEVVKPGASVKLSCKTSGYTFTSNWMHWVKQRPGQGLEWIGEIDPSDSYT
NYNQNFKGKAKLTVDKSTSTAYMEVSSLRSDDTAVYYCARGSNPYYYAMDYWGQGTSV
TVSS

CD19 B4 resurfaced VK

EIVLTQSPAIMSASPGERVTMTCSASSGVNYMHWYQQKPGTSPRRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEPEDAATYYCHQRGSYTFGGGTKLEIK

Anti-Her2

Herceptin VH chain

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT
VSS

Herceptin VL chain

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Anti-CD25

Simulect VK (also known as Basiliximab)

QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQQKPGTSPKRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEAEDAATYYCHQRSSYTFGGGTKLEIK

Simulect VH

QLQQSGTVLARPGASVKMSCKASGYSFTRYWMHWIKQRPGQGLEWIGAIYPGNSDTSY
NQKFEGKAKLTAVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFDFWGQGTTLTVSS

Anti-PSMA

Deimmunised VH ‘1
EVQLVQSGPEVKKPGATVKISCKTSGYTFTEYTIHWVKQAPGKGLEWIGNINPNNGGTTY
NQKFEDKATLTVDKSTDTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTLLTVSS

Deimmunised VK ‘1
DIQMTQSPSSLSTSVGDRVTLTCKASQDVGTAVDWYQQKPGPSPKLLIYWASTRHTGIPS
RFSGSGSGTDFTLTISSLQPEDFADYYCQQYNSYPLTFGPGTKVDIK

Deimmunised VH1 ‘5
EVKLVESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNN
FATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTGVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH2 ‘5
EVKLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH3 ‘5
EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRVTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VH4 ‘5
EVQLVESGGGLVQPGGSLKLSCVASGFTFSNYWMNWVRQAPGKGLEWVAEIRSQSNNF
ATHYAESVKGRFTISRDDSKSIVYLQMNNLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Deimmunised VK1 ‘5
NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFTGSGSATDFTLTISSLQTEDLADYYCGQSYTFPYTFGQGTKLEMK

Deimmunised VK2 ‘5
NIVMTQFPSSMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK3 ‘5

NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDLADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK4 ‘5

NIQMTQFPSAMSASVGDRVTITCKASENVGTYVSWYQQKPDQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFTLTISSLQAEDEADYYCGQSYTFPYTFGQGTKLEIK

Deimmunised VK DI ‘5

NIVMTQFPKSMSASAGERMTLTCKASENVGTYVSWYQQKPTQSPKMLIYGASNRFTGVP
DRFSGSGSGTDFILTISSVQAEDLVDYYCGQSYTFPYTFGGGTKLEMK

Deimmunised VH DI ‘5

EVKLEESGGGLVQPGGSMKISCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKSSVYLQMNSLRAEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**A ‘5

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**B ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**C ‘5

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**D ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVGEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**E ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRFTISRDDSKNTVYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**F ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**H**G ‘5

EVKLVESGGGLVQPGGSLKLSCAASGFTFSNYWMNWVRQASGKGLEWVAEIRSQSNNF
ATHYAESVKGRVIISRDDSKNTAYLQMNSLRTEDTAVYYCTRRWNNFWGQGTTVTVSS

Humanised R**K**A ‘5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**B ‘5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**C ‘5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**D ‘5

DIQMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**E ‘5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKLLIYGASNRFTGVPD
RFTGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**F ‘5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPS
RFSGSGSATDFILTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

Humanised R**K**G ‘5

NIVMTQSPSSVSASVGDRVTITCKASENVGTYVSWYQQKPGTAPKMLIYGASNRFTGVPD
RFTGSGSATDFTLTINNLQPEDFATYYCGQSYTFPYTFGQGTKVEIK

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

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

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

Connection of Linker unit to Ligand unit

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

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

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

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

Methods of Treatment

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

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

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

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

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

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

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

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

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

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

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

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

Drug loading

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

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

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. Higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the Drug Linker (A or B) . 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 (A or B) relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

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

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

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

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

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

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

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

General synthetic routes

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

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

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

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

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

Synthesis route

The Drug Linker compounds of the present invention (A and B) may be synthesised according to the Examples.

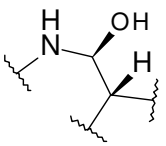
Synthesis of Drug Conjugates

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

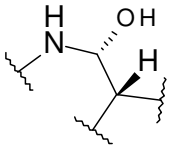
Further Preferences

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

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



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



5

In one embodiment of the present invention, the compound of formula III is A.
In one embodiment of the present invention, the compound of formula III is B.

In one embodiment of the present invention, the Drug Linker unit of formula III is D^L-A.
In one embodiment of the present invention, the Drug Linker unit of formula III is D^L-B.

Examples

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 VWR, U.K. All chemicals were purchased from Aldrich.

Proton NMR chemical shift values were measured on the delta scale at 400 MHz using a Bruker AV400. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; br, broad. Coupling constants are reported in Hz. Column chromatography was performed on an Isolera (Biotage) automated system using normal phase SNAP cartridges.

The LC/MS conditions were as follow:

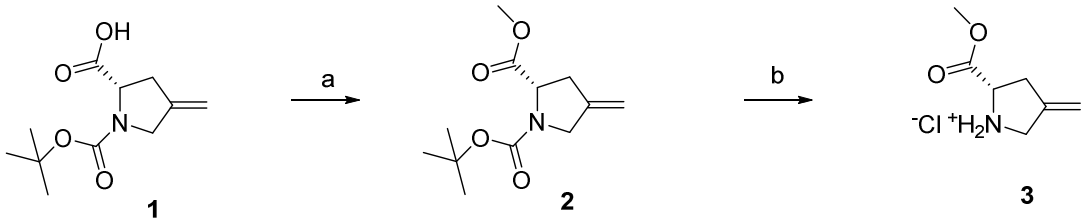
LCMS data were obtained using a Shimadzu Nexera series LC/MS with a Shimadzu LCMS-2020 quadrupole MS, with Electrospray ionisation. Mobile phase A - 0.1% formic acid in water. Mobile phase B - 0.1% formic acid in acetonitrile.

Short run gradient: initial composition was 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.

Long run gradient: initial composition 5% B held over 1 min, then increase from 5% B to 100% B over a 9 min period. The composition was held for 2 min at 100% B, then returned to 5% B in 0.10 minutes and hold there for 3 min. Total gradient run time equals 15 min. Flow rate 0.6 mL/min. Wavelength detection range: 190 to 800 nm. Oven temperature: 50°C. Column: ACE Excel 2 C18-AR, 2 µ, 3.0 x 100mm.

Example 1

(a) (S)-2-(methoxycarbonyl)-4-methylenepyrrolidinium chloride (3)



1 2 3

Commercially available proline derivative (**1**) was obtained from Omegachem

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

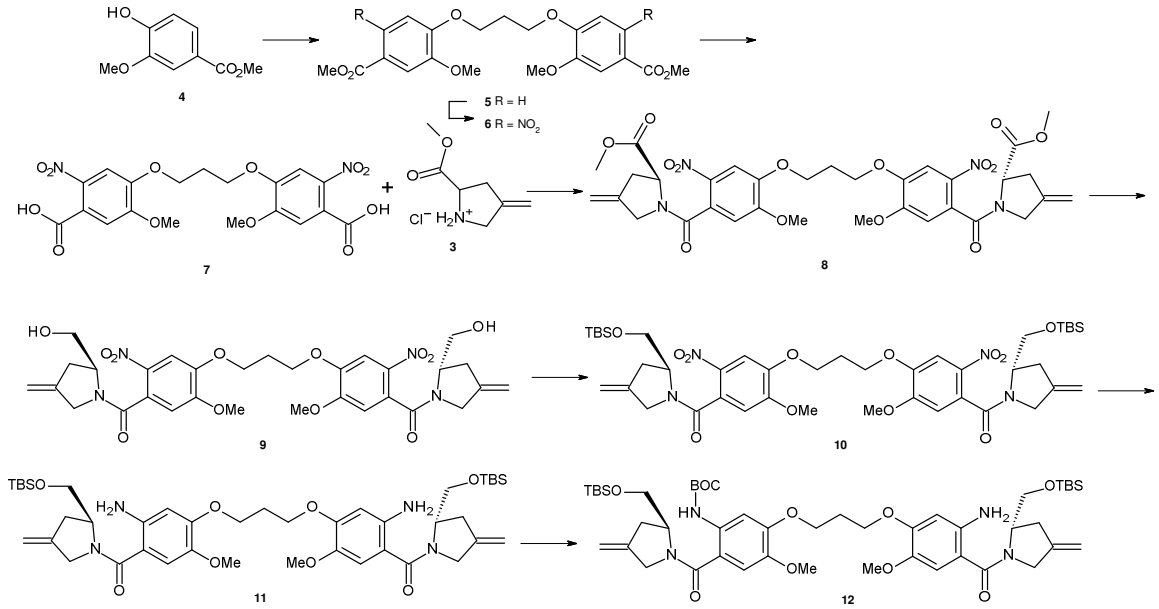
Potassium carbonate (19.92 g, 14 mmol, 3.0 eq.) was added to a stirred solution of the carboxylic acid **1** (10.92 g, 48 mmol, 1.0 eq.) in DMF (270 mL). The resulting white suspension was stirred at room temperature for 30 mins, at which point iodomethane (21.48 g, 9.5 mL, 151 mmol, 3.15 eq.) was added. The reaction mixture was allowed to stir at room temperature for 3 days. The DMF was removed by rotary evaporation under reduced pressure to afford a yellow residue which was partitioned between ethylacetate and water. The organic layer was separated and the aqueous phase was extracted with ethylacetate. The combined organic layers were washed with water brined and dried over magnesium sulphate. The ethylacetate was removed by rotary evaporation under reduced pressure to give the crude product as a yellow oil. The crude product was purified by flash chromatography [85% n-hexane/15% ethylacetate] to afford the product as a colourless oil (10.74 g, 93%).

*(ii) (**S**)-2-(methoxycarbonyl)-4-methylenepyrrolidinium chloride (**3**)*

A solution of 4 M hydrochloric acid in dioxane (63 mL, 254.4 mmol, 4.5 eq.) was added to the Boc protected C-ring fragment **2** (13.67 g, 56.6 mmol, 1.0 eq.) at room temperature. Effervescence was observed indicating liberation of CO_2 and removal of the Boc group. The product precipitated as a white solid and additional dioxane was added to facilitate stirring the reaction mixture was allowed to stir for an hour and then diluted with diethyl ether. The precipitated product was collected by vacuum filtration and washed with

additional diethyl ether. Air drying afforded the desired product as a white powder (9.42 g, 94%).

(b) *tert*-Butyl (5-(3-(5-amino-4-((S)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**12**)



*(i) 1',3'-Bis[2-methoxy-4-(methoxycarbonyl)phenoxy]propane **(5)***

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 **4** (60 g, 329 mmol) and Ph_3P (129.4 g, 494 mmol) in anhydrous THF (800 mL) at 0-5 °C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to stir at 0-5 °C for an additional 1 h after which time a solution of 1,3-propanediol (11.4 mL, 12.0 g, 158 mmol) in THF (12 mL) was added drop-wise over a period of 20 min. The reaction mixture was allowed to warm to room temperature and stirred for 5 days. The resulting white precipitate **3** was collected by vacuum filtration, washed with THF and dried in a vacuum desiccator to constant weight. Yield = 54.68 g (84% based on 1,3-propanediol). Analytical Data: Purity satisfactory by LC/MS 3.20 min (ES+) *m/z* (relative intensity) 427 ($[M + Na]^{+\cdot}$, 10); ^1H NMR (400 MHz, $CDCl_3$) δ δ7.64 (dd, 2H, J = 1.8, 8.3 Hz), 7.54 (d, 2H, J = 1.8 Hz), 6.93 (d, 2H, J = 8.5 Hz), 4.30 (t, 4H, J = 6.1 Hz), 3.90 (s, 6H), 3.89 (s, 6H), 2.40 (p, 2H, J = 6.0 Hz).

(ii) 1',3'-Bis[2-methoxy-4-(methoxycarbonyl)-5-nitrophenoxy]propane (6)

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

*(iii) 1',3'-Bis(4-carboxy-2-methoxy-5-nitrophenoxy) propane (**7**)*

A slurry of the methyl ester **6** (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 **7** was collected and dried thoroughly in a vacuum oven (50 °C). Yield = 54.5 g (87%). Analytical Data: Purity satisfactory by LC/MS 2.65 min (ES+) *m/z* (relative intensity) 489 ($[M + Na]^{+\cdot}$, 30); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (s, 2H), 7.30 (s, 2H), 4.29 (t, 4H, J = 6.0 Hz), 3.85 (s, 6H), 2.30-2.26 (m, 2H).

