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DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] The B7 homology 3 protein (B7-H3) (also known as CD276 and B7RP-2, and referred to herein as "B7-H3") is a type I transmembrane glycoprotein of the immunoglobulin superfamily. Human B7-H3 contains a putative signal peptide, V-like and C-like Ig domains, a transmembrane region and a cytoplasmic domain. Exon duplication in humans results in the expression of two B7-H3 isoforms having either a single IgV-IgC-like domain (2IgB7-H3 isoform) or a IgV-IgC-IgV-IgC-like domain (4IgB7-H3 isoform) containing several conserved cysteine residues. The predominant B7-H3 isoform in human tissues and cell lines is the 4IgB7-H3 isoform (Steinberger et al., J. Immunol. 172(4): 2352-9 (2004)).

[0002] B7-H3 has been reported as having both co-stimulatory and co-inhibitory signaling functions (see, e.g., Chapoval et al., Nat. Immunol. 2: 269-74 (2001); Suh et al., Nat. Immunol. 4: 899-906 (2003); Prasad et al., J. Immunol. 173: 2500-6 (2004); and Wang et al., Eur. J. Immunol. 35: 428-38 (2005)). For example, *in vitro* studies have shown B7-H3's co-stimulatory function since B7-H3 was able to increase proliferation of cytotoxic T-lymphocytes (CTLs) and upregulate interferon gamma (IFN- γ) production in the presence of anti-CD3 antibody to mimic the T cell receptor signal (Chapoval *et al.*, 2001). Moreover, *in vivo* studies using cardiac allografts in B7-H3 $-/-$ mice showed decreased production of key cytokine, chemokine and chemokine receptor mRNA transcripts (e.g., IL-2, IFN- γ , monocyte chemoattractant protein (MCP-1) and IFN-inducible protein (IP)-10) as compared to wild-type control (Wang *et al.*, 2005). In contrast, B7-H3 co-inhibitory function has been observed, for example, in mice where B7-H3 protein inhibited T-cell activation and effector cytokine production (Suh *et al.*, 2003). Although no ligands have been identified for human B7-H3, murine B7-H3 has been found to bind to the triggering receptor expressed on myeloid cells (TREM-) like transcript 2 (TLT-2), a modulator of adaptive and innate immunity cellular responses. Binding of murine B7-H3 to TLT-2 on CD8⁺ T-cells enhances T-cell effector functions such as proliferation, cytotoxicity and cytokine production (Hashiguchi et al., Proc. Nat'l. Acad. Sci. U.S.A. 105(30): 10495-500 (2008)).

[0003] B7-H3 is not constitutively expressed in many immune cells (e.g., natural killer (NK) cells, T-cells, and antigen-presenting cells (APCs)), however, its expression can be induced. Further, the expression of B7-H3 is not restricted to immune cells. B7-H3 transcripts are expressed in a variety of human tissues including colon, heart, liver, placenta, prostate, small intestine, testis, and uterus, as well as osteoblasts, fibroblasts, epithelial cells, and other cells of non-lymphoid lineage, potentially indicating immunological and non-immunological functions (Nygren et al. Front Biosci. 3:989-93 (2011)). However, protein expression in normal tissue is typically maintained at a low level and thus, may be subject to post-transcriptional regulation.

[0004] B7-H3 is also expressed in a variety of human cancers, including prostate cancer, clear cell renal cell carcinoma, glioma, melanoma, lung cancer, non-small cell lung cancer (NSCLC), small cell lung cancer, pancreatic cancer, gastric cancer, acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), ovarian cancer, colorectal cancer, colon cancer, renal cancer, hepatocellular carcinoma, kidney cancer, head and neck cancer, hypopharyngeal squamous cell carcinoma, glioblastoma, neuroblastoma, breast cancer, endometrial cancer, and urothelial cell carcinoma. Although the role of B7-H3 in cancer cells is unclear, its expression may orchestrate signaling events that may protect cancer cells from innate and adaptive immune responses. For example, B7-H3 is overexpressed in high-grade prostatic intraepithelial neoplasia and adenocarcinomas of the prostate, and high expression levels of B7-H3 in these cancerous cells is associated with an increased risk of cancer progression after surgery (Roth et al. Cancer Res. 67(16): 7893-900 (2007)). Further, tumor B7-H3 expression in NSCLC inversely correlated with the number of tumor-infiltrating

lymphocytes and significantly correlated with lymph node metastasis (Sun et al. Lung Cancer 53(2): 143-51 (2006)). The level of circulating soluble B7-H3 in NSCLC patients has also been associated with higher tumor stage, tumor size, lymph node metastasis, and distant metastasis (Yamato et al., Br. J. Cancer 101(10): 1709-16 (2009)).

[0005] B7-H3 may also play an important role in T-cell-mediated antitumor responses in a context dependent manner. For example, gastric cancer tumor cell expression of B7-H3 positively correlated with survival time, infiltration depth, and tissue type (Wu et al., World J. Gastroenterol. 12(3): 457-9 (2006)). Further, high expression of B7-H3 in pancreatic tumor cells was associated with patient survival after surgical resection and significantly correlated with the number of tumor-infiltrating CD8⁺ T-cells (Loos et al., BMC Cancer 9:463 (2009)).

[0006] Antibody drug conjugates (ADC) represent a relatively new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen, including target surface antigens that are overexpressed in the tumor cells.

[0007] There remains a need in the art for anti-B7-H3 antibodies and anti-B7-H3 ADCs that can be used for therapeutic purposes in the treatment of cancer.

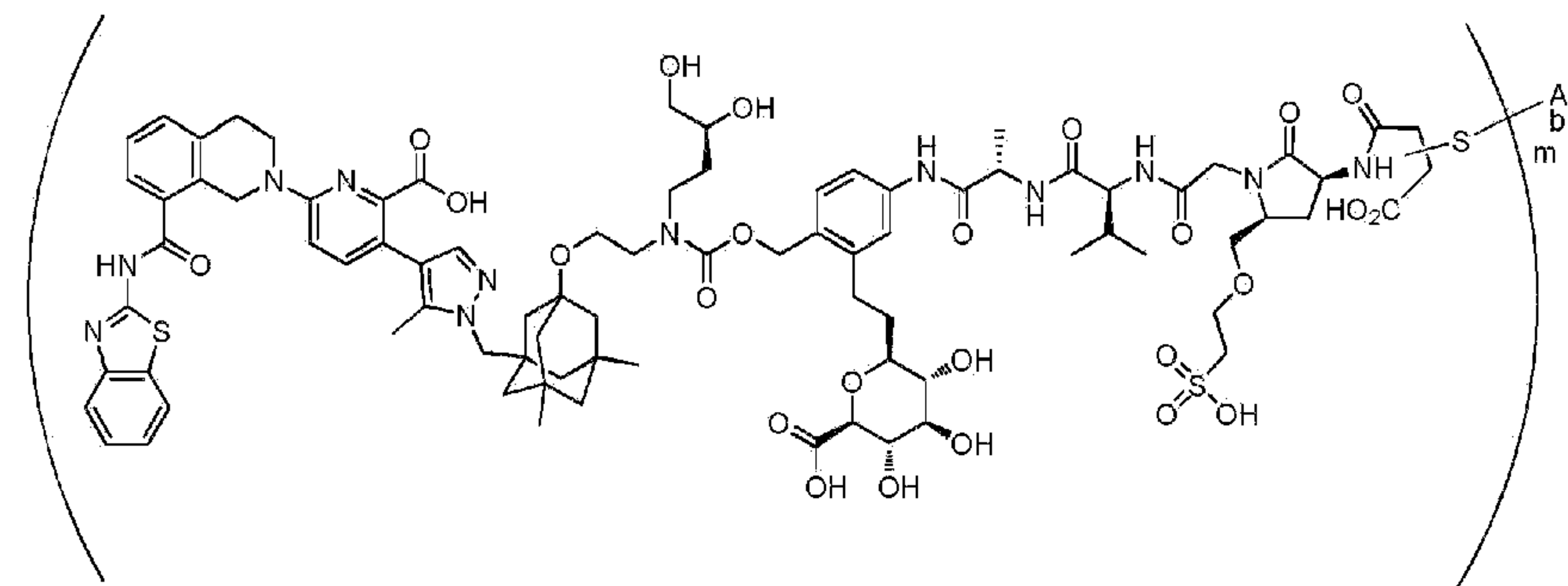
[0008] US 2012/0294796 relates to anti-B7-H3 antibodies and pharmaceutical compositions comprising the same. US 2012/0294796 notes that the anti-B7-H3 antibodies disclosed therein can be conjugated to or associated with a radioactive molecule, toxin (calicheamicin), chemotherapeutic molecule, liposomes or other vesicles containing containing chemotherapeutic compositions. US 2012/0294796 does not however disclose conjugating the anti-B7-H3 antibodies to Bcl-xL inhibitors.

[0009] Deryk Loo et al "abstract 1201: Anti-B7-H3 antibody-drug conjugates as potential therapeutics for solid cancer" disclose anti-B7-H3 auristatin E ADCs. However, they do not disclose conjugating anti-B7-H3 antibodies to Bcl-xL inhibitors.

SUMMARY OF THE INVENTION

[0010] The present invention provides for antibody drug conjugates (ADCs) that specifically bind to human B7-H3. The present invention provides novel ADCs that can selectively deliver Bcl-xL inhibitors to target cancer cells, e.g., B7-H3 expressing cells.

[0011] The present invention provides an anti-hB7-H3 Antibody Drug Conjugate (ADC) comprising the following structure:



wherein m is 2 and Ab is an anti-hB7-H3 antibody, wherein the antibody Ab comprises

a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 35, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 34, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 33; and

a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 38, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 37.

[0012] In one embodiment, the Ab is an anti-hB7-H3 antibody, wherein the anti-hB7H3 antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 147, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 144. In one embodiment, Ab is an anti-hB7-H3 antibody, wherein the anti-hB7-H3 antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 168, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 169.

[0013] In one embodiment, the ADC is huAb13v1-AAA, wherein AAA is a synthon disclosed in Table B, and wherein the conjugated synthon is in open form.

[0014] In one aspect, the present invention provides a pharmaceutical composition comprising an effective amount of the ADC described herein, and a pharmaceutically acceptable carrier.

[0015] In one aspect, the present invention provides a method for treating cancer, comprising administering a therapeutically effective amount of an ADC described herein to a subject in need thereof.

[0016] In one embodiment, the cancer is selected from the group consisting of small cell lung cancer, non small cell lung cancer, breast cancer, ovarian cancer, a glioblastoma, prostate cancer, pancreatic cancer, colon cancer, gastric cancer, melanoma, hepatocellular carcinoma, head and neck cancer, kidney cancer, leukemia, e.g., acute myeloid leukemia (AML), and lymphoma, e.g., non-Hodgkin's lymphoma (NHL).

[0017] In one embodiment, the cancer is a squamous cell carcinoma. In one embodiment, the squamous cell carcinoma is squamous lung cancer or squamous head and neck cancer.

[0018] In one embodiment, the cancer is triple negative breast cancer.

[0019] In one embodiment, the cancer is non-small cell lung cancer.

[0020] In one embodiment, the cancer is characterized as having an activating EGFR mutation. In one embodiment, the activating EGFR mutation is selected from the group consisting of an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

[0021] In one aspect, the present invention provides a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an effective amount of an ADC described herein to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased.

[0022] In one embodiment, the solid tumor is a non-small cell lung carcinoma.

[0023] In one embodiment, the ADC is administered in combination with an additional agent or an additional

therapy.

[0024] In one embodiment, the additional agent is selected from the group consisting of an anti-PD 1 antibody (e.g. pembrolizumab), an anti-PD-L1 antibody (e.g., atezolizumab), an anti-CTLA-4 antibody (e.g. ipilimumab), a MEK inhibitor (e.g. trametinib), an ERK inhibitor, a BRAF inhibitor (e.g. dabrafenib), osimertinib, erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g. dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-2 inhibitor (e.g. venetoclax), a Bcl-xL inhibitor, ibrutinib, a mTOR inhibitor (e.g. everolimus), a PI3K inhibitor (e.g. buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g. lapatinib), a taxane (e.g. docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g. rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g. TDM1), a TRAIL agonist, a proteasome inhibitor (e.g. bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor.

[0025] In one embodiment, the anti-B7-H3 ADC of the invention is administered in combination with venetoclax to a human subject for the treatment of small cell lung cancer (SCLC).

[0026] In one embodiment, the additional therapy is radiation.

[0027] In one embodiment, the additional agent is a chemotherapeutic agent.

In one aspect, the present invention provides a process for the preparation of an ADC as claimed, the process comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27°C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising synthon 2.166 (Table B);

adjusting the pH of the solution to a pH of 7.5 to 8.5;

allowing the reaction to run for 48 to 80 hours to form the ADC;

wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

[0028] In another aspect, the present invention provides an ADC prepared by the process as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029]

Figure 1 is a graphical representation of the epitope grouping of murine anti-B7-H3 hybridoma antibodies as determined by pair-wise binding assays.

Figure 2 depicts an antibody reduction, modification with a maleimide derivative to give a thiosuccinimide intermediate, and subsequent hydrolysis of thiosuccinimide moiety

Figure 5 depicts the MS characterization of light chain and heavy chain of huAb13v1 1) prior to conjugation,

2) after conjugation to a maleimide derivative to give a thiosuccinimide intermediate and 3) post pH 8-mediated hydrolysis of the thiosuccinimide ring.

DETAILED DESCRIPTION OF THE INVENTION

[0030] Aspects of the invention relate to, anti-B7-H3 ADCs, and pharmaceutical compositions thereof. Methods of using the ADCs described herein to detect human B7-H3, to inhibit human B7-H3 activity (*in vitro* or *in vivo*), and to treat cancers are also encompassed by the invention. In certain embodiments, the invention provides anti-B7-H3 ADC compositions comprising the ADCs, methods of making the ADCs, and various methods of using the ADCs.

[0031] It will also be appreciated by skilled artisans that the various ADCs and/or ADC synthons described herein may be in the form of salts, and in certain embodiments, particularly pharmaceutically acceptable salts. The compounds of the present disclosure that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion, e.g., a halide such as a bromide, chloride, or fluoride.

[0032] Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, etc. Base addition salts include those derived from inorganic bases, such as ammonium and alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like.

[0033] In the disclosure below, if both structural diagrams and nomenclature are included and if the nomenclature conflicts with the structural diagram, the structural diagram controls.

[0034] An outline of the Detailed Description of the Invention is provided below:

1. I. Definitions
2. II. Anti-B7-H3 Antibodies
 - II.A. Anti-B7-H3 Chimeric Antibodies
 - II.B. Humanized Anti-B7-H3 Antibodies
3. III. Anti-B7-H3 Antibody Drug Conjugates (ADCs)
 - III.A. Anti-B7-H3 / Bcl-xL Inhibitor ADCs
 - III.A.1. Bcl-xL Inhibitors
 - III.A.2 Bcl-xL Linkers
 - Cleavable Linkers
 - Non-Cleavable Linkers
 - Groups Used to Attach Linkers to Anti-B7-H3 Antibodies
 - Linker Selection Considerations
 - III.A.3. Bcl-xL ADC Synthons

III.A.4 Methods of Synthesis of Bcl-xL ADCs

III.A.5. General Methods for Synthesizing Bcl-xL Inhibitors

III.A.6. General Methods for Synthesizing Synthons

III.A.7. General Methods for Synthesizing Anti-B7-H3 ADCs

III.B. Anti-B7-H3 ADCs: Other Exemplary Drugs for Conjugation

III.C. Anti-B7-H3 ADCs: Other Exemplary Linkers

4. IV. Purification of Anti-B7-H3 ADCs

5. V. Uses of Anti-B7-H3 Antibodies and Anti-B7-H3 ADCs

6. VI. Pharmaceutical Compositions

I. Definitions

[0035] In order that the invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

[0036] The term "anti-B7-H3 antibody" refers to an antibody that specifically binds to B7-H3. An antibody "which binds" an antigen of interest, *i.e.*, B7-H3, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. In a preferred embodiment, the antibody specifically binds to human B7-H3 (hB7-H3). Examples of anti-B7-H3 antibodies are disclosed in the examples below. Unless otherwise indicated, the term "anti-B7-H3 antibody" is meant to refer to an antibody which binds to wild type B7-H3 (e.g., a 4IgB7-H3 isoform of B7-H3) or any variant of B7-H3. The amino acid sequence of wild type human B7-H3 is provided below as SEQ ID NO: 149, where the signal peptide (amino acid residues 1-28) is underlined.

MLRRRGSPGMGVHVGAAALGALWFCLTGALEVPEDPVVALVGTDATLCCSFSPFPGFSLAQLNLIWQ
 LTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASRLRLQVRVVADEGSFTCFVSIIRDFGSAAVSL
 QVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDV
 HSILRVVLGANGTYSCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSFSP
 EPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQGNASRLRLQVRVVADEGSFTC
 FVSIIRDFGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYRGYPEAEVFWQDGQGVPLTGNV
 TTSQMANEQGLFDVHSLRVVLGANGTYSCLVRNPVLQQDAHGSVTITGQPMTFPPEALWVTVGLSVC
 LIALLVALAFVCWRKIKQSCWEENAGAEDQDGEGEKSKTALQPLKHSKEDDGQEIA (SEQ ID
 NO: 149)

Thus, the ADC binds human B7-H3 as defined in SEQ ID NO: 149. The extracellular domain (ECD) of human B7-H3 is provided in SEQ ID NO: 152 (inclusive of a His tag). As such, in one embodiment of the invention, the antibody of ADC binds the ECD of human B7-H3 as described in the ECD of SEQ ID NO: 152.

[0037] The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody or an ADC with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody or ADC is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody or ADC. By way of example, an antibody "binds specifically" to a target if the antibody, when labeled, can be competed away from its target by the corresponding non-labeled antibody. In one

embodiment, an antibody specifically binds to a target, e.g., B7-H3, if the antibody has a K_D for the target of at least about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less (less meaning a number that is less than 10^{-12} , e.g. 10^{-13}). In one embodiment, the term "specific binding to B7-H3" or "specifically binds to B7-H3," as used herein, refers to an antibody or an ADC that binds to B7-H3 and has a dissociation constant (K_D) of 1.0×10^{-7} M or less, as determined by surface plasmon resonance. It shall be understood, however, that the antibody or ADC may be capable of specifically binding to two or more antigens which are related in sequence. For example, in one embodiment, an antibody can specifically bind to both human and a non-human (e.g., mouse or non-human primate) orthologs of B7-H3.

[0038] The term "antibody" refers to an immunoglobulin molecule that specifically binds to an antigen and comprises a heavy (H) chain(s) and a light (L) chain(s). Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY) and class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

[0039] While the term "antibody" is not intended to include antigen binding portions of an antibody (defined below), it is intended, in certain embodiments, to include a small number of amino acid deletions from the carboxy end of the heavy chain(s). In one embodiment, an antibody comprises a heavy chain having 1-5 amino acid deletions the carboxy end of the heavy chain. In one embodiment, an antibody is a monoclonal antibody which is an IgG, having four polypeptide chains, two heavy (H) chains, and two light (L) chains) that can bind to hB7-H3. In one embodiment, an antibody is a monoclonal IgG antibody comprising a lambda or a kappa light chain.

[0040] The term "antigen binding portion" or "antigen binding fragment" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hB7-H3). It has been shown that the antigen binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen binding portion" of an antibody. In certain embodiments of the invention, scFv molecules may be incorporated into a fusion protein. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a

single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R.J., et al. (1994) Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

[0041] An IgG is a class of antibody comprising two heavy chains and two light chains arranged in a Y-shape. An IgG constant domain refers to a heavy or light chain constant domain. Exemplary human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and represented below in Table A.

Table A: Sequences of human IgG heavy chain constant domains and light chain constant domains

Protein	Sequence Identifier	Sequence
Ig gamma-1 constant region	SEQ ID NO:159	ASTKGPSVFPLAPSSKSTSGGTAAAGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFSVMSHEALHNHYT QKSLSLSPGK
Ig gamma-1 constant region mutant	SEQ ID NO:160	ASTKGPSVFPLAPSSKSTSGGTAAAGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFSVMSHEALHNHYT QKSLSLSPGK
Ig Kappa constant region	SEQ ID NO:161	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKKHKVYACEVTHQGLSS PVTKSFNRGEC
Ig Lambda constant region	SEQ ID NO:162	QPKAAPSVTLFPPSSEELQANKATLVCLISDF YPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVE KTVAPTECS

[0042] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds B7-H3 is substantially free of antibodies that specifically bind antigens other than B7-H3). An isolated antibody that specifically binds B7-H3 may, however, have cross-reactivity to other antigens, such as B7-H3 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0043] The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a nonhuman species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. In particular, the term "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')₂, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In other embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

[0044] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0045] The terms "Kabat numbering," "Kabat definitions," and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and, Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

[0046] As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain (HC) and the light chain (LC), which are designated CDR1, CDR2 and CDR3 (or specifically HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3), for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations,

despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J. Mol. Biol. 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

[0047] As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, CDR-L2, and CDR-L3 of light chain and CDR-H1, CDR-H2, and CDR-H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region.

[0048] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

[0049] The term "human acceptor framework", as used herein, is meant to refer to a framework of an antibody or antibody fragment thereof comprising the amino acid sequence of a VH or VL framework derived from a human antibody or antibody fragment thereof or a human consensus sequence framework into which CDR's from a non-human species may be incorporated.

[0050] "Percent (%) amino acid sequence identity" with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software.

Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0051] The term "multivalent antibody" is used herein to denote an antibody comprising two or more antigen binding sites. In certain embodiments, the multivalent antibody may be engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody.

[0052] The term "multispecific antibody" refers to an antibody capable of binding two or more unrelated antigens. In one embodiment, the multispecific antibody is a bispecific antibody that is capable of binding to two unrelated antigens, *e.g.*, a bispecific antibody, or antigen-binding portion thereof, that binds B7-H3 and CD3.

[0053] The term "activity" includes activities such as the binding specificity/affinity of an antibody or ADC for an antigen, for example, an anti-hB7-H3 antibody that binds to an hB7-H3 antigen and/or the neutralizing potency of an antibody, for example, an anti-hB7-H3 antibody whose binding to hB7-H3 inhibits the biological activity of hB7-H3, *e.g.*, inhibition of proliferation of B7-H3 expressing cell lines, *e.g.*, human H146 lung carcinoma cells, human H1650 lung carcinoma cells, or human EBC1 lung carcinoma cells.

[0054] The term "non small-cell lung carcinoma (NSCLC) xenograft assay," as used herein, refers to an *in vivo* assay used to determine whether an anti-B7-H3 antibody or ADC, can inhibit tumor growth (*e.g.*, further growth) and/or decrease tumor growth resulting from the transplantation of NSCLC cells into an immunodeficient mouse. An NSCLC xenograft assay includes transplantation of NSCLC cells into an immunodeficient mouse such that a tumor grows to a desired size, *e.g.*, 200-250 mm³, whereupon the antibody or ADC is administered to the mouse to determine whether the antibody or ADC can inhibit and/or decrease tumor growth. In certain embodiments, the activity of the antibody or ADC is determined according to the percent tumor growth inhibition (%TGI) relative to a control antibody, *e.g.*, a human IgG antibody (or collection thereof) which does not specifically bind tumor cells, *e.g.*, is directed to an antigen not associated with cancer or is obtained from a source which is noncancerous (*e.g.*, normal human serum). In such embodiments, the antibody (or ADC) and the control antibody are administered to the mouse at the same dose, with the same frequency, and via the same route. In one embodiment, the mouse used in the NSCLC xenograft assay is a severe combined immunodeficiency (SCID) mouse and/or an athymic CD-1 nude mouse. Examples of NSCLC cells that may be used in the NSCLC xenograft assay include, but are not limited to, H1299 cells (NCI-H1299 [H-1299] (ATCC® CRL-5803)), H1975 cells (NCI-H1975 cells [H1975] (ATCC® CRL-5908™)), and EBC-1 cells.

[0055] The term "small-cell lung carcinoma (SCLC) xenograft assay," as used herein, refers to an *in vivo* assay used to determine whether an anti-B7-H3 antibody or ADC, can inhibit tumor growth (*e.g.*, further growth) and/or decrease tumor growth resulting from the transplantation of SCLC cells into an immunodeficient mouse. An SCLC xenograft assay includes transplantation of SCLC cells into an immunodeficient mouse such that a tumor grows to a desired size, *e.g.*, 200-250 mm³, whereupon the antibody or ADC is administered to the mouse to determine whether the antibody or ADC can inhibit and/or decrease tumor growth. In certain embodiments, the activity of the antibody or ADC is determined according to the percent tumor growth inhibition (%TGI) relative to a control antibody, *e.g.*, a human IgG antibody (or collection thereof) which does not specifically bind tumor cells, *e.g.*, is directed to an antigen not associated with cancer or is obtained from a source which is noncancerous (*e.g.*, normal human serum). In such embodiments, the antibody (or ADC) and the control antibody are administered to the mouse at the same dose, with the same frequency, and via the same route. In one embodiment, the mouse used in the NSCLC xenograft assay is a severe combined immunodeficiency (SCID) mouse and/or an athymic CD-1 nude mouse. Examples of SCLC cells that may be used in the SCLC xenograft assay include, but are not limited to, H146 cells (NCI-H146 cells [H146] (ATCC® HTB-173™)), and H847 cells (NCI-H847 [H847] (ATCC®

CRL-5846™)). The term "epitope" refers to a region of an antigen that is bound by an antibody or ADC. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

[0056] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnson, B., et al. (1991) *Anal. Biochem.* 198:268-277. In one embodiment, surface plasmon resonance is determined according to the methods described in Example 2.

[0057] The term " k_{on} " or " k_a ", as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex.

[0058] The term " k_{off} " or " k_d ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

[0059] The term " K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction (e.g., huAb13 antibody and B7-H3). K_D is calculated by k_a / k_d .

[0060] The term "competitive binding", as used herein, refers to a situation in which a first antibody competes with a second antibody, for a binding site on a third molecule, e.g., an antigen. In one embodiment, competitive binding between two antibodies is determined using FACS analysis.

[0061] The term "competitive binding assay" is an assay used to determine whether two or more antibodies bind to the same epitope. In one embodiment, a competitive binding assay is a competition fluorescent activated cell sorting (FACS) assay which is used to determine whether two or more antibodies bind to the same epitope by determining whether the fluorescent signal of a labeled antibody is reduced due to the introduction of a non-labeled antibody, where competition for the same epitope will lower the level of fluorescence.

[0062] The term "labeled antibody" as used herein, refers to an antibody, or an antigen binding portion thereof, with a label incorporated that provides for the identification of the binding protein, e.g., an antibody. Preferably, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , or ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates.

[0063] The term "antibody-drug-conjugate" or "ADC" refers to a binding protein, such as an antibody or antigen binding fragment thereof, chemically linked to one or more chemical drug(s) (also referred to herein as agent(s), warhead(s), and payload(s)) that may optionally be therapeutic or cytotoxic agents. In the

present invention an ADC includes an antibody, a drug, (e.g. a cytotoxic drug), and a linker that enables attachment or conjugation of the drug to the antibody. The ADC of the invention has 2 drugs conjugated to the antibody. In the present invention the drug is a Bcl-xL inhibitor.

[0064] The terms "anti-B7-H3 antibody drug conjugate" or "anti-B7-H3 ADC", used interchangeably herein, refer to an ADC comprising an antibody that specifically binds to B7-H3, whereby the antibody is conjugated to one or more chemical agent(s). In one embodiment, the anti-B7-H3 ADC comprises antibody huAb13v1 conjugated to a Bcl-xL inhibitor. In the present invention the anti-B7-H3 ADC binds to human B7-H3 (hB7-H3).

[0065] The term "Bcl-xL inhibitor", as used herein, refers to a compound which antagonizes Bcl-xL activity in a cell. In one embodiment, a Bcl-xL inhibitor promotes apoptosis of a cell by inhibiting Bcl-xL activity.

[0066] The term "drug-to-antibody ratio" or "DAR" refers to the number of drugs, e.g., Bcl-xL inhibitor, attached to the antibody of the ADC. The number of drugs, e.g., Bcl-xL inhibitors, attached to the antibody of the ADC of the present invention is 2.

[0067] The term a "xenograft assay", as used herein, refers to a human tumor xenograft assay, wherein human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immunocompromised mice that do not reject human cells.

[0068] The term "cancer" is meant to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include glioblastoma, acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), non-small cell lung cancer, lung cancer, colon cancer, colorectal cancer, head and neck cancer, breast cancer (e.g., triple negative breast cancer), pancreatic cancer, squamous cell tumors, squamous cell carcinoma (e.g., squamous cell lung cancer or squamous cell head and neck cancer), anal cancer, skin cancer, and vulvar cancer. In one embodiment, the ADCs of the invention are administered to a patient having a tumor(s) that overexpresses B7-H3. In one embodiment, the ADCs of the invention are administered to a patient having a solid tumor which is likely to over-express B7-H3. In one embodiment, the ADCs of the invention are administered to a patient having squamous cell non-small cell lung cancer (NSCLC). In one embodiment, the ADCs of the invention are administered to a patient having solid tumors, including advanced solid tumors. In one embodiment, the ADCs of the invention are administered to a patient having prostate cancer. In one embodiment, the ADCs of the invention are administered to a patient having non-small cell lung cancer. In one embodiment, the ADCs of the invention are administered to a patient having a glioblastoma. In one embodiment, the ADCs of the invention are administered to a patient having colon cancer. In one embodiment, the ADCs of the invention are administered to a patient having head and neck cancer. In one embodiment, the ADCs of the invention are administered to a patient having kidney cancer. In one embodiment, the ADCs of the invention are administered to a patient having clear cell renal cell carcinoma. In one embodiment, the ADCs of the invention are administered to a patient having glioma. In one embodiment, the ADCs of the invention are administered to a patient having melanoma. In one embodiment, the ADCs of the invention are administered to a patient having pancreatic cancer. In one embodiment, the ADCs of the invention are administered to a patient having gastric cancer. In one embodiment, the ADCs of the invention are administered to a patient having ovarian cancer. In one embodiment, the ADCs of the invention are administered to a patient having colorectal cancer. In one embodiment, the ADCs of the invention are administered to a patient having renal cancer. In one embodiment, the ADCs of the invention are administered to a patient having small cell lung cancer. In one embodiment, the ADCs of the invention are administered to a patient having hepatocellular carcinoma. In one embodiment, the ADCs of the invention are administered to a patient having hypopharyngeal squamous

cell carcinoma. In one embodiment, the ADCs of the invention are administered to a patient having neuroblastoma. In one embodiment, the ADCs of the invention are administered to a patient having breast cancer. In one embodiment, the ADCs of the invention are administered to a patient having endometrial cancer. In one embodiment, the ADCs of the invention are administered to a patient having urothelial cell carcinoma. In one embodiment, the ADCs of the invention are administered to a patient having acute myeloid leukemia (AML). In one embodiment, the ADCs of the invention are administered to a patient having non-Hodgkin's lymphoma (NHL).

[0069] The term "B7-H3 expressing tumor," as used herein, refers to a tumor which expresses B7-H3 protein. In one embodiment, B7-H3 expression in a tumor is determined using immunohistochemical staining of tumor cell membranes, where any immunohistochemical staining above background level in a tumor sample indicates that the tumor is a B7-H3 expressing tumor. Methods for detecting expression of B7-H3 in a tumor are known in the art, and include immunohistochemical assays. In contrast, a "B7-H3 negative tumor" is defined as a tumor having an absence of B7-H3 membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

[0070] The terms "overexpress," "overexpression," or "overexpressed" interchangeably refer to a gene that is transcribed or translated at a detectably greater level, usually in a cancer cell, in comparison to a normal cell. Overexpression therefore refers to both overexpression of protein and RNA (due to increased transcription, post transcriptional processing, translation, post translational processing, altered stability, and altered protein degradation), as well as local overexpression due to altered protein traffic patterns (increased nuclear localization), and augmented functional activity, *e.g.*, as in an increased enzyme hydrolysis of substrate. Thus, overexpression refers to either protein or RNA levels. Overexpression can also be by 50%, 60%, 70%, 80%, 90% or more in comparison to a normal cell or comparison cell. In certain embodiments, the anti-B7-H3 ADCs of the invention are used to treat solid tumors likely to overexpress B7-H3.

[0071] The term "gene amplification", as used herein, refers to a cellular process characterized by the production of multiple copies of any particular piece of DNA. For example, a tumor cell may amplify, or copy, chromosomal segments as a result of cell signals and sometimes environmental events. The process of gene amplification leads to the production of additional copies of the gene. In one embodiment, the gene is B7-H3, *i.e.*, "B7-H3 amplification." In one embodiment, the compositions and methods disclosed herein are used to treat a subject having B7-H3 amplified cancer.

[0072] The term "administering" as used herein is meant to refer to the delivery of a substance (*e.g.*, an anti-B7-H3 antibody or ADC) to achieve a therapeutic objective (*e.g.*, the treatment of a B7-H3-associated disorder). Modes of administration may be parenteral, enteral and topical. Parenteral administration is usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0073] The term "combination therapy" or "combination" in the context of a therapeutic method (*e.g.*, a treatment method), as used herein, refers to the administration of two or more therapeutic substances, *e.g.*, an ADC and an additional therapeutic agent. The additional therapeutic agent may be administered concomitant with, prior to, or following the administration of the anti-B7-H3 ADC.

[0074] As used herein, the term "effective amount" or "therapeutically effective amount" refers to the amount of a drug, *e.g.*, an antibody or ADC, which is sufficient to reduce or ameliorate the severity and/or duration of a disorder, *e.g.*, cancer, or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or

therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent). The effective amount of an ADC may, for example, inhibit tumor growth (e.g., inhibit an increase in tumor volume), decrease tumor growth (e.g., decrease tumor volume), reduce the number of cancer cells, and/or relieve to some extent one or more of the symptoms associated with the cancer. The effective amount may, for example, improve disease free survival (DFS), improve overall survival (OS), or decrease likelihood of recurrence.

[0075] As used herein, the term "stable" refers to compounds that possess stability sufficient to allow manufacture and that maintain the integrity of the compound for a sufficient period of time to be useful for the purpose detailed herein.

[0076] As used herein, the following terms are intended to have the following meanings:

The term salt when used in context of "or salt thereof" include salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention

Where a salt is intended to be administered to a patient (as opposed to, for example, being in use in an *in vitro* context), the salt preferably is pharmaceutically acceptable and/or physiologically compatible. The term "pharmaceutically acceptable" is used adjectivally in this patent application to mean that the modified noun is appropriate for use as a pharmaceutical product or as a part of a pharmaceutical product. The term "pharmaceutically acceptable salt" includes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention.

II. Anti-B7-H3 Antibodies

[0077] The anti-B7-H3 antibodies described herein provide the ADCs of the invention with the ability to bind to B7-H3 such that the cytotoxic Bcl-xL drug attached to the antibody may be delivered to the B7-H3-expressing cell, particularly a B7-H3 expressing cancer cell.

[0078] While the term "antibody" is used throughout, it should be noted that antibody fragments (*i.e.*, antigen-binding portions of an anti-B7-H3 antibody) are also included in the invention and may be included in the embodiments (methods and compositions) described throughout. For example, an anti-B7-H3 antibody fragment may be conjugated to the Bcl-xL inhibitors described herein. Thus, it is within the scope of the invention that in certain embodiments, antibody fragments of the anti-B7-H3 antibodies described herein are conjugated to Bcl-xL inhibitors via linkers. In certain embodiments, the anti-B7-H3 antibody binding portion is a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, an scFv, a single domain antibody, or a diabody.

II.A. Anti-B7-H3 Chimeric Antibodies

[0079] A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397. In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454,) by

splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used.

[0080] As described in Example 3, eighteen anti-B7-H3 murine antibodies were identified having high specific binding activity against human and cynomolgus B7-H3. Chimeric antibodies, in the context of a human immunoglobulin constant region, were generated from these eighteen antibodies.

II.B. Humanized Anti-B7-H3 Antibodies

[0081] The chimeric antibodies disclosed herein may be used in the production of humanized anti-B7-H3 antibodies. For example, following the generation and characterization of chimeric anti-B7-H3 antibodies chAb1-chAb18, antibodies chAb3, chAb13, and chAb18 were selected for humanization. Specifically, six different humanized antibodies were created based on chAb3 nine different humanized antibodies were created based on chAb13 (referred to herein as huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, huAb13v9), and ten different humanized antibodies were created based on chAb18. Tables 18 and 19 provide the amino acid sequences of CDR, VH and VL regions of humanized chAb13.

[0082] Generally, humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., [www.ncbi.nlm.nih.gov/entrez-query.fcgi](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi); www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH-05/kuby05.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/about.mrc7/m-ikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html; www.immunologylink.com/; pathbox.wustl.edu/about.hcenter/index.html; www.biotech.ufl.edu/about.hcl/; www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.ac.jp/about.yasuhito/Elisa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/links.html; www.biotech.ufl.edu/about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/about.rek/AEP-Start.html; baserv.uci.kun.nl/about.jraats/links1.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/about.honegger/AHOseminar/Slide01.html; www.cryst.bbk.ac.uk/about.ubcg07s/; www.nimr.mrc.ac.uk/CC/caewg/caewg.htm; www.path.cam.ac.uk/about.mrc7/h-umanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/about.fmolina/Webpages/Pept/spottech.html; www.jerini.de/fr_roducts.htm; www.patents.ibm.com/ibm.html. Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983). Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

[0083] Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat.

No. 5,585,089; Riechmann et al., Nature 332:323 (1988)) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., Nature 321:522 (1986); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Caster et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994); PCT publication WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567.

Humanized anti-B7-H3 antibodies derived from chab13

[0084] The nine different humanized antibodies created based on chAb13 include the following:

1. A) huAb13v1 (VH amino acid sequence set forth in SEQ ID NO: 147 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 144 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
2. B) huAb13v2 (VH amino acid sequence set forth in SEQ ID NO: 146 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 143 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
3. C) huAb13v3 (VH amino acid sequence set forth in SEQ ID NO: 146 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 144 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
4. D) huAb13v4 (VH amino acid sequence set forth in SEQ ID NO: 146 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 145 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
5. E) huAb13v5 (VH amino acid sequence set forth in SEQ ID NO: 147 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 143 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
6. F) huAb13v6 (VH amino acid sequence set forth in SEQ ID NO: 147 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 145 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
7. G) huAb13v7 (VH amino acid sequence set forth in SEQ ID NO: 148 and VH CDR1, CDR2, and CDR3

amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 143 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);

8. H) huAb13v8 (VH amino acid sequence set forth in SEQ ID NO: 148 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 144 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively);
9. I) huAb13v9 (VH amino acid sequence set forth in SEQ ID NO: 148 and VH CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 33, 34, and 35, respectively; and VL amino acid sequence set forth in SEQ ID NO: 145 and VL CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively).

[0085] Thus, one aspect of the present disclosure provides antibodies comprising variable and/or CDR sequences from a humanized antibody derived from chAb13. The present disclosure features anti-B7-H3 antibodies which are derived from chAb13 and have improved characteristics, *e.g.*, improved binding affinity to isolated B7-H3 protein and improved binding to B7-H3 expressing cells, as described in the Examples below. Collectively these novel antibodies are referred to herein as "Ab13 variant antibodies." Generally, the Ab13 variant antibodies retain the same epitope specificity as Ab13.

[0086] Conservative amino acid substitutions may also be made in portions of the antibodies other than, or in addition to, the CDRs. For example, conservative amino acid modifications may be made in a framework region or in the Fc region. A variable region or a heavy or light chain may comprise 1, 2, 3, 4, 5, 1-2, 1-3, 1-4, 1-5, 1-10, 1-15, 1-20, 1-25, or 1-50 conservative amino acid substitutions relative to the anti-B7-H3 antibody sequences provided herein. In certain embodiments, the anti-B7-H3 antibody comprises a combination of conservative and non-conservative amino acid modification.

[0087] To generate and to select CDRs having preferred B7-H3 binding and/or neutralizing activity with respect to hB7-H3, standard methods known in the art for generating antibodies, or antigen binding portions thereof, and assessing the B7-H3 binding and/or neutralizing characteristics of those antibodies, or antigen binding portions thereof, may be used, including but not limited to those specifically described herein.

[0088] In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, or IgD constant region. In certain embodiments, the anti-B7-H3 antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgG constant domain, a human IgM constant domain, a human IgE constant domain, and a human IgA constant domain. In further embodiments, the antibody, or antigen binding portion thereof, has an IgG1 heavy chain constant region, an IgG2 heavy chain constant region, an IgG3 constant region, or an IgG4 heavy chain constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

[0089] In certain embodiments, the anti-B7-H3 antibody binding portion is a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, an scFv, a single domain antibody, or a diabody.

[0090] In certain embodiments, the anti-B7-H3 antibody, or antigen binding portion thereof, is a multispecific antibody, *e.g.* a bispecific antibody.

[0091] Replacements of amino acid residues in the Fc portion to alter antibody effector function have been described (Winter, et al. US Patent Nos. 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

[0092] One embodiment of the invention includes a labeled anti-B7-H3 antibody, or antibody portion thereof, where the antibody is derivatized or linked to one or more functional molecule(s) (e.g., another peptide or protein). For example, a labeled antibody can be derived by functionally linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a pharmaceutical agent, a protein or peptide that can mediate the association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag), and/or a cytotoxic or therapeutic agent selected from the group consisting of a mitotic inhibitor, an antitumor antibiotic, an immunomodulating agent, a vector for gene therapy, an alkylating agent, an antiangiogenic agent, an antimetabolite, a boron-containing agent, a chemoprotective agent, a hormone, an antihormone agent, a corticosteroid, a photoactive therapeutic agent, an oligonucleotide, a radionuclide agent, a topoisomerase inhibitor, a kinase inhibitor, a radiosensitizer, and a combination thereof.

[0093] Useful detectable agents with which an antibody or antibody portion thereof, may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

[0094] In one embodiment, the antibody of the invention is conjugated to an imaging agent. Examples of imaging agents that may be used in the compositions and methods described herein include, but are not limited to, a radiolabel (e.g., indium), an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

[0095] In one embodiment, the antibodies or ADCs are linked to a radiolabel, such as, but not limited to, indium (^{111}In). $^{111}\text{Indium}$ may be used to label the antibodies and ADCs described herein for use in identifying B7-H3 positive tumors. In a certain embodiment, anti-B7-H3 antibodies (or ADCs) described herein are labeled with ^{111}I via a bifunctional chelator which is a bifunctional cyclohexyl diethylenetriaminepentaacetic acid (DTPA) chelate (see US Patent Nos. 5,124,471; 5,434,287; and 5,286,850).

[0096] Another embodiment of the invention provides a glycosylated binding protein wherein the anti-B7-H3 antibody or antigen binding portion thereof comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting

proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* 21 (2005), pp. 11-16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., et al., *Mol. Immunol.* (1993) 30:1361- 1367), or result in increased affinity for the antigen (Wallick, S.C., et al., *Exp. Med.* (1988) 168:1099-1109; Wright, A., et al., *EMBO J.* (1991) 10:2717-2723).

[0097] One aspect of the invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity, but have increased or decreased binding activity, are another object of the invention.

[0098] In still another embodiment, the glycosylation of the anti-B7-H3 antibody or antigen binding portion of the invention is modified. For example, an aglycosylated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861.

[0099] Additionally or alternatively, a modified anti-B7-H3 antibody of the invention can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80.

[0100] Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (*e.g.*, glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

[0101] Differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize

specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

[0102] Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using recombinant techniques, a practitioner may generate antibodies or antigen binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S. patent Publication Nos. 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

[0103] Antibodies may be produced by any of a number of techniques. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0104] Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R.J. Kaufman and P.A. Sharp (1982) Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[0105] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[0106] In a preferred system for recombinant expression of an antibody, or antigen binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV

enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell in a suitable culture medium until a recombinant antibody is synthesized. Recombinant antibodies of the invention may be produced using nucleic acid molecules corresponding to the amino acid sequences disclosed herein. The method can further comprise isolating the recombinant antibody from the culture medium.

[0107] The N- and C-termini of antibody polypeptide chains of the present invention may differ from the expected sequence due to commonly observed post-translational modifications. For example, C-terminal lysine residues are often missing from antibody heavy chains. Dick et al. (2008) *Biotechnol. Bioeng.* 100:1132. N-terminal glutamine residues, and to a lesser extent glutamate residues, are frequently converted to pyroglutamate residues on both light and heavy chains of therapeutic antibodies. Dick et al. (2007) *Biotechnol. Bioeng.* 97:544; Liu et al. (2011) *JBC* 286:11211; Liu et al. (2011) *J. Biol. Chem.* 286:11211.

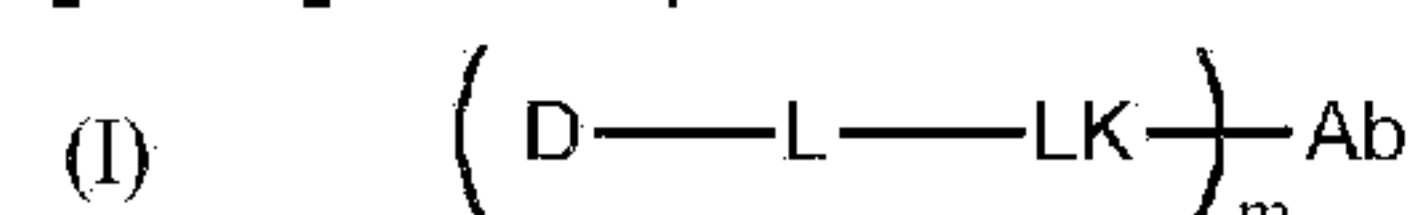
III. Anti-B7-H3 Antibody Drug Conjugates (ADCs)

[0108] Anti-B7-H3 antibodies described herein may be conjugated to a drug moiety to form an anti-B7-H3 Antibody Drug Conjugate (ADC). Antibody-drug conjugates (ADCs) may increase the therapeutic efficacy of antibodies in treating disease, *e.g.*, cancer, due to the ability of the ADC to selectively deliver one or more drug moiety(s) to target tissues, such as a tumor-associated antigen, *e.g.*, B7-H3 expressing tumors. Thus, the invention provides anti-B7-H3 ADCs for therapeutic use, *e.g.*, treatment of cancer.

[0109] Anti-B7-H3 ADCs of the invention comprise an anti-B7-H3 antibody, *i.e.*, an antibody that specifically binds to B7-H3, linked to 2 drug moieties. The specificity of the ADC is defined by the specificity of the antibody, *i.e.*, anti-B7-H3.

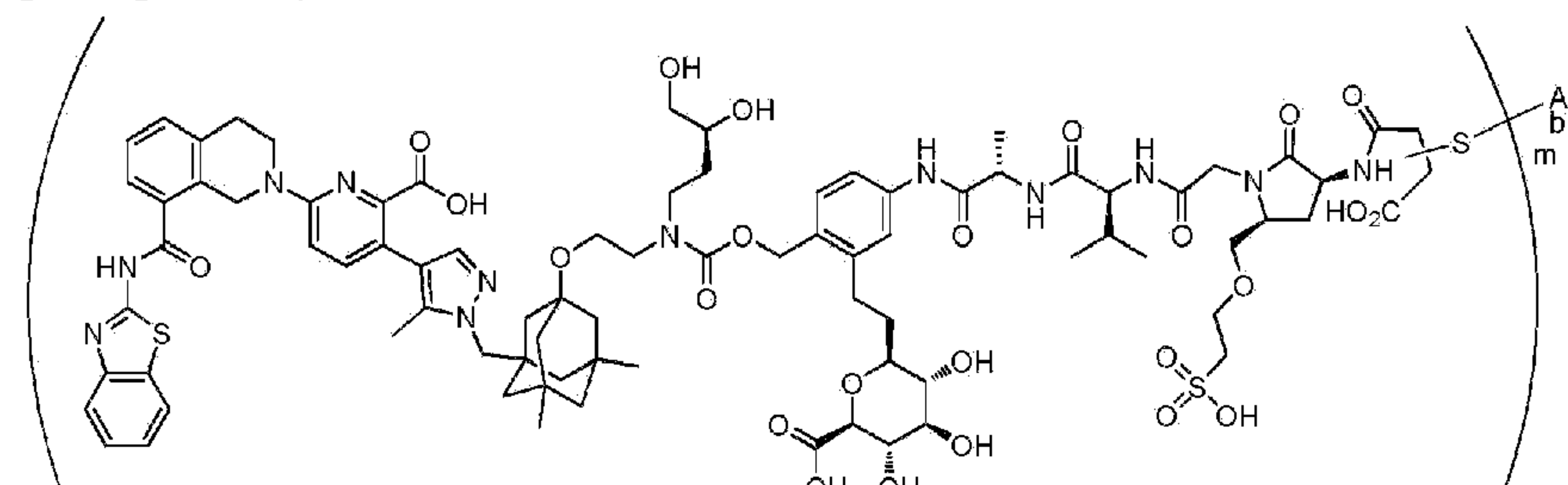
[0110] The terms "drug," "agent," and "drug moiety" are used interchangeably herein. The terms "linked" and "conjugated" are also used interchangeably herein and indicate that the antibody and moiety are covalently linked.

[0111] In the present invention the ADC of the claims has the following general formula (formula I):



wherein Ab is the antibody, (L) is a linker, (D) is a drug, and LK represents a covalent linkage linking linker L to antibody Ab; and m is 2.. The DAR of an ADC is equivalent to the "m" referred to in Formula I.

[0112] In the present invention, the ADC is:



wherein m is 2 and Ab is an anti-hB7-H3 antibody, wherein the antibody Ab comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 35, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 34, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 33; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 38, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 37.

III. A. Anti-B7-H3 ADCs: Bcl-xL Inhibitors, Linkers, Synthons, and Methods of Making Same

[0113] Dysregulated apoptotic pathways have also been implicated in the pathology of cancer. The implication that down-regulated apoptosis (and more particularly the Bcl-2 family of proteins) is involved in the onset of cancerous malignancy has revealed a novel way of targeting this still elusive disease. Research has shown, for example, the anti-apoptotic proteins, Bcl 2 and Bcl-xL, are overexpressed in many cancer cell types. See, Zhang, 2002, Nature Reviews/Drug Discovery 1:101; Kirkin et al., 2004, Biochimica Biophysica Acta 1644:229-249; and Amundson et al., 2000, Cancer Research 60:6101-6110. The effect of this deregulation is the survival of altered cells which would otherwise have undergone apoptosis in normal conditions. The repetition of these defects associated with unregulated proliferation is thought to be the starting point of cancerous evolution.

[0114] The disclosure concerns anti-hB7-H3 ADCs comprising an anti-hB7-H3 antibody conjugated to a drug via a linker, wherein the drug is a Bcl-xL inhibitor as defined in claim 1.

III.A.1. Bcl-xL Inhibitors

[0115] One aspect of the instant disclosure concerns Bcl-xL inhibitors that have low cell permeability. The compounds are generally heterocyclic in nature and include one or more solubilizing groups that impart the compounds with high water solubility and low cell permeability. The solubilizing groups are generally groups that are capable of hydrogen bonding, forming dipole-dipole interactions, and/or that include a polyethylene glycol polymer containing from 1 to 30 units, one or more polyols, one or more salts, or one or more groups that are charged at physiological pH.

[0116] The Bcl-xL inhibitors bind to and inhibit anti-apoptotic Bcl-xL proteins, inducing apoptosis. The ability of specific Bcl-xL inhibitors to bind to and inhibit Bcl-xL activity may be confirmed in standard binding and activity assays, including, for example, the TR-FRET Bcl-xL binding assays described in Tao et al., 2014, ACS Med. Chem. Lett., 5:1088-1093. A specific TR-FRET Bcl-xL binding assay that can be used to confirm Bcl-xL binding is provided in Example 4, below. Typically, Bcl-xL inhibitors useful as inhibitors *per se* and in the ADCs described herein will exhibit a K_i in the binding assay of Example 5 of less than about 1 nM, but may exhibit a significantly lower K_i , for example a K_i of less than about 1, 0.1, or even 0.01 nM.

