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(54) **Title:**
MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO LIGANDS

(57) **Abstract:**
296 Abstract MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO LIGANDS Auristatin peptides, including MeVal-Val-Dil-Dap-Norephedrine (MMAE) and MeVal Val-Dil-Dap-Phe (MMAF), were prepared and attached to Ligands through various linkers, including maleimidocaproyl-val-cit-PAB. The resulting ligand drug conjugates were active in vitro and in vivo. (Figure 1)

Abstract**MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO
LIGANDS**

Auristatin peptides, including MeVal-Val-Dil-Dap-Norephedrine (MMAE) and MeVal-Val-Dil-Dap-Phe (MMAF), were prepared and attached to Ligands through various linkers, including maleimidocaproyl-val-cit-PAB. The resulting ligand drug conjugates were active *in vitro* and *in vivo*.

(Figure 1)

MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO LIGANDS

CONTINUITY

5 This application claims the benefit of U.S. Provisional Patent Application
No. 60/518,534, filed November 6, 2003; U.S. Provisional Patent Application No.
60/557,116, filed March 26, 2004; U.S. Provisional Patent Application No. 60/598,899,
filed August 4, 2004; and U.S. Provisional Patent Application No. 60/622,455, filed
October 27, 2004; the disclosures of which are incorporated by reference herein.

1. FIELD OF THE INVENTION

10 The present invention is directed to a Drug Compound and more
particularly to Drug-Linker-Ligand Conjugates, Drug-Linker Compounds, and Drug-
Ligand Conjugates, to compositions including the same, and to methods for using the
15 same to treat cancer, an autoimmune disease or an infectious disease. The present
invention is also directed to antibody-drug conjugates, to compositions including the
same, and to methods for using the same to treat cancer, an autoimmune disease or an
infectious disease. The invention also relates to methods of using antibody-drug
conjugate compounds for *in vitro*, *in situ*, and *in vivo* diagnosis or treatment of
20 mammalian cells, or associated pathological conditions.

2. BACKGROUND OF THE INVENTION

Improving the delivery of drugs and other agents to target cells, tissues and
tumors to achieve maximal efficacy and minimal toxicity has been the focus of
25 considerable research for many years. Though many attempts have been made to develop
effective methods for importing biologically active molecules into cells, both *in vivo* and
in vitro, none has proved to be entirely satisfactory. Optimizing the association of the
drug with its intracellular target, while minimizing intercellular redistribution of the drug,
e.g., to neighboring cells, is often difficult or inefficient.

30 Most agents currently administered to a patient parenterally are not
targeted, resulting in systemic delivery of the agent to cells and tissues of the body where
it is unnecessary, and often undesirable. This may result in adverse drug side effects, and

often limits the dose of a drug (e.g., chemotherapeutic (anti-cancer), cytotoxic, enzyme inhibitor agents and antiviral or antimicrobial drugs) that can be administered. By comparison, although oral administration of drugs is considered to be a convenient and economical mode of administration, it shares the same concerns of non-specific toxicity to unaffected cells once the drug has been absorbed into the systemic circulation. Further complications involve problems with oral bioavailability and residence of drug in the gut leading to additional exposure of gut to the drug and hence risk of gut toxicities. Accordingly, a major goal has been to develop methods for specifically targeting agents to cells and tissues. The benefits of such treatment include avoiding the general physiological effects of inappropriate delivery of such agents to other cells and tissues, such as uninfected cells. Intracellular targeting may be achieved by methods, compounds and formulations which allow accumulation or retention of biologically active agents, i.e. active metabolites, inside cells.

Monoclonal antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, e.g., drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz and Springer (1997) *Adv. Drg. Del. Rev.* 26:151-172; U.S. Patent No. 4975278) theoretically allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, while systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin *et al.*, 1986, *Lancet* pp. (Mar. 15, 1986):603-05; Thorpe, 1985, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological And Clinical Applications*, A. Pinchera *et al.* (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland *et al.*, 1986, *Cancer Immunol. Immunother.* 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland *et al.*, 1986, *supra*). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Kerr *et al.*, 1997, *Bioconjugate Chem.* 8(6):781-784; Mandler *et al.* (2000) *Jour. of the Nat. Cancer Inst.* 92(19):1573-1581; Mandler *et al.* (2000) *Bioorganic & Med. Chem.*

Letters 10:1025-1028; Mandler *et al.* (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode *et al.* (1998) Cancer Res. 58:2928; Hinman *et al.* (1993) Cancer Res. 53:3336-3342). The toxins may affect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition (Meyer, D.L. and Senter, P.D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" in Annual Reports in Medicinal Chemistry, Vol 38 (2003) Chapter 23, 229-237). Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ¹¹¹In or ⁹⁰Y radioisotope bound by a thiourea linker-chelator (Wiseman *et al.* (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman *et al.* (2002) Blood 99(12):4336-42; Witzig *et al.* (2002) J. Clin. Oncol. 20(10):2453-63; Witzig *et al.* (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; U.S. Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The same maytansinoid drug moiety, DM1, was linked through a non-disulfide linker, SMCC, to a mouse murine monoclonal antibody, TA.1 (Chari *et al.* (1992) Cancer Research 52:127-131). This conjugate was reported to be 200-fold less potent than the corresponding disulfide linker conjugate. The SMCC linker was considered therein to be "noncleavable."

Several short peptidic compounds have been isolated from the marine mollusc *Dolabella auricularia* and found to have biological activity (Pettit *et al.* (1993) Tetrahedron 49:9151; Nakamura *et al.* (1995) Tetrahedron Letters 36:5059-5062; Sone *et al.* (1995) Jour. Org Chem. 60:4474). Analogs of these compounds have also been prepared, and some were found to have biological activity (for a review, see Pettit *et al.* (1998) Anti-Cancer Drug Design 13:243-277). For example, auristatin E (U.S. Patent No. 5635483) is a synthetic analogue of the marine natural product Dolastatin 10, an agent that inhibits tubulin polymerization by binding to the same domain on tubulin as the anticancer drug vincristine (G. R. Pettit, (1997) Prog. Chem. Org. Nat. Prod. 70:1-79).
Dolastatin 10, auristatin PE, and auristatin E are linear peptides having four amino acids, three of which are unique to the dolastatin class of compounds, and a C-terminal amide.

The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to: (i) chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas); (ii) cAC10 which is specific to CD30 on hematological malignancies (Klussman, *et al.* (2004), Bioconjugate Chemistry 15(4):765-773; Doronina *et al.* (2003) Nature Biotechnology 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco *et al.* (2003) Blood 102(4):1458-1465; U.S. Publication 2004/0018194; (iii) anti-CD20 antibodies such as RITUXAN® (WO 04/032828) for the treatment of CD20-expressing cancers and immune disorders; (iv) anti-EphB2 antibodies 2H9 and anti-IL-8 for treatment of colorectal cancer (Mao, *et al.* (2004) Cancer Research 64(3):781-788); (v) E-selectin antibody (Bhaskar *et al.* (2003) Cancer Res. 63:6387-6394); and (vi) other anti-CD30 antibodies (WO 03/043583).

Auristatin E conjugated to monoclonal antibodies are disclosed in Senter *et al.*, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004.

Despite *in vitro* data for compounds of the dolastatin class and its analogs, significant general toxicities at doses required for achieving a therapeutic effect compromise their efficacy in clinical studies. Accordingly, there is a clear need in the art for dolastatin/auristatin derivatives having significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

The ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). A panel of anti-ErbB2 antibodies has been characterized using the human breast tumor cell line SKBR3 (Hudziak *et al.*, (1989) *Mol. Cell. Biol.* 9(3):1165-1172. Maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF- α (U.S. Patent No. 5677171). The anti-ErbB2 antibodies discussed in Hudziak *et al.* are further characterized in Fendly *et al.* (1990) *Cancer Research* 50:1550-1558; Kotts *et al.* (1990) *In vitro* 26(3):59A; Sarup *et al.* (1991) *Growth Regulation* 1:72-82; Shepard *et al.* J. (1991) *Clin. Immunol.* 11(3):117-127; Kumar *et al.* (1991) *Mol. Cell. Biol.* 11(2):979-986; Lewis *et al.* (1993) *Cancer Immunol. Immunother.* 37:255-263; Pietras *et al.* (1994) *Oncogene* 9:1829-1838; Vitetta *et al.* (1994) *Cancer Research* 54:5301-5309; Sliwkowski *et al.* (1994) *J. Biol. Chem.* 269(20):14661-14665; Scott *et al.* (1991) *J. Biol. Chem.* 266:14300-5; D'souza *et al.* *Proc. Natl. Acad. Sci.* (1994) 91:7202-7206; Lewis *et al.* (1996) *Cancer Research* 56:1457-1465; and Schaefer *et al.* (1997) *Oncogene* 15:1385-1394.

Other anti-ErbB2 antibodies with various properties have been described in Tagliabue *et al.* *Int. J. Cancer* 47:933-937 (1991); McKenzie *et al.* *Oncogene* 4:543-548 (1989); Maier *et al.* *Cancer Res.* 51:5361-5369 (1991); Bacus *et al.* *Molecular Carcinogenesis* 3:350-362 (1990); Stancovski *et al.* *Proc. Natl. Acad. Sci. USA* 88:8691-8695 (1991); Bacus *et al.* *Cancer Research* 52:2580-2589 (1992); Xu *et al.* *Int. J. Cancer* 53:401-408 (1993); WO94/00136; Kasprzyk *et al.* *Cancer Research* 52:2771-2776 (1992); Hancock *et al.* (1991) *Cancer Res.* 51:4575-4580; Shawver *et al.* (1994) *Cancer Res.* 54:1367-1373; Arteaga *et al.* (1994) *Cancer Res.* 54:3758-3765; Harwerth *et al.* (1992) *J. Biol. Chem.* 267:15160-15167; U.S. Patent No. 5783186; and Klapper *et al.* (1997) *Oncogene* 14:2099-2109.

Homology screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (U.S. Patent No. 5,183,884; U.S. Patent No. 5,480,968; KraU.S. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:9193-9197) and ErbB4 (EP 599274; Plowman *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:1746-1750; and Plowman *et al.*

(1993) Nature 366:473-475). Both of these receptors display increased expression on at least some breast cancer cell lines.

HERCEPTIN® (Trastuzumab) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay ($K_d =$ 5 nM) to the extracellular domain of the human epidermal growth factor receptor2 protein, HER2 (ErbB2) (U.S. Patent No. 5821337; U.S. Patent No. 6054297; U.S. Patent No. 6407213; U.S. Patent No. 6639055; Coussens L, *et al.* (1985) Science 230:1132-9; Slamon DJ, *et al.* (1989) Science 244:707-12). Trastuzumab is an IgG1 kappa antibody that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. Trastuzumab binds to the HER2 antigen and thus. inhibits the growth of cancerous cells. Because Trastuzumab is a humanized antibody, it minimizes any HAMA response in patients. The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary, CHO) suspension culture. The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185kDa, which is structurally related to the epidermal growth factor receptor. HER2 protein overexpression is observed in 25%–30% of primary breast cancers and can be determined using an immunohistochemistry based assessment of fixed tumor blocks (Press MF, *et al.* (1993) Cancer Res 53:4960-70. Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2 (Hudziak RM, *et al.* (1989) Mol Cell Biol 9:1165-72; Lewis GD, *et al.* (1993) Cancer Immunol Immunother; 37:255-63; Baselga J, *et al.* (1998) Cancer Res. 58:2825-2831). Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity, ADCC (Hotelling TE, *et al.* (1996) [abstract]. Proc. Annual Meeting Am Assoc Cancer Res; 37:471; Pegram MD, *et al.* (1997) [abstract]. Proc Am Assoc Cancer Res; 38:602). *In vitro*, Trastuzumab mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2. HERCEPTIN® as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease.

HERCEPTIN® in combination with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have not received chemotherapy for their metastatic disease. HERCEPTIN® is clinically active in

patients with ErbB2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga et al, (1996) J. Clin. Oncol. 14:737-744).

The murine monoclonal anti-HER2 antibody inhibits the growth of breast cancer cell lines that overexpress HER2 at the 2+ and 3+ ($1-2 \times 10^6$ HER2 receptors per cell) level, but has no activity on cells that express lower levels of HER2 (Lewis *et al.*, (1993) Cancer Immunol. Immunother. 37:255-263). Based on this observation, antibody 4D5 was humanized (huMAb4D5-8, rhuMAb HER2, U.S. Patent No. 5821337; Carter *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89: 4285-4289) and tested in breast cancer patients whose tumors overexpress HER2 but who had progressed after conventional chemotherapy (Cobleigh *et al.*, (1999) J. Clin. Oncol. 17: 2639-2648).

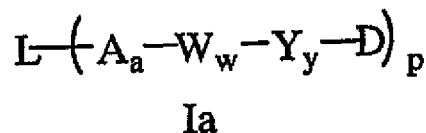
Although HERCEPTIN is a breakthrough in treating patients with ErbB2-overexpressing breast cancers that have received extensive prior anti-cancer therapy, some patients in this population fail to respond or respond only poorly to HERCEPTIN treatment.

Therefore, there is a significant clinical need for developing further HER2-directed cancer therapies for those patients with HER2-overexpressing tumors or other diseases associated with HER2 expression that do not respond, or respond poorly, to HERCEPTIN treatment.

The recitation of any reference in this application is not an admission that the reference is prior art to this application.

3. SUMMARY OF THE INVENTION

In one aspect, the present invention provides Drug-Linker-Ligand compounds having the Formula Ia:



or a pharmaceutically acceptable salt or solvate thereof

wherein,

L- is a Ligand unit;

-A_a-W_w-Y_y- is a Linker unit (LU), wherein the Linker unit includes:

-A- is a Stretcher unit,

a is 0 or 1,

each -W- is independently an Amino Acid unit,

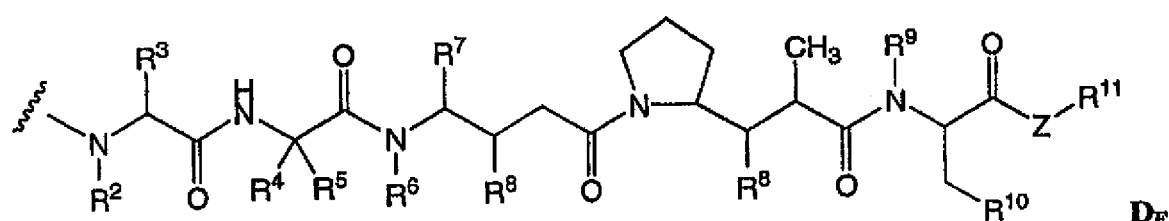
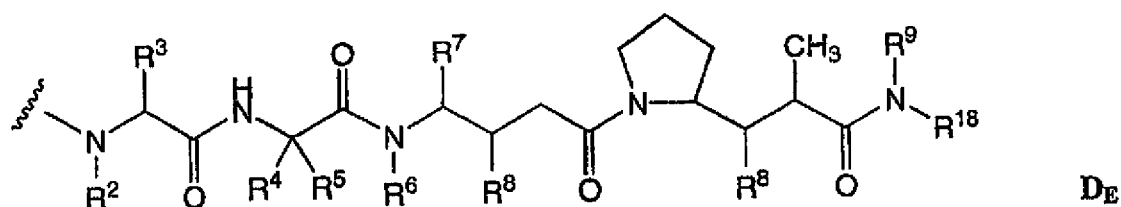
w is an integer ranging from 0 to 12,

-Y- is a Spacer unit, and

y is 0, 1 or 2;

p ranges from 1 to about 20; and

-D is a Drug unit having the Formulas D_E and D_F:



wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R^7 is selected from H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl-(C_3 - C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1 - C_8 alkyl, C_3 - C_8 carbocycle and O-(C_1 - C_8 alkyl);

R^9 is selected from H and C_1 - C_8 alkyl;

R^{10} is selected from aryl or C_3 - C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_8 alkyl;

R^{11} is selected from H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_8 alkyl;

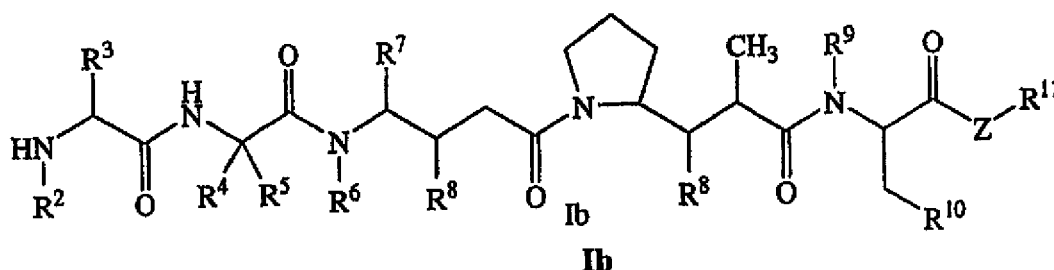
R^{14} is H or C_1 - C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_n-COOH$; where; n is an integer ranging from 0 to 6; and

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2$ -(C_3 - C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2$ -(C_3 - C_8 carbocycle).

In another aspect, Drug Compounds having the Formula Ib are provided:



or pharmaceutically acceptable salts or solvates thereof,

wherein:

R^2 is selected from hydrogen and $-C_1$ - C_8 alkyl;

R^3 is selected from hydrogen, $-C_1$ - C_8 alkyl, $-C_3$ - C_8 carbocycle, aryl, $-C_1$ - C_8 alkyl-aryl, $-C_1$ - C_8 alkyl-(C_3 - C_8 carbocycle), $-C_3$ - C_8 heterocycle and $-C_1$ - C_8 alkyl-(C_3 - C_8 heterocycle);

R^4 is selected from hydrogen, $-C_1-C_8$ alkyl, $-C_3-C_8$ carbocycle, $-aryl$, $-C_1-C_8$ alkyl- $aryl$, $-C_1-C_8$ alkyl- $(C_3-C_8$ carbocycle), $-C_3-C_8$ heterocycle and $-C_1-C_8$ alkyl- $(C_3-C_8$ heterocycle) wherein R^5 is selected from $-H$ and $-methyl$; or R^4 and R^5 jointly, have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from $-H$, $-C_1-C_8$ alkyl and $-C_3-C_8$ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R^6 is selected from H and $-C_1-C_8$ alkyl;

R^7 is selected from H , $-C_1-C_8$ alkyl, $-C_3-C_8$ carbocycle, $aryl$, $-C_1-C_8$ alkyl- $aryl$, $-C_1-C_8$ alkyl- $(C_3-C_8$ carbocycle), $-C_3-C_8$ heterocycle and $-C_1-C_8$ alkyl- $(C_3-C_8$ heterocycle);

each R^8 is independently selected from H , $-OH$, $-C_1-C_8$ alkyl, $-C_3-C_8$ carbocycle and $-O-(C_1-C_8$ alkyl);

R^9 is selected from H and $-C_1-C_8$ alkyl;

R^{10} is selected from $aryl$ group or $-C_3-C_8$ heterocycle;

Z is $-O-$, $-S-$, $-NH-$, or $-NR^{12}-$, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H , C_1-C_{20} alkyl, $aryl$, $-C_3-C_8$ heterocycle, $-(R^{13}O)_m-$, R^{14} , or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is $-C_2-C_8$ alkyl;

R^{14} is H or $-C_1-C_8$ alkyl;

each occurrence of R^{15} is independently H , $-COOH$, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H , $-C_1-C_8$ alkyl, or $-(CH_2)_n-COOH$; and

n is an integer ranging from 0 to 6.

The compounds of Formula (Ib) are useful for treating cancer, an autoimmune disease or an infectious disease in a patient or useful as an intermediate for the synthesis of a Drug-Linker, Drug-Linker-Ligand Conjugate, and Drug-Ligand Conjugate having a cleavable Drug unit.

In another aspect, compositions are provided including an effective amount of a Drug-Linker-Ligand Conjugate and a pharmaceutically acceptable carrier or vehicle.

In still another aspect, the invention provides pharmaceutical compositions comprising an effective amount of a Drug-Linker Compound and a pharmaceutically acceptable carrier or vehicle.

5 In still another aspect, the invention provides compositions comprising an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate and a pharmaceutically acceptable carrier or vehicle.

In yet another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

10 In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

15 In still another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

20 In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

25 In still another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

30 In another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

In another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

5 In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective
10 amount of a Drug-Linker-Ligand Conjugate.

In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

15 In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount
20 of a Drug-Linker-Ligand Conjugate.

In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

25 In yet another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need
30 thereof an effective amount of a Drug-Linker-Ligand Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need

thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

5 In still another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

10 In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

In still another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

15 In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

20 In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

25 In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

30 In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

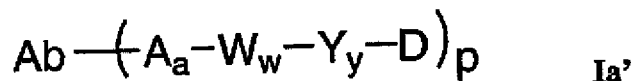
5 In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

10 In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate.

In another aspect, a Drug Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker Compound having a cleavable Drug unit from the Drug-Ligand Conjugate.