*(iv) Dimethyl 1,1'-(4,4'-(propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitrobenzoyl))(2**S**,2'**S**)-bis(4-methylenepyrrolidine-2-carboxylate) (**8**)*

A catalytic amount of anhydrous DMF (2.4 mL) was added to a stirred suspension of oxalyl chloride (14.7 g, 9.8 mL, 115.8 mmol, 3 eq.) and dimer core **7** (18 g, 38.6 mmol, 1 eq.) in anhydrous DCM (500 mL) at room temperature. Vigorous effervescence was observed after the addition of DMF and the reaction mixture was allowed to stir for 18 h in a round bottom flask fitted with a calcium chloride drying tube. The resulting clear solution was evaporated under reduced pressure and the solid triturated with ether. The solid product was collected by vacuum filtration, washed with additional ether and dried *in vacuo* at 40 °C for 1.5 h. This solid was then added portion wise to a suspension of the C-ring **3** (15.1 g, 84.9 mmol, 2.2 eq.) and TEA (19.5 g, 27 ml, 119.6 mmol, 5 eq.) in dry DCM (375 mL),

maintaining the temperature between -40 and -50 °C with the aid of a dry ice/acetonitrile bath. The reaction mixture was allowed to stir at -40 °C for 1h and then allowed to warm to room temperature at which point LCMS indicated the complete consumption of the starting material. The reaction mixture was diluted with additional DCM and washed sequentially with aqueous hydrochloric acid (1M, 2 x 200 mL), saturated aqueous sodium bicarbonate (2 x 250 mL), water (250 mL), brine (250 mL), dried ($MgSO_4$). DCM was removed by rotary evaporation under reduced pressure to afford the product as a yellow foam (25.72 g, 94 %). Analytical Data: RT 1.59 min; MS (ES^+) *m/z* (relative intensity) 713 ($[M + H]^{+\cdot}$, 100)

*(v) ((Propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((**S**)-2-(hydroxymethyl)-4-methylenepyrrolidin-1-yl)methanone) (**9**)*

Solid lithium borohydride (3.18 g, 146 mmol, 3 eq.) was added in one portion to a solution of the ester **8** (34.72 g, 48.7 mmol, 1 eq.) in dry THF (350 mL) under a nitrogen atmosphere at 0 ºC (ice bath). The reaction mixture was allowed to stir at 0 °C for 30 mins and then allowed to warm to room temperature at which point precipitation of an orange gum was observed. The reaction mixture was allowed to stir at room temperature for a futher 2 hours and then cooled in an ice bath and treated with water to give a yellow suspension. Hydrochloric acid (1M) was carefully added until effervescence ceased. The reaction mixture was extracted with ethylacetate (x 4) and the combined organic layers were washed with water (x 1), brine (x 1) and dried ($MgSO_4$). Ethylacetate was removed by rotary evaporation under reduced pressure to give a yellow foam. Purification by flash column chromatography [gradient elution DCM/MeOH 0% to 5% in 1% increments] gave the product as a pale yellow foam (23.1 g, 72%). Analytical Data: RT 1.23 min; MS (ES^+) *m/z* (relative intensity) 657 ($[M + H]^{+\cdot}$, 100)

*(vi) ((Propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitro-4,1-phenylene))bis(((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidin-1-yl)methanone) (**10**)*

A solution of the *bis*-alcohol **9** (10 g, 15.2 mmol, 1 eq.), *t*-butyldimethylsilylchloride (5.97 g, 39.6 mmol, 2.6 eq.) and imidazole (5.38 g, 79 mmol, 5.2 eq.) in dry DMF (80 ml) was stirred at room temperature for 3h. The reaction mixture was poured into water (500 mL) to give a yellow precipitate. The mixture was extracted with DCM (4 x 100 mL) and the combined extracts were washed with water and brine, dried ($MgSO_4$) and evaporated under reduced pressure to give a viscous yellow oil. Purification by column chromatography [biotage isolera, gradient elution hexane 60%/EtOAc 40% to EtOAc 100%, 8 column volumes 100 g snap ultra® cartridge] gave the product as a yellow foam (11.8 g,

88%). Analytical Data: RT 2.20 min; MS (ES^+) *m/z* (relative intensity) 885 ($[M + H]^{+\cdot}$, 100), 907 ($[M + Na]^{+\cdot}$, 50)

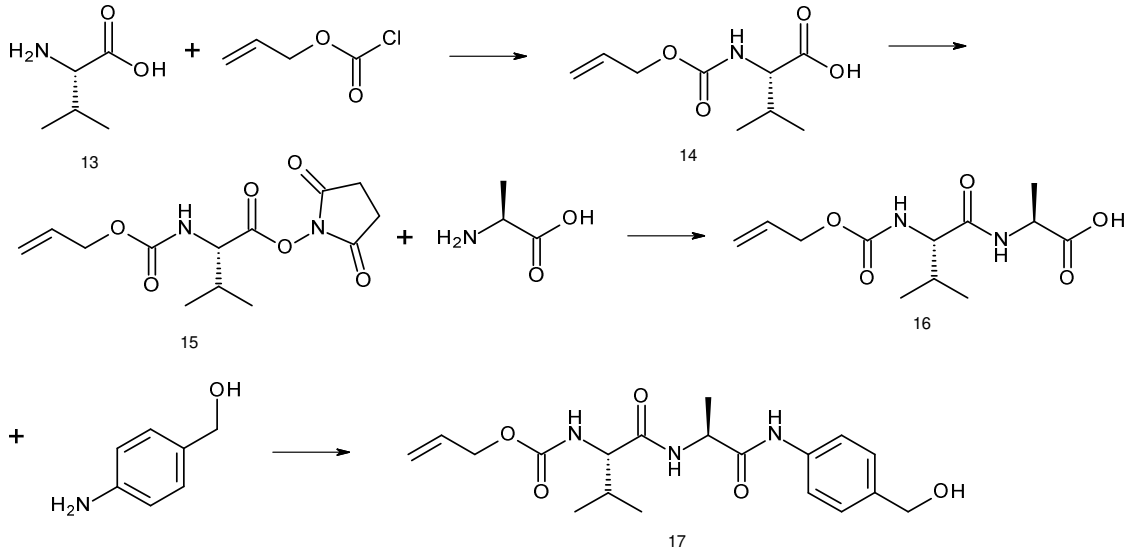
*(vii) ((Propane-1,3-diylbis(oxy))bis(2-amino-5-methoxy-4,1-phenylene))bis(((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidin-1-yl)methanone) (**11**)*

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

*(viii) tert-Butyl (5-(3-(5-amino-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**12**)*

A solution of the *bis*-aniline **11** (3.27 g, 3.96 mmol) and di-*t*-butyldicarbonate (0.85 g, 3.96 mmol) in dry THF (125 mL) were heated under reflux for 24h. The reaction mixture was cooled and the solvent evaporated under reduced pressure. The residue was purified by flash column chromatography [*n*-hexane/EtOAc 50/50 v/v to EtOAc 100% in 10% increments then EtOAc/MeOH 98/2 v/v] to give the desired product as a yellow foam (1.63 g, 44%). Analytical Data: RT 2.28 min; MS (ES^+) *m/z* (relative intensity) 925 ($[M + H]^{+\cdot}$, 70), 947 ($[M + Na]^{+\cdot}$, 100)

(c) Alloc-Val-Ala-PABOH (**17**)



*(i) Alloc-Val-OH (**14**)*

Allyl chloroformate (41 g, 36.2 mL, 0.34 mol, 1.2 eq.) was added dropwise to a stirred solution of L-valine **13** (33.25 g, 0.28 mol, 1 eq.) and potassium carbonate (58.9 g, 0.426 mol, 1.5 eq.) in water (650 mL) and THF (650 mL). The reaction mixture was stirred at room temperature for 18h. The THF was evaporated under reduced pressure and the remaining solution was extracted with diethyl ether (or MTBE) (x 2). The aqueous portion was acidified to pH 2 with conc. HCl and extracted with DCM (x 3). The combined organic extracts were washed with brine (x 1), dried ($MgSO_4$) and evaporated under reduced pressure to give a colourless oil (57.1 g). This was used in the next step without further purification.

*(ii) Alloc-Val-OSu (**15**)*

To a stirred solution of compound **14** (57.1 g, 0.28 mol, 1 eq.) and N-hydroxysuccinimide (32.68 g, 0.28 mol, 1 eq.) in dry THF (800 mL) was added dicyclohexylcarbodiimide (58.6 g, 0.28 mol, 1 eq.). The reaction mixture was stirred at room temperature for 18h. The reaction mixture was filtered. The solid was washed with THF and the combined filtrate was concentrated under reduced pressure. The oil/solid residue was re-dissolved in DCM and left to stand at 0 °C for 30 min. The suspension was filtered washing with cold DCM. Evaporation of the filtrate under reduced pressure gave the succinimide ester as a white solid which was used in the next step without further purification.

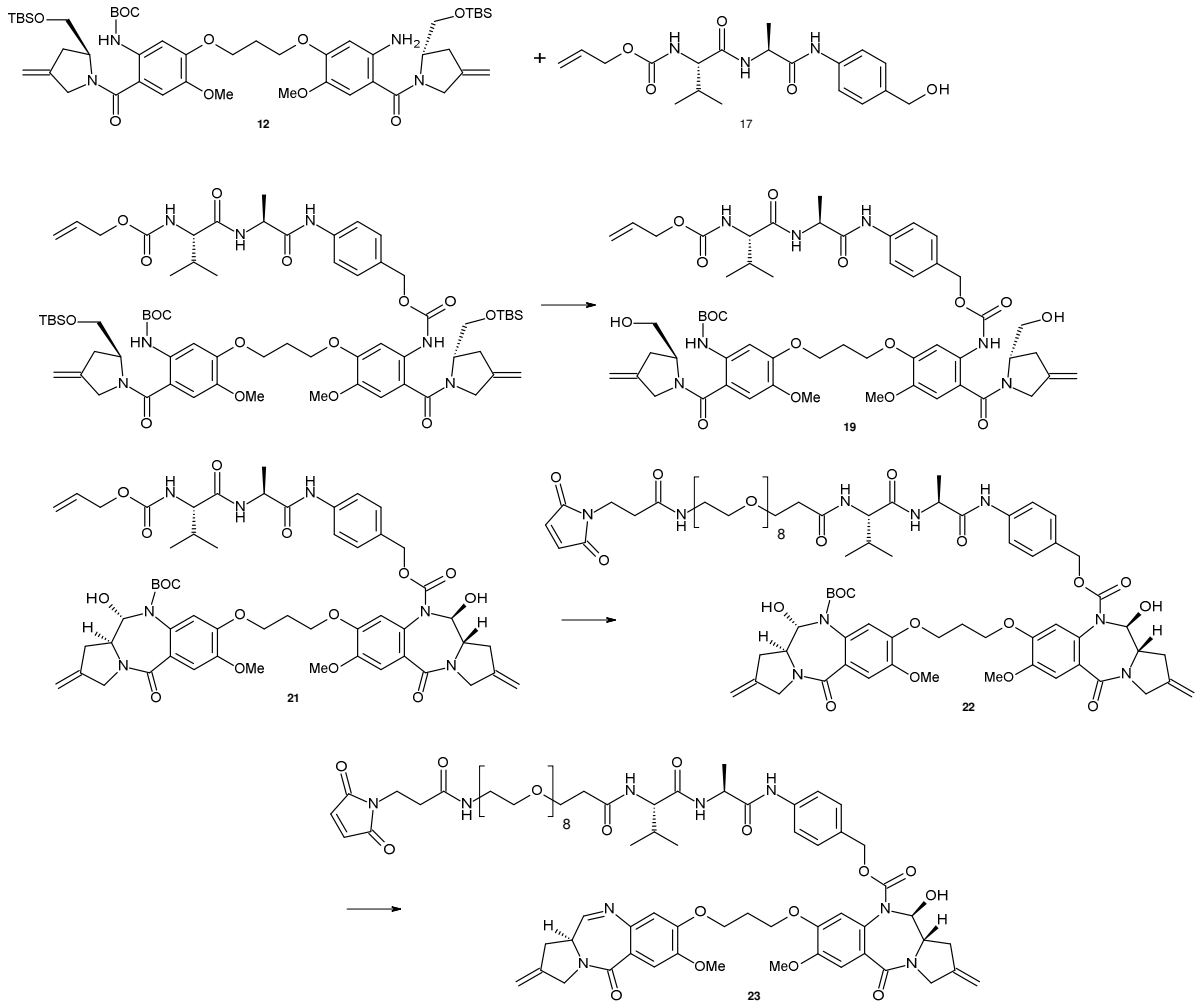
*(iii) Alloc-Val-Ala-OH (**16**)*

A solution of Alloc-Val-OSu **15** (11.67 g, 39.0 mmol, 1 eq.) in THF (50 mL) was added to a solution of H-Ala-OH (3.66 g, 41.08 mmoL, 1.05 eq.) and $NaHCO_3$ (3.61 g, 43.03 mmol, 1.1 eq.) in THF (100 mL) and H_2O (100 mL). The mixture was stirred at room temperature for 72h and the THF was evaporated under reduced pressure. The pH was adjusted to 3 - 4 with citric acid to precipitate a white gum. This was extracted with ethylacetate (6 x 150 mL) and the combined extracts were washed with H_2O (200 mL), brine (200 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. Trituration with diethyl ether (xs) afforded the pure product as a white powder (7.93 g, 74%). Analytical Data: RT 2.17 min; MS (ES^+) *m/z* (relative intensity) 295 ($[M + Na]^{+\cdot}$, 63), 273 ($[M + 1]^{+\cdot}$, 60).

*(iv) Alloc-Val-Ala-PABOH (**17**)*

EEDQ (4.79 g, 19.3 mmol, 1.05 eq.) was added to a solution of *p*-aminobenzyl alcohol (2.38 g, 19.3 mmol, 1.05 eq.) and Alloc-Val-Ala-OH **16** (5.02 g, 18.4 mmol, 1.0eq) in dry THF (100 mL). The mixture was stirred at room temperature for 72h. The solvent was evaporated under reduced pressure to give a pale brown solid. The solid was triturated with diethyl ether and filtered washing with an excess of diethyl ether. This afforded the product as a white solid (6.2 g, 89%). Analytical Data: RT 2.50 min; MS (ES^+) *m/z* (relative intensity) 400.6 ($[M + Na]^{+\cdot}$, 50), 378.6 ($[M + 1]^{+\cdot}$, 60).