[0117] Bcl-xL inhibitory activity may also be confirmed in standard cell-based cytotoxicity assays, such as the FL5.12 cellular and Molt-4 cytotoxicity assays described in Tao et al., 2014, ACS Med. Chem. Lett., 5:1088-1093. A specific Molt-4 cellular cytotoxicity assay that may be used to confirm Bcl-xL inhibitory activity of specific Bcl-xL inhibitors that are able to permeate cell membranes is provided in Examples 5 and 6, below. Typically, such cell-permeable Bcl-xL inhibitors will exhibit an EC_{50} of less than about 500 nM in the Molt-4 cytotoxicity assay of Examples 5 and 6, but may exhibit a significantly lower EC_{50} , for example an EC_{50} of

less than about 250, 100, 50, 20, 10 or even 5 nM.

[0118] Owing to the presence of solubilizing groups, many of the Bcl-xL inhibitors described herein are expected to exhibit low or very low cell permeability, and therefore will not yield significant activity in certain cellular assays due to the inability of the compound to traverse the cell membrane, including the Molt-4 cellular toxicity assay of Examples 5 and 6. Bcl-xL inhibitory activity of compounds that do not freely traverse cell membranes may be confirmed in cellular assays with permeabilized cells. The process of mitochondrial outer-membrane permeabilization (MOMP) is controlled by the Bcl-2 family proteins. Specifically, MOMP is promoted by the pro-apoptotic Bcl-2 family proteins Bax and Bak which, upon activation oligomerize on the outer mitochondrial membrane and form pores, leading to release of cytochrome c (cyt c). The release of cyt c triggers formation of the apoptosome which, in turn, results in caspase activation and other events that commit the cell to undergo programmed cell death (see, Goldstein et al., 2005, *Cell Death and Differentiation* 12:453-462). The oligomerization action of Bax and Bak is antagonized by the anti-apoptotic Bcl-2 family members, including Bcl-2 and Bcl-xL. Bcl-xL inhibitors, in cells that depend upon Bcl-xL for survival, can cause activation of Bax and/or Bak, MOMP, release of cyt c and downstream events leading to apoptosis. The process of cyt c release can be measured via western blot of both mitochondrial and cytosolic fractions of cells and used as a proxy measurement of apoptosis in cells.

[0119] As a means of detecting Bcl-xL inhibitory activity and consequent release of cyt c for Bcl-xL inhibitors with low cell permeability, the cells can be treated with an agent that causes selective pore formation in the plasma, but not mitochondrial, membrane. Specifically, the cholesterol/phospholipid ratio is much higher in the plasma membrane than the mitochondrial membrane. As a result, short incubation with low concentrations of the cholesterol-directed detergent digitonin selectively permeabilizes the plasma membrane without significantly affecting the mitochondrial membrane. This agent forms insoluble complexes with cholesterol leading to the segregation of cholesterol from its normal phospholipid binding sites. This action, in turn, leads to the formation of holes about 40-50 Å wide in the lipid bilayer. Once the plasma membrane is permeabilized, cytosolic components able to pass over digitonin-formed holes can be washed out, including the cytochrome C that was released from mitochondria to cytosol in the apoptotic cells (Campos, 2006, *Cytometry A* 69(6):515-523).

[0120] Typically, Bcl-xL inhibitors will yield an EC₅₀ of less than about 10 nM in the Molt-4 cell permeabilized cyt c assay of Examples 5 and 6, although the compounds may exhibit significantly lower EC₅₀s, for example, less than about 5, 1, or even 0.5 nM. As demonstrated in Example 6, Bcl-xL inhibitors having low or very low cell permeability that do not exhibit activity in the standard Molt-4 cellular toxicity assay with non-permeabilized cells exhibit potent functional activity, as measured by release of cyt c, in cellular cytotoxicity assays with permeabilized cells. In addition to cytochrome c release, mitochondria undergoing apoptosis frequently lose their transmembrane mitochondrial membrane potential (Bouchier-Hayes et al., 2008, *Methods* 44(3): 222-228). JC-1 is a cationic carbocyanine dye that accumulates in mitochondria and fluoresces red when mitochondria are healthy and is lost when the mitochondrial membrane is compromised (percentage depolarization; Smiley et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88: 3671-3675; Reers et al., 1991: *Biochemistry*, 30: 4480-4486). This loss in signal can be detected in permeabilized cells using a fluorimeter (excitation 545 nm and emission of 590 nm) and is therefore fully quantitative, enhancing both reproducibility and throughput. Typically, Bcl-xL inhibitors will yield an EC₅₀ of less than about 10 nM in the Molt-4 cell permeabilized JC-1 assay of Examples 5 and 6, although the compounds may exhibit significantly lower EC₅₀s, for example, less than about 5, 1, 0.5 or even 0.05 nM. As demonstrated in Example 6, Bcl-xL inhibitors having low or very low cell permeability that do not exhibit activity in the standard Molt-4 cellular toxicity assay with non-permeabilized cells exhibit potent functional activity, as measured by their loss of transmembrane mitochondrial membrane potential in the JC-1 assay, in cellular cytotoxicity assays with permeabilized cells. Low permeability Bcl-xL inhibitors also exhibit potent activity when administered to cells in the form of ADCs

[0121] Although many of the Bcl-xL inhibitors of the present disclosure selectively or specifically inhibit Bcl-xL over other anti-apoptotic Bcl-2 family proteins, selective and/or specific inhibition of Bcl-xL is not necessary. The Bcl-xL inhibitors and ADCs comprising the compounds may also, in addition to inhibiting Bcl-xL, inhibit one or more other anti-apoptotic Bcl-2 family proteins, such as, for example, Bcl-2. In some embodiments, the Bcl-xL inhibitors and/or ADCs are selective and/or specific for Bcl-xL. By specific or selective is meant that the particular Bcl-xL inhibitor and/or ADC binds or inhibits Bcl-xL to a greater extent than Bcl-2 under equivalent assay conditions. In specific embodiments, the Bcl-xL inhibitors and/or ADCs exhibit in the range of about 10-fold, 100-fold, or even greater specificity or selectivity for Bcl-xL than Bcl-2 in binding assays.

III.A.2. Bcl-xL Linkers

[0122] In the ADCs described herein, the Bcl-xL inhibitors are linked to the antibody by way of linkers.

[0123] As will be appreciated by skilled artisans, the linkers link the Bcl-xL inhibitors to the antibody by forming a covalent linkage to the Bcl-xL inhibitor at one location and a covalent linkage to antibody at another. The covalent linkages are formed by reaction between functional groups on the linker and functional groups on the inhibitors and antibody. As used herein, the expression "linker" is intended to include (i) unconjugated forms of the linker that include a functional group capable of covalently linking the linker to a Bcl-xL inhibitor and a functional group capable of covalently linking the linker to an antibody; (ii) partially conjugated forms of the linker that include a functional group capable of covalently linking the linker to an antibody and that is covalently linked to a Bcl-xL inhibitor, or *vice versa*; and (iii) fully conjugated forms of the linker that is covalently linked to both a Bcl-xL inhibitor and an antibody.

III.A.3. Bcl-xL ADC Synthons

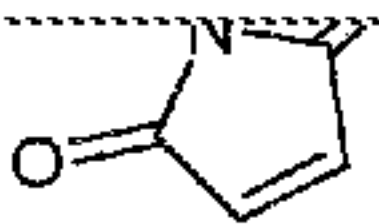
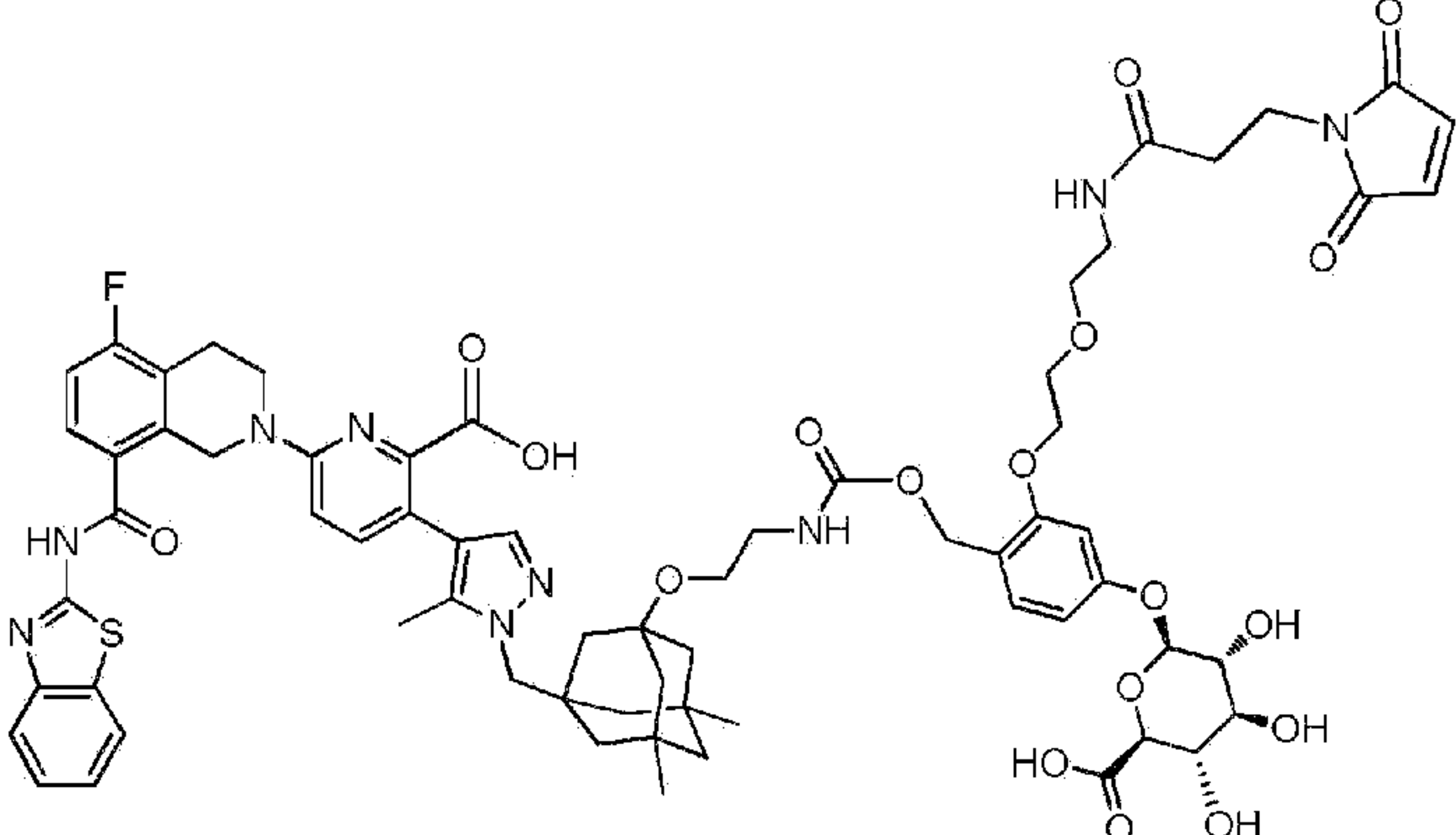
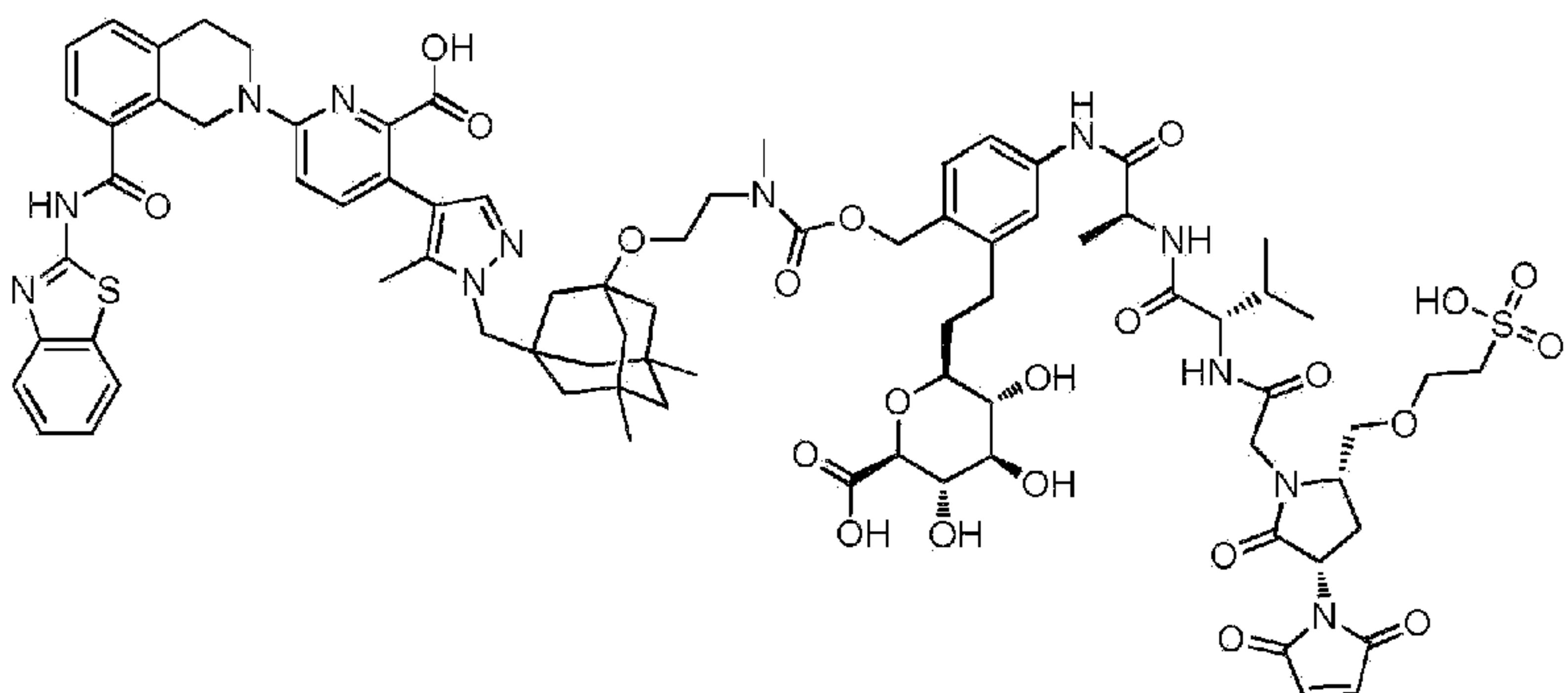
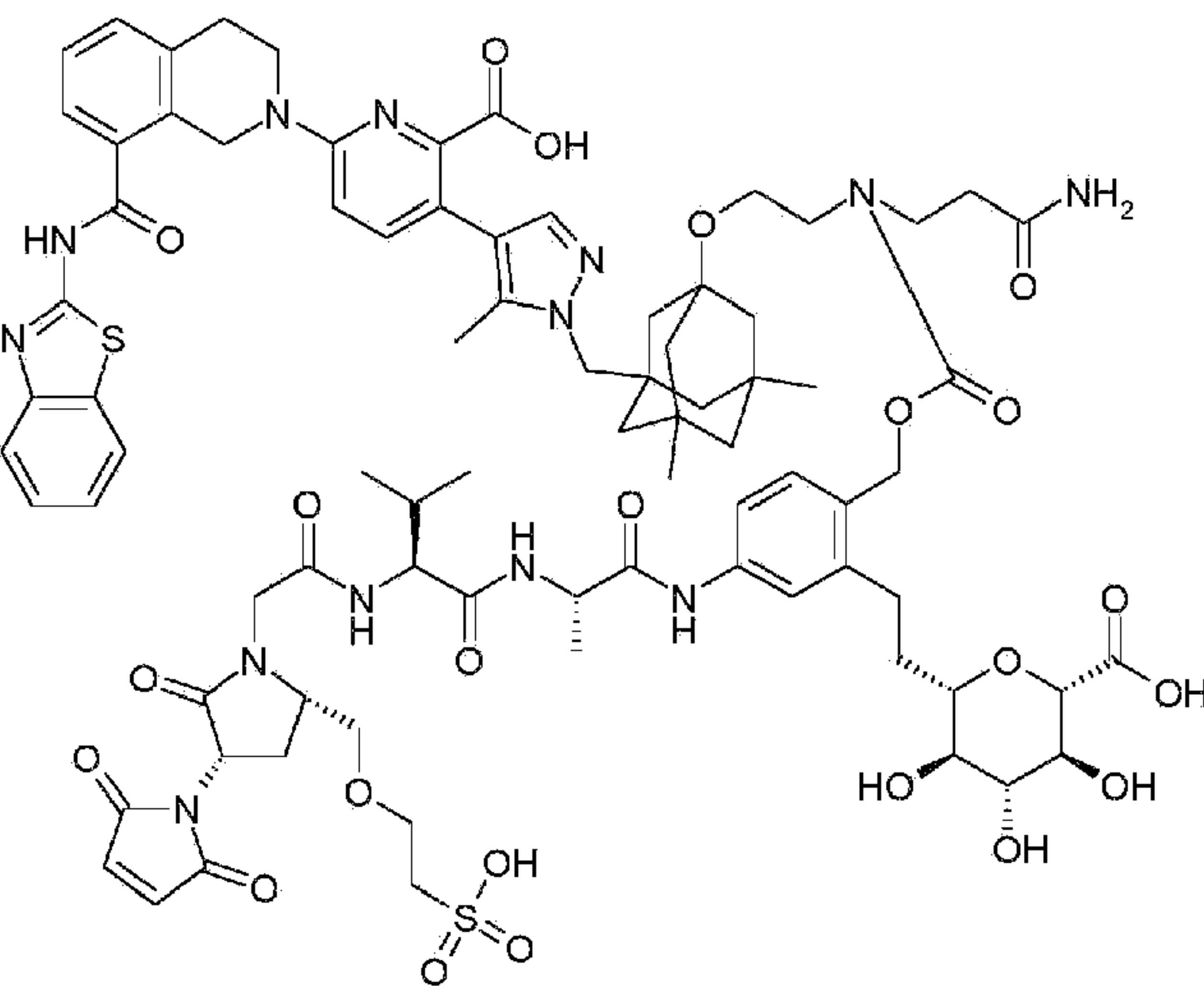
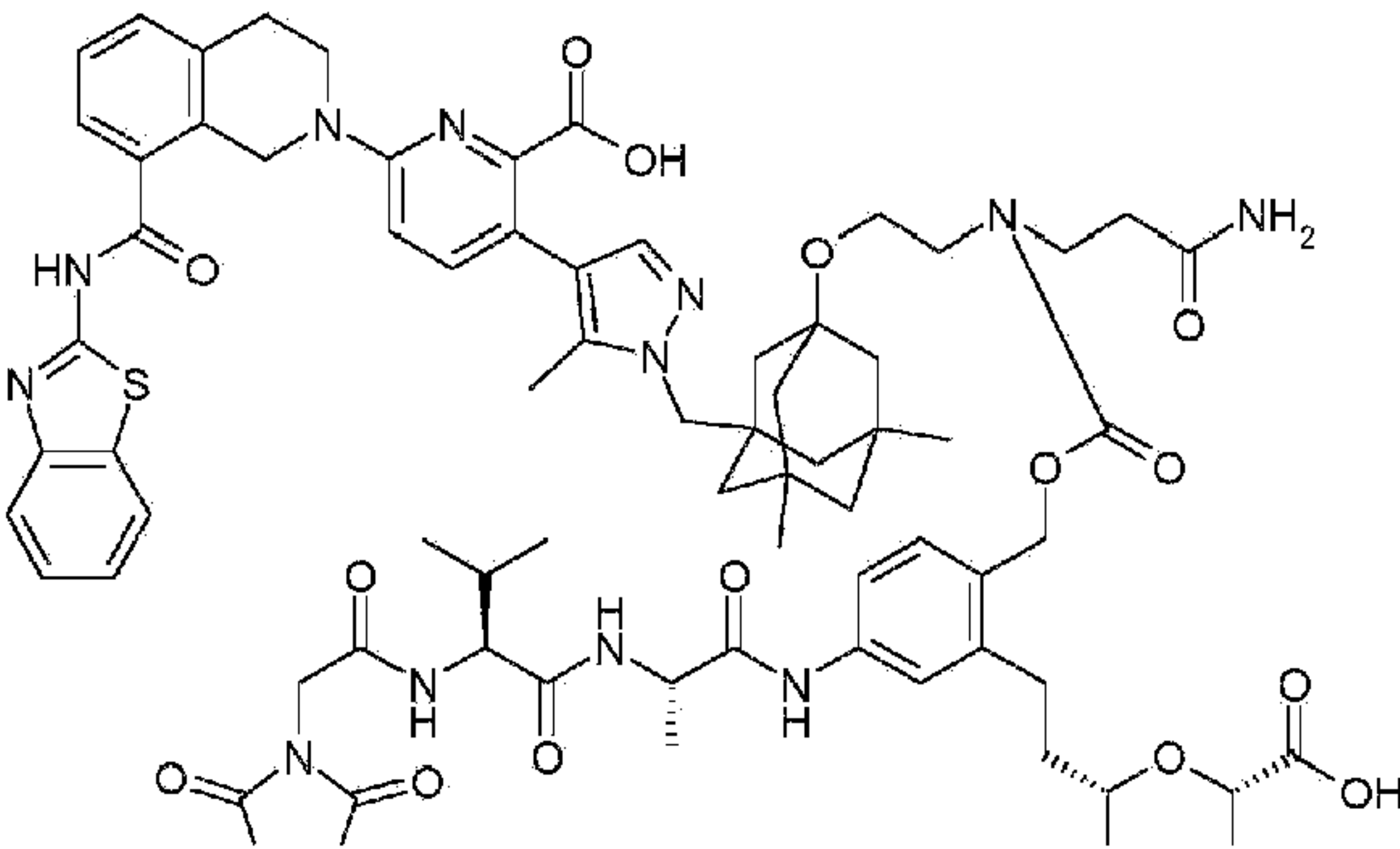
[0124] Antibody-Drug Conjugate synthons are synthetic intermediates used to form ADCs. The synthons are generally compounds according to structural formula (III):

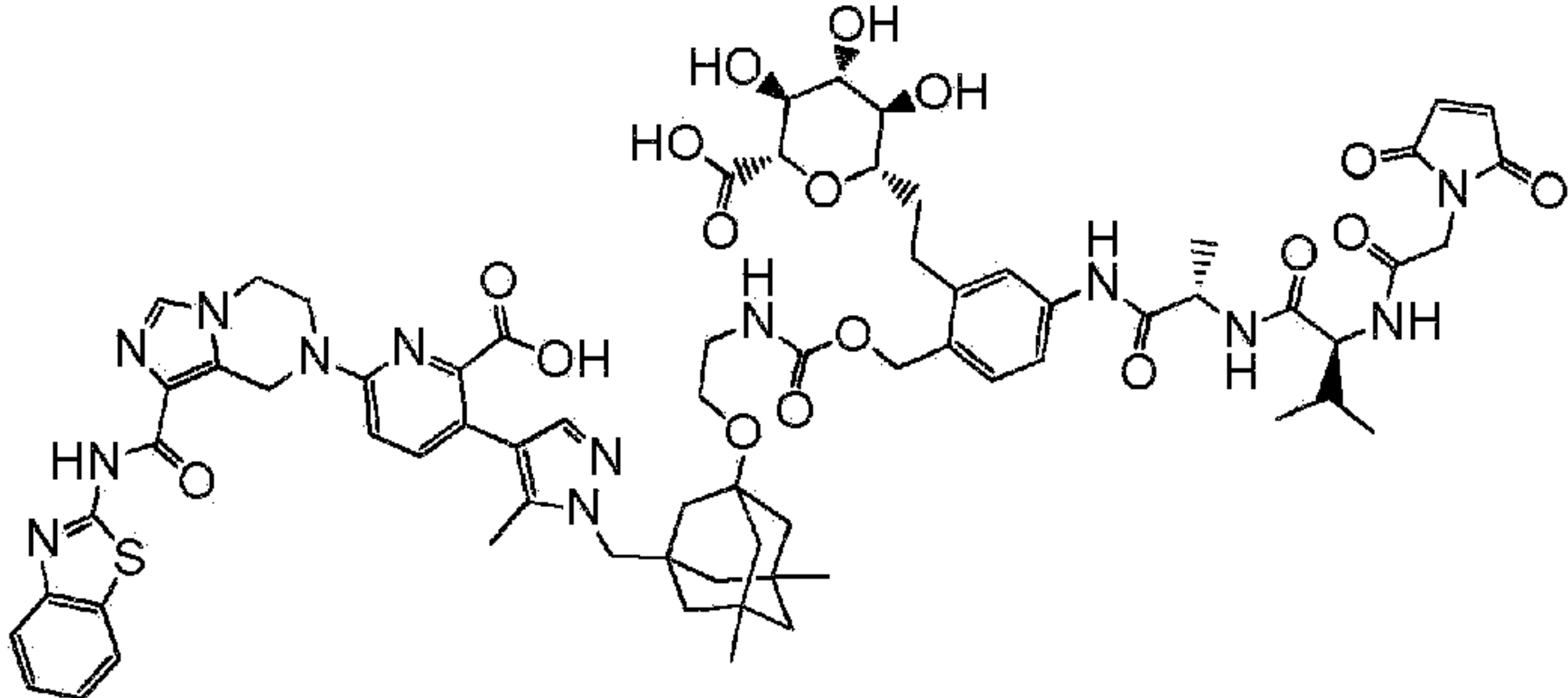
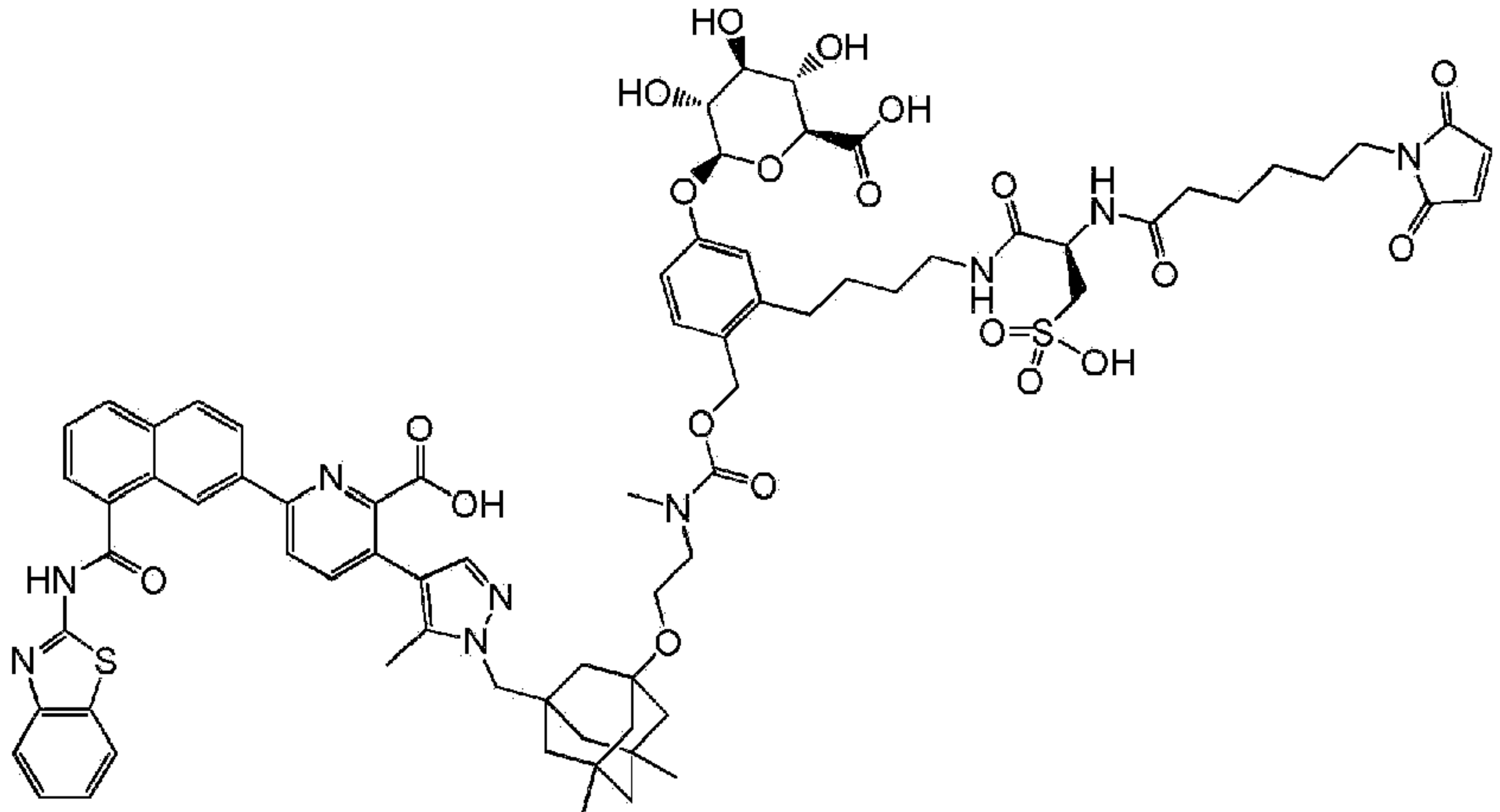
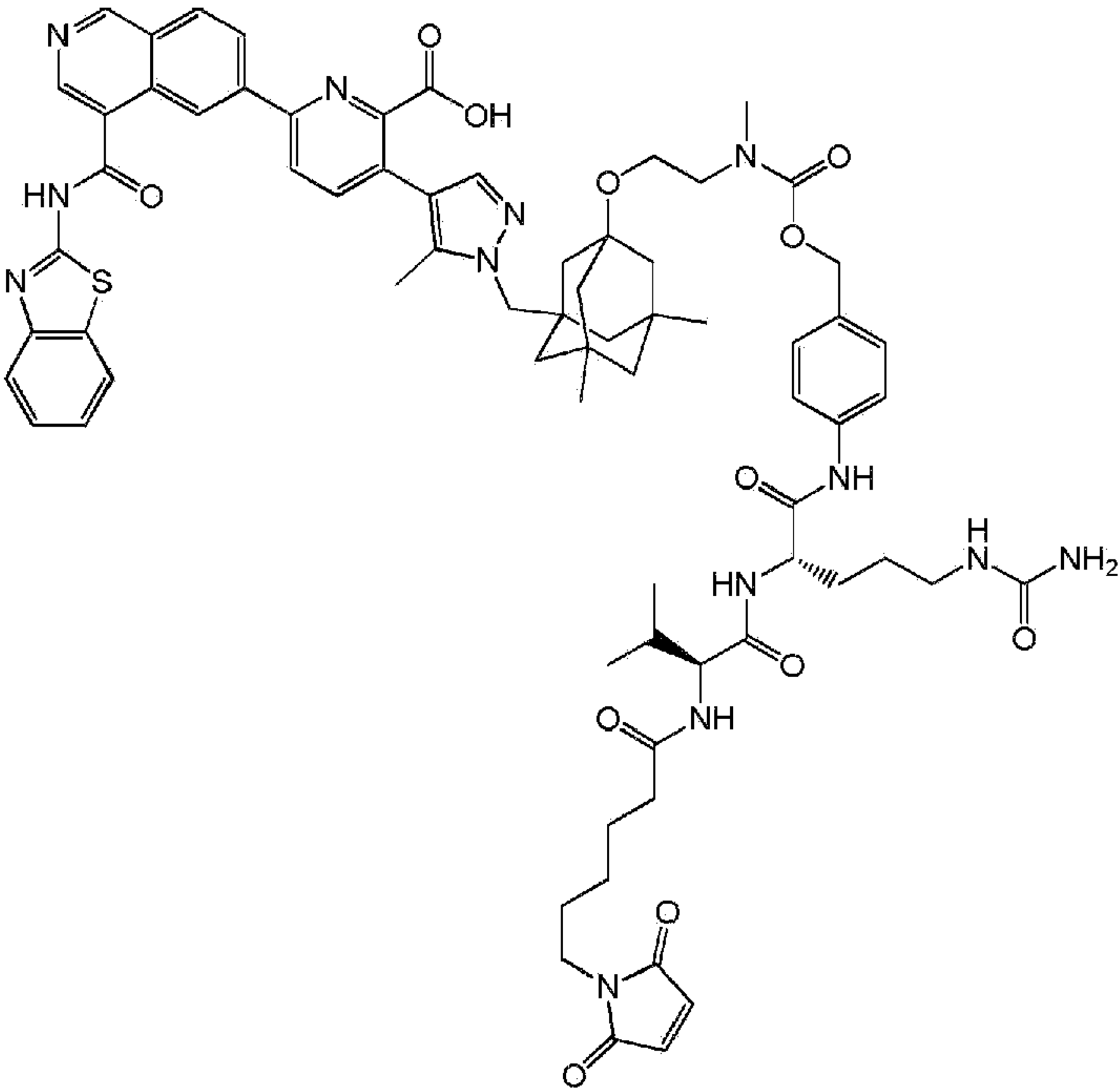
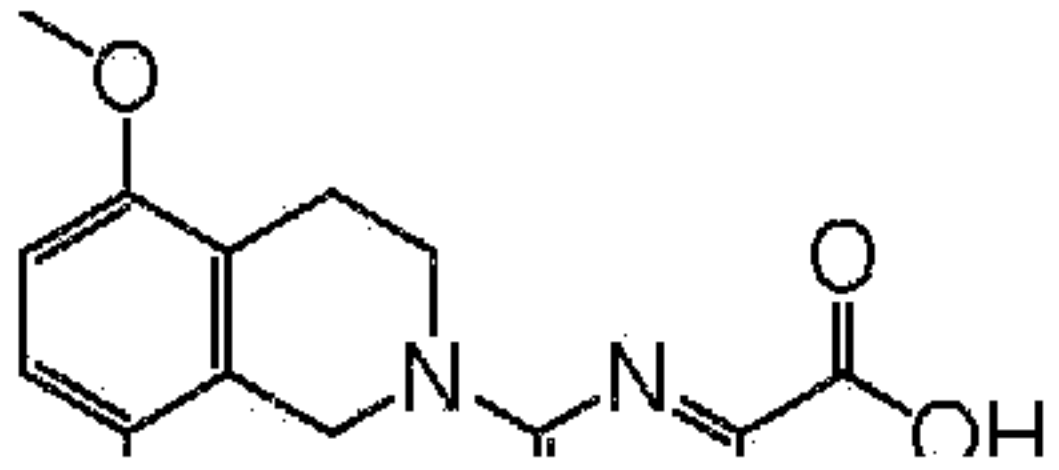
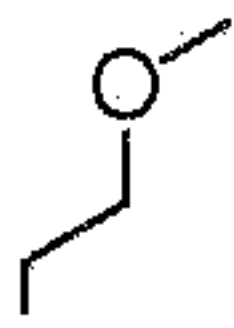
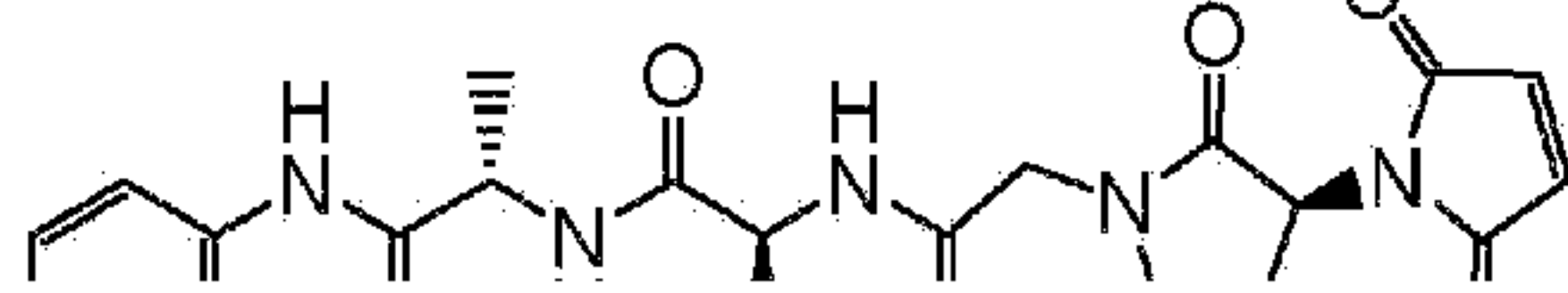


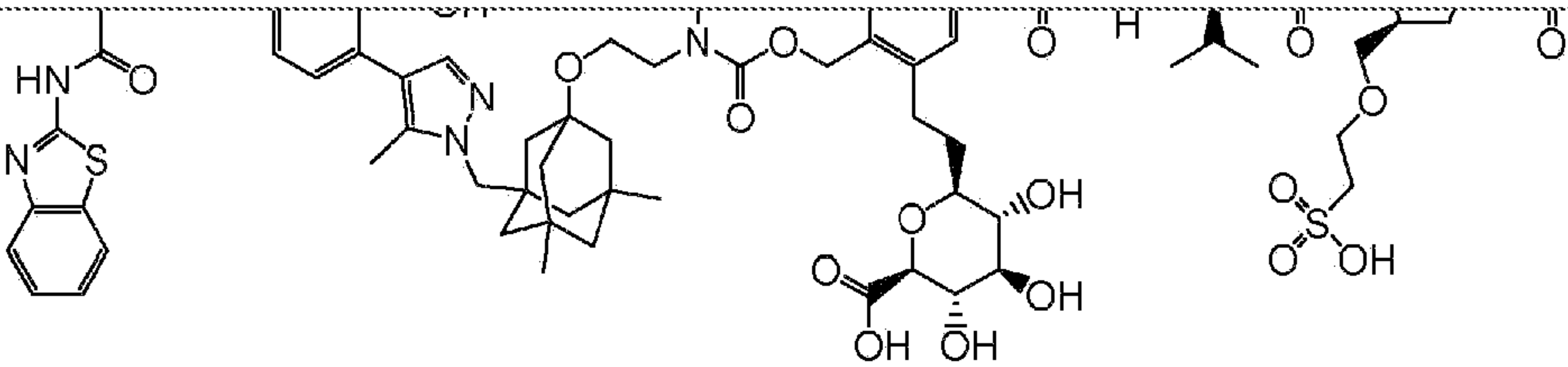
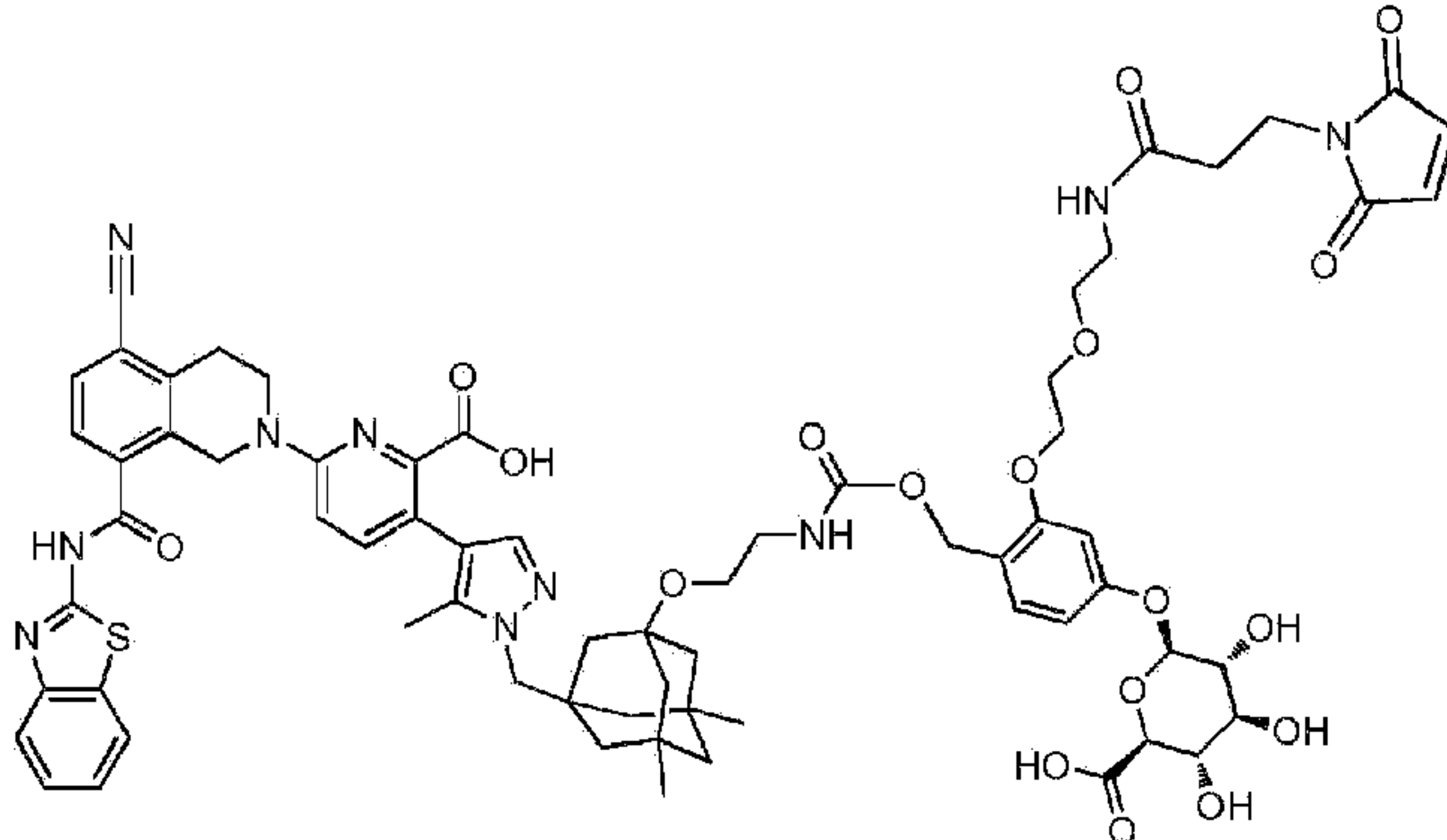
or a pharmaceutically acceptable salt thereof, wherein D is a Bcl-xL inhibitor as previously described, L is a linker as previously described, and R^x is a reactive group suitable for linking the synthon to an antibody.

[0125] Synthons used in the Examples of the present application are listed below in Table B. The synthon of the present invention is Example No. 2.166 (AAA).

Table B

Example No.	Synthon Code	Synthon Structure
		
2.177 (control)	LB	
2.178 (control)	WD	
2.179 (control)	ZZ	
2.180 (control)	ZT	

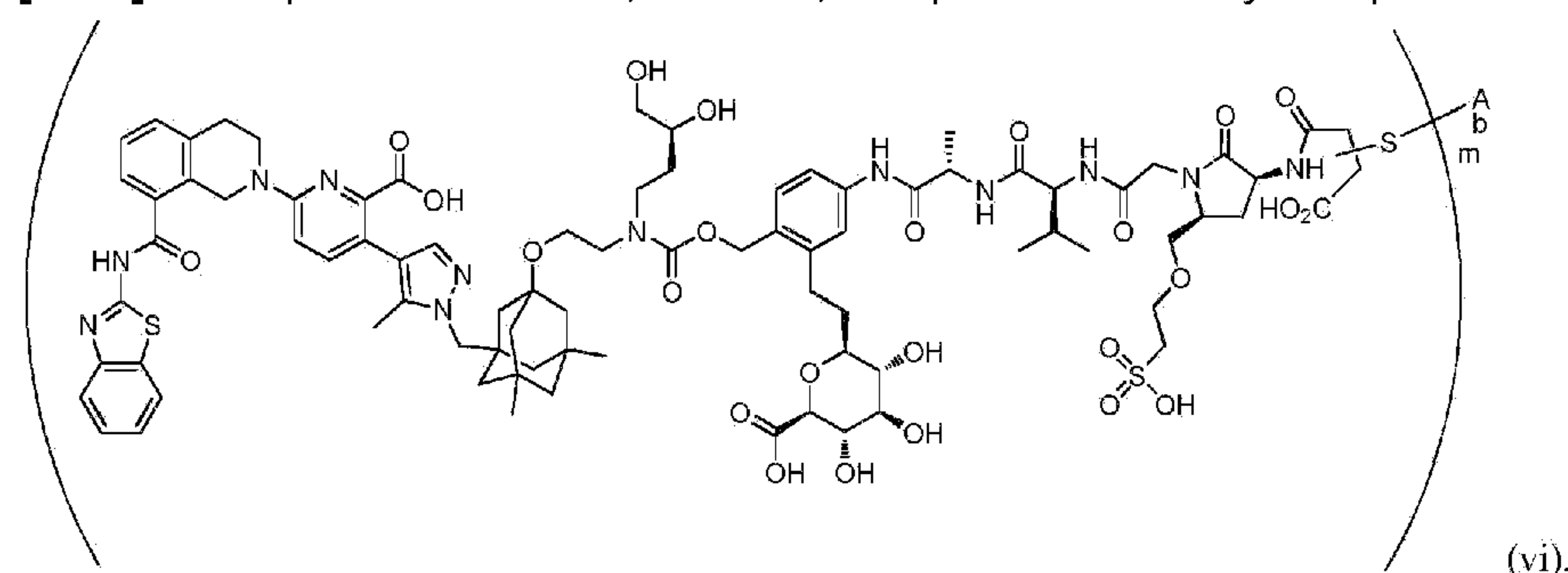
Example No.	Synthon Code	Synthon Structure
		
2.181 (control)	XW	
2.182 (control)	SE	
2.183 (control)	SR	
		  

Example No.	Synthon Code	Synthon Structure
2.184 (control)	YG	
2.185 (control)	KZ	

[0126] In the present invention the synthon is 2.166 or a pharmaceutically acceptable salt thereof. The compound name of the synthon is provided below:

6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)-4-(((S)-2-((S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid;

[0127] In the present invention, the ADC, or a pharmaceutically acceptable salt thereof, is:



wherein m is 2, Ab is either an anti-hB7-H3 antibody, wherein the anti-hB7-H3 antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 35, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 34, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 33; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 38, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 37; or an anti-hB7-H3 antibody, wherein the anti-hB7H3 antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 147, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 144; or an anti-hB7-H3 antibody, wherein the anti-hB7-H3 antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 168, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 169.

[0128] Bcl-xL inhibitors, including warheads and synthons, and methods of making the same, are described in US 2016/0339117 (AbbVie Inc.).

III.A.4. Methods of Synthesis of Bcl-xL ADCs

[0129] The Bcl-xL inhibitors and synthons described herein may be synthesized using standard, known techniques of organic chemistry. Specific methods for synthesizing exemplary Bcl-xL inhibitors and synthons that may be useful for guidance are provided in the Examples section.

[0130] Examples of the foregoing Bcl-xL inhibitors, linkers, and synthons thereof, as well as methods of making the same, can be found in US Patent Publication No. US 2016/0339117.

[0131] Specific methods for synthesizing exemplary ADCs described herein are provided in the Examples section.

III.A.7. General Methods for Synthesizing Anti-B7-H3 ADCs

[0132] The present invention also discloses a process to prepare an anti-B7-H3 ADC of the invention comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising synthon 2.166 (Table B);

adjusting the pH of the solution to a pH of 7.5 to 8.5;

allowing the reaction to run for 48 to 80 hours to form the ADC;

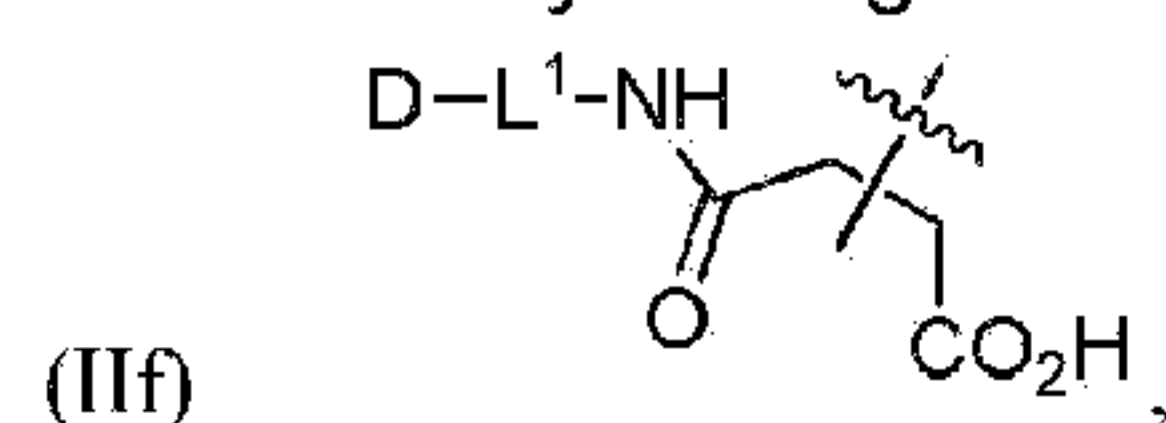
wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

[0133] In certain embodiments, the antibody is huAb13v1.

[0134] The present invention is also directed to an anti-B7-H3 ADC prepared by the above-described process.

[0135] In certain embodiments, the anti-B7-H3 ADC disclosed in the present application is formed by contacting an antibody that binds an hB7-H3 cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the drug-linker synthon covalently links to the antibody through a maleimide moiety as shown in formulae (II f).



wherein D is the Bcl-xL inhibitor drug and L¹ is the portion of the linker not formed from the maleimide; and wherein the drug-linker synthon is example 2.166 (Table B), or a pharmaceutically acceptable salt thereof.

IV. Purification of Anti-B7-H3 ADCs

[0136] Purification of the ADCs may be achieved in such a way that ADCs having certain DARs are collected. For example, HIC resin may be used to separate high drug loaded ADCs from ADCs having optimal drug to antibody ratios (DARs), *e.g.* a DAR of 4 or less. A hydrophobic resin is added to an ADC mixture such that undesired ADCs, *i.e.*, higher drug loaded ADCs, bind the resin and can be selectively removed from the mixture. Separation of the ADCs may be achieved by contacting an ADC mixture (*e.g.*, a mixture comprising a drug loaded species of ADC of 4 or less and a drug loaded species of ADC of 6 or more) with a hydrophobic resin, wherein the amount of resin is sufficient to allow binding of the drug loaded species which is being removed from the ADC mixture. The resin and ADC mixture are mixed together, such that the ADC species being removed (*e.g.*, a drug loaded species of 6 or more) binds to the resin and can be separated from the other ADC species in the ADC mixture. The amount of resin used in the method is based on a weight ratio between the species to be removed and the resin, where the amount of resin used does not allow for significant binding of the drug loaded species that is desired. Thus, methods may be used to reduce the average DAR to less than 4. Further, the purification methods described herein may be used to isolate ADCs having any desired range of drug loaded species, *e.g.*, a drug loaded species of 4 or less, a drug loaded species of 3 or less, a drug loaded species of 2 or less, a drug loaded species of 1 or less.

[0137] Certain species of molecule(s) binds to a surface based on hydrophobic interactions between the species and a hydrophobic resin. In one embodiment of the present disclosure, the method refers to a purification process that relies upon the intermixing of a hydrophobic resin and a mixture of ADCs, wherein the amount of resin added to the mixture determines which species (*e.g.*, ADCs with a DAR of 6 or more) will bind. Following production and purification of an antibody from an expression system (*e.g.*, a mammalian expression system), the antibody is reduced and coupled to a drug through a conjugation reaction. The resulting ADC mixture often contains ADCs having a range of DARs, *e.g.*, 1 to 8.. The ADC mixture may be purified using a process, such as, but not limited to, a batch process, such that ADCs having a drug loaded species of 2 are selected and separated from ADCs having a higher drug load.

[0138] The ADC separation method described herein method may be performed using a batch purification method. The batch purification process generally includes adding the ADC mixture to the hydrophobic resin in a vessel, mixing, and subsequently separating the resin from the supernatant. For example, in the context of batch purification, a hydrophobic resin may be prepared in or equilibrated to the desired equilibration buffer. A slurry of the hydrophobic resin may thus be obtained. The ADC mixture may then be contacted with the slurry to adsorb the specific species of ADC(s) to be separated by the hydrophobic resin. The solution comprising the desired ADCs that do not bind to the hydrophobic resin material may then be separated from the slurry, *e.g.*, by filtration or by allowing the slurry to settle and removing the supernatant. The resulting slurry can be subjected to one or more washing steps. In order to elute bound ADCs, the salt concentration can be decreased.

[0139] Alternatively, purification may be performed using a circulation process, whereby the resin is packed in a container and the ADC mixture is passed over the hydrophobic resin bed until the specific species of ADC(s) to be separated have been removed. The supernatant (containing the desired ADC species) is then pumped from the container and the resin bed may be subjected to washing steps.