15 In another aspect, a Drug-Linker Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker-Ligand Conjugate.

In another aspect, compounds having having Formula Ia' are provided:



or a pharmaceutically acceptable salt or solvate thereof, wherein:

20 Ab includes an antibody including one which binds to to CD30, CD40, CD70, and Lewis Y antigen,

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

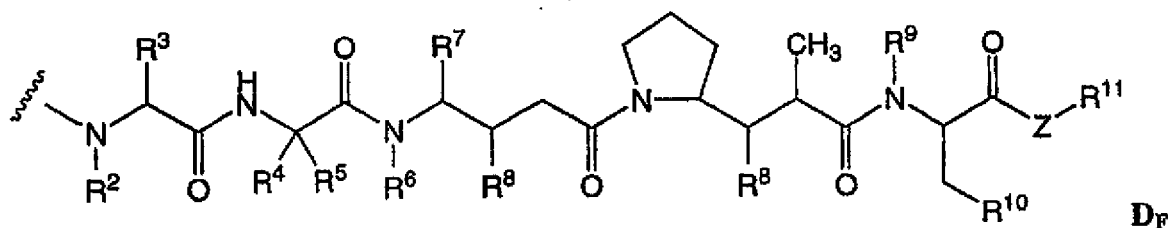
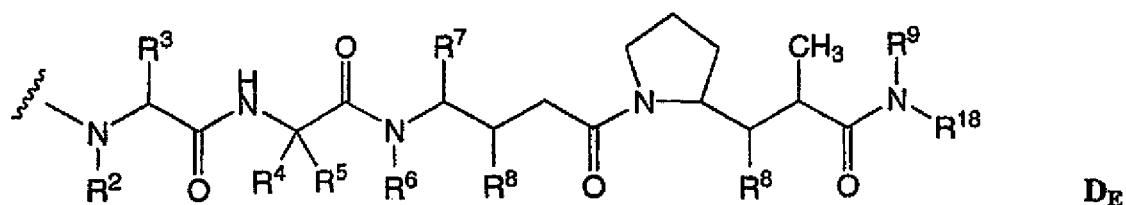
w is an integer ranging from 0 to 12,

25 Y is a Spacer unit, and

y is 0, 1 or 2,

p ranges from 1 to about 20, and

D is a Drug unit selected from Formulas D_E and D_F:



- 5 wherein, independently at each location:
 R^2 is selected from H and C_1-C_8 alkyl;
 R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-
 aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$
 heterocycle);
 10 R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-
 aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$
 heterocycle);
 R^5 is selected from H and methyl;
 or R^4 and R^5 jointly form a carbocyclic ring and have the formula
 15 $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8
 carbocycle and n is selected from 2, 3, 4, 5 and 6;
 R^6 is selected from H and C_1-C_8 alkyl;
 R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-
 aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$
 20 heterocycle);
 each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8
 carbocycle and O- $(C_1-C_8$ alkyl);
 R^9 is selected from H and C_1-C_8 alkyl;
 R^{10} is selected from aryl or C_3-C_8 heterocycle;
 25 Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$,
or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_8 alkyl;

5 R^{14} is H or C_1 - C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$,
 $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_n$ -
COOH;

10 R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8$
heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment, Ab is not an antibody which binds to an ErbB receptor
or which binds to one or more of receptors (1)-(35):

15 (1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank
accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank
accession no. NM_012449);

20 (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor,
mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34
(sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b,
25 Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG,
Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-
like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B,
Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

5 (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein,
10 Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

15 (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);

(15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing
20 phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

25 (20) IL20R α (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) Ephb2R (Genbank accession no. NM_004442);

- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- 5 (27) CD22 (Genbank accession no. NP-001762.1);
- (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);
- 10 (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);
- 15 (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);
- 20 (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);
- 25 (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); or

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

5 In still another aspect, the invention provides pharmaceutical compositions comprising an effective amount of a Drug-Linker-Antibody Conjugate and a pharmaceutically acceptable carrier or vehicle.

 In still another aspect, the invention provides compositions comprising an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit (moiety)
10 from the Drug-Antibody Conjugate and a pharmaceutically acceptable carrier or vehicle.

 In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

 In another aspect, the invention provides methods for killing or inhibiting
15 the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

 In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-
20 Antibody Conjugate.

 In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

 In another aspect, the invention provides methods for killing or inhibiting
25 the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

 In another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a
30 cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

5 In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

10 In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

15 In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

20 In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

25 In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

30 In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to

a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

5 In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

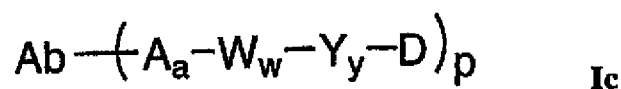
10 In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

15 In another aspect, a Drug Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker Compound having a cleavable Drug unit from the Drug-Antibody Conjugate.

20 In another aspect, a Drug-Linker Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker-Antibody Conjugate.

In one aspect, the present invention provides Drug-Linker-Antibody Conjugates (also referred to as antibody-drug conjugates) having Formula Ic:



25 or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to one or more of the antigens (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

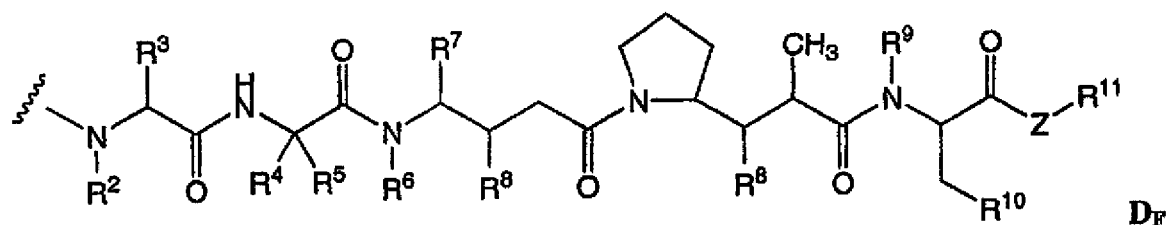
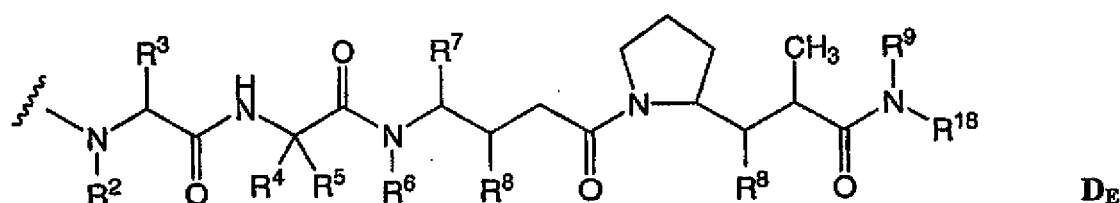
30 (3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

- (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);
- (6) Napi3b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);
- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);
- (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);
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- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);
- (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);

- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
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- (22) Ephb2R (Genbank accession no. NM_004442);
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- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP_001762.1);
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- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor
- 15 that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
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- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus
- 30 erythematosus, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); or

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1);

- 5 A is a Stretcher unit,
 a is 0 or 1,
 each W is independently an Amino Acid unit,
 w is an integer ranging from 0 to 12,
 Y is a Spacer unit, and
 10 y is 0, 1 or 2,
 p ranges from 1 to about 20, and
 D is a Drug moiety selected from Formulas D_E and D_F:



wherein the wavy line of D_E and D_F indicates the covalent attachment site to A, W, or Y, and independently at each location:

- 20 R² is selected from H and C₁-C₈ alkyl;
 R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
 R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
 25 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula

$-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

5 R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$ heterocycle);

10 each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O- $(C_1-C_8$ alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R_{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1-C_8 alkyl;

15 R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-$, R^{14} , or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C^1-C^8 alkyl;

20 each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C^3-C_8$ heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and

25 n is an integer ranging from 0 to 6.

In another aspect, the antibody of the antibody-drug conjugate (ADC) of the invention specifically binds to a receptor encoded by an ErbB2 gene.

In another aspect, the antibody of the antibody-drug conjugate is a humanized antibody selected from huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (Trastuzumab).

In another aspect, the invention includes an article of manufacture comprising an antibody-drug conjugate compound of the invention; a container; and a

package insert or label indicating that the compound can be used to treat cancer characterized by the overexpression of an ErbB2 receptor.

5 In another aspect, the invention includes a method for the treatment of cancer in a mammal, wherein the cancer is characterized by the overexpression of an ErbB2 receptor and does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody, comprising administering to the mammal a therapeutically effective amount of an antibody-drug conjugate compound of the invention.

10 In another aspect, a substantial amount of the drug moiety is not cleaved from the antibody until the antibody-drug conjugate compound enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate, and the drug moiety is cleaved from the antibody when the antibody-drug conjugate does enter the cell.

15 In another aspect, the bioavailability of the antibody-drug conjugate compound or an intracellular metabolite of the compound in a mammal is improved when compared to a drug compound comprising the drug moiety of the antibody-drug conjugate compound, or when compared to an analog of the compound not having the drug moiety.

In another aspect, the drug moiety is intracellularly cleaved in a mammal from the antibody of the compound, or an intracellular metabolite of the compound.

20 In another aspect, the invention includes a pharmaceutical composition comprising an effective amount of the antibody-drug conjugate compound of the invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient. The composition may further comprise a therapeutically effective amount of chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, and a DNA binder.

25 In another aspect, the invention includes a method for killing or inhibiting the proliferation of tumor cells or cancer cells comprising treating tumor cells or cancer cells with an amount of the antibody-drug conjugate compound of the invention, or a pharmaceutically acceptable salt or solvate thereof, being effective to kill or inhibit the proliferation of the tumor cells or cancer cells.

30 In another aspect, the invention includes a method of inhibiting cellular proliferation comprising exposing mammalian cells in a cell culture medium to an antibody drug conjugate compound of the invention, wherein the antibody drug conjugate

compound enters the cells and the drug is cleaved from the remainder of the antibody drug conjugate compound; whereby proliferation of the cells is inhibited.

5 In another aspect, the invention includes a method of treating cancer comprising administering to a patient a formulation of an antibody-drug conjugate compound of the invention and a pharmaceutically acceptable diluent, carrier or excipient.

In another aspect, the invention includes an assay for detecting cancer cells comprising:

- (a) exposing cells to an antibody-drug conjugate compound of the invention; and
- 10 (b) determining the extent of binding of the antibody-drug conjugate compound to the cells.

The invention will best be understood by reference to the following detailed description of the exemplary embodiments, taken in conjunction with the accompanying drawings, figures, and schemes. The discussion below is descriptive,
15 illustrative and exemplary and is not to be taken as limiting the scope defined by any appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an *in vivo*, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous Karpas-299 ALCL xenografts.
20

Figure 2 shows an *in vivo*, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous L540cy. For this study there were 4 mice in the untreated group and 10 in each of the treatment groups.

Figures 3a and 3b show *in vivo* efficacy of cBR96-mcMMAF in
25 subcutaneous L2987. The filled triangles in Figure 3a and arrows in Figure 3b indicate the days of therapy.

Figures 4a and 4b show *in vitro* activity of cAC10-antibody-drug conjugates against CD30⁺ cell lines.

Figures 5a and 5b show *in vitro* activity of cBR96-antibody-drug
30 conjugates against Le^y cell lines.

Figures 6a and 6b show *in vitro* activity of c1F6-antibody-drug conjugates against CD70⁺ renal cell carcinoma cell lines.

Figure 7 shows an *in vitro*, cell proliferation assay with SK-BR-3 cells treated with antibody drug conjugates (ADC): —●— Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, —○— Trastuzumab-MC-MMAF, 4.1 MMAF/Ab, and —Δ— Trastuzumab-MC-MMAF, 4.8 MMAF/Ab, measured in Relative Fluorescence Units (RLU) versus $\mu\text{g/ml}$ concentration of ADC. H = Trastuzumab where H is linked via a cysteine [cys].

Figure 8 shows an *in vitro*, cell proliferation assay with BT-474 cells treated with ADC: —●— Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, —○— Trastuzumab-MC-MMAF, 4.1 MMAF/Ab, and —Δ— Trastuzumab-MC-MMAF, 4.8 MMAF/Ab.

Figure 9 shows an *in vitro*, cell proliferation assay with MCF-7 cells treated with ADC: —●— Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, —○— Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab, and —Δ— Trastuzumab-MC-MMAF, 4.1 MMAF/Ab.

Figure 10 shows an *in vitro*, cell proliferation assay with MDA-MB-468 cells treated with ADC: —●— Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab, —○— Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab, and —Δ— Trastuzumab-MC-vc-PAB-MMAF, 3.7 MMAF/Ab.

Figure 11 shows a plasma concentration clearance study after administration of H-MC-vc-PAB-MMAF-TEG and H-MC-vc-PAB-MMAF to Sprague-Dawley rats: The administered dose was 2 mg of ADC per kg of rat. Concentrations of total antibody and ADC were measured over time. (H = Trastuzumab).

Figure 12 shows a plasma concentration clearance study after administration of H-MC-vc-MMAE to Cynomolgus monkeys at different doses: 0.5, 1.5, 2.5, and 3.0 mg/kg administered at day 1 and day 21. Concentrations of total antibody and ADC were measured over time. (H = Trastuzumab).

Figure 13 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with: Vehicle, Trastuzumab-MC-vc-PAB-MMAE ($1250 \mu\text{g/m}^2$) and Trastuzumab-MC-vc-PAB-MMAF ($555 \mu\text{g/m}^2$). (H = Trastuzumab).

Figure 14 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with 10

mg/kg (660 $\mu\text{g}/\text{m}^2$) of Trastuzumab-MC-MMAE and 1250 $\mu\text{g}/\text{m}^2$ Trastuzumab-MC-vc-PAB-MMAE.

Figure 15 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with Vehicle and 650 $\mu\text{g}/\text{m}^2$ trastuzumab-MC-MMAF.

Figure 16 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with Vehicle and 350 $\mu\text{g}/\text{m}^2$ of four trastuzumab-MC-MMAF conjugates where the MMAF/trastuzumab (H) ratio is 2, 4, 5.9 and 6.

Figure 17 shows the Group mean change, with error bars, in animal (rat) body weights (Mean $\pm$ SD) after administration of Vehicle, trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF.

Figure 18 shows the Group mean change in animal (rat) body weights (Mean $\pm$ SD) after administration of 9.94 mg/kg H-MC-vc-MMAF, 24.90 mg/kg H-MC-vc-MMAF, 10.69 mg/kg H-MC(Me)-vc-PAB-MMAF, 26.78 mg/kg H-MC(Me)-vc-PAB-MMAF, 10.17 mg/kg H-MC-MMAF, 25.50 mg/kg H-MC-MMAF, and 21.85 mg/kg H-MC-vc-PAB-MMAF. H = trastuzumab. The MC linker is attached via a cysteine of trastuzumab for each conjugate.

Figure 19 shows the Group mean change, with error bars, in Sprague Dawley rat body weights (Mean $\pm$ SD) after administration of trastuzumab (H)-MC-MMAF at doses of 2105, 3158, and 4210 $\mu\text{g}/\text{m}^2$. The MC linker is attached via a cysteine of trastuzumab for each conjugate.

4. DETAILED DESCRIPTION OF THE EXEMPLARY EMBODIMENTS

4.1 DEFINITIONS AND ABBREVIATIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. Described in terms of its structure, an antibody typically has a Y-shaped protein consisting of four amino acid chains, two heavy and two light. Each antibody has primarily two regions: a variable region and a constant region. The variable region, located on the ends of the arms of the Y, binds to and interacts with the target antigen. This variable region includes a complementary determining region (CDR) that recognizes and binds to a specific binding site on a particular antigen. The constant region, located on the tail of the Y, is recognized by and interacts with the immune system (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody.

The term "antibody" as used herein, also refers to a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, *i.e.*, a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (*e.g.*, IgG, IgE, IgM, IgD, and IgA), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin. In another aspect, the antibodies are polyclonal, monoclonal, bispecific, human, humanized or chimeric antibodies, single chain antibodies, Fv, Fab fragments, F(ab') fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's, and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, U.S. Patent No. 4816567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature*, 352:624-628 and Marks *et al.* (1991) *J. Mol. Biol.*, 222:581-597, for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent mp/ 4816567; and Morrison *et al.* (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855).

Various methods have been employed to produce monoclonal antibodies (MAbs). Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare MAbs uses genetic engineering including recombinant DNA techniques. Monoclonal antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For

example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen.

As noted, murine antibodies can be used. While useful for diagnostics and short-term therapies, murine antibodies cannot be administered to people long-term without increasing the risk of a deleterious immunogenic response. This response, called Human Anti-Mouse Antibody (HAMA), occurs when a human immune system recognizes the murine antibody as foreign and attacks it. A HAMA response can cause toxic shock or even death.

Chimeric and humanized antibodies reduce the likelihood of a HAMA response by minimizing the nonhuman portions of administered antibodies. Furthermore, chimeric and humanized antibodies have the additional benefit of activating secondary human immune responses, such as antibody dependent cellular cytotoxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof.

The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

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

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:6497-6501 (1985) and Yamamoto *et al.*, (1986) *Nature*, 319:230-234 (Genebank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat p185neu. Preferred ErbB2 is native sequence human ErbB2.

Antibodies to ErbB receptors are available commercially from a number of sources, including, for example, Santa Cruz Biotechnology, Inc., California, USA.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand may be a native sequence human ErbB ligand such as epidermal growth factor (EGF) (Savage *et al.* (1972) *J. Biol. Chem.*, 247:7612-7621); transforming growth factor alpha (TGF- α) (Marquardt *et al.* (1984) *Science* 223:1079-1082); amphiregulin also known as schwannoma or keratinocyte autocrine growth factor (Shoyab *et al.* (1989) *Science* 243:1074-1076; Kimura *et al.*, *Nature*, 348:257-260 (1990); and Cook *et al.*, *Mol. Cell. Biol.*, 11:2547-2557 (1991)); betacellulin (Shing *et al.*, *Science*, 259:1604-1607 (1993); and Sasada *et al.*, *Biochem. Biophys. Res. Commun.*, 190:1173 (1993)); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama *et al.*, *Science*, 251:936-939 (1991)); epiregulin (Toyoda *et al.*, *J. Biol. Chem.*, 270:7495-7500 (1995); and Komurasaki *et al.*, *Oncogene*, 15:2841-2848 (1997)); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway *et al.*, *Nature*, 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang *et al.*, *Proc. Natl. Acad. Sci.*, 94:9562-9567 (1997)); neuregulin-4 (NRG-4) (Harari *et al.*, *Oncogene*, 18:2681-89 (1999)) or cripto (CR-1) (Kannan *et al.*, *J. Biol. Chem.*, 272(6):3330-3335 (1997)). ErbB ligands which bind EGFR include EGF, TGF- α , amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins. The

ErbB ligand may also be a synthetic ErbB ligand. The synthetic ligand may be specific for a particular ErbB receptor, or may recognize particular ErbB receptor complexes. An example of a synthetic ligand is the synthetic heregulin/EGF chimera biregulin (see, for example, Jones *et al.*, (1999) *FEBS Letters*, 447:227-231, which is incorporated by reference).

"Heregulin" (HRG) refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Patent No. 5641869 or Marchionni *et al.*, *Nature*, 362:312-318 (1993). Examples of heregulins include heregulin- α , heregulin- β 1, heregulin- β 2 and heregulin- β 3 (Holmes *et al.*, *Science*, 256:1205-1210 (1992); and U.S. Patent No. 5641869); neu differentiation factor (NDF) (Peles *et al.*, *Cell* 69: 205-216 (1992)); acetylcholine receptor-inducing activity (ARIA) (Falls *et al.* (1993) *Cell* 72:801-815); glial growth factors (GGFs) (Marchionni *et al.*, *Nature*, 362:312-318 (1993)); sensory and motor neuron derived factor (SMDF) (Ho *et al.*, *J. Biol. Chem.*, 270:14523-14532 (1995)); γ -heregulin (Schaefer *et al.*, *Oncogene*, 15:1385-1394 (1997)). The term includes biologically active fragments and/or amino acid sequence variants of a native sequence HRG polypeptide, such as an EGF-like domain fragment thereof (*e.g.*, HRG β 1177-244).

"ErbB hetero-oligomer" is a noncovalently associated oligomer comprising at least two different ErbB receptors. An "ErbB dimer" is a noncovalently associated oligomer that comprises two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand. ErbB oligomers, such as ErbB dimers, can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski *et al.*, *J. Biol. Chem.*, 269(20):14661-14665 (1994), for example. Examples of such ErbB hetero-oligomers include EGFR-ErbB2 (also referred to as HER1/HER2), ErbB2-ErbB3 (HER2/HER3) and ErbB3-ErbB4 (HER3/HER4) complexes. Moreover, the ErbB hetero-oligomer may comprise two or more ErbB2 receptors combined with a different ErbB receptor, such as ErbB3, ErbB4 or EGFR (ErbB1). Other proteins, such as a cytokine receptor subunit (*e.g.*, gp130) may be included in the hetero-oligomer.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide, *e.g.*, tumor-associated antigen receptor, derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino

acid sequence of naturally-occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide.

5 Ordinarily, amino acid sequence variants will possess at least about 70% homology with at least one receptor binding domain of a native ligand, or with at least one ligand binding domain of a native receptor, such as a tumor-associated antigen, and preferably, they will be at least about 80%, more preferably, at least about 90% homologous with such receptor or ligand binding domains. The amino acid sequence variants possess
10 substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

"Sequence identity" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Methods and computer
15 programs for the alignment are well known in the art. One such computer program is "Align 2," authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors
20 (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, (1991) *Annu. Rev. Immunol.* 9:457-92. To assess ADCC
25 activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Patent No. 5500362 or 5821337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al.*, *Proc. Natl. Acad.*
30 *Sci. USA*, 95:652-656 (1998).

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor)

and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof.

5 Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See review M. in *Daëron, Annu. Rev. Immunol.*, 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.*, 9:457-92 (1991); Capel *et al.*, *Immunomethods*,
10 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.*, 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. (Guyer *et al.*, *J. Immunol.*, 117:587 (1976) and Kim *et al.*, *J. Immunol.*, 24:249 (1994)).

15 “Complement dependent cytotoxicity” or “CDC” refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (*e.g.*, an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, *e.g.*, as described in Gazzano-Santoro *et al.*, *J.*
20 *Immunol. Methods*, 202:163 (1996), may be performed.

The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated
25 in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of,
30 the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.* (1991)

Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National

Institutes of Health, Bethesda, MD). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

5 The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al. supra*) and/or those residues from a "hypervariable loop" (*e.g.*,
10 residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk (1987) *J. Mol. Biol.*, 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

15 Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

20 Fv is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six
25 hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

30 The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains

bear at least one free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

5 The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH
10 and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (VH) connected to a
15 variable light domain (VL) in the same polypeptide chain (VH - VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

20 "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat,
25 rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the
30 humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will

comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.* (1986) *Nature*, 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-329; and Presta, (1992) *Curr. Op. Struct. Biol.*, 2:593-596.

5 Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as described in Table 3 of U.S. Patent No. 5821337 expressly incorporated herein by reference; humanized 520C9 (WO 93/21319) and humanized 2C4 antibodies as described herein below.

10 An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will
15 be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated
20 antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

25 An antibody "which binds" an antigen of interest is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen.

30 An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is a tumor cell, *e.g.*, a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and

nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells.

A "disorder" is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemia and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The term "substantial amount" refers to a majority, *i.e.* >50% of a population, of a collection or a sample.