(d) 4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl (11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**23**)



*(i) tert-butyl (5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**18**)*

Triethylamine (0.38 g, 0.53 mL, 3.8 mmol, 2.2 eq.) was added to a stirred solution of the mono-boc protected *bis*-aniline (**12**) (1.6 g, 1.72 mmol, 1.0 eq.) and triphosgene (0.184 g, 0.62 mmol, 0.36 eq.) in dry THF (25 mL) under a nitrogen atmosphere at room

temperature. The reaction mixture was heated to 40°C, after 5 min a sample was treated with methanol and analysed by LCMS as the methyl carbamate. Analytical Data: RT 2.32 min; MS (ES^+) *m/z* (relative intensity) 983 ($[M + H]^{+\cdot}$, 55), 1005 ($[M + Na]^{+\cdot}$, 100)
A solution/suspension of the benzyl-alcohol (**17**) (1.52 g, 2.35 mmol, 1.4 eq.) and triethylamine (0.26 g, 0.36 mL 2.6 mmol, 1.5 eq.) in dry THF (40 mL) was run in from a dropping funnel to the freshly prepared isocyanate. The reaction mixture was stirred at 40°C for 2.5h. The reaction mixture was allowed to cool, filtered and the filtrate evaporated to dryness to afford the crude product as a yellow oil which was purified by flash column chromatography [*n*-hexane/EtOAc 50/50 v/v] which gave the product as a yellow glass (1.192 g). The mixed fractions were purified by flash column chromatography [$CHCl_3$/MeOH 0% to 1%] to give a further amount of product (0.22 g). The material was combined to give the product as a yellow foam (1.41 g, 63%). Analytical Data: RT 2.27 min; MS (ES^+) *m/z* (relative intensity) 1328 ($[M + H]^{+\cdot}$,30), 1350 ($[M + Na]^{+\cdot}$, 100)

(ii) tert-butyl (5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (19)
A 1.0M solution of TBAF in THF (2.34 mL, 2.34 mmol, 2.2 eq.) was added to a solution of the *bis*-TBS compound (**18**) (1.41 g, 1.06 mmol, 1.0 eq.) in anhydrous THF (12 mL). The mixture was stirred at room temperature for 30 min., the solvent was removed under reduced pressure and the residue purified by flash column chromatography [$CHCl_3$/MeOH 0% to 4% in 1% increments] to give the desired product as a white foam (0.98 g, 84%). Analytical Data: RT 1.62 min; MS (ES^+) *m/z* (relative intensity) 1100 ($[M + H]^{+\cdot}$,60), 1122 ($[M + Na]^{+\cdot}$, 100)

*(iii) 4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**20**)*
IBX (45 wt%, 1.3 g, 2.09 mmol, 2.4 eq.) was added to a solution of the *bis*-alcohol **19** (0.959 g, 0.87 mmol, 1.0 eq.) in anhydrous DMSO (25 mL). The solution was stirred at 30°C for 18h. LCMS analysis indicated the presence of a small amount of partially cyclised material. A further portion of IBX (45 wt%, 0.049 g, 0.17 mmol, 0.2 eq.) was added and the reaction was continued for a further 18h. The reaction mixture was poured into water (200

mL) and the resultant precipitate was collected by filtration washing with water. The precipitate was dissolved in DCM (150 mL) and washed with saturated $NaHCO_3$ (100 mL), water (100 mL) and brine (100 mL). The organic portion was dried ($MgSO_4$) and evaporated to give a white solid. Purification by flash column chromatography [$CHCl_3$/MeOH 0% to 4% in 1% increments] gave the product as a white solid (0.696 g, 73%). Analytical Data: RT 1.55 min; MS (ES^+) *m/z* (relative intensity) 1096 ($[M + H]^{+\cdot}$,20), 1118 ($[M + Na]^{+\cdot}$, 100)

*(iv) 4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl (11S,11aS)-8-(3-(((11S,11aS)-10-(tert-butoxycarbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**21**)*

$Pd(PPh_3)_4$ (14 mg, 12.28 µmol, 0.02 eq.) was added to a solution of the cyclised product **20** (0.673 g, 0.61 mmol, 1.0 eq.) and pyrrolidine (55 mg, 63 µL, 0.8 mmol, 1.25 eq.)in anhydrous DCM (30 mL). The solution was stirred at room temperature for 30 min. The reaction mixture was diluted with DCM (70 mL) and washed with saturated NH_4Cl (100 mL), saturated brine (100 mL), dried ($MgSO_4$) and evaporated to give an off white foam. The product was triturated with diethyl ether and dried to give the product (0.62 g, 100%) which was used without further purification. Analytical Data: RT 1.16 min; MS (ES^+) *m/z* (relative intensity) 1012 ($[M + H]^{+\cdot}$,80), 1034 ($[M + Na]^{+\cdot}$, 20)

*(v) tert-butyl (11S,11aS)-8-(3-(((11S,11aS)-10-(((4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**22**)*

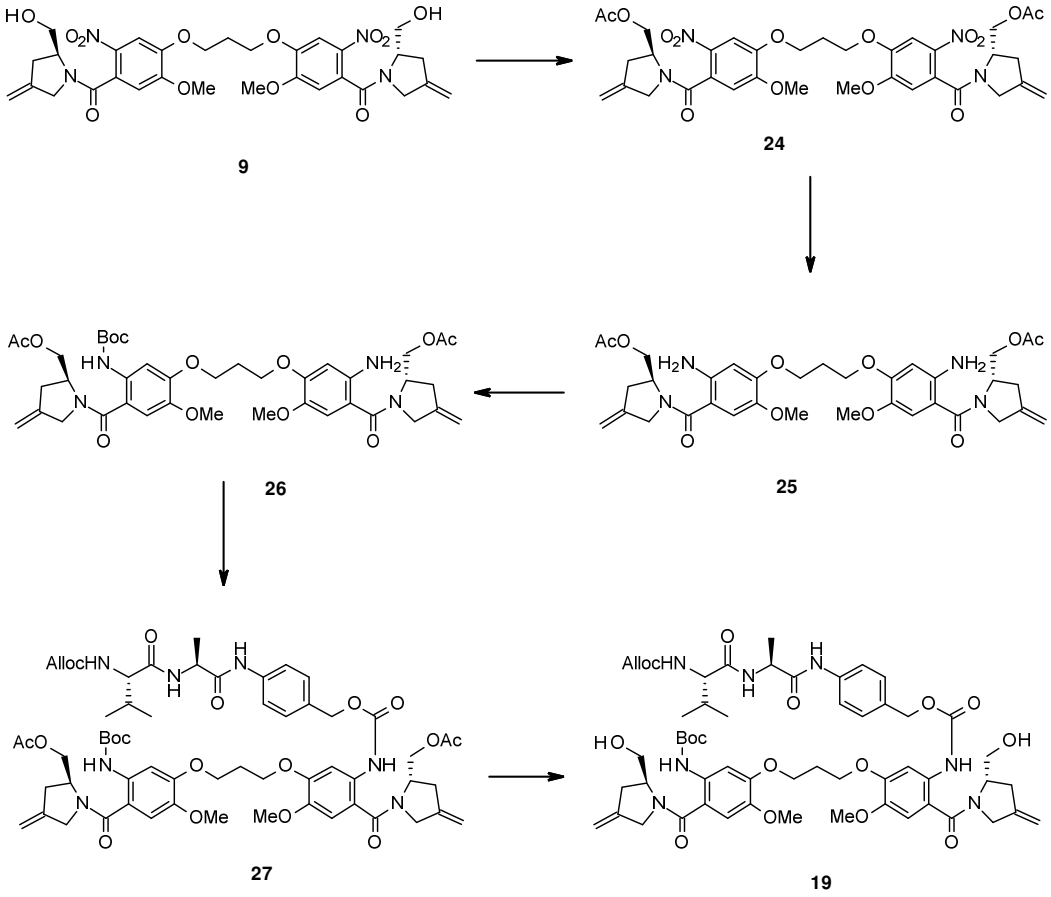
EDCI.HCl (0.13 g, 0.66 mmol, 1.1 eq.) was added to a cloudy solution of compound **21** (0.61 g, 0.6 mmol, 1.0 eq.) and Mal-dPEG$_8$®-OH (0.393 g, 0.66 mmol, 1.1 eq.) in $CHCl_3$ (25 mL). The clear solution was stirred at room temperature for 1.5h., diluted with $CHCl_3$ (100 mL) washed with brine (2 x 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow foam. Purification by flash column chromatography [$CHCl_3$/MeOH 0% to 6% in 1% increments gave the product as a white foam (0.786 g, 82%). Analytical Data: RT 1.44 min; MS (ES^+) *m/z* (relative intensity) 1586 ($[M + H]^{+\cdot}$,40), 1609 ($[M + Na]^{+\cdot}$, 100)

*(vi) 4-((2S,5S)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl (11S,11aS)-11-hydroxy-7-methoxy-8-(3-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**23**)*

Ice cold 95% $TFA_{(aq)}$ solution (10 mL) was added to the Boc protected compound **22** (0.759 g, 0.48 mmol, 1.0eq) which had been cooled to 0°C (ice bath). The yellow solution was stirred at 0°C for 1h. The reaction mixture was poured onto ice/water (200 mL) and the mixture was basified to pH 8 with solid $NaHCO_3$. The mixture was extracted with DCM (4 x 50 mL) and the combined extracts washed with brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by flash column chromatography [$CHCl_3$/MeOH 0% to 8% in 1% increments] to give a pale yellow foam (0.445 g, 65%). Analytical Data: RT 1.37 min; MS (ES^+) *m/z* (relative intensity) 1468 ($[M + H]^{+\cdot}$,40)

Example 2

Alternate synthesis of tert-butyl (5-(3-(5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)-4-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (19)



*(i)((2S,2'S)-(4,4'-(propane-1,3-diylbis(oxy))bis(5-methoxy-2-nitrobenzoyl)) bis(4-methylenepyrrolidine-1,2-diyl))bis(methylene) diacetate (**24**)*

A solution of acetyl chloride (21.1 mL, 23.3 g, 297 mmol) in DCM (100 mL) was added drop-wise over a period of 20 min to a stirred solution of bis-alcohol (**9**) (75 g, 114 mmol) and triethylamine (34.7 g, 343 mmol) in anhydrous DCM (900 mL) at 0-5 °C under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for a further 60 mins. The reaction mixture was washed with ice cold 0.5M HCl (500 mL), saturated aqueous sodium hydrogen carbonate (250 mL), brine (100 mL) and dried ($MgSO_4$). Removal of the solvent by rotary evaporation gave a pale yellow foam which was used in the next step without further purification. Yield = 66.7 g (79%). Analytical Data:

Purity satisfactory by LC/MS (7.60 min (ES+) *m/z* (relative intensity) 741.2 ([*M* + 1]$^{+\cdot}$, 60) 763.3 ([*M* + Na]$^{+\cdot}$, 100)); ^{1}H NMR (400 MHz, $CDCl_3$) δ δ7.73 (s, 2H), 6.83 (s,2H), 5.12 (d, 2H, *J* = 12 Hz), 5.02 (s, 2H), 4.89 (s, 2H), 4.79 (m, 2H), 4.61 (m, 1H), 4.35 (m, 6H), 3.98 (s, 6H), 3.87 (d, 1H, *J* = 4.0 Hz), 3.76 (m, 3H), 2.87-2.83 (m, 2H), 2.56-2.43 (m, 4H), 2.05 (s, 4H), 1.96 (s, 2H).

*(ii) ((2S,2'S)-(4,4'-(propane-1,3-diylbis(oxy))bis(2-amino-5-methoxybenzoyl)) bis(4-methylenepyrrolidine-1,2-diyl))bis(methylene) diacetate (**25**)*

A 10% solution of formic acid in methanol (500 mL) was added in one go, via a separating funnel, to a solution of the bis-alcohol **(24)** (66 g, 0.09 mol) in methanol (1000 mL) containing zinc* (145 g, 2.22 mol) at room temperature. The temperature of the reaction mixture rapidly rose to 42°C and was then cooled back to room temperature with the aid of a cold water bath. The excess zinc was removed by filtering through a short bed of celite, which was then washed with ethyl acetate (100 mL). The filtrate was diluted with ethyl acetate (1400 mL) and washed with saturated sodium hydrogen carbonate (1500 mL), water (500 mL), brine (100 mL) and dried ($MgSO_4$). Removal of the solvent by rotary evaporation gave a yellow solid which was purified by column chromatography (4% methanol / DCM) to give the product as a pale yellow foam. Yield = 38.1 g (63%). Analytical Data: Purity satisfactory by LC/MS (6.61 min (ES+) *m/z* (relative intensity) 681.2 ([*M* + 1]$^{+\cdot}$, 100)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 2H), 6.31 (s, 2H), 5.02 (bs, 2H), 4.97 (bs, 2H), 4.80 (s, 2H), 4.33-4.10 (m, 16H), 3.78 (s, 3H), 2.78 (m, 2H), 2.46 (m, 2H), 2.34 (m, 2H), 2.04 (s, 6H).

*(iii) ((S)-1-(4-(3-(4-((S)-2-(acetoxymethyl)-4-methylenepyrrolidine-1-carbonyl)-5-((tert-butoxycarbonyl)amino)-2-methoxyphenoxy)propoxy)-2-amino-5-methoxybenzoyl)-4-methylenepyrrolidin-2-yl)methyl acetate (**26**)*

Boc anhydride (21.6 g, 31.7 mmol) was added to a solution of the diamine (**25**) (6.92 g 31.7 mmol) in THF (200 mL) at room temperature. The resulting solution was then heated at reflux for 3 hours, cooled and evaporated to dryness under reduced pressure. The resulting residue was purified by column chromatography (70-100% ethyl acetate / hexane) to give the product as a pale yellow solid. Yield = 8.4 g (34%).Analytical Data: Purity satisfactory by LC/MS (3min run) (1.64 min (ES+) *m/z* (relative intensity) 781.2 ([*M* + Na]$^{+\cdot}$, 30)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (bs, 1H), 7.87 (s, 1H), 6.80 (s, 1H), 6.73 (s, 1H), 5.02 (m, 3H), 4.79 (3H), 4.34-4.09 (m, 14H), 3.83 (s, 3H), 3.78 (s, 3H), 2.81-2.74 (m, 2H), 2.48-2.36 (m, 4H), 2.04 (m, 7H), 1.49 (s, 9H).