[0140] Alternatively, a flow through process may be used to purify an ADC mixture to arrive at a composition comprising a majority of ADCs having a certain desired DAR. In a flow through process, resin is packed in a

container, e.g., a column, and the ADC mixture is passed over the packed resin such that the desired ADC species does not substantially bind to the resin and flows through the resin, and the undesired ADC species is bound to the resin. A flow through process may be performed in a single pass mode (where the ADC species of interest are obtained as a result of a single pass through the resin of the container) or in a multi-pass mode (where the ADC species of interest are obtained as a result of multiple passes through the resin of the container). The flow through process is performed such that the weight of resin selected binds to the undesired ADC population, and the desired ADCs (e.g., DAR 2-4) flow over the resin and are collected in the flow through after one or multiple passes.

[0141] Following a flow through process, the resin may be washed with a one or more washes following in order to further recover ADCs having the desired DAR range (found in the wash filtrate). For example, a plurality of washes having decreasing conductivity may be used to further recover ADCs having the DAR of interest. The elution material obtained from the washing of the resin may be subsequently combined with the filtrate resulting from the flow through process for improved recovery of ADCs having the DAR of interest.

[0142] The aforementioned batch, circulation, and flow through process purification methods are based on the use of a hydrophobic resin to separate high vs. low drug loaded species of ADC. Hydrophobic resin comprises hydrophobic groups which interact with the hydrophobic properties of the ADCs. Hydrophobic groups on the ADC interact with hydrophobic groups within the hydrophobic resin. The more hydrophobic a protein is the stronger it will interact with the hydrophobic resin.

[0143] Hydrophobic resin normally comprises a base matrix (e.g., cross-linked agarose or synthetic copolymer material) to which hydrophobic ligands (e.g., alkyl or aryl groups) are coupled. Many hydrophobic resins are available commercially. Examples include, but are not limited to, Phenyl Sepharose™ 6 Fast Flow with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™. t-Butyl Supports (Bio-Rad, California); WP Hi-Propyl (C₃)™ (J. T. Baker, New Jersey); and Toyopearl™ ether, hexyl, phenyl or butyl (TosoHaas, PA). In one embodiment, the hydrophobic resin is a butyl hydrophobic resin. In another embodiment, the hydrophobic resin is a phenyl hydrophobic resin. In another embodiment, the hydrophobic resin is a hexyl hydrophobic resin, an octyl hydrophobic resin, or a decyl hydrophobic resin. In one embodiment, the hydrophobic resin is a methacrylic polymer having n-butyl ligands (e.g. TOYOPEARL® Butyl-600M).

[0144] Further methods for purifying ADC mixtures to obtain a composition having a desired DAR are described in U.S. Application No. 14/210,602 (U.S. Patent Appln. Publication No. US 2014/0286968).

[0145] In certain embodiments of the invention, ADCs described herein having a DAR2 are purified from ADCs having higher or lower DARs. Such purified DAR2 ADCs are referred to herein as "E2". Purification methods for achieving a composition having E2 anti-B7-H3 ADCs. In one embodiment, of the invention provides a composition comprising an ADC mixture, wherein at least 75% of the ADCs are anti-B7H3 ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 80% of the ADCs are anti-B7H3 ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 85% of the ADCs are anti-B7H3 ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 90% of the ADCs are anti-B7H3 ADCs (like those described herein) having a DAR2.

V. Uses of Anti-B7-H3 Antibodies and Anti-B7-H3 ADCs

[0146] The ADCs of the invention preferably are capable of neutralizing human B7-H3 activity both *in vivo* and *in vitro*. Accordingly, such ADCs of the invention can be used to inhibit hB7-H3 activity, e.g., in a cell culture containing hB7-H3, in human subjects or in other mammalian subjects having B7-H3 with which an antibody of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting hB7-H3 activity comprising contacting hB7-H3 with an ADC of the invention such that hB7-H3 activity is inhibited. For example, in a cell culture containing, or suspected of containing hB7-H3, an antibody or antibody portion of the ADC of the invention can be added to the culture medium to inhibit hB7-H3 activity in the culture.

[0147] In another embodiment, of the invention a method for reducing hB7-H3 activity in a subject, advantageously from a subject suffering from a disease or disorder in which B7-H3 activity is detrimental. The invention provides methods for reducing B7-H3 activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject an ADC of the invention such that B7-H3 activity in the subject is reduced. Preferably, the B7-H3 is human B7-H3, and the subject is a human subject. Alternatively, the subject can be a mammal expressing a B7-H3 to which antibodies of the invention are capable of binding. Still further the subject can be a mammal into which B7-H3 has been introduced (e.g., by administration of B7-H3 or by expression of a B7-H3 transgene). ADCs of the invention can be administered to a human subject for therapeutic purposes. Moreover, ADCs of the invention can be administered to a non-human mammal expressing a B7-H3 with which the antibody is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

[0148] As used herein, the term "a disorder in which B7-H3 expression is detrimental" is intended to include diseases and other disorders in which the presence of B7-H3 in a subject suffering from the disorder has been shown to be expressed, or has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to the disorder. For example, the ADCs of the invention may be used to target tumor cells that are expressing B7-H3. Non-limiting examples of disorders that can be treated with the ADCs of the invention, include, but are not limited to, a variety of cancers including, but not limited to, small cell lung cancer, non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, lung cancer, a glioma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, leukemia, e.g., acute myeloid leukemia (AML), lymphoma, e.g., non-Hodgkin's lymphoma (NHL), and kidney cancer. Other examples of cancer that may be treated using the compositions and methods disclosed herein include squamous cell carcinoma (e.g., squamous lung cancer or squamous head and neck cancer), triple negative breast cancer, non-small cell lung cancer, colorectal cancer, and mesothelioma. In one embodiment, the ADCs disclosed herein are used to treat a solid tumor, e.g., inhibit growth of or decrease size of a solid tumor, overexpressing B7-H3 or which is B7-H3 positive. In one embodiment, the invention is directed to the treatment of squamous lung cancer associated with B7-H3 expression. In another embodiment, the ADCs disclosed herein are used to treat triple negative breast cancer (TNBC). Diseases and disorders described herein may be treated by anti-B7-H3 ADCs of the invention, as well as pharmaceutical compositions comprising such anti-B7-H3 ADCs.

[0149] In certain embodiments, the cancer may be characterized as having EGFR overexpression. In one embodiment, the ADCs of the invention may be used to treating cancer associated with an activating EGFR mutation. Examples of such mutations include, but are not limited to, an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

[0150] In certain embodiments, the ADCs disclosed herein are administered to a subject in need thereof in order to treat advanced solid tumor types likely to exhibit elevated levels of B7-H3. Examples of such tumors include, but are not limited to, small cell lung cancer, breast cancer, ovarian cancer, head and neck

squamous cell carcinoma, non-small cell lung cancer, triple negative breast cancer, colorectal carcinoma, and glioblastoma multiforme.

[0151] In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an anti-B7-H3 ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. In certain embodiments, the solid tumor is a non-small cell lung carcinoma or a glioblastoma. In further embodiments, the solid tumor is a B7-H3-expressing solid tumors. In further embodiments, the solid tumor is an B7-H3 overexpressing solid tumors. In certain embodiments the anti-B7-H3 ADCs described herein are administered to a subject having glioblastoma multiforme, alone or in combination with an additional agent, *e.g.*, radiation and/or temozolomide.

[0152] In certain embodiments the anti-B7-H3 ADCs described herein are administered to a subject having small cell lung cancer, alone or in combination with an additional agent, *e.g.*, ABT-199 (venetoclax).

[0153] In certain embodiments the anti-B7-H3 ADCs described herein are administered to a subject having non-small cell lung cancer, alone or in combination with an additional agent, *e.g.*, a taxane. In certain embodiments the anti-B7-H3 ADCs described herein are administered to a subject having breast cancer, alone or in combination with an additional agent, *e.g.*, a taxane. In certain embodiments the anti-B7-H3 ADCs described herein are administered to a subject having ovarian cancer, alone or in combination with an additional agent, *e.g.*, a taxane.

[0154] Other combination therapies which are included in the invention are the administration of an anti-B7-H3 ADC with an agent selected from the group consisting of an anti-PD1 antibody (*e.g.* pembrolizumab), an anti-PD-L1 antibody (*e.g.*, atezolizumab), an anti-CTLA-4 antibody (*e.g.* ipilimumab), a MEK inhibitor (*e.g.* trametinib), an ERK inhibitor, a BRAF inhibitor (*e.g.* dabrafenib), osimertinib, erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (*e.g.* dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-xL inhibitor, a Bcl-2 inhibitor (*e.g.* venetoclax), ibrutinib, a mTOR inhibitor (*e.g.* everolimus), a PI3K inhibitor (*e.g.* buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (*e.g.* lapatinib), a taxane (*e.g.*, docetaxel, paclitaxel, nab-paclitaxel), venetoclax, an ADC comprising an auristatin, an ADC comprising a PBD (*e.g.* rovalpituzumab tesirine), an ADC comprising a maytansinoid (*e.g.* TDM1), a TRAIL agonist, a proteasome inhibitor (*e.g.* bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor.

[0155] Combination therapies include administration of an ADC of the invention prior to, concurrently with, or following administration of an additional therapeutic agent, including those described above.

[0156] In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor which was identified as an B7-H3 expressing or B7-H3 overexpressing tumor, said method comprising administering an anti-B7-H3 ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. Methods for identifying B7-H3 expressing tumors (*e.g.*, B7-H3 overexpressing tumors) are known in the art, and include FDA-approved tests and validation assays. For example, the B7-H3 assay is a qualitative immunohistochemical (IHC) kit system used to identify B7-H3 expression in normal and neoplastic tissues routinely-fixed for histological evaluation. In addition, PCR-based assays may also be used for identifying B7-H3 overexpressing tumors. The amplified PCR products may be subsequently analyzed, for example, by gel electrophoresis using standard methods known in the art to determine the size of the PCR products. Such tests may be used to identify tumors that may be treated with the methods and compositions described herein.

[0157] Any of the methods for gene therapy available in the art can be used according to the invention. For

general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926- 932 (1993); and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy is provided in US20050042664 A1.

[0158] In another aspect, this application features a method of treating (e.g., curing, suppressing, ameliorating, delaying or preventing the onset of, or preventing recurrence or relapse of) or preventing a B7-H3-associated disorder, in a subject. The method includes: administering to the subject an B7-H3 binding agent, e.g., an anti-B7-H3 antibody or fragment thereof as described herein, in an amount sufficient to treat or prevent the B7-H3-associated disorder. The B7-H3 antagonist, e.g., the anti-B7-H3 antibody or fragment thereof, can be administered to the subject, alone or in combination with other therapeutic modalities as described herein.

[0159] Antibodies or ADCs of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the antibodies of the invention or antigen binding portion thereof can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, e.g., an agent which affects the viscosity of the composition.

[0160] It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

[0161] The combination therapy can include one or more B7-H3 antagonists, e.g., anti-B7-H3 antibodies or fragments thereof, formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents (e.g., systemic anti-inflammatory agents), anti-fibrotic agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, mitotic inhibitors, antitumor antibiotics, immunomodulating agents, vectors for gene therapy, alkylating agents, antiangiogenic agents, antimetabolites, boron-containing agents, chemoprotective agents, hormones, antihormone agents, corticosteroids, photoactive therapeutic agents, oligonucleotides, radionuclide agents, topoisomerase inhibitors, kinase inhibitors, or radiosensitizers, as described in more herein.

[0162] In a particular embodiment, the anti-B7-H3 binding proteins described herein are used in combination with an anti-cancer agent or an antineoplastic agent. The terms "anti-cancer agent" and "antineoplastic agent" refer to drugs used to treat malignancies, such as cancerous growths. Drug therapy may be used alone, or in combination with other treatments such as surgery or radiation therapy. Several classes of drugs may be used in cancer treatment, depending on the nature of the organ involved. For example, breast cancers are commonly stimulated by estrogens, and may be treated with drugs which inactive the sex hormones. Similarly, prostate cancer may be treated with drugs that inactivate androgens, the male sex hormone. Anti-cancer agents that may be used in conjunction with the anti-B7-H3 ADCs of the invention

include, among others, an anti-PDl antibody (e.g., pembrolizumab), an anti-PD-Ll antibody (e.g., atezolizumab), an anti-CTLA-4 antibody (e.g., ipilimumab), a MEK inhibitor (e.g., trametinib), an ERK inhibitor, a BRAF inhibitor (e.g., dabrafenib), osimertinib (AZD9291), erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g., dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-xL inhibitor, a Bcl-2 inhibitor (e.g., venetoclax), ibrutinib, a mTOR inhibitor (e.g., everolimus), a PI3K inhibitor (e.g., buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g., lapatinib), Herceptin, a taxane (e.g., docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g., rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g., TDM1), a TRAIL agonist, a proteasome inhibitor (e.g., bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor, as well as the following agents:

Anti-Cancer Agent	Comments	Examples
Antibodies	Antibodies which bind IGF-1R (insulin-like growth factor type 1 receptor), which is expressed on the cell surface of most human cancers	A12 (fully humanized mAb)
		19D12 (fully humanized mAb)
		Cp751-871 (fully humanized mAb)
		H7C10 (humanized mAb)
		alphaIR3 (mouse)
		ScFV/FC (mouse/human chimera)
		EM/164 (mouse)
	Antibodies which bind EGFR; Mutations affecting EGFR expression or activity could result in cancer	Matuzumab (EMD72000)
		Erbitux® / Cetuximab (Imclone)
		Vectibix® / Panitumumab (Amgen)
	Antibodies which bind cMET (Mesenchymal epithelial transition factor); a member of the MET family of receptor tyrosine kinases	mAb 806
		Nimotuxumab (TheraCIM)
		AVEO (AV299) (AVEO)
		AMG102 (Amgen)
		5D5 (OA-5d5) (Genentech)
		H244G11 (Pierre Fabre)
	Anti-ErbB3 antibodies	Ab #14 (MM 121-14)
		Herceptin® (Trastuzumab; Genentech)
		1B4C3; 2D1D12 (U3 Pharma AG)
Small Molecules Targeting IGF1R	Insulin-like growth factor type 1 receptor which is expressed on the cell surface of many human cancers	NVP-AEW541-A
		BMS-536,924 (1H-benzimidazol-2-yl)-1H-pyridin-2-one)
		BMS-554,417
		Cycloligan

Anti-Cancer Agent	Comments	Examples
		TAE226
		PQ401
Small Molecules Targeting EGFR	EGFR; Overexpression or mutations affecting EGFR expression or activity could result in cancer	Iressa® / Gefitinib (AstraZeneca)
		CI-1033 (PD 183805) (Pfizer)
		Lapatinib (GW-572016) (GlaxoSmithKline)
		Tykerb® / Lapatinib Ditosylate (Smith Kline Beecham)
		Tarceva ® / Erlotinib HCL (OSI-774) (OSI Pharma)
		PKI-166 (Novartis)
		PD-158780
		EKB-569
		Tyrphostin AG 1478 (4-(3-Chloroanillino)-6,7-dimethoxyquinazoline)
		PHA665752
Small Molecules Targeting cMET	cMET (Mesenchymal epithelial transition factor); a member of the MET family of receptor tyrosine kinases)	ARQ 197
Antimetabolites		Flourouracil (5-FU)
		Capecitabine / XELODA® (HLR Roche) 5-Trifluoromethyl-2'-deoxyuridine
		Methotrexate sodium (Trexall) (Barr)
		Raltitrexed/ Tomudex® (AstraZeneca)
		Pemetrexed / Alimta® (Lilly)
		Tegafur
		Cytosine Arabinoside (Cytarabine, Ara-C) /
		Thioguanine® (GlaxoSmithKline)
		5 -azacytidine
		6-mercaptopurine (Mercaptopurine, 6-MP)
		Azathioprine / Azasan® (AAIPHARMA LLC)
		6-thioguanine (6-TG) / Purinethol® (TEVA)

Anti-Cancer Agent	Comments	Examples
		Pentostatin / Nipent® (Hospira Inc.)
		Fludarabine phosphate / Fludara® (Bayer Health Care)
		Cladribine (2-CdA, 2-chlorodeoxyadenosine) / Leustatin® (Ortho Biotech)
Alkylating agents	An alkylating antineoplastic agent is an alkylating agent that attaches an alkyl group to DNA. Since cancer cells generally proliferate unrestrictively more than do healthy cells they are more sensitive to DNA damage, and alkylating agents are used clinically to treat a variety of tumors.	Ribonucleotide Reductase Inhibitor (RNR)
		Cyclophosphamide / Cytosan (BMS)
		Neosar (TEVA)
		Ifosfamide / Mitoxana® (ASTA Medica)
		Thiotepa (Bedford, Abraxis, Teva)
		BCNU→ 1,3-bis(2-chloroethyl)-1-nitrosourea CCNU1, -(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (methyl CCNU)
		Hexamethylmelamine (Altretamine, HMM) / Hexalen® (MGI Pharma Inc.)
		Busulfan / Myleran (GlaxoSmithKline)
		Procarbazine HCL/ Matulane (Sigma Tau Pharmaceuticals, Inc.)
		Dacarbazine (DTIC)
		Chlorambucil / Leukara® (SmithKline Beecham)
		Melphalan / Alkeran® (GlaxoSmithKline)
		Cisplatin (Cisplatinum, CDDP) / Platinol (Bristol Myers)
		Carboplatin / Paraplatin (BMS)
		Oxaliplatin /Eloxitan® (Sanofi-Aventis US)
Topoisomerase	Topoisomerase inhibitors are chemotherapy agents designed to interfere with the action of	Doxorubicin HCL / Doxil® (Alza)

Anti-Cancer Agent	Comments	Examples
inhibitors	topoisomerase enzymes (topoisomerase I and II), which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle.	Daunorubicin citrate / Daunoxome® (Gilead)
		Mitoxantrone HCL / Novantrone (EMD Serono)
		Actinomycin D
		Etoposide / Vepesid® (BMS)/ Etopophos®
		(Hospira, Bedford, Teva Parenteral, Etc.)
		Topotecan HCL / Hycamtin® (GlaxoSmithKline)
		Teniposide (VM-26) / Vumon® (BMS)
		Irinotecan HCL(CPT-II) / Camptosar®
		(Pharmacia & Upjohn)
Microtubule		Vincristine / Oncovin® (Lilly)
targeting agents	Microtubules are one of the components of the cytoskeleton. They have diameter of ~24 nm and length varying from several micrometers to possibly millimeters in axons of nerve cells. Microtubules serve as structural components within cells and are involved in many cellular processes including mitosis, cytokinesis, and vesicular transport.	Vinblastine sulfate / Velban®(discontinued) (Lilly)
		Vinorelbine tartrate / Navelbine® (PierreFabre)
		Vindesine sulphate / Eldisine® (Lilly)
		Paclitaxel / Taxol® (BMS)
		Docetaxel / Taxotere® (Sanofi Aventis US)
		Nanoparticle paclitaxel (ABI-007) /
		Abraxane® (Abraxis BioScience, Inc.)
		Ixabepilone / IXEMPRA™ (BMS)
Kinase inhibitors		Imatinib mesylate / Gleevec (Novartis)
		Sunitinib malate / Sutent® (Pfizer)
		Sorafenib tosylate / Nexavar® (Bayer)
		Nilotinib hydrochloride monohydrate /
		Tasigna® (Novartis), Osimertinib,

Anti-Cancer Agent	Comments	Examples
		Cobimetinib, Trametinib, Dabrafenib,
		Dinaciclib
Protein synthesis inhibitors	Induces cell apoptosis	L-asparaginase / Elspar® (Merck & Co.)
Immunotherapeutic agents	Induces cancer patients to exhibit immune responsiveness	Alpha interferon
		Angiogenesis Inhibitor / Avastin® (Genentech)
		IL-2→ Interleukin 2 (Aldesleukin) / Proleukin ® (Chiron)
		IL-12→ Interleukin 12
	Antibody / small molecule immune checkpoint modulators	Anti-CTLA-4 and PR-1 therapies
		Yervoy® (ipilimumab; Bristol-Myers Squibb)
		Opdivo® (nivolumab; Bristol-Myers Squibb)
		Keytrada® (pembrolizumab; Merck)
Hormones	Hormone therapies associated with menopause and aging seek to increase the amount of certain hormones in your body to compensate for age- or disease-related hormonal declines. Hormone therapy as a cancer treatment either reduces the level of specific hormones or alters the cancer's ability to use these hormones to grow and spread.	Toremifene citrate / Fareston® (GTX, Inc.)
		Fulvestrant / Faslodex® (AstraZeneca)
		Raloxifene HCL / Evista® (Lilly)
		Anastrozole / Arimidex® (AstraZeneca)
		Letrozole / Femara® (Novartis)
		Fadrozole (CGS 16949A)
		Exemestane / Aromasin® (Pharmacia & Upjohn)
		Leuprolide acetate / Eligard® (QTL USA)
		Lupron® (TAP Pharm)
		Goserelin acetate / Zoladex® (AstraZeneca)
		Triptorelin pamoate / Trelstar® (Watson Labs)
		Buserelin / Suprefact® (Sanofi Aventis)
		Nafarelin / Synarel® (Pfizer)
		Cetrorelix / Cetrotide®

Anti-Cancer Agent	Comments	Examples
		(EMD Serono)
		Bicalutamide / Casodex® (AstraZeneca)
		Nilutamide / Nilandron® (Aventis Pharm.)
		Megestrol acetate / Megace® (BMS)
		Somatostatin Analogs (Octreotide acetate / Sandostatin® (Novartis)
Glucocorticoids	Anti-inflammatory drugs used to reduce swelling that causes cancer pain.	Prednisolone
		Dexamethasone / Decadron® (Wyeth)
Aromatase inhibitors	Includes imidazoles	Ketoconazole
mTOR inhibitors	the mTOR signaling pathway was originally discovered during studies of the immunosuppressive agent rapamycin. This highly conserved pathway regulates cell proliferation and metabolism in response to environmental factors, linking cell growth factor receptor signaling via phosphoinositide-3-kinase(PI-3K) to cell growth, proliferation, and angiogenesis.	Sirolimus (Rapamycin) / Rapamune® (Wyeth)
		Temsirolimus (CCI-779) / Torisel® (Wyeth)
		Deforolimus (AP23573) / (Ariad Pharm.)
		Everolimus (RAD001) / Certican® (Novartis)

[0163] In addition to the above anti-cancer agents, the anti-B7-H3 ADCs described herein may be administered in combination with the agents described herein.

[0164] In particular embodiments, the anti-B7-H3 ADCs can be administered alone or with another anti-cancer agent which acts in conjunction with or synergistically with the antibody to treat the disease associated with B7-H3 activity. Such anti-cancer agents include, for example, agents well known in the art (e.g., cytotoxins, chemotherapeutic agents, small molecules and radiation). Examples of anti-cancer agents include, but are not limited to, Panorex (Glaxo-Wellcome), Rituxan (IDEC/Genentech/Hoffman la Roche), Mylotarg (Wyeth), Campath (Millennium), Zevalin (IDEC and Schering AG), Bexxar (Corixa/GSK), Erbitux (Imclone/BMS), Avastin (Genentech) and Herceptin (Genentech/Hoffman la Roche). Other anti-cancer agents include, but are not limited to, those disclosed in U.S. Patent No. 7,598,028 and International Publication No. WO2008/100624. One or more anti-cancer agents may be administered either simultaneously or before or after administration of an antibody or antigen binding portion thereof of the invention.

[0165] In particular embodiments of the invention, the anti-B7-H3 ADCs described herein can be used in a combination therapy with an apoptotic agent, such as a Bcl-xL inhibitor or a Bcl-2 (B-cell lymphoma 2) inhibitor (e.g., ABT-199 (venetoclax)) to treat cancer, such as leukemia, in a subject. In one embodiment, the anti-B7-H3 ADCs described herein can be used in a combination therapy with a Bcl-xL inhibitor for treating cancer. In one embodiment, the anti-B7-H3 ADCs described herein can be used in a combination therapy with venetoclax for treating cancer.

[0166] In particular embodiments of the invention, the anti-B7-H3 ADCs described herein can be used in a combination therapy with an inhibitor of NAMPT (see examples of inhibitors in US 2013/0303509; AbbVie, Inc.) to treat a subject in need thereof. NAMPT (also known as pre-B-cell-colony-enhancing factor (PBEF) and visfatin) is an enzyme that catalyzes the phosphoribosylation of nicotinamide and is the rate-limiting enzyme in one of two pathways that salvage NAD. In one embodiment of the invention, anti-B7-H3 ADCs described herein are administered in combination with a NAMPT inhibitor for the treatment of cancer in a subject.

[0167] In particular embodiments of the invention, the anti-B7-H3 ADCs described herein can be used in a combination therapy with SN-38, which is the active metabolite of the topoisomerase inhibitor irinotecan.

[0168] In other embodiments of the invention, the anti-B7-H3 ADCs described herein can be used in a combination therapy with a PARP (poly ADP ribose polymerase) inhibitor, e.g., veliparib, to treat cancer, including breast, ovarian and non-small cell lung cancers.

[0169] Further examples of additional therapeutic agents that can be co-administered and/or formulated with anti-B7-H3 ADCs described herein, include, but are not limited to, one or more of: inhaled steroids; beta-agonists, e.g., short-acting or long-acting beta-agonists; antagonists of leukotrienes or leukotriene receptors; combination drugs such as ADVAIR; IgE inhibitors, e.g., anti-IgE antibodies (e.g., XOLAIR®, omalizumab); phosphodiesterase inhibitors (e.g., PDE4 inhibitors); xanthines; anticholinergic drugs; mast cell-stabilizing agents such as cromolyn; IL-4 inhibitors; IL-5 inhibitors; eotaxin/CCR3 inhibitors; antagonists of histamine or its receptors including H1, H2, H3, and H4, and antagonists of prostaglandin D or its receptors (DP1 and CRTH2). Such combinations can be used to treat, for example, asthma and other respiratory disorders. Other examples of additional therapeutic agents that can be co-administered and/or formulated with anti-B7-H3 ADCs described herein, include, but are not limited to, one or more of, an anti-PD1 antibody (e.g., pembrolizumab), an anti-PD-L1 antibody (e.g., atezolizumab), an anti-CTLA-4 antibody (e.g., ipilimumab), a MEK inhibitor (e.g., trametinib), an ERK inhibitor, a BRAF inhibitor (e.g., dabrafenib), osimertinib (AZD9291), erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g., dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-xL inhibitor, a Bcl-2 inhibitor (e.g., venetoclax), ibrutinib, a mTOR inhibitor (e.g., everolimus), a PI3K inhibitor (e.g., buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g., lapatinib), Herceptin, a taxane (e.g., docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g., rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g., TDM1), a TRAIL agonist, a proteasome inhibitor (e.g., bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor. Additional examples of therapeutic agents that can be co-administered and/or formulated with one or more anti-B7-H3 antibodies or fragments thereof include one or more of: TNF antagonists (e.g., a soluble fragment of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kD TNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBREX)); TNF enzyme antagonists, e.g., TNF converting enzyme (TACE) inhibitors; muscarinic receptor antagonists; TGF-beta antagonists; interferon gamma; perfenidone; chemotherapeutic agents, e.g., methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, e.g., CCI-779; COX2 and cPLA2 inhibitors; NSAIDs; immunomodulators; p38 inhibitors, TPL-2, MK-2 and NFkB inhibitors, among others.

[0170] Other preferred combinations are cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-31, interferons, EMAP-II, GM-CSF, FGF, EGF, PDGF, and endothelin-1, as well as the receptors of these cytokines and growth factors. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA, CTLA-4, PD-1, or their ligands including CD154 (gp39 or CD40L).

[0171] Preferred combinations of therapeutic agents may interfere at different points in the inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, adalimumab, (HUMIRA; D2E7; PCT Publication No. WO 97/29131 and U.S. Patent No. 6,090,382), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), and also TNF converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 4.

[0172] The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0173] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0174] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an ADC is 0.1-20 mg/kg, more preferably 1-10 mg/kg. In one embodiment, the dose of the ADC described herein is 1 to 6 mg/kg, including the individual doses recited therein, e.g., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, and 6 mg/kg. In another embodiment, the dose of the ADC described herein is 1 to 200 µg/kg, including the individual doses recited therein, e.g., 1 µg/kg, 2 µg/kg, 3 µg/kg, 4 µg/kg, 5 µg/kg, 10 µg/kg, 20 µg/kg, 30 µg/kg, 40 µg/kg, 50 µg/kg, 60 µg/kg, 80 µg/kg, 100 µg/kg, 120 µg/kg, 140 µg/kg, 160 µg/kg, 180 µg/kg and 200 µg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0175] In one embodiment, an anti-B7-H3 ADC is administered to a subject in need thereof, e.g., a subject having cancer, at a dose of 0.1 to 30 mg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, e.g., a subject having cancer, as an ADC at a dose of 1 to 15 mg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, e.g., a subject having cancer, as an ADC at a dose of 1 to 10 mg/kg. In another embodiment, the anti-B7-H3

antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 2 to 3. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 1 to 4 mg/kg.

[0176] In one embodiment, an anti-B7-H3 ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 1 to 200 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 150 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 100 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 90 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 80 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 70 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 5 to 60 µg/kg. In another embodiment, the anti-B7-H3 antibody is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 10 to 80 µg/kg.

[0177] Doses described above may be useful for the administration of anti-B7-H3 disclosed herein.

[0178] Given their ability to bind to human B7-H3, the anti-human B7-H3 antibodies, or portions thereof (as well as ADCs thereof) can be used to detect human B7-H3 (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. In one aspect, the invention provides a method for detecting human B7-H3 in a biological sample comprising contacting a biological sample with an antibody, or antibody portion and detecting either the antibody (or antibody portion) bound to human B7-H3 or unbound antibody (or antibody portion), to thereby detect human B7-H3 in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ³H, ¹⁴C, ³⁵S, ⁹⁰Y, ⁹⁹Tc, ¹¹¹In, ¹²⁵I, ¹³¹I, ¹⁷⁷Lu, ¹⁶⁶Ho, or ¹⁵³Sm.

[0179] Alternative to labeling the antibody, human B7-H3 can be assayed in biological fluids by a competition immunoassay utilizing rhB7-H3 standards labeled with a detectable substance and an unlabeled anti-human B7-H3 antibody. In this assay, the biological sample, the labeled rhB7-H3 standards and the anti-human B7-H3 antibody are combined and the amount of labeled rhB7-H3 standard bound to the unlabeled antibody is determined. The amount of human B7-H3 in the biological sample is inversely proportional to the amount of labeled rhB7-H3 standard bound to the anti-B7-H3 antibody. Similarly, human B7-H3 can also be assayed in biological fluids by a competition immunoassay utilizing rhB7-H3 standards labeled with a detectable substance and an unlabeled anti-human B7-H3 antibody.

VI. Pharmaceutical Compositions

[0180] The invention also provides pharmaceutical compositions comprising an ADC of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising ADCs of the invention are

for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research.. In another embodiment, the pharmaceutical composition comprises one or more ADCs of the invention and one or more prophylactic or therapeutic agents other than ADCs of the invention for treating a disorder in which B7-H3 activity is detrimental. Preferably, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

[0181] The ADCs of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the ADC.

[0182] Various delivery systems are known and can be used to administer ADCs of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (*e.g.*, intranasal and oral routes). In addition, pulmonary administration can be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Pat. Nos. 6,019,968, 5,985, 320, 5,985,309, 5,934, 272, 5,874,064, 5,855,913, 5,290, 540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903. In one embodiment a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0183] In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (*e.g.*, Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than an antibody of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder

or one or more symptoms thereof.

[0184] In another embodiment, the prophylactic or therapeutic agent of the invention can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U. S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0185] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U. S. Pat. No. 4,526, 938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning et al., 1996, "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song et al., 1995, "Antibody Mediated Lung Targeting of Long- Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397, Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760.

[0186] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

[0187] If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable

gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, *e.g.*, gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0188] If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gel caps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

[0189] The method of the invention may comprise pulmonary administration, *e.g.*, by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, *e.g.*, U.S. Pat. Nos. 6,019, 968, 5,985, 320, 5, 985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903. In a specific embodiment, a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

[0190] The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (*e.g.*, by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (*e.g.*, in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) before use.

[0191] The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (*e.g.*, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (*e.g.*, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (*e.g.*, as a sparingly soluble salt).

[0192] The methods of the invention encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0193] Generally, the ingredients of compositions are supplied either separately or mixed together in unit

dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0194] In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (*e.g.*, with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2° C. and 8° C. in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2° C. and 8° C. in its original container.

[0195] The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the antibodies and antibody-portions of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (*e.g.*, antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (*i.e.* greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (see WO2004078140, US2006104968 incorporated herein by

reference).

[0196] The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[0197] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

[0198] The ADCs of the invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0199] In certain embodiments, an ADC of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0200] In other embodiments, an ADC of the invention may be conjugated to a polymer-based species such that said polymer-based species may confer a sufficient size upon said antibody or antibody portion of the invention such that said antibody or antibody portion of the invention benefits from the enhanced

permeability and retention effect (EPR effect) (See also PCT Publication No. WO2006/042146A2 and U.S. Publication Nos. 2004/0028687A1, 2009/0285757A1, and 2011/0217363A1, and U.S. Patent No. 7,695,719.

[0201] Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an ADC of the invention is formulated with and/or co-administered with one or more additional therapeutic agents that are useful for treating disorders in which B7-H3 activity is detrimental. For example, an ADC of the invention may be formulated and/or co-administered with one or more additional antibodies that bind other targets (e.g., antibodies that bind cytokines or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0202] In certain embodiments, an ADC to B7-H3 or fragment thereof is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044.

EXAMPLES

Example 1: Synthesis of Exemplary Bcl-xL Inhibitors

[0203] This example provides synthetic methods for exemplary Bcl-xL inhibitory compounds. Bcl-xL inhibitors and synthons were named using ACD/Name 2012 release (Build 56084, 05 April 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 October 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA), or ChemDraw® Professional Ver. 15.0.0.106. Bcl-xL inhibitor and synthon intermediates were named with ACD/Name 2012 release (Build 56084, 05 April 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 October 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA), or ChemDraw® Professional Ver. 15.0.0.106.

1.1.1 3-bromo-5,7-dimethyladamantanecarboxylic acid

[0204] Into a 50 mL round-bottomed flask at 0 °C, was added bromine (16 mL). Iron powder (7 g) was added, and the reaction was stirred at 0 °C for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g) was added. The mixture was warmed up to room temperature and stirred for 3 days. A mixture of ice and concentrated HCl was poured into the reaction mixture. The resulting suspension was treated twice with Na₂SO₃ (50 g in 200 mL water) and extracted three times with dichloromethane. The combined organics were washed with 1N aqueous HCl, dried over sodium sulfate, filtered, and concentrated to give the title compound.

1.1.2 3-bromo-5,7-dimethyladamantanemethanol

[0205] To a solution of Example 1.1.1 (15.4 g) in tetrahydrofuran (200 mL) was added BH₃ (1M in tetrahydrofuran, 150 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched by adding methanol dropwise. The mixture was then concentrated under vacuum, and the residue was balanced between ethyl acetate (500 mL) and 2N aqueous HCl (100 mL). The aqueous layer was further extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over sodium sulfate, and filtered. Evaporation of the solvent gave the title compound.

1.1.3 1-((3-bromo-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-1H-pyrazole

[0206] To a solution of Example 1.1.2 (8.0 g) in toluene (60 mL) was added 1H-pyrazole (1.55 g) and cyanomethylenetriethylphosphorane (2.0 g), and the mixture was stirred at 90 °C overnight. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (10:1 heptane:ethyl acetate) to give the title compound. MS (ESI) m/e 324.2 (M+H)⁺.

1.1.4 2-([3,5-dimethyl-7-(1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}] dec-1-yl]oxy)ethanol

[0207] To a solution of Example 1.1.3 (4.0 g) in ethane-1,2-diol (12 mL) was added triethylamine (3 mL). The mixture was stirred at 150 °C under microwave conditions (Biotage Initiator) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, dried over sodium sulfate, and filtered. Evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, followed by 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 305.2 (M+H)⁺.

1.1.5 2-([3,5-dimethyl-7-[(5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl]oxy)ethanol

[0208] To a cooled (-78 °C) solution of Example 1.1.4 (6.05 g) in tetrahydrofuran (100 mL) was added n-BuLi (40 mL, 2.5M in hexane), and the mixture was stirred at -78 °C for 1.5 hours. Iodomethane (10 mL) was added through a syringe, and the mixture was stirred at -78 °C for 3 hours. The reaction mixture was then quenched with aqueous NH₄Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over sodium sulfate, the solution was filtered and concentrated, and the residue was purified by silica gel column chromatography, eluting with 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 319.5 (M+H)⁺.

1.1.6 1-([3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo [3.3.1.1^{3,7}]dec-1-yl]methyl)-4-iodo-5-methyl-1H-pyrazole

[0209] To a solution of Example 1.1.5 (3.5 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g), and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was diluted with ethyl acetate (600 mL) and washed with aqueous NaHSO₃, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 445.3 (M+H)⁺.

1.1.9 6-fluoro-3-bromopicolinic acid

[0210] A slurry of 6-amino-3-bromopicolinic acid (25 g) in 400 mL 1:1 dichloromethane/chloroform was added to nitrosonium tetrafluoroborate (18.2 g) in dichloromethane (100 mL) at 5 °C over 1 hour. The resulting mixture was stirred for another 30 minutes, then warmed to 35 °C and stirred overnight. The reaction was cooled to room temperature, and then adjusted to pH 4 with aqueous NaH₂PO₄ solution. The resulting solution was extracted three times with dichloromethane, and the combined extracts were washed with brine, dried over sodium sulfate, filtered and concentrated to provide the title compound.

1.1.10 Tert-butyl 3-bromo-6-fluoropicolinate

[0211] Para-toluenesulfonyl chloride (27.6 g) was added to a solution of Example 1.1.9 (14.5 g) and pyridine (26.7 mL) in dichloromethane (100 mL) and tert-butanol (80 mL) at 0 °C. The reaction was stirred for 15 minutes, and then warmed to room temperature, and stirred overnight. The solution was concentrated and partitioned between ethyl acetate and aqueous Na₂CO₃ solution. The layers were separated, and the aqueous layer extracted with ethyl acetate. The organic layers were combined, rinsed with aqueous Na₂CO₃ solution and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound.

1.1.11 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[0212] To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 1.1.10 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL), and the mixture was stirred at 50 °C for 24 hours. The mixture was then diluted with ethyl acetate (500 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in hexane, to give the title compound. MS (ESI) m/e 448.4 (M+H)⁺.

1.2.1 methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[0213] To a solution of Example 1.1.11 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL), and the mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by silica gel chromatography, eluting with 20% ethyl acetate in hexane, provided the title compound.

1.2.2 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[0214] To a solution of Example 1.2.1 (2.25 g) in tetrahydrofuran (30 mL) and water (10 mL) was added

Example 1.1.6 (2.0 g), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane (329 mg), tris(dibenzylideneacetone)dipalladium(0) (206 mg) and potassium phosphate tribasic (4.78 g). The mixture was refluxed overnight, cooled and diluted with ethyl acetate (500 mL). The resulting mixture was washed with water and brine, and the organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes followed by 5% methanol in dichloromethane, to provide the title compound.

1.2.3 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[0215] To a cold solution of Example 1.2.2 (3.32 g) in dichloromethane (100 mL) in an ice-bath was sequentially added triethylamine (3 mL) and methanesulfonyl chloride (1.1 g). The reaction mixture was stirred at room temperature for 1.5 hours and diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound.

1.2.4 methyl 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[0216] To a solution of Example 1.2.3 (16.5 g) in N,N-dimethylformamide (120 mL) was added sodium azide (4.22 g). The mixture was heated at 80 °C for 3 hours, cooled, diluted with ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes, to provide the title compound.

1.2.5 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[0217] To a solution of Example 1.2.4 (10 g) in a mixture of tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2g). The mixture was stirred at room temperature overnight and neutralized with 2% aqueous HCl. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate (800 mL), and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound.

1.2.6 tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[0218] A mixture of Example 1.2.5 (10 g), benzo[d]thiazol-2-amine (3.24 g), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (5.69 g) and N,N-diisopropylethylamine (5.57 g) in N,N-dimethylformamide (20 mL) was heated at 60 °C for 3 hours, cooled and diluted with ethyl acetate. The resulting mixture was washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in dichloromethane to give the title compound.

1.2.7 tert-butyl 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-

yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[0219] To a solution of Example 1.2.6 (2.0 g) in tetrahydrofuran (30 mL) was added Pd/C (10%, 200 mg). The mixture was stirred under a hydrogen atmosphere overnight. The insoluble material was filtered off and the filtrate was concentrated to provide the title compound.

1.2.8 tert-butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[(2,2,7,7-tetramethyl-10,10-dioxido-3,3-diphenyl-4,9-dioxa-10 λ ⁶-thia-13-aza-3-silapentadecan-15-yl)oxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylate

[0220] To a solution of Example 1.2.7 (500 mg) in N,N-dimethylformamide (8 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (334 mg). The reaction was stirred at room temperature overnight and methylamine (0.3 mL) was added to quench the reaction. The resulting mixture was stirred for 20 minutes and purified by reverse-phase chromatography using an Analogix system (C18 column), eluting with 50-100% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound.

1.2.9 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[0221] Example 1.2.8 (200 mg) in dichloromethane (5 mL) was treated with trifluoroacetic acid (2.5 mL) overnight. The reaction mixture was concentrated and purified by reverse phase chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. ¹H NMR (500 MHz, dimethyl sulfoxide-*d*₆) δ ppm 12.86 (s, 1H), 8.32 (s, 2H), 8.02 (d, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.51 (d, 1H), 7.40-7.49 (m, 2H), 7.31-7.39 (m, 2H), 7.27 (s, 1H), 6.95 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.15-3.25 (m, 2H), 3.03-3.13 (m, 2H), 3.00 (t, 2H), 2.79 (t, 2H), 2.09 (s, 3H), 1.39 (s, 2H), 1.22-1.34 (m, 4H), 0.94-1.18 (m, 6H), 0.85 (s, 6H). MS (ESI) *m/e* 854.1 (M+H)⁺.

1.20.1 2-((3,5-dimethyl-7-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethanol

[0222] To a solution of Example 1.1.6 (9 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (827 mg) in acetonitrile (60 mL) was added triethylamine (10 mL) and pinacolborane (6 mL). The mixture was stirred at reflux overnight, cooled and used directly in the next step. MS (ESI) *m/e* 445.4 (M+H)⁺.

1.20.2 tert-butyl 6-chloro-3-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[0223] To a solution of tert-butyl 3-bromo-6-chloropicolinate (5.92 g) in tetrahydrofuran (60 mL) and water

(30 mL) was added the crude Example 1.20.1 (4.44 g), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxo-6-phosphaadamante (1.5 g), tris(dibenzylideneacetone)dipalladium(0) (927 mg) and K_3PO_4 (22 g). The mixture was stirred at reflux overnight, cooled, diluted with ethyl acetate (800 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptane followed by 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 531.1 (M+H)⁺.

1.43.1 tert-butyl 3-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(methoxycarbonyl)naphthalen-2-yl)picolinate

[0224] To a solution of methyl 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthoate (2.47 g) in 1,4-dioxane (40 mL) and water (20 mL) was added Example 1.20.2 (4.2 g), bis(triphenylphosphine)palladium(II)dichloride (556 mg), and cesium fluoride (3.61 g), and the reaction was stirred at reflux overnight. The mixture was diluted with ethyl acetate (400 mL) and washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane followed by 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 680.7 (M+H)⁺.

1.43.2 tert-butyl 3-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(methoxycarbonyl)naphthalen-2-yl)picolinate

[0225] To a cooled (0 °C) solution of Example 1.43.1 (725 mg) in dichloromethane (10 mL) and triethylamine (0.5 mL) was added methanesulfonyl chloride (0.249 mL), and the mixture was stirred for 4 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent gave the title product, which was used in the next reaction without further purification. MS (ESI) m/e 759.9 (M+H)⁺.

1.43.3 tert-butyl 3-(1-(((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(methoxycarbonyl)naphthalen-2-yl)picolinate

[0226] To a solution of Example 1.43.2 (4.2 g) in N,N-dimethylformamide (30 mL) was added sodium azide (1.22 g), and the mixture was stirred for 96 hours. The reaction mixture was diluted with ethyl acetate (600 mL), washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent provided the title compound. MS (ESI) m/e 705.8 (M+H)⁺.

1.43.4 7-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1-naphthoic acid

[0227] To a solution of Example 1.43.3 (3.5 g) in tetrahydrofuran/methanol/water (2:1:1, 30 mL) was added lithium hydroxide monohydrate (1.2 g), and the mixture was stirred overnight. The reaction mixture was acidified with 1N aqueous HCl and was diluted with ethyl acetate (600 mL), washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent provided the title compound. MS (ESI) m/e 691.8 (M+H)⁺.

1.43.5 tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinate

[0228] To a solution of Example 1.43.4 (870 mg) in N,N-dimethylformamide (10 mL) was added benzo[d]thiazol-2-amine (284 mg), fluoro-N,N,N',N'- tetramethylformamidinium hexafluorophosphate (499 mg) and N,N-diisopropylethylamine (488 mg). The mixture was stirred at 60 °C for 3 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent provided the title compound. MS (ESI) m/e 824.1 (M+H)⁺.

1.43.6 tert-butyl 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinate

[0229] To a solution of Example 1.43.5 (890 mg) in tetrahydrofuran (30 mL) was added Pd/C (90 mg). The mixture was stirred under 1 atmosphere of hydrogen overnight. The reaction mixture was filtered, and the catalyst was washed with ethyl acetate. The solvent was evaporated to provide the title compound. MS (ESI) m/e 798.1 (M+H)⁺.

1.43.7 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[0230] To a solution of Example 1.43.6 (189 mg) in N,N-dimethylformamide (6 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (106 mg). The mixture was stirred for 4 days. The mixture was diluted with ethyl acetate (300 mL) and washed with water and brine and dried over sodium sulfate. After filtration and evaporation of the solvent, the residue was dissolved in trifluoroacetic acid (10 mL) and sat overnight. The trifluoroacetic acid was evaporated under vacuum, and the residue was dissolved in dimethyl sulfoxide/methanol (1:1, 6 mL). The mixture was purified by reverse phase HPLC (Gilson system), eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to give the title compound. ¹H NMR (400 MHz, dimethyl sulfoxide-*d*₆) δ ppm 13.09 (s, 1H), 9.02 (s, 1H), 8.31-8.43 (m, 3H), 8.16-8.26 (m, 3H), 7.93-8.08 (m, 3H), 7.82 (d, 1H), 7.66-7.75 (m, 1H), 7.46-7.55 (m, 2H), 7.37 (t, 1H), 3.90 (s, 3H), 3.17-3.28 (m, 2H), 3.07-3.16 (m, 2H), 2.82 (t, 2H), 2.24 (s, 3H), 1.44 (s, 2H), 0.99-1.37 (m, 12H), 0.87 (s, 6H). MS (ESI) m/e 849.1 (M+H)⁺.

1.85 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-[(3-(2-[(3S)-3,4-dihydroxybutyl]amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W2.85)

[0231] To a solution of Example 1.2.7 (213 mg) in dichloromethane (2 mL) was added (S)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (42 mg). After stirring at room temperature for 30 minutes, sodium triacetoxyborohydride (144 mg) was added. The reaction mixture was stirred at room temperature overnight. Trifluoroacetic acid (2 mL) was added and stirring was continued overnight. The reaction mixture was concentrated, and the residue was purified by reverse-phase HPLC using a Gilson system, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and

freeze-dried to provide the title compound. ^1H NMR (400 MHz, dimethyl sulfoxide- d_6) δ ppm 12.86 (s, 1H), 8.22 (d, 2H), 8.05 - 8.01 (m, 1H), 7.79 (d, 1H), 7.61 (d, 1H), 7.53 - 7.41 (m, 3H), 7.36 (td, 2H), 7.28 (s, 1H), 6.95 (d, 1H), 4.95 (s, 2H), 3.88 (t, 2H), 3.82 (s, 2H), 3.26 - 2.94 (m, 7H), 2.10 (s, 3H), 1.84 - 1.75 (m, 1H), 1.52-1.63 (m, 1H), 1.45 - 1.23 (m, 6H), 1.19 - 0.96 (m, 7H), 0.86 (s, 6H). MS (ESI) m/e 834.3 ($M+H$) $^+$.

Example 2: Synthesis of Exemplary Synthons

[0232] This example provides synthetic methods for exemplary synthons useful to make ADCs.

2.49.1 3-(1-(((1*r*,3*r*)-3-(2-(((4-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1*H*-pyrazol-4-yl)-6-(8-(benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)picolinic acid

[0233] A solution of Example 1.2.9 (0.045 g) (9*H*-fluoren-9-yl)methyl ((*S*)-3-methyl-1-(((*S*)-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (0.043 g) and *N,N*-diisopropylethylamine (0.041 mL) were stirred together in *N,N*-dimethylformamide (1 mL) at room temperature. After stirring overnight, diethylamine (0.024 mL) was added to the reaction, and stirring was continued for 2 hours. The reaction was quenched with trifluoroacetic acid then purified by reverse phase HPLC using a Gilson system, eluting with 10-75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

2.119.1 (3*R*,7*aS*)-3-phenyltetrahydropyrrolo[1,2-*c*]oxazol-5(3*H*)-one

[0234] A mixture of (*S*)-5-(hydroxymethyl)pyrrolidin-2-one (25g), benzaldehyde (25.5g) and para-toluenesulfonic acid monohydrate (0.50 g) in toluene (300 mL) was heated to reflux using a Dean-Stark trap under a drying tube for 16 hours. The reaction was cooled to room temperature, and the solvent was decanted from the insoluble materials. The organic layer was washed with saturated aqueous sodium bicarbonate mixture (2x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with 35/65 heptane/ethyl acetate, to give the title compound. MS (DCI) m/e 204.0 ($M+H$) $^+$.

2.119.2 (3*R*,6*R*,7*aS*)-6-bromo-3-phenyltetrahydropyrrolo[1,2-*c*]oxazol-5(3*H*)-one

[0235] To a cold (-77 °C) mixture of Example 2.119.1 (44.6 g) in tetrahydrofuran (670 mL) was added lithium bis(trimethylsilyl)amide (1.0M in hexanes, 250 mL) dropwise over 40 minutes, keeping $T_{\text{rxn}} < -73$ °C. The reaction was stirred at -77 °C for 2 hours, and bromine (12.5 mL) was added dropwise over 20 minutes, keeping $T_{\text{rxn}} < -64$ °C. The reaction was stirred at -77 °C for 75 minutes and was quenched by the addition of 150 mL cold 10% aqueous sodium thiosulfate mixture to the -77 °C reaction. The reaction was warmed to room temperature and partitioned between half-saturated aqueous ammonium chloride mixture and ethyl acetate. The layers were separated, and the organic layer was washed with water and brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 80/20, 75/25, and 70/30 heptane/ethyl acetate to give the title

compound. MS (DCI) m/e 299.0 and 301.0 (M+NH₃+H)⁺.

2.119.4 (3R,6S,7aS)-6-azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[0236] To a mixture of Example 2.119.2 (19.3 g) in N,N-dimethylformamide (100 mL) was added sodium azide (13.5 g). The reaction was heated to 60 °C for 2.5 hours. The reaction was cooled to room temperature and quenched by the addition of water (500 mL) and ethyl acetate (200 mL). The layers were separated, and the organic layer was washed brine. The combined aqueous layers were back-extracted with ethyl acetate (50 mL). The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 78/22 heptane/ethyl acetate, to give the title compound. MS (DCI) m/e 262.0 (M+NH₃+H)⁺.

2.119.5 (3R,6S,7aS)-6-amino-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[0237] To a mixture of Example 2.119.4 (13.5 g) in tetrahydrofuran (500 mL) and water (50 mL) was added polymer-supported triphenylphosphine (55 g). The reaction was mechanically stirred overnight at room temperature. The reaction was filtered through diatomaceous earth, eluting with ethyl acetate and toluene. The mixture was concentrated under reduced pressure, dissolved in dichloromethane (100 mL), dried with sodium sulfate, then filtered and concentrated to give the title compound, which was used in the subsequent step without further purification. MS (DCI) m/e 219.0 (M+H)⁺.