The term "intracellular metabolite" refers to a compound resulting from a metabolic process or reaction inside a cell on an antibody drug conjugate (ADC). The metabolic process or reaction may be an enzymatic process such as proteolytic cleavage of a peptide linker of the ADC, or hydrolysis of a functional group such as a hydrazone, ester, or amide. Intracellular metabolites include, but are not limited to, antibodies and free drug which have undergone intracellular cleavage after entry, diffusion, uptake or transport into a cell.

The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction inside a cell on an Drug-Ligand Conjugate, a Drug-Linker-Ligand Conjugate, an an antibody drug conjugate (ADC) or the like whereby the covalent

attachment, *e.g.*, the linker, between the drug moiety (D) and the antibody (Ab) is broken, resulting in the free drug dissociated from the antibody inside the cell. The cleaved moieties of the Drug-Ligand Conjugate, a Drug-Linker-Ligand Conjugate or ADC are thus intracellular metabolites.

5 The term "bioavailability" refers to the systemic availability (*i.e.*, blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

10 The term "cytotoxic activity" refers to a cell-killing, cytostatic or anti-proliferation effect of an antibody drug conjugate compound or an intracellular metabolite of an antibody drug conjugate compound. Cytotoxic activity may be expressed as the IC_{50} value which is the concentration (molar or mass) per unit volume at which half the cells survive.

15 The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises one or more cancerous cells. 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 squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer ("NSCLC"), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, 20 kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

25 An "ErbB2-expressing cancer" is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer.

30 A cancer "characterized by excessive activation" of an ErbB2 receptor is one in which the extent of ErbB2 receptor activation in cancer cells significantly exceeds the level of activation of that receptor in non-cancerous cells of the same tissue type. Such excessive activation may result from overexpression of the ErbB2 receptor and/or

greater than normal levels of an ErbB2 ligand available for activating the ErbB2 receptor in the cancer cells. Such excessive activation may cause and/or be caused by the malignant state of a cancer cell. In some embodiments, the cancer will be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression of an ErbB2 receptor is occurring which results in such excessive activation of the ErbB2 receptor. Alternatively, or additionally, the cancer may be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression an ErbB2 ligand is occurring in the cancer which attributes to excessive activation of the receptor. In a subset of such cancers, excessive activation of the receptor may result from an autocrine stimulatory pathway.

A cancer which "overexpresses" an ErbB2 receptor is one which has significantly higher levels of an ErbB2 receptor at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB2 receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB2 protein present on the surface of a cell (*e.g.*, via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB2-encoding nucleic acid in the cell, *e.g.*, via fluorescent *in situ* hybridization (FISH; see WO 98/45479), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Overexpression of the ErbB2 ligand, may be determined diagnostically by evaluating levels of the ligand (or nucleic acid encoding it) in the patient, *e.g.*, in a tumor biopsy or by various diagnostic assays such as the IHC, FISH, southern blotting, PCR or *in vivo* assays described above. One may also study ErbB2 receptor overexpression by measuring shed antigen (*e.g.*, ErbB2 extracellular domain) in a biological fluid such as serum (see, *e.g.*, U.S. Patent No. 4933294; WO 91/05264; U.S. Patent No. 5401638; and Sias *et al.*, (1990) *J. Immunol. Methods*, 132: 73-80). Aside from the above assays, various other *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.*, a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.*, by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The tumors overexpressing HER2 are rated by immunohistochemical scores corresponding to the number of copies of HER2 molecules expressed per cell, and can be determined biochemically: 0 = 0-10,000 copies/cell, 1+ = at least about 200,000 copies/cell, 2+ = at least about 500,000 copies/cell, 3+ = about $1-2 \times 10^6$ copies/cell.

5 Overexpression of HER2 at the 3+ level, which leads to ligand-independent activation of the tyrosine kinase (Hudziak *et al.*, (1987) *Proc. Natl. Acad. Sci. USA*, 84:7159-7163), occurs in approximately 30% of breast cancers, and in these patients, relapse-free survival and overall survival are diminished (Slamon *et al.*, (1989) *Science*, 244:707-712; Slamon *et al.*, (1987) *Science*, 235:177-182).

10 Conversely, a cancer which is "not characterized by overexpression of the ErbB2 receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB2 receptor compared to a noncancerous cell of the same tissue type.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is
15 intended to include radioactive isotopes (*e.g.*, ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{60}Co , and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogs and derivatives thereof. In one aspect, the term is not intended to include radioactive isotopes.

20 A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine,
25 triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; TLK 286 (TELCYTA™); acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®),
30 acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; calystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic

analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, 5 nimustine, and ranimustine; bisphosphonates, such as clodronate; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)) and anthracyclines such as annamycin, AD 32, alcarubicin, daunorubicin, dexrazoxane, DX- 10 52-1, epirubicin, GPX-100, idarubicin, KRN5500, menogaril, dynemicin, including dynemicin A, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, 15 ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, and deoxydoxorubicin), esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; 20 folic acid analogues such as denopterin, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, and testolactone; anti-adrenals 25 such as aminogluthethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid (leucovorin); aceglatone; anti-folate anti-neoplastic agents such as ALIMTA®, LY231514 pemetrexed, dihydrofolate reductase inhibitors such as methotrexate, anti-metabolites such as 5-fluorouracil (5-FU) and its prodrugs such as UFT, S-1 and capecitabine, and thymidylate synthase inhibitors and glycinamide ribonucleotide 30 formyltransferase inhibitors such as raltitrexed (TOMUDEX^{RM}, TDX); inhibitors of dihydropyrimidine dehydrogenase such as eniluracil; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid;

gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids and taxanes, *e.g.*, TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Illinois), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; platinum; platinum analogs or platinum-based analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine (VELBAN®); etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); vinca alkaloid; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a

1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in adherent cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as gene therapy vaccines, for example,

- 5 ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine;
PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase I inhibitor; ABARELIX®
rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

- As used herein, the term "EGFR-targeted drug" refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such
- 10 agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Patent No. 4943533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBITUX®) and reshaped human 225 (H225) (see, WO 96/40210,
- 15 Imclone Systems Inc.); antibodies that bind type II mutant EGFR (U.S. Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in U.S. Patent No. 5891996; and human antibodies that bind EGFR, such as ABX-EGF (see WO 98/50433, Abgenix). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP 659,439A2, Merck Patent GmbH).
- 20 Examples of small molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen).

- A "tyrosine kinase inhibitor" is a molecule which inhibits to some extent tyrosine kinase activity of a tyrosine kinase such as an ErbB receptor. Examples of such
- 25 inhibitors include the EGFR-targeted drugs noted in the preceding paragraph as well as quinazolines such as PD 153035, 4-(3-chloroanilino)quinazoline, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706, and pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d]pyrimidines, curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide), tyrphostines
- 30 containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (*e.g.*, those that bind to ErbB-encoding nucleic acid); quinoxalines (U.S. Patent No. 5,804,396); tryphostins (U.S. Patent No. 5804396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-ErbB inhibitors such as CI-1033 (Pfizer); Affinitac

(ISIS 3521; Isis/Lilly); Imatinib mesylate (Gleevec; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxanib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: U.S. Patent No. 5804396; WO 99/09016 (American Cyanamid); WO 98/43960 (American Cyanamid); WO 97/38983 (Warner Lambert); WO 99/06378 (Warner Lambert); WO 99/06396 (Warner Lambert); WO 96/30347 (Pfizer, Inc); WO 96/33978 (Zeneca); WO 96/3397 (Zeneca); and WO 96/33980 (Zeneca).

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. In one embodiment, the anti-angiogenic factor is an antibody that binds to Vascular Endothelial Growth Factor (VEGF).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources

or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically or hydrolytically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as including the anti-CD30, CD40, CD70 or Lewis Y antibodies and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic

acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism.

5 The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or
10 secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being
15 linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking can be accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers can be used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are
20 used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened
25 for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include,
30 but are not limited to arthritis (rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis; chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, systemic

scleroderma and sclerosis, responses associated with inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis), and IBD with co-segregate of pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, and/or episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN, idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset diabetes, multiple sclerosis (MS) such as spino-optical MS, allergic encephalomyelitis, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis, agranulocytosis, vasculitis (including Large Vessel vasculitis (including Polymyalgia Rheumatica and Giant Cell (Takayasu's) Arteritis), Medium Vessel vasculitis (including Kawasaki's Disease and Polyarteritis Nodosa), CNS vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS)), aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet disease, Castleman's syndrome, Goodpasture's Syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson syndrome, solid organ transplant rejection (including pretreatment for high panel reactive antibody titers, IgA deposit in tissues, and rejection arising from renal transplantation, liver transplantation, intestinal transplantation, cardiac transplantation, etc.), graft versus host disease (GVHD), pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's

disease, stiff-man syndrome, immune complex nephritis, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), thrombocytopenia (as developed by myocardial infarction patients, for example), including autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM), including pediatric IDDM, and Sheehan's syndrome; autoimmune hepatitis, Lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré Syndrome, Berger's Disease (IgA nephropathy), primary biliary cirrhosis, celiac sprue (gluten enteropathy), refractory sprue with co-segregate dermatitis herpetiformis, cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, monoclonal gammopathy of uncertain/unknown significance (MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS; autism, inflammatory myopathy, and focal segmental glomerulosclerosis (FSGS).

"Alkyl" is C₁-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH₃), ethyl (Et, -CH₂CH₃), 1-propyl (n-Pr, n-propyl, -CH₂CH₂CH₃), 2-propyl (i-Pr, i-propyl, -CH(CH₃)₂), 1-butyl (n-Bu, n-butyl, -CH₂CH₂CH₂CH₃), 2-methyl-1-propyl (i-Bu, i-butyl, -CH₂CH(CH₃)₂), 2-butyl (s-Bu, s-butyl, -CH(CH₃)CH₂CH₃), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH₃)₃), 1-pentyl (n-pentyl, -CH₂CH₂CH₂CH₂CH₃), 2-pentyl (-CH(CH₃)CH₂CH₂CH₃), 3-pentyl (-CH(CH₂CH₃)₂), 2-methyl-2-butyl (-C(CH₃)₂CH₂CH₃), 3-methyl-2-butyl (-CH(CH₃)CH(CH₃)₂), 3-methyl-1-butyl (-CH₂CH₂CH(CH₃)₂), 2-methyl-1-butyl (-CH₂CH(CH₃)CH₂CH₃), 1-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₃), 2-hexyl (-CH(CH₃)CH₂CH₂CH₂CH₃), 3-hexyl (-CH(CH₂CH₃)(CH₂CH₂CH₃)), 2-methyl-2-

pentyl ($-\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3-methyl-2-pentyl ($-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 4-methyl-2-pentyl ($-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}(\text{CH}_3)_2$), 3-methyl-3-pentyl ($-\text{C}(\text{CH}_3)(\text{CH}_2\text{CH}_3)_2$), 2-methyl-3-pentyl ($-\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}(\text{CH}_3)_2$), 2,3-dimethyl-2-butyl ($-\text{C}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$), 3,3-dimethyl-2-butyl ($-\text{CH}(\text{CH}_3)\text{C}(\text{CH}_3)_3$).

5 "Alkenyl" is $\text{C}_2\text{-C}_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, *i.e.* a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl ($-\text{CH}=\text{CH}_2$), allyl ($-\text{CH}_2\text{CH}=\text{CH}_2$), cyclopentenyl ($-\text{C}_5\text{H}_7$), and 5-hexenyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$).

10 "Alkynyl" is $\text{C}_2\text{-C}_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, *i.e.* a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic ($-\text{C}\equiv\text{CH}$) and propargyl ($-\text{CH}_2\text{C}\equiv\text{CH}$).

15 "Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene ($-\text{CH}_2-$), 1,2-ethyl ($-\text{CH}_2\text{CH}_2-$), 1,3-propyl ($-\text{CH}_2\text{CH}_2\text{CH}_2-$), 1,4-butyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$), and the like.

20 "Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene ($-\text{CH}=\text{CH}-$).

25 "Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene ($-\text{C}\equiv\text{C}-$), propargyl ($-\text{CH}_2\text{C}\equiv\text{C}-$), and 4-pentynyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}\equiv\text{CH}-$).

30 "Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Some aryl groups are represented in the exemplary

structures as "Ar". Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, *e.g.*, the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, *e.g.*, the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O⁻, -OR, -SR, -S⁻, -NR₂, -NR₃, =NR, -CX₃, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO₂, =N₂, -N₃, NC(=O)R, -C(=O)R, -C(=O)NR₂, -SO₃⁻, -SO₃H, -S(=O)₂R, -OS(=O)₂OR, -S(=O)₂NR, -S(=O)R, -OP(=O)(OR)₂, -P(=O)(OR)₂, -PO₃⁻, -PO₃H₂, -C(=O)R, -C(=O)X, -C(=S)R, -CO₂R, -CO₂⁻, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR₂, -C(=S)NR₂, -C(=NR)NR₂, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C₂-C₁₈ alkyl, C₆-C₂₀ aryl, C₃-C₁₄ heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, *e.g.*, nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β -carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolyl, pyrazolidinyl, pyrazolyl, piperazinyl, indolyl, isoindolyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole,

position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β -carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetidedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, *e.g.*, arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

"Linker", "Linker Unit", or "link" means a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety.

In various embodiments, a linker is specified as LU. Linkers include a divalent radical such as an alkylidyl, an arylidyl, a heteroarylidyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (*e.g.*, polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (*e.g.*, polyethyleneamino, JeffamineTM); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

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

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

5 "Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, *e.g.*, melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

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

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist
15 in optically active forms, *i.e.*, they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or *R* and *S*, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes *d* and *l* or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or *l* meaning that the compound is levorotatory.
20 A compound prefixed with (+) or *d* is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no
25 stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

30 Examples of a "patient" include, but are not limited to, a human, rat, mouse, guinea pig, monkey, pig, goat, cow, horse, dog, cat, bird and fowl. In an exemplary embodiment, the patient is a human.

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic group can be unsubstituted or substituted with one or

more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, $-aryl$, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2$, $-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, $-OH$, $-halogen$, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H , $-C_1-C_8$ alkyl and $aryl$.

5 The term " C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms.

Representative " C_1-C_8 alkyl" groups include, but are not limited to, $-methyl$, $-ethyl$, $-n-propyl$, $-n-butyl$, $-n-pentyl$, $-n-hexyl$, $-n-heptyl$, $-n-octyl$, $-n-nonyl$ and $-n-decyl$; while branched C_1-C_8 alkyls include, but are not limited to, $-isopropyl$, $-sec-butyl$, $-isobutyl$, $-tert-butyl$, $-isopentyl$, $2-methylbutyl$, unsaturated C_1-C_8 alkyls include, but are not limited to, $-vinyl$, $-allyl$, $-1-butenyl$, $-2-butenyl$, $-isobutylenyl$, $-1-pentenyl$, $-2-pentenyl$, $-3-methyl-1-butenyl$, $-2-methyl-2-butenyl$, $-2,3-dimethyl-2-butenyl$, $1-hexyl$, $2-hexyl$, $3-hexyl$, $-acetylenyl$, $-propynyl$, $-1-butynyl$, $-2-butynyl$, $-1-pentynyl$, $-2-pentynyl$, $-3-methyl-1-butynyl$. $methyl$, $ethyl$, $propyl$, $isopropyl$, $n-butyl$, $isobutyl$, $sec-butyl$, $tert-butyl$, $n-pentyl$, $isopentyl$, $neopentyl$, $n-hexyl$, $isohexyl$, $2-methylpentyl$, $3-methylpentyl$, $2,2-dimethylbutyl$, $2,3-dimethylbutyl$, $2,2-dimethylpentyl$, $2,3-dimethylpentyl$, $3,3-dimethylpentyl$, $2,3,4-trimethylpentyl$, $3-methylhexyl$, $2,2-dimethylhexyl$, $2,4-dimethylhexyl$, $2,5-dimethylhexyl$, $3,5-dimethylhexyl$, $2,4-dimethylpentyl$, $2-methylheptyl$, $3-methylheptyl$, $n-heptyl$, $isoheptyl$, $n-octyl$, and $isooctyl$. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, $-aryl$, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2$, $-NHC(O)R'$, $-SO_3R'$, $-S(O)_2R'$, $-S(O)R'$, $-OH$, $-halogen$, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; where each R' is independently selected from H , $-C_1-C_8$ alkyl and $aryl$.

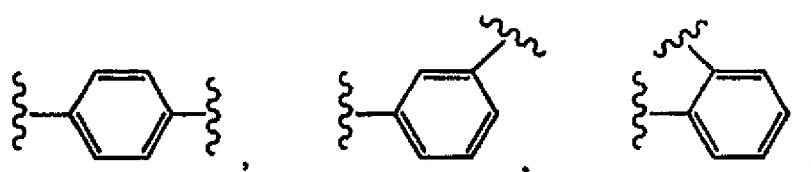
25 A " C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, $-cyclopropyl$, $-cyclobutyl$, $-cyclopentyl$, $-cyclopentadienyl$, $-cyclohexyl$, $-cyclohexenyl$, $-1,3-cyclohexadienyl$, $-1,4-cyclohexadienyl$, $-cycloheptyl$, $-1,3-cycloheptadienyl$, $-1,3,5-cycloheptatrienyl$, $-cyclooctyl$, and $-cyclooctadienyl$. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, $-aryl$, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2$, $-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, $-OH$, $-$

halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; where each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

A " C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

5 A " C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula $-(CH_2)_{1-10}-$. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene and decalene.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), $-aryl$, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2$, $-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, $-OH$, $-halogen$, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently
15 selected from H, $-C_1-C_8$ alkyl and aryl.

A " C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be
20 unsubstituted or substituted with up to seven groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), $-aryl$, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2$, $-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, $-OH$, $-halogen$, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.
25

" C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8

heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; wherein each R' is independently selected from H, -C₁-C₈ alkyl and aryl.

An "Exemplary Compound" is a Drug Compound or a Drug-Linker Compound.

An "Exemplary Conjugate" is a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate or a Drug-Linker-Ligand Conjugate.

In some embodiments, the Exemplary Compounds and Exemplary Conjugates are in isolated or purified form. As used herein, "isolated" means separated from other components of (a) a natural source, such as a plant or animal cell or cell culture, or (b) a synthetic organic chemical reaction mixture. As used herein, "purified" means that when isolated, the isolate contains at least 95 %, and in another aspect at least 98%, of Exemplary Compound or Exemplary Conjugate by weight of the isolate.

Examples of a "hydroxyl protecting group" include, but are not limited to, methoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ether, benzyl ether, p-methoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, triisopropyl silyl ether, t-butyl dimethyl silyl ether, triphenylmethyl silyl ether, acetate ester, substituted acetate esters, pivaloate, benzoate, methanesulfonate and p-toluenesulfonate.

"Leaving group" refers to a functional group that can be substituted by another functional group. Such leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

The phrase "pharmaceutically acceptable salt," as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an Exemplary Compound or Exemplary Conjugate. The Exemplary Compounds and Exemplary Conjugates contain at least one amino group, and accordingly acid addition salts can be formed with this amino group. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate,

succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule
5 such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound.

Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt
10 can have one or more charged atoms and/or one or more counterion.

"Pharmaceutically acceptable solvate" or "solvate" refer to an association of one or more solvent molecules and a compound of the invention, *e.g.*, an Exemplary Compound or Exemplary Conjugate. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol,
15 DMSO, ethyl acetate, acetic acid, and ethanolamine.

The following abbreviations are used herein and have the indicated definitions: AE is auristatin E, Boc is *N*-(*t*-butoxycarbonyl), cit is citrulline, dap is dolaproine, DCC is 1,3-dicyclohexylcarbodiimide, DCM is dichloromethane, DEA is diethylamine, DEAD is diethylazodicarboxylate, DEPC is diethylphosphorylcyanide,
20 DIAD is diisopropylazodicarboxylate, DIEA is *N,N*-diisopropylethylamine, dil is dolaisoleuine, DMAP is 4-dimethylaminopyridine, DME is ethyleneglycol dimethyl ether (or 1,2-dimethoxyethane), DMF is *N,N*-dimethylformamide, DMSO is dimethylsulfoxide, doe is dolaphenine, dov is *N,N*-dimethylvaline, DTNB is 5,5'-dithiobis(2-nitrobenzoic acid), DTPA is diethylenetriaminepentaacetic acid, DTT is dithiothreitol, EDCI is 1-(3-
25 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EEDQ is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ES-MS is electrospray mass spectrometry, EtOAc is ethyl acetate, Fmoc is *N*-(9-fluorenylmethoxycarbonyl), gly is glycine, HATU is *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOBt is 1-hydroxybenzotriazole, HPLC is high pressure liquid
30 chromatography, ile is isoleucine, lys is lysine, MeCN (CH₃CN) is acetonitrile, MeOH is methanol, Mtr is 4-anisylidiphenylmethyl (or 4-methoxytrityl), nor is (1*S*, 2*R*)-(+)-norephedrine, PAB is *p*-aminobenzyl, PBS is phosphate-buffered saline (pH 7.4), PEG is polyethylene glycol, Ph is phenyl, Pnp is *p*-nitrophenyl, MC is 6-maleimidocaproyl, phe

is L-phenylalanine, PyBrop is bromo *tris*-pyrrolidino phosphonium hexafluorophosphate, SEC is size-exclusion chromatography, Su is succinimide, TBTU is *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyluronium tetrafluoroborate, TFA is trifluoroacetic acid, TLC is thin layer chromatography, UV is ultraviolet, and val is valine.

5 The following linker abbreviations are used herein and have the indicated definitions: Val Cit is a valine-citrulline, dipeptide site in protease cleavable linker; PAB is *p*-aminobenzylcarbamoyl; (Me)vc is *N*-methyl-valine citrulline, where the linker peptide bond has been modified to prevent its cleavage by cathepsin B; MC(PEG)6-OH is maleimidocaproyl- polyethylene glycol; SPP is *N*-Succinimidyl 4-(2-pyridylthio)
10 pentanoate; and SMCC is *N*-Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1 carboxylate.

 The terms "treat" or "treatment," unless otherwise indicated by context, refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder,
15 such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can
20 also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

 In the context of cancer, the term "treating" includes any or all of:
25 preventing growth of tumor cells, cancer cells, or of a tumor; preventing replication of tumor cells or cancer cells, lessening of overall tumor burden or decreasing the number of cancerous cells, and ameliorating one or more symptoms associated with the disease.