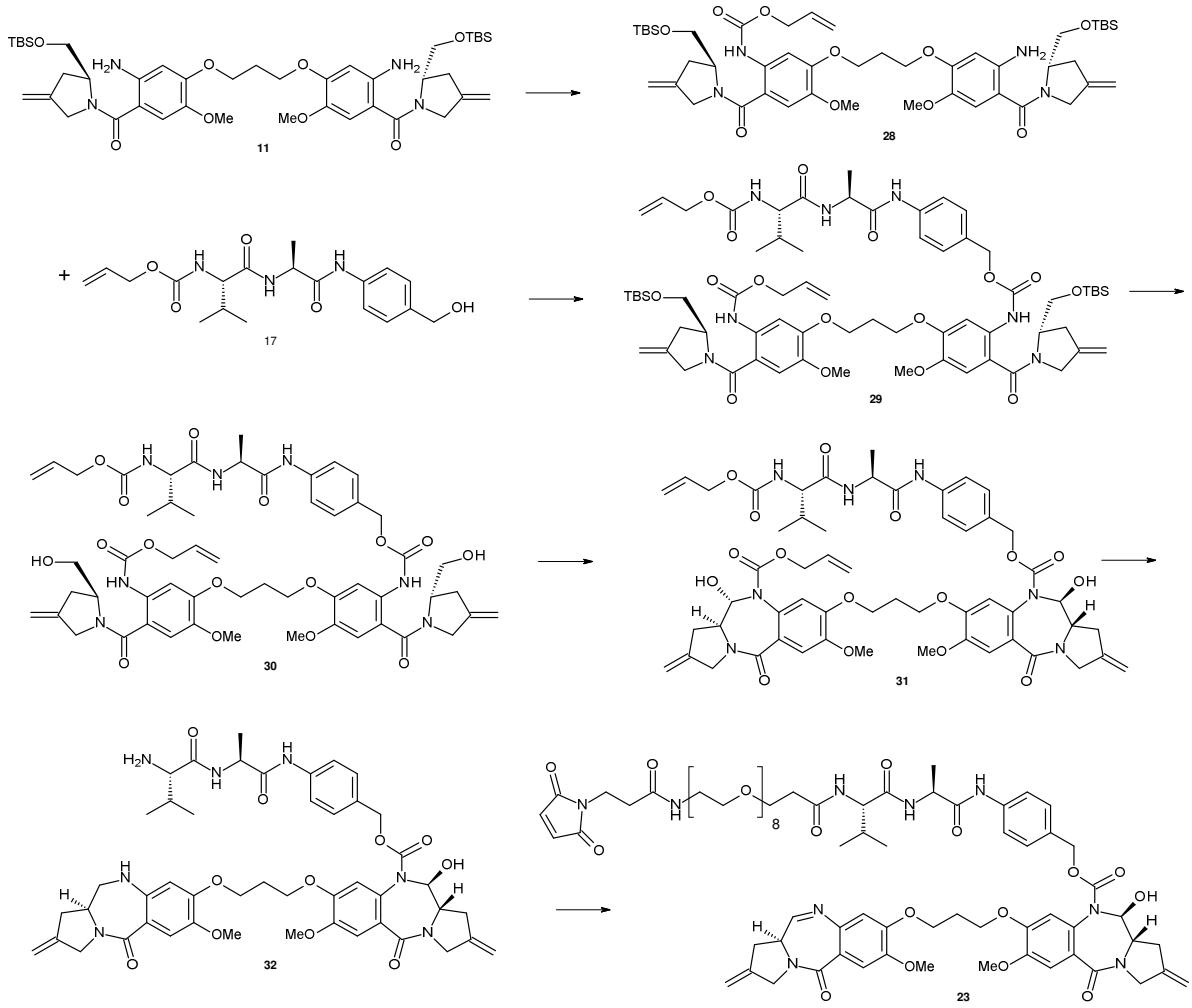
*(iv) ((S)-1-(4-(3-(4-((S)-2-(acetoxymethyl)-4-methylenepyrrolidine-1-carbonyl)-5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl) oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-2-((tert-butoxycarbonyl)amino)-5-methoxybenzoyl)-4-methylenepyrrolidin-2-yl)methyl acetate (**27**)*

Triethyl amine (0.57 g, 5.6 mmol) was added in one go to a solution of the amine (**26**) (2 g, 2.56 mmol) and triphosgene (0.27 g, 0.92 mmol) in THF (30 mL) under nitrogen. The resulting mixture was heated at 40°C for 5 min. A small aliquot was quenched with methanol, and LCMS indicated complete conversion to the methyl carbamate (*m/z* 983, M+1). A slurry of SG3366 (2.25 g, 3.48 mmol) and triethyl amine (0.39 g, 3.84 mmol) in THF (50 mL) was added in one go and the resulting mixture heated at 40°C for 4 hours. After cooling, the white solid was removed by filtration and the filtrate evaporated to dryness under reduced pressure, and purified by column chromatography (1-3% methanol / DCM) to give the product as a pale yellow solid. Yield = 2.1 g (69%). Analytical Data: Purity satisfactory by LC/MS (8.26 min (ES+) *m/z* (relative intensity) 1184.3 ([M+1]$^+$, 70), 1206.3 ([*M* + Na]$^{+\cdot}$, 100)); ^{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).

*(v) ((S)-1-(4-(3-(4-((S)-2-(acetoxymethyl)-4-methylenepyrrolidine-1-carbonyl)-5-((((4-((S)-2-((S)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl) oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-2-((tert-butoxycarbonyl)amino)-5-methoxybenzoyl)-4-methylenepyrrolidin-2-yl)methyl acetate (**19**)*

Potassium carbonate (1.16 g, 8.44 mmol) was dissolved in water (8.4 mL) and added to a solution of the diacetate (**27**) (2.0 g, 1.69 mmol) in methanol (40 mL). The resulting mixture was stirred at 25°C for 30 mins, then evaporated to dryness under reduced pressure. The resulting residue was taken up in water (100 mL), acidified (pH 3) with 1M citric acid and extracted with ethyl acetate (3 x 100 mL). The combined extracts were washed with water (100 mL), brine (30 mL) and dried ($MgSO_4$). Removal of the solvent under reduced pressure left the product as an off-white solid which was used in the next step without further purification. Yield = 1.6 g (87%). **Analytical Data:** Purity satisfactory by LC/MS (7.32 min (ES+) *m/z* (relative intensity) 1100.7 ([M+1]$^+$, 50), 1122.3 ([*M* + Na]$^{+\cdot}$, 100)); ^{1}H NMR (400 MHz, DMSO-d_6) δ9.98 (bs, 1H), 9.09 (bs, 1H), 8.73 (bs, 1H), 8.14 (d, *J* = 8 Hz, 1H), 7.59 (d, *J* = 8 Hz, 2H), 7.33 (d, *J* = 8 Hz, 2H), 7.21 (m, 3H), 6.90 (bs, 2H), 5.91 (m, 1H), 5.30 (d, *J* = 4 Hz, 1H), 5.19 (d, *J* = 4 Hz, 1H), 5.00 (m, 6H), 4.70 – 4.35 (m, 6H), 4.15 – 3.88 (m, 12H), 3.77 (s, 3H), 3.67 (s, 3H), 2.82 – 2.67 (m, 2H), 2.42 (m, 3H), 2.21 (t, *J* = 4Hz, 2H), 1.98 (m, 6H), 1.42 (s, 9H), 1.31 (d, *J* = 8 Hz, 3H), 0.90 (d, *J* = 4 Hz, 3H), 0.84 (d, *J* = 4Hz, 3H).

Example 3



*(a) Allyl (5-(3-(5-amino-4-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**28**)*

A solution of allyl chloroformate (1.05 g, 0.9 mL, 8.7 mmol, 0.9 eq.) was added drop wise to a solution of *bis* aniline (**11**)(8.02 g, 9.7 mmol, 1 eq.) and pyridine (1.15 g, 1.2 mL, 14.55 mmol, 1.9 eq.) in dry DCM (350 mL) at -78 °C (dry ice/acetone bath). The reaction mixture was stirred at -78°C for 1 hour and then allowed to reach room temperature. The reaction mixture was washed with saturated aqueous copper sulphate solution (250 mL), water (250 mL), saturated sodium bicarbonate (250 mL), brine (250 mL) and dried ($MgSO_4$). Rotary evaporation under reduced pressure afforded the crude product. Purification by flash chromatography [50% *n*-hexane/50% ethyl acetate, to 20% *n*-

hexane/80% ethyl acetate to 100% ethyl acetate to 1% methanol/99% ethyl acetate] gave the *bis*-alloc product (2.066 g), the desired *mono*-alloc product (4.33 g, 49%) and recovered *bis*-aniline (1.96 g). Analytical Data: RT 2.26 min; MS (ES^+) *m/z* (relative intensity) 909 ($[M + 1]^{+\cdot}$, 100); 931 ($[M + Na]^{+\cdot}$, 100)

*(b) 4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-(3-(5-(((allyloxy)carbonyl)amino)-4-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**29**)*

Triethylamine (1.22 g, 1.7 mL, 12.1 mmol, 2.2 eq.) was added to a stirred solution of the *mono*-boc protected *bis*-aniline (**28**) (5.0 g, 5.5 mmol, 1.0 eq.) and triphosgene (0.59 g, 1.98 mmol, 0.36 eq.) in dry THF (75 mL) under a nitrogen atmosphere at room temperature. The reaction mixture was heated to 40 °C, after 5 min a sample was treated with methanol and analysed by LCMS as the methyl carbamate. Analytical Data: RT 2.30 min; MS (ES^+) *m/z* (relative intensity) 967 ($[M + H]^{+\cdot}$, 25), 989 ($[M + Na]^{+\cdot}$, 100)

A solution/suspension of the benzyl-alcohol (**17**) (3.11 g, 8.25 mmol, 1.5 eq.) and triethylamine (0.83 g, 1.1 mL 2.6 mmol, 1.5 eq.) in dry THF (75 mL) was run in from a dropping funnel to the freshly prepared isocyanate. The reaction mixture was stirred at 40°C for 5h. then overnight at room temperature The reaction mixture was allowed to cool, filtered and the filtrate evaporated to dryness to afford the crude product as a yellow oil which was purified by flash column chromatography [50% *n*-hexane/50% ethyl acetate to 40% *n*-hexane/60% ethyl acetate] which gave the product as a yellow glass (1.25 g, 17%). Analytical Data: RT 2.26 min; MS (ES^+) *m/z* (relative intensity) 1312 ($[M + H]^{+\cdot}$, 25), 1335 ($[M + Na]^{+\cdot}$, 35)

*(c) 4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (5-(3-(5-(((allyloxy)carbonyl)amino)-4-((**S**)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((S)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**30**)*

A 1.0M solution of TBAF in THF (5.2 mL, 5.2 mmol, 2.2 eq.) was added to a solution of the *bis* TBS compound (**29**) (3.096 g, 2.36 mmol, 1.0 eq.) in anhydrous THF (25 mL). The mixture was stirred at room temperature for 30 min., the solvent was removed under reduced pressure and the residue purified by flash column chromatography [ethyl acetate/methanol 0% to 6% in 1% increments] which gave the desired product as a white

foam (1.91 g, 75%). Analytical Data: RT 1.56 min; MS (ES^+) *m/z* (relative intensity) 1084 ([*M* + H]$^{+\cdot}$, 100), 1106 ([*M* + Na]$^{+\cdot}$, 90)

*(d) Allyl (11**S**,11a**S**)-8-(3-(((11**S**,11a**S**)-10-(((4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**31**)*

IBX (45 wt%, 2.06 g, 3.3 mmol, 2.4 eq.) was added to a solution of the *bis*-alcohol **30** (1.49 g, 1.38 mmol, 1.0 eq.) in anhydrous DMSO (40 mL). The solution was stirred at 30°C for 18h. LCMS analysis indicated the presence of a small amount of partially cyclised material. A further portion of IBX (45 wt%, 0.171 g, 0.275 mmol, 0.2 eq.) was added and the reaction was continued for a further 24h. The reaction mixture was poured into water (200 mL) and the resultant precipitate was collected by filtration washing with water. The precipitate was dissolved in DCM (150 mL) and washed with saturated $NaHCO_3$ (100 mL), water (100 mL) and brine (100 mL). The organic portion was dried ($MgSO_4$) and evaporated to give a white solid. Purification by flash column chromatography [$CHCl_3$/MeOH 0% to 3% in 1% increments] gave the product as a white solid (1.06 g, 72%). Analytical Data: RT 6.88 min; MS (ES^+) *m/z* (relative intensity) 1080 ([*M* + H]$^{+\cdot}$, 50), 1102 ([*M* + Na]$^{+\cdot}$, 100)

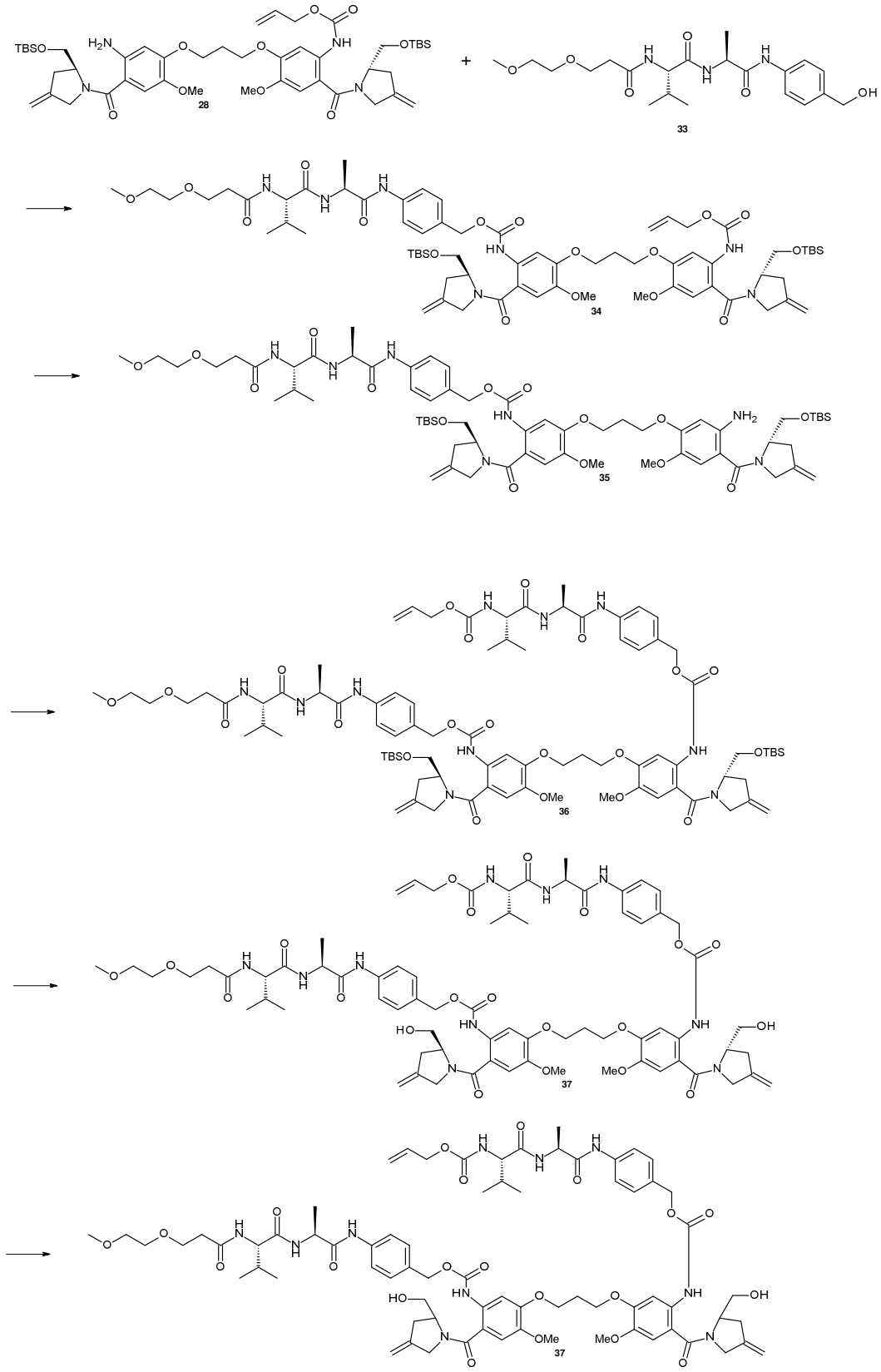
*(e) 4-((**S**)-2-((**S**)-2-amino-3-methylbutanamido)propanamido)benzyl (11**S**,11a**S**)-11-hydroxy-7-methoxy-8-(3-(((**S**)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**32**)*