2.119.6 (3R,6S,7aS)-6-(dibenzylamino)-3-phenyltetrahydropyrrolo[1,2-c] oxazol-5(3H)-one

[0238] To a mixture of Example 2.119.5 (11.3 g) in N,N-dimethylformamide (100 mL) was added potassium carbonate (7.0 g), potassium iodide (4.2 g), and benzyl bromide (14.5 mL). The reaction was stirred at room temperature overnight and quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 10 to 15% ethyl acetate in heptane to give a solid that was triturated with heptane to give the title compound. MS (DCI) m/e 399.1 (M+H)⁺.

2.119.7 (3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)pyrrolidin-2-one

[0239] To a mixture of Example 2.119.6 (13 g) in tetrahydrofuran (130 mL) was added *paratoluene* sulfonic acid monohydrate (12.4 g) and water (50 mL), and the reaction was heated to 65 °C for 6 days. The reaction was cooled to room temperature and quenched by the addition of saturated aqueous sodium bicarbonate and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The waxy solids were triturated with heptane (150 mL) to give the title compound. MS (DCI) m/e 311.1 (M+H)⁺.

2.119.8 (3S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(dibenzylamino)pyrrolidin-2-one

[0240] To a mixture of Example 2.119.7 (9.3 g) and 1H-imidazole (2.2 g) in N,N-dimethylformamide was added tert-butylchlorodimethylsilane (11.2 mL, 50 weight % in toluene), and the reaction mixture was stirred overnight. The reaction mixture was quenched by the addition of water and ethyl ether. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to give the title compound. MS (DCI) m/e 425.1 (M+H)⁺.

2.119.9 tert-butyl 2-((3S,5S)-5-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl)acetate

[0241] To a cold (0 °C) mixture of Example 2.119.8 (4.5 g) in tetrahydrofuran (45 mL) was added 95% sodium hydride (320 mg) in two portions. The cold mixture was stirred for 40 minutes, and tert-butyl 2-bromoacetate (3.2 mL) was added. The reaction was warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 5-12% ethyl acetate in heptane, to give the title compound. MS (DCI) m/e 539.2 (M+H)⁺.

2.119.10 tert-butyl 2-((3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)-2-oxopyrrolidin-1-yl)acetate

[0242] To a mixture of Example 2.119.9 (5.3 g) in tetrahydrofuran (25 mL) was added tetrabutylammonium fluoride (11 mL, 1.0M in 95/5 tetrahydrofuran /water). The reaction was stirred at room temperature for one hour and then quenched by the addition of saturated aqueous ammonium chloride mixture, water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to give the title compound. MS (DCI) m/e 425.1 (M+H)⁺.

2.119.11 tert-butyl 2-((3S,5S)-5-((2-((4-((tert-butyl)dimethylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl)acetate

[0243] To a mixture of Example 2.119.10 (4.7 g) in dimethyl sulfoxide (14 mL) was added a mixture of 4-((tert-butyl)diphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (14.5 g) in dimethyl sulfoxide (14 mL). Potassium carbonate (2.6 g) and water (28 µL) were added, and the reaction was heated at 60 °C under nitrogen for one day. The reaction was cooled to room temperature, and then quenched by the addition of brine mixture, water and diethyl ether. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 15-25% ethyl acetate in heptane, to give the title compound. MS (ESI⁺) m/e 871.2 (M+H)⁺.

2.119.12 tert-butyl 2-((3S,5S)-3-amino-5-((2-((4-((tertbutyl)dimethylsilyl)oxy)-2,2-

dimethylbutoxy)sulfonyl)ethoxy)methyl)-2-oxopyrrolidin-1-yl)acetate

[0244] Example 2.119.11 (873 mg) was dissolved in ethyl acetate (5 mL) and methanol (15 mL), and palladium hydroxide on carbon, 20% by wt (180 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere (30 psi) at room temperature for 30 hours, then at 50 °C for one hour. The reaction was cooled to room temperature, filtered, and concentrated to give the desired product. MS (ESI+) m/e 691.0 (M+H)⁺.

2.119.13 **4-(((3S,5S)-1-(2-(tert-butoxy)-2-oxoethyl)-5-((2-((4-((tert-butyldimethylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-2-oxopyrrolidin-3-yl)amino)-4-oxobut-2-enoic acid**

[0245] Maleic anhydride (100 mg) was dissolved in dichloromethane (0.90 mL), and a mixture of Example 2.119.12 (650 mg) in dichloromethane (0.90 mL) was added dropwise, then heated at 40 °C for 2 hours. The reaction mixture was directly purified by silica gel chromatography, eluting with a gradient of 1.0-2.5% methanol in dichloromethane containing 0.2% acetic acid. After concentrating the product-bearing fractions, toluene (10 mL) was added, and the mixture was concentrated again to give the title compound. MS (ESI-) m/e 787.3 (M-H)⁻.

2.119.14 **tert-butyl** **2-((3S,5S)-5-((2-((4-((tert-butyldimethylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxopyrrolidin-1-yl)acetate**

[0246] Example 2.119.13 (560 mg) was slurried in toluene (7 mL), and triethylamine (220 µL) and sodium sulfate (525 mg) were added. The reaction was heated at reflux under a nitrogen atmosphere for 6 hours, and the reaction mixture was stirred at room temperature overnight. The reaction was filtered, and the solids were rinsed with ethyl acetate. The eluent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 45/55 heptane/ethyl acetate to give the title compound.

2.119.15 **2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetic acid**

[0247] Example 2.119.14 (1.2 g) was dissolved in trifluoroacetic acid (15 mL) and heated to 65-70 °C under nitrogen overnight. The trifluoroacetic acid was removed under reduced pressure. The residue was dissolved in acetonitrile (2.5 mL) and purified by preparative reverse-phase liquid chromatography on a Luna C18(2) AXIA column (250 x 50 mm, 10µm particle size) using a gradient of 5-75% acetonitrile containing 0.1% trifluoroacetic acid in water over 30 minutes, to give the title compound. MS (ESI-) m/e 375.2 (M-H)⁻.

2.119.16 **3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid**

[0248] The title compound was prepared by substituting Example 1.43.7 for Example 1.2.9 in Example 2.49.1. MS (ESI-) m/e 1252.4 (M-H)⁻.

2.119.17 **N-({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-N-{4-[[[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfoethyl)carbamoyl}oxy)methyl]phenyl}-N5-carbamoyl-L-ornithinamide**

[0249] Example 2.119.15 (7 mg) was dissolved in N,N-dimethylformamide (0.15 mL), and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (9 mg) and N,N-diisopropylethylamine (7 µL) were added. The mixture was stirred for 3 minutes at room temperature and added to a mixture of Example 2.119.16 (28 mg) and N,N-diisopropylethylamine (15 µL) in N,N-dimethylformamide (0.15 mL). After 1 hour, the reaction was diluted with N,N-dimethylformamide/water 1/1 (1.0 mL) and purified by reverse-phase chromatography (C18 column), eluting with 5-75% acetonitrile in 0.1% TFA water, to provide the title compound. ¹H NMR (500 MHz, dimethyl sulfoxide-*d*₆) δ ppm 9.95 (s, 1H), 9.02 (s, 1H), 8.37 (d, 1H), 8.22 (m, 2H), 8.18 (m, 2H), 8.08 (m, 2H), 8.03 (m, 1H), 7.96 (br d, 1H), 7.81 (d, 1H), 7.70 (t, 1H), 7.61 (br m, 3H), 7.48 (m, 2H), 7.37 (t, 1H), 7.27 (br m, 2H), 7.08 (s, 2H), 4.99 (br d, 3H), 4.68 (t, 1H), 4.39 (m, 1H), 4.20 (m, 2H), 4.04 (m, 1H), 3.87 (br d, 2H), 3.74 (br m, 1H) 3.65 (br t, 2H), 3.48 (br m, 4H), 3.43 (br m, 2H), 3.26 (br m, 2H), 3.00 (br m, 2H), 2.80 (m, 1H), 2.76 (m, 1H), 2.66 (br m, 2H), 2.36 (br m, 1H), 2.22 (s, 3H), 2.00 (m, 1H), 1.87 (m, 1H), 1.69 (br m, 1H), 1.62 (br m, 1H), 1.40 (br m, 4H), 1.31-1.02 (m, 10 H), 0.96 (m, 2H), 0.85 (m, 12H). MS (ESI-) m/e 1610.3 (M-H)⁻.

2.123 Synthesis of (6S)-2,6-anhydro-6-(2-{2-[[[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfoethyl)carbamoyl}oxy)methyl]-5-{[N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl]amino)phenyl]ethyl)-L-gulonic acid (Synthon SZ)

2.123.1 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydropyran-2-one

[0250] To a mixture of (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (75 g) in dimethyl sulfoxide (400 mL) at 0 °C was added acetic anhydride (225 mL). The mixture was stirred for 16 hours at room temperature before it was cooled to 0 °C. A large volume of water was added, and stirring was stopped so that the reaction mixture was allowed to settle for 3 hours (the crude lactone migrated to the bottom of the flask). The supernatant was removed, and the crude mixture was diluted with ethyl acetate and was washed 3 times with water, neutralized with saturated aqueous mixture of NaHCO₃, and washed again twice with water. The organic layer was then dried over magnesium sulfate, filtered and concentrated to give the title compound. MS (ESI) m/e 561 (M+Na)⁺.

2.123.2 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-2-ethynyl-tetrahydro-2H-pyran-2-ol

[0251] To a mixture of ethynyltrimethylsilane (18.23 g) in tetrahydrofuran (400 mL) under nitrogen and chilled in a dry ice/acetone bath (internal temp -65 °C) was added 2.5M BuLi in hexane (55.7 mL) dropwise,

keeping the temperature below -60 °C. The mixture was stirred in a cold bath for 40 minutes, followed by an ice-water bath (internal temp rose to 0.4°C) for 40 minutes, and finally cooled to -75°C again. A mixture of Example 2.123.1 (50 g) in tetrahydrofuran (50 mL) was added dropwise, keeping the internal temperature below -70 °C. The mixture was stirred in a dry ice/acetone bath for additional 3 hours. The reaction was quenched with saturated aqueous NaHCO₃ mixture (250 mL). The mixture was allowed to warm to room temperature, extracted with ethyl acetate (3x 300 mL), dried over MgSO₄, filtered, and concentrated in *vacuo* to give the title compound. MS (ESI) m/e 659 (M+Na)⁺.

2.123.3 trimethyl(((3S,4R,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)ethynyl)silane

[0252] To a mixed mixture of Example 2.123.2 (60 g) in acetonitrile (450 mL) and dichloromethane (150 mL) at -15 °C in an ice-salt bath was added triethylsilane (81 mL) dropwise, followed by addition of boron trifluoride diethyl ether complex (40.6 mL) at such a rate that the internal temperature did not exceed -10 °C. The mixture was then stirred at -15 °C to -10 °C for 2 hours. The reaction was quenched with saturated aqueous NaHCO₃ mixture (275 mL) and stirred for 1 hour at room temperature. The mixture was then extracted with ethyl acetate (3 x 550 mL). The extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified by flash chromatography eluting with a gradient of 0% to 7% ethyl acetate/petroleum ether to give the title compound. MS (ESI) m/e 643 (M+Na)⁺.

2.123.4 (2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-(benzyloxymethyl)-6-ethynyl-tetrahydro-2H-pyran

[0253] To a mixed mixture of Example 2.123.3 (80 g) in dichloromethane (200 mL) and methanol (1000 mL) was added 1N aqueous NaOH mixture (258 mL). The mixture was stirred at room temperature for 2 hours. The solvent was removed. The residue was then partitioned between water and dichloromethane. The extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated to give the title compound. MS (ESI) m/e 571 (M+Na)⁺.

2.123.5 (2R,3R,4R,5S)-2-(acetoxymethyl)-6-ethynyl-tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0254] To a mixture of Example 2.123.4 (66 g) in acetic anhydride (500 mL) cooled by an ice/water bath was added boron trifluoride diethyl ether complex (152 mL) dropwise. The mixture was stirred at room temperature for 16 hours, cooled with an ice/water bath and neutralized with saturated aqueous NaHCO₃ mixture. The mixture was extracted with ethyl acetate (3x500 mL), dried over Na₂SO₄, filtered, and concentrated in *vacuo*. The residue was purified by flash chromatography eluting with a gradient of 0% to 30% ethyl acetate/petroleum ether to give the title compound. MS (ESI) m/e 357 (M+H)⁺.

2.123.6 (3R,4R,5S,6R)-2-ethynyl-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol

[0255] To a mixture of Example 2.123.5 (25 g) in methanol (440 mL) was added sodium methanolate (2.1 g). The mixture was stirred at room temperature for 2 hours, and then neutralized with 4 M HCl in dioxane. The solvent was removed, and the residue was adsorbed onto silica gel and loaded onto a silica gel column. The column was eluted with a gradient of 0 to 100% ethyl acetate/petroleum ether then 0% to 12%

methanol/ethyl acetate to give the title compound. MS (ESI) m/e 211 ($M+Na$)⁺.

2.123.7 (2S,3S,4R,5R)-6-ethynyl-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-carboxylic acid

[0256] A three-necked round bottom flask was charged with Example 2.123.6 (6.00 g), KBr (0.30 g), tetrabutylammonium bromide (0.41 g) and 60 mL of saturated aqueous NaHCO₃ mixture. TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl, 0.15 g) in 60 mL dichloromethane was added. The mixture was stirred vigorously and cooled in an ice-salt bath to -2 °C internal temperature. A mixture of brine (12 mL), aqueous NaHCO₃ mixture (24 mL) and NaOCl (154 mL) was added dropwise such that the internal temperature was maintained below 2 °C. The pH of the reaction mixture was maintained in the 8.2-8.4 range with the addition of solid Na₂CO₃. After a total of 6 hours, the reaction mixture was cooled to 3 °C internal temperature and ethanol (~20 mL) was added dropwise. The mixture was stirred for ~ 30 minutes. The mixture was transferred to a separatory funnel, and the dichloromethane layer was discarded. The pH of the aqueous layer was adjusted to 2-3 using 1 M aqueous HCl. The aqueous layer was then concentrated to dryness to afford a solid. Methanol (100 mL) was added to the dry solid, and the slurry was stirred for ~30 minutes. The mixture was filtered over a pad of diatomaceous earth, and the residue in the funnel was washed with ~100 mL of methanol. The filtrate was concentrated under reduced pressure to obtain the title compound.

2.123.8 (2S,3S,4R,5R)-methyl 6-ethynyl-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate

[0257] A 500 mL three-necked round bottom flask was charged with a suspension of Example 2.123.7 (6.45 g) in methanol (96 mL) and was cooled in an ice-salt-bath with internal temperature of - 1 °C. Neat thionyl chloride (2.79 mL) was carefully added. The internal temperature kept rising throughout the addition but did not exceed 10 °C. The reaction was allowed to slowly warm up to 15-20 °C over 2.5 hours. After 2.5 hours, the reaction was concentrated to give the title compound.

2.123.9 (3S,4R,5S,6S)-2-ethynyl-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0258] To Example 2.123.8 (6.9 g) as a mixture in N,N-dimethylformamide (75 mL) was added 4-(dimethylamino)pyridine (0.17 g) and acetic anhydride (36.1 mL). The suspension was cooled in an ice-bath and pyridine (18.04 mL) was added via syringe over 15 minutes. The reaction was allowed to warm to room temperature overnight. Additional acetic anhydride (12 mL) and pyridine (6 mL) were added and stirring was continued for an additional 6 hours. The reaction was cooled in an ice-bath and 250 mL of saturated aqueous NaHCO₃ mixture was added and stirred for 1 hour. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The organic extract was washed twice with saturated CuSO₄ mixture, dried, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 50% ethyl acetate/petroleum ether to give the title compound. ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 5.29 (t, 1H), 5.08 (td, 2H), 4.48 (dd, 1H), 4.23 (d, 1H), 3.71 (s, 3H), 3.04 (d, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 4H).

2.123.10 2-iodo-4-nitrobenzoic acid

[0259] A 3L fully jacketed flask equipped with a mechanical stirrer, temperature probe and an addition funnel under a nitrogen atmosphere, was charged with 2-amino-4-nitrobenzoic acid (69.1 g, Combi-Blocks) and sulfuric acid, 1.5 M aqueous (696 mL). The resulting suspension was cooled to 0 °C internal temperature,

and a mixture of sodium nitrite (28.8 g) in water (250 mL) was added dropwise over 43 minutes with the temperature kept below 1 °C. The reaction was stirred at ca. 0 °C for 1 hour. A mixture of potassium iodide (107 g) in water (250 mL) was added dropwise over 44 minutes with the internal temperature kept below 1 °C. (Initially addition was exothermic and there was gas evolution). The reaction was stirred 1 hour at 0 °C. The temperature was raised to 20 °C and then stirred at ambient temperature overnight. The reaction mixture became a suspension. The reaction mixture was filtered, and the collected solid was washed with water. The wet solid (~ 108 g) was stirred in 10 % sodium sulfite (350 ml, with ~ 200 mL water used to wash in the solid) for 30 minutes. The suspension was acidified with concentrated hydrochloric acid (35 mL), and the solid was collected by filtration and washed with water. The solid was slurried in water (1L) and refiltered, and the solid was left to dry in the funnel overnight. The solid was then dried in a vacuum oven for 2 hours at 60 °C. The resulting solid was triturated with dichloromethane (500 mL), and the suspension was filtered and washed with additional dichloromethane. The solid was air-dried to give the title compound

2.123.11 (2-iodo-4-nitrophenyl)methanol

[0260] A flame-dried 3 L 3-necked flask was charged with Example 2.123.10 (51.9 g) and tetrahydrofuran (700 mL). The mixture was cooled in an ice bath to 0.5 °C, and boranetetrahydrofuran complex (443 mL, 1M in THF) was added dropwise (gas evolution) over 50 minutes, reaching a final internal temperature of 1.3 °C. The reaction mixture was stirred for 15 minutes, and the ice bath was removed. The reaction was left to come to ambient temperature over 30 minutes. A heating mantle was installed, and the reaction was heated to an internal temperature of 65.5 °C for 3 hours, and then allowed to cool to room temperature while stirring overnight. The reaction mixture was cooled in an ice bath to 0 °C and quenched by dropwise addition of methanol (400 mL). After a brief incubation period, the temperature rose quickly to 2.5 °C with gas evolution. After the first 100 mL are added over ~ 30 minutes, the addition was no longer exothermic, and the gas evolution ceased. The ice bath was removed, and the mixture was stirred at ambient temperature under nitrogen overnight. The mixture was concentrated to a solid, dissolved in dichloromethane/methanol and adsorbed on to silica gel (~ 150 g). The residue was loaded on a plug of silica gel (3000 mL) and eluted with dichloromethane to give the title compound.

2.123.12 (4-amino-2-iodophenyl)methanol

[0261] A 5 L flask equipped with a mechanical stirrer, heating mantle controlled by a JKEM temperature probe and a condenser was charged with Example 2.123.11 (98.83 g) and ethanol (2 L). The reaction was stirred rapidly, and iron (99 g) was added, followed by a mixture of ammonium chloride (20.84 g) in water (500 mL). The reaction was heated over the course of 20 minutes to an internal temperature of 80.3 °C, where it began to reflux vigorously. The mantle was dropped until the reflux calmed. Thereafter, the mixture was heated to 80 °C for 1.5 hour. The reaction was filtered hot through a membrane filter, and the iron residue was washed with hot 50% ethyl acetate/methanol (800 mL). The eluent was passed through a diatomaceous earth pad, and the filtrate was concentrated. The residue was partitioned between 50% brine (1500 mL) and ethyl acetate (1500 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (400 mL x 3). The combined organic layers were dried over sodium sulfate, filtered and concentrated to give the title compound, which was used without further purification.

2.123.13 4-(((tert-butyldimethylsilyl)oxy)methyl)-3-iodoaniline

[0262] A 5 L flask with a mechanical stirrer was charged with Example 2.123.12 (88 g) and dichloromethane

(2 L). The suspension was cooled in an ice bath to an internal temperature of 2.5 °C, and tert-butylchlorodimethylsilane (53.3 g) was added portion-wise over 8 minutes. After 10 minutes, 1H-imidazole (33.7 g) was added portionwise to the cold reaction. The reaction was stirred 90 minutes while the internal temperature rose to 15 °C. The reaction mixture was diluted with water (3 L) and dichloromethane (1 L). The layers were separated, and the organic layer was dried over sodium sulfate, filtered, and concentrated to an oil. The residue was purified by silica gel chromatography (1600 g silica gel), eluting a gradient of 0 - 25% ethyl acetate in heptane, to give the title compound as an oil.

2.123.14 (S)-2-(((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanoic acid

[0263] To a mixture of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoic acid (6.5 g) in dimethoxyethane (40 mL) was added (S)-2-aminopropanoic acid (1.393 g) and sodium bicarbonate (1.314 g) in water (40 mL). Tetrahydrofuran (20 mL) was added to aid solubility. The resulting mixture was stirred at room temperature for 16 hours. Aqueous citric acid (15%, 75 mL) was added, and the mixture was extracted with 10% 2-propanol in ethyl acetate (2 x 100 mL). A precipitate formed in the organic layer. The combined organic layers were washed with water (2 x 150 mL). The organic layer was concentrated under reduced pressure and then triturated with diethyl ether (80 mL). After brief sonication, the title compound was collected by filtration. MS (ESI) m/e 411 (M+H)⁺.

2.123.15 (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-(4-(((tertbutyldimethylsilyl)oxy)methyl)-3-iodophenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

[0264] A mixture of Example 2.123.13 (5.44 g) and Example 2.123.14 (6.15 g) in a mixture of dichloromethane (70 mL) and methanol (35.0 mL) was added ethyl 2-ethoxyquinoline-1(2H)-carboxylate (4.08 g), and the reaction was stirred overnight. The reaction mixture was concentrated and loaded onto silica gel, eluting with a gradient of 10% to 95% heptane in ethyl acetate followed by 5% methanol in dichloromethane. The product-containing fractions were concentrated, dissolved in 0.2% methanol in dichloromethane (50 mL), loaded onto silica gel and eluted with a gradient of 0.2% to 2% methanol in dichloromethane. The product containing fractions were collected to give the title compound. MS (ESI) m/e 756.0 (M+H)⁺.

2.123.16 (2S,3S,4R,5S,6S)-2-((5-(((S)-2-(((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amin o)-3-methylbutanamido)propanamido)-2-(((tertbutyldimethylsilyl)oxy)methyl)phenyl)ethynyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0265] A mixture of Example 2.123.9 (4.500 g), Example 2.123.15 (6.62 g), copper(I) iodide (0.083 g) and bis(triphenylphosphine)palladium(II) dichloride (0.308 g) were combined in vial and degassed. N,N-dimethylformamide (45 mL) and N-ethyl-N-isopropylpropan-2-amine (4.55 mL) were added, and the reaction vessel was flushed with nitrogen and stirred at room temperature overnight. The reaction was partitioned between water (100 mL) and ethyl acetate (250 mL). The layers were separated, and the organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 5% to 95% ethyl acetate in heptane. The product containing fractions were collected, concentrated and purified by silica gel chromatography, eluting with a gradient of 0.25% to 2.5% methanol in dichloromethane to give the title compound. MS (ESI) m/e 970.4 (M+H)⁺.

2.123.17 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((tertbutyldimethylsilyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0266] Example 2.123.16 (4.7 g) and tetrahydrofuran (95 mL) were added to 5% Pt/C (2.42 g, wet) in a 50 mL pressure bottle and shaken for 90 minutes at room temperature under 50 psi of hydrogen. The reaction was filtered and concentrated to give the title compound. MS (ESI) m/e 974.6 (M+H)⁺.

2.123.18 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0267] A mixture of Example 2.123.17 (5.4 g) in tetrahydrofuran (7 mL), water (7 mL) and glacial acetic acid (21 mL) was stirred overnight at room temperature. The reaction was diluted with ethyl acetate (200 mL) and washed with water (100 mL), saturated aqueous NaHCO₃ mixture (100 mL), brine (100 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 0.5% to 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 860.4 (M+H)⁺.

2.123.19 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[0268] To a mixture of Example 2.123.18 (4.00 g) and bis(4-nitrophenyl) carbonate (2.83 g) in acetonitrile (80 mL) was added N-ethyl-N-isopropylpropan-2-amine (1.22 mL) at room temperature. After stirring overnight, the reaction was concentrated, dissolved in dichloromethane (250 mL) and washed with saturated aqueous NaHCO₃ mixture (4 x 150 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The resulting foam was purified by silica gel chromatography, eluting with a gradient of 5% to 75% ethyl acetate in hexanes to give the title compound. MS (ESI) m/e 1025.5 (M+H)⁺.

2.166 Synthesis of 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)-4-((S)-2-((S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (Synthon AAA)

[0269] The title compound was prepared by substituting Example 2.167.1 for Example 2.119.16 in Example 2.119.17. ¹H NMR (500 MHz, dimethyl sulfoxide-d₆) δ ppm 9.86 (br d, 1H), 8.17 (br d, 1H), 8.04 (m, 2H), 7.78 (d, 1H), 7.61 (d, 1H), 7.51 (br d, 1H), 7.49-7.39 (m, 4H), 7.36 (m, 2H), 7.29 (s, 1H), 7.21 (d, 1H), 7.07 (s, 2H), 6.95 (d, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.64 (t, 1H), 4.36 (m, 1H), 4.19 (m, 1H), 4.16 (d, 1H), 4.01 (d, 1H), 3.88 (br t, 2H), 3.82 (br m, 3H), 3.75 (br m, 1H), 3.64 (t, 2H), 3.54 (d, 2H), 3.47 (m, 4H), 3.43 (br m, 4H), 3.23 (br m, 5H), 3.13 (t, 1H), 3.10 (br m, 1H), 3.01 (br m, 2H), 2.93 (t, 1H), 2.83-2.68 (m, 3H), 2.37 (m,

1H), 2.08 (s, 3H), 1.99 (br m, 2H), 1.85 (m, 1H), 1.55 (br m, 1H), 1.37 (br m, 1H), 1.28 (br m, 6H), 1.10 (br m, 7H), 0.93 (br m, 1H), 0.88-0.69 (m, 12H). MS (ESI) m/e 1713.6 (M-H)⁻.

Alternative Synthesis of 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (Synthon AAA)

2.167.1 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[0270] Example 2.167.1 was prepared by substituting Example 2.123.19 for (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate and substituting Example 1.85 for Example 1.2.9 in Example 2.49.1. MS (ESI) m/e 1355.5 (M-H)⁻.

Example 3: Generation of Mouse Anti-B7-H3 Monoclonal Antibodies by Mouse Hybridoma Technology

[0271] B7-H3 specific antibodies were raised using mouse hybridomas technology. Specifically, a mouse fibroblast cell line (3T12) expressing full length human B7-H3 as well as recombinant human or mouse B7-H3-ECD-human Fc fusion proteins were used as immunogens, the sequences of which are provided in Table 1. Human HCT116 cell lines expressing human B7-H3 were used for determining anti-sera titer and for screening antigen-specific antibodies. Cell lines were exposed to approximately 3000 mREM of gamma source radiation prior to immunization. Two different strains of mice were immunized in the hock with dosages containing 5×10^6 cells/mouse/injection or 10 ug of protein/mouse/injection in the presence of Gerbu MM adjuvant (Cooper-Casey Corporation, Valley Center, CA, US) for both primary and boost immunizations. To increase immune response to mouse B7-H3, the mice were further boosted with a mixture of human and mouse B7-H3-ECD-human Fc proteins for the final boosts. Briefly, the antigens were prepared in PBS as follows: 200×10^6 cells/mL or 400 ug/mL protein. The calculated volume of antigen was transferred to a sterile microcentrifuge tube and equal volume of Gerbu MM was then added. The solution was mixed by gently vortexing for 1 minute. The adjuvant-antigen solution was then drawn into a proper syringe for animal injection. A total of 25 μ L of the mixture was injected into the hock of each leg of the mouse. Each animal was boosted 3 times before serum titer was determined for the groups. All animals were given 2 additional boosts with an equal mixture of mouse B7-H3-ECD-human Fc and human B7-H3-ECD-human Fc proteins in adjuvant before fusion.

Table 1: Amino acid sequences of recombinant proteins used for immunization or screening

Protein	Amino Acid Sequence
Human full length B7-H3	<u>MLRRRGSPGMGVHVGAAALGALWFCLTGALEVQVPEDPVVALVGTDATLCCSFSP</u> <u>EPGFSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTY</u> <u>SCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYRGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSVTITGQPM</u> <u>TFPPEALWVTVGLSVCLIALLLVALAFVCWRKIKQSCEENAGAEDQDGE</u> <u>GEGSKTALQPLKHSDSKEDDGQEIA</u> (SEQ ID NO: 149)
Human B7-H3-ECD (fc fusion)	<u>MLRRRGSPGMGVHVGAAALGALWFCLTGALEVQVPEDPVVALVGTDATLCCSFSP</u> <u>EPGFSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTY</u> <u>SCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYRGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSVTITGQPM</u> <u>TFAAADKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH</u> <u>EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE</u> <u>SNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQK</u> <u>SLSLSPGK</u> (SEQ ID NO: 150)
Mouse B7-H3-ECD (fc fusion)	<u>MLRGWGGPSVGVCVRTALGVLCCLTGAVEVQVSEDPVVALVDTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYS</u> <u>SNRTALFPDLLVQGNASLRLQ</u> <u>RVRVTDEGSYTCFVSIQDFD</u> <u>SAAVSLQVAAPYSKPSMTLEPNKDLRPG</u> <u>NMVTITCSSYQGYPEAEVFWKDQGVPLTGNVTTSQMANERGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSVTITGQPLTFAAADKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPE</u> <u>VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN</u> <u>GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMH</u> <u>EALHNHYTQKSLSLSPGK</u> (SEQ ID NO: 151)
Human B7-H3-ECD (His tag)	<u>MEFGLSWLFLVAILKGVQCGALEVQVPEDPVVALVGTDATLCCSFSP</u> <u>EPGFSGFSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTY</u> <u>SCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYRGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSVTITGQPM</u> <u>THHHHHH</u> (SEQ ID NO: 152)
Mouse B7-H3-ECD (His tag)	<u>MEFGLSWLFLVAILKGVQCVEVQVSEDPVVALVDTDATLRCSFS</u> <u>PEPGFSGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYS</u> <u>SNRTALFPDLLVQGNASLRLQ</u> <u>RVRVTDEGSYTCFVSIQDFD</u> <u>SAAVSLQVAAPYSKPSMTLEPNKDLRPG</u> <u>NMVTITCSSYQGYPEAEVFWKDQGVPLTGNVTTSQMANERGLFDVHSVLRVVLGANGTYS</u> <u>CLVRNPVLQQDAHGSVTITGQPLTFHHHHHH</u> (SEQ ID NO: 153)
Cyno B7-H3-ECD (his tag)	<u>MLHRRGSPGMGVHVGAAALGALWFCLTGALEVQVPEDPVVALVGTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFLDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYRGYPEAEVFWQDGQGAPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSITITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSFS</u> <u>PEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFLDLLAQGNASLRLQ</u> <u>RVRVADEGSFTCFVSIRDFGSA</u> <u>AVSLQVAAPYSKPSMTLEPNKDLRPGD</u> <u>TVTITCSSYRGYPEAEVFWQDGQGAPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTY</u> <u>SCLVRNPVLQQDAHGSVTITGQPM</u> <u>TFAAAHHHHHHHH</u> (SEQ ID NO: 154)

Protein	Amino Acid Sequence
Note: leader sequence, Fc, and His sequences are underlined	

Hybridoma fusion and screening

[0272] Cells of murine myeloma cell line (NS-0, ECACC No. 85110503) were cultured to reach the log phase stage right before fusion. Popliteal and inguinal lymph nodes were removed from each mouse and single cell suspensions were prepared sterilely. Lymphocytes were fused with myeloma cells (E. Harlow, D. Lane, Antibody: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998); Kohler G. and Milstein C., "Continuous cultures of fused cell secreting antibody of predefined specificity," Nature, 256:495-497 (1975); BTX Harvard Apparatus (Holliston, MA, US) ECM 2001 technical manual). Fused hybrid cells were dispensed into 96-well plates in DMEM/10% FBS/HAT media. Supernatants from surviving hybridoma colonies were subjected to cell-based screening using human cell lines expressing the recombinant human B7-H3. Briefly, a human cell line expressing the human B7-H3 was thawed and directly dispensed into 96 well (black with clear bottom for imaging) plates at 50,000 cells/well in growth media and incubated for 2 days at 37 °C to reach 50% confluency. Hybridoma supernatants (50 µL/well) were transferred to respective plates and incubated at room temperature for 30 minutes. Media was removed from each well and goat anti-mouse IgG-AF488 (Invitrogen, No. A11029, Grand Island, NY, US) was used for detection using the InCell Analyzer 2000 (GE). Hits were expanded and binding was confirmed by FACS using a different human cell line or a mouse cell line expressing the human B7-H3 and goat anti-mouse IgG-PE for detection. Species specificity was determined using the ELISA format according to the following procedure. ELISA plates were coated with human B7-H3-ECD-human Fc, cynomolgous B7-H3-ECD-his, or mouse B7-H3-ECD-human Fc proteins overnight at room temperature. Plates were washed and hybridoma supes (100 µL) was added to each well, and incubated at room temperature for 1 hour. Plates were washed, donkey anti-mouse IgG-HRP (Jackson Immunochemicals, No. 115-035-071, West Grove, PA, US) was used for detection, and binding ODs were observed at 650 nm.

[0273] A selection of hits were subcloned using the MoFlo (Beckman, Indianapolis, IN, US) by depositing a single cell per well into 96 well cell culture plates to ensure clonality of the cell line. Resulting colonies were screened for specificity by FACS using mouse 3T12 fibroblast cell lines expressing the human B7-H3, cynomolgous B7-H3 or mouse B7-H3. Isotype of each monoclonal antibody was determined using the Mouse Monoclonal Isotyping Kit (Roche, No. 11-493-027-001, Indianapolis, IN, USA). Hybridoma clones producing antibodies that showed high specific binding activity against human and cynomolgus B7-H3 antigen were subcloned and purified (Table 2).

Table 2: List of Anti-B7-H3 antibodies generated using mouse hybridoma technology

Clone Name	Species / Isotype	FACS Binding (EC ₅₀ nM)		
		Human B7-H3	Cynomolgous B7-H3	Mouse B7-H3
Ab1	mouse IgG1/k	2.10	1.79	299.0
Ab2	mouse IgG1/k	1.70	1.50	1.00
Ab3	mouse IgG1/k	1.66	1.42	0.94
Ab4	mouse IgG2b/k	4.06	3.10	1.75
Ab5	mouse IgG1/k	2.71	1.91	6.01
Ab6	mouse IgG1/k	1.59	1.53	No binding
Ab7	mouse IgG1/k	3.22	2.67	67.13

Clone Name	Species / Isotype	FACS Binding (EC ₅₀ nM)		
		Human B7-H3	Cynomolgous B7-H3	Mouse B7-H3
Ab8	mouse IgG1/k	3.83	8.63	193.0
Ab9	mouse IgG1/k	4.49	259.0	0.72
Ab10	mouse IgG2b/k	3.97	4.46	3.80
Ab11	mouse IgG1/k	23.40	2.03	568.60
Ab12	mouse IgG1/k	3.88	6.71	8.72
Ab13	mouse IgG1/k	1.94	4.12	25.80
Ab14	mouse IgG1/k	3.03	2.97	102.2
Ab15	mouse IgG1/k	5.37	6.52	4.61
Ab16	mouse IgG1/k	3.94	4.28	318.7
Ab17	mouse IgG2b/k	2.75	2.60	2.39
Ab18	mouse IgG1/k	5.98	6.49	No binding

Example 4: *In Vitro* Characterization of Anti-B7-H3 Mouse Monoclonal Antibodies.

[0274] The binding affinity of the purified anti-B7-H3 monoclonal antibodies was determined by surface plasma resonance. Table 3 shows the association rate constants (k_a) dissociation rate constants (k_d) and equilibrium dissociation constants (K_D) for a series of mouse hybridoma derived anti-B7-H3 monoclonal antibodies (mAbs) binding to the soluble ECDs of human B7-H3 and cyno B7-H3. The binding kinetics were derived from SPR measurements using a Biacore T200 instrument and a mAb capture approach (as described in the materials and methods below).

Table 3: Biacore kinetics of anti-B7-H3 mouse hybridoma antibodies binding to human and cynomolgus monkey B7-H3.

<i>huB7-H3</i>				<i>cynoB7-H3</i>		
<i>Murine Antibody Name</i>	k_a (1/MS)	k_d (1/s)	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)
Ab17	5.4E+05	1.9E-05	3.4E-11	5.1E+05	1.0E-05	1.9E-11
Ab18	2.1E+05	3.6E-05	1.7E-10	2.4E+05	2.9E-05	1.2E-10
Ab15	8.0E+04	3.4E-05	4.3E-10	7.7E+04	7.0E-05	9.1E-10
Ab4	6.9E+05	1.1E-03	1.6E-09	5.4E+05	9.6E-04	1.8E-09
Ab8	5.8E+04	9.9E-05	1.7E-09	1.6E+05	2.6E-04	1.7E-09
Ab10	4.1E+04	1.9E-04	4.6E-09	2.0E+05	4.2E-03	2.0E-08
Ab12	3.8E+04	2.5E-04	6.7E-09	5.5E+04	1.0E-05	1.8E-10
Ab5	1.3E+06	1.2E-02	9.2E-09	1.4E+06	2.8E-01	2.0E-07
Ab14	1.1E+05	1.4E-03	1.3E-08	6.9E+05	3.0E-03	4.3E-09
Ab9	6.6E+04	1.1E-03	1.7E-08	poor kinetic fit		
Ab13	3.3E+05	5.8E-03	1.7E-08	4.4E+05	3.7E-03	8.4E-09
Ab3	5.2E+05	1.0E-02	1.9E-08	3.8E+05	1.0E-02	2.6E-08

<i>huB7-H3</i>				<i>cynoB7-H3</i>		
<i>Murine Antibody Name</i>	k_a (1/MS)	k_d (1/s)	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)
Ab16	1.4E+05	3.2E-03	2.4E-08	7.5E+05	5.6E-03	7.5E-09
Ab2	1.2E+05	2.9E-03	2.4E-08	2.3E+05	1.1E-02	5.0E-08
Ab11	2.0E+04	8.9E-04	4.5E-08	2.7E+04	7.2E-05	2.6E-09
Ab6	1.2E+04	1.0E-02	8.4E-07	2.8E+04	1.2E-02	4.1E-07
Ab1		no observable binding			no observable binding	
Ab7		little observable binding			little observable binding	

[0275] Pair-wise binding assays performed on Biacore T200 SPR instruments were used to determine the relative epitope grouping for the murine anti-B7-H3 mAbs as described in the methods below. Figure 1 shows an epitope grouping depiction, which describes the relative human B7-H3 epitope diversity and overlap for a series of anti-B7-H3 mAbs identified herein. Epitope groups are represented as individual ovals, some of which overlap with each other. Antibodies in different epitope groups can bind to B7-H3 simultaneously and likely bind to different epitopes while antibodies within a given epitope group cannot bind to B7-H3 simultaneously and likely bind to overlapping epitopes. The grouping information was derived from a simultaneous binding assay as described in materials and methods. Ab3, Ab4, Ab5, Ab11, Ab12, and Ab8 groupings were ambiguous.

Materials and methods: Binding kinetics

[0276] Biacore T200 SPR instruments were used to measure the binding kinetics of human B7-H3 (analyte) binding to various mAbs (ligands). The assay format was Fc-based capture via immobilized anti-mouse (Fc) (Pierce 31170) or immobilized anti-human (Fc) (Pierce 31125). A standard amine coupling protocol was employed to immobilize the capture reagents via primary amines to the carboxy-methyl (CM) dextran surface of CM5 sensorchips (Biacore); capture antibodies were coupled to a level of approximately 5000RU. For binding kinetic measurements the assay buffer was HBS-EP+ (Biacore): 10 mM Hepes, pH7.4, 150 mM NaCl, 3 mM EDTA, 0.05% polysorbate 20. During the assay, all measurements were referenced against the capture surface alone. Each assay cycle consisted of the following steps: 1) Capture of ligand to approximately 50RU; 2) Analyte injection over both reference and test surface, 240 μ L at 80 μ L/min, after which the dissociation was monitored for 900 seconds at 80 μ L/min; 3) Regeneration of capture surface with low pH glycine. For kinetic determinations analyte injections were 3-point, 9-fold dilution series of 900 nM, 100 nM and 11.11 nM, buffer only injections were included for secondary referencing. Data were processed and fit to a 1:1 binding model using Biacore T200 Evaluation Software to determine the binding kinetic rate constants, k_a (on-rate) and k_d (off-rate), and the equilibrium dissociation constant (affinity, K_D).

Materials and methods: Epitope grouping

[0277] Pair-wise binding assays performed on Biacore T200 SPR instruments were used to determine the relative epitope grouping for a series of anti-B7-H3 mAbs. The assay format was Fc-based capture via immobilized anti-mouse(Fc) (Pierce 31170) or immobilized anti-human (Fc) (Pierce 31125). A standard

amine coupling protocol was employed to immobilize the capture reagents via primary amines to the carboxy-methyl (CM) dextran surface of CM5 sensorchips (Biacore); capture antibodies were coupled to a level of approximately 2000RU. Epitope grouping measurements were done at 12 °C (low temperature allows for grouping information on fast off-rate mAbs), the assay buffer was HBS-EP+ (Biacore): 10 mM Hepes, pH7.4, 150 mM NaCl, 3 mM EDTA, 0.05% polysorbate 20. Each assay cycle consisted of the following steps in a four flowcell system: 1) separate test mAbs were captured in flowcells 2, 3 & 4 (flowcell 1 was reference, no test mAb); 2) all 4 flowcells were then blocked by injection with isotype control mAb or isotype mAb cocktail at 50 µg/mL; 3) all 4 flowcells were then injected with antigen or buffer only (buffer only is for double referencing, done for each mAb pair individually); 4) all 4 flowcells were then injected with 2nd test mAb at 10 µg/mL; 5) all 4 flowcells were then regenerated with glycine, pH1.5. The assay was done for each test mAb pair in reciprocal orientations. Simultaneous binding was evaluated examining the ratio of the 2nd test mAb response to the Ag response (RU_{mAb2}/RU_{Ag}); if this ratio was equal to or greater than 0.2 the interaction was scored as a simultaneous binder. From this pair-wise binding assay data a "venn" style diagram was constructed manually to depict relative epitope groupings.

Example 5: Generation of Anti-hB7-H3 Chimeric Antibodies

[0278] Following the identification of mouse anti-B7-H3 hybridoma antibodies, heavy and light chain variable regions (VH and VL) corresponding to the secreted antibodies were determined from cells using reverse transcriptase-polymerase chain reaction (RT-PCR). Murine variable regions were expressed in mammalian host cells in the context of a human immunoglobulin constant region to provide chimeric antibodies. Table 4 below provides the variable region amino acid sequences for the mouse chimerized hybridomas.

Table 4: Variable region amino acid sequences of anti-B7-H3 antibodies from mouse hybridomas

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
1	chAb2	VH		QVQLQQPGAELVKPGASVKLSCKASGY TFTSYWMHWVKQRPGQGLEWIGMIHPD SGTTNYNEKFRSKATLTVDKSSSTAYM QLSSLTSEDSAVYYCAVYYGSTYWYFD VWGTGTTVTVSS
2	chAb2	CDR-H1	Residues 26-35 of SEQ ID NO:1	GYTFTSYWMH
3	chAb2	CDR-H2	Residues 50-66 of SEQ ID NO:1	MIHPDSGTTNYNEKFRS
4	chAb2	CDR-H3	Residues 99-109 of SEQ ID NO:1	YYGSTYWYFDV
5	chAb2	VL		DVVMTQTPLSLPVSLGDQAYISCRSSQ SLVHINGNTYLHWYRQKPCQSPKLLIY KVSNRFSGVDPDRFSGSGSGTDETLKIS RVEAEDLGVIYFCSSQSTHFPFTFGSGTK LEIK
6	chAb2	CDR-L1	Residues 24-39 of SEQ ID NO:5	RSSQSLVHINGNTYLH
7	chAb2	CDR-L2	Residues 55-61 of SEQ ID NO:5	KVSNRFS
8	chAb2	CDR-L3	Residues 94-102 of SEQ ID NO:5	SQSTHFPFT

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
9	chAb3	VH		QVQLQQPGAELVKPGASVKLSCKASGY TFSSYWMHWVKQRPGQGLEWIGLIHPD SGSTNYNEMFKNKATLTVDRSSSTAYV QLSSLTSEDSAVYFCAGGGRLYFDYWG QGTTLTVSS
10	chAb3	CDR-H1	Residues 26-35 of SEQ ID NO:9	GYTFSSYWMH
11	chAb3	CDR-H2	Residues 50-66 of SEQ ID NO:9	LIHPDSGSTNYNEMFKN
12	chAb3	CDR-H3	Residues 99-106 of SEQ ID NO:9	GGRLYFDY
13	chAb3	VL		DVVMTQTPLSLPVSLGDQASISCRSSQ SLVHSNGDITYLRWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKIT RVEAEDLGVIYFCSSQSTHVPYTFGGGTK LEIK
14	chAb3	CDR-L1	Residues 24-39 of SEQ ID NO:13	RSSQSLVHSNGDITYLR
7	chAb3	CDR-L2	Residues 55-61 of SEQ ID NO:13	KVSNRFS
15	chAb3	CDR-L3	Residues 94-102 of SEQ ID NO:13	SQSTHVPYT
16	chAb4	VH		QVQLQQPGAELVKPGASVKLSCKASGY SFTSYWMHWVKQRPGQGLEWIGMIHPN SGSNNYNEKFKSKATLTVDKSSNTAYM QLSSLTSEDSAVYYCARRLGLHFDYWG QGTTLTVSS
17	chAb4	CDR-H1	Residues 26-35 of SEQ ID NO:16	GYSFTSYWMH
18	chAb4	CDR-H2	Residues 50-66 of SEQ ID NO:16	MIHPNSGSNNYNEKFKS
19	chAb4	CDR-H3	Residues 99-106 of SEQ ID NO:16	RLGLHFDY
20	chAb4	VL		DIVMTQSQKEMSTPVGDRVSI ~~TCK~~ ASQ NVGTAVAWYQQKPGQSPKLLIY ~~S~~ ASNR YTGVPDRFTGSGSGTDFTLTISNMQSE DLADYFCQQYSSYPYT ~~F~~ GGGTKLEIK
21	chAb4	CDR-L1	Residues 24-34 of SEQ ID NO:20	KASQNVGTAVA
22	chAb4	CDR-L2	Residues 50-56 of SEQ ID NO:20	SASNRYT
23	chAb4	CDR-L3	Residues 89-97 of SEQ ID NO:20	QQYSSYPYT

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
24	chAb18	VH		QVQLQQSAAELARPGASVKMSCKASGY SFTSYTIHWVKQRPGQGLEWIGYINPN SRNTDYNQKFKDETTLTADRSSSTAYM QLISLTSEDSAVYYCARYSGSTPYWYF DVWGAGTTVTVSS
25	chAb18	CDR-H1	Residues 26-35 of SEQ ID NO:24	GYSFTSYTIH
26	chAb18	CDR-H2	Residues 50-66 of SEQ ID NO:24	YINPNSRNTDYNQKFKD
27	chAb18	CDR-H3	Residues 99-110 of SEQ ID NO:24	YSGSTPYWYFDV
28	chAb18	VL		QIVLTQSPAILSASPGEKVTMTCRASS SVSYMNWYQQKPGSSPKPWIYATSNLA SGVPARFSVSVSGTSHSLTISRVEAED AATYYCQQWSSNPLTFGAGTKLELK
29	chAb18	CDR-L1	Residues 24-33 of SEQ ID NO:28	RASSSVSYMN
30	chAb18	CDR-L2	Residues 49-55 of SEQ ID NO:28	ATSNLAS
31	chAb18	CDR-L3	Residues 88-96 of SEQ ID NO:28	QQWSSNPLT
32	chAb13	VH		DVQLQESGPDLVKPSQSLSLTCTVTGY SITSGYSWHWIRQFPGNKLEWMGYIHS SGSTNYNPSLKSRLSINRDTSKNQFFL QLNSVTTEDTATYYCAGYDDYFEYWGQ GTTTLTVSS
33	chAb13	CDR-H1	Residues 26-36 of SEQ ID NO:32	GYSITSGYSWH
34	chAb13	CDR-H2	Residues 51-66 of SEQ ID NO:32	YIHSSGSTNYNPSLKS
35	chAb13	CDR-H3	Residues 99-105 of SEQ ID NO:32	YDDYFEY
36	chAb13	VL		DIVMTQSQKFMSTSVGDRVSVTCKASQ NVGFNVAWYQQKPGQSPKALIYSASYR YSGVPDRFTGSGSGTDFTLTISNVQSE DLAEYFCQQYNSYPFTFGSGTKLEIK
37	chAb13	CDR-L1	Residues 24-34 of SEQ ID NO:36	KASQNVGFNVA
38	chAb13	CDR-L2	Residues 50-56 of SEQ ID NO:36	SASYRYS
182	chAb13	CDR-L3	Residues 89-97 of SEQ ID NO:36	QQYNSYPFT
				EVKLVESGGGLVKPGGSLKLSCAASGF TFSSYGMSWVROTPEKRLEWVATISGG

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
48	chAb14	VH		GTNTYYPDSVEGRFTISRDNÄKNFLYL QMSSLRSEDTALYYCARHYGSQTMDYW GQGTSVTVSS
49	chAb14	CDR-H1	Residues 26-35 of SEQ ID NO:48	GFTFSSYGMS
50	chAb14	CDR-H2	Residues 50-66 of SEQ ID NO:48	TISGGGTNTYYPDSVEG
51	chAb14	CDR-H3	Residues 99-107 of SEQ ID NO:48	HYGSQTMDY
52	chAb14	VL		DIQMTQSPASLSASVGETVTITCRTSG NIHNYL TWYQQKQGKSPQLLVYNÄKTL ADGVPSRFSGSGSGTQFSLKINSLOPE DFGSYYCQHFWSIMWTFGGGTKLEIK
53	chAb14	CDR-L1	Residues 24-34 of SEQ ID NO:52	RTSGNIHNYLT
54	chAb14	CDR-L2	Residues 50-56 of SEQ ID NO:52	NAKTLAD
55	chAb14	CDR-L3	Residues 89-97 of SEQ ID NO:52	QHFWSIMWT
56	chAb6	VH		QVQLQQSGAELMKPGASVKISCKATGY TFSRYWIEWVKQRPGHGLEWIGEILPG SGSTNYNEKFKGKATFTADTSSNTAYM QVSSLTSEDSÄVHYCARRGYGYVPYAL DYWGQGTSVTVSS
57	chAb6	CDR-H1	Residues 26-35 of SEQ ID NO:56	GYTFSRYWIE
58	chAb6	CDR-H2	Residues 50-66 of SEQ ID NO:56	EILPGSGSTNYNEKFKG
59	chAb6	CDR-H3	Residues 99-110 of SEQ ID NO:56	RGYGYVPYALDY
60	chAb6	VL		EIQMTQTSSLSASLGDRVTISCRASQ DISNSL N WYQQKPDGTVNLLIYYTSRL YSGVPSRFSGSGSGTDYSLTISNLEQE DIATYFCQQGNTLPYTFGGGTKLEIK
61	chAb6	CDR-L1	Residues 24-34 of SEQ ID NO:60	RASQDISNSLN
62	chAb6	CDR-L2	Residues 50-56 of SEQ ID NO:60	YTSRLYS
63	chAb6	CDR-L3	Residues 89-97 of SEQ ID NO:60	QQGNTLPYT
64	chAb11	VH		EVKLVESGGGLVQPGGSLRLSCATSGF TFTNYYSWVRQPPGKALEWLG FIRNK ÄNDYTTEYSÄSVKGRFTISRDNÄQSIL VIQOMNITLRÄPDSÄTVYCARFÄDÄNDFÄ

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
				YWGQGTLLVTVSA
65	chAb11	CDR-H1	Residues 26-35 of SEQ ID NO:64	GFTFTNYYMS
66	chAb11	CDR-H2	Residues 50-68 of SEQ ID NO:64	FIRNKANDYTTEYSASVKG
67	chAb11	CDR-H3	Residues 101-109 of SEQ ID NO:64	ESPGNPFAY
68	chAb11	VL		DIVMTQSPSSSLTVTAGEKVTMTCKSSQ SLLNSGTQKNFLT TWYQQKPGQPPKLLI YWASTRESGVPDRETGSGSGTDFTLTIT SSVQAEDLAVYFCQNDYIYPLTEGAGT KLELK
69	chAb11	CDR-L1	Residues 24-40 of SEQ ID NO:68	KSSQSLLNSGTQKNFLT
70	chAb11	CDR-L2	Residues 56-62 of SEQ ID NO:68	WASTRES
71	chAb11	CDR-L3	Residues 95-103 of SEQ ID NO:68	QNDYIYPLT
72	chAb16	VH		EVKLVESGGGLVQPGGSLKLSCAASGF DFFSRYWMSWVRQAPGKGLEWIGEPD SSTINYPSTSLKDKFIISRDNKNTLYL QMSKVRSEDTALYYCARPGFGNYIYAM DYWGQGTSTVTVSS
73	chAb16	CDR-H1	Residues 26-35 of SEQ ID NO:72	GFDFSRYWMS
74	chAb16	CDR-H2	Residues 50-66 of SEQ ID NO:72	EINPDSSTINYPSTSLKD
75	chAb16	CDR-H3	Residues 99-110 of SEQ ID NO:72	PGFGNYIYAMDY
76	chAb16	VL		DIQMTQTTSSSLASLGDRVTINCRASQ DISNFLNWKYQQKPDGTVKLLIYYTSRL YLGVPSTRFSGSGSGTDYSLTISNLEQE DIATYFCQQGNTLPPTFGGGTKLEIK
77	chAb16	CDR-L1	Residues 24-34 of SEQ ID NO:76	RASQDISNFLN
78	chAb16	CDR-L2	Residues 50-56 of SEQ ID NO:76	YTSRLYL
79	chAb16	CDR-L3	Residues 89-97 of SEQ ID NO:76	QQGNTLPPT

Example 6: Binding Characterization of Chimeric Anti-B7-H3 Antibodies

[0279] To generate purified chimeric antibodies, expression vectors were transiently transfected into HEK293 6E suspension cell cultures in a ratio of 60% to 40% light to heavy chain construct. 1 mg/ml of polyethylenimine (PEI) or 2.6 μ L/mL of Expifectamine was used to transfect the cells. Cell supernatants were harvested after five days in shaking flasks, spun down to pellet cells, and filtered through 0.22 μ m filters to separate IgG from culture contaminants. Antibody-containing supernatants were purified on Akta Pure using protein A mAb SelectSure. Columns were equilibrated in PBS pH 7.4, supernatants were then passed through the column and a wash was performed with PBS pH 7.4. IgG were eluted with 0.1 M acetic acid pH 3.5 and collected in several aliquots. Fractions containing IgG were pooled and dialyzed in PBS overnight at 4 °C. Anti-B7-H3 chimeric antibodies that were successfully expressed were characterized for the ability to bind the B7-H3 overexpressing human non-small cell lung cancer cell line NCI-H1650 (ATCC® No. CRL-5883) by FACS using the methods described below. Table 5 summarizes the binding properties of the chimeric anti-B7-H3 antibodies.