 In the context of an autoimmune disease, the term "treating" includes any or all of: preventing replication of cells associated with an autoimmune disease state
30 including, but not limited to, cells that produce an autoimmune antibody, lessening the autoimmune-antibody burden and ameliorating one or more symptoms of an autoimmune disease.

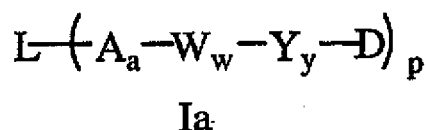
In the context of an infectious disease, the term "treating" includes any or all of: preventing the growth, multiplication or replication of the pathogen that causes the infectious disease and ameliorating one or more symptoms of an infectious disease.

The following cytotoxic drug abbreviations are used herein and have the indicated definitions: MMAE is mono-methyl auristatin E (MW 718); MMAF is N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine (MW 731.5); MMAF-DMAEA is MMAF with DMAEA (dimethylaminoethylamine) in an amide linkage to the C-terminal phenylalanine (MW 801.5); MMAF-TEG is MMAF with tetraethylene glycol esterified to the phenylalanine; MMAF-NtBu is N-t-butyl, attached as an amide to C-terminus of MMAF; AEVB is auristatin E valeryl benzylhydrazone, acid labile linker through the C-terminus of AE (MW 732); and AFP is Monoamide of p-phenylene diamine with C-terminal Phenylalanine of Auristatin F (MW 732).

4.2 THE COMPOUNDS OF THE INVENTION

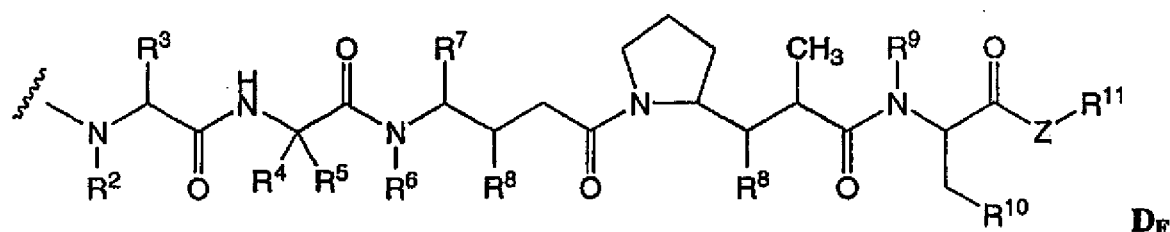
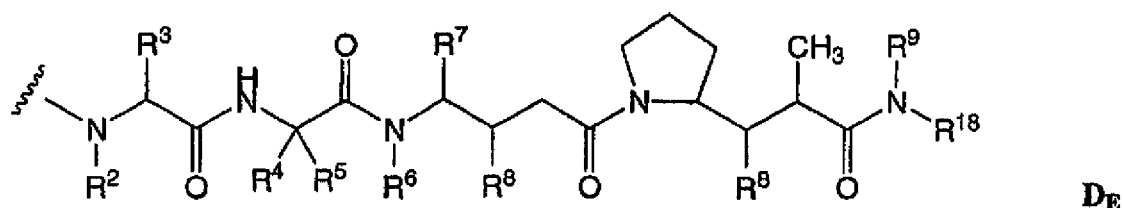
4.2.1 THE COMPOUNDS OF FORMULA (Ia)

In one aspect, the invention provides Drug-Linker-Ligand Conjugates having Formula Ia:



- or a pharmaceutically acceptable salt or solvate thereof
- wherein,
- L- is a Ligand unit;
- A_a-W_w-Y_y- is a Linker unit (LU), wherein the Linker unit includes:
- A- is a Stretcher unit,
- a is 0 or 1,
- each -W- is independently an Amino Acid unit,
- w is an integer ranging from 0 to 12,
- Y- is a Spacer unit, and
- y is 0, 1 or 2;
- p ranges from 1 to about 20; and

-D is a Drug unit having the Formulas D_E and D_F:



wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O-(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, -(R¹³O)_m-R¹⁴, or -(R¹³O)_m-CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

5 R¹³ is C₂-C₈ alkyl;

R¹⁴ is H or C₁-C₈ alkyl;

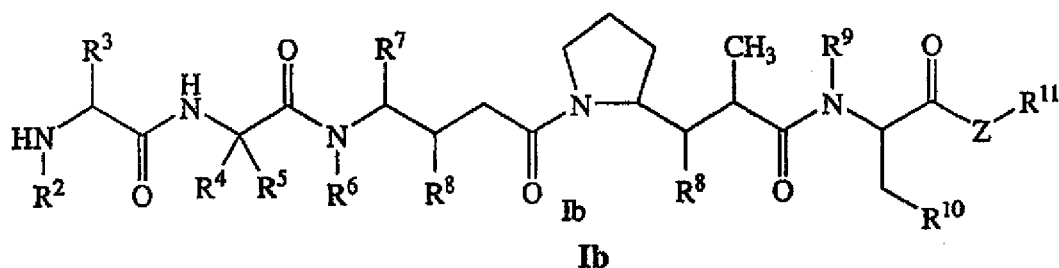
each occurrence of R¹⁵ is independently H, COOH, -(CH₂)_n-N(R¹⁶)₂, -(CH₂)_n-SO₃H, or -(CH₂)_n-SO₃-C₁-C₈ alkyl;

10 each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl, or -(CH₂)_n-COOH;

R¹⁸ is selected from -C(R⁸)₂-C(R⁸)₂-aryl, -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ heterocycle), and -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ carbocycle); and

n is an integer ranging from 0 to 6.

15 In another embodiment, the present invention provides Drug Compounds having the Formula Ib:



or pharmaceutically acceptable salts or solvates thereof,

wherein:

20 R² is selected from hydrogen and -C₁-C₈ alkyl;

R³ is selected from hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle);

25 R⁴ is selected from hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, -aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from -H and -methyl; or R⁴ and R⁵ jointly, have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from -H, -C₁-C₈ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R^6 is selected from H and $-C_1-C_8$ alkyl;

R^7 is selected from H, $-C_1-C_8$ alkyl, $-C_3-C_8$ carbocycle, aryl, $-C_1-C_8$ alkyl-aryl, $-C_1-C_8$ alkyl- $(C_3-C_8$ carbocycle), $-C_3-C_8$ heterocycle and $-C_1-C_8$ alkyl- $(C_3-C_8$ heterocycle);

5 each R^8 is independently selected from H, $-OH$, $-C_1-C_8$ alkyl, $-C_3-C_8$ carbocycle and $-O-(C_1-C_8$ alkyl);

R^9 is selected from H and $-C_1-C_8$ alkyl;

R^{10} is selected from aryl group or $-C_3-C_8$ heterocycle;

Z is $-O-$, $-S-$, $-NH-$, or $-NR^{12}-$, wherein R^{12} is C_1-C_8 alkyl;

10 R^{11} is selected from H, C_1-C_{20} alkyl, aryl, $-C_3-C_8$ heterocycle, $-(R^{13}O)_m-$, R^{14} , or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is $-C_2-C_8$ alkyl;

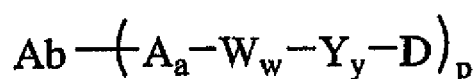
R^{14} is H or $-C_1-C_8$ alkyl;

15 each occurrence of R^{15} is independently H, $-COOH$, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, $-C_1-C_8$ alkyl, or $-(CH_2)_n-COOH$; and

n is an integer ranging from 0 to 6.

20 In yet another embodiment, the invention provides Drug-Linker-Ligand Conjugates having the Formula Ia':



Formula Ia'

or pharmaceutically acceptable salts or solvates thereof.

25 wherein:

Ab is an antibody,

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

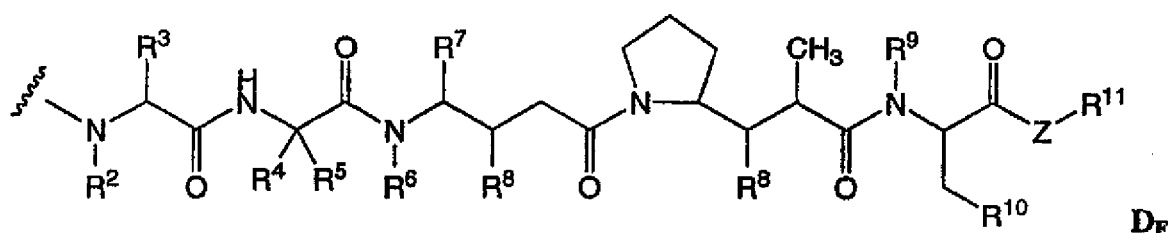
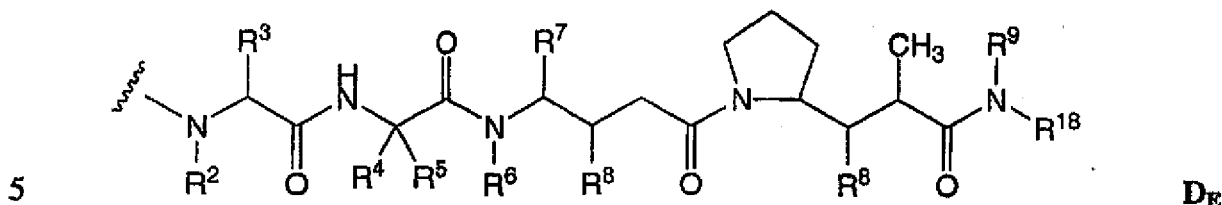
30 w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

y is 0, 1 or 2,

p ranges from 1 to about 20, and

D is a Drug moiety selected from Formulas D_E and D_F:



wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;

10 R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

15 R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

20 R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

25 each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O-(C₁-C₈ alkyl);

R^9 is selected from H and C_1 - C_8 alkyl;

R^{10} is selected from aryl or C_3 - C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_8 alkyl;

R^{11} is selected from H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$,

5 or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_8 alkyl;

R^{14} is H or C_1 - C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$,

10 $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_n$ -COOH;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2$ -(C_3 - C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2$ -(C_3 - C_8 carbocycle); and

15 n is an integer ranging from 0 to 6.

Ab is any antibody covalently attached to one or more drug units. Ab includes an antibody which binds to CD30, CD40, CD70, Lewis Y antigen. In another embodiment, Ab does not include an antibody which binds to an ErbB receptor or to one or more of receptors

20 (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank

25 accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34
30 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Sema5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-

like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

5 (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

10 (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

15 (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);

20 (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

25 (18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20R α (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) Ephb2R (Genbank accession no. NM_004442);

30 (23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763);

(26) BAFF-R (Genbank accession no. NP_443177.1);

(27) CD22 (Genbank accession no. NP-001762.1);

(28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);

(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); and/or

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

In one embodiment -Ww- is -Val-Cit-.

In another embodiment, R³, R⁴ and R⁷ are independently isopropyl or sec-butyl and R⁵ is -H. In an exemplary embodiment, R³ and R⁴ are each isopropyl, R⁵ is -H, and R⁷ is sec-butyl. In yet another embodiment, R² and R⁶ are each methyl, and R⁹ is -H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is $-H$, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is $-H$.

In one embodiment, Z is $-O-$ or $-NH-$.

5 In one embodiment, R^{10} is aryl

In an exemplary embodiment, R^{10} is -phenyl.

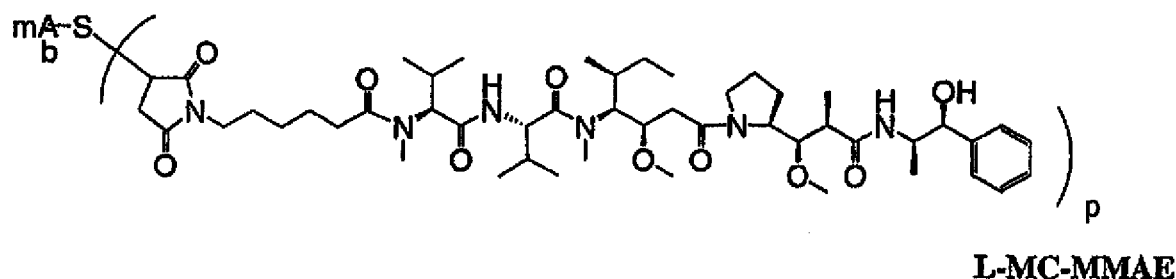
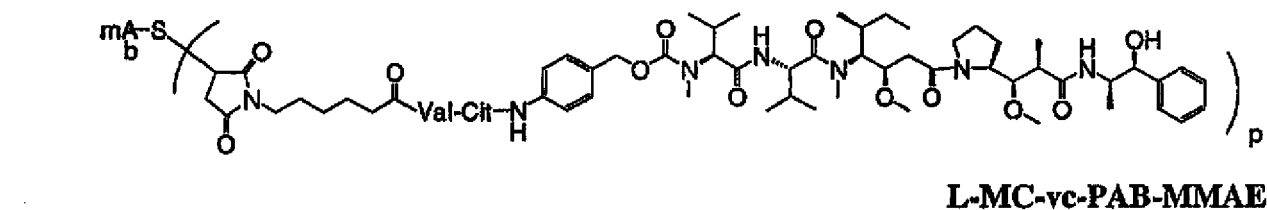
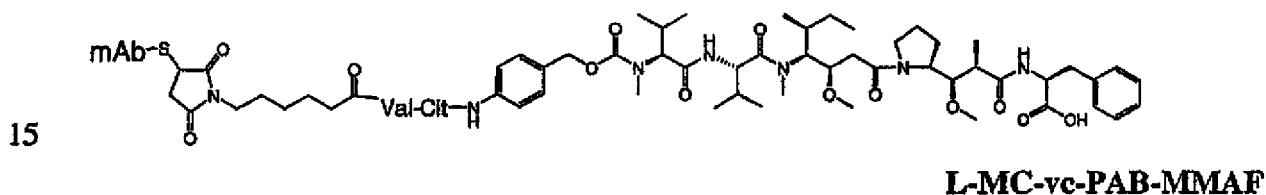
In an exemplary embodiment, when Z is $-O-$, R^{11} is $-H$, methyl or t-butyl.

In one embodiment, when Z is $-NH$, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

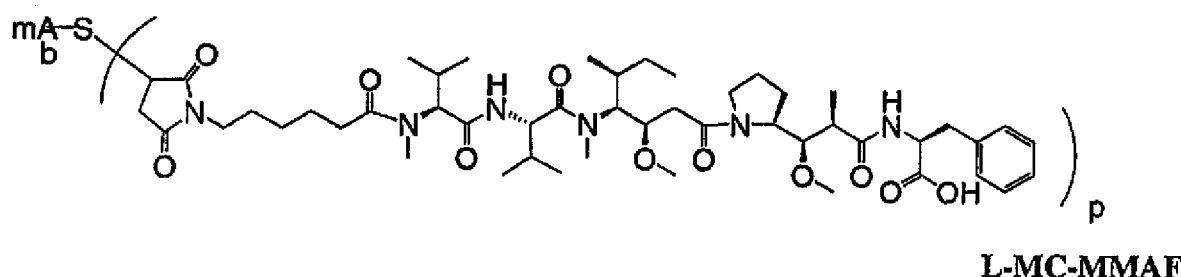
10 In another embodiment, when Z is $-NH$, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

In one aspect, Ab is cAC10, cBR96, cS2C6, c1F6, c2F2, hAC10, hBR96, hS2C6, h1F6, and h2F2.

Exemplary embodiments of Formula Ia have the following structures:



or

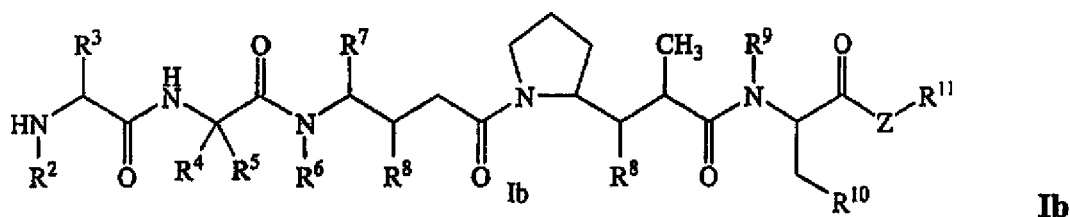


wherein L is an antibody, Val is valine, and Cit is citrulline.

The drug loading is represented by p, the average number of drug molecules per antibody in a molecule (e.g., of Formula Ia, Ia' and Ic). Drug loading may range from 1 to 20 drugs (D) per Ligand (eg. Ab or mAb). Compositions of Formula Ia and Formula Ia' include collections of antibodies conjugated with a range of drugs, from 1 to 20. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Ligand-Drug-Conjugates in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous Ligand-Drug-conjugates where p is a certain value from Ligand-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

4.2.2 THE DRUG COMPOUNDS OF FORMULA (Ib)

In another aspect, the present invention provides Drug Compounds having the Formula (Ib):



or a pharmaceutically acceptable salt or solvate thereof,

wherein:

R² is selected from -hydrogen and -C₁-C₈ alkyl;

R³ is selected from -hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from -hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, -aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-

C₈ heterocycle) wherein R⁵ is selected from -H and -methyl; or R⁴ and R⁵ jointly, have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from -H, -C₁-C₈ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

5 R⁶ is selected from -H and -C₁-C₈ alkyl;

R⁷ is selected from -H, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle);

10 each R⁸ is independently selected from -H, -OH, -C₁-C₈ alkyl, -C₃-C₈ carbocycle and -O-(C₁-C₈ alkyl);

R⁹ is selected from -H and -C₁-C₈ alkyl;

R¹⁰ is selected from aryl group or -C₃-C₈ heterocycle;

Z is -O-, -S-, -NH-, or -NR¹²-, wherein R¹² is C₁-C₈ alkyl;

15 R¹¹ is selected from -H, C₁-C₂₀ alkyl, aryl, -C₃-C₈ heterocycle, -(R¹³O)_m-R¹⁴, or -(R¹³O)_m-CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is -C₂-C₈ alkyl;

R¹⁴ is -H or -C₁-C₈ alkyl;

20 each occurrence of R¹⁵ is independently -H, -COOH, -(CH₂)_n-N(R¹⁶)₂, -(CH₂)_n-SO₃H, or -(CH₂)_n-SO₃-C₁-C₈ alkyl;

each occurrence of R¹⁶ is independently -H, -C₁-C₈ alkyl, or -(CH₂)_n-COOH; and

n is an integer ranging from 0 to 6.

25 In one embodiment, R³, R⁴ and R⁷ are independently isopropyl or sec-butyl and R⁵ is -H. In an exemplary embodiment, R³ and R⁴ are each isopropyl, R⁵ is -H, and R⁷ is sec-butyl.

In another embodiment, R² and R⁶ are each methyl, and R⁹ is -H.

In still another embodiment, each occurrence of R⁸ is -OCH₃.

30 In an exemplary embodiment, R³ and R⁴ are each isopropyl, R² and R⁶ are each methyl, R⁵ is -H, R⁷ is sec-butyl, each occurrence of R⁸ is -OCH₃, and R⁹ is -H.

In one embodiment, Z is -O- or -NH-.

In one embodiment, R¹⁰ is aryl

In an exemplary embodiment, R¹⁰ is -phenyl.

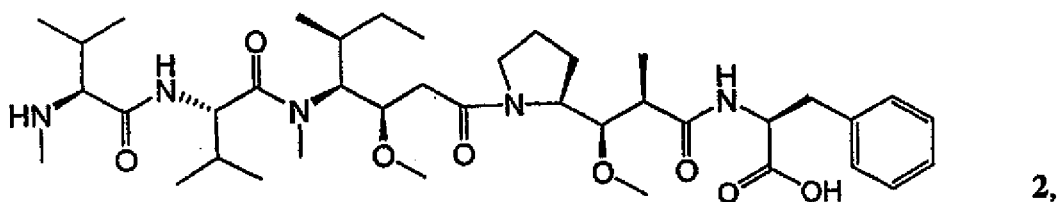
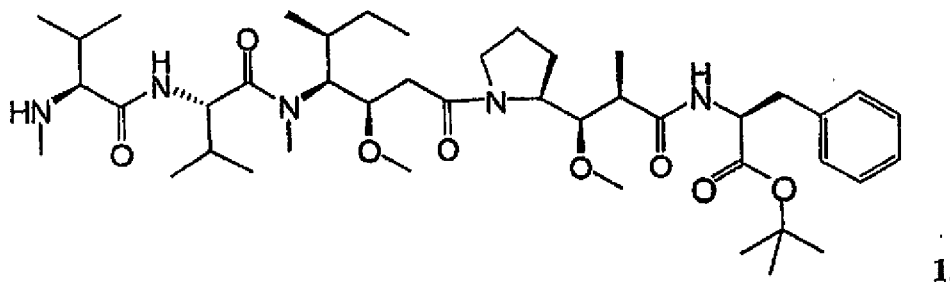
In an exemplary embodiment, when Z is -O-, R¹¹ is -H, methyl or t-butyl.

In one embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -
(CH₂)_n-N(R¹⁶)₂, and R¹⁶ is -C₁-C₈ alkyl or -(CH₂)_n-COOH.

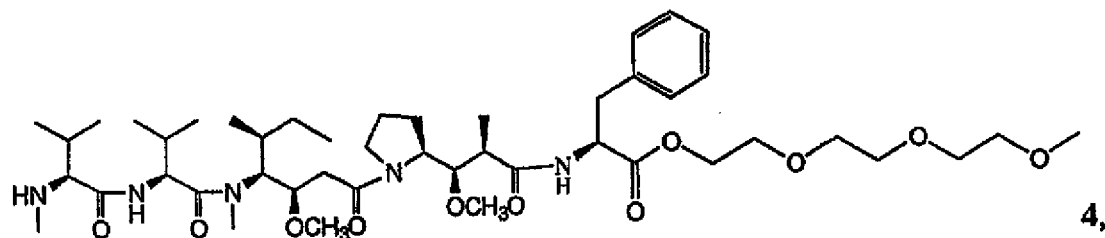
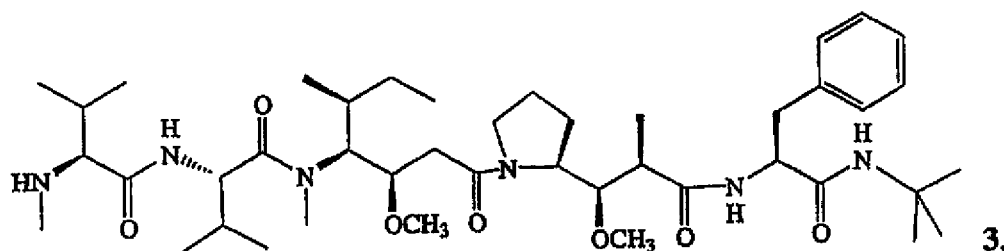
In another embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -

5 (CH₂)_n-SO₃H.