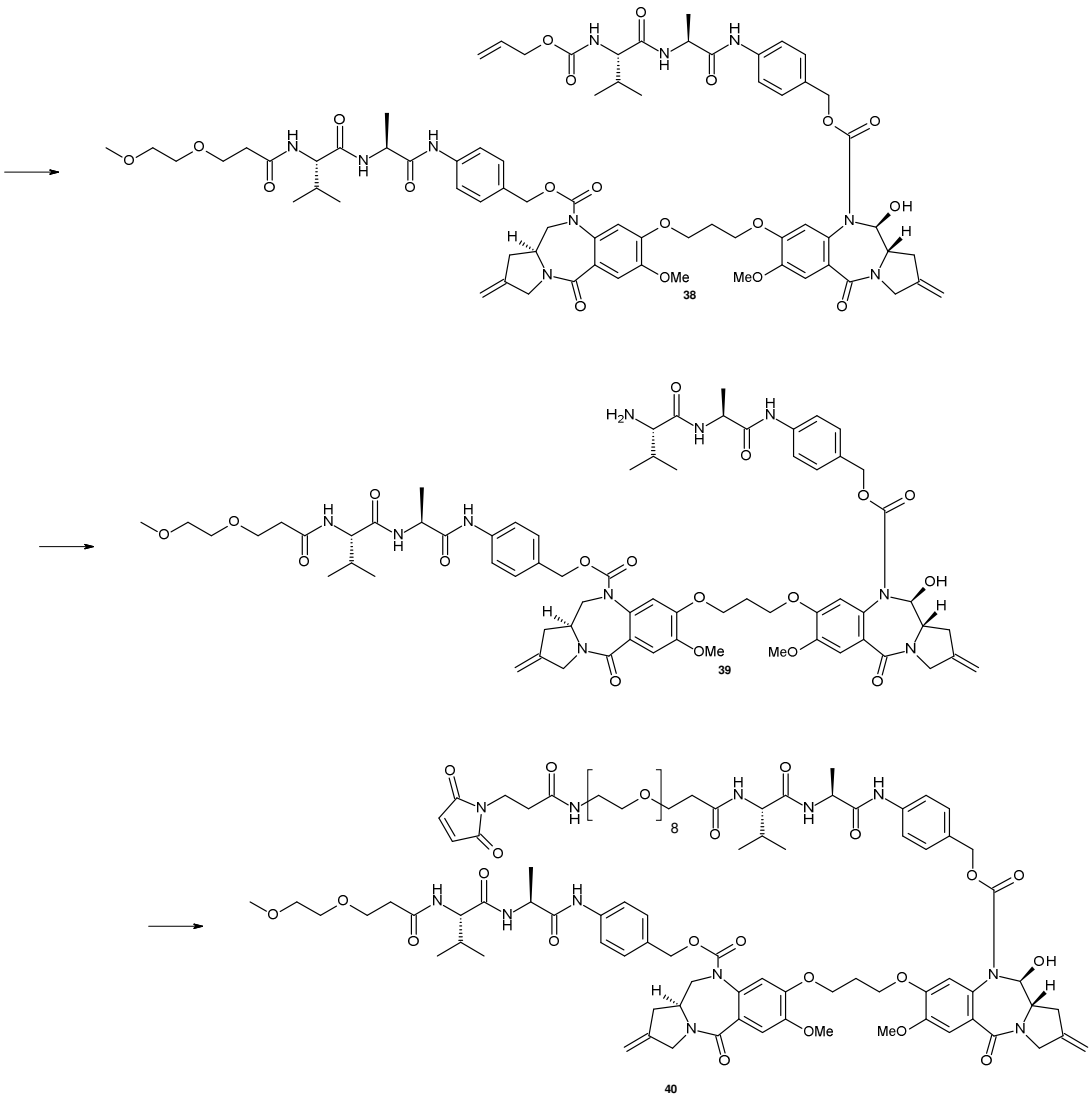
$Pd(PPh_3)_4$ (44 mg, 38.5 µmol, 0.04 eq.) was added to a solution of the cyclised product **31** (1.04 g, 0.96 mmol, 1.0 eq.) and pyrrolidine (0.171 mg, 196 µL, 2.4 mmol, 2.5 eq.) in anhydrous DCM (30 mL). The solution was stirred at room temperature for 30 min. The reaction mixture was diluted with DCM (30 mL) and washed with saturated NH_4Cl (100 mL), saturated brine (100 mL), dried ($MgSO_4$) and evaporated to give an off white foam. The product was triturated with diethyl ether and dried to give the product (0.86 g, 100%) which was used without further purification. Analytical Data: RT 1.10 min; MS (ES^+) *m/z* (relative intensity) 894 ([*M* + H]$^{+\cdot}$, 30)

*(f) 4-((2**S**,5**S**)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl (11**S**,11a**S**)-11-hydroxy-7-methoxy-8-(3-(((**S**)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**23**)*

EDCI.HCl (0.203 g, 1.06 mmol, 1.1 eq.) was added to a solution of compound **32** (0.86 g, 0.96 mmol, 1.0 eq.) and Mal-dPEG$_8$®-OH (0.57 g, 0.96 mmol, 1.1 eq.) in dry DCM (30 mL) and $CHCl_3$ (to give a clear solution). The clear solution was stirred at room temperature for 18h. then a further portion of EDCI.HCl (0.037 g, 0.19 mmol, 0.2 eq.) was added and reaction continued for a further 24h. The reaction mixture was diluted with DCM (70 mL) washed with water (100 mL), brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow foam. Purification by flash column chromatography [$CHCl_3$/MeOH 0% to 6% in 1% increments] gave the product as an off white foam (0.56 g, 40%). Analytical Data: RT 6.13 min; MS (ES^+) *m/z* (relative intensity) 1468 ($[M + H]^{+\cdot}$, 20)

Example 4





*(a) Allyl (2-((**R**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-5-(3-(4-((**R**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-5-((((4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-4-methoxyphenyl)carbamate (**34**)*

Triethylamine (0.049 g, 0.07 mL, 0.48 mmol, 2.2 eq.) was added to a stirred solution of the *mono*-alloc protected *bis*-aniline (**23**) (0.2 g, 0.22 mmol, 1.0 eq.) and triphosgene (0.024 g, 0.079 mmol, 0.36 eq.) in dry THF (5 mL) under an argon atmosphere at room temperature. The reaction mixture was heated to 40°C, after 5 min a sample was treated with methanol and analysed by LCMS as the methyl carbamate. Analytical Data: RT 2.27 min; MS (ES^+) *m/z* (relative intensity) 967 ($[M + H]^{+\cdot}$, 80), 989 ($[M + Na]^{+\cdot}$, 100)

A solution/suspension of the benzyl-alcohol (**33**) (0.121 g, 0.29 mmol, 1.3 eq.) and triethylamine (0.029 g, 0.04 mL 0.29 mmol, 1.3 eq.) in dry THF (5 mL) was run in from a dropping funnel to the freshly prepared isocyanate. The reaction mixture was stirred at 40°C for 4h. then overnight at room temperature The reaction mixture was allowed to cool, filtered and the filtrate evaporated to dryness to afford the crude product which was purified by flash column chromatography [Biotage Isolera™ $CHCl_3$/MeOH 2% to 4%, gradient elution]. This gave the product (0.237 g, 79%). Analytical Data: RT 2.19 min; MS (ES^+) *m/z* (relative intensity) 1358 ($[M + H]^{+\cdot}$, 30), 1380 ($[M + Na]^{+\cdot}$, 15)

*(b) 4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl (5-(3-(5-amino-4-((**R**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-2-methoxyphenoxy)propoxy)-2-((**R**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-4-methoxyphenyl)carbamate (**35**)*

$Pd(PPh_3)_4$ (0.3 g, 0.25 mmol, 0.06 eq.) was added to a solution of the alloc protected intermediate **34** (5.89 g, 4.3 mmol, 1.0 eq.) and pyrrolidine (0.46 g, 530 µL, 6.5 mmol, 1.5 eq.) in anhydrous DCM (50 mL). The solution was stirred at room temperature for 1h. The reaction mixture was diluted with DCM and washed with saturated NH_4Cl, saturated brine, dried ($MgSO_4$) and evaporated to give crude product. The product was purified by flash column chromatography [Biotage Isolera™ DCM/MeOH 1% to 3%] to give the product (4.53 g, 83%) which had an overall purity of 80% and was used without further purification. Analytical Data: RT 2.10 min; MS (ES^+) *m/z* (relative intensity) 1275 ($[M + H]^{+\cdot}$, 40).

*(c) 4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (2-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-5-(3-(4-((**S**)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenepyrrolidine-1-carbonyl)-5-((((4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-4-methoxyphenyl)carbamate (**36**)*

Triethylamine (0.35 g, 48 µL, 0.34 mmol, 2.2 eq.) was added to a stirred solution of the aniline (**35**) (0.2 g, 0.157 mmol, 1.0 eq.) and triphosgene (0.017 g, 57 µmol, 0.36 eq.) in dry THF (5 mL) under an argon atmosphere at room temperature. The reaction mixture was heated to 40°C, after 5 min a sample was treated with methanol and analysed by LCMS as the methyl carbamate. Analytical Data: RT 2.15 min; MS (ES^+) *m/z* (relative intensity) 1333 ($[M + H]^{+\cdot}$, 40), 1354 ($[M + Na]^{+\cdot}$, 35).

A solution/suspension of the benzyl-alcohol (**17**) (0.071 g, 0.19 mmol, 1.2 eq.) and triethylamine (19 mg, 26 µL 0.19 mmol, 1.2 eq.) in dry THF (5 mL) was run in from a dropping funnel to the freshly prepared isocyanate. The reaction mixture was stirred at 40°C for 4h. then overnight at room temperature The reaction mixture was filtered and the filtrate evaporated to dryness to afford the crude product which was purified by flash column chromatography [Biotage Isolera™ $CHCl_3$/MeOH 2% to 3%, gradient elution] which gave the product (0.152 g, 58%). Analytical Data: RT 2.12 min; MS (ES^+) *m/z* (relative intensity) 1677 ($[M + H]^{+\cdot}$, 30), 1700 ($[M + Na]^{+\cdot}$, 100).

*(d) 4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (2-((**S**)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-5-(3-(4-((**S**)-2-(hydroxymethyl)-4-methylenepyrrolidine-1-carbonyl)-5-((((4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)amino)-2-methoxyphenoxy)propoxy)-4-methoxyphenyl)carbamate (**37**)*

A 1.0M solution of TBAF in THF (3.4 mL, 3.4 mmol, 2.0 eq.) was added to a solution of the *bis* TBS compound (**36**) (2.86 g, 1.7 mmol, 1.0 eq.) in anhydrous THF (30 mL) under an argon atmosphere. The mixture was stirred at room temperature for 6h., the reaction mixture was diluted with $CHCl_3$ and washed with water, brine, dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow solid. The residue was purified by flash column chromatography [Biotage Isolera™ $CHCl_3$/MeOH, gradient elution with the product eluting at 4% MeOH] which gave the desired product (1.365 g) and mixed fractions which were further purified by flash column chromatography [$CHCl_3$/MeOH 1% to 5%] to give further product (0.562 g) this gave a combined yield of desired product (1.93 g, 75%). Analytical Data: RT 1.55 min; MS (ES^+) *m/z* (relative intensity) 1449 ($[M + 1]^{+\cdot}$, 25); 1471 ($[M + Na]^{+\cdot}$, 20).

*(e) 4-((**S**)-2-((**S**)-2-(((allyloxy)carbonyl)amino)-3-methylbutanamido)propanamido)benzyl (11**S**,11a**S**)-11-hydroxy-8-(3-(((**S**)-10-(((4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**38**)*

IBX (45 wt%, 0.236 g, 0.38 mmol, 2.2 eq.) was added to a solution of the *bis*-alcohol **37** (0.25 g, 0.17 mmol, 1.0 eq.) in anhydrous DMSO (12 mL). The solution was stirred at 30°C for 3.5d. The reaction mixture was poured into water (100 mL) and the resultant precipitate was collected by filtration washing with water. The precipitate was extracted with DCM (5 x

30 mL) and the combined fractions were washed with saturated $NaHCO_3$ (60 mL), water (60 mL) and brine (60 mL). The organic portion was dried ($MgSO_4$) and evaporated to give crude product. Purification by flash column chromatography [$CHCl_3$/MeOH 1% to 5%] gave the product as a white solid (0.158 g, 64%). Analytical Data: RT 1.53 min; MS (ES^+) *m/z* (relative intensity) 1445 ([*M* + 1]$^{+\cdot}$, 20); 1467 ([*M* + Na]$^{+\cdot}$, 30).