TABLE 5: *In vitro* characterization of B7-H3 chimeric antibodies

Chimeric Ab Name	Isotype	Parental Hybridoma	FACS binding (EC ₅₀ nM)
chAb2	hulgG1/k	Ab2	0.10
chAb3	hulgG1/k	Ab3	0.61
chAb4	hulgG1/k	Ab4	0.56
chAb18	hulgG1/k	Ab18	1.14
chAb13	hulgG1/k	Ab13	1.53
chAb11	hulgG1/k	Ab11	1.12
chAb6	hulgG1/k	Ab6	0.33
chAb16	hulgG1/k	Ab16	0.27
chAb14	hulgG1/k	Ab14	0.81

FACS binding methods

[0280] Cells were harvested from flasks when approximately 80% confluent using Gibco® Cell Dissociation Buffer. Cells were washed once in PBS/1% FBS (FACS buffer) then resuspended at 2.5x10⁶ cells/mL in FACS buffer. 100 μ L of cells/well were added to a round bottom 96-well plate. 10 μ L of a 10x concentration of mAb/ADC (final concentrations are indicated the figures). Wells were washed twice with FACS buffer and resuspended in 50 μ L of secondary Ab (AlexaFluor 488) diluted in FACS buffer. The plate was incubated at 4°C for one hour and washed twice with FACS buffer. Cells were resuspended in 100 μ L of PBS/1% formaldehyde and analyzed on a Becton Dickinson LSRII flow cytometer. Data was analyzed using WinList flow cytometry analysis software.

Example 7: Characterization of Chimeric Anti-B7-H3 Antibodies as Bcl-xL Inhibiting Antibody Drug Conjugates

[0281] Nine anti-B7-H3 chimeric antibodies were conjugated to the Bcl-xL inhibiting (Bcl-xLi) synthon CZ (Example 2.1) using conjugation Method A described below. The resulting ADCs (anti-B7-H3 antibodies conjugated to synthon CZ) were tested for binding to cell surface human B7-H3 by FACS (as described in Example 6) and for cell cytotoxicity in cell lines expressing B7-H3. Of the nine antibodies, three antibodies (chAb2, chAb6, and chAb16) precipitated following conjugation to synthon CZ and showed weak cytotoxicity

in cells expressing human B7-H3. Table 6 provides cell surface binding and cytotoxicity activity of anti-B7-H3 chimera ADCs against breast cancer cell HCC38 expressing human B7-H3.

TABLE 6: *In vitro* characterization of B7-H3 chimeric-CZ conjugates

ADC Name	Conjugation observation	FACS Binding Human B7-H3 EC ₅₀ nM	Cytotoxicity (HCC38 cell line IC ₅₀ nM)	Conjugation Method	DAR by MS	% agg by SEC
chAb2-CZ	Precipitates	2.60	4.77	A	1.0	5.9
chAb3-CZ	Clear	0.65	0.17	A	4.6	6.3
chAb4-CZ	Clear	0.54	0.26	A	1.4	5.7
chAb18-CZ	Clear	1.78	0.28	A	2.3	4.3
chAb13-CZ	Clear	1.49	0.18	A	3.8	8.3
chAb11-CZ	Clear	1.12	2.34	A	3.2	5.6
chAb6-CZ	Precipitates	0.56	80.98	A	1.3	0.5
chAb16-CZ	Precipitates	0.62	21.89	A	0.9	2.3
chAb14-CZ	Clear	0.50	2.01	A	1.4	1.9

Materials and methods: Conjugation of Bcl-xL inhibitory ADCs

[0282] ADCs were synthesized using one of the methods described below. Exemplary ADCs were synthesized using one of nine exemplary methods, described below.

[0283] Method A. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to a solution of antibody (10 mg/mL, 1 mL) preheated to 37 °C. The reaction mixture was kept at 37 °C for 1 hour. The solution of reduced antibody was added to a solution of synthon (3.3 mM, 0.160 mL in DMSO) and gently mixed for 30 minutes. The reaction solution was loaded onto a desalting column (PD10, washed with Dulbecco's phosphate-buffered saline [DPBS] 3x before use), followed by DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

[0284] Method B. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) preheated to 37 °C. The reaction mixture was kept at 37 °C for 1 hour. The solution of reduced antibody was adjusted to pH=8 by adding boric buffer (0.05 mL, 0.5 M, pH 8), added to a solution of synthon (3.3 mM, 0.160 mL in DMSO) and gently mixed for 4 hours. The reaction solution was loaded onto a desalting column (PD10, washed with DPBS 3x before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

[0285] Method C. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid

handling system equipped with an 1235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

[0286] A Pall Filter plate 5052 was pre-wet with 100 μ L 1x DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8 well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8 tip Varispan arm, equipped with 1 mL conductive tips, was used to aspirate the resin (250 μ L) and transfer to a 96-well filter plate. A vacuum was applied for 2 cycles to remove most of the buffer. Using the MDT, 150 μ L of 1xPBS was aspirated and dispensed to the 96-well filter plate holding the resin. A vacuum was applied, removing the buffer from the resin. The rinse/vacuum cycle was repeated 3 times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 μ L of 5x DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 μ L) DPBS was prepared as described above for Conditions A and preloaded into a 96 well plate. The solutions of reduced antibody were transferred to the filter plate wells containing the resin, and the mixture was mixed with the MDT by repeated aspiration/dispensation of a 100 μ L volume within the well for 45 seconds per cycle. The aspiration/dispensation cycle was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied to the filter plate for 2 cycles, thereby removing excess antibody. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed 150 μ L of DPBS to the filter plate wells containing resin -bound antibody, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. After the last vacuum cycle, 100 μ L of 1x DPBS was dispensed to the wells containing the resin-bound antibody. The MDT then collected 30 μ L each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the filter plate containing resin-bound antibody in DPBS. The wells containing the conjugation mixture were mixed with the MDT by repeated aspiration/dispensation of a 100 μ L volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon to waste. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 μ L) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 μ L of 10x DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed 100 μ L of IgG Elution Buffer 3.75 (Pierce) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 μ L of 5x DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

[0287] Method D. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid handling system equipped with an 1235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

[0288] A Pall Filter plate 5052 was prewet with 100 μ L 1x DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8-well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8

tip Varispan arm, equipped with 1 mL conductive tips, was used to aspirate the resin (250 μ L) and transfer to a 96-well filter plate. A vacuum was applied to the filter plate for 2 cycles to remove most of the buffer. The MDT aspirated and dispensed 150 μ L of DPBS to the filter plate wells containing the resin. The wash and vacuum sequence was repeated two more times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 μ L of 5x DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 μ L) DPBS was prepared as described above for Conditions A and dispensed into the 96-well plate. The MDT then collected 30 μ L each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the plate loaded with reduced antibody in DPBS. The mixture was mixed with the MDT by twice repeated aspiration/dispensation of a 100 μ L volume within the well. After five minutes, the conjugation reaction mixture (230 μ L) was transferred to the 96-well filter plate containing the resin. The wells containing the conjugation mixture and resin were mixed with the MDT by repeated aspiration/dispensation of a 100 μ L volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon and protein to waste. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 μ L) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 μ L of 10x DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed 100 μ L of IgG Elution Buffer 3.75 (P) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 μ L of 5x DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

[0289] Method E. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of reduced antibody cooled to room temperature and was added to a solution of synthon (10 mM, 0.040 mL in DMSO) followed by addition of boric buffer (0.1 mL, 1M, pH 8). The reaction solution was let to stand for 3 days at room temperature, loaded onto a desalting column (PD10, washed with DPBS 3x5mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

[0290] Method F. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system. The solution of antibody (10 mg/mL) was preheated to 37 °C and aliquoted to a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37°C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37 °C for 75 minutes. The solution of reduced antibody was transferred to an unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL in DMSO) were added to the wells with reduced antibodies and treated for 15 minutes. The reaction solutions were loaded onto a platform (8 x 12) of desalting columns (NAP5, washed with DPBS 4x before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4 °C.

[0291] Method G. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system. The solution of antibody (10 mg/mL) was preheated to 37 °C and aliquoted onto a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37 °C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37 °C for 75 minutes. The solutions of reduced antibody were transferred to an

unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL/well in DMSO) were added to the wells with reduced antibodies followed by addition of boric buffer (pH=8, 0.03 mL/well) and treated for 3 days. The reaction solutions were loaded onto a platform (8 x 12) of desalting columns (NAP5, washed with DPBS 4x before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4 °C.

[0292] *Method H.* A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of synthon (10 mM, 0.40 mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2 - 2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in phase A (phase A: 1.5 M ammonium sulphate, 25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as "E2") and DAR4 (also referred to as "E4") eluted upon applying gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The purified ADC solutions were filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

[0293] *Method I.* A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of synthon (10 mM, 0.40 mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2 - 2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto a butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in Phase A (phase A: 1.5 M ammonium sulphate, 25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as "E2") and DAR 4 (also referred to as "E4") eluted upon applying a gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The ADC solutions were treated with boric buffer (0.1 mL, 1M, pH8). The reaction solution was let stand for 3 days at room temperature, then loaded onto a desalting column (PD10, washed with DPBS 3x5mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

DAR and aggregation of ADCs

[0294] The DAR and percentage aggregation of ADCs synthesized were determined by LC-MS and size exclusion chromatography (SEC), respectively.

LC-MS general methodology

[0295] LC-MS analysis was performed using an Agilent 1100 HPLC system interfaced to an Agilent LC/MSD TOF 6220 ESI mass spectrometer. The ADC was reduced with 5 mM (final concentration) Bond-Breaker® TCEP solution (Thermo Scientific, Rockford, IL), loaded onto a Protein Microtrap (Michrom Bioresources, Auburn, CA) desalting cartridge, and eluted with a gradient of 10% B to 75% B in 0.2 minutes at ambient temperature. Mobile phase A was H₂O with 0.1% formic acid (FA), mobile phase B was acetonitrile with 0.1% FA, and the flow rate was 0.2 mL/min. Electrospray-ionization time-of-flight mass spectra of the co-eluting light and heavy chains were acquired using Agilent MassHunter™ acquisition software. The extracted

intensity vs. m/z spectrum was deconvoluted using the Maximum Entropy feature of MassHunter software to determine the mass of each reduced antibody fragment. DAR was calculated from the deconvoluted spectrum by summing intensities of the naked and modified peaks for the light chain and heavy chain, normalized by multiplying intensity by the number of drugs attached. The summed, normalized intensities were divided by the sum of the intensities, and the summing results for two light chains and two heavy chains produced a final average DAR value for the full ADC.

[0296] Thiosuccinimide hydrolysis of a bioconjugate can be monitored by electrospray mass spectrometry, since the addition of water to the conjugate results in an increase of 18 Daltons to the observable molecular weight of the conjugate. When a conjugate is prepared by fully reducing the interchain disulfides of a human IgG1 antibody and conjugating the maleimide derivative to each of the resulting cysteines, each light chain of the antibody will contain a single maleimide modification and each heavy chain will contain three maleimide modifications, as described in Figure 2. Upon complete hydrolysis of the resulting thiosuccinimides, the mass of the light chain will therefore increase by 18 Daltons, while the mass of each heavy chain will increase by 54 Daltons. This is illustrated in Figure 5 with the conjugation and subsequent hydrolysis of an exemplary maleimide drug-linker (synthon TX, molecular weight 1736 Da) to the fully reduced huAb13v1 antibody.

Size exclusion chromatography general methodology

[0297] Size exclusion chromatography was performed using a Shodex KW802.5 column in 0.2 M potassium phosphate pH 6.2 with 0.25 mM potassium chloride and 15% IPA at a flow rate of 0.75 ml/min. The peak area absorbance at 280 nm was determined for each of the high molecular weight and monomeric eluents by integration of the area under the curve. The % aggregate fraction of the conjugate sample was determined by dividing the peak area absorbance at 280 nM for the high molecular weight eluent by the sum of the peak area absorbances at 280 nM of the high molecular weight and monomeric eluents multiplied by 100%.

In vitro cell viability assay methods

[0298] The tumor cell lines HCC38 (breast cancer), NCI-H1650 (NSCLC) and NCI-H847 (small cell lung cancer cell line) were obtained from American Type Culture Collection (ATCC). Cells were grown in 96-well culture plates using recommended growth media overnight at a density of 5×10^3 (HCC38) or 20×10^3 (NCI-H847) or 40×10^3 (NCI-H1650) per well. The following day, treatments were added in fresh media to triplicate wells. Cellular viability was determined 5 days later using the CellTiter-Glo Luminescent Cell Viability Assay kit (Promega), as directed in the manufacturer's protocol. Cell viability was assessed as percentage of control untreated cells.

Example 8: *In vivo* Efficacy of Anti-B7-H3 Antibody Drug Conjugates

[0299] Of the nine chimeric antibodies tested *in vitro* conjugated to CZ synthons, four showed subnanomolar cytotoxicity (Table 6). chAb3-CZ, chAb18-CZ, and chAb13-CZ achieved DARS ranging from 2.6 to 4.2 (see Table 7) and were assessed for anti-tumor activity in a mouse small cell lung cancer cell line xenograft model NCI-H146, of human origin, using the methods described below. Antibody MSL109 (an IgG1 antibody that binds to cytomegalovirus (CMV) glycoprotein H) was used as a control, both as a naked antibody and as an ADC (conjugated to the same synthon (CZ) as the chAb3, chAb18, and chAb13 antibodies). MSL109 is an isotype matched non-targeting control. The methods of this xenograft assay are described below. The

results are presented in Table 7. The results show that each of the anti-B7-H3 Bcl-xL inhibiting ADCs were able to significantly inhibit tumor growth relative to the naked antibody control (MSL109) or non-target specific Bcl-xL ADC control (MSL109-CZ).

Table 7: *In vivo* efficacy of chimeric anti-B7-H3 antibody as Bcl-xL drug conjugates

ADC	Conjugation Method	DAR	Dose ^[a] /route/ regimen	Number of mice	TGI _{max} (%)	TGD (%)
MSL109	-	-	10mg/kg/IP/QDx1	8	0	0
MSL109-CZ	A	4.2	10mg/kg/IP/QDx1	8	34	10
chAb3-CZ	A	3.5	10mg/kg/IP/QDx1	8	87	109
chAb18-CZ	A	2.6	10mg/kg/IP/QDx1	8	90	100
chAb13-CZ	A	3.7	10mg/kg/IP/QDx1	8	81	104
[a] dose is given in mg/kg/day						

Evaluation of efficacy in xenograft models methods

[0300] NCI-H146 cells, NCI-1650 cells, and EBC-1 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured as monolayers in RPMI-1640 (NCI-H146, NCI-H1650) or MEM (EBC-1) culture media (Invitrogen, Carlsbad, CA) that was supplemented with 10 % Fetal Bovine Serum (FBS, Hyclone, Logan, UT). To generate xenografts, 5×10^6 viable cells were inoculated subcutaneously into the right flank of immune deficient female SCID/bg mice (Charles River Laboratories, Wilmington, MA) respectively. The injection volume was 0.2 mL and composed of a 1:1 mixture of S MEM and Matrigel (BD, Franklin Lakes, NJ). Tumors were size matched at approximately 200 mm³. Antibodies and conjugates were formulated in 0.9% sodium chloride for injection and injected intraperitoneally. Injection volume did not exceed 200 µL. Therapy began within 24 hours after size matching of the tumors. Mice weighed approximately 22 g at the onset of therapy. Tumor volume was estimated two to three times weekly. Measurements of the length (L) and width (W) of the tumor were taken via electronic caliper and the volume was calculated according to the following equation: $V = L \times W^2/2$. Mice were euthanized when tumor volume reached 3,000 mm³ or skin ulcerations occurred. Eight mice were housed per cage. Food and water were available ad libitum. Mice were acclimated to the animal facilities for a period of at least one week prior to commencement of experiments. Animals were tested in the light phase of a 12-hour light: 12-hour dark schedule (lights on at 06:00 hours). As described above, human IgG control antibody (MSL109) was used as a negative control agent.

[0301] To refer to efficacy of therapeutic agents, parameters of amplitude (TGI_{max}), durability (TGD) of therapeutic response are used. TGI_{max} is the maximum tumor growth inhibition during the experiment. Tumor growth inhibition is calculated by $100 \times (1 - T_v/C_v)$ where T_v and C_v are the mean tumor volumes of the treated and control groups, respectively. TGD or tumor growth delay is the extended time of a treated tumor needed to reach a volume of 1 cm³ relative to the control group. TGD is calculated by $100 \times (T_t/C_t - 1)$ where T_t and C_t are the median time periods to reach 1 cm³ of the treated and control groups, respectively.

Example 9: Humanization of Anti-B7-H3 Antibody chAb18

[0302] Anti-B7-H3 chimeric antibody chAb18 was selected for humanization based on its binding characteristics and favorable properties as an ADC, including its properties when conjugated to a Bcl-xL inhibitor (described above as exemplary conjugate CZ).

[0303] Humanized antibodies were generated based on the variable heavy (VH) and variable light (VL) CDR sequences of chAb18. Specifically, human germline sequences were selected for constructing CDR-grafted, humanized chAb18 antibodies, where the CDR domains of the VH and VL chains were grafted onto different human heavy and light chain acceptor sequences. Based on the alignments with the VH and VL sequences of monoclonal antibody chAb18, the following human sequences were selected as acceptors:

- IGHV1-69*06 and IGHJ6*01 for constructing heavy chain acceptor sequences
- IGKV1-9*01 and IGKJ2*01 for constructing light chain acceptor sequences
- IGKV6-21*01and IGKJ2*01 as backup acceptor for constructing light chain

[0304] Thus, the VH and VL CDRs of chAb18 were grafted into said acceptor sequences.

[0305] To generate humanized antibodies, framework back-mutations were identified and introduced into the CDR-grafted antibody sequences by *de novo* synthesis of the variable domain, or mutagenic oligonucleotide primers and polymerase chain reactions, or both by methods well known in the art. Different combinations of back mutations and other mutations were constructed for each of the CDR-grafts as described below. Residue numbers for these mutations are based on the Kabat numbering system.

[0306] For heavy chains huAb 18VH.1, one or more of the following Vernier and VH/VL interfacing residues were back mutated as follows: L46P, L47W, G64V, F71H. Additional mutations include the following: Q1E, N60A, K64Q, D65G. For light chains huAb18VL.1, one or more of the following Vernier and VH/VL interfacing residues were back mutated as follows: A43S, L46P, L47W, G64V, G66V, F71H. For light chains huAb18VL.2, one or more of the following Vernier and VH/VL interfacing residues were back mutated as follows: L46P, L47W, K49Y, G64V, G66V, F71H.

[0307] The variable region and CDR amino acid sequences of the humanized antibodies are described in Table 8 below.

TABLE 8: VH and VL amino acid sequences of humanized versions of chAb18

SEQ ID NO:	Clone	Protei n Region	Residues	Amino Acid Sequence
116	huAb18VH.1	VH		EVQLVQSGAEVKKPGSSVKVSCKAS GYSFTSYTIHWRQAPGQGLEWMGY INPNSRNTDYNQKFKDRVTITADKS TSTAYMELSSLRSEDTA VYYCARYS GSTPYWYFDVWGQGT TTVTVSS
25	huAb18VH.1	CDR-H1	Residues 26-35 of SEQ ID NO:116	GYSFTSYTIH
26	huAb18VH.1	CDR-H2	Residues 50-66 of SEQ ID NO:116	YINPNSRNTDYNQKFKD
27	huAb18VH.1	CDR-H3	Residues 99-110 of SEQ ID NO:116	YSGSTPYWYFDV

SEQ ID NO:	Clone	Protei n Region	Residues	Amino Acid Sequence
117	huAb18VH.1a	VH		EVQLVQSGAEVKKPGSSVKVSCKAS GYSFTSYTIHWVRQAPGQGLEWIGY INPNSRNTDYNQKFKDRTTLTADRS TSTAYMELSSLRSEDVAVYYCARYS GSTPYWYFDVWGQGTTITVSS
25	huAb18VH.1a	CDR-H1	Residues 26-35 of SEQ ID NO:117	GYSFTSYTIH
26	huAb18VH.1a	CDR-H2	Residues 50-66 of SEQ ID NO:117	YINPNSRNTDYNQKFKD
27	huAb18VH.1a	CDR-H3	Residues 99-110 of SEQ ID NO:117	YSGSTPYWYFDV
118	huAb18VH.1b	VH		EVQLVQSGAEVKKPGSSVKVSCKAS GYSFTSYTIHWVRQAPGQGLEWMGY INPNSRNTDYAQKFQGRVTLTADKS TSTAYMELSSLRSEDVAVYYCARYS GSTPYWYFDVWGQGTTITVSS
25	huAb18VH.1b	CDR-H1	Residues 26-35 of SEQ ID NO:118	GYSFTSYTIH
119	huAb18VH.1b	CDR-H2	Residues 50-66 of SEQ ID NO:118	YINPNSRNTDYAQKFQG
27	huAb18VH.1b	CDR-H3	Residues 99-110 of SEQ ID NO:118	YSGSTPYWYFDV
120	huAb18VL.1	VL		DIQLTQSPSFLSASVGDRTITCRA SSSVSYMNWYQQKPGKAPKLLIYAT SNLASGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQWSSNPLTFGQG TKLEIK
29	huAb18VL.1	CDR-L1	Residues 24-33 of SEQ ID NO:120	RASSSVSYMN
30	huAb18VL.1	CDR-L2	Residues 49-55 of SEQ ID NO:120	ATSNLAS
31	huAb18VL.1	CDR-L3	Residues 88-96 of SEQ ID NO:120	QQWSSNPLT
121	huAb18VL.1a	VL		DIQLTQSPSFLSASVGDRTITCRA SSSVSYMNWYQQKPGKSPKPWIYAT SNLASGVPSRFSVSVSGTEHTLTIS SLQPEDFATYYCQQWSSNPLTFGQG TKLEIK
29	hu18AbVL.1a	CDR-L1	Residues 24-33 of SEQ ID NO:121	RASSSVSYMN
30	huAb18VL.1a	CDR-L2	Residues 49-55 of SEQ ID NO:121	ATSNLAS
31	huAb18VL.1a	CDR-L3	Residues 88-96 of SEQ ID NO:121	QQWSSNPLT
122	huAb18VL.1b	VL		DIQLTQSPSFLSASVGDRTITCRA

SEQ ID NO:	Clone	Protei n Region	Residues	Amino Acid Sequence
				SSSVSYMNWYQQKPGKAPKRWIYAT SNLASGVPSRFSVSGSGTEHTLTIS SLQPEDFATYYCQQWSSNPLTFGQG TKLEIK
29	huAb18VL.1b	CDR-L1	Residues 24-33 of SEQ ID NO:122	RASSSVSYMN
30	huAb18VL.1b	CDR-L2	Residues 49-55 of SEQ ID NO:122	ATSNLAS
31	huAb18VL.1b	CDR-L3	Residues 88-96 of SEQ ID NO:122	QQWSSNPLT
123	huAb18VL.2	VL		EIVLTQSPDFQSVTPKEKVTITCRA SSSVSYMNWYQQKPDQSPKLLIKAT SNLASGVPSRFSVSGSGTDFTLTIN SLEAEDAATYYCQQWSSNPLTFGQG TKLEIK
29	huAb18VL.2	CDR-L1	Residues 24-33 of SEQ ID NO:123	RASSSVSYMN
30	huAb18VL.2	CDR-L2	Residues 49-55 of SEQ ID NO:123	ATSNLAS
31	huAb18VL.2	CDR-L3	Residues 88-96 of SEQ ID NO:123	QQWSSNPLT
124	huAb18VL.2a	VL		EIVLTQSPDFQSVTPKEKVTITCRA SSSVSYMNWYQQKPDQSPKRWIYAT SNLASGVPSRFSVSVSGTDHTLTIN SLEAEDAATYYCQQWSSNPLTFGQG TKLEIK
29	huAb18VL.2a	CDR-L1	Residues 24-33 of SEQ ID NO:124	RASSSVSYMN
30	huAb18VL.2a	CDR-L2	Residues 49-55 of SEQ ID NO:124	ATSNLAS
31	huAb18VL.2a	CDR-L3	Residues 88-96 of SEQ ID NO:124	QQWSSNPLT

[0308] Humanized variable regions of the murine monoclonal Ab18 (described above) were cloned into IgG expression vectors for functional characterization:

- Humanized Ab18VH.1 (huAb 18VH.1) is a CDR-grafted, humanized Ab18 VH containing IGHV1-69*06 and IGHJ6*01 framework sequences. It also contains a Q1E change to prevent pyroglutamate formation. The variable and CDR sequences of huAb18VH.1 are described in Table 8.
- Humanized Ab18VH1.a (huAb18VH.1a) is a humanized design based on huAb 18VH.1 and contains 4 proposed framework back-mutations: M48I, V67T, L69I, K73R. The variable and CDR sequences of huAb18VH.1a are described in Table 8.
- Humanized Ab18VH1.b (huAb 18VH.1b) is a humanized design based on huAb 18VH.1 and huAb18VH.1a and contains 1 proposed framework back-mutation L69I and 3 HCDR2 germlining changes N60A, K64Q, D65G. The variable and CDR sequences of huAb18VH.1b are described in Table 8.

- Humanized Ab18VL.1 (huAb 18VL.1) is a CDR-grafted, humanized Ab18 VL containing IGKV1-9*01 and IGKJ2*01 framework sequences. The variable and CDR sequences of huAb18VL.1 are described in Table 8.
- Humanized Ab18VL.1a (huAb 18VL.1a) is a humanized design based on huAb 18VL.1 and contains 6 proposed framework back-mutations: A43S, L46P, L47W, G64V, G66V, F71H. The variable and CDR sequences of huAb18VL.11 are described in Table 8.
- Humanized Ab18VL.1b (huAb 18VL.1b) is a humanized design based on huAb 18VL.1 and huAb18VL.1a contains 4 proposed framework back-mutations: L46P, L47W, G64V, F71H. The variable and CDR sequences of huAb18VL.1b are described in Table 8.
- Humanized Ab18VL.2 (huAb18VL.2) is a CDR-grafted, humanized Ab18 VL containing IGKV6-21*01 and IGKJ2*01 framework sequences. The variable and CDR sequences of huAb18VL.2 are described in Table 8.
- Humanized Ab18VL.2a (huAb18VL.2a) is a humanized design based on huAb18VL.2 and contains 6 proposed framework back-mutations: L46P, L47W, K49Y, G64V, G66V, F71H. The variable and CDR sequences of huAb18VL.2a are described in Table 8.

[0309] Thus, the humanization of chAb18 resulted in 10 humanized antibodies, including huAb18v1, huAb18v2, huAb18v3, huAb18v4, huAb18v5, huAb18v6, huAb18v7, huAb18v8, huAb18v9, and huAb18v10. The variable and heavy light chains for each of these humanized versions of Ab18 are provided below:

Table 9: Anti-B7-H3 Ab18 humanized antibodies

huAb18v1	huAb18 VH1 / huAb18VL1
huAb 18v2	huAb18 VH1b / huAb18VL1
huAb18v3	huAb18 VH1a / huAbVL1a
huAb 18v4	huAb18 VH1b / huAb18VL1a
huAb 18v5	huAn18 VH1/huAb18VL2
huAb 18v6	huAb18 VH1b/ huAb18VL2
huAb 18v7	huAb18 VH1b/ huAb18 VL2a
huAb 18v8	huAb18 VH1a/ huAb18 VL1b
huAb 18v9	huAb18 VH1a/ huAb18 VL2a
huAb18v10	huAb18 VH1b/huAb18 VL1b

Example 10: *In vitro* Characterization of Anti-B7-H3 chAb18 Humanized Variants

[0310] The humanization of chAb18 generated 10 variants (described above in Table 9) that retained binding to human and cyno B7-H3 as assessed by FACS (the method of which is described above in Example 6). These variants were further characterized for binding by SPR and were successfully conjugated to the Bcl-xL inhibitor synthon CZ using Method A (described above) and assessed for cell cytotoxicity as described in Example 7. Table 10 summarizes the *in vitro* characteristics of the various humanized Ab18 variants. The parental chAb18 from which the variants were derived was also tested as a comparator. All humanized variants had similar binding properties as assessed by biacore, and retained binding activity to cell surface expressed as conjugates with the CZ synthon. The cytotoxicity of all of the variants as CZ synthons were similar to the chAb18 from which they were derived.

TABLE 10: *In vitro* characterization of humanized anti-B7-H3 Ab18 variants

Variant name	Sequence Numbers	DAR by MS	% agg by SEC	FACS Binding to hu B7-H3)	Affinity of naked mAbs (Biacore, K_D)	Cytotoxicity (HCC38 Cell line IC ₅₀)
chAb18-CZ	24, 28	2.3		1.14	1.70E-10	0.28
huAb18v1-CZ	116, 120	2.6	3.3	1.27	5.20E-10	0.39
huAb 18v2-CZ	118, 120	1.8	3.3	2.25	6.90E-10	1.19
huAb18v3-CZ	117,121	2.4	3.6	1.27	2.30E-10	0.32
huAb 18v4-CZ	118, 121	2.5	5.5	0.90	5.70E-10	0.29
huAb18v5-CZ	116, 123	3.4	4.2	2.91	2.30E-10	0.12
huAb 18v6-CZ	118, 123	3.4	3.5	2.09	2.00E-10	0.14
huAb 18v7-CZ	118, 124	4.3	3.6	1.92	4.00E-10	0.03
huAb 18v8-CZ	117, 122	2.6	3.5	1.98	2.50E-10	1.3
huAb 18v9-CZ	117, 124	2.4	3.8	1.58	3.80E-10	0.9
huAb18v10-CZ	118, 122	2.7	3.2	1.19	2.50E-10	0.57

[0311] Humanized chAb18 variants were conjugated to the CZ synthon and tested for cytotoxicity in HCC38 cell line. As described in Table 10, most humanized antibodies showed potent cytotoxicity, similar to those observed with control antibody chAb18.

Example 11: *In Vivo* Efficacy of Humanized Ab18 Variants as Bcl-xL Inhibitor ADCs

[0312] Six of the humanized chAb18 variants were selected based on *in vitro* cytotoxicity results described in Example 10. Specifically, antibodies huAb18v1, huAb18v3, huAb18v4, huAb18v6, huAb18v7, and huAb18v9 were each conjugated to the CZ synthon (to form an anti-B7-H3 CZ ADC) for evaluation in an *in vivo* xenograft model of small cell lung cancer (using NCI-H146 cells), as described in Example 8. Single dose treatment of the tumor bearing mice resulted in tumor growth inhibition and tumor growth delay and the results are summarized in Table 11. Ab095 was used as a negative control for the effect of administering IgG, as it is an isotype matched non-target specific antibody raised against tetanus toxoid. See Larrick et al., 1992, Immunological Reviews 69-85. Mice were administered 6 mg/kg of the ADC intraperitoneally QDx1.

Table 11: *In vivo* efficacy of anti-B7-H3 ADCs (humanized chAb18-CZ variants)

ADC	Conjugation Method	DAR by MS	Dose ^[a] //route/ regimen	Number of mice	TGI _{max} (%)	TGD (%)
AB095	-	n/a	6mg/kg/IP/QDx1	8	0	0
huAb18v1-CZ	A	2.6	6mg/kg/IP/QDx1	8	79	45

ADC	Conjugation Method	DAR by MS	Dose ^[a] //route/ regimen	Number of mice	TGI _{max} (%)	TGD (%)
huAb18v3-CZ	A	2.4	6mg/kg/IP/QDx1	8	81	39
huAB 18v4-CZ	A	2.5	6mg/kg/IP/QDx1	8	85	48
huAB 18v6-CZ	A	3.4	6mg/kg/IP/QDx1	8	86	45
huAb 18v7-CZ	A	4.3	6mg/kg/IP/QDx1	8	87	42
huAb 18v9-CZ	A	2.4	6mg/kg/IP/QDx1	8	83	35
[a] dose is given in mg/kg/day						

[0313] As described in Table 11, each of the tested humanized antibodies was able to inhibit tumor growth in the mouse xenograft model.

Example 12: Humanization of Anti-B7-H3 Antibody chAb3

[0314] Anti-B7-H3 chimeric antibody chAb3 was selected for humanization based on its favorable properties as a Bcl-xL inhibiting (Bcl-xLi) conjugate. Humanized antibodies were generated based on the variable heavy (VH) and variable light (VL) CDR sequences of chAB3. Specifically, human germline sequences were selected for constructing CDR-grafted, humanized chAb3 antibodies where the CDR domains of the VH and VL chains of chAb3 were grafted onto different human heavy and light chain acceptor sequences. Based on the alignments with the VH and VL sequences of monoclonal antibody chAb3 the following human sequences were selected as acceptors:

- IGHV1-69*06 and IGHJ6*01 for constructing heavy chain acceptor sequences
- IGKV2-28*01 and IGKJ4*01 for constructing light chain acceptor sequences

IGHV1-69*06 IGHJ6
QVQLVQSGAEVKKPGSSVKVCKASggtfssyaisWVRQAPGQGLEWMGgiipifgtanyaqkfqgRVTI
TADKSTSTAYMELSSLRSEDTAVYYCARxxxxxxxxWGQGTTVTVSS (SEQ ID NO: 174);
where xxxxxxxx represents the CDR-H3 region.

IGKV2-28*01 IGKJ4
DIVMTQSPLSLPVTPGEPASISCrssqslhsgnyldWYLQKPGQSPQLLIYlgnsrasGVPDRFSGSGS
GTDFTLKISRVEAEDVGVYYCxxxxxxxxFGGGTKVEIK (SEQ ID NO: 175);
where xxxxxxxx represents the CDR-L3 region.

[0315] By grafting the corresponding VH and VL CDRs of chAb3 into said acceptor sequences, CDR-grafted, humanized, and modified VH and VL sequences were prepared. To generate humanized antibodies with potential framework back-mutations, the mutations were identified and introduced into the CDR-grafted antibody sequences by *de novo* synthesis of the variable domain, or mutagenic oligonucleotide primers and polymerase chain reactions, or both. Different combinations of back mutations and other mutations are constructed for each of the CDR-grafts as follows. Residue numbers for these mutations are based on the

Kabat numbering system.

[0316] The amino acid sequences of the various humanized heavy and light chain variable regions are described below in Table 12.

[0317] For heavy chains huAb3VH.1, one or more of the following Vernier and VH/VL interfacing residues were back mutated as follows: M48I, V67A, I69L, A71V, K73R, M80V, Y91F, R94G. For light chains huAb31 VL.1, one or more of the following Vernier and VH/VL interfacing residues were back mutated as follows: I2V, Y87F.

[0318] The following humanized variable regions of the murine monoclonal chAb3 antibody were cloned into IgG expression vectors for functional characterization:

- Humanized Ab3 VH.1 (huAb3VH.1) is a CDR-grafted, humanized Ab3 VH containing IGHV1-69*06 and IGHJ6*01 framework sequences. It also contains a Q1E change to prevent pyroglutamate formation.
- Humanized Ab3 VH.1a (huAb3VH.1a) is a humanized design based on huAb3VH.1 and contains 8 proposed framework back-mutations: M48I, V67A, I69L, A71V, K73R, M80V, Y91F, R94G.
- Humanized Ab3 VH.1b (huAb3VH.1b) is a humanized design between huAb3VH.1 and huAb3VH.1a and contains 6 proposed framework back-mutations: M48I, V67A, I69L, A71V, K73R, R94G.
- Humanized Ab3 VL.1 (huAb3VL.1) is a CDR-grafted, humanized Ab3 VL containing IGKV2-28*01 and IGKJ4*01 framework sequences.
- Humanized Ab3 VL.1a (huAb3VL.1a) is a humanized design based on huAb3VL.1 and contains 2 proposed framework back-mutations: I2V, Y87F.
- Humanized Ab3 VL.1b (huAb3VL.1b) is a humanized design contains only 1 proposed framework back-mutations: I2V.

[0319] The variable region and CDR amino acid sequences of the foregoing humanized antibodies are described in Table 12 below.

TABLE 12: VH and VL sequences of humanized versions of chAb3

SEQ ID NO:	Clone	Prote in Regio n	Residues	Amino Acid Sequence
125	huAb3VH.1	VH		EVQLVQSGAEVKKPGSSVKVS CKASGYTFSSYWMHWVRQAPG QGLEWMGLIHPDSGSTNYNEM FKNRVTITADKSTSTAYMELS SLRSEDNAVYYCARGGRLYFD YWGQGTITVTVSS
10	huAb3VH.1	CDR-H1	Residues 26-35 of SEQ ID NO:125	GYTFSSYWMH
11	huAb3VH.1	CDR-H2	Residues 50-66 of SEQ ID NO:125	LIHPDSGSTNYNEMFKN
12	huAb3VH.1	CDR-H3	Residues 99-106 of SEQ ID NO:125	GGRLYFDY
126	huAb3VH.1a	VH		EVQLVQSGAEVKKPGSSVKVS CKASGYTFSSYWMHWVRQAPG QGLEWIGLIHPDSGSTNYNEM FKNRATLTVDNSTSTAYVELS

SEQ ID NO:	Clone	Prote in Regio n	Residues	Amino Acid Sequence
				SLRSEDTAVYFCAG GGRLYFD YWGQGTITVTVSS
10	huAb3VH.1a	CDR-H1	Residues 26-35 of SEQ ID NO:126	GYTFSSYWMH
11	huAb3VH.1a	CDR-H2	Residues 50-66 of SEQ ID NO:126	LIHPDSGSTNYNEMFKN
12	huAb3VH.1a	CDR-H3	Residues 99-106 of SEQ ID NO:126	GGRLYFDY
127	huAb3VH.1b	VH		EVQLVQSGAEVKKPGSSVKVS CKAS GYTFSSYWMH WVRQAPG QGLEWIG LIHPDSGSTNYNEM FKN RATLTVD RSTSTAYMELS SLRSEDTAVYYCAG GGRLYFD YWGQGTITVTVSS
10	huAb3VH.1b	CDR-H1	Residues 26-35 of SEQ ID NO:127	GYTFSSYWMH
11	huAb3VH.1b	CDR-H2	Residues 50-66 of SEQ ID NO:127	LIHPDSGSTNYNEMFKN
12	huAb3VH.1b	CDR-H3	Residues 99-106 of SEQ ID NO:127	GGRLYFDY
128	huAb3VL.1	VL		DIVMTQSPLSLPVTPGEPASI SCR SSQSLVHSNGD TYLRWYL QKPGQSPQLLIY KVSNRFS GV PDRFSGSGSGTDFTLKISRVE AEDVGVYYC SQSTHVPY TFGG GTKVEIK
14	huAb3VL.1	CDR-L1	Residues 24-39 of SEQ ID NO:128	RSSQSLVHSNGD TYLR
7	huAb3VL.1	CDR-L2	Residues 55-61 of SEQ ID NO:128	KVSNRFS
15	huAb3VL.1	CDR-L3	Residues 94-102 of SEQ ID NO:128	SQSTHVPYT
129	huAb3VL.1a	VL		DVVMTQSPLSLPVTPGEPASI SCR SSQSLVHSNGD TYLRWYL QKPGQSPQLLIY KVSNRFS GV PDRFSGSGSGTDFTLKISRVE AEDVGVYFC SQSTHVPY TFGG GTKVEIK
14	huAb3VL.1a	CDR-L1	Residues 24-39 of SEQ ID NO:129	RSSQSLVHSNGD TYLR
7	huAb3VL.1a	CDR-L2	Residues 55-61 of SEQ ID NO:129	KVSNRFS
15	huAb3VL.1a	CDR-L3	Residues 94-102 of SEQ ID NO:129	SQSTHVPYT
130	huAb3VL.1b	VL		DVVMTQSPLSLPVTPGEPASI SCR SSQSLVHSNGD TYLRWYL QKPGQSPQLLIY KVSNRFS GV PDRFSGSGSGTDFTLKISRVE AEDVGVYFC SQSTHVPY TFGG GTKVEIK

SEQ ID NO:	Clone	Prote in Regio n	Residues	Amino Acid Sequence
				QKPGQSPQLLIYKVSNRFSGV PDRFSGSGSGTDFTLKISRVE AEDVGVYYCSQSTHVPYTFGG GTKVEIK
14	huAb3VL.1b	CDR-L1	Residues 24-39 of SEQ ID NO:130	RSSQSLVHSNGDTYLR
7	huAb3VL.1b	CDR-L2	Residues 55-61 of SEQ ID NO:130	KVSNRFS
15	huAb3VL.1b	CDR-L3	Residues 94-102 of SEQ ID NO:130	SQSTHVPYT

[0320] The humanization of chAb3 resulted in 6 humanized antibodies, including huAb3v1, huAb3v2, huAb3v3, huAb3v4, huAb18v5, and huAb3v6. The variable and heavy light chains for each of these humanized versions of Ab18 are provided below in Table 13.

Table 13: Humanized Ab3 antibodies

huAb3v1	huAb3 VH1 / huAb3 VL1
huAb3v2	huAb3 VH1b / huAb3 VL1
huAb3v3	huAb3 VH1a / huAb3 VL1a
huAb3v4	huAb3 VH1 / huAb3 VL1b
huAb3v5	huAb3 VH1b / huAb3 VL1b
huAb3v6	huAb3 VH1a / huAb3 VL1b

Example 13: *In vitro* characterization of chAb3 humanized variants

[0321] The humanization of chAb3 generated 6 variants (described in Table 13) that retained binding to human B7-H3 as assessed by FACS (as described in Example 6). These variants were further characterized for binding by SPR and as ADCs conjugated to the Bcl-xL inhibitor synthon (linker warhead) CZ. The humanized Ab3 antibodies were also assessed for cell cytotoxicity (using the assay described above in Example 7). Table 14 summarizes *in vitro* characteristics of chAb3 humanized variants. An ADC comprising chAb3 conjugated to synthon CZ was used as a control.

TABLE 14: *In vitro* characterization of humanized variants of chAb3

ADC	Seq. Id. Number	Conjugati on Method	DAR by MS	% agg by SEC	FACS (Binding to hu B7-H3) EC ₅₀ (nM)	Affinity of naked mAbs (Biacore, K _D)	Cytotoxicity (HCC38 Cell line IC ₅₀) (nM)
chAb3-CZ	9, 13	A	3.8		0.61	1.90E-08	0.17
huAb3v1-CZ	125, 128	A	3.6	3.3	1.45	5.20E-10	0.53
huAb3v2-CZ	127, 128	A	3.8	10.1	0.73	6.90E-10	0.13
huAb3v3-CZ	126, 129	A	3.6	2.5	1.68	2.30E-10	9.22

ADC	Seq. Id. Number	Conjugation Method	DAR by MS	% agg by SEC	FACS (Binding to hu B7-H3) EC ₅₀ (nM)	Affinity of naked mAbs (Biacore, K _D)	Cytotoxicity (HCC38 Cell line IC ₅₀) (nM)
huAb3v4-CZ	125, 130	A	3.1	3.1	n/a	5.70E-10	n/a
huAb3v5-CZ	127, 130	A	3.1	5.9	0.85	2.30E-10	0.17
huAb3v6-CZ	126, 130	A	3.3	4.9	1.78	2.00E-10	0.13

Example 14: *In vivo* Efficacy of chAb3 Humanized Variants as Bcl-xL ADCs

[0322] Two of the humanized variants (huAb3v2 and huAb3v6) were selected based on potent *in vitro* cytotoxicity as CZ conjugates and acceptable aggregation properties for evaluation in an *in vivo* murine xenograft model of small cell lung cancer cells (NCI-H146 cells) as described in materials and methods in Example 8. Single dose treatment of tumor bearing mice resulted in tumor growth inhibition and tumor growth delay for both humanized antibodies conjugated to an exemplary Bcl-xL inhibitor, and the results are summarized in Table 15.

Table 15: *In vivo* efficacy of humanized chAb3-CZ variants

ADC	Conjugation Method	DAR	Dose ^[a] /route/regimen	Number of mice	TGI _{max} (%)	TGD (%)
AB095	-		6mg/kg/IP/QDx1	8	0	0
huAb3v2-CZ	A	3.8	6mg/kg/IP/QDx1	8	83	52
huAb3v6-CZ	A	3.3	6mg/kg/IP/QDx1	8	91	88

[a] dose is given in mg/kg/day

Example 15: Modifications of the CDRs of Humanized Variant Antibody huAb3v2

[0323] huAb3v2 showed favorable binding and cell killing properties. An examination of the variable region amino acid sequences of huAb3v2, however, revealed potential deamidation and/or isomerization sites.

[0324] The amino acid sequences of huAb3 variable regions are described below, including the light chain (huAb3VL1) and the heavy chain (huAb3VH1b). The potential deamidation and/or isomerization sites in CDRs of the VH (CDR2 at amino acids "ds" and VL (CDR1 at amino acids "ng") are italicized and were thus engineered to improve antibody manufacturing. The CDRs are described in lower case letters in the sequences below.

[0325] To make huAb3v2 variants lacking these potential deamidation and/or isomerization sites, each of the amino acids indicated below (x and z; representing the potential sites in the CDR1 of the VL and the CDR2 of the VH) were mutagenized. The resulting 30 VL variants were paired with the original huAb3v2 VH and tested for binding. The resulting 29 VH variants were paired with the original huAb3v2 VL and tested for

binding. Successful VH variants were combined and tested with productive VL variants harboring changes in LCDR1 to make the final humanized variants lacking the potential deamidation and/or isomerization sites in CDRs. The amino acid sequences of the variants are provided in Table 16 below. The full length amino acid sequences of the heavy chain and light chain of the huAb3v2 variant, huAb3v2.5 are provided in SEQ ID NOs: 170 and 171, respectively. The full length amino acid sequences of the heavy chain and light chain of the huAb3v2 variant, huAb3v2.6 are provided in SEQ ID NOs: 172 and 173, respectively.

huAb3 VL1
DIVMTQSP~~LSLPVTPGEPASIS~~CrssqslvhsngdtylrWYLQKPGQSPQLLIYkvsnrfsGVPDRFSGSGS
GTDFTLKISRVEAEDVGVYYCsqsthvpytFGGGTKVEIK (SEQ ID NO: 128)

xg (15 variants) (SEQ ID NO: 178)

nz (15 variants) (SEQ ID NO: 179)

huAb3 VH1b
EVQLVQSGAEVKKPGSSVKVSCKASgytfs~~sywmh~~WVRQAPGQGLEWIGlihpdsgstnynemfknRAT
LTVDRSTSTAYMELSSLRSEDTAVYYCAGggrlyfdyWGQGTTVTVSS (SEQ ID NO: 127)

(15 variants) xs (SEQ ID NO: 180)

(14 variants) dz (SEQ ID NO: 181)

where (for both the VL and VH),

x = All amino acids, except: M, C, N, D, or Q.

z = All amino acids, except: M, C, G, S, N, or P.

Proposed framework back mutations are underlined (see Example 12).