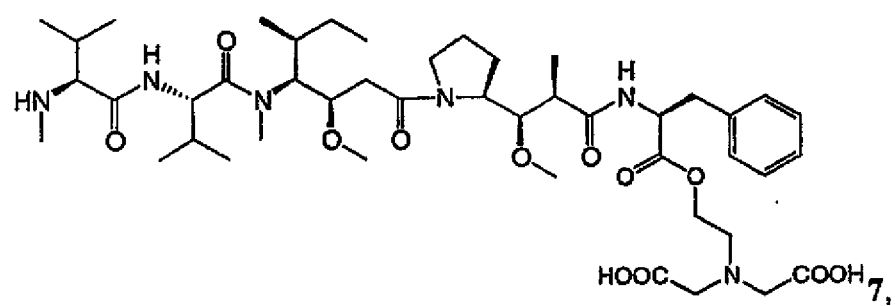
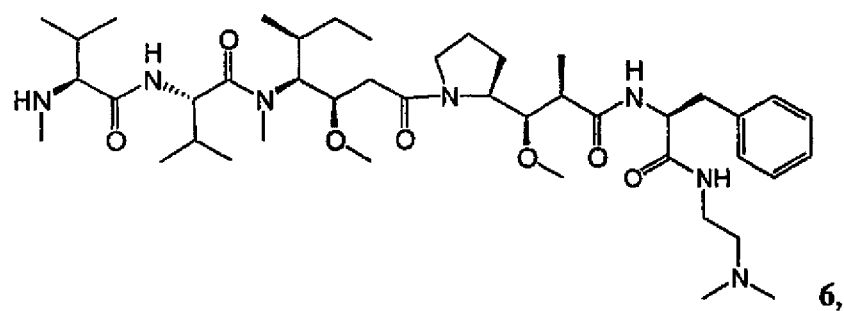
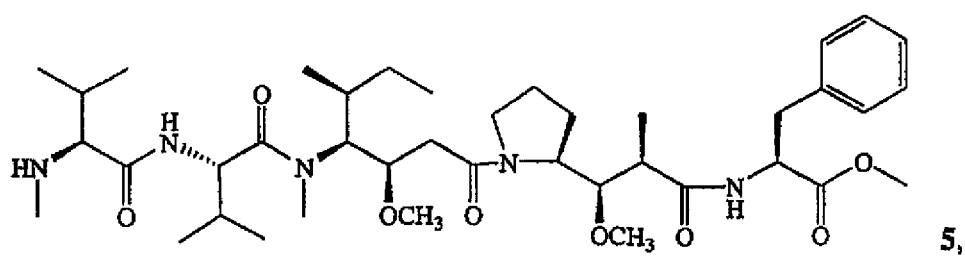
Illustrative Compounds of Formula (Ib), each of which may be used as drug moieties (D) in ADC, include compounds having the following structures:



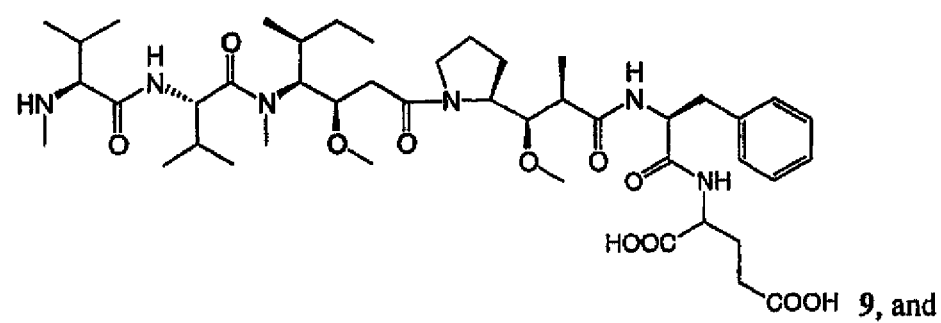
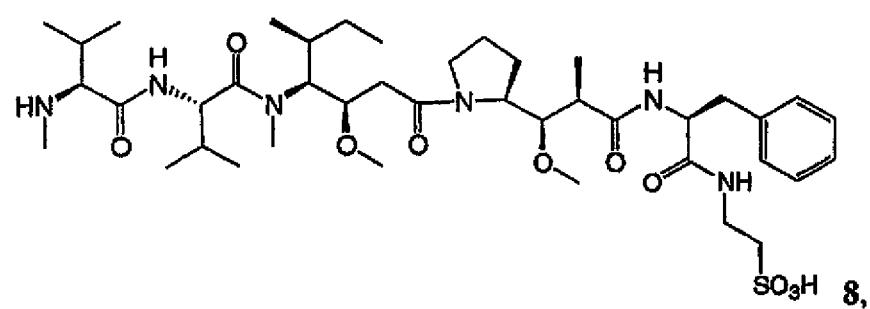
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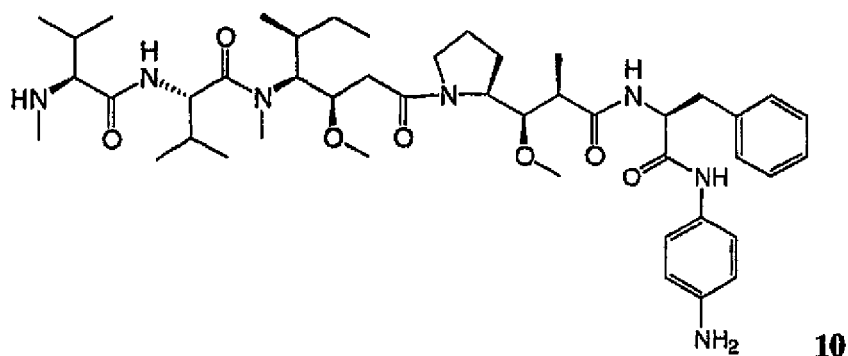


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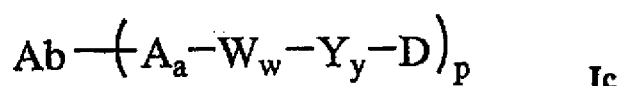




and pharmaceutically acceptable salts or solvates thereof.

5 THE COMPOUNDS OF FORMULA (Ic)

In another aspect, the invention provides antibody-drug conjugate compounds (ADC) having Formula Ic:



comprising an antibody covalently attached to one or more drug units (moieties). The antibody-drug conjugate compounds include pharmaceutically acceptable salts or solvates thereof.

Formula Ic compounds are defined wherein:

Ab is an antibody which binds to one or more tumor-associated antigen receptors (1)-(35):

- 15 (1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);
- (2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);
- (3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);
- 20 (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);
- (6) Napi3b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, 25 Genbank accession no. NM_006424);

- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);
- 5 (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank
- 10 accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);
- 15 (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr
- 20 virus receptor) or Hs.73792 Genbank accession no. M26004);
- (15) CD79b (CD79B, CD79 β , Igb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no.
- 25 NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
- 30 (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);

- (25) GEDA (Genbank accession no. AY260763;
- (26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);
- (27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);
- 5 (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor
- 10 that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No.
- 15 NP_002111.1);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);
- 20 (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus
- 25 erythematosus, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); and
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation
- 30 associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

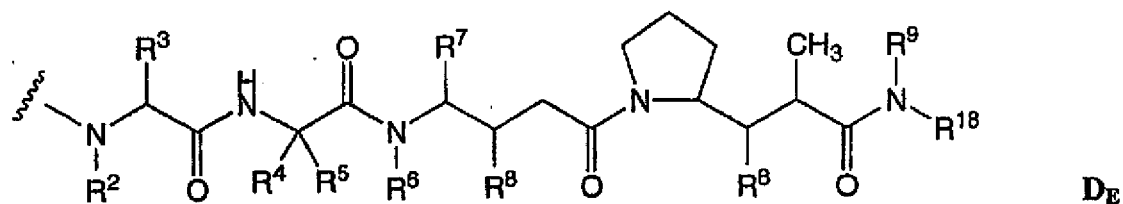
Y is a Spacer unit, and

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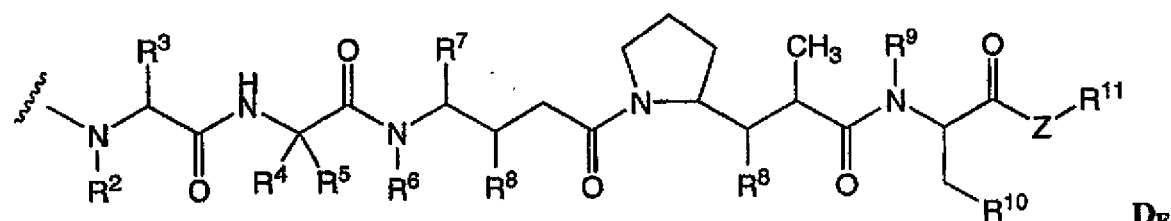
y is 0, 1 or 2,

p ranges from 1 to about 8, and

D is a Drug moiety selected from Formulas D_E and D_F:



D_E



D_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to A, W, or Y, and independently at each location:

R² is selected from H and C₁-C₈ alkyl;

15 R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

20

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$ heterocycle);

5 each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O- $(C_1-C_8$ alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1-C_8 alkyl;

10 R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

15 each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and

20 n is an integer ranging from 0 to 6.

In one embodiment -Ww- is -Val-Cit-.

In another embodiment, R^3 , R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is -H. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is -H, and R^7 is sec-butyl.

25 In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is -H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is -H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is -H.

In one embodiment, Z is -O- or -NH-.

30 In one embodiment, R^{10} is aryl.

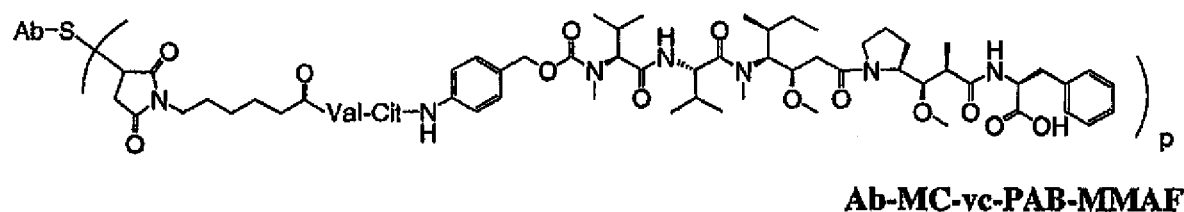
In an exemplary embodiment, R^{10} is -phenyl.

In an exemplary embodiment, when Z is -O-, R^{11} is -H, methyl or t-butyl.

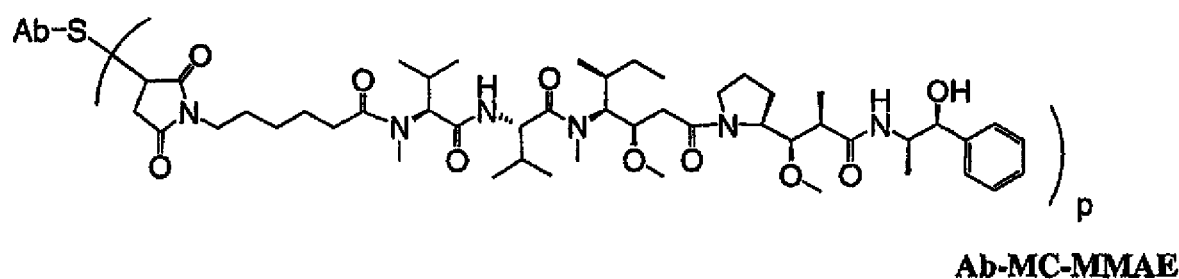
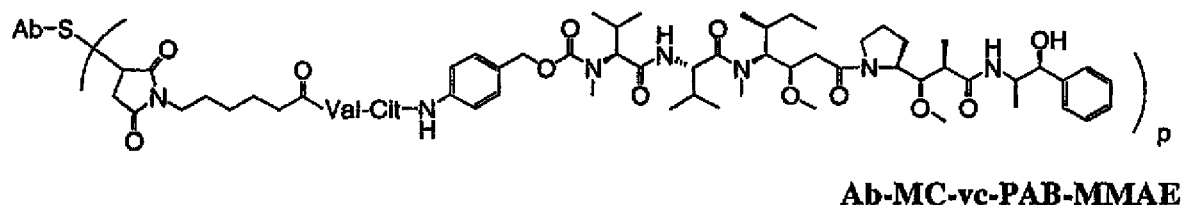
In one embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -
(CH₂)_n-N(R¹⁶)₂, and R¹⁶ is -C₁-C₈ alkyl or -(CH₂)_n-COOH.

In another embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -
(CH₂)_n-SO₃H.

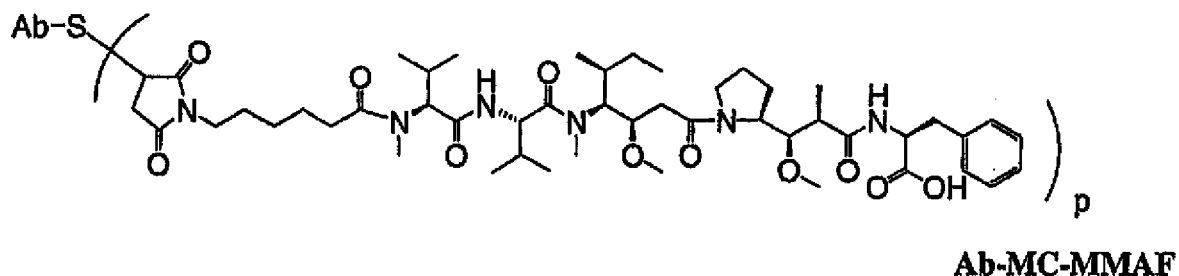
5 Exemplary embodiments of Formula Ic ADC have the following
structures:



10



15



20 wherein Ab is an antibody which binds to one or more tumor-associated antigen receptors
(1)-(35); Val is valine; and Cit is citrulline.

The drug loading is represented by p, the average number of drugs per
antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drugs (D) per

antibody (Ab or mAb). Compositions of ADC of Formula I include collections of antibodies conjugated with a range of drugs, from 1 to 20. The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as UV/visible spectroscopy, mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached.

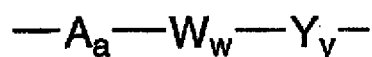
Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the drug-linker intermediate or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds of the invention exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT). Additionally, the antibody must be subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate, or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by dual ELISA antibody assay, specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass

spectroscopy, and separated by HPLC, *e.g.*, hydrophobic interaction chromatography ("Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate", Hamblett, K.J., et al, Abstract No. 624, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; "Controlling the Location of Drug Attachment in Antibody-Drug Conjugates", Alley, S.C., et al, Abstract No. 627, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). Thus, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

4.3 THE LINKER UNIT

A "Linker unit" (LU) is a bifunctional compound which can be used to link a Drug unit and an Ligand unit to form Drug-Linker-Ligand Conjugates, or which are useful in the formation of immunoconjugates directed against tumor associated antigens. Such immunoconjugates allow the selective delivery of toxic drugs to tumor cells. In one embodiment, the Linker unit of the Drug-Linker Compound and Drug-Linker-Ligand Conjugate has the formula:



wherein:

-A- is a Stretcher unit;

a is 0 or 1;

each -W- is independently an Amino Acid unit;

w is independently an integer ranging from 0 to 12;

-Y- is a Spacer unit; and

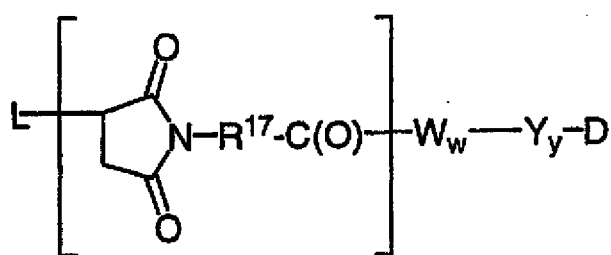
y is 0, 1 or 2.

In the Drug-Linker-Ligand Conjugate, the Linker is capable of linking the Drug moiety and the Ligand unit.

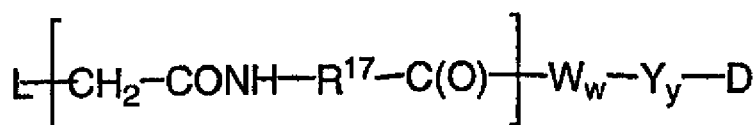
4.3.1 THE STRETCHER UNIT

The Stretcher unit (-A-), when present, is capable of linking a Ligand unit to an amino acid unit (-W-). In this regard a Ligand (L) has a functional group that can form a bond with a functional group of a Stretcher. Useful functional groups that can be present on a ligand, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl (-SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl. In one aspect, the Ligand functional groups are sulfhydryl and amino. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of a Ligand. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of a Ligand using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent.

In one embodiment, the Stretcher unit forms a bond with a sulfur atom of the Ligand unit. The sulfur atom can be derived from a sulfhydryl group of a Ligand. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas IIIa and IIIb, wherein L-, -W-, -Y-, -D, w and y are as defined above, and R¹⁷ is selected from -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-, -O-(C₁-C₈ alkyl)-, -arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r, and -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging from 1-10. It is to be understood from all the exemplary embodiments of Formula Ia, such as III-VI, that even where not denoted expressly, from 1 to 20 drug moieties are linked to a Ligand (p = 1-20).

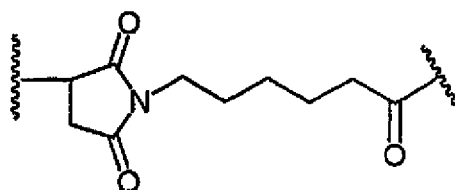


IIIa

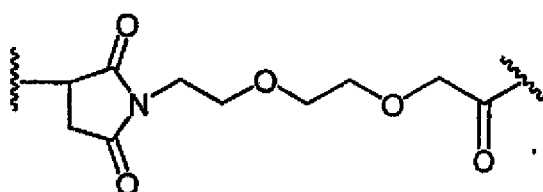


IIIb

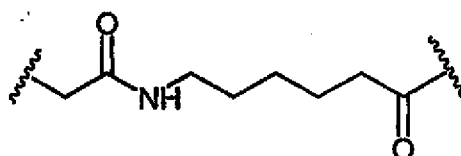
An illustrative Stretcher unit is that of Formula **IIIa** wherein R^{17} is $-(CH_2)_5-$:



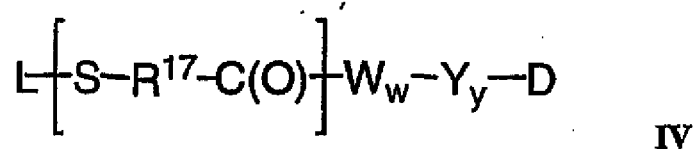
Another illustrative Stretcher unit is that of Formula **IIIa** wherein R^{17} is $-(CH_2CH_2O)_r-CH_2-$; and r is 2:



Still another illustrative Stretcher unit is that of Formula **IIIb** wherein R^{17} is $-(CH_2)_5-$:

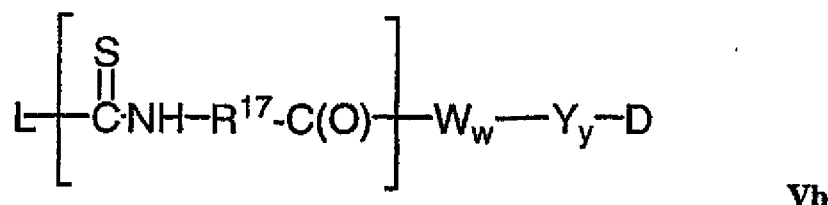
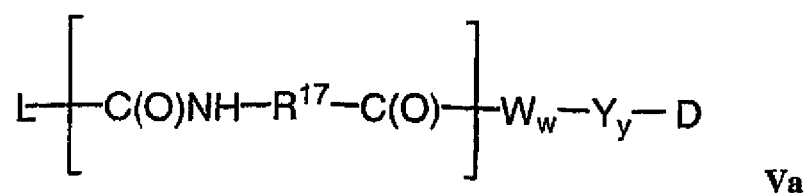


In another embodiment, the Stretcher unit is linked to the Ligand unit via a disulfide bond between a sulfur atom of the Ligand unit and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted within the square brackets of Formula IV, wherein R^{17} , L -, $-W$ -, $-Y$ -, $-D$, w and y are as defined above.

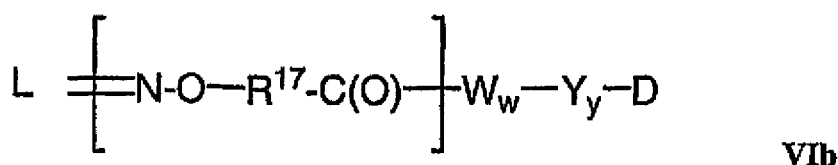
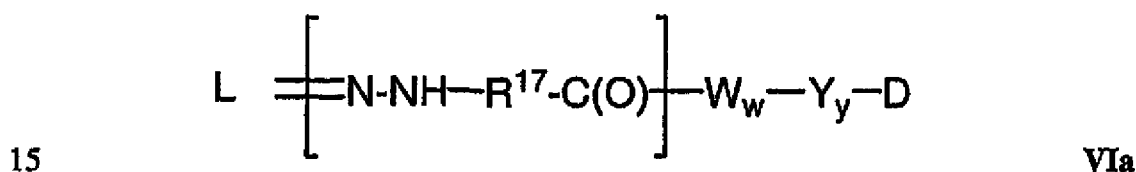


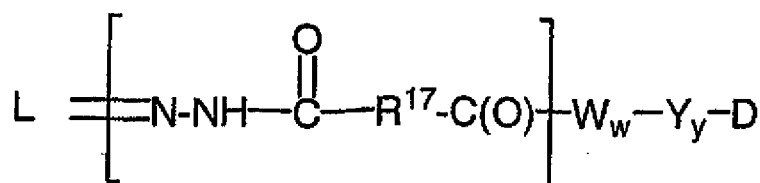
In yet another embodiment, the reactive group of the Stretcher contains a reactive site that can form a bond with a primary or secondary amino group of a Ligand. Example of these reactive sites include, but are not limited to, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates.

Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas Va and Vb, wherein $-R^{17}$ -, L -, $-W$ -, $-Y$ -, $-D$, w and y are as defined above;



- 5 In yet another aspect, the reactive group of the Stretcher contains a reactive site that is reactive to a modified carbohydrate's ($-\text{CHO}$) group that can be present on a Ligand. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting ($-\text{CHO}$) unit of the oxidized carbohydrate can be condensed with a Stretcher that contains a functionality such as a hydrazide, an oxime, 10 a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide such as those described by Kaneko, T. *et al.* (1991) Bioconjugate Chem 2:133-41. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas VIa, VIb, and VIc, wherein $-R^{17}$ -, L -, $-W$ -, $-Y$ -, $-D$, w and y are as defined above.



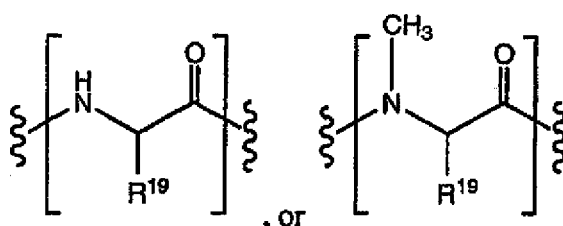


VIc

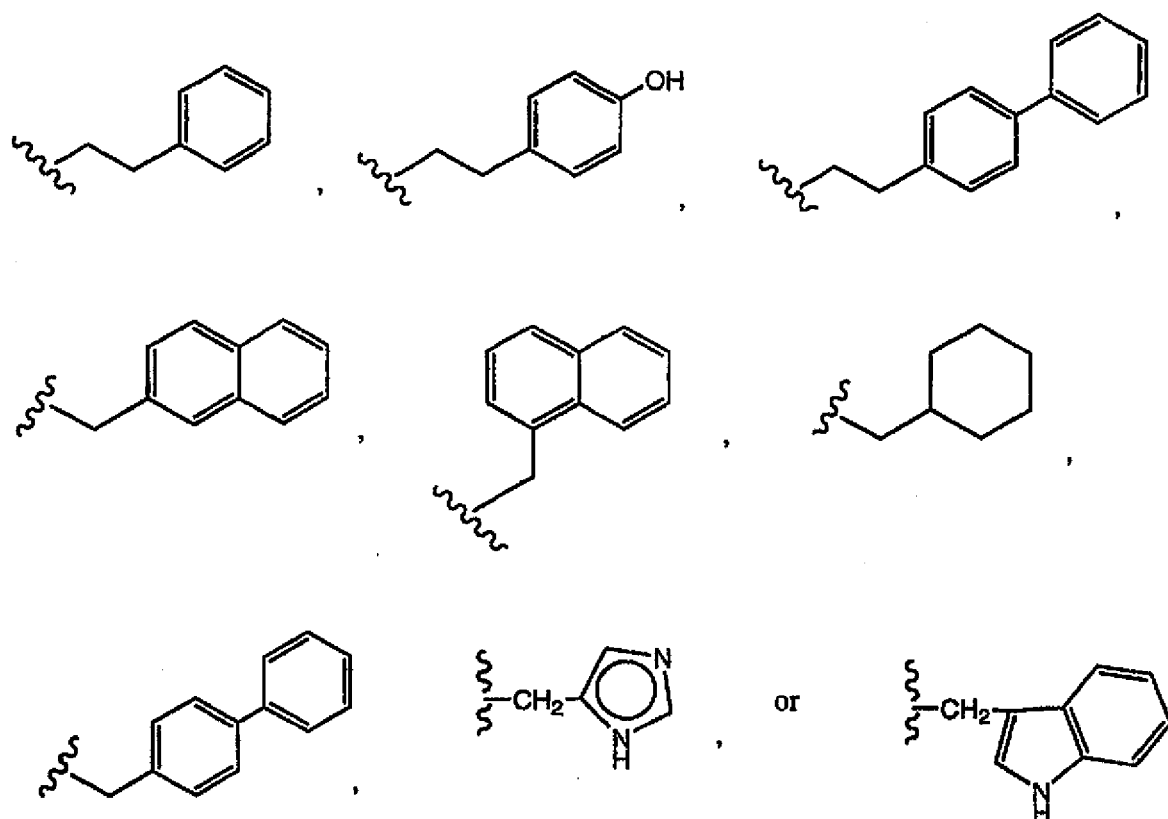
4.3.2 THE AMINO ACID UNIT

The Amino Acid unit (-W-), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the Drug moiety if the Spacer unit is absent, and links the Ligand unit to the Drug unit if the Stretcher unit and Spacer unit are absent.

W_w- is a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each -W- unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 to 12:

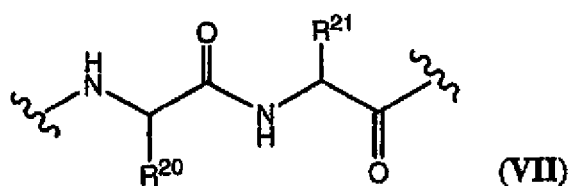


wherein R¹⁹ is hydrogen, methyl, isopropyl, isobutyl, *sec*-butyl, benzyl, *p*-hydroxybenzyl, -CH₂OH, -CH(OH)CH₃, -CH₂CH₂SCH₃, -CH₂CONH₂, -CH₂COOH, -CH₂CH₂CONH₂, -CH₂CH₂COOH, -(CH₂)₃NHC(=NH)NH₂, -(CH₂)₃NH₂, -(CH₂)₃NHCOCH₃, -(CH₂)₃NHCHO, -(CH₂)₄NHC(=NH)NH₂, -(CH₂)₄NH₂, -(CH₂)₄NHCOCH₃, -(CH₂)₄NHCHO, -(CH₂)₃NHCONH₂, -(CH₂)₄NHCONH₂, -CH₂CH₂CH(OH)CH₂NH₂, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



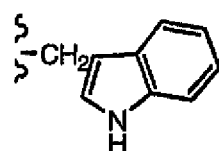
The Amino Acid unit can be enzymatically cleaved by one or more enzymes, including a tumor-associated protease, to liberate the Drug unit (-D), which in one embodiment is protonated *in vivo* upon release to provide a Drug (D).

5 Illustrative W_w units are represented by formulas (VII)-(IX):



wherein R^{20} and R^{21} are as follows:

R^{20}	R^{21}
benzyl	$(CH_2)_4NH_2$;
methyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_3NHCONH_2$;
benzyl	$(CH_2)_3NHCONH_2$;
isobutyl	$(CH_2)_3NHCONH_2$;
sec-butyl	$(CH_2)_3NHCONH_2$;

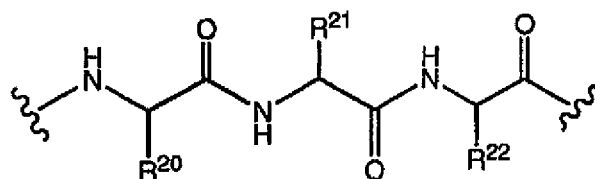


benzyl

benzyl



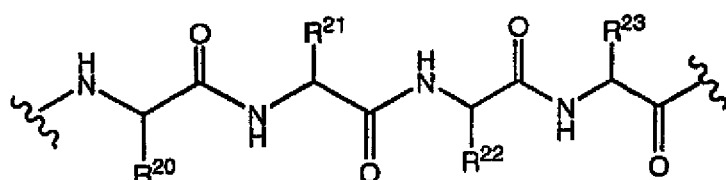
methyl; and



(VIII)

wherein R^{20} , R^{21} and R^{22} are as follows:

R^{20}	R^{21}	R^{22}
benzyl	benzyl	$(\text{CH}_2)_4\text{NH}_2$;
isopropyl	benzyl	$(\text{CH}_2)_4\text{NH}_2$; and
H	benzyl	$(\text{CH}_2)_4\text{NH}_2$;



(IX)

wherein R^{20} , R^{21} , R^{22} and R^{23} are as follows:

R^{20}	R^{21}	R^{22}	R^{23}
H	benzyl	isobutyl	H; and
methyl	isobutyl	methyl	isobutyl.

Exemplary Amino Acid units include, but are not limited to, units of formula (VII) where: R^{20} is benzyl and R^{21} is $-(\text{CH}_2)_4\text{NH}_2$; R^{20} isopropyl and R^{21} is $-(\text{CH}_2)_4\text{NH}_2$; R^{20} isopropyl and R^{21} is $-(\text{CH}_2)_3\text{NHCONH}_2$. Another exemplary Amino Acid unit is a unit of formula (VIII) wherein R^{20} is benzyl, R^{21} is benzyl, and R^{22} is $-(\text{CH}_2)_4\text{NH}_2$.

Useful $-\text{W}_w-$ units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease.

In one embodiment, a $-W_w-$ unit is that whose cleavage is catalyzed by cathepsin B, C and D, or a plasmin protease.

In one embodiment, $-W_w-$ is a dipeptide, tripeptide, tetrapeptide or pentapeptide.

5 When R^{19} , R^{20} , R^{21} , R^{22} or R^{23} is other than hydrogen, the carbon atom to which R^{19} , R^{20} , R^{21} , R^{22} or R^{23} is attached is chiral.

Each carbon atom to which R^{19} , R^{20} , R^{21} , R^{22} or R^{23} is attached is independently in the (S) or (R) configuration.

10 In one aspect of the Amino Acid unit, the Amino Acid unit is valine-citrulline. In another aspect, the Amino Acid unit is phenylalanine-lysine (*i.e.* flk). In yet another aspect of the Amino Acid unit, the Amino Acid unit is N-methylvaline-citrulline. In yet another aspect, the Amino Acid unit is 5-aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepepotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepepotic acid.

15 In certain embodiments, the Amino Acid unit can comprise natural amino acids. In other embodiments, the Amino Acid unit can comprise non-natural amino acids.

4.3.3 THE SPACER UNIT

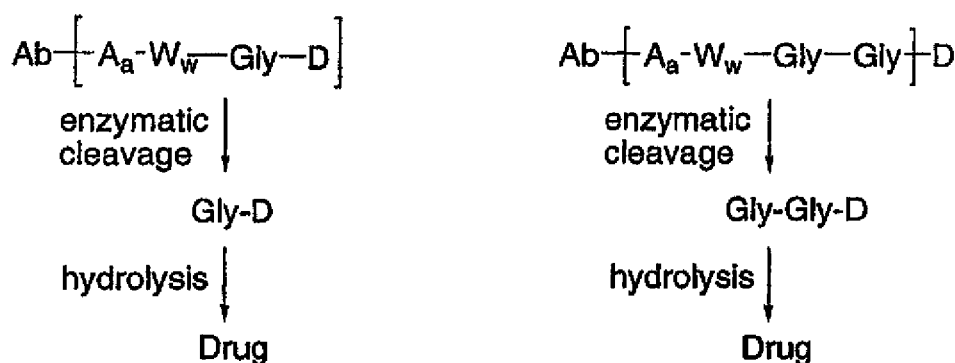
20 The Spacer unit ($-Y-$), when present, links an Amino Acid unit to the Drug moiety when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the Drug moiety when the Amino Acid unit is absent. The Spacer unit also links the Drug moiety to the Ligand unit when both the Amino Acid unit and Stretcher unit are absent.

25 Spacer units are of two general types: self-immolative and non self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to the Drug moiety after cleavage, particularly enzymatic, of an Amino Acid unit from the Drug-Linker-Ligand Conjugate or the Drug-Linker Compound. Examples of a non self-immolative Spacer unit include, but are not limited to a (glycine-glycine) Spacer unit and a glycine Spacer unit (both depicted in Scheme 1) (*infra*). When
30 an Exemplary Compound containing a glycine-glycine Spacer unit or a glycine Spacer unit undergoes enzymatic cleavage via a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease, a glycine-glycine-Drug moiety or a glycine-Drug moiety is cleaved from $L-A_n-W_w-$. In one embodiment, an

independent hydrolysis reaction takes place within the target cell, cleaving the glycine-Drug moiety bond and liberating the Drug.

In another embodiment, -Y- is a p-aminobenzyl alcohol (PAB) unit (see Schemes 2 and 3) whose phenylene portion is substituted with Q_m wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, - nitro or -cyano; and m is an integer ranging from 0-4.

Scheme 1

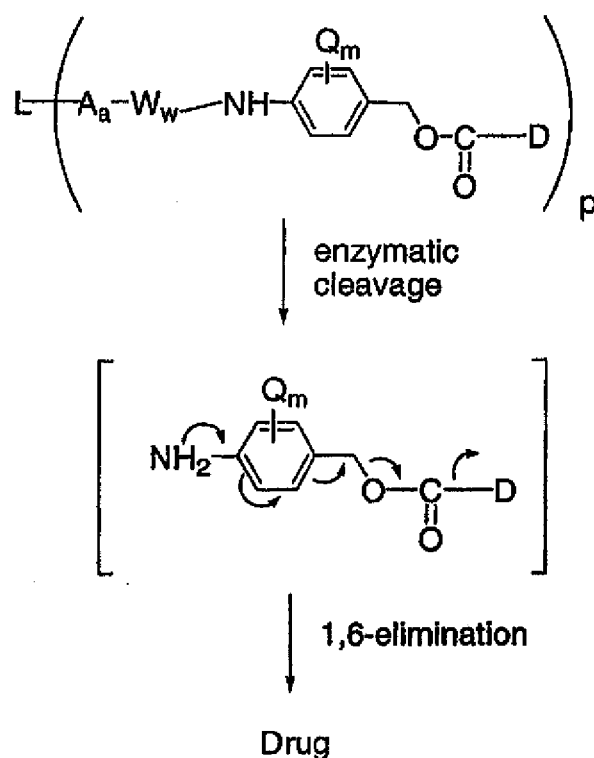


In one embodiment, a non self-immolative Spacer unit (-Y-) is -Gly-Gly-. In another embodiment, a non self-immolative the Spacer unit (-Y-) is -Gly-.

10 In one embodiment, a Drug-Linker Compound or a Drug-Linker Ligand Conjugate is provided in which the Spacer unit is absent (y=0), or a pharmaceutically acceptable salt or solvate thereof.

Alternatively, an Exemplary Compound containing a self-immolative Spacer unit can release -D without the need for a separate hydrolysis step. In this
 15 embodiment, -Y- is a PAB group that is linked to -W_w- via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group. Without being bound by any particular theory or mechanism, Scheme 2 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via a carbamate or carbonate group espoused by Toki *et al.* (2002) J Org. Chem. 67:1866-1872.

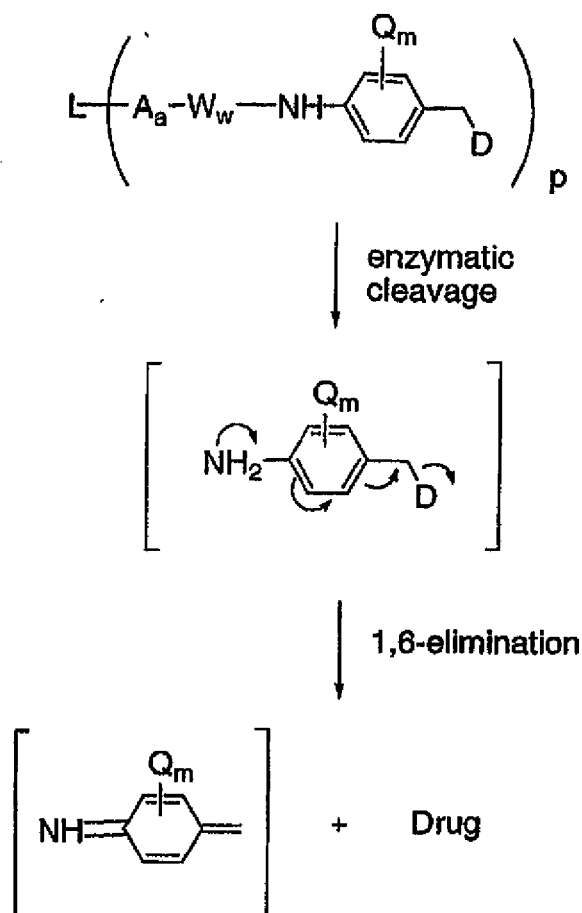
Scheme 2



wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

- 5 Without being bound by any particular theory or mechanism, Scheme 3 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via an ether or amine linkage.

Scheme 3

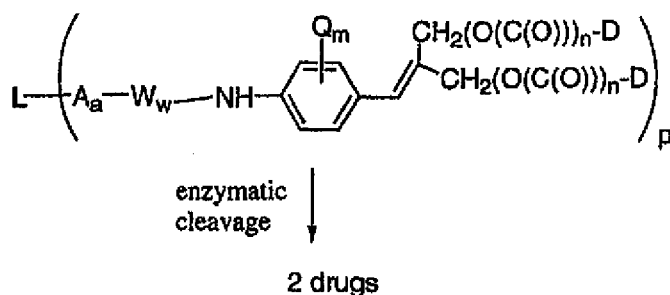


wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, - nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

- 5 Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay *et al.* (1999) *Bioorg. Med. Chem. Lett.* 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-
- 10 aminobutyric acid amides (Rodrigues *et al.*, *Chemistry Biology*, 1995, 2, 223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, *et al.*, *J. Amer. Chem. Soc.*, 1972, 94, 5815) and 2-aminophenylpropionic acid amides (Amsberry, *et al.*, *J. Org. Chem.*, 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the α-position of glycine (Kingsbury, *et al.*, *J. Med. Chem.*, 1984, 27, 1447)
- 15 are also examples of self-immolative spacer useful in Exemplary Compounds.

In one embodiment, the Spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted in Scheme 4, which can be used to incorporate and release multiple drugs.

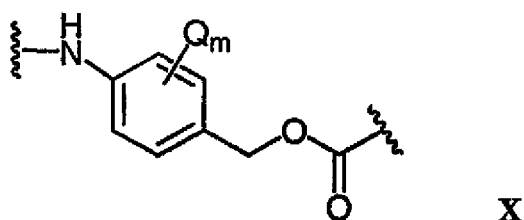
Scheme 4



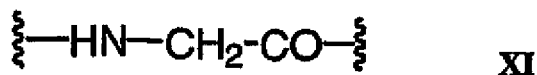
wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

In one embodiment, the -D moieties are the same. In yet another embodiment, the -D moieties are different.

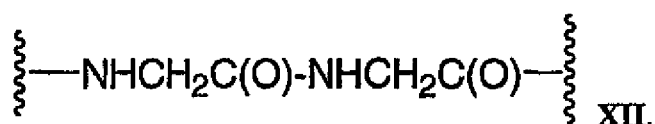
10 In one aspect, Spacer units (-Y_Y-) are represented by Formulas (X)-(XII):



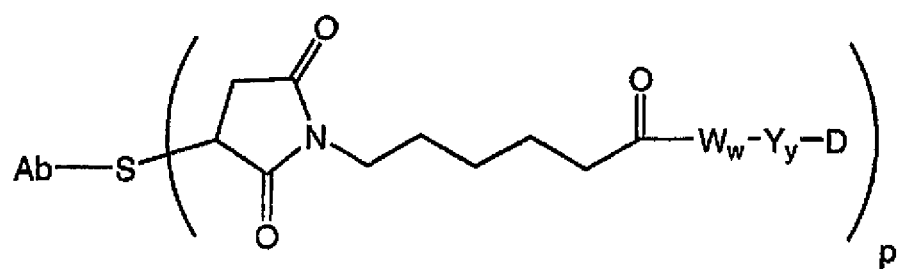
wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4;



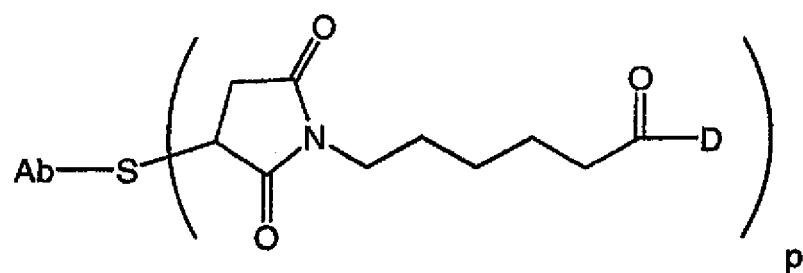
15 and



Embodiments of the Formula Ia' and Ic antibody-drug conjugate compounds include:

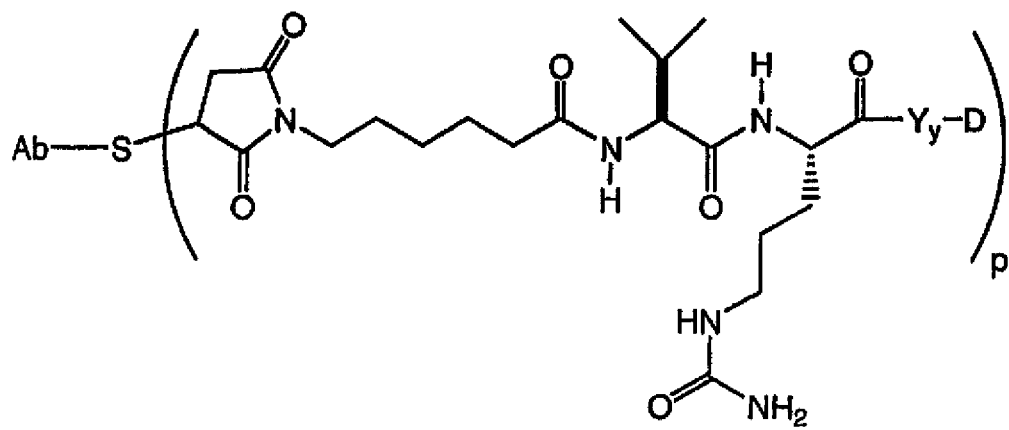
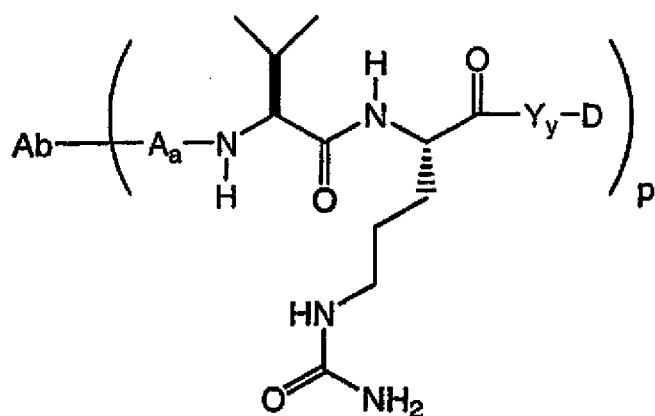


and,

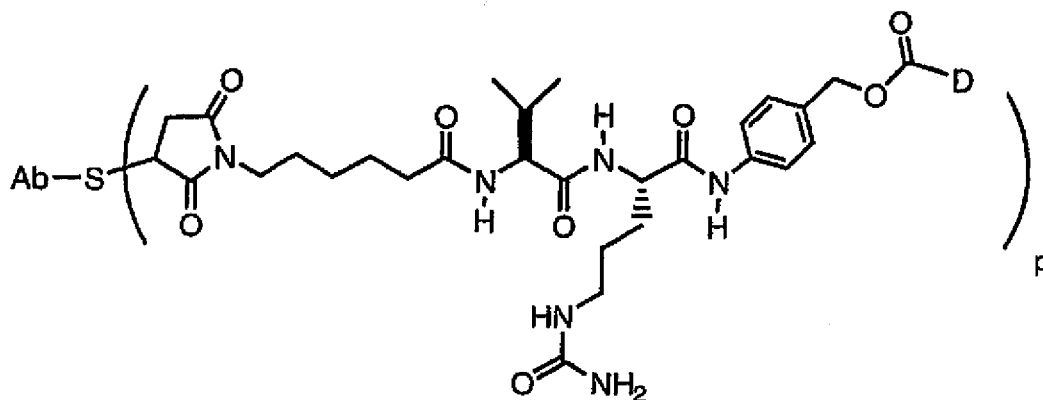


wherein w and y are each 0,

5



, and

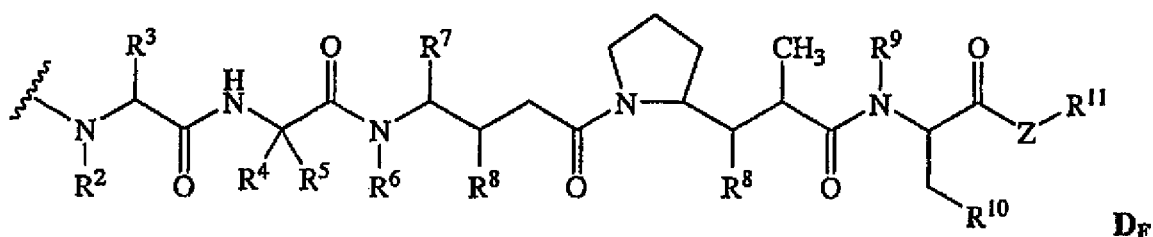
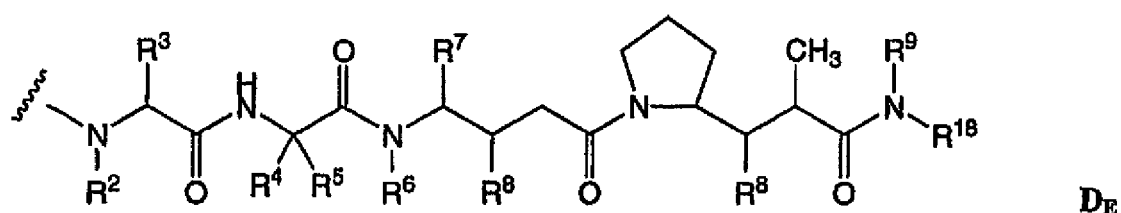


4.4 THE DRUG UNIT (MOIETY)

The drug moiety (D) of the antibody drug conjugates (ADC) are of the dolastatin/auristatin type (U.S. Patent Nos. 5635483; 5780588) which have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke *et al.* (2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) and have anticancer (U.S. Patent No. 5663149) and antifungal activity (Pettit *et al.* (1998) *Antimicrob. Agents Chemother.* 42:2961-2965)

D is a Drug unit (moiety) having a nitrogen atom that can form a bond with the Spacer unit when $y=1$ or 2, with the C-terminal carboxyl group of an Amino Acid unit when $y=0$, with the carboxyl group of a Stretcher unit when w and $y=0$, and with the carboxyl group of a Drug unit when a , w , and $y=0$. It is to be understood that the terms "drug unit" and "drug moiety" are synonymous and used interchangeably herein.

In one embodiment, -D is either formula D_E or D_F :



wherein, independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$ heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$ heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl- $(C_3-C_8$ carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl- $(C_3-C_8$ heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and $O-(C_1-C_8$ alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment, R^3 , R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is -H. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is H, and R^7 is sec-butyl.

In another embodiment, R^2 and R^6 are each methyl, and R^9 is H.

5 In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is H.

In one embodiment, Z is $-O-$ or $-NH-$.

In one embodiment, R^{10} is aryl

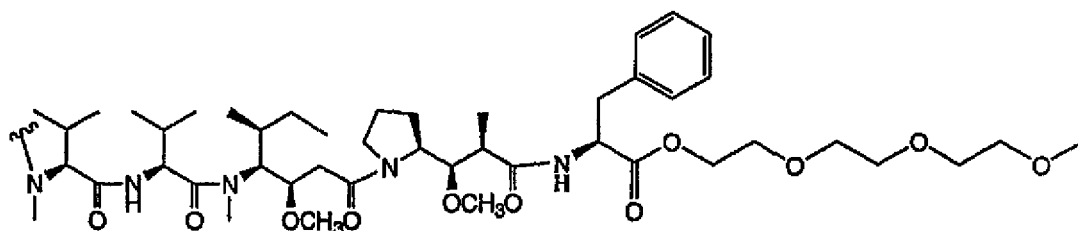
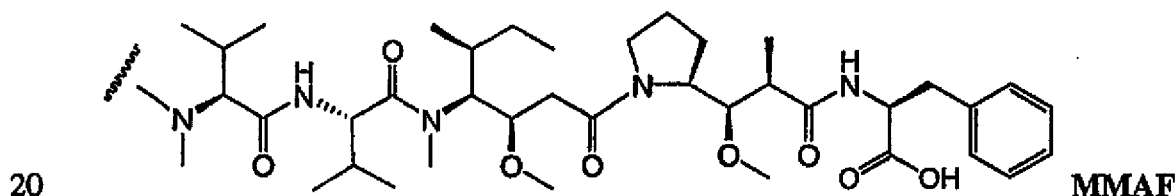
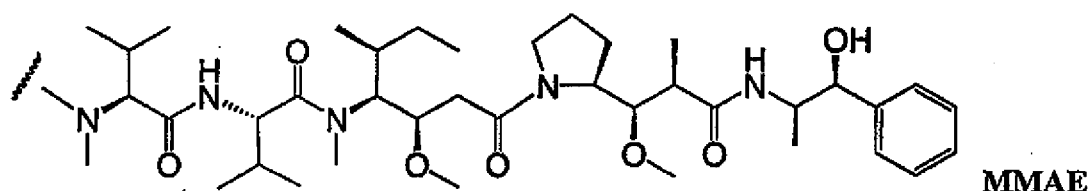
10 In an exemplary embodiment, R^{10} is -phenyl.

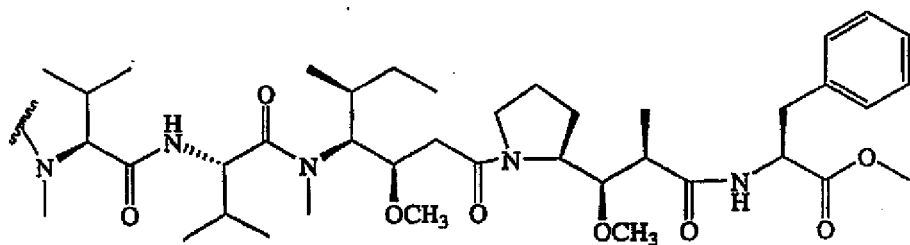
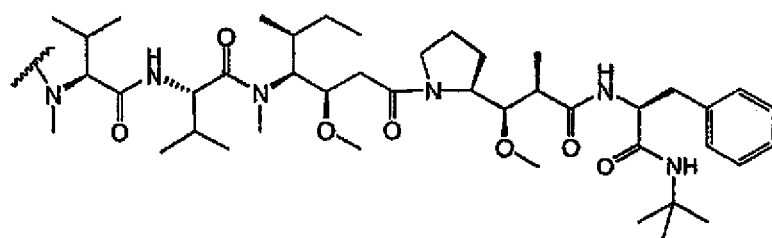
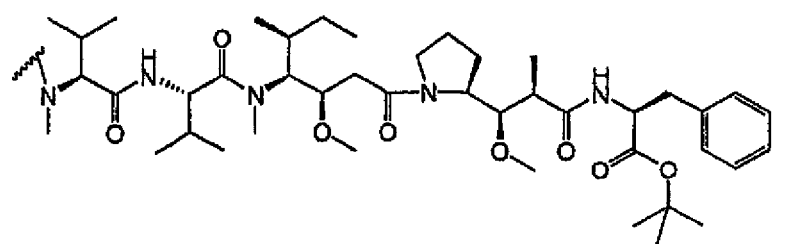
In an exemplary embodiment, when Z is $-O-$, R^{11} is H, methyl or t-butyl.

In one embodiment, when Z is $-NH$, R^{11} is $-\text{CH}(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

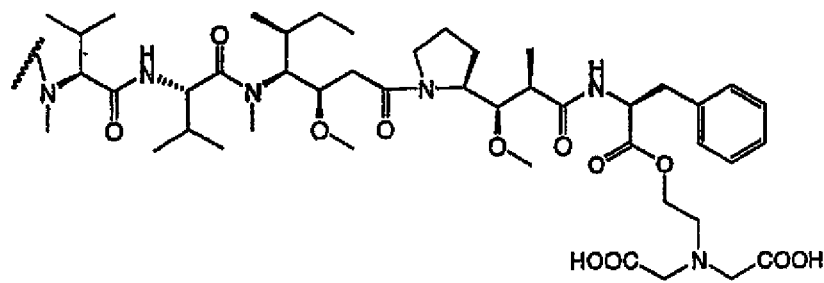
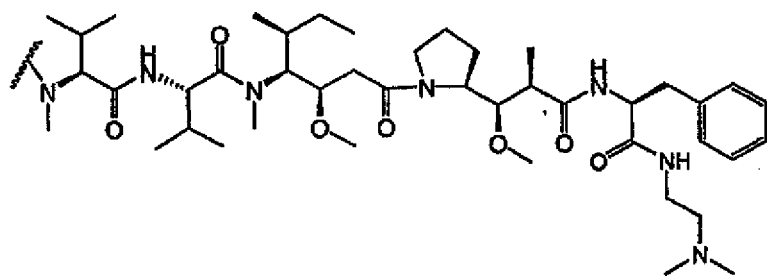
15 In another embodiment, when Z is $-NH$, R^{11} is $-\text{CH}(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

Illustrative Drug units (-D) include the drug units having the following structures:

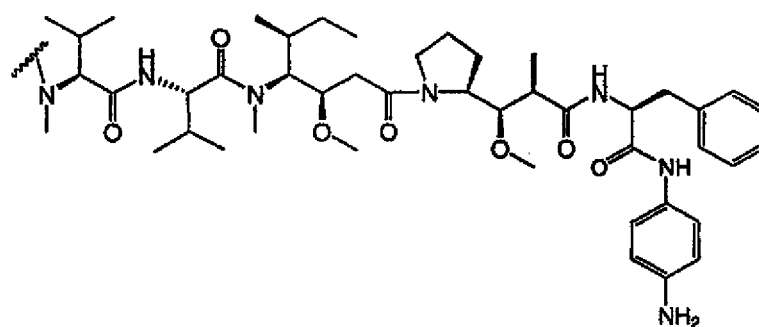
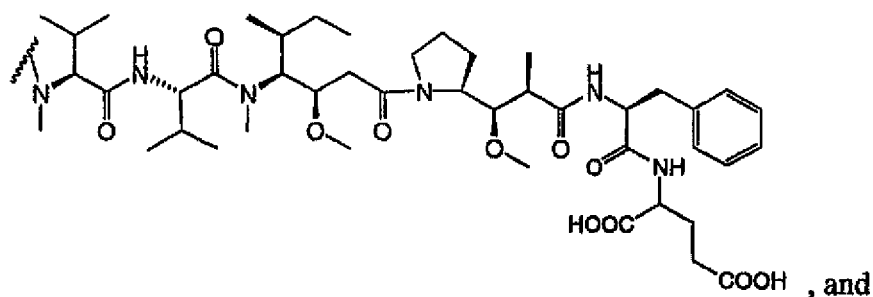
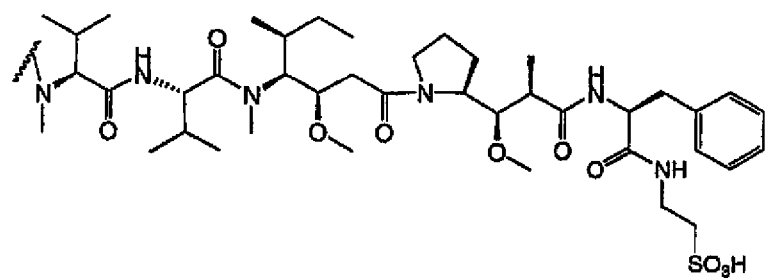




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and pharmaceutically acceptable salts or solvates thereof.

In one aspect, hydrophilic groups, such as but not limited to triethylene glycol esters (TEG), as shown above, can be attached to the Drug Unit at R¹¹. Without
10 being bound by theory, the hydrophilic groups assist in the internalization and non-agglomeration of the Drug Unit.

4.5 THE LIGAND UNIT

The Ligand unit (L-) includes within its scope any unit of a Ligand (L) that
15 binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population. A Ligand is a molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. In one aspect, the Ligand unit acts to deliver the Drug unit to the particular target cell population with which the Ligand unit

reacts. Such Ligands include, but are not limited to, large molecular weight proteins such as, for example, full-length antibodies, antibody fragments, smaller molecular weight proteins, polypeptide or peptides, lectins, glycoproteins, non-peptides, vitamins, nutrient-transport molecules (such as, but not limited to, transferrin), or any other cell binding molecule or substance.

A Ligand unit can form a bond to a Stretcher unit, an Amino Acid unit, a Spacer Unit, or a Drug Unit. A Ligand unit can form a bond to a Linker unit via a heteroatom of the Ligand. Heteroatoms that may be present on a Ligand unit include sulfur (in one embodiment, from a sulfhydryl group of a Ligand), oxygen (in one embodiment, from a carbonyl, carboxyl or hydroxyl group of a Ligand) and nitrogen (in one embodiment, from a primary or secondary amino group of a Ligand). These heteroatoms can be present on the Ligand in the Ligand's natural state, for example a naturally-occurring antibody, or can be introduced into the Ligand via chemical modification.

In one embodiment, a Ligand has a sulfhydryl group and the Ligand bonds to the Linker unit via the sulfhydryl group's sulfur atom.

In yet another aspect, the Ligand has one or more lysine residues that can be chemically modified to introduce one or more sulfhydryl groups. The Ligand unit bonds to the Linker unit via the sulfhydryl group's sulfur atom. The reagents that can be used to modify lysines include, but are not limited to, N-succinimidyl S-acetylthioacetate (SATA) and 2-Iminothiolane hydrochloride (Traut's Reagent).

In another embodiment, the Ligand can have one or more carbohydrate groups that can be chemically modified to have one or more sulfhydryl groups. The Ligand unit bonds to the Linker Unit, such as the Stretcher Unit, via the sulfhydryl group's sulfur atom.

In yet another embodiment, the Ligand can have one or more carbohydrate groups that can be oxidized to provide an aldehyde (-CHO) group (see, for *e.g.*, Laguzza, *et al.*, *J. Med. Chem.* 1989, 32(3), 548-55). The corresponding aldehyde can form a bond with a Reactive Site on a Stretcher. Reactive sites on a Stretcher that can react with a carbonyl group on a Ligand include, but are not limited to, hydrazine and hydroxylamine. Other protocols for the modification of proteins for the attachment or association of Drug Units are described in Coligan *et al.*, *Current Protocols in Protein Science*, vol. 2, John Wiley & Sons (2002), incorporated herein by reference.

Useful non-immunoreactive protein, polypeptide, or peptide Ligands include, but are not limited to, transferrin, epidermal growth factors ("EGF"), bombesin, gastrin, gastrin-releasing peptide, platelet-derived growth factor, IL-2, IL-6, transforming growth factors ("TGF"), such as TGF- α and TGF- β , vaccinia growth factor ("VGF"),
5 insulin and insulin-like growth factors I and II, lectins and apoprotein from low density lipoprotein.

Useful polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Various procedures well known in the art may be used for the production of polyclonal antibodies to an antigen-of-
10 interest. For example, for the production of polyclonal antibodies, various host animals can be immunized by injection with an antigen of interest or derivative thereof, including but not limited to rabbits, mice, rats, and guinea pigs. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete) adjuvant, mineral gels such as
15 aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Useful monoclonal antibodies are homogeneous populations of antibodies
20 to a particular antigenic determinant (*e.g.*, a cancer cell antigen, a viral antigen, a microbial antigen, a protein, a peptide, a carbohydrate, a chemical, nucleic acid, or fragments thereof). A monoclonal antibody (mAb) to an antigen-of-interest can be prepared by using any technique known in the art which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited
25 to, the hybridoma technique originally described by Köhler and Milstein (1975, *Nature* 256, 495-497), the human B cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today* 4: 72), and the EBV-hybridoma technique (Cole *et al.*, 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and IgD and any subclass
30 thereof. The hybridoma producing the mAbs of use in this invention may be cultivated *in vitro* or *in vivo*.

Useful monoclonal antibodies include, but are not limited to, human monoclonal antibodies, humanized monoclonal antibodies, antibody fragments, or

chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (*e.g.*, Teng *et al.*, 1983, *Proc. Natl. Acad. Sci. USA.* 80, 7308-7312; Kozbor *et al.*, 1983, *Immunology Today* 4, 72-79; and Olsson *et al.*, 1982, *Meth. Enzymol.* 92, 3-16).

5 The antibody can also be a bispecific antibody. Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein *et al.*, 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light
10 chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Similar procedures are disclosed in International Publication No. WO 93/08829, and in Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991).

 According to a different approach, antibody variable domains with the
15 desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light chain binding, present in at least one of the fusions.
20 Nucleic acids with sequences encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the
25 optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

 In an embodiment of this approach, the bispecific antibodies have a hybrid
30 immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the

presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation (International Publication No. WO 94/04690) which is incorporated herein by reference in its entirety.

For further details for generating bispecific antibodies see, for example,
5 Suresh *et al.*, *Methods in Enzymology*, 1986, 121:210; Rodrigues *et al.*, 1993, *J. of Immunology* 151:6954-6961; Carter *et al.*, 1992, *Bio/Technology* 10:163-167; Carter *et al.*, 1995, *J. of Hematotherapy* 4:463-470; Merchant *et al.*, 1998, *Nature Biotechnology* 16:677-681. Using such techniques, bispecific antibodies can be prepared for use in the treatment or prevention of disease as defined herein.

10 Bifunctional antibodies are also described, in European Patent Publication No. EPA 0 105 360. As disclosed in this reference, hybrid or bifunctional antibodies can be derived either biologically, *i.e.*, by cell fusion techniques, or chemically, especially with cross-linking agents or disulfide-bridge forming reagents, and may comprise whole antibodies or fragments thereof. Methods for obtaining such hybrid antibodies are
15 disclosed for example, in International Publication WO 83/03679, and European Patent Publication No. EPA 0 217 577, both of which are incorporated herein by reference.

The antibody can be a functionally active fragment, derivative or analog of an antibody that immunospecifically binds to cancer cell antigens, viral antigens, or microbial antigens or other antibodies bound to tumor cells or matrix. In this regard,
20 "functionally active" means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies that recognize the same antigen that the antibody from which the fragment, derivative or analog is derived recognized. Specifically, in an exemplary embodiment the antigenicity of the idiotype of the immunoglobulin molecule can be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR
25 sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art (*e.g.*, the BIA core assay) (See, for *e.g.*, Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md; Kabat E *et al.*, 1980, *J. of*
30 *Immunology* 125(3):961-969).

Other useful antibodies include fragments of antibodies such as, but not limited to, F(ab')₂ fragments, which contain the variable region, the light chain constant region and the CH1 domain of the heavy chain can be produced by pepsin digestion of the

antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Other useful antibodies are heavy chain and light chain dimers of antibodies, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent No. 4946778; Bird, 1988, *Science* 242:423-42; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward *et al.*, 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are useful antibodies. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal and human immunoglobulin constant regions. (*See, e.g.*, Cabilly *et al.*, U.S. Patent No. 4816567; and Boss *et al.*, U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (*See, e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Publication No. WO 87/02671; European Patent Publication No. 184,187; European Patent Publication No. 171496; European Patent Publication No. 173494; International Publication No. WO 86/01533; U.S. Patent No. 4816567; European Patent Publication No. 12,023; Berter *et al.*, 1988, *Science* 240:1041-1043; Liu *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.*, 1987, *Cancer. Res.* 47:999-1005; Wood *et al.*, 1985, *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, 1985, *Science* 229:1202-1207; Oi *et al.*, 1986, *BioTechniques* 4:214; U.S. Patent No. 5225539; Jones *et al.*, 1986, *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.*, 1988, *J. Immunol.* 141:4053-4060; each of which is incorporated herein by reference in its entirety.

Completely human antibodies are particularly desirable and can be produced using transgenic mice that are incapable of expressing endogenous

immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies. See, *e.g.*, U.S. Patent Nos. 5625126; 5633425; 5569825; 5661016; 5545806; each of which is incorporated herein by reference in its entirety. Other human antibodies can be obtained commercially from, for example, Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA).

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.* (1994) *Biotechnology* 12:899-903). Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991); Quan, M. P. and Carter, P. 2002. *The rise of monoclonal antibodies as therapeutics*. In *Anti-IgE and Allergic Disease*, Jardieu, P. M. and Fick Jr., R. B, eds., Marcel Dekker, New York, NY, Chapter 20, pp. 427-469).