*(f) 4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl (11S,11aS)-11-hydroxy-8-(3-(((11S,11aS)-11-hydroxy-10-(((4-((10S,13S)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**39**)*

$Pd(PPh_3)_4$ (8 mg, 6.9 µmol, 0.06 eq.) was added to a solution of the cyclised product **38** (0.158 g, 0.109 mmol, 1.0 eq.) and pyrrolidine (0.01 g, 12 µL, 0.15 mmol, 1.5 eq.) in anhydrous DCM (10 mL). The solution was stirred at room temperature for 15 min. The reaction mixture was diluted with $CHCl_3$ and washed with saturated sodium bicarbonate solution, saturated brine, dried ($MgSO_4$) and evaporated to give crude product. The product was triturated with diethyl ether (x 3) and dried to give the product (0.136 g, 100%) which was used without further purification. Analytical Data: RT 1.21 min; MS (ES^+) *m/z* (relative intensity) 1361 ([*M* + 1]$^{+\cdot}$, 50); 1384 ([*M* + Na]$^{+\cdot}$, 10).

*(g) 4-((2**S**,5**S**)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl (11**S**,11a**S**)-11-hydroxy-8-(3-(((11**S**,11a**S**)-11-hydroxy-10-(((4-((10**S**,13**S**)-10-isopropyl-13-methyl-8,11-dioxo-2,5-dioxa-9,12-diazatetradecan-14-amido)benzyl)oxy)carbonyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepin-8-yl)oxy)propoxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-10(5H)-carboxylate (**40**)*

A solution of compound **39** (0.136 g, 0.1 mmol, 1.0 eq.), Mal-$dPEG_8$®-OH (0.066 g, 0.11 mmol, 1.1 eq.) and EDCI.HCl (0.022 g, 0.11 mmol, 1.1 eq.) in dry DCM (10 mL) and MeOH (1 drop) was stirred at room temperature for 1.45h. The reaction mixture was diluted with $CHCl_3$ and washed with water, brine, dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product. Purification by flash column chromatography [$CHCl_3$/MeOH 1% to 9%] gave the product as a white solid (0.123 g, 63%). Analytical Data: $[\alpha]^{21}_D$ = +112.5° (*c* = 0.4, hplc $CHCl_3$); RT 6.37 min; MS (ES^+) *m/z* (relative intensity) 1936 ([*M* + 1]$^{+\cdot}$, 35); 1958 ([*M* + Na]$^{+\cdot}$, 15).

Example 5 - Conjugation

Conjugate trastuzumab-23

A 50 mM solution of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in phosphate-buffered saline pH 7.4 (PBS) was added (50 molar equivalent/antibody, 35 micromoles, 700 µL) to a 24.14 mL solution of antibody, trastuzumab, (105 mg, 700 nanomoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4.35 mg/mL. The reduction mixture was heated at +37°C for 3 hours (or until full reduction is observed by UHPLC) in an incubator with gentle (<150 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS pH 7.4 and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 10 molar equivalent/antibody, 7 micromoles, 140 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (< 150 rpm) shaking at an antibody concentration of 2.3 mg/mL (or more DHAA added and reaction left for longer until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS pH 7.4, 1 mM EDTA for a final antibody concentration of 1.0-1.5 mg/mL. Compound 23 (SG3400) was added as a DMSO solution (10 molar equivalent/antibody, 1 micromole, in 1.0 mL DMSO) to 9 mL of this reoxidised antibody solution (15 mg, 100 nanomoles) for a 10% (*v/v*) final DMSO concentration. The solution was mixed for 1.5 hours at room temperature, then the conjugation was quenched by addition of *N*-acetyl cysteine (4 micromoles, 40 µL at 100 mM), diluted to >50 mL in PBS and conjugate trastuzumab-23 was purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered and analysed. UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 mm x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of conjugate trastuzumab-A at 280 nm and 330 nm (Compound A specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of compound 23, consistent with a drug-per-antibody ratio (DAR) of 1.71 molecules of compound 23 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel G3000SWXL 5 µm 7.8 x 300 mm column (with a 7 µm 6.0 x 40 mm guard column) eluting with sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of conjugate trastuzumab-23 at

280 nm shows a monomer purity of 94%. UHPLC SEC analysis gives a concentration of final conjugate trastuzumab-23 at 0.84 mg/mL in 15 mL, obtained mass of conjugate trastuzumab-23 is 12.7 mg (84% yield).

Conjugate trastuzumab-40

A 50 mM solution of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in phosphate-buffered saline pH 7.4 (PBS) was added trastuzumab, (50 molar equivalent/antibody, 50 micromoles, 1.0 mL) to a 34.5 mL solution of antibody (150 mg, 1.0 micromole) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) at a final antibody concentration of 4.35 mg/mL. The reduction mixture was heated at +37°C for 3 hours (or until full reduction is observed by UHPLC) in an incubator with gentle (<150 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 50 molar equivalent/antibody, 50 micromoles, 1.0 mL) in DMSO was added and the reoxidation mixture was allowed to react for 2 hours at room temperature (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC) with gentle (< 150 rpm) shaking at an antibody concentration of 2-3 mg/mL. The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1 mM EDTA to a final antibody concentration of ~1.5 mg/mL. Compound 40 was added as a DMSO solution (10 molar equivalent/antibody, 1 micromole, in 0.9 mL DMSO) to 9 mL of this reoxidised antibody solution (15 mg, 100 nanomoles). The solution was mixed for 1.25 hours at room temperature, after which the conjugation reaction was quenched by addition of *N*-acetyl cysteine (4 micromoles, 40 µL at 100 mM) and diluted to >50 mL in PBS. The conjugation mixture was purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered, analysed and stored at +4 °C. The reduction and reoxidation steps are monitored by comparison of the relative amounts of individual light and heavy chains with full length antibody as observed by UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile. UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of conjugate trastuzumab-B at 280 nm and 330 nm (Compound 40 specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound 40, consistent with a drug-per-antibody ratio (DAR) of 1.68 molecules of Compound 40 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of conjugate trastuzumab-40 at 280 nm shows a monomer purity of 93% with no impurity detected. UHPLC SEC analysis gives a concentration of final conjugate trastuzumab-B at 0.74 mg/mL in 17 mL, obtained mass of conjugate trastuzumab-40 is 12.5 mg (83% yield).

Conjugate R347-23

A 50 mM solution of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in phosphate-buffered saline pH 7.4 (PBS) was added (42 molar equivalent/antibody, 56 micromoles, 1.12 mL at 50 mM) to a 14.09 mL solution of antibody (200 mg, 1.33 micromoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4.0 mg/mL. The reduction mixture was heated at +25°C for 24 hours (or until full reduction observed by UHPLC) in an incubator with gentle (<100 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* Tangential Flow Filtration unit (TFF) using mPES, MidiKros® 30 kDa fiber filter with 115 cm^2 surface area, into a reoxidation buffer containing PBS pH 7.4 and 1 mM EDTA to remove all the excess reducing agent. The reduced antibody was centrifuged for 3 min at 4000 rpm and then filtered using 0.45 µM membrane filter. A 50 mM solution of dehydroascorbic acid (DHAA, 15 molar equivalent/antibody, 20 micromoles, 400 µL at 50 mM) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature with gentle (<100 rpm) shaking at an antibody concentration of 2.5 mg/mL (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC). The reoxidation mixture was centrifuged for 3 min at 4000 rpm and then sterile-filtered using 0.2 µM membrane filter. Compound 23 was added as a DMSO solution (10 molar equivalent/antibody, 13.3 micromoles, in 6.6 mL DMSO) to 80 mL of this reoxidised antibody solution (200 mg, 1.33 micromoles) for a 10% (*v/v*) final DMSO concentration. The solution was shaken for 3 hours at +25°C and then the conjugation was quenched with *N*-acetyl cysteine (72.3 micromoles, 0.72 mL at 100 mM).

Excess free drug was removed *via* Tangential Flow Filtration unit (TFF) using mPES, MidiKros® 30 kDa fiber filter with 115 cm^2 surface area, into buffer containing PBS pH 7.4. Extent of free drug removal was monitored by UHPLC-RP using neat conjugate. After complete removal of free drug, ADC were formulated onto 25 mM Histidine, 200 mM

Sucrose, pH 6.0, *via* TFF using mPES, MidiKros® 30 kDa fiber filter with 115 cm^2 surface area. The whole process of R347 conjugation with Compound 23 was repeated with 400 mg antibody and also purified using TFF. ADC from both batches were combined and then filtered using Mustang filter under sterile atmosphere and then further stored at -78°C.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm and 330 nm (Compound 23 specific) shows a mixture of light and heavy chains attached to several molecules of Compound 23, consistent with a drug-per-antibody ratio (DAR) of 1.71 molecules of Compound 23 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ADC at 280 nm shows a monomer purity of greater than 97%. UHPLC SEC analysis gives a concentration of final ADC at 1.92 mg/mL in 265 mL, obtained mass of ADC is 509 mg (85% yield).

Conjugate R347-40

A 50 mM solution of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in phosphate-buffered saline pH 7.4 (PBS) was added (50 molar equivalent/antibody, 20 micromoles, 0.4 mL) to a 13.25 mL solution of antibody (60 mg, 0.4 micromoles) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) at a final antibody concentration of 4.5 mg/mL. The reduction mixture was heated at +37°C for 3 hours (or until full reduction is observed by UHPLC) in an incubator with gentle (<150 rpm) shaking. After cooling down to room temperature, the reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 12 molar equivalent/antibody, 4.8 micromoles, 96 µL) in DMSO was added and the reoxidation mixture was allowed to react for 17 hours at room temperature (or until full reoxidation of the cysteine thiols to reform the inter-chain cysteine disulfides is observed by UHPLC) with gentle (< 150 rpm) shaking at an antibody concentration of ~1.6 mg/mL. The reoxidation mixture was then sterile-filtered and diluted in a conjugation buffer containing PBS and 1 mM EDTA to a final antibody concentration of ~1.5 mg/mL. Compound 40 was added as a DMSO solution (11 molar equivalent/antibody, 0.44 micromoles, in 0.45 mL DMSO) to 4.05

mL of this reoxidised antibody solution (6 mg, 40 nanomoles). The solution was mixed for 1.25 hours at room temperature, after which the conjugation reaction was quenched by addition of *N*-acetyl cysteine (1.76 micromoles, 17.6 µL at 100 mM). The conjugation mixture was purified by spin filtration with PBS using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered, analysed and stored at +4 °C.

The reduction and reoxidation steps are monitored by comparison of the relative amounts of individual light and heavy chains with full length antibody as observed by UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile. UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate R347-40 at 280 nm and 330 nm (Conjugate 40 specific) shows unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to a single molecule of Compound 40, consistent with a drug-per-antibody ratio (DAR) of 1.86 molecules of Compound 40 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel G3000SWXL 5 µm 7.8 x 300 mm column (with a 7 µm 6.0 x 40 mm guard column) eluting with sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of Conjugate R347-40 at 280 nm shows a monomer purity of 97% with no impurity detected. UHPLC SEC analysis gives a concentration of final Conjugate R347-40 at 0.87 mg/mL in 5.5 mL, obtained mass of Conjugate R347-40 is 4.8 mg (80% yield).

Conjugate HLL2-23

A 50 mM solution of DTT (Dithiothreitol) in phosphate-buffered saline pH 7.4 (PBS) was added (40 molar equivalent/antibody, 40 micromoles, 825µL) to a 37.5 mL solution of antibody HLL2 (150mg, 1 micromol) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4 mg/mL. The reduction mixture was incubated at room temperature overnight with gentle (135 rpm) shaking. The reduced antibody was buffer-exchanged against PBS+1mM EDTA (to remove the excess of DTT) using TFF (Tangential Flow Filtration, Spectrum Labs 115 cm^2 hollow fibre cassette with 50kDa molecular weight cut off). The sample was filtered using a 0.4µm syringe filter to remove any debris from the TFF step and antibody concentration brought to 1.5mg/mL before reoxidation. A 50 mM solution of dehydroascorbic acid (DHAA,

15 molar equivalent/antibody, 13.9 micromoles, 0.28 mL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature under gentle (< 150 rpm) shaking. The reoxidation mixture was then sterile-filtered; 139mg of antibody (92.6mL as 1.5 mg/mL solution) was obtained, 14mL of which was taken forward for conjugation with Compound 23 (estimated ca. 21 mg antibody, 0.14 micromoles). Compound 23 was added as a DMSO solution (10 molar equivalent/antibody, 0.33 micromoles in 0.133 mL DMSO) to 14 mL of the reoxidised antibody solution. The conjugation mixture was topped with 1.27ml of DMSO to bring the final DMSO concentration to 10% (*v/v*) and incubated for 3 hours at room temperature under gentle agitation (135rpm). Free drug was then removed from the antibody-drug conjugate by extensive diafiltration in PBS using a spin filter device (Amicon Ultra-30K centrifugal filter, Millipore). The resulting conjugation mixture was sterile-filtered and analysed by UHPLC.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 214 nm (ADC) and 330 nm (Compound 23 specific) shows a mixture of heavy chains either unconjugated or attached to 1 molecule of Compound 23, consistent with a drug-per-antibody ratio (DAR) of 1.64 molecules of Compound 23 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ADC at 280 nm shows a monomer purity greater than 97%. UHPLC SEC analysis gives a concentration of final ADC at 2.06 mg/mL in 10 mL, obtained mass of ADC is 20.6 mg.