Table 16: Variable region sequences of huAb3v2 antibody variants

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
131	huAb3v2.1	VH		EVQLVQSGAEVKKPGSSVKVSCK AS <u>GYTFSSYWMH</u> WVRQAPGQGLE WIG <u>LIHPW</u> SGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGG <u>RLYFDY</u> WGQGTTVTV SS
10	huAb3v2.1	CDR-H1	Residues 26-35 of SEQ ID NO:131	GYTFSSYWMH
132	huAb3v2.1	CDR-H2	Residues 50-66 of SEQ ID NO:131	LIHPWSGSTNYNEMFKN
12	huAb3v2.1	CDR-H3	Residues 99-106 of SEQ ID NO:131	GGRLYFDY
133	huAb3v2.1	VL		DIVMTQSP ~~LSLPVTPGEPASIS~~ <u>RSSQSLVHSSGDTYL</u> RWYLQKPG QSPQLLIY <u>KVSNRFS</u> GVPDRFSG SGSGTDFTLKISRVEAEDVGVYY C <u>SQSTHVPYT</u> FGGGTKVEIK
134	huAb3v2.1	CDR-L1	Residues 24-39 of SEQ ID NO:133	RSSQSLVHSSGDTYLR
7	huAb3v2.1	CDR-L2	Residues 55-61 of SEQ ID NO:133	KVSNRFS

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
15	huAb3v2.1	CDR-L3	Residues 94-102 of SEQ ID NO:133	SQSTHVPYT
131	huAb3v2.2	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPWSGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.2	CDR-H1	Residues 26-35 of SEQ ID NO:131	GYTFSSYWMH
132	huAb3v2.2	CDR-H2	Residues 50-66 of SEQ ID NO:131	LIHPWSGSTNYNEMFKN
12	huAb3v2.2	CDR-H3	Residues 99-106 of SEQ ID NO:131	GGRLYFDY
135	huAb3v2.2	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNRDITYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
136	huAb3v2.2	CDR-L1	Residues 24-39 of SEQ ID NO:135	RSSQSLVHSNRDITYLR
7	huAb3v2.2	CDR-L2	Residues 55-61 of SEQ ID NO:135	KVSNRFS
15	huAb3v2.2	CDR-L3	Residues 94-102 of SEQ ID NO:135	SQSTHVPYT
131	huAb3v2.3	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPWSGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.3	CDR-H1	Residues 26-35 of SEQ ID NO:131	GYTFSSYWMH
132	huAb3v2.3	CDR-H2	Residues 50-66 of SEQ ID NO:131	LIHPWSGSTNYNEMFKN
12	huAb3v2.3	CDR-H3	Residues 99-106 of SEQ ID NO:131	GGRLYFDY
137	huAb3v2.3	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNQDITYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
138	huAb3v2.3	CDR-L1	Residues 24-39 of SEQ ID NO:137	RSSQSLVHSNQDITYLR
7	huAb3v2.3	CDR-L2	Residues 55-61 of SEQ ID NO:137	KVSNRFS

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
15	huAb3v2.3	CDR-L3	Residues 94-102 of SEQ ID NO:137	SQSTHVPYT
139	huAb3v2.4	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPESGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.4	CDR-H1	Residues 26-35 of SEQ ID NO:139	GYTFSSYWMH
140	huAb3v2.4	CDR-H2	Residues 50-66 of SEQ ID NO:139	LIHPESGSTNYNEMFKN
12	huAb3v2.4	CDR-H3	Residues 99-106 of SEQ ID NO:139	GGRLYFDY
133	huAb3v2.4	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSSGDTYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
134	huAb3v2.4	CDR-L1	Residues 24-39 of SEQ ID NO:133	RSSQSLVHSSGDTYLR
7	huAb3v2.4	CDR-L2	Residues 55-61 of SEQ ID NO:133	KVSNRFS
15	huAb3v2.4	CDR-L3	Residues 94-102 of SEQ ID NO:133	SQSTHVPYT
139	huAb3v2.5	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPESGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.5	CDR-H1	Residues 26-35 of SEQ ID NO:139	GYTFSSYWMH
140	huAb3v2.5	CDR-H2	Residues 50-66 of SEQ ID NO:139	LIHPESGSTNYNEMFKN
12	huAb3v2.5	CDR-H3	Residues 99-106 of SEQ ID NO:139	GGRLYFDY
135	huAb3v2.5	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNRDITYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
136	huAb3v2.5	CDR-L1	Residues 24-39 of SEQ ID NO:135	RSSQSLVHSNRDITYLR
7	huAb3v2.5	CDR-L2	Residues 55-61 of SEQ ID NO:135	KVSNRFS

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
15	huAb3v2.5	CDR-L3	Residues 94-102 of SEQ ID NO:135	SQSTHVPYT
139	huAb3v2.6	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPESGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.6	CDR-H1	Residues 26-35 of SEQ ID NO:139	GYTFSSYWMH
140	huAb3v2.6	CDR-H2	Residues 50-66 of SEQ ID NO:139	LIHPESGSTNYNEMFKN
12	huAb3v2.6	CDR-H3	Residues 99-106 of SEQ ID NO:139	GGRLYFDY
137	huAb3v2.6	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNQDTYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
138	huAb3v2.6	CDR-L1	Residues 24-39 of SEQ ID NO:137	RSSQSLVHSNQDTYLR
7	huAb3v2.6	CDR-L2	Residues 55-61 of SEQ ID NO:137	KVSNRFS
15	huAb3v2.6	CDR-L3	Residues 94-102 of SEQ ID NO:137	SQSTHVPYT
141	huAb3v2.7			EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPISGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTVSS
10	huAb3v2.7	CDR-H1	Residues 26-35 of SEQ ID NO:141	GYTFSSYWMH
142	huAb3v2.7	CDR-H2	Residues 50-66 of SEQ ID NO:141	LIHPISGSTNYNEMFKN
12	huAb3v2.7	CDR-H3	Residues 99-106 of SEQ ID NO:141	GGRLYFDY
133	huAb3v2.7			DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSSGDTYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
134	huAb3v2.7	CDR-L1	Residues 24-39 of SEQ ID NO:133	RSSQSLVHSSGDTYLR
7	huAb3v2.7	CDR-L2	Residues 55-61 of SEQ ID NO:133	KVSNRFS

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
15	huAb3v2.7	CDR-L3	Residues 94-102 of SEQ ID NO:133	SQSTHVPYT
141	huAb3v2.8	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPISGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTV SS
10	huAb3v2.8	CDR-H1	Residues 26-35 of SEQ ID NO:141	GYTFSSYWMH
142	huAb3v2.8	CDR-H2	Residues 50-66 of SEQ ID NO:141	LIHPISGSTNYNEMFKN
12	huAb3v2.8	CDR-H3	Residues 99-106 of SEQ ID NO:141	GGRLYFDY
135	huAb3v2.8	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNRDITYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
136	huAb3v2.8	CDR-L1	Residues 24-39 of SEQ ID NO:135	RSSQSLVHSNRDITYLR
7	huAb3v2.8	CDR-L2	Residues 55-61 of SEQ ID NO:135	KVSNRFS
15	huAb3v2.8	CDR-L3	Residues 94-102 of SEQ ID NO:135	SQSTHVPYT
141	huAb3v2.9	VH		EVQLVQSGAEVKKPGSSVKVSCK ASGYTFSSYWMHWVRQAPGQGLE WIGLIHPISGSTNYNEMFKNRAT LTVDRSTSTAYMELSSLRSEDTA VYYCAGGGRLYFDYWGQGTITVTV SS
10	huAb3v2.9	CDR-H1	Residues 26-35 of SEQ ID NO:141	GYTFSSYWMH
142	huAb3v2.9	CDR-H2	Residues 50-66 of SEQ ID NO:141	LIHPISGSTNYNEMFKN
12	huAb3v2.9	CDR-H3	Residues 99-106 of SEQ ID NO:141	GGRLYFDY
137	huAb3v2.9	VL		DIVMTQSPFLSLPVTPEGEPASISC RSSQSLVHSNQDITYLRWYLQKPG QSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVIY CSQSTHVPYTFGGGTKVEIK
138	huAb3v2.9	CDR-L1	Residues 24-39 of SEQ ID NO:137	RSSQSLVHSNQDITYLR
7	huAb3v2.9	CDR-L2	Residues 55-61 of SEQ ID NO:137	KVSNRFS

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
15	huAb3v2.9	CDR-L3	Residues 94-102 of SEQ ID NO:137	SQSTHVPYT

Example 16: *In vitro* characterization of huAb3v2 Variants

[0326] Removal of potential deamidation and/or isomerization sites (described in Example 15) generated only 6 variants that retained binding to both human and cyno B7-H3 exogenously expressed on mouse 3T12 fibroblasts as assessed by FACS (as described in the methods of Example 6).

[0327] These new anti-B7-H3 antibodies were further characterized for binding by SPR and conjugated to the Bcl-xLi synthon CZ and assessed for cell cytotoxicity (using the methods described in Example 7). Table 17 provides *in vitro* characteristics of six huAb3v2 humanized variants.

Table 17: *In vitro* characterization of humanized huAb3v2 variants, including naked antibodies and ADCs

ADC	Sequence number	Conjugation Method	DAR by MS	% agg by SEC	ELISA hB7-H3 EC ₅₀ nM	FACS (EC ₅₀ nM)		Affinity of naked mAbs (Biacore, K _D)	Cytotoxicity (H847 Cell line IC ₅₀) (nM)
						hB7-H3	cyB7-H3		
huAb3v2-CZ	127, 128	A	3.5		0.44	5.11	2.87	2.30E-09	1.49
huAb3v2.2-CZ	131, 135	A	0.7	1.8	0.10	5.29	3.68	Poor fit	26.7
huAb3v2.3-CZ	131, 137	A	1.1	1.5	0.11	6.50	4.03	Poor fit	--
huAb3v2.5-CZ	139, 135	A	3.4	15.6	0.13	5.14	4.86	5.30E-09	1.57
huAb3v2.6-CZ	139, 137	A	3.3	15	0.09	5.64	3.31	5.80E-08	1.70
huAb3v2.8-CZ	141, 135	A	2.0	5.7	0.14	3.94	3.01	Poor fit	2.36
huAb3v2.9-CZ	141, 137	A	2.7	4.3	0.16	6.16	4.64	Poor fit	2.30

[0328] As described in Table 17, the results showed that all six huAb3v2 variants had similar binding properties to cells expressing human or cynoB7-H3 as compared to the parental huAb3v2. Of the six huAb3v2 variants, four antibodies (huAb3v2.5, huAb3v2.6, huAb3v2.8, huAb3v2.9) showed potent cytotoxicity in H847 cells when conjugated to exemplary Bcl-xLi synthon CZ.

Example 17: Humanization of Anti-B7-H3 Antibody chAb13

[0329] The anti-B7-H3 chimeric antibody chAb13 was selected for humanization based on its binding characteristics and favorable properties as an ADC (conjugated to a Bcl-xL inhibitor).

[0330] Prior to humanization, chAb13 was modified in order to minimize potential deamidation in the light chain CDR3 (QQYNSYPFT (SEQ ID NO: 182); potential deamidation site is indicated as residues "NS" (italicized)). Point mutations in the amino acid position corresponding to "N" and/or "S" within the light chain CDR3 of chAb13 were introduced, resulting in 30 variants. Antibodies containing these CDR3 light chain variants were then screened for their ability to retain the binding characteristics of chAb13. Variants comprising a CDR3 having a tryptophan (W) point mutation instead of the serine "S" in the "NS" motif (*i.e.*, QQYNWYPFT (SEQ ID NO: 39)) retained the binding features of the parent chAb13 antibody. The substitution of the S residue with a W residue within the CDR3 was surprising given the structural differences between serine and tryptophan as well as the significant role the CDR3 plays in antigen binding.

[0331] Humanized antibodies were generated based on the variable heavy (VH) and variable light (VL) CDR sequences of chAb13, including the "NW" light chain CDR3. Specifically, human germline sequences were selected for constructing CDR-grafted, humanized chAb13 antibodies, where the CDR domains of the VH and VL chains were grafted onto different human heavy and light chain acceptor sequences. Based on the alignments with the VH and VL sequences of monoclonal antibody chAb13, the following human sequences were selected as acceptors:

- IGHV4-b*01(0-1) and IGHJ6*01 for constructing heavy chain acceptor sequences
- IGKV1-39*01 and IGKJ2*01 for constructing light chain acceptor sequences
- IGHV4-b_IGHJ6
QVQLQESGPGLVKPSSETLSLTCAVSgysissgyywgWIRQPPGKGLEWIGsiyhsgstyynpslksRVTISV
DTSKNQFSLKLSSVTAADTAVYYCARxxxxxxxWGQGTTVTVSS (SEQ ID NO: 176);
where xxxxxxx represents the CDR-H3 region.

IGKV1-39_IGKJ2
DIQMTQSPSSLSASVGDRVTITCrasqsisssylhWYQQKPGKAPKLLIYaasslqsGVPSRFSGSGSGTD
FTLTISLQPEDFATYYCxxxxxxxxxFGQGTKLEIK (SEQ ID NO: 177);
where xxxxxxxxx represents the CDR-L3 region.

[0332] By grafting the "NW" light chain CDR3 and the remaining five corresponding VH and VL CDRs of chAb13 into said acceptor sequences, CDR-grafted, humanized, and modified VH and VL sequences were prepared. To generate humanized antibodies with potential framework back-mutations, mutations were identified and introduced into the CDR-grafted antibody sequences by *de novo* synthesis of the variable domain, or mutagenic oligonucleotide primers and polymerase chain reactions, or both by methods well known in the art. Different combinations of back mutations and other mutations were constructed for each of the CDR-grafts as follows. Residue numbers for these mutations are based on the Kabat numbering system.

[0333] The following humanized variable regions of the murine monoclonal chAb13 antibodies were cloned into IgG expression vectors for functional characterization:

- Humanized Ab13 VH.1 (huAb13VH.1) is a CDR-grafted, humanized Ab13 VH containing IGHV4-b*01(0-1) and IGHJ6*01 framework sequences. It also contains a Q1E change to prevent pyroglutamate formation.
- Humanized Ab13 VH.1 (huAb13 VH.1a) is a humanized design based on huAb13VH.1 and contains 9 proposed framework back-mutation(s): S25T, P40F, K43N, I48M, V67I, T68S, V71R, S79F, R94G.
- Humanized Ab13 VH.1b (huAb13VH.1b) is an intermediate design between on huAb13VH.1 and huAb13VH.1a and contains 4 proposed framework back-mutation(s): K43N, I48M, V67I, V71R.
- Humanized Ab13 VL.1 (huAb13VL.1) is a CDR-grafted, humanized Ab13 VL containing IGKV1-39*01

and IGHJ6*01 framework sequences.

- Humanized Ab13 VL.1a (huAb13VL.1a) is a humanized design based on huAb13VL.1 and contains 4 proposed framework back-mutation(s): A43S, L46A, T85E, Y87F.
- Humanized Ab13 VL.1b (huAb13VL.1b) is an intermediate design between on huAb13VL.1 and huAb13VL.1a and contains 1 proposed framework back-mutation(s): Y87F.

[0334] The variable region and CDR amino acid sequences of the foregoing are described in Table 18 below.

Table 18: Amino acid variable region sequences of humanized Ab13

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
143	huAb13VL.1	VL		DIQMTQSPSSLSASVGDRVTIT CKASQNVGFNVAWYQQKPGKAP KLLIY SASYRYS GVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQYNWYPFTFGQGTKLEIK
37	huAb13VL.1	CDR-L1	Residues 24-34 of SEQ ID NO:143	KASQNVGFNVA
38	huAb13VL.1	CDR-L2	Residues 50-56 of SEQ ID NO:143	SASYRYS
39	huAb13VL.1	CDR-L3	Residues 89-97 of SEQ ID NO:143	QQYNWYPFT
144	huAb13VL.1a	VL		DIQMTQSPSSLSASVGDRVTIT CKASQNVGFNVAWYQQKPGKSP KALIIY SASYRYS GVPSRFSGSG SGTDFTLTISSLQPEDFAEYFC QQYNWYPFTFGQGTKLEIK
37	huAb13VL.1a	CDR-L1	Residues 24-34 of SEQ ID NO:144	KASQNVGFNVA
38	huAb13VL.1a	CDR-L2	Residues 50-56 of SEQ ID NO:144	SASYRYS
39	huAb13VL.1a	CDR-L3	Residues 89-97 of SEQ ID NO:144	QQYNWYPFT
146	huAb13VH.1	VH		EVQLQESGPGLVKPSSETLSLTC AVSGYSITSGYSWHWIRQPPGK GLEWIGYIHSSGSTNYNPSLKS RVTISVDTSKNQFSLKLSSVTA ADTAVYYCARYDDY FEY WGQGT TVTVSS
33	huAb13VH.1	CDR-H1	Residues 26-36 of SEQ ID NO:146	GYSITSGYSWH
34	huAb13VH.1	CDR-H2	Residues 51-66 of SEQ ID NO:146	YIHSSGSTNYNPSLKS
35	huAb13VH.1	CDR-H3	Residues 99-105 of SEQ ID NO:146	YDDYFEY

SEQ ID NO:	Clone	Protein Region	Residues	Amino Acid Sequence
145	huAB13VL.1b	VL		DIQMTQSPSSLSASVGDRVTIT CKASQNVGFNVAWYQQKPGKAP KLLIY SASYRY SGVPSRFSGSG SGTDFTLTISSLQPEDFATYFC QQYNWYPFTFGQGTKLEIK
37	huAb13VL.1b	CDR-L1	Residues 24-34 of SEQ ID NO:145	KASQNVGFNVA
38	huAb13VL.1b	CDR-L2	Residues 50-56 of SEQ ID NO:145	SASYRYS
39	huAb13VL.1b	CDR-L3	Residues 89-97 of SEQ ID NO:145	QQYNWYPFT
147	huAb13VH.1a	VH		EVQLQESGPGLVKPSSETLSLTC AVTGYSITSGYSWHWIRQFPNG GLEWMGYIHSSGSTNYNPSLKS RISISRDTSKNQFFLKLSSVTA ADTAVYYCAGYDDY FEY WGQGT TVTVSS
33	huAb13VH.1a	CDR-H1	Residues 26-36 of SEQ ID NO:147	GYSITSGYSWH
34	huAb13VH.1a	CDR-H2	Residues 51-66 of SEQ ID NO:147	YIHSSGSTNYNPSLKS
35	huAb13VH.1a	CDR-H3	Residues 99-105 of SEQ ID NO:147	YDDYFEY
148	huAb13VH.1b	VH		EVQLQESGPGLVKPSSETLSLTC AVSGYSITSGYSWHWIRQPPGN GLEWMGYIHSSGSTNYNPSLKS RITISRDTSKNQFSLKLSSVTA ADTAVYYCARYDDY FEY WGQGT TVTVSS
33	huAb13VH.1b	CDR-H1	Residues 26-36 of SEQ ID NO:148	GYSITSGYSWH
34	huAb13VH.1b	CDR-H2	Residues 51-66 of SEQ ID NO:148	YIHSSGSTNYNPSLKS
35	huAb13VH.1b	CDR-H3	Residues 99-105 of SEQ ID NO:148	YDDYFEY

Example 18: Generation of huAb13 variants

[0335] The 3 VH and 3 VL region amino acid sequences of humanized Ab13 variants described in Table 18 were paired together to generate 9 huAb13 variants described in Table 19. The full length amino acid sequences of the heavy chain and light chain of the huAb13v1 variant, huAb13v1 are provided in SEQ ID NOs: 168 and 169, respectively.

Table 19: Variable region sequences of engineered huAb13 variants

SEQ ID NO:	Clone	Protein Region	Residues	Amino acid sequence
147	huAb13v1	VH		EVQLQESGPGGLVKPSETLSLTCAV TGYSITSGYSWHWIRQFPGNGLEW MGYIHSSGSTNYNPSLKSRLSISR DTSKNQFFLKLSSVTAADTAVYYC AGYDDYFEYWGQGTITVTVSS
33	huAb13v1	CDR-H1	Residues 26-36 of SEQ ID NO:147	GYSITSGYSWH
34	huAb13v1	CDR-H2	Residues 51-66 of SEQ ID NO:147	YIHSSGSTNYNPSLKS
35	huAb13v1	CDR-H3	Residues 99-105 of SEQ ID NO:147	YDDYFEY
144	huAb13v1	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKSPKALI YSASYRYSQVPSRFSGSGSGTDFT LTISSLQPEDFAEYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v1	CDR-L1	Residues 24-34 of SEQ ID NO:144	KASQNVGFNVA
38	huAb13v1	CDR-L2	Residues 50-56 of SEQ ID NO:144	SASYRYS
39	huAb13v1	CDR-L3	Residues 89-97 of SEQ ID NO:144	QQYNWYPFT
146	huAb13v2	VH		EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGKGLEW IGYIHSSGSTNYNPSLKSRLTISV DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTITVTVSS
33	huAb13v2	CDR-H1	Residues 26-36 of SEQ ID NO:146	GYSITSGYSWH
34	huAb13v2	CDR-H2	Residues 51-66 of SEQ ID NO:146	YIHSSGSTNYNPSLKS
35	huAb13v2	CDR-H3	Residues 99-105 of SEQ ID NO:146	YDDYFEY
143	huAb13v2	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSQVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYNWYPF TFGQGTKLEIK
37	huAb13v2	CDR-L1	Residues 24-34 of SEQ ID NO:143	KASQNVGFNVA
38	huAb13v2	CDR-L2	Residues 50-56 of SEQ ID NO:143	SASYRYS
39	huAb13v2	CDR-L3	Residues 89-97 of SEQ ID NO:143	QQYNWYPFT

SEQ ID NO:	Clone	Protein Region	Residues	Amino acid sequence
146	huAb13v3	VH		EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGKGLEW IGYIHSSGSTNYNPSLKS RVTISV DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTTVTVSS
33	huAb13v3	CDR-H1	Residues 26-36 of SEQ ID NO:146	GYSITSGYSWH
34	huAb13v3	CDR-H2	Residues 51-66 of SEQ ID NO:146	YIHSSGSTNYNPSLKS
35	huAb13v3	CDR-H3	Residues 99-105 of SEQ ID NO:146	YDDYFEY
144	huAb13v3	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKSPKALI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFAEYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v3	CDR-L1	Residues 24-34 of SEQ ID NO:144	KASQNVGFNVA
38	huAb13v3	CDR-L2	Residues 50-56 of SEQ ID NO:144	SASYRYS
39	huAb13v3	CDR-L3	Residues 89-97 of SEQ ID NO:144	QQYNWYPFT
146	huAb13v4	VH		EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGKGLEW IGYIHSSGSTNYNPSLKS RVTISV DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTTVTVSS
33	huAb13v4	CDR-H1	Residues 26-36 of SEQ ID NO:146	GYSITSGYSWH
34	huAb13v4	CDR-H2	Residues 51-66 of SEQ ID NO:146	YIHSSGSTNYNPSLKS
35	huAb13v4	CDR-H3	Residues 99-105 of SEQ ID NO:146	YDDYFEY
145	huAb13v4	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v4	CDR-L1	Residues 24-34 of SEQ ID NO:145	KASQNVGFNVA
38	huAb13v4	CDR-L2	Residues 50-56 of SEQ ID NO:145	SASYRYS
39	huAb13v4	CDR-L3	Residues 89-97 of SEQ ID NO:145	QQYNWYPFT

SEQ ID NO:	Clone	Protein Region	Residues	Amino acid sequence
147	huAb13v5	VH		EVQLQESGPGGLVKPSETLSLTCAV TGYSITSGYSWHWIRQFPGNGLEW MGYIHSSGSTNYNPSLKSRLSISR DTSKNQFFLKLSSVTAADTAVYYC AGYDDYFEYWGQGTITVTVSS
33	huAb13v5	CDR-H1	Residues 26-36 of SEQ ID NO:147	GYSITSGYSWH
34	huAb13v5	CDR-H2	Residues 51-66 of SEQ ID NO:147	YIHSSGSTNYNPSLKS
35	huAb13v5	CDR-H3	Residues 99-105 of SEQ ID NO:147	YDDYFEY
143	huAb13v5	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYNWYPF TFGQGTKLEIK
37	huAb13v5	CDR-L1	Residues 24-34 of SEQ ID NO:143	KASQNVGFNVA
38	huAb13v5	CDR-L2	Residues 50-56 of SEQ ID NO:143	SASYRYS
39	huAb13v5	CDR-L3	Residues 89-97 of SEQ ID NO:143	QQYNWYPFT
147	huAb13v6	VH		EVQLQESGPGGLVKPSETLSLTCAV TGYSITSGYSWHWIRQFPGNGLEW MGYIHSSGSTNYNPSLKSRLSISR DTSKNQFFLKLSSVTAADTAVYYC AGYDDYFEYWGQGTITVTVSS
33	huAb13v6	CDR-H1	Residues 26-36 of SEQ ID NO:147	GYSITSGYSWH
34	huAb13v6	CDR-H2	Residues 51-66 of SEQ ID NO:147	YIHSSGSTNYNPSLKS
35	huAb13v6	CDR-H3	Residues 99-105 of SEQ ID NO:147	YDDYFEY
145	huAb13v6	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v6	CDR-L1	Residues 24-34 of SEQ ID NO:145	KASQNVGFNVA
38	huAb13v6	CDR-L2	Residues 50-56 of SEQ ID NO:145	SASYRYS
39	huAb13v6	CDR-L3	Residues 89-97 of SEQ ID NO:145	QQYNWYPFT

SEQ ID NO:	Clone	Protein Region	Residues	Amino acid sequence
148	huAb13v7	VH		EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGNGLEW MGYIHSSGSTNYNPSLKSRLTISR DTSKNQFSLKLSSVTAAADTAVYYC ARYDDYFEYWGQGTTVTVSS
33	huAb13v7	CDR-H1	Residues 26-36 of SEQ ID NO:148	GYSITSGYSWH
34	huAb13v7	CDR-H2	Residues 51-66 of SEQ ID NO:148	YIHSSGSTNYNPSLKS
35	huAb13v7	CDR-H3	Residues 99-105 of SEQ ID NO:148	YDDYFEY
143	huAb13v7	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYNWYPF TFGQGTKLEIK
37	huAb13v7	CDR-L1	Residues 24-34 of SEQ ID NO:143	KASQNVGFNVA
38	huAb13v7	CDR-L2	Residues 50-56 of SEQ ID NO:143	SASYRYS
39	huAb13v7	CDR-L3	Residues 89-97 of SEQ ID NO:143	QQYNWYPFT
148	huAb13v8	VH		EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGNGLEW MGYIHSSGSTNYNPSLKSRLTISR DTSKNQFSLKLSSVTAAADTAVYYC ARYDDYFEYWGQGTTVTVSS
33	huAb13v8	CDR-H1	Residues 26-36 of SEQ ID NO:148	GYSITSGYSWH
34	huAb13v8	CDR-H2	Residues 51-66 of SEQ ID NO:148	YIHSSGSTNYNPSLKS
35	huAb13v8	CDR-H3	Residues 99-105 of SEQ ID NO:148	YDDYFEY
144	huAb13v8	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKSPKALI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFAEYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v8	CDR-L1	Residues 24-34 of SEQ ID NO:144	KASQNVGFNVA
38	huAb13v8	CDR-L2	Residues 50-56 of SEQ ID NO:144	SASYRYS
39	huAb13v8	CDR-L3	Residues 89-97 of SEQ ID NO:144	QQYNWYPFT

SEQ ID NO:	Clone	Protein Region	Residues	Amino acid sequence
148	huAb13v9	VH		EVQLQESGPGLVKPSSETLSLTCAV SGYSITSGYSWHWIRQPPGNGLEW MGYIHSSGSTNYNPSLKSRTISR DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTITVTVSS
33	huAb13v9	CDR-H1	Residues 26-36 of SEQ ID NO:148	GYSITSGYSWH
34	huAb13v9	CDR-H2	Residues 51-66 of SEQ ID NO:148	YIHSSGSTNYNPSLKS
35	huAb13v9	CDR-H3	Residues 99-105 of SEQ ID NO:148	YDDYFEY
145	huAb13v9	VL		DIQMTQSPSSLSASVGDRVTITCK ASQNVGFNVAWYQQKPGKAPKLLI YSASYRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYFCQQYNWYPF TFGQGTKLEIK
37	huAb13v9	CDR-L1	Residues 24-34 of SEQ ID NO:145	KASQNVGFNVA
38	huAb13v9	CDR-L2	Residues 50-56 of SEQ ID NO:145	SASYRYS
39	huAb13v9	CDR-L3	Residues 89-97 of SEQ ID NO:145	QQYNWYPFT

Example 19: Characterization of huAb13 VL.1a Humanized Variants

[0336] Nine huAb13 variants described in Examples 17 and 18 were generated and tested for binding to B7-H3 by FACS (according to methods described in Example 6). Six variants did not bind to human B7-H3. The remaining three variants were further characterized for binding by SPR and conjugated (via Method A) to the Bcl-xL inhibitor (specifically the linker warhead (or synthon) CZ) and assessed for cell cytotoxicity (according to methods described in Example 7). Table 20 provides the *in vitro* characteristics of these variants.

Table 20: *In vitro* characterization of huAb13 VL.1a variants conjugated to synthon (or linker payload) CZ

Variant name	Sequence Number	DAR by MS	% agg by SEC	ELISA hB7-H3 EC ₅₀ nM	FACS nM) (EC ₅₀		Affinity of naked mAbs (Biacore, K _D)	Cytotoxicity H847 Cell line IC ₅₀) (nM)
					hB7-H3	cyB7-H3		
chAb13-CZ	32, 36	-	-	0.26	6.27	18.35	5.7E-09	-
huAb13v1-CZ	147, 144	4.0	5.1	0.12	6.01	10.0	6.2E-09	0.09
huAb13v5-CZ	147, 143	3.4	2.4	0.19	5.21	10.59	Poor fit	1.60
huAb 13v6-CZ	147, 145	3.6	7.3	0.14	5.83	12.95	Poor fit	0.84

[0337] HuAb13v1 was selected for further study due in part to its potent and superior cytotoxicity against H847 cells and similar binding characteristics as chAb13 from which it was derived. In contrast, huAb13v5 and huAb13v6 showed poor fit kinetics in Biacore experiments suggesting their binding properties are more divergent from the parental chAb13 than huAb13v1 and have reduced activity in the cell killing assay.

Example 20: *In vitro* potency of Selected Humanized B7-H3 Antibodies with Exemplary Bcl-xL Inhibitor Linker Warheads (Synthons)

[0338] Humanized antibodies huAb13v1, huAb3v2.5 and huAb3v2.6 were selected to be conjugated with several Bcl-xL inhibitor payloads (or synthons) at a 3 mg scale using Methods A, E or G, as described in Example 7. The anti-tumor activity of these ADCs was tested in cytotoxicity assays using the NCI-H1650 non-small cell lung cancer cell line as described in Example 7. As control, the *in vitro* anti-tumor activity of ADCs comprising the non-targeting antibody MSL109 (a monoclonal antibody that binds to the CMV glycoprotein H conjugated to Bcl-xL inhibitor payloads (or synthons) was also evaluated. The results are described in Table 21.

Table 21: *In vitro* tumor cell cytotoxicity of selected humanized B7-H3 ADCs with exemplary Bcl-xL inhibitor linker warheads (synthons)

ADC	Conjugation Method	DAR by MS	%agg by SEC	ADC conc (mg/ml)	EC ₅₀ nM H1650
huAb13v1-CZ	G	4	3.9	3	0.18
huAb13v1-TX	G	3.6	2.8	2.6	0.22
huAb13v1-TV	G	2.4	3	3.9	0.43
huAb13v1-AAA	G	2	20.2	2.7	0.37
huAb13v1-AAD	G	3.7	3.3	2.7	0.21
huAb13v1-WD	E	3	5.4	5.8	0.45
huAb13v1-LB	A	2.2	21.9	3.7	>133
huAb13v1-ZT	G	2.4	10.6	1.7	0.3
huAb13v1-ZZ	G	1.4	20.3	2.5	0.42
huAb13v1-XW	G	4.3	6.3	2.6	0.86
huAb13v1-SE	A	3.7	4	5.4	0.63
huAb13v1-SR	A	2.6	49.5	4.5	0.59
huAb13v1-YG	E	3.3	2.1	3.8	0.33
huAb13v1-KZ	A	2.8	16.8	3.5	178.8
huAb3v2.5-CZ	G	3.3	15.6	3.6	0.40
huAb3v2.5-TX	G	3.3	8.9	2.9	0.62
huAb3v2.5-TV	G	3.7	10.4	3.5	0.53
huAb3v2.5-YY	G	2.3	16.2	3.2	0.71
huAb3v2.5-AAA	G	2	14.8	3.3	0.85
huAb3v2.5-AAD	G	3.4	11.3	3.7	0.49

ADC	Conjugation Method	DAR by MS	%agg by SEC	ADC conc (mg/ml)	EC ₅₀ nM H1650
huAb3v2.5-WD	E	2.8	11.5	5.4	0.83
huAb3v2.5-LB	A	2.2	24.4	3.9	2.59
huAb3v2.5-ZT	G	1.6	20.1	3.3	0.95
huAb3v2.5-ZZ	G	1.2	19.4	3.7	1.1
huAb3v2.5-XW	G	3.9	16.4	3.4	2.18
huAb3v2.5-SE	A	3.7	10.6	5.4	0.85
huAb3v2.5-SR	A	1.8	48.5	5.1	0.59
huAb3v2.5-YG	E	4	8.6	3.3	0.71
huAb3v2.5-KZ	A	2.6	24.5	3.4	0.87
huAb3v2.6-CZ	G	3.4	15	3.6	0.40
huAb3v2.6-TX	G	3.2	10.4	3.4	0.47
huAb3v2.6-TV	G	3.3	10.7	3.8	0.52
huAb3v2.6-YY	G	2.2	19.9	3.4	0.72
huAb3v2.6-AAA	G	1.9	20.2	3.6	1.24
huAb3v2.6-AAD	G	3.4	11.9	3.7	0.85
huAb3v2.6-WD	E	3.1	12.4	5.3	0.79
huAb3v2.6-LB	A	2.4	27.2	3.9	2.07
huAb3v2.6-ZT	G	1.7	21.6	3.7	1.11
huAb3v2.6-ZZ	G	1.2	20.7	3.5	1.35
huAb3v2.6-XW	G	4	16.8	3.2	2.4
huAb3v2.6-SE	A	3.6	11.8	5.7	1.01
huAb3v2.6-SR	A	2.5	48.2	5.2	0.71
huAb3v2.6-YG	E	3.7	9.9	4.8	0.68
huAb3v2.6-KZ	A	3.5	26.1	3.6	5.52
MSL109-CZ	G	3.2	0.5	3.7	19.50
MSL109-TX	G	3.5	0.7	3	20.00
MSL109-TV	G	3.6	0	2.6	31.13
MSL109-YY	G	2.9	0	1.8	26.53
MSL109-AAA	G	1.9	13.7	3.2	23.52
MSL109-AAD	G	3	0.4	3.8	>67
MSL109-WD	E	2.9	0	7.06	18.22
MSL109-LB	A	1.8	0	4.2	9.88
MSL109-ZT	G	2.3	7.5	2.2	>67
MSL109-ZZ	G	1.4	15	3.5	>67
MSL109-XW	G	3.3	3.7	3.2	>67
MSL109-SE	A	3.6	33.4	6.0	29.56
MSL109-SR	A	1.8	2.3	3.8	53.29
MSL109-YG	E	3.1	13.2	4.0	19.93

ADC	Conjugation Method	DAR by MS	%agg by SEC	ADC conc (mg/ml)	EC ₅₀ nM H1650
MSL109-KZ	A	2.5	18	4.3	50.16

[0339] In contrast to the low anti-tumor activity exhibited by the ADCs comprising the non-targeting antibody MSL109 conjugated to a Bcl-xL inhibitor payload, the B7-H3-targeting ADCs exhibited greater tumor cell killing, which reflects the antigen-dependent delivery of the B7-H3-targeting ADCs to the B7-H3-expressing tumor cells.

[0340] The anti-tumor activity of two of these ADCs was tested in cytotoxicity assays using the NCI-H146 small cell lung cancer cell line as described in Example 7. The results are described in Table 22.

Table 22: *In vitro* tumor cell cytotoxicity of selected humanized B7-H3 ADCs with exemplary Bcl-xL inhibitor synthons.

ADC	Conjugation Method	DAR	% agg by SEC	ADC conc (mg/ml)	EC ₅₀ nM H146
huAb13v1-AAA E2	I	2	3.3	11.6	2
huAb13v1-WD E2	I	2	4.5	14.5	2

[0341] huAb13v1-AAA E2 and huAb13v1-WD E2 were tested for cytotoxicity using H146 cells. Both conjugates show potent and comparable cytotoxicity.

Example 21: *In vivo* analysis of anti-B7-H3 ADCs

[0342] Humanized anti-B7-H3 antibodies huAb13v1, huAb3v2.5 and huAb3v2.6 were selected to be conjugated with several Bcl-xL inhibitor payloads and were evaluated in xenograft models of small cell lung cancer (H146) as conjugates using a number of Bcl-xL inhibitor warheads (synthons) using the methods described in Example 7 and Example 8. The results are summarized in Table 23 and Table 24.

Table 23: *In vivo* efficacy of humanized anti-B7-H3 ADCs

ADC	Conjugation Method	DAR	Dose ^[a] /route/regimen	Number of mice	TGI _{max} (%)	TGD (%)
AB095	-	n/a	6mg/kg/IP/QDx1	8	0	0
huAb3v2.5-CZ	A	3.5	6mg/kg/IP/QDx1	8	92	122
huAb3v2.6-CZ	A	3.4	6mg/kg/IP/QDx1	8	93	130
huAb3v2.9-CZ	A	2.8	6mg/kg/IP/QDx1	8	94	135
huAb3v2.9-TX	E	1.7	6mg/kg/IP/QDx1	8	93	109
huAb3v2.6-TX	E	2.7	6mg/kg/IP/QDx1	8	92	130
huAb3v2.5-TX	E	2.5	6mg/kg/IP/QDx1	8	86	89

ADC	Conjugation Method	DAR	Dose ^[a] /route/regimen	Number of mice	TGI _{max} (%)	TGD (%)
[a] dose is given in mg/kg/day						

Table 24: *In vivo* efficacy of humanized anti-B7-H3 ADCs

ADC	Conjugation Method	DAR	Dose ^[a] /route/regimen	Number of mice	TGI _{max} (%)
AB095	-	n/a	6mg/kg/IP/QDx1	8	0
huAb3v2.5-AAA	E	2.3	6mg/kg/IP/QDx1	8	65
huAb3v2.5-XW	E	3.1	6mg/kg/IP/QDx1	8	51
huAb3v2.6-AAA	E	3.5	6mg/kg/IP/QDx1	8	47
huAb3v2.6-XW	E	4.0	6mg/kg/IP/QDx1	8	43
huAb13v1-AAA	E	3.5	6mg/kg/IP/QDx1	8	76
huAb13v1-XW	E	4.2	6mg/kg/IP/QDx1	8	35
huAb13v1-TX E2	I	2	6mg/kg/IP/QDx1	8	88
[a] dose is given in mg/kg/day					

[0343] Humanized anti-B7-H3 antibody huAb13v1 was conjugated with the Bcl-xL inhibitor synthon WD and evaluated in a xenograft model of the B7-H3-positive small cell lung cancer (H1650) as conjugates using the methods described in Example 7 and Example 8. As control, the *in vivo* anti-tumor activity of a non-targeting IgG isotype matched antibody (AB095) was also evaluated. The results are summarized in Table 25.

Table 25: *In vivo* efficacy of humanized anti-B7-H3 ADC huAb13v1-WD in H1650

ADC	DAR/Conjugation Method	Dose mg/kg/day	route/regimen	TGI _{max} (%)	TGD (%)
AB095 ^(a)	N.A.	10	IP/QDx1	0	0
huAb13v1-WD-E2	2/1	1	IP/QDx1	46*	47*
huAb13v1-WD-E2	2/1	3	IP/QDx1	48*	47*
huAb13v1-WD-E2	2/1	10	IP/QDx1	62*	77*
(a) IgG1 mAb					
* = p < 0.05 as compared to control treatment (AB095)					
‡ = p < 0.05 as compared to the most active partner in a drug combination					
N.A. = not applicable					

[0344] In contrast to the lack of activity observed using the non-targeting IgG isotype-matched antibody Ab095, the B7-H3-targeting Bcl-xL ADCs exhibited tumor growth inhibition (TGI) and tumor growth delay (TGD), as shown in Tables 24 and 25, reflecting the antigen-dependent delivery of the B7-H3-targeting ADCs which deliver the Bcl-xL inhibitor to the B7-H3-expressing tumor cells in this xenograft mouse model.

As an additional control, the *in vivo* anti-tumor activity of ADCs comprising the non-targeting antibody MSL109 conjugated with Bcl-xL inhibitor synthons was evaluated in the xenograft model of the B7-H3-positive small cell lung cancer (H1650). The activity of these ADCs was compared that of the non-targeting IgG isotype matched antibody, AB095, as control. As shown in Table 26, the ADCs comprising the non-targeting antibody MSL109 conjugated with Bcl-xL inhibitor synthons exhibited very modest tumor growth inhibition and low or no tumor growth delay. In contrast, the B7-H3-targeting Bcl-xL ADCs (as shown in Table 25) exhibited, much greater tumor growth inhibition (TGI) and tumor growth delay (TGD), reflecting the antigen-dependent delivery of these ADCs to B7-H3-expressing cells in this mouse xenograft model.

Table 26. *In vivo* efficacy of non-targeting (MSL109) BCL-xL inhibiting ADCs in NCI-H1650 model of NSCLC

Treatment	Dose ^[a] /route/regimen	Growth Inhibition	
		TGI _{max} (%)	TGD (%)
MSL109 [†] -H	3/IP/Q4Dx6	18*	0
MSL109 [†] -H	10/IP/Q4Dx6	43*	20*
MSL109 [†] -H	30/IP/Q4Dx6	8	0
MSL109 [†] -CZ	3/IP/Q4Dx6	29*	0
MSL109 [†] -CZ	3/IP/Q7Dx6	18*	0
MSL109 [†] -CZ	10/IP/Q4Dx6	32*	16
MSL109 [†] -CZ	30/IP/Q4Dx6	32*	12
† Non-targeting antibody			
[a] dose is given in mg/kg/day			
* = p < 0.05 as compared to control treatment (AB095)			
Q4Dx6 indicates one dose every 4 days for a total of 6 doses			

Example 22: B7-H3 Combination Therapy

[0345] The anti-tumor activity of huAb13v1 as CZ or TX conjugates as purified DAR2 (E2) conjugates were characterized in xenograft models of non-small cell lung cancer (H1650, H1299, H1975, and EBC1) of human origin using the methods described in Example 8. The anti-tumor activity was assessed as monotherapy and in combination with docetaxel (H1650, H1299, H1975, and EBC1). The results are presented in Table 27.

Table 27: *In vivo* efficacy of humanized huAb13v1 anti-B7-H3 conjugates as monotherapy and in combination with docetaxel

ADC	DAR/Conjugation Method	Dose mg/kg/day	route/regimen	TGI _{max} (%)	TGD (%)
EBC1					
AB095	-	10	Q4Dx6/IP	0	0
huAb13v1-TX E2	2/I	10	Q4Dx6/IP	58	67
Docetaxel	-	7.5	QDx1/IV	85	80
huAb13v1-TXE2 + Docetaxel	2/I	10+7.5	Q4Dx6/IP+Q Dx1/IV	140	140

NCI-H1299					
AB095		10	Q4Dx6/IP	0	0
huAb13v1-TX E2	2/I	10	Q4Dx6/IP	80	24
Docetaxel	-	7.5	QDx1/IV	87	48
huAb13v1-TXE2 +Docetaxel	2/I	10+7.5	Q4Dx6/IP+Q D _x 1/IV	97	83
NCI-H1975					
AB095		10	Q4Dx6/IP	0	0
huAb13v1-TX E2	2/I	10	Q4Dx6/IP	52	62
Docetaxel		7.5	QDx1/IV	81	77
huAb13v1-TXE2 +Docetaxel	2/I	10+7.5	Q4Dx6/IP+Q D _x 1/IV	92	108
NCI-H1650					
AB095	-	8	Q7Dx6/IP	0	0
huAb13v1-CZ	2/I	10	QDx1/IP	80	100
Docetaxel	-	7.5	QDx1/IV	84	143
huAb13v1-CZ +Docetaxel	-	10+7.5	QDx1/IP+Q D _x 1/IV	99	>600
NCI-H1650					
AB095 ^(a)	N.A.	10	IP/ Q14Dx3	0	0
DTX	N.A.	7.5	IV/Q14Dx3	80*	158*
huAb13v1-WD E2	2/I	10	IP/ Q14Dx3	67*	83*
huAb13v1-WD E2 + DTX)	2/I + N.A.	10 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	98*¥	>717*¥
huAb13v1-WD E2	2/.	3	IP/ Q14Dx3	56*	75*
huAb13v1-WD E2 + DTX)	2/I + N.A.	3 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	99*¥	>717*¥
huAb13v1-WD E2	2/I	1	IP/ Q14Dx3	60*	67*
huAb13v1-WD E2 + DTX	2/I + N.A	1 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	88*¥	467*¥
huAb13v1-AAA E2	2/I	10	IP/ Q14Dx3	63*	117*
huAb13v1-AAA E2 + DTX	2/I + N.A	10 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	99*¥	>717*¥
huAb13v1-AAA E2	2/I	3	IP/ Q14Dx3	60*	117*
huAb13v1-AAA E2 + DTX	2/I + N.A	3 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	99*¥	>717*¥
huAb13v1-AAA E2	2/I	1	IP/ Q14Dx3	50*	67*

NCI-H1650					
huAb13v1-AAA E2 + DTX	2/I + N.A	1 + 7.5	IP/ Q14Dx3 + IV/Q14Dx3	92*¥	>717*¥
(a) IgG1 mAb					
* = p < 0.05 as compared to control treatment (AB095)					
¥ = p < 0.05 as compared to the most active partner in a drug combination					
N.A. = not applicable					

[0346] The results presented in Table 27 demonstrate that above, huAb13v1 as CZ, TX, WD or AAA purified DAR2 (E2) conjugates inhibited the growth of all four NSCLC xenograft models as monotherapy. In addition, huAb13v1 as CZ, TX, WD or AAA purified DAR2 (E2) conjugates effectively combined with docetaxel to produce more sustained tumor growth inhibition. This is most dramatically illustrated in the H1650 xenograft model where the combination therapy resulted in a TGD of between 467% and >717%, whereas the individual monotherapies resulted in TGD in the range of 67% -158%. These results support the clinical utility of Bcl-xL inhibitor (Bcl-xLi) ADCs to be dosed in combination with chemotherapy.

SEQUENCE SUMMARY

[0347]

SEQ ID NO:	Description	Amino Acid Sequence
1	chAb2 VH amino acid sequence	QVQLQQPGAELVKPGASVKLSCKA SGYTFTSYWMHWVKQRPGQGLEWI GMIHPDSGTTNYNEKFRSKATLTV DKSSSTAYMQLSSLTSEDSAVYYC AVYYGSTYWFYFDVWGTTTVTVSS
2	chAb2 VH CDR1 amino acid sequence	GYTFTSYWMH
3	chAb2 VH CDR2 amino acid sequence	MIHPDSGTTNYNEKFRS
4	chAb2 VH CDR3 amino acid sequence	YYGSTYWFYFDV
5	chAb2 VL amino acid sequence	DVVMTQTPLSLPVSLGDQAYISCR SSQSLVHINGNTYLHWYRQKPGQS PKLLIYKVSNRFSGVPDFRFSGSGS GTDFTLKISRVEAEDLGVYFCSSQ THFPFTFGSGTKLEIK
6	chAb2 VL CDR1 amino acid sequence	RSSQSLVHINGNTYLH
7	chAb2, chAb3, chAb10, huAb3VL.1, huAb3VL.1a, huAb3VL.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VL CDR2 amino acid sequence	KVSNRFS
8	chAb2 VL CDR3 amino acid sequence	SQSTHFPFT
9	chAb3 VH amino acid sequence	QVQLQQPGAELVKPGASVKLSCKA SGYTFFSYWMHWVKQRPGQGLEWI GLIHPDSGSTNYNEMFKNKATLTV

SEQ ID NO:	Description	Amino Acid Sequence
		DRSSSTAYVQLSSLTSEDSAVYFC AGGGRLYFDYWGQGTTLTVSS
10	chAb3, huAb3VH.1, huAb3VH.1a, huAb3VH.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR1 amino acid sequence	GYTFSSYWMH
11	chAb3, huAb3VH.1, huAb3VH.1a, and huAb3VH.1b VH CDR2 amino acid sequence	LIHPDSGSTNYNEMFKN
12	chAb3, huAb3VH.1, huAb3VH.1a, huAb3VH.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR3 amino acid sequence	GGRLYFDY
13	chAb3 VL amino acid sequence	DVVMTQTPLSLPVSLGDQASISCR SSQSLVHSNGDTYLRWYLQKPGQS PKLLIYKVSNRFSGVPDRFSGSGS GTDFTLKITRVEAEDLGVIYFCSSQS THVPYTFGGGTKLEIK
14	chAb3, huAb3VL.1, huAb3VL.1a, and huAb3VL.1b VL CDR1 amino acid sequence	RSSQSLVHSNGDTYLR
15	chAb3, huAb3VL.1, huAb3VL.1a, huAb3VL.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VL CDR3 amino acid sequence	SQSTHVPYT
16	chAb4 VH amino acid sequence	QVQLQQPGAELVKPGASVKLSCKA SGYSFTSYWMHWVKQRPQGGLWEI GMIHPNSGSNNYNEKFKSKATLTV DKSSNTAYMQLSSLTSEDSAVYYC ARRLGLHFDYWGQGTTLTVSS
17	chAb4 VH CDR1 amino acid sequence	GYSFTSYWMH
18	chAb4 VH CDR2 amino acid sequence	MIHPNSGSNNYNEKFKS
19	chAb4 VH CDR3 amino acid sequence	RLGLHFDY
20	chAb4 VL amino acid sequence	DIVMTQSQKFMSTPVGDRVSITCK ASQNVGTAWAWYQQKPGQSPKLLI YSASNRYTGVPDRFTGSGSGTDFT LTISNMQSEDLADYFCQQYSSYPY TFGGGTKLEIK
21	chAb4 VL CDR1 amino acid sequence	KASQNVGTAVA
22	chAb4 VL CDR2 amino acid sequence	SASNRYT
23	chAb4 VL CDR3 amino acid sequence	QQYSSYPY
24	chAb18 VH amino acid sequence	QVQLQQSAAELARPGASVKMSCKA SGYSFTSYTIHWVKQRPQGGLWEI GYINPNSRNTDYNQKFKDETTLTA DRSSSTAYMQLISLTSEDSAVYYC ARYSGSTPYWYFDVWGAGTTVTVS S

SEQ ID NO:	Description	Amino Acid Sequence
25	chAb18, huAb18VH.1, huAb18VH.1a, and huAb18VH.1b VH CDR1 amino acid sequence	GYSFTSYTIH
26	chAb18, huAb18VH.1, and huAb18VH.1a VH CDR2 amino acid sequence	YINPNSRNTDYNQKFKD
27	chAb18, huAb18VH.1, huAb18VH.1a, and huAb18VH.1b VH CDR3 amino acid sequence	YSGSTPYWYFDV
28	chAb18 VL amino acid sequence	QIVLTQSPAILSASPGEKVTMTCR ASSSVSYMNWYQQKPGSSPKPWIY ATSNLASGVPARFSVSVSGTSHSL TISRVEAEDAATYYCQQWSSNPLT FGAGTKLELK
29	chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR1 amino acid sequence	RASSSVSYMN
30	chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR2 amino acid sequence	ATSNLAS
31	chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR3 amino acid sequence	QQWSSNPLT
32	chAb13 VH amino acid sequence	DVQLQESGPDLVKPSQSLSLTCTV TGYSITSGYSWHWIRQFPGNKLEW MGYIHSSGSTNYNPSLKSRI SINR DTSKNQFFLQLNSVTTEDTATYYC AGYDDYFEYWGQGTTLTVSS
33	chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VH CDR1 amino acid sequence	GYSITSGYSWH
34	chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VH CDR2 amino acid sequence	YIHSSGSTNYNPSLKS
35	chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VH CDR3 amino acid sequence	YDDYFEY
36	chAb13 VL amino acid sequence	DIVMTQSQKFMSTSVGDRVSVTCK ASQNVGFNVAWYQQKPGQSPKALI YSASYRYSQVPDRFTGSGSGTDEF LTISNVQSEDLAEYFCQQYNSYPF TFGSGTKLEIK
37	chAb13, huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3,	KASQNVGFNVA

SEQ ID NO:	Description	Amino Acid Sequence
	huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR1 amino acid sequence	
38	chAb13, huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR2 amino acid sequence	SASYRYS
39	huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR3 amino acid sequence	QQYNWYPFT
40	chAb12 VH amino acid sequence	EVQLVESGGGLVKPGGSLKLSCAA SGFTFSSYAMSWVRQTPEKRLEWV ATISSGTNYTYYPDSVKGRFTISR DNAKNTLYLQMTSLRSEDTAMYVC ARQGRYSWIAYWGQGTTLTVSA
41	chAb12 VH CDR1 amino acid sequence	GFTFSSYAMS
42	chAb12 VH CDR2 amino acid sequence	TISSGTNYTYYPDSVKG
43	chAb12 VH CDR3 amino acid sequence	QGRYSWIAY
44	chAb12 VL amino acid sequence	DIVLTQSPASLAVSLGQRATISCR ASKSVSTSDYSYMHWNQKPGQPP KLLIYLASNLESGVPARFSGSGSG TDFTLNHPVEEEDAATYYCQHSR ELLTFGAGTKLELK
45	chAb12 VL CDR1 amino acid sequence	RASKSVSTSDYSYMH
46	chAb12 and chAb17 VL CDR2 amino acid	LASNLES
47	chAb12 VL CDR3 amino acid sequence	QHSRELLT
48	chAb14 VH amino acid sequence	EVKLVESGGGLVKPGGSLKLSCAA SGFTFSSYGMSWVRQTPEKRLEWV ATISGGGTNTTYYPDSVEGRFTISR DNAKNFLYLQMSSLRSEDTALYYC ARHYGSQTMDYWGQGTSTVTVSS
49	chAb14 and chAb8 VH CDR1 amino acid sequence	GFTFSSYGMS
50	chAb14 VH CDR2 amino acid sequence	TISGGGTNTTYYPDSVEG
51	chAb14 VH CDR3 amino acid sequence	HYGSQTMDY
52	chAb14 VL amino acid sequence	DIQMTQSPASLSASVGETVTITCR TSGNIHNYLTWYQQKQKSPQLLV YNAKTLADGVPSRFSGSGSGTQFS LKINSLQPEDFGSYVCQHFWSIMW TFGGGTKLEIK
53	chAb14 VL CDR1 amino acid sequence	RTSGNIHNYLT
54	chAb14 VL CDR2 amino acid sequence	NAKTLAD