Conjugate AntiCD79b-23

A 50 mM solution of DL-dithiothreitol (DTT) in phosphate-buffered saline pH 7.4 (PBS) was added (80 molar equivalent/antibody, 53.3 micromoles, 1.07 mL) to a 25 mL solution of antibody CD79b (100mg, 667 nmol) in reduction buffer containing PBS and 1 mM ethylenediaminetetraacetic acid (EDTA) and a final antibody concentration of 4 mg/mL. The reduction mixture was allowed to react at room temperature overnight with gentle shaking. The reduced antibody was buffer exchanged, *via* spin filter centrifugation, into a reoxidation buffer containing PBS and 1 mM EDTA to remove all the excess reducing agent. A 50 mM solution of dehydroascorbic acid (DHAA, 15 molar equivalent/antibody,

9.28 micromoles, 185 µL) in DMSO was added and the reoxidation mixture was allowed to react for 16 hours at room temperature under gentle (< 150 rpm) shaking. The reoxidation mixture was then sterile-filtered; Compound 23 was added as a DMSO solution (15 molar equivalent/antibody, 1.8 micromoles in 1.0 mL DMSO) to 9 mL of this reoxidised antibody solution (18 mg, 120 nanomoles) for a 10% (*v/v*) final DMSO concentration and a final antibody concentration of 1.8 mg/mL in PBS + 1 mM EDTA. The solution was mixed for 2 hours at room temperature under gentle agitation (135 rpm), then the conjugation was quenched by addition of *N*-acetyl cysteine (7.2 micromoles, 72 µL at 100 mM), then purified by spin filtration using a 15 mL Amicon Ultracell 50 kDa MWCO spin filter, sterile-filtered and analysed.

UHPLC analysis on a Shimadzu Prominence system using a Phenomenex Aeris 3.6u XB-C18 150 x 2.1 mm column eluting with a gradient of water and acetonitrile on a reduced sample of Conjugate at 280 nm (ADC) and 330 nm (Compound 23 specific) unconjugated light chains and a mixture of unconjugated heavy chains and heavy chains attached to 1 or 2 molecules of Compound 23, consistent with a drug-per-antibody ratio (DAR) of 2.08 molecules of Compound 23 per antibody.

UHPLC analysis on a Shimadzu Prominence system using a Tosoh Bioscience TSKgel SuperSW mAb HTP 4 µm 4.6 x 150 mm column (with a 4 µm 3.0 x 20 mm guard column) eluting with 0.3 mL/minute sterile-filtered SEC buffer containing 200 mM potassium phosphate pH 6.95, 250 mM potassium chloride and 10% isopropanol (*v/v*) on a sample of ADC at 280 nm shows a monomer purity of greater than 98%. UHPLC SEC analysis gives a concentration of final ADC at 1.62 mg/mL in 7.6 mL, obtained mass of ADC is 12.28 mg.

Example 5 – *in vitro* testing

Medium from sub-confluent (80-90% confluency) cell culture in a T75 flask was aspirated and the flask rinsed with PBS (about 20ml) and emptied. Trypsin-EDTA (5ml) was added, the flask returned to the 37°C gassed incubator for up to about 5 minutes, then rapped sharply to dislodge and dissociate cells from the plastic. The cell suspension was transferred to a sterile 50ml screw-top centrifuge tube, diluted with growth medium to a final volume of 15ml, then centrifuged (400g for 5 min). The supernatant was aspirated and the pellet re-suspended in 10ml culture medium. Repeated pipetting may be necessary to produce monodisperse cell suspensions. The cell concentration and viability are measured of trypan blue cell stained cells, using a haemocytometer. Cells were diluted

to $2x10^5$/ml, dispensed (50µl /well) into 96 well flat bottom plates and incubated overnight before use.

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

ADC dilution was dispensed (50µl/ well) into 4 replicate wells of the 96-well plate, containing 50µl cell suspension seeded the previous day. Control wells received 50µl cell culture medium.

The 96-well plate containing cells and ADCs was incubated at 37°C in a CO_2-gassed incubator for the exposure time.

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

Cell Line	Description	ADC Exposure	Cell growth medium
SKBR3	Breast carcinoma	4 days	McCoys with Glutamax, 10% FBS
BT474	Breast carcinoma	5 days	DMEM with glutamax, 10% FBS
NCIN87	Gastric carcinoma	7 days	RPMI 1640 with glutamax, 10% FBS

ADC	**DAR**	**IC_{50} (µg/ml) in:**		
		BT474	**NCI-N87**	**SKBR3**
trastuzumab-40	1.68	>1	0.019	0.019
trastuzumab-23	1.71	>1	0.030	0.022

Testing of *AntiCD79b-23*

The concentration and viability of cells from a sub-confluent (80-90% confluency) T75 flask are measured by trypan blue staining, and counted using the LUNA-II™ Automated Cell Counter. Cells were diluted to $2x10^5$/ml, dispensed (50 µl /well) into 96-well flat-bottom plates.

A stock solution (1 ml) of antibody drug conjugate (ADC) (20 µg/ml) was made by dilution of filter-sterilised ADC into cell culture medium. A set of 8x 10-fold dilutions of stock ADC were made in a 24-well plate by serial transfer of 100 µl into 900 µl of cell culture medium. ADC dilution was dispensed (50 µl/ well) into 4 replicate wells of the 96-well plate, containing 50 µl cell suspension seeded the previously. Control wells received 50 µl cell culture medium. The 96-well plate containing cells and ADCs was incubated at 37°C in a CO_2-gassed incubator for the exposure time.

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

ADC incubation times were 4 days with WSUDLCL2 (B-cell non-Hodgkin lymphoma) and SUDHL4 (B-lymphocyte), 5 days for Granta519 (B-cell non-Hodgkin lymphoma) and 6 days for BJAB (Burkitt lymphoma). WSUDLCL2 and SUDHL4 were cultured in RPMI 1640 with Glutamax + 10% (v/v) HyClone™ Fetal Bovine Serum, Granta519 in DMEM + Glutamax with 10% (v/v) HyClone™ Fetal Bovine Serum and BJAB in RPMI 1640 + Glutamax with 20% (v/v) HyClone™ Fetal Bovine Serum.

ADC	**EC_{50} (µg/ml) in:**			
	SUDHL4	**WSUDLCL2**	**GRANTA519**	**BJAB**
AntiCD79b-23	0.05387	0.9268	0.04957	0.003158

Example 6

Mice

Female severe combined immune-deficient mice (Fox Chase SCID®, C.B-17/Icr-*Prkdcscid*, Charles River) were ten weeks old with a body weight (BW) range of 16.2 to 21.9 grams on Day 1 of the study. The animals were fed *ad libitum* water (reverse osmosis, 1 ppm Cl), and NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% crude protein, 5.0% crude fat, and 5.0% crude fibre. The mice were housed on irradiated Enricho'cobs ™ Laboratory Animal Bedding in static micro-isolators on a 12-hour light cycle at 20–22°C (68–72°F) and 40–60% humidity. CR Discovery Services specifically complies with the recommendations of the *Guide for Care and Use of Laboratory Animals* with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care. The animal care and use program at CR Discovery Services is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), which assures compliance with accepted standards for the care and use of laboratory animals.

In Vivo Implantation and Tumour Growth

Xenografts were initiated with BT474 human breast carcinomas maintained at CR Discovery Services by serial subcutaneous transplantation into the SCID mice (see above). On the day of tumour implant, each test mouse received a 1-mm^3 BT474 fragment implanted subcutaneously in the right flank, and tumour growth was monitored as the average size approached the target range of 100 to 150 mm^3. Thirty-three days after tumour implantation, designated as Day 1 of the study, the animals were sorted into nine groups each consisting of ten mice with individual tumour volumes of 75 to 144 mm^3 and group mean tumour volumes of 111 to 112 mm^3. Tumours were measured in two dimensions using calipers, and volume was calculated using the formula:

$$\text{Tumour Volume } (mm^3) = 0.5(w^2 \times l)$$

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

Treatment 1

Treatment began on Day 1 in groups of mice (n=10) with established subcutaneous BT474 tumors (75–196 mm^3). Trastuzumab-23 was administered intravenously once on Day 1 (qd x 1) at two dosages (0.3 and 1 mg/kg). A vehicle-treated group served as the control group for efficacy analysis. Tumors were measured twice per week until the study was ended on Day 60. Each mouse was euthanized when its tumor reached the endpoint volume of 800 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

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

Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. All regimens were acceptably tolerated.

The median TTE for vehicle-treated controls was 44.4 days, establishing a maximum possible TGD of 15.6 days (35%) for the 60-day study. ADC regimens resulted in the maximum possible TGD, produced survival benefit that was statistically significantly different from vehicle-treated controls ($P < 0.01$) and could not be distinguished based on logrank analysis ($P > 0.05$). Differences within ADC treatments were only evident in the MTV on the final day and numbers and types of regression responses produced by each regimen.

Trastuzumab-23 at 1 mg/kg produced four partial regressions (PRs). The results are illustrated in Figure 1.

Treatment 2

Treatment began on Day 1 in groups of mice (n = 9 or 10) with established subcutaneous BT474 tumors (108-196 mm^3). Trastuzumab-40 was administered intravenously once on Day 1 (qd x 1) at two dosages (0.3 and 1 mg/kg). A vehicle-treated group served as the control group for efficacy analysis. Tumors were measured twice per week until the study

was ended on Day 62. Each mouse was euthanized when its tumor reached the endpoint volume of 1000 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

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

Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects.

All regimens were acceptably tolerated. The median TTE for vehicle-treated controls was 52.9 days, establishing a maximum possible TGD of 9.1 days (17%) for the 62-day study. ADC treatment resulted in the maximum possible TGD, however only the 1 mg/kg treatment produced survival benefit that was statistically significantly different from vehicle-treated controls ($P < 0.001$).

Trastuzumab-40, at 1 mg/kg produced four partial regressions (PRs) and one complete regression (CR) which remained a tumour-free survivor (TFS) at study end. The results are shown in Figure 2.

Example 7

Tumor Cell Culture

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

In Vivo Implantation and Tumor Growth

The NCI-N87 cells used for implantation were harvested during log phase growth and resuspended in phosphate buffered saline (PBS) containing 50% Matrigel™ (BD Biosciences). On the day of tumor implant, each test mouse (SCID mice as in Example 6) was injected subcutaneously in the right flank with 1×10^7 cells (0.1 mL cell suspension),

and tumor growth was monitored as the average size approached the target range of 100 to 150 mm^3. Eleven days later, designated as Day 1 of the study, mice were sorted according to calculated tumor size into eleven groups each consisting of ten animals with individual tumor volumes ranging from 88 to 144 mm^3 and group mean tumor volumes of 119–121 mm3. Tumors were measured in two dimensions using calipers, and volume was calculated using the formula:

$$\text{Tumour Volume } (mm^3) = 0.5\ (w^2 \times l)$$

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

Treatment 1

Treatment began on Day 1 in groups of mice (n=10) with established subcutaneous NCI-N87 tumors (88-144 mm^3). Trastuzumab-23 was administered intravenously once on Day 1 (qd x 1) at two dosages (0.3 and 1 mg/kg). A vehicle-treated group served as the control group for efficacy analysis. Tumors were measured twice per week until the study was ended on Day 81. Each mouse was euthanized when its tumor reached the endpoint volume of 800 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

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

Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. All regimens were acceptably tolerated.

The median TTE for vehicle-treated controls was 53.4 days, establishing a maximum possible TGD of 27.6 days (52%) for the 81-day study. Trastuzumab-23 tested at 1 mg/kg produced survival benefit that was statistically significantly different from vehicle-treated controls ($P < 0.001$) and resulted in the maximum possible TGD. At 0.3 mg/kg, the median TTE was 80.5 days, which corresponds to TGD of 27.1 days (51%).

Trastuzumab-23, at 1 mg/kg produced one partial regressions (PR). The results are illustrated in Figure 3.

Treatment 2

Treatment began on Day 1 in groups of mice (n=10) with established subcutaneous NCI-N87 tumors (75-126 mm^3). Trastuzumab-40 was administered intravenously once on Day 1 (qd x 1) at two dosages (0.3 and 1 mg/kg). A vehicle-treated group served as the control group for efficacy analysis. Tumors were measured twice per week until the study was ended on Day 83. Each mouse was euthanized when its tumor reached the endpoint volume of 800 mm^3 or on the final day, whichever came first. The time to endpoint (TTE) was calculated for each mouse.

Treatment outcome was determined from percent tumor growth delay (%TGD), defined as the percent increase in median TTE for treated versus control mice, with differences between groups deemed statistically significant at $P \leq 0.05$ using logrank survival analysis. Mice were monitored for complete regression (CR) and partial regression (PR) responses. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. Treatment tolerability was assessed by body weight measurements and frequent observation for signs of treatment-related side effects. All regimens were acceptably tolerated.

The median TTE for vehicle-treated controls was 44.9 days, establishing a maximum possible TGD of 38.1 days (85%) for the 83-day study. Trastuzumab-40 (0.3mg/kg) had a median TTE of 54.2 days corresponding to a TGD of 9.3 days (21%). Trastuzumab-40 (1mg/kg) had a median TTE of 61.6 days corresponding to a TGD of 16.7 days (37%).

The results are illustrated in Figure 4.

Example 8 – Toxicity studies/Therapeutic Index

Rat study:

A single dose toxicity study was used to determine the maximum tolerated dose (MTD) and safety profile of Trastuzumab-23. Male Sprague Dawley rats (Harlan, Inc) were dosed once by slow bolus intravenous injection via the tail vein with vehicle control (25 mM Histidine-HCl, 7% sucrose, 0.02% Polysorbate 80, pH 6.0) or test material (Trastuzumab-23). Parameters evaluated during the study included mortality, physical examinations, cageside observations, body weights, body weight changes, clinical pathology (clinical chemistry,

hematology, and coagulation), and gross pathology findings. All animals were terminated on Study Day (SD) 29.

Group	Treatment	Dose Route	Dose (mg/kg)	Frequency	Male Rats Main Study N
1	Control	IV	0	Single	5
7	Trastuzumab-23	IV	3	Single	5
8	Trastuzumab-23	IV	4	Single	5
9	Trastuzumab-23	IV	5	Single	5
10	Trastuzumab-23	IV	6	Single	5
13	Trastuzumab-23	IV	7	Single	5

Control = 25 mM Histidine-HCl, 7% sucrose, 0.02% Polysorbate 80, pH 6.0

Tolerability was determined based on toxicity end points, including body weight loss (>10%) and bone marrow suppression. Based on minimal adverse findings at the high dose, the maximum tolerated dose (MTD) in the rat after a single dose of Trastuzumab-23 was determined to be >7mg/kg, which was the highest dose level evaluated.

Therapeutic Index

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

Thus for conjugates of compound 23, the therapeutic index is the MTD of greater than 7 mg/kg divided by the MED which is less than 1 mg/kg (see Fig 3 at 28 days), giving a Therapeutic Index of greater than 7.

Cynomolgus Macaque Study:

A Single-Dose Toxicity study was performed in male Cynomolgus macaques monkeys (Macaca fascicularis) of Cambodian origin following a single intravenous (IV) bolus injection of ADC-SG3400. The study was conducted in 2 phases. In phase 1, animals (n=1) were treated at dose levels of 1, 3, or 6 mg/kg to determine the optimal dose level to explore in phase 2. Animals were dosed by slow bolus intravenous injection via the saphenous vein with vehicle control (25 mM Histidine, 200 mM Sucrose, pH 6.0) or test material (Trastuzumab-23). Based on observations of significant body weight loss at 6

mg/kg, a dose level of 4.5 mg/kg was chosen for phase 2 of the study. In phase 2, animals (n=3) were administered a single dose of 4.5 mg/kg Trastuzumab-23 on Day 1 and necropsied on Days 71 or 72.

Group	Treatment	Dose Route	Dose (mg/kg)	Frequency	Male Cyno Main Study N
1	Trastuzumab-23	IV	1	Single	1
2	Trastuzumab-23	IV	3	Single	1
3	Trastuzumab-23	IV	6	Single	1
4	Trastuzumab-23	IV	4.5	Single	3

Tolerability was determined based on toxicity end points, including body weight loss (>10%) and bone marrow suppression. There was no unscheduled mortality in any animal administered Trastuzumab-23. The major findings were body weight loss and bone marrow suppression at the highest tested dose of 6 mg/kg. Based on these data, and minimal signs of toxicity at next lowest dose, the MTD of Trastuzumab-23 in cynos was 4.5 mg/kg.

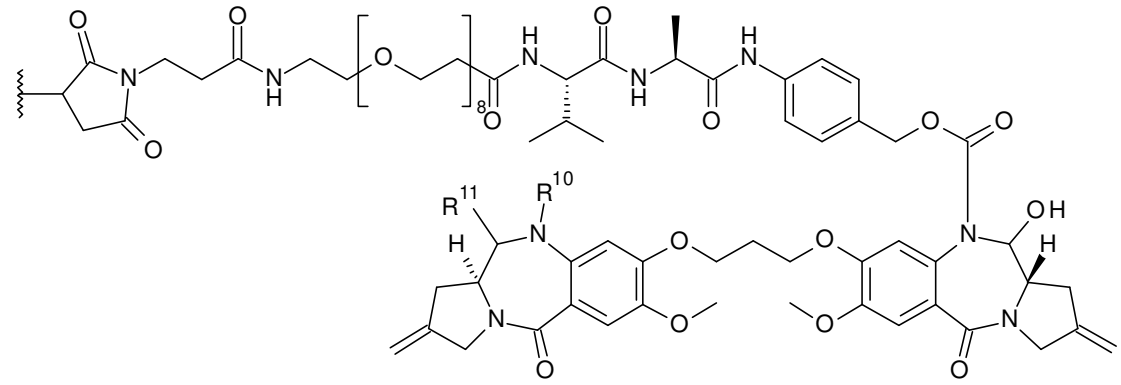
All documents and other references mentioned above are herein incorporated by reference.

-CLAIMS

1. A conjugate of formula I:

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

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



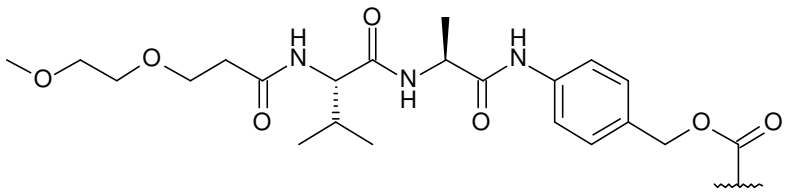
(II)

wherein

either:

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

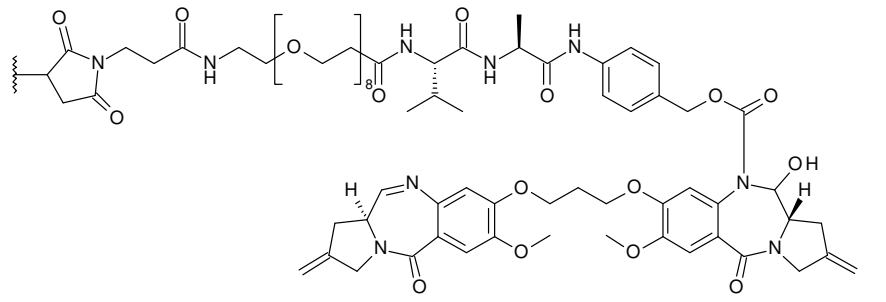
(b) R^{11} is OH, and R^{10} is:



;

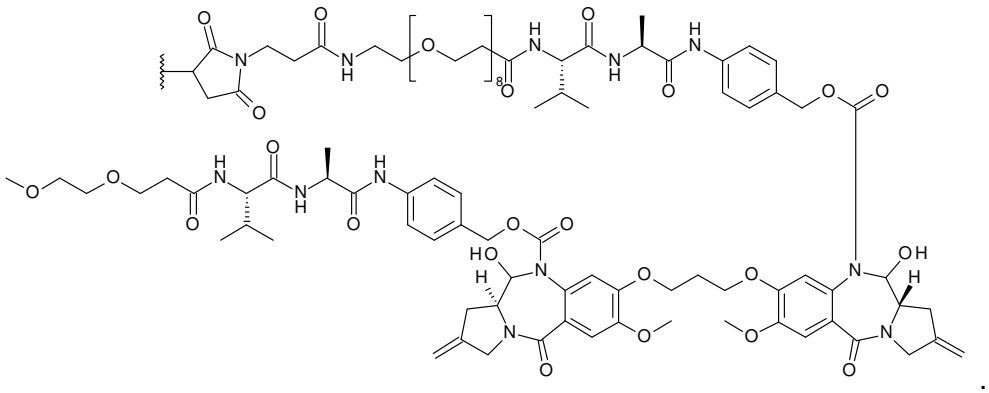
p is an integer of from 1 to 20.

2. A conjugate according to claim 1, wherein D^L is D^L-A:



.

3. A conjugate according to claim 1, wherein D^L is D^L-B:



.

4. A conjugate according to any one of claims 1 to 3, wherein the Ligand Unit is an antibody or an active fragment thereof.

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

6. The conjugate according to claim 4 wherein the antibody or antibody fragment is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(88):

(1) BMPR1B;
(2) E16;
(3) STEAP1;
(4) 0772P;
(5) MPF;
(6) Napi3b;
(7) Sema 5b;
(8) PSCA hlg;
(9) ETBR;
(10) MSG783;
(11) STEAP2;
(12) TrpM4;
(13) CRIPTO;
(14) CD21;
(15) CD79b;
(16) FcRH2;
(17) HER2;
(18) NCA;

(19) MDP;

(20) IL20R-alpha;

(21) Brevican;

(22) EphB2R;

(23) ASLG659;

(24) PSCA;

(25) GEDA;

(26) BAFF-R;

(27) CD22;

(28) CD79a;

(29) CXCR5;

(30) HLA-DOB;

(31) P2X5;

(32) CD72;

(33) LY64;

(34) FcRH1;

(35) IRTA2;

(36) TENB2;

(37) PSMA – FOLH1;

(38) SST;

(38.1) SSTR2;

(38.2) SSTR5;

(38.3) SSTR1;

(38.4)SSTR3;

(38.5) SSTR4;

(39) ITGAV;

(40) ITGB6;

(41) CEACAM5;

(42) MET;

(43) MUC1;

(44) CA9;

(45) EGFRvIII;

(46) CD33;

(47) CD19;

(48) IL2RA;

(49) AXL;

(50) CD30 - TNFRSF8;

(51) BCMA - TNFRSF17;

(52) CT Ags – CTA;

(53) CD174 (Lewis Y) - FUT3;

(54) CLEC14A;

(55) GRP78 – HSPA5;

(56) CD70;

(57) Stem Cell specific antigens;

(58) ASG-5;

(59) ENPP3;

(60) PRR4;

(61) GCC – GUCY2C;

(62) Liv-1 – SLC39A6;

(63) 5T4;

(64) CD56 – NCMA1;

(65) CanAg;

(66) FOLR1;

(67) GPNMB;

(68) TIM-1 – HAVCR1;

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

(70) B7-H4 – VTCN1;

(71) PTK7;

(72) CD37;

(73) CD138 – SDC1;

(74) CD74;

(75) Claudins – CLs;

(76) EGFR;

(77) Her3;

(78) RON - MST1R;

(79) EPHA2;

(80) CD20 – MS4A1;

(81) Tenascin C – TNC;

(82) FAP;

(83) DKK-1;

(84) CD52;

(85) CS1 - SLAMF7;

(86) Endoglin – ENG;

(87) Annexin A1 – ANXA1;
(88) V-CAM (CD106) - VCAM1.

7. The conjugate according to any one of claims 4 to 6 wherein the antibody or antibody fragment is a cysteine-engineered antibody.

8. The conjugate according to any one of claims 1 to 7 wherein p is an integer from 1 to 8.

9. The conjugate according to claim 8, wherein p is 1, 2, 3, or 4.

10. A composition comprising a mixture of conjugates according to any one of claims 1 to 9, wherein the average p in the mixture of conjugate compounds is about 1 to about 8.

11. The conjugate according to any one of claims 1 to 9, for use in therapy.

12. A pharmaceutical composition comprising the conjugate of any one of claims 1 to 9 and a pharmaceutically acceptable diluent, carrier or excipient.

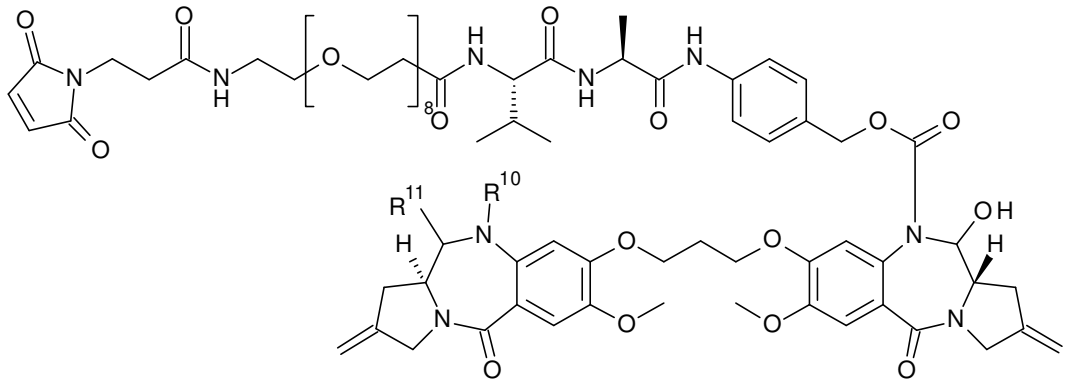
13. The conjugate according to any one of claims 1 to 9 or the pharmaceutical composition according to claim 12, for use in the treatment of a proliferative disease in a subject.

14. The conjugate for use according to claim 13, wherein the disease treated is cancer.

15. Use of a conjugate according to any one of claims 1 to 9 in the manufacture of a medicament for the treatment of a proliferative disease.

16. The use according to claim 15, wherein the proliferative disease is a cancer.

17. A compound of formula III:



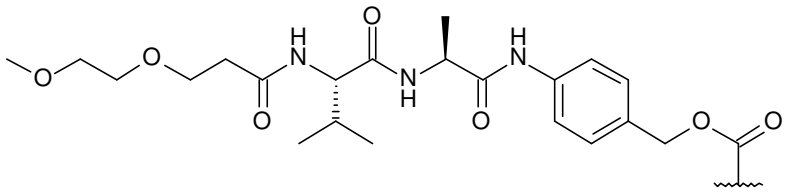
(III)

wherein

either:

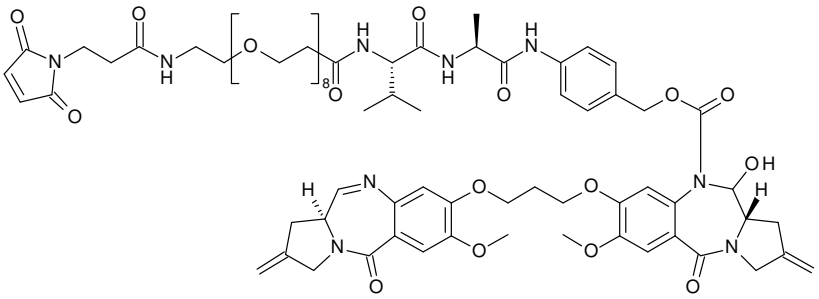
(a) R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(b) R^{11} is OH, and R^{10} is:



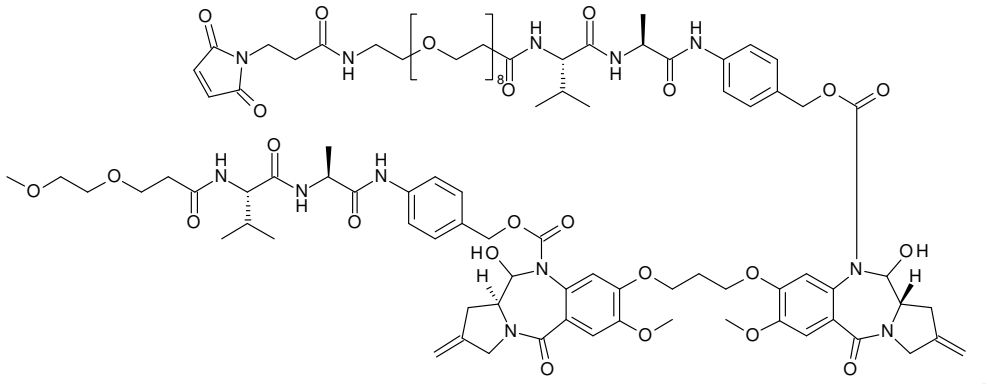
.

18. A compound according to claim 17 which is A:



.

19. A compound according to claim 17 which is B:



.

20. A method of synthesis of a conjugate according to any one of claims 1 to 9 comprising the step of conjugating a compound according to any one of claims 17 to 19 with a cell-binding agent.