SEQ ID NO:	Description	Amino Acid Sequence
55	chAb14 VL CDR3 amino acid sequence	QHFWSIMVT
56	chAb6 VH amino acid sequence	QVQLQQSGAELMKPGASVKISCKA TGYTFSRYWIEWVKQRPGHGLEWI GEILPGSGSTNYNEKFKGKATFTA DTSSNTAYMQVSSLTSEDSAVHYC ARRGYGYVPYALDYWGQGTSVTVS S
57	chAb6 VH CDR1 amino acid sequence	GYTFSRYWIE
58	chAb6 VH CDR2 amino acid sequence	EILPGSGSTNYNEKFKG
59	chAb6 VH CDR3 amino acid sequence	RGYGYVPYALDY
60	chAb6 VL amino acid sequence	EIQMTQTSSLSASLGDRVTISCR ASQDISNSLNWYQQKPDGTVNLLI YYTSRLYSGVPSRFSGSGSGTDYS LTISNLEQEDIATYFCQQGNTLPY TFGGGTKLEIK
61	chAb6 VL CDR1 amino acid sequence	RASQDISNSLN
62	chAb6 VL CDR2 amino acid sequence	YTSRLYS
63	chAb6 VL CDR3 amino acid sequence	QQGNTLPYT
64	chAb11 VH amino acid sequence	EVKLIVESGGGLVQPGGSLRLSCAT SGFTFTNYYMSWVRQPPGKALEWL GFIRNKANDYTTEYSASVKGRFTI SRDNSQSILYLQMNTLRAEDSATY YCAR ~~ES~~ PGNPFAYWGQGTLVTVSA
65	chAb11 VH CDR1 amino acid sequence	GFTFTNYYMS
66	chAb11 VH CDR2 amino acid sequence	FIRNKANDYTTEYSASVKG
67	chAb11 VH CDR3 amino acid sequence	ESPGNPFAY
68	chAb11 VL amino acid sequence	DIVMTQSPSSLTVTAGEKVTMTCK SSQSLLNSGTQKNFLT ~~WY~~ QQKPGQ PPKLLIYWASTRESGVPDRFTGSG SGTDFTLTISSVQAEDLAVYFCQN DYIYPLTFGAGTKLELK
69	chAb11 VL CDR1 amino acid sequence	KSSQSLLNSGTQKNFLT
70	chAb11 VL CDR2 amino acid sequence	WASTRES
71	chAb11 VL CDR3 amino acid sequence	QNDYIYPLT
72	chAb16 VH amino acid sequence	EVKLLESGGGLVQPGGSLKLSCAA SGFDF ~~S~~ RYWMSWVRQAPGKGLEWI GEINPDSSTINYTPSLKDKFIISR DNAKNTLYLQMSKVRSEDTALYYC ARPGFGNYIYAMDYWGQGTSVTVS S
73	chAb16 VH CDR1 amino acid sequence	GFDFSRYWMS
74	chAb16 VH CDR2 amino acid sequence	EINPDSSTINYTPSLKD

SEQ ID NO:	Description	Amino Acid Sequence
75	chAb16 VH CDR3 amino acid sequence	PGFGNYIYAMDY
76	chAb16 VL amino acid sequence	DIQMTQTSSLSASLGDRVTINCR ASQDISNFLNWWYQQKPDGTVKLLI YYTSRLYLGVPSSRFSGSGSGTDYS LTISNLEQEDIATYFCQQGNTLPP TFGGGTKLEIK
77	chAb16 VL CDR1 amino acid sequence	RASQDISNFLN
78	chAb16 VL CDR2 amino acid sequence	YTSRLYL
79	chAb16 VL CDR3 amino acid sequence	QQGNTLPPT
80	chAb10 VH amino acid sequence	DVQLQESGPGGLVKPSQSLSLTCTV TGYSITSDYAWNWIHQFPGNRLEW MGHINYSGITNYPNPSLKSRLSITR DTSKNQFFLQLYSVTTEDTATYFC ARRSLFYFYYGSSLYAMDYWGQGTS VTVSS
81	chAb10 VH CDR1 amino acid sequence	GYSITSDYAWN
82	chAb10 VH CDR2 amino acid sequence	HINYSGITNYPNPSLKS
83	chAb10 VH CDR3 amino acid sequence	RSLFYFYYGSSLYAMDY
84	chAb10 VL amino acid sequence	DVVMTQSPFSLPVS LGDQASISCR SSQSLVHSNGNTYLHWYLQKPGQS PKLLIYKVSNRFSQVPDRFSGSGS GTDFTLKISRVEAEDLGVYFCSQS THVPWTFGGGTKLEIK
85	chAb10 VL CDR1 amino acid sequence	RSSQSLVHSNGNTYLH
86	chAb10 VL CDR3 amino acid sequence	SQSTHVPWT
87	chAb7 VH amino acid sequence	EVQLVESGENLVKPGGSLKLSCAA SGFSFRGYGMSWVRQTPDKRLEWV AAISTGGNYTYYPDSVQGRFTISR DNANNTLYLQMSSLKSEDTAMYYC ARRGGNYAGFAYWGQGLTVTSA
88	chAb7 VH CDR1 amino acid sequence	GFSFRGYGMS
89	chAb7 VH CDR2 amino acid sequence	AISTGGNYTYYPDSVQG
90	chAb7 VH CDR3 amino acid sequence	RGGNYAGFAY
91	chAb7 VL amino acid sequence	DIQMTQSPASLSVSVGETVTITCR PSENIYSNLAWYQQKQKSPQLLV YAATNLADGVPSRFSGSGSGTQYS LKINSLQSEDFGTYYCQHFWGTPF TFGSGTKLEIK
92	chAb7 VL CDR1 amino acid sequence	RSENIYSNLA
93	chAb7 and chAb8 VL CDR2 amino acid	AATNLAD
94	chAb7 VL CDR3 amino acid sequence	QHFWGTPFT
95	chAb8 VH amino acid sequence	EVKLVESGGGLVKPGGSLKLSCAA

SEQ ID NO:	Description	Amino Acid Sequence
		SGFTFSSYGMSWVRQTPEKRLEWV ATISGGGNYTYCPDSVKGRFTISR DNAKNNLYLQMSSLRSEDALYYC TRQRGYDYHYAMD ~~F~~ WGQTSVTVS S
96	chAb8 VH CDR2 amino acid sequence	TISGGGNYTYCPDSVKG
97	chAb8 VH CDR3 amino acid sequence	QRGYDYHYAMD ~~F~~
98	chAb8 VL amino acid sequence	DIQMTQSPASLSVSVGETVTITCR ASENIYSNLAWHQQKQKSPQLLV YAATNLADGVPSRFSGNGSDTQYS LKINSLQSEDFGSYFCQ ~~N~~ FWGTSW TFGGGTKLEIK
99	chAb8 VL CDR1 amino acid sequence	RASENIYSNLA
100	chAb8 VL CDR3 amino acid sequence	Q ~~N~~ FWGTSW ~~T~~
101	chAb17 VH amino acid sequence	EVKLVESGGGLVQPGGSLKLSCAA SGFTFSSYIMSWVRQTPEKRLEWV ASIVSSNITYYPDSMKGRFTISR NARNILYLQMSSLKSEDTAMYYCA RSGTRAWFAYWGQGLVTVSA
102	chAb17 VH CDR1 amino acid sequence	GFTFSSYIMS
103	chAb17 VH CDR2 amino acid sequence	SIVSSNITYYPDSMKG
104	chAb17 VH CDR3 amino acid sequence	SGTRAWFAY
105	chAb17 VL amino acid sequence	DIVLTQSPASLAVSLGQRATISCR ASKSVSTSA ~~S~~ YMH ~~W~~ YQQKPGQPP KLLIY ~~L~~ ASN ~~L~~ ESGVPARFSGSGSG TDFTLN ~~I~~ HPVEEEDAATYYCQ ~~H~~ SR ELPYTFGGGTKLEIK
106	chAb17 VL CDR1 amino acid sequence	RASKSVSTSA ~~S~~ YMH
107	chAb17 VL CDR3 amino acid sequence	QHSREL ~~P~~ YT
108	chAb5 VH amino acid sequence	QVQLQQPGDELVKPGASVKLSCKT SGYTFTTDWMH ~~W~~ VKQRPQGGL ~~E~~ WI GMIHPNSGTTNYNEKFKSKAALT ~~V~~ DKSSSTACMQLSLTS ~~E~~ DSAVYYC AR ~~S~~ YWK ~~W~~ YFDVWGTGTTVT ~~V~~ SS
109	chAb5 VH CDR1 amino acid sequence	GYTFTTDWMH
110	chAb5 VH CDR2 amino acid sequence	MIHPNSGTTNYNEKFKS
111	chAb5 VH CDR3 amino acid sequence	SYWK ~~W~~ YFDV
112	chAb5 VL amino acid sequence	QIVLTQSPA ~~I~~ MSASLGEEITLTCS ASSSVSYM ~~H~~ WYQQKSGTSPKLLIY STSNLASGVPSRFSGSGSGTFYSL TISSVEAEDSADYYCHQ ~~W~~ TSYMYT FGGGTKLEIK
113	chAb5 VL CDR1 amino acid sequence	SASSSVSYM ~~H~~

SEQ ID NO:	Description	Amino Acid Sequence
114	chAb5 VL CDR2 amino acid sequence	STSNLAS
115	chAb5 VL CDR3 amino acid sequence	HQWTSYMYT
116	huAb18VH.1, huAb18v1, and huAb18v5 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYSF TSYTI HWVRQAPGQGLEWM GYINPNSRNTDYN QKFK DRVTITA DKSTSTAYMELSSLRSEDTAVYYC ARYSG STPYWYFD VWGQGTITVTVS S
117	huAb18VH.1a, huAb18v3, huAb18v8, and huAb18v9 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYSF TSYTI HWVRQAPGQGLEWI GYINPNSRNTDYN QKFK DR TT LTA DRSTSTAYMELSSLRSEDTAVYYC ARYSG STPYWYFD VWGQGTITVTVS S
118	huAb18VH.1b, huAb18v2, huAb18v4, huAb18v6, huAb18v7, and huAb18v10 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYSF TSYTI HWVRQAPGQGLEWM GYINPNSRNTDY AQKFQ GRVTLTA DKSTSTAYMELSSLRSEDTAVYYC ARYSG STPYWYFD VWGQGTITVTVS S
119	huAb18VH.1b VH CDR2 amino acid sequence	YINPNSRNTDYAQKFQG
120	huAb18VL.1, huAb18v1, and huAb18v2 VL amino acid sequence	DIQLTQSPSFLSASVGDRV TI TCR ASSSVSYMN WYQQKPGKAPK LLI Y ATSNLAS GVPSRFS SGSG SGTEFTL TISSLQPEDFATYYC QQWSSNPLT FGQGTKLEIK
121	huAb18VL.1a, huAb18v3, and huAb18v4 VL amino acid sequence	DIQLTQSPSFLSASVGDRV TI TCR ASSSVSYMN WYQQKPGKSPK PW IY ATSNLAS GVPSRFSVSVSGTEHTL TISSLQPEDFATYYC QQWSSNPLT FGQGTKLEIK
122	huAb18VL.1b, huAb18v8, and huAb18v10 VL amino acid sequence	DIQLTQSPSFLSASVGDRV TI TCR ASSSVSYMN WYQQKPGKAPK PW IY ATSNLAS GVPSRFSVSGSGTEHTL TISSLQPEDFATYYC QQWSSNPLT FGQGTKLEIK
123	huAb18VL.2, huAb18v5, and huAb18v6 VL amino acid sequence	EIVLTQSPDFQSVTPKEK VT ITCR ASSSVSYMN WYQQKPDQSPK LLI K ATSNLAS GVPSRFS SGSG SGTDFTL TINSLEAEDAATYYC QQWSSNPLT FGQGTKLEIK
124	huAb18VL.2a, huAb18v7, and huAb18v9 VL amino acid sequence	EIVLTQSPDFQSVTPKEK VT ITCR ASSSVSYMN WYQQKPDQSPK PW IY ATSNLAS GVPSRFSVSVSGTDHTL TIN ST LEAEDAATYYC QQWSSNPLT

SEQ ID NO:	Description	Amino Acid Sequence
		TINDDEAEADATTTTCQGWSSNFTT FGQGTKLEIK
125	huAb3VH.1, huAb3v1, and huAb3v4 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYWMHWVRQAPGQGLEWM GLIHPDSGSTNYNEMFKNRVTITA DKSTSTAYMELSSLRSEDTAVYYC ARGGRLYFDYWGQGTTVTVSS
126	huAb3VH.1a, huAb3v3, and huAb3v6 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYWMHWVRQAPGQGLEWI GLIHPDSGSTNYNEMFKNRATLTV DRSTSTAYVELSSLRSEDTAVYFC AGGGRLYFDYWGQGTTVTVSS
127	huAb3VH.1b, huAb3v2, and huAb3v5 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYWMHWVRQAPGQGLEWI GLIHPDSGSTNYNEMFKNRATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDYWGQGTTVTVSS
128	huAb3VL.1, huAb3v1, and huAb3v2 VL amino acid sequence	DIVMTQSPLSLPVTPGEPASISCR SSQSLVHSNGDTYLRWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIK
129	huAb3VL.1a and huAb3v3 VL amino acid sequence	DVVMTQSPLSLPVTPGEPASISCR SSQSLVHSNGDTYLRWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYFCQS THVPYTFGGGTKVEIK
130	huAb3VL.1b, huAb3v4, huAb3v5, and huAb3v6 VL amino acid sequence	DVVMTQSPLSLPVTPGEPASISCR SSQSLVHSNGDTYLRWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIK
131	huAb3v2.1, huAb3v2.2, and huAb3v2.3 VH amino acid sequence	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYWMHWVRQAPGQGLEWI GLIHPWSGSTNYNEMFKNRATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDYWGQGTTVTVSS
132	huAb3v2.1, huAb3v2.2, and huAb3v2.3 VH CDR2 amino acid sequence	LIHPWSGSTNYNEMFKN
133	huAb3v2.1, huAb3v2.4, and huAb3v2.7 VL amino acid sequence	DIVMTQSPLSLPVTPGEPASISCR SSQSLVHSSGDTYLRWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIK

SEQ ID NO:	Description	Amino Acid Sequence
134	huAb3v2.1, huAb3v2.4, and huAb3v2.7 VL CDR1 amino acid sequence	RSSQSLVHSSGDTYLR
135	huAb3v2.2, huAb3v2.5, and huAb3v2.8 VL amino acid sequence	DIVMTQSP ~~LS~~ LPVTPGEPASISCR SSQSLVHSNRDTYLR WYLQKPGQS PQLLIY KVSNRFS GVDPDRFSGSGS GTDFTLKISRVEAEDVGVYYC CSQS THVPYTF GGG TKVEIK
136	huAb3v2.2, huAb3v2.5, and huAb3v2.8 VL CDR1 amino acid sequence	RSSQSLVHSNRDTYLR
137	huAb3v2.3, huAb3v2.6, and huAb3v2.9 VL amino acid sequence	DIVMTQSP ~~LS~~ LPVTPGEPASISCR SSQSLVHSNQDTYLR WYLQKPGQS PQLLIY KVSNRFS GVDPDRFSGSGS GTDFTLKISRVEAEDVGVYYC CSQS THVPYTF GGG TKVEIK
138	huAb3v2.3, huAb3v2.6, and huAb3v2.9 VL CDR1 amino acid sequence	RSSQSLVHSNQDTYLR
139	huAb3v2.4, huAb3v2.5, and huAb3v2.6 VH amino acid sequence	EVQLVQSGAEVKKPGSSSVKVSCKA SGYTFSSYWMH WVRQAPGQGLEWI GLIHPESGSTNYNEMFKN RATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDY WGQGT TVTVSS
140	huAb3v2.4, huAb3v2.5, and huAb3v2.6 VH CDR2 amino acid sequence	LIHPESGSTNYNEMFKN
141	huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH amino acid sequence	EVQLVQSGAEVKKPGSSSVKVSCKA SGYTFSSYWMH WVRQAPGQGLEWI GLIHPISGSTNYNEMFKN RATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDY WGQGT TVTVSS
142	huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR2 amino acid sequence	LIHPISGSTNYNEMFKN
143	huAb13VL.1, huAb13v2, huAb13v5, and huAb13v7 VL amino acid sequence	
144	huAb13VL.1a, huAb13v1, huAb13v3, and huAb13v8 VL amino acid sequence	DIQMTQSPSSLSASVGDRV ITCK ASQNVGFNVAWY QQKPGKAPKLLI YSASYRYS GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC QQYNWYPF TFGQGTKLEIK DIQMTQSPSSLSASVGDRV ITCK ASQNVGFNVAWY QQKPGKSPKALI YSASYRYS GVPSRFSGSGSGTDFT LTISSLQPEDFAEYFC QQYNWYPF TFGQGTKLEIK DIQMTQSPSSLSASVGDRV ITCK ASQNVGFNVAWY QQKPGKAPKLLI YSASYRYS GVPSRFSGSGSGTDFT LTISSLQPEDFATYFC QQYNWYPF

SEQ ID NO:	Description	Amino Acid Sequence
		TFGQGTKLEIK
145	huAB13VL.1b, huAb13v4, huAb13v6, and huAb13v9 VL amino acid sequence	
146	huAb13VH.1, huAb13v2, huAb13v3, and huAb13v4 VH amino acid sequence	EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPGKGLEW IGYIHSSGSTNYNPSLKSRVTISV DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTTVTVSS
147	huAb13VH.1a, huAb13v1, huAb13v5, and huAb13v6 VH amino acid sequence	EVQLQESGPGGLVKPSETLSLTCAV TGYSTITSGYSWHWIRQFPNGLEW MGYIHSSGSTNYNPSLKSRISISR DTSKNQFFLKLSSVTAADTAVYYC AGYDDYFEYWGQGTTVTVSS
148	huAb13VH.1b, huAb13v7, huAb13v8, and huAb13v9 VH amino acid sequence	EVQLQESGPGGLVKPSETLSLTCAV SGYSITSGYSWHWIRQPPNGLEW MGYIHSSGSTNYNPSLKSRITISR DTSKNQFSLKLSSVTAADTAVYYC ARYDDYFEYWGQGTTVTVSS
149	B7-H3 amino acid sequence (human)	MLRRRGSPGMGVHVGAAALGALWFC LTGALEVQVPEDPVVALVGTDATL CCSFSPGPGFSLAQLNLIWQLTDT KQLVHSFAEGQDQGSAYANRTALF PDLLAQGNASLRLQVRVVADEGSF TCFVSIRDFGSAAVSLQVAAPYSK PSMTLEPNKDLRPGDTVITITCSSY QGYPEAEVFWQDGQGVPLTGNVTT SQMANEQGLFDVHSILRVVLGANG TYSCLVRNPVLQQDAHSSVTITPQ RSPTGAVEVQVPEDPVVALVGTD TLRCSFSPEPGFSLAQLNLIWQLT DTKQLVHSFTEGRDQGSAYANRTA LFPDLLAQGNASLRLQVRVVADEG SFTCFVSIRDFGSAAVSLQVAAPY SKPSMTLEPNKDLRPGDTVITITCS SYRGYPEAEVFWQDGQGVPLTGNV TTSQMANEQGLFDVHSVLRVVLGA NGTYSCLVRNPVLQQDAHGSVTIT GQPMTFPPEALWTVGLSVCLIAL LVALAFVCWRKIKQSCEENAGAE DQDGEGEGSKTALQPLKHSDSKED DGQEIA
150	Human B7-H3-ECD (fc fusion) Note: Fc sequence is underlined	MLRRRGSPGMGVHVGAAALGALWFC LTGALEVQVPEDPVVALVGTDATL CCSFSPGPGFSLAQLNLIWQLTDT KQLVHSFAEGQDQGSAYANRTALF PDLLAQGNASLRLQVRVVADEGSF TCFVSIRDFGSAAVSLQVAAPYSK PSMTLEPNKDLRPGDTVITITCSSY QGYPEAEVFWQDGQGVPLTGNVTT SQMANEQGLFDVHSILRVVLGANG TYSCLVRNPVLQQDAHSSVTITPQ RSPTGAVEVQVPEDPVVALVGTD TLRCSFSPEPGFSLAQLNLIWQLT DTKOLVHSFTEGRDOGSAYANRTA

SEQ ID NO:	Description	Amino Acid Sequence
		<u>LFPDLLAQGNASLRLQVRVVADEG</u> <u>SFTCFVSIRDFGSAAVSLQVAAPY</u> <u>SKPSMTLEPNKDLRPGDTVITITCS</u> <u>SYRGYPEAEVFWQDGQGVPLTGNV</u> <u>TTSQMANEQGLFDVHSVLRVVLGA</u> <u>NGTYSCLVRNPVLQQDAHGSVTIT</u> <u>GQPMTFAAADKHTCPCPAPEAE</u> <u>GAPSVFLFPPKPKDTLMISRTPEV</u> <u>TCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLT</u> <u>VLHQDWLNGKEYKCKVSNKALPAP</u> <u>IEKTIISKAKGQPREPQVYTLPPSR</u> <u>EEMTKNQVSLTCLVKGFYPSDIAV</u> <u>EWESNGQPENNYKTPPVLDSDGS</u> <u>FFLYSKLTVDKSRWQQGNVFSCSV</u> <u>MHEALHNHYTQKSLSLSPGK</u>
151	Mouse B7-H3-ECD (fc fusion) Note: Fc sequence is underlined	<u>MLRGWGGPSVGVCVRTALGVLCLC</u> <u>LTGAVEVQVSEDPVVALVDTDATL</u> <u>RCSFSPEPGFSLAQLNLIWQLTDT</u> <u>KQLVHSFTEGRDQGSAYSNRTALF</u> <u>PDLLVQGNASLRLQVRVVTDEGSY</u> <u>TCFVSIQDFDSAAVSLQVAAPYSK</u> <u>PSMTLEPNKDLRPGNMVTITCSSY</u> <u>QGYPEAEVFWKDGQGVPLTGNVTT</u> <u>SQMANERGLFDVHSVLRVVLGANG</u> <u>TYSCLVRNPVLQQDAHGSVTITGQ</u> <u>PLTFAAADKHTCPCPAPEAEGA</u> <u>PSVFLFPPKPKDTLMISRTPEVTC</u> <u>VVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVL</u> <u>HQDWLNGKEYKCKVSNKALPAPIE</u> <u>KTISKAKGQPREPQVYTLPPSREE</u> <u>MTKNQVSLTCLVKGFYPSDIAVEW</u> <u>ESNGQPENNYKTPPVLDSDGSFF</u> <u>LYSKLTVDKSRWQQGNVFSCSVMH</u> <u>EALHNHYTQKSLSLSPGK</u>
152	Human B7-H3-ECD (his tag)	<u>MEFGLSWLFLVAILKGVQC</u> GALEV QVPEDPVVALVGTDATLCCSFSPE PGFSLAQLNLIWQLTDTKQLVHSF AEGQDQGSAYANRTALFPDLLAQG NASLRLQVRVVADEGSFTCFVSIR DFGSAAVSLQVAAPYSKPSMTLEP NKDLRPGDTVITITCSSYQGYPEAE VFWQDGQGVPLTGNVTTSQMANEQ GLFDVHSILRVVLGANGTYSCLVR NPVLQQDAHSSVTITPQRSPTGAV EVQVPEDPVVALVGTDATLRCSFS PEPGFSLAQLNLIWQLTDTKQLVH SFTEGRDQGSAYANRTALFPDLLA QGNASLRLQVRVVADEGSFTCFVS IRDFGSAAVSLQVAAPYSKPSMTL EPNKDLRPGDTVITITCSSYRGYPE AEVFWQDGQGVPLTGNVTTSQMAN EQGLEFDVHSVLRVVLGANGTYSCL VRNPVLQQDAHGSVTITGQPMTHH HHHH
153	Mouse B7-H3-ECD (his tag)	<u>MEFGLSWLFLVAILKGVQC</u> VEVQV SEDPVVALVDTDATLRCSFSPEPG FSLAQLNLIWQLTDTKQLVHSFTE GRDQGSAYSNRTALFPDLLVQGNA STRLQVRVVTDEGSYTCFVSTQDF

SEQ ID NO:	Description	Amino Acid Sequence
		DSAAVSLQVAAPYSKPSMTLEPNK DLRPGNMVTITCSSYQGYPEAEVF WKDGQGVPLTGNVTTSQMANERGL FDVHSVLRVVLGANGTYSCLVRNP VLQQDAHGSVTITGQPLTFHHHHH H
154	Cynomolgus B7-H3-ECD (his tag)	MLHRRGSPGMGVHVGAAALGALWFC LTGALEVQVPEDPVVALVGTDATL RCSFSPEPGFSLAQLNLIWQLTDT KQLVHSFTEGRDQGSAYANRTALF LDLLAQGNASLRLQRRVADEGSF TCFVSIRDFGSAAVSLQVAAPYSK PSMTLEPNKDLRPGDTVITITCSSY RGYPEAEVFWQDGQGAPLTGNVTT SQMANEQGLEFDVHSVLRVVLGANG TYSCLVRNPVLQQDAHGSITITPQ RSPTGAVEVQVPEDPVVALVGTD TLRCSF SPEPGFSLAQLNLIWQLTDTKQLV HSFTEGRDQGSAYANRTALFLDLL AQGNASLRLQRRVADEGSFTCFV SIRDFGSAAVSLQVAAPYSKPSMT LEPNKDLRPGDTVITITCSSYRGYP EAEVFWQDGQGAPLTGNVTTSQMA NEQGLEFDVHSVLRVVLGANGTYS CLVRNPVLQQDAHGSVTITGQPMTF AAAHHHHHHHH
155	Amino acid sequence of IGHV1-69*06	QVQLVQSGAEVKKPGSSVKVSCKA SGGTFSSYAISWVRQAPGGLEWM GGIIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYC AR
156	Amino acid sequence of IGHJ6*01	WGQGTTVTVSS
157	Amino acid sequence of IGKV1-9*01	DIQLTQSPSFLSASVGDRVITICR ASQGISSYLAWYQQKPGKAPKLLI YAASTLQSGVPSRFSGSGSGTEFT LTISSLQPEDFATYYCQQLNSYPP
158	Amino acid sequence of IGKJ2*01	FGQGTKLEIK
159	Ig gamma-1 constant region	ASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTCTPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
160	Ig gamma-1 constant region mutant	ASTKGPSVFPLAPSSKSTSGGTAA

SEQ ID NO:	Description	Amino Acid Sequence
		LGCLVKDYFPEPVTVSWNSGALT'S GVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
161	Ig Kappa constant region	RTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYLSSTL TLISKADYEKKVYACEVTHQGLSS PVTKSFNRGEC
162	Ig Lambda constant region	QPKAAPSVTLFPPSSEELQANKAT LVCLISDFYPGAVTVAWKADSSPV KAGVETTTPSKQSNNKYAASSYLS LTPEQWKSHRYSQCQVTHEGSTVE KTVAPTECS
163	Amino acid sequence of IGKV6-21*01	EIVLTQSPDFQSVTPKEKVTITCR ASQSIGSSLHWYQQKPDQSPKLLI KYASQSESGVPSRFSGSGSGTDFT LTINSLEAEDAATYYCHQSSSLPX
164	Amino acid sequence of IGKV2-28*01	DIVMTQSPPLSLPVTPGEPASISCR SSQSLIHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCMQA LQTPP
165	Amino acid sequence of IGKJ4*01	FGGGTKVEIK
166	Amino acid sequence of IGHV-b*01(0-1)	QVQLQESGPGLVKPSSETLSLTCAV SGYSISSGYWGWIRQPPGKGLEW IGSIYHSGSTYYNPSLKSRVTISV DTSKNQFSLKLSSVTAADTAVYYC AR
167	Amino acid sequence of IGKv1-39*01	DIQMTQSPSSLSASVGDRVITITCR ASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSYSTPP
168	Amino acid sequence of huAb13v1 heavy chain Note: Ig gamma-1 constant region mutant sequence is underlined.	EVQLQESGPGLVKPSSETLSLTCAV TGYSITSGYSWHWIRQFPNGLEW MGYIHSSGSTNYNPSLKSRISISR DTSKNQFFLKLSSVTAADTAVYYC AGYDDYFEYWGQGT ^{TVTVSS} ASTK GPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHT

SEQ ID NO:	Description	Amino Acid Sequence
		<u>FPAVLQSSGLYSLSSVVTVPSSSL</u> <u>GTQTYICNVNHKPSNTKVDKKVEP</u> <u>KSCDKTHTCPPCPAPEAAGGPSVF</u> <u>LFPPKPKDTLMISRTPEVTCVVVD</u> <u>VSHEDPEVKFNWYVDGVEVHNAKT</u> <u>KPREEQYNSTYRVVSVLTVLHQDW</u> <u>LNGKEYKCKVSNKALPAPIEKTIS</u> <u>KAKGQPREPQVYTLPPSREEMTKN</u> <u>QVSLTCLVKGFYPSDIAVEWESNG</u> <u>QPENNYKTTTPVLDSDGSFFLYSK</u> <u>LTVDKSRWQQGNVFSCSVMHEALH</u> <u>NHYTQKSLSLSPGK</u>
169	Amino acid sequence of huAb13v1 light chain	<u>DIQMTQSPSSLSASVGDRVTITCK</u> <u>ASQNVGFNVAWYQQKPGKSPKALI</u> <u>YSASYRYSGVPSRFSGSGSGTDEFT</u> <u>LTISSLQPEDFAEYFCQQYNWYPF</u> <u>TFGQGTKLEIKRTVAAPSVFI</u> <u>FPP</u> <u>SDEQLKSGTASVVCLLNNFYPREA</u> <u>KVQWKVDNALQSGNSQESVTEQDS</u> <u>KDSTYSLSSSTLTLSKADYEKHKVY</u> <u>ACEVTHQGLSSPVTKSFNRGEC</u>
170	Amino acid sequence of huAb3v2.5 heavy chain	<u>EVQLVQSGAEVKKPGSSVKVSCKA</u> <u>SGYTFSSYWMHWVRQAPGQGLEWI</u> <u>GLIHPESGSTNYNEMFKNRATLTV</u> <u>DRSTSTAYMELSSLRSEDTAVYYC</u> <u>AGGGRLYFDYWGQGT</u> <u>TVTVSSAST</u> <u>KGPSVFPLAPSSKSTSGGTAALGC</u> <u>LVKDYFPEPVTVSWNSGALTSGVH</u> <u>T</u> <u>FPAVLQSSGLYSLSSVVTVPSSS</u> <u>LGTQTYICNVNHKPSNTKVDKKVE</u> <u>PKSCDKTHTCPPCPAPEAAGGPSV</u> <u>FLFPPKPKDTLMISRTPEVTCVVV</u> <u>DVSHEDPEVKFNWYVDGVEVHNAK</u> <u>TKPREEQYNSTYRVVSVLTVLHQD</u> <u>WLNGKEYKCKVSNKALPAPIEKT</u> <u>I</u> <u>SKAKGQPREPQVYTLPPSREEMTK</u> <u>NQVSLTCLVKGFYPSDIAVEWESN</u> <u>GQPENNYKTTTPVLDSDGSFFLYS</u> <u>KLTVDKSRWQQGNVFSCSVMHEAL</u> <u>HNHYTQKSLSLSPGK</u>
171	Amino acid sequence of huAb3v2.5 light chain	<u>DIVMTQSPPLSLPVTPGEPASISCR</u> <u>SSQSLVHSNRDTYLRWYLQKPGQS</u> <u>PQLLIYKVSNRFSGVPDRFSGSGS</u> <u>GTDEFTLKISRVEAEDVGVYYCSQS</u> <u>THVPYTFGGGTKVEIKRTVAAPSV</u> <u>F</u> <u>IFPPSDEQLKSGTASVVCLLNNF</u> <u>YPREAKVQWKVDNALQSGNSQESV</u> <u>TEQDSKDSTYSLSSSTLTLSKADYE</u> <u>KHKVYACEVTHQGLSSPVTKSFNR</u> <u>GEC</u>
172	Amino acid sequence of huAb3v2.6 heavy chain	<u>EVQLVQSGAEVKKPGSSVKVSCKA</u> <u>SGYTFSSYWMHWVRQAPGQGLEWI</u> <u>GLIHPESGSTNYNEMFKNRATLTV</u> <u>DRSTSTAYM</u> <u>ELSSLRSEDTAVYYCAGGGRLYFD</u> <u>YWGQGT</u> <u>TVTVSSASTKGPSVFPLA</u> <u>PSSKSTSGGTAALGCLVKDYFPEP</u>

SEQ ID NO:	Description	Amino Acid Sequence
	Note: Ig gamma-1 constant region mutant sequence is underlined.	<u>VTVSWNSGALTSGVHTFFPAVLQSS</u> <u>GLYSLSSVVTVPSSSLGTQTYICN</u> <u>VNHKPSNTKVDKKVEPKSCDKTHT</u> <u>CPPCPAPEAAGGPSVFLFPPKPKD</u> <u>TLMISRTPEVTCVVVDVSHEDPEV</u> <u>KFNWYVDGVEVHNAKTKPREEQYN</u> <u>STYRVVSVLTVLHQDWLNGKEYKC</u> <u>KVSNKALPAPIEKTISKAKGQPRE</u> <u>PQVYTLPPSREEMTKNQVSLTCLV</u> <u>KGFYPSDIAVEWESNGQPENNYKT</u> <u>TPPVLDSDGSFFLYSKLTVDKSRW</u> <u>QQGNVFSCSVMHEALHNHYTQKSL</u> <u>SLSPGK</u>
173	Amino acid sequence of huAb3v2.6 light chain	DIVMTQSP ¹ LSLPVTPGEPASISCR SSQSLVHSNQDTYLRWYLQKPGQS PQLLIYKVS ² NRFS ³ GV ⁴ PDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIKRTVAAPSV <u>EIEPPSDEQLKSGTASVVCLLNNE</u> <u>YPREAKVQWKVDNALQSGNSQESV</u> <u>TEQDSKDS</u> TYSLSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNR <u>GEC</u>
174	Amino acid sequence of IGHV1-69*06_IGHJ6	QVQLVQSGAEVKKPGSSVKVSCKA SGGTFFSYAISWVRQAPGQGLEWM GGIIPITFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYC ARXXXXXXXXXWGQGTTVTVSS
175	Amino acid sequence of IGKV2-28*01_IGKJ4	DIVMTQSP ¹ LSLPVTPGEPASISCR SSQSL ² LHSGNYLDWYLQKPGQS PQLLIYLGSNRASGV ⁴ PDRFSGSGS GTDFTLKISRVEAEDVGVYYCXXX XXXXXXFGGGTKVEIK
176	Amino acid sequence of IGHV4-b_IGHJ6	QVQLQESGPGLVKPSETLSLTCAV SGYSISSGYWGWIRQPPGKGLEW IGSIYHSGSTYYNPSL ³ KSRVTISV DTSKNQFSLKLSSVTAADTAVYYC ARXXXXXXXXXWGQGTTVTVSS
177	Amino acid sequence of IGKV1-39_IGKJ2	DIQMTQSP ¹ SSLSASVGDRVTITCR ASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCXXXXXXXXX XFGQGTKLEIK
178	Amino acid sequence of huAb3 VL1 variants Note: X can be any amino acid except: M, C, N, D, or Q	DIVMTQSP ¹ LSLPVTPGEPASISCR SSQSLVHSXGDTYLRWYLQKPGQS PQLLIYKVS ² NRFS ³ GV ⁴ PDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIK
179	Amino acid sequence of huAb3 VL1 variants Note:	

SEQ ID NO:	Description	Amino Acid Sequence
	X can be any amino acid except: M, C, G, S, N, or P	DIVMTQSPFLSLPVTGPGEPAISCR SSQSLVHSNXTYLRWYLQKPGQS PQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQS THVPYTFGGGTKVEIK
180	Amino acid sequence of huAb3 VH1b variants Note: X can be any amino acid except: M, C, N, D, or Q	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFESSYWMHWVRQAPGQGLEWI GLIHFXSGSTNYNEMFKNRATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDYWGQGTTVTVSS
181	Amino acid sequence of huAb3 VH1b variants Note: X can be any amino acid except: M, C, G, S, N, or P	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFESSYWMHWVRQAPGQGLEWI GLIHFDXGSTNYNEMFKNRATLTV DRSTSTAYMELSSLRSEDTAVYYC AGGGRLYFDYWGQGTTVTVSS
182	chAb13 VL CDR3 amino acid sequence	QQYNSYPFT

SEQUENCE LISTING

[0348]

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- <130> 117813-12620
- <140>
- <141>
- <150> US 62/347,476
- <151> 2016-06-08
- <150> US 62/366,511
- <151> 2016-07-25
- <160> 182
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- <210> 1
- <211> 120
- <212> PRT
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- <220>
- <223> Synthetic: chAb2 VH amino acid sequence

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Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Met Ile His Pro Asp Ser Gly Thr Thr Asn Tyr Asn Glu Lys Phe
 50 55 60

Arg Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Ala Val Tyr Tyr Gly Ser Thr Tyr Trp Tyr Phe Asp Val Trp Gly Thr
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 2

<211> 10

<212> PRT

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<223> Synthetic: chAb2 VH CDR1 amino acid sequence

<400> 2

Gly Tyr Thr Phe Thr Ser Tyr Trp Met His
 1 5 10

<210> 3

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb2 VH CDR2 amino acid sequence

<400> 3

Met Ile His Pro Asp Ser Gly Thr Thr Asn Tyr Asn Glu Lys Phe Arg
 1 5 10 15

Ser

<210> 4

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb2 VH CDR3 amino acid sequence

<400> 4

Tyr Tyr Gly Ser Thr Tyr Trp Tyr Phe Asp Val

1 - - - 5 - - - 10 -

<210> 5

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb2 VL amino acid sequence

<400> 5

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Tyr Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ile
20 25 30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Arg Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Phe Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 6

<211> 16

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<223> Synthetic: chAb2 VL CDR1 amino acid sequence

<400> 6

Arg Ser Ser Gln Ser Leu Val His Ile Asn Gly Asn Thr Tyr Leu His
1 5 10 15

<210> 7

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb2, chAb3, chAb10, huAb3VL.1, huAb3VL.1a, huAb3VL.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VL CDR2 amino acid sequence

<400> 7

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 8

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb2 VL CDR3 amino acid sequence

<400> 8

Ser	Gln	Ser	Thr	His	Phe	Pro	Phe	Thr
1				5				

<210> 9

<211> 117

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<213> Artificial Sequence

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<223> Synthetic: chAb3 VH amino acid sequence

<400> 9

Gln	Val	Gln	Leu	Gln	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala
1			5					10						15	

Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
		20					25						30		

Trp	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
	35					40						45			

Gly	Leu	Ile	His	Pro	Asp	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Glu	Met	Phe
	50					55				60					

Lys	Asn	Lys	Ala	Thr	Leu	Thr	Val	Asp	Arg	Ser	Ser	Ser	Thr	Ala	Tyr
65					70				75					80	

Val	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
			85					90						95	

Ala	Gly	Gly	Gly	Arg	Leu	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Leu	Thr	Val	Ser	Ser
			115	

<210> 10

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<223> Synthetic: chAb3, huAb3VH.1, huAb3VH.1a, huAb3VH.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR1 amino acid sequence

<400> 10

Gly	Tyr	Thr	Phe	Ser	Ser	Tyr	Trp	Met	His
1				5				10	

<210> 11

<211> 17

<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic: chAb3, huAb3VH.1, huAb3VH.1a, and huAb3VH.1b VH CDR2 amino acid sequence

<400> 11
Leu Ile His Pro Asp Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe Lys
1 5 10 15

Asn

<210> 12
<211> 8
<212> PRT
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<220>
<223> Synthetic: chAb3, huAb3VH.1, huAb3VH.1a, huAb3VH.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR3 amino acid sequence

<400> 12
Gly Gly Arg Leu Tyr Phe Asp Tyr
1 5

<210> 13
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb3 VL amino acid sequence

<400> 13
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Thr Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 14
<211> 16
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<220>
<223> Synthetic: chAb3, huAb3VL.1, huAb3VL.1a, and huAb3VL.1b VL CDR1 amino acid sequence

<400> 14

Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser	Asn	Gly	Asp	Thr	Tyr	Leu	Arg
1				5					10					15	

<210> 15

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb3, huAb3VL.1, huAb3VL.1a, huAb3VL.1b, huAb3v2.1, huAb3v2.2, huAb3v2.3, huAb3v2.4, huAb3v2.5, huAb3v2.6, huAb3v2.7, huAb3v2.8, and huAb3v2.9 VL CDR3 amino acid sequence

<400> 15

Ser	Gln	Ser	Thr	His	Val	Pro	Tyr	Thr
1				5				

<210> 16

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VH amino acid sequence

<400> 16

Gln	Val	Gln	Leu	Gln	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala
1				5					10					15	

Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Met	Ile	His	Pro	Asn	Ser	Gly	Ser	Asn	Asn	Tyr	Asn	Glu	Lys	Phe
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

50

55

60

Lys	Ser	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Asn	Thr	Ala	Tyr
65					70					75				80	

Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85						90					95	

Ala	Arg	Arg	Leu	Gly	Leu	His	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Leu	Thr	Val	Ser	Ser
			115	

<210> 17

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VH CDR1 amino acid sequence

<400> 17

Gly Tyr Ser Phe Thr Ser Tyr Trp Met His
1 5 10

<210> 18

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VH CDR2 amino acid sequence

<400> 18

Met Ile His Pro Asn Ser Gly Ser Asn Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15

Ser

<210> 19

<211> 8

<212> PRT

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<223> Synthetic: chAb4 VH CDR3 amino acid sequence

<400> 19

Arg Leu Gly Leu His Phe Asp Tyr
1 5

<210> 20

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VL amino acid sequence

<400> 20

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Pro Val Gly
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Met Gln Ser
65 70 75 80

Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Ser Ser Tyr Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 21

<211> 11

<212> PRT

<213> Artificial Sequence

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<223> Synthetic: chAb4 VL CDR1 amino acid sequence

<400> 21

Lys	Ala	Ser	Gln	Asn	Val	Gly	Thr	Ala	Val	Ala
1				5					10	

<210> 22

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VL CDR2 amino acid sequence

<400> 22

Ser	Ala	Ser	Asn	Arg	Tyr	Thr
1			5			

<210> 23

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb4 VL CDR3 amino acid sequence

<400> 23

Gln	Gln	Tyr	Ser	Ser	Tyr	Pro	Tyr	Thr
1			5					

<210> 24

<211> 121

<212> PRT

<213> Artificial Sequence

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<223> Synthetic: chAb18 VH amino acid sequence

<400> 24

Gln	Val	Gln	Leu	Gln	Gln	Ser	Ala	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala
1			5						10					15	

Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Ser	Tyr
			20					25					30		

Thr	Ile	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35				40						45			

Gly	Tyr	Ile	Asn	Pro	Asn	Ser	Arg	Asn	Thr	Asp	Tyr	Asn	Gln	Lys	Phe
	50					55					60				

Lys	Asp	Glu	Thr	Thr	Leu	Thr	Ala	Asp	Arg	Ser	Ser	Ser	Thr	Ala	Tyr
65					70				75						80

Met	Gln	Leu	Ile	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala Arg Tyr Ser Gly Ser Thr Pro Tyr Trp Tyr Phe Asp Val Trp Gly
100 105 110

Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 25
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<220>
<223> Synthetic: chAb18, huAb18VH.1, huAb18VH.1a, and huAb18VH.1b VH CDR1 amino acid sequence

<400> 25
Gly Tyr Ser Phe Thr Ser Tyr Thr Ile His
1 5 10

<210> 26
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb18, huAb18VH.1, and huAb18VH.1a VH CDR2 amino acid sequence

<400> 26
Tyr Ile Asn Pro Asn Ser Arg Asn Thr Asp Tyr Asn Gln Lys Phe Lys
1 5 10 15

Asp

<210> 27
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb18, huAb18VH.1, huAb18VH.1a, and huAb18VH.1b VH CDR3 amino acid sequence

<400> 27
Tyr Ser Gly Ser Thr Pro Tyr Trp Tyr Phe Asp Val
1 5 10

<210> 28
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb18 VL amino acid sequence

<400> 28
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Val Ser
50 55 60

Val Ser Gly Thr Ser His Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 29
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR1 amino acid sequence

<400> 29
Arg Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

<210> 30
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR2 amino acid sequence

<400> 30
Ala Thr Ser Asn Leu Ala Ser
1 5

<210> 31
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<220>
<223> Synthetic: chAb18, huAb18VL.1, huAb18VL.1a, huAb18VL.1b, huAb18VL.2, and huAb18VL.2a, VL CDR3 amino acid sequence

<400> 31
Gln Gln Trp Ser Ser Asn Pro Leu Thr
1 5

<210> 32
<211> 116
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic: chAb13 VH amino acid sequence

<400> 32

Asp Val Gln Leu Gln Glu Ser Gly Pro Asp Leu Val Lys Pro Ser Gln
1 5 10 15

Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Ser Trp His Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
35 40 45

Met Gly Tyr Ile His Ser Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Ile Ser Ile Asn Arg Asp Thr Ser Lys Asn Gln Phe Phe
65 70 75 80

Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Gly Tyr Asp Asp Tyr Phe Glu Tyr Trp Gly Gln Gly Thr Thr Leu
100 105 110

Thr Val Ser Ser
115

<210> 33

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VH CDR1 amino acid sequence

<400> 33

Gly Tyr Ser Ile Thr Ser Gly Tyr Ser Trp His
1 5 10

<210> 34

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9VH CDR2 amino acid sequence

<400> 34

Tyr Ile His Ser Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 35

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb13, huAb13Vh.1, huAb13Vh.1a, huAb13Vh.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VH CDR3 amino acid sequence

<400> 35
Tyr Asp Asp Tyr Phe Glu Tyr
1 5

<210> 36
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb13 VL amino acid sequence

<400> 36
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Phe Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Phe
85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 37
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb13, huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR1 amino acid sequence

<400> 37
Lys Ala Ser Gln Asn Val Gly Phe Asn Val Ala
1 5 10

<210> 38
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb13, huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR2 amino acid sequence

<400> 38
Asp Val Asp Met Ser Val Met Val Glu

Ser Ala Ser Tyr Arg Tyr Ser
1 5

<210> 39

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VL.1, huAb13VL.1a, huAb13VL.1b, huAb13v1, huAb13v2, huAb13v3, huAb13v4, huAb13v5, huAb13v6, huAb13v7, huAb13v8, and huAb13v9 VL CDR3 amino acid sequence

<400> 39

Gln Gln Tyr Asn Trp Tyr Pro Phe Thr
1 5

<210> 40

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VH amino acid sequence

<400> 40

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Ser Gly Thr Asn Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Arg Tyr Ser Trp Ile Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 41

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VH CDR1 amino acid sequence

<400> 41

Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
1 5 10

<210> 42

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VH CDR2 amino acid sequence

<400> 42

Thr	Ile	Ser	Ser	Gly	Thr	Asn	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 43

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VH CDR3 amino acid sequence

<400> 43

Gln	Gly	Arg	Tyr	Ser	Trp	Ile	Ala	Tyr
1				5				

<210> 44

<211> 110

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VL amino acid sequence

<400> 44

Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1				5					10					15	

Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Lys	Ser	Val	Ser	Thr	Ser
			20					25					30		

Asp	Tyr	Ser	Tyr	Met	His	Trp	Asn	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro
		35					40					45			

Lys	Leu	Leu	Ile	Tyr	Leu	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro	Ala
	50					55					60				

Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His
65					70					75					80

Pro	Val	Glu	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Ser	Arg
				85					90					95	

Glu	Leu	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
			100					105					110

<210> 45

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VL CDR1 amino acid sequence

<400> 45

Arg	Ala	Ser	Lys	Ser	Val	Ser	Thr	Ser	Asp	Tyr	Ser	Tyr	Met	His
1				5					10					15

<210> 46

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 and chAb17 VL CDR2 amino acid sequence

<400> 46

Leu	Ala	Ser	Asn	Leu	Glu	Ser
1				5		

<210> 47

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb12 VL CDR3 amino acid sequence

<400> 47

Gln	His	Ser	Arg	Glu	Leu	Leu	Thr
1				5			

<210> 48

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb14 VH amino acid sequence

<400> 48

Glu	Val	Lys	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly
1				5					10					15	

Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Gly	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val
	35					40						45			

Ala	Thr	Ile	Ser	Gly	Gly	Gly	Thr	Asn	Thr	Tyr	Tyr	Pro	Asp	Ser	Val
	50					55				60					

Glu	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Phe	Leu	Tyr
65					70					75				80	

Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys
			85					90						95	

Ala	Arg	His	Tyr	Glu	Ser	Gln	Thr	Met	Asn	Tyr	Trp	Glu	Gln	Glu	Thr
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Met Arg Met Tyr Gly Ser Ser Thr Met Asp Tyr Asp Gly Ser Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 49
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 and chAb8 VH CDR1 amino acid sequence

<400> 49
Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser
1 5 10

<210> 50
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VH CDR2 amino acid sequence

<400> 50
Thr Ile Ser Gly Gly Gly Thr Asn Thr Tyr Tyr Pro Asp Ser Val Glu
1 5 10 15

Gly

<210> 51
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VH CDR3 amino acid sequence

<400> 51
His Tyr Gly Ser Gln Thr Met Asp Tyr
1 5

<210> 52
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VL amino acid sequence

<400> 52
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Gly Asn Ile His Asn Tyr
20 25 30

Leu Thr Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35 40 45

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Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Ile Met Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 53
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VL CDR1 amino acid sequence

<400> 53
Arg Thr Ser Gly Asn Ile His Asn Tyr Leu Thr
1 5 10

<210> 54
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VL CDR2 amino acid sequence

<400> 54
Asn Ala Lys Thr Leu Ala Asp
1 5

<210> 55
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb14 VL CDR3 amino acid sequence

<400> 55
Gln His Phe Trp Ser Ile Met Trp Thr
1 5

<210> 56
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VH amino acid sequence

<400> 56
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Arg Tyr
20 25 30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val His Tyr Cys
85 90 95

Ala Arg Arg Gly Tyr Gly Tyr Val Pro Tyr Ala Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 57
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VH CDR1 amino acid sequence

<400> 57
Gly Tyr Thr Phe Ser Arg Tyr Trp Ile Glu
1 5 10

<210> 58
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VH CDR2 amino acid sequence

<400> 58
Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15
Gly

<210> 59
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VH CDR3 amino acid sequence

<400> 59
Arg Gly Tyr Gly Tyr Val Pro Tyr Ala Leu Asp Tyr
1 5 10

<210> 60

<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VL amino acid sequence

<400> 60
Glu Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Ser
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Asn Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser Arg Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 61
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VL CDR1 amino acid sequence

<400> 61
Arg Ala Ser Gln Asp Ile Ser Asn Ser Leu Asn
1 5 10

<210> 62
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VL CDR2 amino acid sequence

<400> 62
Tyr Thr Ser Arg Leu Tyr Ser
1 5

<210> 63
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb6 VL CDR3 amino acid sequence

<400> 63

Gln Gln Gly Asn Thr Leu Pro Tyr Thr
 1 5

<210> 64

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VH amino acid sequence

<400> 64

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr Asn Tyr
 20 25 30

Tyr Met Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
 35 40 45

Gly Phe Ile Arg Asn Lys Ala Asn Asp Tyr Thr Thr Glu Tyr Ser Ala
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile
 65 70 75 80

Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr
 85 90 95

Tyr Cys Ala Arg Glu Ser Pro Gly Asn Pro Phe Ala Tyr Trp Gly Gln
 100 105 110

Gly Thr Leu Val Thr Val Ser Ala
 115 120

<210> 65

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VH CDR1 amino acid sequence

<400> 65

Gly Phe Thr Phe Thr Asn Tyr Tyr Met Ser
 1 5 10

<210> 66

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VH CDR2 amino acid sequence

<400> 66

Phe Ile Arg Asn Lys Ala Asn Asp Tyr Thr Thr Glu Tyr Ser Ala Ser
 1 5 10 15

Val Lys Gly

<210> 67

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VH CDR3 amino acid sequence

<400> 67

Glu Ser Pro Gly Asn Pro Phe Ala Tyr
1 5

<210> 68

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VL amino acid sequence

<400> 68

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Thr Gln Lys Asn Phe Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Asn
85 90 95

Asp Tyr Ile Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 69

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VL CDR1 amino acid sequence

<400> 69

Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Thr Gln Lys Asn Phe Leu
1 5 10 15

Thr

<210> 70

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VL CDR2 amino acid sequence

<400> 70

Trp	Ala	Ser	Thr	Arg	Glu	Ser
1				5		

<210> 71

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb11 VL CDR3 amino acid sequence

<400> 71

Gln	Asn	Asp	Tyr	Ile	Tyr	Pro	Leu	Thr
1				5				

<210> 72

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb16 VH amino acid sequence

<400> 72

Glu	Val	Lys	Leu	Leu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10						15	

Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asp	Phe	Ser	Arg	Tyr
	20							25					30		

Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
	35					40						45			

Gly	Glu	Ile	Asn	Pro	Asp	Ser	Ser	Thr	Ile	Asn	Tyr	Thr	Pro	Ser	Leu
	50					55					60				

Lys	Asp	Lys	Phe	Ile	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr
65				70					75					80	

Leu	Gln	Met	Ser	Lys	Val	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys
			85					90						95	

Ala	Arg	Pro	Gly	Phe	Gly	Asn	Tyr	Ile	Tyr	Ala	Met	Asp	Tyr	Trp	Gly
			100					105					110		

Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser
	115						120	

<210> 73

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAbl6 VH CDR1 amino acid sequence

<400> 73

Gly	Phe	Asp	Phe	Ser	Arg	Tyr	Trp	Met	Ser
1				5				10	

<210> 74

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAbl6 VH CDR2 amino acid sequence

<400> 74

Glu	Ile	Asn	Pro	Asp	Ser	Ser	Thr	Ile	Asn	Tyr	Thr	Pro	Ser	Leu	Lys
1				5					10					15	

Asp

<210> 75

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAbl6 VH CDR3 amino acid sequence

<400> 75

Pro	Gly	Phe	Gly	Asn	Tyr	Ile	Tyr	Ala	Met	Asp	Tyr
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

1				5							10
---	--	--	--	---	--	--	--	--	--	--	----

<210> 76

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb16 VL amino acid sequence

<400> 76

Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly
1				5					10					15	

Asp	Arg	Val	Thr	Ile	Asn	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Asn	Phe
			20					25					30		

Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu	Ile
		35					40					45			

Tyr	Tyr	Thr	Ser	Arg	Leu	Tyr	Leu	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					

Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	Asn	Leu	Glu	Gln
65					70					75				80	

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 77

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb16 VL CDR1 amino acid sequence

<400> 77

Arg Ala Ser Gln Asp Ile Ser Asn Phe Leu Asn
1 5 10

<210> 78

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb16 VL CDR2 amino acid sequence

<400> 78

Tyr Thr Ser Arg Leu Tyr Leu
1 5

<210> 79

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb16 VL CDR3 amino acid sequence

<400> 79

Gln Gln Gly Asn Thr Leu Pro Pro Thr
1 5

<210> 80

<211> 125

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VH amino acid sequence

<400> 80

Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Asp
20 25 30

Tyr Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Arg Leu Glu Trp
35 40 45

Met Gly His Ile Asn Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys Asn Gln Phe Phe
65 70 75 80

Leu Gln Leu Tyr Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Arg Arg Ser Leu Phe Tyr Tyr Tyr Gly Ser Ser Leu Tyr Ala Met
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120 125

<210> 81

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VH CDR1 amino acid sequence

<400> 81

Gly Tyr Ser Ile Thr Ser Asp Tyr Ala Trp Asn
1 5 10

<210> 82

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VH CDR2 amino acid sequence

<400> 82

His Ile Asn Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 83

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VH CDR3 amino acid sequence

<400> 83

Arg Ser Leu Phe Tyr Tyr Tyr Gly Ser Ser Leu Tyr Ala Met Asp Tyr
1 5 10 15

<210> 84

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VL amino acid sequence

<400> 84

Asp Val Val Met Thr Gln Ser Pro Phe Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 85

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VL CDR1 amino acid sequence

<400> 85

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
1 5 10 15

<210> 86

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb10 VL CDR3 amino acid sequence

<400> 86

Ser Gln Ser Thr His Val Pro Trp Thr
1 5

<210> 87

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VH amino acid sequence

<400> 87

Glu Val Gln Leu Val Glu Ser Gly Glu Asn Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Arg Gly Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val

35

40

45

Ala Ala Ile Ser Thr Gly Gly Asn Tyr Thr Tyr Tyr Pro Asp Ser Val

505560

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn Thr Leu Tyr

65707580

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys

859095

Ala Arg Arg Gly Gly Asn Tyr Ala Gly Phe Ala Tyr Trp Gly Gln Gly

100105110

Thr Leu Val Thr Val Ser Ala

115

<210> 88

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VH CDR1 amino acid sequence

<400> 88

Gly Phe Ser Phe Arg Gly Tyr Gly Met Ser

1510

<210> 89

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VH CDR2 amino acid sequence

<400> 89

Ala Ile Ser Thr Gly Gly Asn Tyr Thr Tyr Tyr Pro Asp Ser Val Gln

151015

Gly

<210> 90

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VH CDR3 amino acid sequence

<400> 90

Arg Gly Gly Asn Tyr Ala Gly Phe Ala Tyr

1510

<210> 91

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VL amino acid sequence

<400> 91

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Val	Ser	Val	Gly
1				5					10					15	

Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Pro	Ser	Glu	Asn	Ile	Tyr	Ser	Asn
			20					25					30		

Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Val
		35					40					45			

Tyr	Ala	Ala	Thr	Asn	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Ser
65					70					75				80	

Glu	Asp	Phe	Gly	Thr	Tyr	Tyr	Cys	Gln	His	Phe	Trp	Gly	Thr	Pro	Phe
				85					90					95	

Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105		

<210> 92

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VL CDR1 amino acid sequence

<400> 92

Arg	Pro	Ser	Glu	Asn	Ile	Tyr	Ser	Asn	Leu	Ala
1				5					10	

<210> 93

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 and chAb8 VL CDR2 amino acid sequence

<400> 93

Ala	Ala	Thr	Asn	Leu	Ala	Asp
1				5		

<210> 94

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb7 VL CDR3 amino acid sequence

<400> 94

Gln	His	Phe	Trp	Gly	Thr	Pro	Phe	Thr
1				5				

<210> 95
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb8 VH amino acid sequence

<400> 95
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45

Ala Thr Ile Ser Gly Gly Gly Asn Tyr Thr Tyr Cys Pro Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Asn Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Thr Arg Gln Arg Gly Tyr Asp Tyr His Tyr Ala Met Asp Phe Trp Gly
 100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 96
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb8 VH CDR2 amino acid sequence

<400> 96
Thr Ile Ser Gly Gly Gly Asn Tyr Thr Tyr Cys Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 97
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb8 VH CDR3 amino acid sequence

<400> 97
Gln Arg Gly Tyr Asp Tyr His Tyr Ala Met Asp Phe
1 5 10

<210> 98

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb8 VL amino acid sequence

<400> 98

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Val	Ser	Val	Gly
1				5					10					15	

Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asn	Ile	Tyr	Ser	Asn
			20					25					30		

Leu	Ala	Trp	His	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Val
		35				40						45			

Tyr	Ala	Ala	Thr	Asn	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Asn	Gly	Ser	Asp	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Ser
65					70					75				80	

Glu	Asp	Phe	Gly	Ser	Tyr	Phe	Cys	Gln	Asn	Phe	Trp	Gly	Thr	Ser	Trp
				85					90					95	

Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105		

<210> 99

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb8 VL CDR1 amino acid sequence

<400> 99

Arg	Ala	Ser	Glu	Asn	Ile	Tyr	Ser	Asn	Leu	Ala
1				5					10	

<210> 100

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb8 VL CDR3 amino acid sequence

<400> 100

Gln	Asn	Phe	Trp	Gly	Thr	Ser	Trp	Thr
1				5				

<210> 101

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VH amino acid sequence

<400> 101

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ile Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Val Ser Ser Asn Ile Thr Tyr Tyr Pro Asp Ser Met Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65 70 75 80

Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Ser Gly Thr Arg Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ala
115

<210> 102

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VH CDR1 amino acid sequence

<400> 102

Gly Phe Thr Phe Ser Ser Tyr Ile Met Ser
1 5 10

<210> 103

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VH CDR2 amino acid sequence

<400> 103

Ser Ile Val Ser Ser Asn Ile Thr Tyr Tyr Pro Asp Ser Met Lys Gly
1 5 10 15

<210> 104

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VH CDR3 amino acid sequence

<400> 104

Ser Gly Thr Arg Ala Trp Phe Ala Tyr
-

1 5

<210> 105

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VL amino acid sequence

<400> 105

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30

Ala Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 106

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VL CDR1 amino acid sequence

<400> 106

Arg Ala Ser Lys Ser Val Ser Thr Ser Ala Tyr Ser Tyr Met His
1 5 10 15

<210> 107

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb17 VL CDR3 amino acid sequence

<400> 107

Gln His Ser Arg Glu Leu Pro Tyr Thr
1 5

<210> 108

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VH amino acid sequence

<400> 108

Gln Val Gln Leu Gln Gln Pro Gly Asp Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Thr Asp
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Met Ile His Pro Asn Ser Gly Thr Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Ala Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Cys
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Tyr Trp Lys Trp Tyr Phe Asp Val Trp Gly Thr Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 109

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VH CDR1 amino acid sequence

<400> 109

Gly Tyr Thr Phe Thr Thr Asp Trp Met His
1 5 10

<210> 110

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VH CDR2 amino acid sequence

<400> 110

Met Ile His Pro Asn Ser Gly Thr Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15

Ser

<210> 111

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VH CDR3 amino acid sequence

<400> 111

Ser Tyr Trp Lys Trp Tyr Phe Asp Val
1 5

<210> 112

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VL amino acid sequence

<400> 112

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly
1 5 10 15

Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Leu Leu Ile Tyr
35 40 45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser Ser Val Glu Ala Glu
65 70 75 80

Asp Ser Ala Asp Tyr Tyr Cys His Gln Trp Thr Ser Tyr Met Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 113

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VL CDR1 amino acid sequence

<400> 113

Ser Ala Ser Ser Ser Val Ser Tyr Met His
1 5 10

<210> 114

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VL CDR2 amino acid sequence

<400> 114

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 115

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: chAb5 VL CDR3 amino acid sequence

<400> 115

His	Gln	Trp	Thr	Ser	Tyr	Met	Tyr	Thr
1				5				

<210> 116

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VH.1, huAb18v1, and huAb18v5 VH amino acid sequence

<400> 116

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Ser	Tyr
			20					25					30		

Thr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			

Gly	Tyr	Ile	Asn	Pro	Asn	Ser	Arg	Asn	Thr	Asp	Tyr	Asn	Gln	Lys	Phe
	50					55					60				

Lys	Asp	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala	Arg	Tyr	Ser	Gly	Ser	Thr	Pro	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	Gly
			100					105					110		

Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser
		115					120	

<210> 117

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VH.1a, huAb18v3, huAb18v8, and huAb18v9 VH amino acid sequence

<400> 117

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Ser	Tyr
			20					25					30		

Thr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly Tyr Ile Asn Pro Asn Ser Arg Asn Thr Asp Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Thr Thr Leu Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Ser Gly Ser Thr Pro Tyr Trp Tyr Phe Asp Val Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 118

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VH.1b, huAb18v2, huAb18v4, huAb18v6, huAb18v7, and huAb18v10 VH amino acid sequence

<400> 118

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30

Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Tyr Ile Asn Pro Asn Ser Arg Asn Thr Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Leu Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Ser Gly Ser Thr Pro Tyr Trp Tyr Phe Asp Val Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 119

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VH.1b VH CDR2 amino acid sequence

<400> 119

Tyr Ile Asn Pro Asn Ser Arg Asn Thr Asp Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 120

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VL.1, huAb18v1, and huAb18v2 VL amino acid sequence

<400> 120

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Phe	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		

Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr
		35					40					45			

Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser
	50					55					60				

Gly	Ser	Gly	Thr	Glu	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu
65					70					75					80

Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105	

<210> 121

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VL.1a, huAb18v3, and huAb18v4 VL amino acid sequence

<400> 121

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Phe	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		

Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Lys	Pro	Trp	Ile	Tyr
		35					40					45			

Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Val	Ser
	50					55					60				

Val	Ser	Gly	Thr	Glu	His	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu
65					70					75					80

Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105	

<210> 122

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VL.1b, huAb18v8, and huAb18v10 VL amino acid sequence

<400> 122

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Phe	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		

Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Pro	Trp	Ile	Tyr
		35					40					45			

Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Val	Ser
	50					55					60				

Gly	Ser	Gly	Thr	Glu	His	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu
65					70					75					80

Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105	

<210> 123

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VL.2, huAb18v5, and huAb18v6 VL amino acid sequence

<400> 123

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Asp	Phe	Gln	Ser	Val	Thr	Pro	Lys
1				5					10					15	

Glu	Lys	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		

Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Lys
		35					40					45			

Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser
	50					55					60				

Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Asn	Ser	Leu	Glu	Ala	Glu
65					70					75					80

Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105	

<210> 124

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb18VL.2a, huAb18v7, and huAb18v9 VL amino acid sequence

<400> 124

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Asp	Phe	Gln	Ser	Val	Thr	Pro	Lys
1				5					10					15	

Glu	Lys	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		

Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gln	Ser	Pro	Lys	Pro	Trp	Ile	Tyr
		35					40					45			

Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Val	Ser
	50					55					60				

Val	Ser	Gly	Thr	Asp	His	Thr	Leu	Thr	Ile	Asn	Ser	Leu	Glu	Ala	Glu
65					70					75					80

Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105	

<210> 125

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VH.1, huAb3vl, and huAb3v4 VH amino acid sequence

<400> 125

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			

Gly	Leu	Ile	His	Pro	Asp	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Glu	Met	Phe
	50					55					60				

Lys	Asn	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70				75						80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala	Arg	Gly	Gly	Arg	Leu	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Val	Thr	Val	Ser	Ser
				115

<210> 126

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VH.1a, huAb3v3, and huAb3v6 VH amino acid sequence

<400> 126

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Leu	Ile	His	Pro	Asp	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Glu	Met	Phe
	50					55					60				

Lys	Asn	Arg	Ala	Thr	Leu	Thr	Val	Asp	Arg	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Val	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Phe	Cys
				85					90					95	

Ala	Gly	Gly	Gly	Arg	Leu	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Val	Thr	Val	Ser	Ser
			115	

<210> 127

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VH.1b, huAb3v2, and huAb3v5 VH amino acid sequence

<400> 127

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Leu	Ile	His	Pro	Asp	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Glu	Met	Phe
	50					55					60				

Lys	Asn	Arg	Ala	Thr	Leu	Thr	Val	Asp	Arg	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala	Gly	Gly	Gly	Arg	Leu	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Val Thr Val Ser Ser
115

<210> 128

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VL.1, huAb3vl, and huAb3v2 VL amino acid sequence

<400> 128

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 129

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VL.1a and huAb3v3 VL amino acid sequence

<400> 129

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 130

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3VL.1b, huAb3v4, huAb3v5, and huAb3v6 VL amino acid sequence

<400> 130

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 131

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.1, huAb3v2.2, and huAb3v2.3 VH amino acid sequence

<400> 131

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile His Pro Trp Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe
50 55 60

Lys Asn Arg Ala Thr Leu Thr Val Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Gly Gly Gly Arg Leu Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr

100 105 110

Val Thr Val Ser Ser
115

<210> 132
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.1, huAb3v2.2, and huAb3v2.3 VH CDR2 amino acid sequence

<400> 132
Leu Ile His Pro Trp Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe Lys
1 5 10 15

Asn

<210> 133
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.1, huAb3v2.4, and huAb3v2.7 VL amino acid sequence

<400> 133
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Ser Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 134
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.1, huAb3v2.4, and huAb3v2.7 VL CDR1 amino acid sequence

<400> 134
Arg Ser Ser Gln Ser Leu Val His Ser Ser Gly Asp Thr Tyr Leu Arg
1 5 10 15

<210> 135

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.2, huAb3v2.5, and huAb3v2.8 VL amino acid sequence

<400> 135

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5				10				15			

Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		

Asn	Arg	Asp	Thr	Tyr	Leu	Arg	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				

Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70					75					80

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Ser
				85				90						95	

Thr	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105						110	

<210> 136

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.2, huAb3v2.5, and huAb3v2.8 VL CDR1 amino acid sequence

<400> 136

Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser	Asn	Arg	Asp	Thr	Tyr	Leu	Arg
1				5					10					15	

<210> 137

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.3, huAb3v2.6, and huAb3v2.9 VL amino acid sequence

<400> 137

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5				10					15		

Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		

Asn	Gln	Asp	Thr	Tyr	Leu	Arg	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

```

      50              55              60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65              70              75              80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
      85              90              95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
      100             105             110

```

<210> 138

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.3, huAb3v2.6, and huAb3v2.9 VL CDR1 amino acid sequence

<400> 138

```

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gln Asp Thr Tyr Leu Arg
1              5              10              15

```

<210> 139

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb3v2.4, huAb3v2.5, and huAb3v2.6 VH amino acid sequence

<400> 139

```

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1              5              10              15

```

```

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
      20              25              30

```

```

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
      35              40              45

```

```

Gly Leu Ile His Pro Glu Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe
      50              55              60

```

```

Lys Asn Arg Ala Thr Leu Thr Val Asp Arg Ser Thr Ser Thr Ala Tyr
      65              70              75              80

```

```

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
      85              90              95

```

```

Ala Gly Gly Gly Arg Leu Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
      100             105             110

```

```

Val Thr Val Ser Ser
      115

```

<210> 140

<211> 17

<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.4, huAb3v2.5, and huAb3v2.; VH CDR2 amino acid sequence

<400> 140
Leu Ile His Pro Glu Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe Lys
1 5 10 15

Asn

<210> 141
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH amino acid sequence

<400> 141
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
 20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Leu Ile His Pro Ile Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe
 50 55 60

Lys Asn Arg Ala Thr Leu Thr Val Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Gly Gly Gly Arg Leu Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Val Thr Val Ser Ser
 115

<210> 142
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: huAb3v2.7, huAb3v2.8, and huAb3v2.9 VH CDR2 amino acid sequence

<400> 142
Leu Ile His Pro Ile Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe Lys
1 5 10 15

Asn

<210> 143
<211> 107
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VL.1, huAb13v2, huAb13v5, and huAb13v7 VL amino acid sequence

<400> 143

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Phe	Asn
			20					25					30		

Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile
		35					40					45			

Tyr	Ser	Ala	Ser	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80

Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Tyr	Asn	Trp	Tyr	Pro	Phe
				85					90					95	

Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105		

<210> 144

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VL.1a, huAb13v1, huAb13v3, and huAb13v8 VL amino acid sequence

<400> 144

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Phe	Asn
			20					25					30		

Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Lys	Ala	Leu	Ile
		35					40					45			

Tyr	Ser	Ala	Ser	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80

Glu	Asp	Phe	Ala	Glu	Tyr	Phe	Cys	Gln	Gln	Tyr	Asn	Trp	Tyr	Pro	Phe
				85					90					95	

Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105		

<210> 145

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAB13VL.1b, huAb13v4, huAb13v6, and huAb13v9 VL amino acid sequence

<400> 145

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Phe Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Tyr Asn Trp Tyr Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 146

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VH.1, huAb13v2, huAb13v3, and huAb13v4 VH amino acid sequence

<400> 146

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Ser Trp His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile His Ser Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Asp Asp Tyr Phe Glu Tyr Trp Gly Gln Gly Thr Thr Val
100 105 110

Thr Val Ser Ser
115

<210> 147

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VH.1a, huAb13v1, huAb13v5, and huAb13v6 VH amino acid sequence

<400> 147

```

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1          5          10          15

Thr Leu Ser Leu Thr Cys Ala Val Thr Gly Tyr Ser Ile Thr Ser Gly
          20          25          30

Tyr Ser Trp His Trp Ile Arg Gln Phe Pro Gly Asn Gly Leu Glu Trp
          35          40          45

Met Gly Tyr Ile His Ser Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
          50          55          60

Lys Ser Arg Ile Ser Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Phe
65          70          75          80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
          85          90          95

Ala Gly Tyr Asp Asp Tyr Phe Glu Tyr Trp Gly Gln Gly Thr Thr Val
          100          105          110

Thr Val Ser Ser
          115

```

<210> 148

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: huAb13VH.1b, huAb13v7, huAb13v8, and huAb13v9 VH amino acid sequence

<400> 148

```

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1          5          10          15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
          20          25          30

Tyr Ser Trp His Trp Ile Arg Gln Pro Pro Gly Asn Gly Leu Glu Trp
          35          40          45

Met Gly Tyr Ile His Ser Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
          50          55          60

Lys Ser Arg Ile Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65          70          75          80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
          85          90          95

Ala Arg Tyr Asp Asp Tyr Phe Glu Tyr Trp Gly Gln Gly Thr Thr Val
          100          105          110

Thr Val Ser Ser
          115

```

<210> 149
 <211> 534
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (1)..(534)
 <223> B7-H3 amino acid sequence (human)

<400> 149
 Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
 1 5 10 15
 Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
 20 25 30
 Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
 35 40 45
 Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
 50 55 60
 Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
 65 70 75 80
 Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
 85 90 95
 Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
 100 105 110
 Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
 115 120 125
 Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
 130 135 140
 Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
 145 150 155 160
 Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
 165 170 175
 Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
 180 185 190
 Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
 195 200 205
 Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
 210 215 220
 Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
 225 230 235 240
 Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
 245 250 255
 Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
 260 265 270
 Glu Ser Glu Phe Ser Ser Ala Glu Ser Ser Tyr Thr Thr Glu Tyr Thr

Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
 275 280 285
 Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
 290 295 300
 Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
 305 310 315 320
 Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
 325 330 335
 Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
 340 345 350
 Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
 355 360 365
 Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser
 370 375 380
 Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
 385 390 395 400
 Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
 405 410 415
 Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
 420 425 430
 Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
 435 440 445
 Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
 450 455 460
 Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu
 465 470 475 480
 Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
 485 490 495
 Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
 500 505 510
 Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
 515 520 525
 Asp Gly Gln Glu Ile Ala
 530
 <210> 150
 <211> 692
 <212> PRT
 <213> Homo sapiens
 <220>
 <221> misc_feature
 <222> (1)..(692)
 <223> Human B7-H3-ECD (fc fusion)
 <400> 150
 Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
 1 5 10 15

1 5 10 15
 Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
 20 25 30
 Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
 35 40 45
 Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
 50 55 60
 Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
 65 70 75 80
 Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
 85 90 95
 Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
 100 105 110
 Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
 115 120 125
 Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
 130 135 140
 Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
 145 150 155 160
 Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
 165 170 175
 Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
 180 185 190
 Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
 195 200 205
 Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
 210 215 220
 Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
 225 230 235 240
 Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
 245 250 255
 Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
 260 265 270
 Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
 275 280 285
 Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
 290 295 300
 Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
 305 310 315 320
 Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
 325 330 335
 Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
 340 345 350

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Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
355                      360                      365

Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser
370                      375                      380

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385                      390                      395                      400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
405                      410                      415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
420                      425                      430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
435                      440                      445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Ala Ala
450                      455                      460

Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu
465                      470                      475                      480

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
485                      490                      495

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
500                      505                      510

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
515                      520                      525

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
530                      535                      540

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
545                      550                      555                      560

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
565                      570                      575

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
580                      585                      590

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
595                      600                      605

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
610                      615                      620

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
625                      630                      635                      640

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
645                      650                      655

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
660                      665                      670

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
675                      680                      685

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Ser Pro Gly Lys
690

<210> 151

<211> 474

<212> PRT

<213> Mus musculus

<220>

<221> misc_feature

<222> (1)..(474)

<223> Mouse B7-H3-ECD (fc fusion)

<400> 151

Met Leu Arg Gly Trp Gly Gly Pro Ser Val Gly Val Cys Val Arg Thr
1 5 10 15

Ala Leu Gly Val Leu Cys Leu Cys Leu Thr Gly Ala Val Glu Val Gln

20

25

30

Val Ser Glu Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu
35 40 45

Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr
65 70 75 80

Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Thr Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp
115 120 125

Phe Asp Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Lys Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Leu Thr Phe Ala Ala Ala Asp Lys Thr His Thr Cys Pro Pro Cys
245 250 255

Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro
 260 265 270
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 275 280 285
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 290 295 300
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 305 310 315 320
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 325 330 335
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 340 345 350
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 355 360 365
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
 370 375 380
 Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 385 390 395 400
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 405 410 415
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 420 425 430
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 435 440 445
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 450 455 460
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 465 470

<210> 152

<211> 460

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<222> (1)..(460)

<223> Human B7-H3-ECD (his tag)

<400> 152

Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly
 1 5 10 15
 Val Gln Cys Gly Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val
 20 25 30
 Ala Leu Val Gly Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu
 35 40 45

Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp
 50 55 60
 Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser
 65 70 75 80
 Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly
 85 90 95
 Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser
 100 105 110
 Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser
 115 120 125
 Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro
 130 135 140
 Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser
 145 150 155 160
 Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly
 165 170 175
 Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln
 180 185 190
 Gly Leu Phe Asp Val His Ser Ile Leu Arg Val Val Leu Gly Ala Asn
 195 200 205
 Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala
 210 215 220
 His Ser Ser Val Thr Ile Thr Pro Gln Arg Ser Pro Thr Gly Ala Val
 225 230 235 240
 Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp
 245 250 255
 Ala Thr Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala
 260 265 270
 Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His
 275 280 285
 Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr
 290 295 300
 Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu
 305 310 315 320
 Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser
 325 330 335
 Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro
 340 345 350
 Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro
 355 360 365
 Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu
 370 375 380

Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn
385 390 395 400

Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His
405 410 415

Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu
420 425 430

Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile
435 440 445

Thr Gly Gln Pro Met Thr His His His His His His
450 455 460

<210> 153

<211> 241

<212> PRT

<213> Mus musculus

<220>

<221> misc_feature

<222> (1)..(241)

<223> Mouse B7-H3-ECD (his tag)

<400> 153

Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly
1 5 10 15

Val Gln Cys Val Glu Val Gln Val Ser Glu Asp Pro Val Val Ala Leu
20 25 30

Val Asp Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly
35 40 45

Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
50 55 60

Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr
65 70 75 80

Ser Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Val Gln Gly Asn Ala
85 90 95

Ser Leu Arg Leu Gln Arg Val Arg Val Thr Asp Glu Gly Ser Tyr Thr
100 105 110

Cys Phe Val Ser Ile Gln Asp Phe Asp Ser Ala Ala Val Ser Leu Gln
115 120 125

Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys
130 135 140

Asp Leu Arg Pro Gly Asn Met Val Thr Ile Thr Cys Ser Ser Tyr Gln
145 150 155 160

Gly Tyr Pro Glu Ala Glu Val Phe Trp Lys Asp Gly Gln Gly Val Pro
165 170 175

Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Arg Gly Leu
180 185 190

Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr
195 200 205

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly
210 215 220

Ser Val Thr Ile Thr Gly Gln Pro Leu Thr Phe His His His His His
225 230 235 240

His

<210> 154

<211> 473

<212> PRT

<213> Macaca fascicularis

<220>

<221> misc_feature

<222> (1)..(473)

<223> Cynomolgus B7-H3-ECD (his tag)

<400> 154

Met Leu His Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr
65 70 75 80

Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Leu Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Ala Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
 210 215 220
 Pro Val Leu Gln Gln Asp Ala His Gly Ser Ile Thr Ile Thr Pro Gln
 225 230 235 240
 Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
 245 250 255
 Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
 260 265 270
 Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
 275 280 285
 Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
 290 295 300
 Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Leu Asp Leu Leu Ala Gln
 305 310 315 320
 Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
 325 330 335
 Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
 340 345 350
 Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
 355 360 365
 Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser
 370 375 380
 Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
 385 390 395 400
 Gly Ala Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
 405 410 415
 Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
 420 425 430
 Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
 435 440 445
 Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Ala Ala
 450 455 460
 Ala His His His His His His His His
 465 470

<210> 155

<211> 98

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGHV1-69*06

<400> 155

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Thr

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 156

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGHJ6*01

<400> 156

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 157

<211> 96

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKV1-9*01

<400> 157

Asp Ile Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Asn Ser Tyr Pro Pro
85 90 95

<210> 158

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKJ2*01

<400> 158

Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
1				5					10

<210> 159

<211> 330

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Ig gamma-1 constant region

<400> 159

Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys
1				5					10					15	

Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr
			20					25					30		

Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser
		35					40					45			

Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser
	50					55					60				

Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr
65					70				75					80	

Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys
			85						90					95	

Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys
			100					105					110		

Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro
		115				120						125			

Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys
	130					135					140				

Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp
145					150					155					160

Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu
			165						170					175	

Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu
			180					185					190		

His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn
		195				200						205			

Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
	210					215					220				

Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu
225					230					235					240

Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr
			245						250					255	

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 160

<211> 330

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Ig gamma-1 constant region mutant

<400> 160

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 161

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Ig Kappa constant region

<400> 161

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 162

<211> 105

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Ig Lambda constant region

<400> 162

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
1 5 10 15

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
20 25 30

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
35 40 45

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
50 55 60

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
65 70 75 80

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
85 90 95

Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 163

<211> 96

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKV6-21*01

<220>

<221> misc_feature

<222> (96)..(96)

<223> Xaa can be any naturally occurring amino acid

<400> 163

Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Ser
20 25 30

Leu His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Ser Ser Ser Leu Pro Xaa
85 90 95

<210> 164

<211> 101

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKV2-28*01

<400> 164

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
 85 90 95

Leu Gln Thr Pro Pro
 100

<210> 165

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKJ4*01

<400> 165

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 1 5 10

<210> 166

<211> 98

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGHV-b*01(0-1)

<400> 166

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Ser Gly
 20 25 30

Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
 35 40 45

Ile Gly Ser Ile Tyr His Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu
 50 55 60

Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
 65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 167

<211> 96

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of IGKv1-39*01

<400> 167

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr
			20					25					30		

Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile
		35					40					45			

Tyr	Ala	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80

Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Pro
				85					90					95	

<210> 168

<211> 446

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb13v1 heavy chain

<400> 168

Glu	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Glu
1				5					10					15	

Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Thr	Gly	Tyr	Ser	Ile	Thr	Ser	Gly
			20					25					30		

Tyr	Ser	Trp	His	Trp	Ile	Arg	Gln	Phe	Pro	Gly	Asn	Gly	Leu	Glu	Trp
		35					40					45			

Met	Gly	Tyr	Ile	His	Ser	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Pro	Ser	Leu
	50					55					60				

Lys	Ser	Arg	Ile	Ser	Ile	Ser	Arg	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Phe
65					70					75					80

Leu	Lys	Leu	Ser	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala	Gly	Tyr	Asp	Asp	Tyr	Phe	Glu	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val
			100						105					110	

Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala
							115					125			

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
 180 185 190
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 195 200 205
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 210 215 220
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
 225 230 235 240
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 245 250 255
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 260 265 270
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 275 280 285
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
 290 295 300
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
 305 310 315 320
 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 325 330 335
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 340 345 350
 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 355 360 365
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 370 375 380
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 385 390 395 400
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
 405 410 415
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 420 425 430
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> 169

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb13v1 light chain

<400> 169

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Phe	Asn
			20					25					30		

Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Lys	Ala	Leu	Ile
		35					40					45			

Tyr	Ser	Ala	Ser	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80

Glu	Asp	Phe	Ala	Glu	Tyr	Phe	Cys	Gln	Gln	Tyr	Asn	Trp	Tyr	Pro	Phe
				85					90					95	

Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala
			100					105						110	

Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
		115					120					125			

Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130					135					140				

Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145					150					155					160

Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
				165					170					175	

Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
			180					185					190		

Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
		195					200					205			

Phe	Asn	Arg	Gly	Glu	Cys
	210				

<210> 170

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3v2.5 heavy chain

<400> 170

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Met	Met	Val	Met	Val	Val	Gln	Ala	Ser	Gln	Gln	Gln	Tyr	Gln	Met	Val
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

```

trp met his trp val arg gln ala pro gly gln gly leu glu trp ile
  35                      40                      45

Gly leu ile his pro glu ser gly ser thr asn tyr asn glu met phe
  50                      55                      60

lys asn arg ala thr leu thr val asp arg ser thr ser thr ala tyr
  65                      70                      75                      80

met glu leu ser ser leu arg ser glu asp thr ala val tyr tyr cys
      85                      90                      95

ala gly gly gly arg leu tyr phe asp tyr trp gly gln gly thr thr
      100                      105                      110

val thr val ser ser ala ser thr lys gly pro ser val phe pro leu
      115                      120                      125

ala pro ser ser lys ser thr ser gly gly thr ala ala leu gly cys
      130                      135                      140

leu val lys asp tyr phe pro glu pro val thr val ser trp asn ser
      145                      150                      155                      160

gly ala leu thr ser gly val his thr phe pro ala val leu gln ser
      165                      170                      175

ser gly leu tyr ser leu ser ser val val thr val pro ser ser ser
      180                      185                      190

leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn
      195                      200                      205

thr lys val asp lys lys val glu pro lys ser cys asp lys thr his
      210                      215                      220

thr cys pro pro cys pro ala pro glu ala ala gly gly pro ser val
      225                      230                      235                      240

phe leu phe pro pro lys pro lys asp thr leu met ile ser arg thr
      245                      250                      255

pro glu val thr cys val val val asp val ser his glu asp pro glu
      260                      265                      270

val lys phe asn trp tyr val asp gly val glu val his asn ala lys
      275                      280                      285

thr lys pro arg glu glu gln tyr asn ser thr tyr arg val val ser
      290                      295                      300

val leu thr val leu his gln asp trp leu asn gly lys glu tyr lys
      305                      310                      315                      320

cys lys val ser asn lys ala leu pro ala pro ile glu lys thr ile
      325                      330                      335

ser lys ala lys gly gln pro arg glu pro gln val tyr thr leu pro
      340                      345                      350

pro ser arg glu glu met thr lys asn gln val ser leu thr cys leu
      355                      360                      365

```

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 171

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3v2.5 light chain

<400> 171

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Arg Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 172

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3v2.6 heavy chain

<400> 172

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
 20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Leu Ile His Pro Glu Ser Gly Ser Thr Asn Tyr Asn Glu Met Phe
 50 55 60

Lys Asn Arg Ala Thr Leu Thr Val Asp Arg Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Gly Gly Gly Arg Leu Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
 225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 173

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3v2.6 light chain

<400> 173

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gln Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Met Val Val Ser Met Met Phe Gln Gln Gln Met Tyr Val Glu Val Thr

Tnr His Val Pro Tyr Tnr Phe Gly Gly Gly Tnr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 174

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic:IGHV1-69*06_IGHJ6

<220>

<221> misc_feature

<222> (99)..(106)

<223> Xaa can be any naturally occurring amino acid; this section represents the CDR-H3 region

<400> 174

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 175

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: IGKV2-28*01_IGKJ4

<220>

<221> misc_feature

<222> (94)..(102)

<223> Xaa can be any naturally occurring amino acid ; this section represents the CDR-L3 region

<400> 175

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5				10					15		

Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
			20					25					30		

Asn	Gly	Tyr	Asn	Tyr	Leu	Asp	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Gln	Leu	Leu	Ile	Tyr	Leu	Gly	Ser	Asn	Arg	Ala	Ser	Gly	Val	Pro
	50					55					60				

Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70					75					80

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Xaa	Xaa	Xaa
				85				90						95	

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

<210> 176

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic:IGHV4-b_IGHJ6

<220>

<221> misc_feature

<222> (99)..(105)

<223> Xaa can be any naturally occurring amino acid; this section represents the CDR-H3 region

<400> 176

Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Glu
1				5				10					15		

Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Ser	Gly	Tyr	Ser	Ile	Ser	Ser	Gly
			20					25					30		

Tyr	Tyr	Trp	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp
		35					40					45			

Ile	Gly	Ser	Ile	Tyr	His	Ser	Gly	Ser	Thr	Tyr	Tyr	Asn	Pro	Ser	Leu
	50					55					60				

Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Gly Gln Gly Thr Thr Val
100 105 110

Thr Val Ser Ser
115

<210> 177

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: IGKV1-39_IGKJ2

<220>

<221> misc_feature

<222> (89)..(97)

<223> Xaa can be any naturally occurring amino acid; this section represents the CDR-L3 region

<400> 177

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 178

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3 VL1 variants

<220>

<221> misc_feature

<222> (33)..(33)

<223> X can be any naturally occurring amino acid, except M, C, N, D or Q

<400> 178

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Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1           5           10           15

```

```

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
          20           25           30

```

```

Xaa Gly Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35           40           45

```

```

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50           55           60

```

```

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65           70           75           80

```

```

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
          85           90           95

```

```

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
          100          105          110

```

<210> 179

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3 VL1 variants

<220>

<221> misc_feature

<222> (34)..(34)

<223> X can be any naturally occurring amino acid except M, C, G, S, N, or P

<400> 179

```

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1           5           10           15

```

```

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
          20           25           30

```

```

Asn Xaa Asp Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35           40           45

```

```

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50           55           60

```

```

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65           70           75           80

```

```

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
          85           90           95

```

```

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
          100          105          110

```

<210> 180

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3 VH1b variants

<220>

<221> misc_feature

<222> (54)..(54)

<223> X can be any naturally occurring amino acid, except M, C, N, D or Q

<400> 180

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Leu	Ile	His	Pro	Xaa	Ser	Gly	Ser	Thr	Asn	Tyr	Asn	Glu	Met	Phe
	50					55					60				

Lys	Asn	Arg	Ala	Thr	Leu	Thr	Val	Asp	Arg	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala	Gly	Gly	Gly	Arg	Leu	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		

Val	Thr	Val	Ser	Ser
			115	

<210> 181

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence of huAb3 VH1b variants

<220>

<221> misc_feature

<222> (55)..(55)

<223> X can be any naturally occurring amino acid except M, C, G, S, N, or P

<400> 181

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly Leu Ile His Pro Asp Xaa Gly Ser Thr Asn Tyr Asn Glu Met Phe
50 55 60

Lys Asn Arg Ala Thr Leu Thr Val Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Gly Gly Gly Arg Leu Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 182
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: chAb13 VL CDR3 amino acid sequence

<400> 182
Gln Gln Tyr Asn Ser Tyr Pro Phe Thr
1 5

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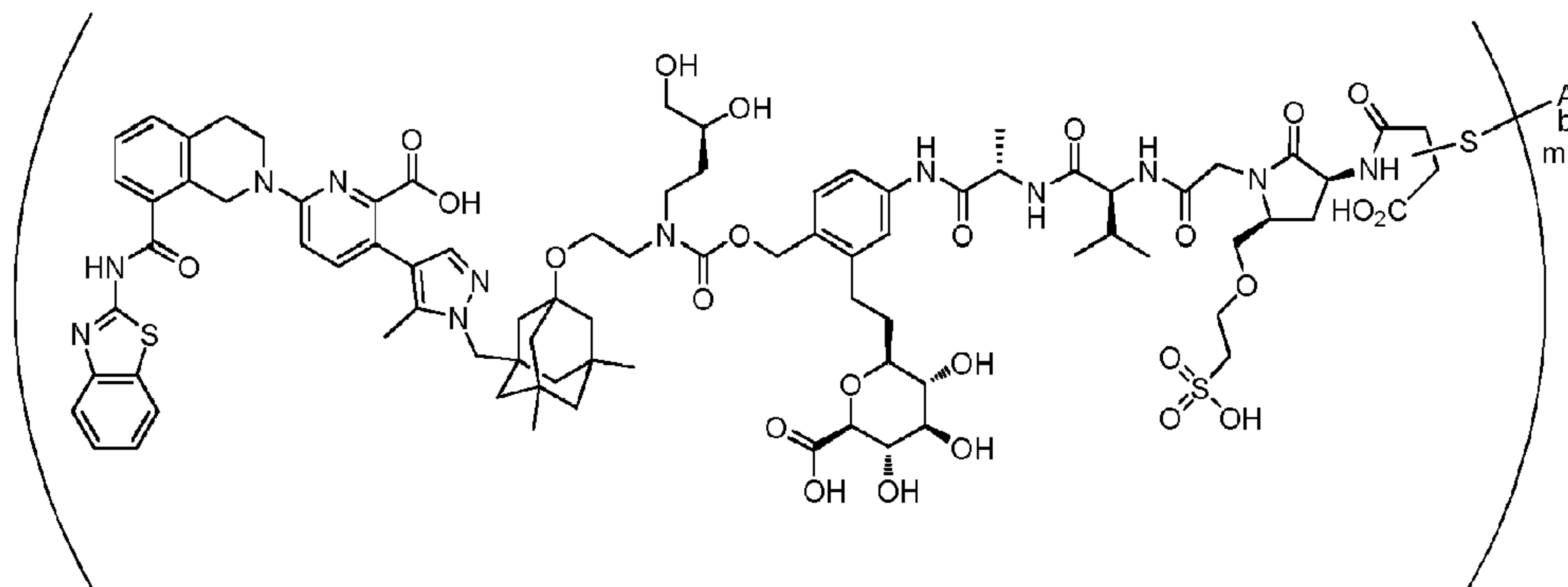
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Patentkrav

1. Anti-human B7H3 (hB7-H3) antistof-lægemiddelkonjugat (ADC), der omfatter følgende struktur:



- 5 hvor m er 2, og Ab er et anti-hB7-H3-antistof, hvor antistoffet Ab omfatter et tungkæde-CDR3-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 35, et tungkæde-CDR2-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 34, og et tungkæde-CDR1-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 33; og
- 10 et letkæde-CDR3-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 39, et letkæde-CDR2-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 38, og et letkæde-CDR1-domæne omfattende aminosyresekvensen, der er anført i SEQ ID NO: 37.
- 15 **2.** Antistof-lægemiddelkonjugat ifølge krav 1, hvor antistoffet omfatter en variabel tungkædereion omfattende SEQ ID NO: 147 og en variabel letkædereion omfattende SEQ ID NO: 144.
- 3.** Antistof-lægemiddelkonjugat ifølge krav 1, hvor antistoffet omfatter en tung kæde
- 20 omfattende SEQ ID NO: 168 og en let kæde omfattende SEQ ID NO: 169.

DRAWINGS

Figure 1

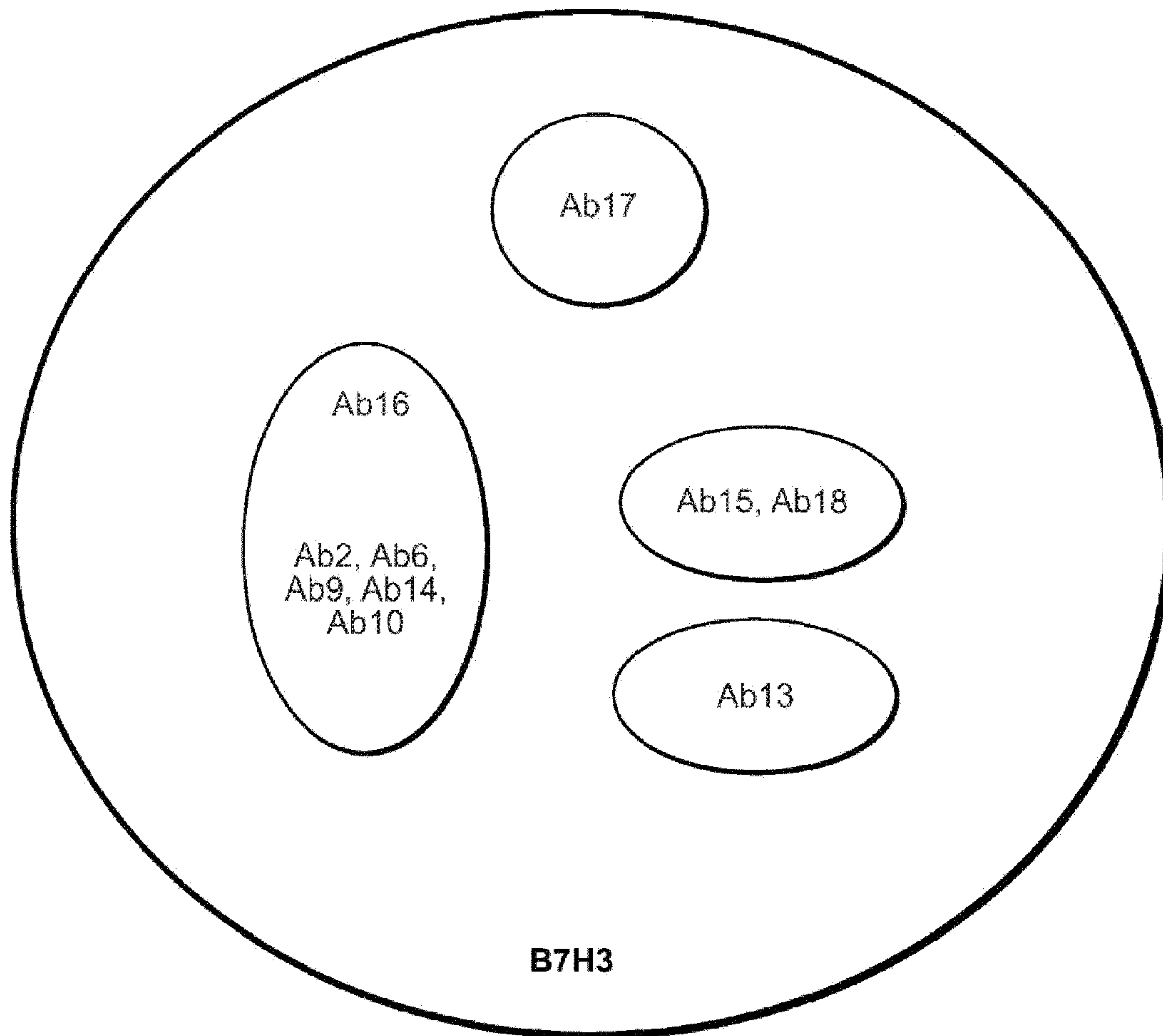
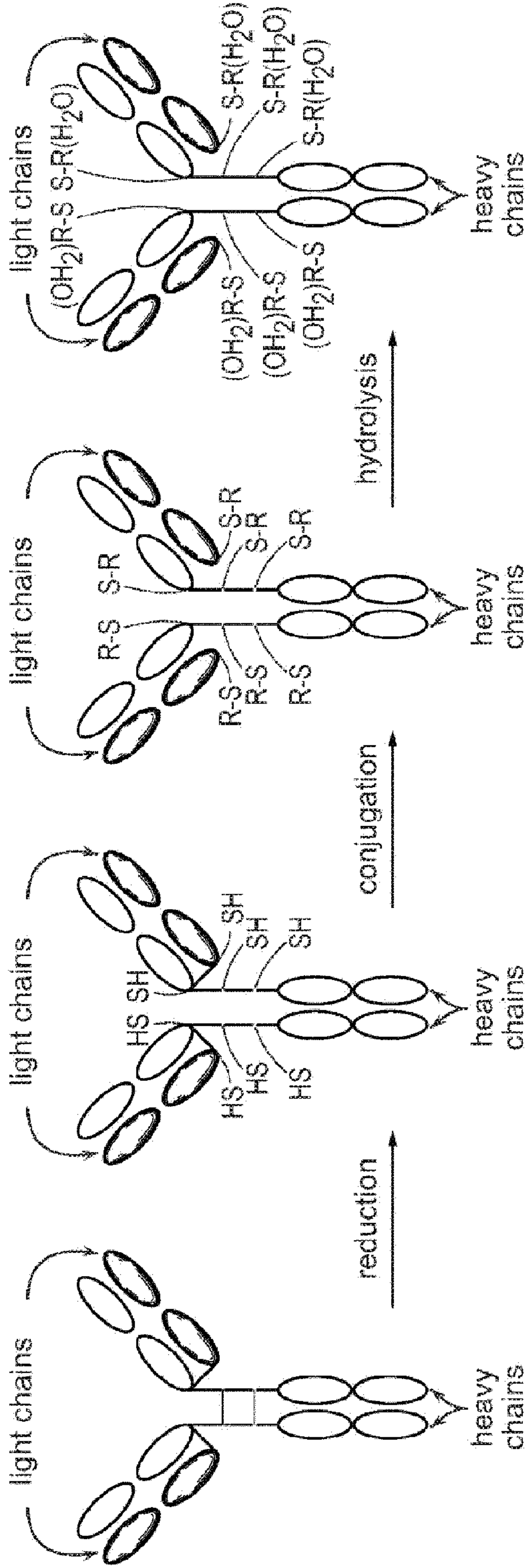


Figure 2



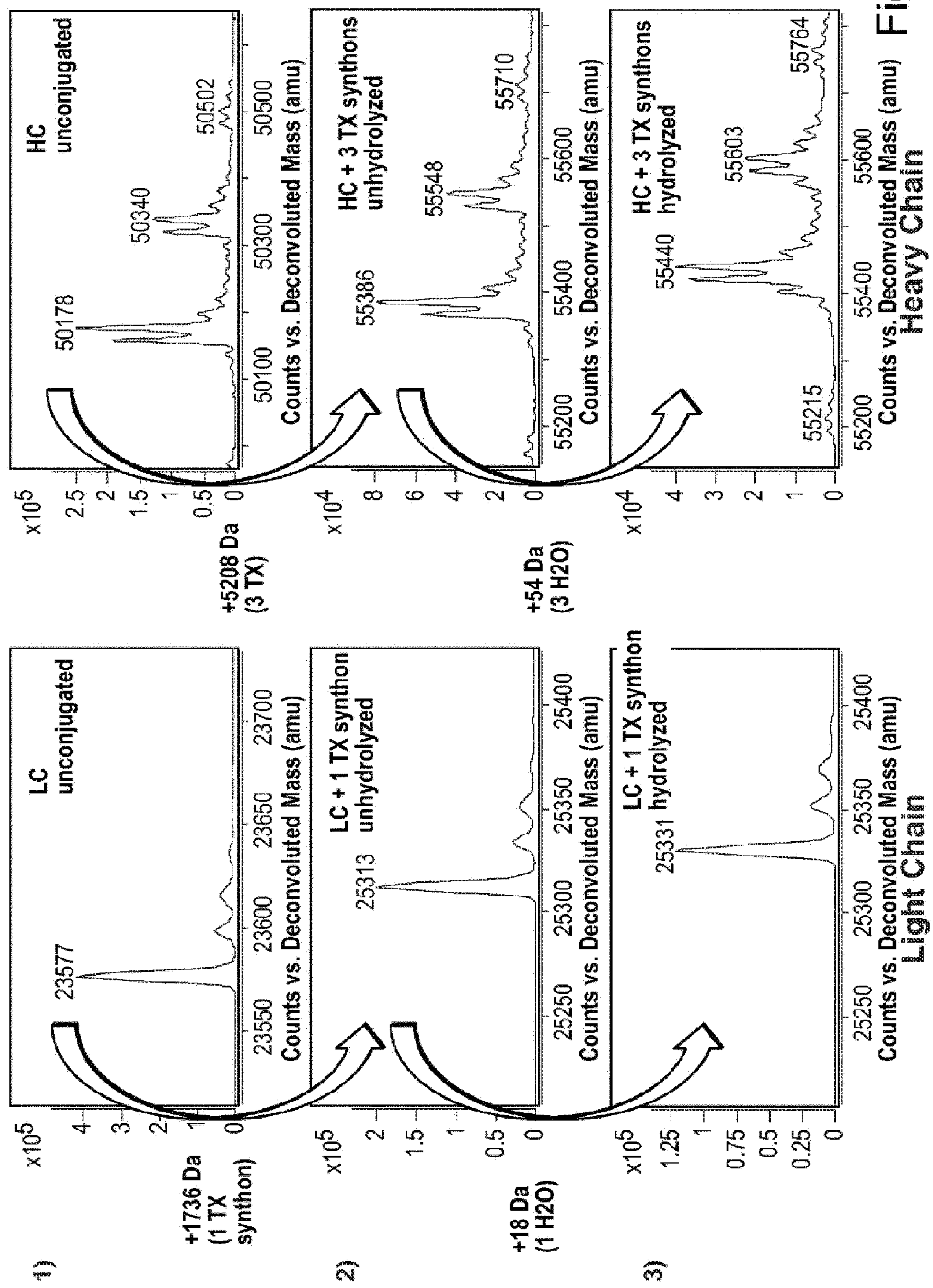


Figure 5