In other embodiments, the antibody is a fusion protein of an antibody, or a functionally active fragment thereof, for example in which the antibody is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the antibody. Preferably, the antibody or fragment thereof is covalently linked to the other protein at the N-terminus of the constant domain.

Antibodies include analogs and derivatives that are either modified, *i.e.*, by the covalent attachment of any type of molecule as long as such covalent attachment permits the antibody to retain its antigen binding immunospecificity. For example, but not by way of limitation, the derivatives and analogs of the antibodies include those that have been further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular antibody unit or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, etc. Additionally, the analog or derivative can contain one or more unnatural amino acids.

The antibodies include antibodies having modifications (*e.g.*, substitutions, deletions or additions) in amino acid residues that interact with Fc receptors. In particular, antibodies include antibodies having modifications in amino acid residues identified as involved in the interaction between the anti-Fc domain and the FcRn receptor (see, *e.g.*, International Publication No. WO 97/34631, which is incorporated herein by reference in its entirety). Antibodies immunospecific for a cancer cell antigen can be obtained commercially, for example, from Genentech (San Francisco, CA) or produced by any method known to one of skill in the art such as, *e.g.*, chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a cancer cell antigen can be obtained, *e.g.*, from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing.

In a specific embodiment, known antibodies for the treatment or prevention of cancer can be used. Antibodies immunospecific for a cancer cell antigen can be obtained commercially or produced by any method known to one of skill in the art such as, *e.g.*, recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a cancer cell antigen can be obtained, *e.g.*, from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing. Examples of antibodies available for the treatment of cancer include, but are not limited to, humanized anti-HER2 monoclonal antibody, HERCEPTIN® (trastuzumab; Genentech) for the treatment of patients with metastatic breast cancer; RITUXAN® (rituximab; Genentech) which is a chimeric anti-CD20 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma; OvaRex (AltaRex

Corporation, MA) which is a murine antibody for the treatment of ovarian cancer; Panorex (Glaxo Wellcome, NC) which is a murine IgG_{2a} antibody for the treatment of colorectal cancer; Cetuximab Erbitux (Imclone Systems Inc., NY) which is an anti-EGFR IgG chimeric antibody for the treatment of epidermal growth factor positive cancers, such as head and neck cancer; Vitaxin (MedImmune, Inc., MD) which is a humanized antibody for the treatment of sarcoma; Campath I/H (Leukosite, MA) which is a humanized IgG₁ antibody for the treatment of chronic lymphocytic leukemia (CLL); Smart MI95 (Protein Design Labs, Inc., CA) which is a humanized anti-CD33 IgG antibody for the treatment of acute myeloid leukemia (AML); LymphoCide (Immunomedics, Inc., NJ) which is a humanized anti-CD22 IgG antibody for the treatment of non-Hodgkin's lymphoma; Smart ID10 (Protein Design Labs, Inc., CA) which is a humanized anti-HLA-DR antibody for the treatment of non-Hodgkin's lymphoma; Oncolym (Techniclone, Inc., CA) which is a radiolabeled murine anti-HLA-Dr10 antibody for the treatment of non-Hodgkin's lymphoma; Allomune (BioTransplant, CA) which is a humanized anti-CD2 mAb for the treatment of Hodgkin's Disease or non-Hodgkin's lymphoma; Avastin (Genentech, Inc., CA) which is an anti-VEGF humanized antibody for the treatment of lung and colorectal cancers; Epratuzamab (Immunomedics, Inc., NJ and Amgen, CA) which is an anti-CD22 antibody for the treatment of non-Hodgkin's lymphoma; and CEAcide (Immunomedics, NJ) which is a humanized anti-CEA antibody for the treatment of colorectal cancer.

Other antibodies useful in the treatment of cancer include, but are not limited to, antibodies against the following antigens: CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE -4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MUC1-KLH (breast cancer), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), PSA (prostate), IL-2 receptor (T-cell leukemia and lymphomas), CD20 (non-Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia), CD22 (lymphoma), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD40 (lymphoma), mucin (carcinomas), P21 (carcinomas), MPG (melanoma), and Neu oncogene product (carcinomas). Some specific, useful antibodies

include, but are not limited to, BR96 mAb (Trail, P. A., Willner, D., Lasch, S. J., Henderson, A. J., Hofstead, S. J., Casazza, A. M., Firestone, R. A., Hellström, I., Hellström, K. E., "Cure of Xenografted Human Carcinomas by BR96-Doxorubicin Immunoconjugates" *Science* 1993, 261, 212-215), BR64 (Trail, P. A., Willner, D., Knipe, J., Henderson, A. J., Lasch, S. J., Zoeckler, M. E., Trailsmith, M. D., Doyle, T. W., King, H. D., Casazza, A. M., Braslawsky, G. R., Brown, J. P., Hofstead, S. J., (Greenfield, R. S., Firestone, R. A., Mosure, K., Kadow, D. F., Yang, M. B., Hellstrom, K. E., and Hellstrom, I. "Effect of Linker Variation on the Stability, Potency, and Efficacy of Carcinoma-reactive BR64-Doxorubicin Immunoconjugates" *Cancer Research* 1997, 57, 100-105, mAbs against the CD40 antigen, such as S2C6 mAb (Francisco, J. A., Donaldson, K. L., Chace, D., Siegall, C. B., and Wahl, A. F. "Agonistic properties and *in vivo* antitumor activity of the anti-CD-40 antibody, SGN-14" *Cancer Res.* 2000, 60, 3225-3231), mAbs against the CD70 antigen, such as 1F6 mAb and 2F2 mAb, and mAbs against the CD30 antigen, such as AC10 (Bowen, M. A., Olsen, K. J., Cheng, L., Avila, D., and Podack, E. R. "Functional effects of CD30 on a large granular lymphoma cell line YT" *J. Immunol.*, 151, 5896-5906, 1993; Wahl *et al.*, 2002 *Cancer Res.* 62(13):3736-42). Many other internalizing antibodies that bind to tumor associated antigens can be used and have been reviewed (Franke, A. E., Sievers, E. L., and Scheinberg, D. A., "Cell surface receptor-targeted therapy of acute myeloid leukemia: a review" *Cancer Biother Radiopharm.* 2000, 15, 459-76; Murray, J. L., "Monoclonal antibody treatment of solid tumors: a coming of age" *Semin Oncol.* 2000, 27, 64-70; Breitling, F., and Dubel, S., *Recombinant Antibodies*, John Wiley, and Sons, New York, 1998).

In certain embodiments, the antibody is not Trastuzumab (full length, humanized anti-HER2 (MW 145167)), HerceptinF(ab')₂ (derived from anti-HER2 enzymatically (MW 100000)), 4D5 (full-length, murine antiHER2, from hybridoma), rhu4D5 (transiently expressed, full-length humanized antibody), rhuFab4D5 (recombinant humanized Fab (MW 47738)), 4D5Fc8 (full-length, murine antiHER2, with mutated FcRn binding domain), or Hg ("Hingeless" full-length humanized 4D5, with heavy chain hinge cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated)).

In another specific embodiment, known antibodies for the treatment or prevention of an autoimmune disease are used in accordance with the compositions and methods of the invention. Antibodies immunospecific for an antigen of a cell that is

responsible for producing autoimmune antibodies can be obtained from any organization (e.g., a university scientist or a company) or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. In another embodiment, useful antibodies are immunospecific for the treatment of

5 autoimmune diseases include, but are not limited to, Anti-Nuclear Antibody; Anti-ds DNA; Anti-ss DNA, Anti-Cardiolipin Antibody IgM, IgG; Anti-Phospholipid Antibody IgM, IgG; Anti-SM Antibody; Anti-Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody; Thyroglobulin Antibody; Anti-SCL-70; Anti-Jo; Anti-U₁RNP; Anti-La/SSB; Anti SSA; Anti-SSB; Anti-Perital Cells Antibody; Anti-Histones; Anti-
10 RNP; C-ANCA; P-ANCA; Anti centromere; Anti-Fibrillarin, and Anti-GBM Antibody.

In certain embodiments, useful antibodies can bind to both a receptor or a receptor complex expressed on an activated lymphocyte. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member, a TNF receptor superfamily member, an integrin, a cytokine receptor, a chemokine receptor, a major
15 histocompatibility protein, a lectin, or a complement control protein. Non-limiting examples of suitable immunoglobulin superfamily members are CD2, CD3, CD4, CD8, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-1, and ICOS. Non-limiting examples of suitable TNF receptor superfamily members are CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, TNF-R1, TNFR-2, RANK, TACI, BCMA,
20 osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3. Non-limiting examples of suitable integrins are CD11a, CD11b, CD11c, CD18, CD29, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD103, and CD104. Non-limiting examples of suitable lectins are C-type, S-type, and I-type lectin.

In one embodiment, the Ligand binds to an activated lymphocyte that is
25 associated with an autoimmune disease.

In another specific embodiment, useful Ligands immunospecific for a viral or a microbial antigen are monoclonal antibodies. The antibodies may be chimeric, humanized or human monoclonal antibodies. As used herein, the term "viral antigen" includes, but is not limited to, any viral peptide, polypeptide protein (e.g., HIV gp120,
30 HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g., gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide,

polypeptide, protein, saccharide, polysaccharide, or lipid molecule (*e.g.*, a bacterial, fungi, pathogenic protozoa, or yeast polypeptide including, *e.g.*, LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response.

Antibodies immunospecific for a viral or microbial antigen can be
5 obtained commercially, for example, from BD Biosciences (San Francisco, CA),
Chemicon International, Inc. (Temecula, CA), or Vector Laboratories, Inc. (Burlingame,
CA) or produced by any method known to one of skill in the art such as, *e.g.*, chemical
synthesis or recombinant expression techniques. The nucleotide sequence encoding
antibodies that are immunospecific for a viral or microbial antigen can be obtained, *e.g.*,
10 from the GenBank database or a database like it, literature publications, or by routine
cloning and sequencing.

In a specific embodiment, useful Ligands are those that are useful for the
treatment or prevention of viral or microbial infection in accordance with the methods
disclosed herein. Examples of antibodies available useful for the treatment of viral
15 infection or microbial infection include, but are not limited to, SYNAGIS (MedImmune,
Inc., MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal
antibody useful for the treatment of patients with RSV infection; PRO542 (Progenics)
which is a CD4 fusion antibody useful for the treatment of HIV infection; OSTAVIR
(Protein Design Labs, Inc., CA) which is a human antibody useful for the treatment of
20 hepatitis B virus; PROTOVIR (Protein Design Labs, Inc., CA) which is a humanized IgG₁
antibody useful for the treatment of cytomegalovirus (CMV); and anti-LPS antibodies.

Other antibodies useful in the treatment of infectious diseases include, but
are not limited to, antibodies against the antigens from pathogenic strains of bacteria
(*Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*
25 *meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium*
perfringens, *Clostridium tetani*, *Hemophilus influenzae*, *Klebsiella pneumoniae*,
Klebsiella ozaenas, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*,
Escherichia coli, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Aeromonas*
hydrophila, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*,
30 *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*,
Salmonella typhimurium, *Treponema pallidum*, *Treponema pertenue*, *Treponema*
carateneum, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*,
Mycobacterium tuberculosis, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella*

abortus, *Brucella suis*, *Brucella melitensis*, *Mycoplasma* spp., *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia* spp.); pathogenic fungi (*Coccidioides immitis*, *Aspergillus fumigatus*, *Candida albicans*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*); protozoa (*Entamoeba histolytica*, *Toxoplasma gondii*, *Trichomonas tenax*, *Trichomonas hominis*, *Trichomonas vaginalis*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*); or Helminths (*Enterobius vermicularis*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Trichinella spiralis*, *Strongyloides stercoralis*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma haematobium*, and hookworms).

Other antibodies useful in this invention for treatment of viral disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxviridae, Herpesviridae, Herpes Simplex virus 1, Herpes Simplex virus 2, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis E virus, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, and Human Immunodeficiency Virus.

In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Antibodies which comprise Ab in Formula Ic antibody drug conjugates (ADC) and which may be useful in the treatment of cancer include, but are not limited to, antibodies against tumor-associated antigens (TAA). Such tumor-associated antigens are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. Examples of TAA include (1)-(35), but are

not limited to TAA (1)-(35) listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s). Tumor-associated antigens targetted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the corresponding sequences listed (SEQ ID NOS: 1-35) or the sequences identified in the cited references. In some embodiments, TAA having amino acid sequence variants exhibit substantially the same biological properties or characteristics as a TAA having the sequence found in the corresponding sequences listed (SEQ ID NOS: 1-35). For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure specifically recited herein are expressly incorporated by reference.

TUMOR-ASSOCIATED ANTIGENS (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203, ten Dijke, P., *et al.* Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Claim 2); WO2003042661 (Claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (Claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (Claim 6); WO2003024392 (Claim 2; Fig 112); WO200298358 (Claim 1; Page 183); WO200254940 (Page 100-101); WO200259377 (Page 349-350); WO200230268 (Claim 27; Page 376); WO200148204 (Example; Fig 4)

NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1 -

Cross-references: MIM:603248; NP_001194.1; NM_001203_1

502 aa

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MLLSAGKLVNVTGKKEDGESTAPTFRPKVLRCKCHHCFEDSVNNICSTDGYCFTMIEED
DSGLPVVTSGLGLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVD
GPIHHRALLISVTVCSLLLVLIILFCYFRYKRQETRPRYSIGLEQDETYIPPGESLRDLI
EQSQSSGSGSGLPLLVQRTIAKQIQMVKQIGKGRYGEVWMGKWRGEKVAVKVFFFTTEEAS
WFRETEIYQTVLMRHNILGFIAADIKGTGSGWTQLYLIITDYHENGSLYDYLKSTTLDAKS
MLKLAYSSVSGLCHEIFSTQGKPAIAHRDLKSKNILVKKNGTCCIALDLGLAVKFLSD
TNEVDIPNTRVGTKRYMPPEVLDESINRNHFQSYIMADMYSGFLILWEVARRCVSGGIV
EEYQLPHYDLVPSDFSYEDMREIVCIKKLRPSFPNRWSSDECLRQMGKLMTECWAHNPAS
RLTALRVKKTAKMSSESQDIKL

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(SEQ ID NO: 1)

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H.W., *et al.* (1992) J. Biol. Chem. 267 (16):11267-11273);

5 WO2004048938 (Example 2); WO2004032842 (Example IV); WO2003042661 (Claim 12); WO2003016475 (Claim 1); WO200278524 (Example 2); WO200299074 (Claim 19; Page 127-129); WO200286443 (Claim 27; Pages 222, 393); WO2003003906 (Claim 10; Page 293); WO200264798 (Claim 33; Page 93-95); WO200014228 (Claim 5; Page 133-136); US2003224454 (Fig 3); WO2003025138 (Claim 12; Page 150);

10 NP_003477 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 /pid=NP_003477.3 - Homo sapiens

Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1
507 aa

15 MAGAGPKRRALAAPAAEEKEEAREKMLAAKSADGSAPAGEGEGVTLQRNITLLNGVAITV
GTIIGSGIFVTPTGVLKEAGSPGLALVWAAACGVFSIVGALCYAELGTTISKSGGDYAYM
LEVYGSLLPAFLKLWIELLIIRPSSQYIVALVFATYLLKPLFPPTCPVPEEAAKLVACLCVL
LLTAVNCYSVKAATRVQDAFAAAKLLALALIILLGFVQIGKGVVSNLDPNFSFEGTKLDV
GNIVLALYSGLFAYGGWNYLNFVTEEMINPYRNLPLAIIISLPITVTLVYVLTNLAYFTTL
20 STEQMLSSSEAVAVDFGNYHLGVMSWIIIPVFVGLSCFGSVNGSLFTSSRLFFVGSREGHLP
SILSMIHPQLLTVPVPSLVFTCVMTLLYAFSKDIFSVINFFSFFNWLCVALAIGMIWLRH
RKPELERPIKVNLLALPVFFILACLFLIIVSFWKTFVECGIGFTIILSGLPVYFFGVWVKN
KPKWLLQGIFSTTVLCQKLMQVVPQET

(SEQ ID NO: 2)

25

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449

Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., *et al.* (1999) Proc. Natl. Acad. Sci. USA. 96 (25):14523-14528); WO2004065577 (Claim 6); WO2004027049 (Fig 1L);

30 EP1394274 (Example 11); WO2004016225 (Claim 2); WO2003042661 (Claim 12); US2003157089 (Example 5); US2003185830 (Example 5); US2003064397 (Fig 2); WO200289747 (Example 5; Page 618-619); WO2003022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A);

NP_036581 six transmembrane epithelial antigen of the prostate

35 Cross-references: MIM:604415; NP_036581.1; NM_012449_1
339 aa

MESRKDITNQEELWKMKPRRNLEEDDYLHKDTGETSMLKRPVLLHLHQTAHADEFDCPSE
 LQHTQELFFQWHLPIKIAAIIASLTFLYTLREVIHPLATSHQQYFYKIPILVINKVLP
 VSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKMWLTKQPGLLSFFFAVLHAIYSL
 SYPMRRSYRYKLLNWAYQQVQONKEDAWIEHDVWRMEIYVSLGIVGLAILALLAVTSIPS
 5 VSDSLTWREFHYIQSKLGIVSLLGLTIHALIFAWNKWIDIKQFVWYTPPTFMIAVFLPIV
 VLIFKSILEFLPCLRKILKIRHGWEDVTINKTEICSQL

(SEQ ID NO 3)

10 (4) 0772P (CA125, MUC16, Genbank accession no. AF361486
 J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Claim 14);
 WO200292836 (Claim 6; Fig 12); WO200283866 (Claim 15; Page 116-121);
 US2003124140 (Example 16); US2003091580 (Claim 6); WO200206317 (Claim 6;
 Page 400-408);

15 Cross-references: GI:34501467; AAK74120.3; AF361486_1
 6995 aa

PVTSLITPGLVITTD RMGISREPGTSSTSNLSSTSHERLTTLEDTVDTTEAMQPSTHTAVT
 NVRTSISGHESQSSVLS DSETPKATSPMGTTYTMGETSVSISTSDFFETSRIOIEPTSSL
 TSGLRETSSSERISSATEGSTVLSEVP SGATTEVSRTEVISSRGTSMSGPDQFTISPDIS
 20 TEAITRLSTSPIMTESAESAITIETGSPGATSEGTLLDSTTTFWSGTHSTASPGFSHS
 EMTTLMSTRTPGDVPWPSLPSVEEASSVSSSLSPAMTSTSFSTLPESISSSPHPVTALL
 TLGPVKTTDMLRTSSEPETSSPPNLSSTSAEILATSEVTKDREKIHPSNTFVVNVGTVI
 YKHLSPSSVLADLVTTKPTSPMATTSTLGNTSVSTSTPAFPETMMTQPTSSLTSGLRIS
 TSQETSSATERSASLSGMPTGATTKVSRTEALSLGRTSTPGPAQSTISPEISTETITRIS
 25 TPLTTTGSAMTITPKTGHS GASSQGTFTLDTSRASWPGTHSAATHRS PHSGMTTPMSR
 GPEDVSWPSRPSVEKTSPPSSLVLSAVTSPSPLYSTPSESSHSSPLRVTS LFTPVMMKT
 TDMLDTSLEPVTTSPPSMNITSDESLATSKATMETRAIQLSENTAVTQMGTISARQEFYS
 SYFGLPEPSKVTSPPVTSSTIKDIVSTTIPASSEITRIEMESTSTLTPTPRETSTSQBIH
 SATKPSTVPYKALTSATIEDSMTQVMSSSRGSPDQSTMSQDISTEVITRLSTSPIKTES
 30 TEMTITTQTGSPGATSRGTLTLDSTTFMSGTHSTASQGFSHSQMTALMSRTPGEVPWLS
 HPSVEEASSASFSLSPPVMTSSSPVSSLTLPDSIHSSSLPVTSLLTSGLVKTTTELLGTSSE
 PETSSPPNLSSTSAEILATTEVTTDTEKLEMTNVVTS GYTHESPSSVLADSVTTKATSSM
 GITYPTGDTNVLTSTPAFSDTSRIQTKSKLSLT PGLMETSISEETSSATEKSTVLSSVPT
 GATTEVSRTEAIISSRTSIPGPAQSTMSSDTSMETITRISTPLTRKESTDMAITPKTGPS
 35 GATSQGTFTLDSSSTASWPGTHSATTQRFPRSVVTPMSRGPEDVSWPSPLSVEKNSPPS
 SLVSSSSVTSPSPLYSTPSGSSHSPVPVTS LFTSIMMKATDMLDASLEPETTSAPNMNI
 TDES LAASKATTETEAHV FENTAASHVETTSATEELYSSSPGFSEPTKVISPVTSSS
 IRDNMVSTTMPGSSGITRIEIESMSSLT PGLRETRTSQDITSSSTETSTVLYKMPSGATPE
 VSRTEVMPSSRTSIPGPAQSTMSLDISDEVVTRLSTSPIMTESAEITITTQTGYSLATSQ
 40 VTLP LGTSMFTLSGTHSTMSQGLSHSEMTNLMSRGPELSWTS PRFVETTRSSSSSLTSLP
 LTTSLSPVSSLTLLDSSPSSPLPVTS LILPGLVKTTTEVLDTSSEPKTSSSPNLSSTSV EIP
 ATSEIMTDTEKIHPSNTAVAKVRTSSSVHESHSSVLADSETTITIPSMGITSAVEDTTV

FTSNPAFSETRRIPTTEPTFSLTPGFRETSTSEETTSITETSAVLFGVPTSATTEVSMTEI
 MSSNRTHIPDSQSTMSPDIIITEVITRLSSSSMMSESTQMTITTTQKSSPGATAQSTLTLA
 TTTAPLARHSTVPPRFLHSEMTTLMRSRSPENPSWKSSPFVEKTSSSSSLLSLPVTTSPTS
 VSSTLPQSIPISSSSFSVTSLLTPGMVKTTDTSTEPGTSLSPNLSGTSVEILAASEVTTDTE
 5 KIHPSSSMAVTNVGTTSSGHELYSSVSIHSEPSKATYPVGTTPSSMAETSISTSMMPANFET
 TGFEAEFPFSLTSGLRKTNMSLDTSSTVPTNTPSSPGSTHLLQSSKTDFTSSAKTSSPDW
 PPASQYTEIPVDIITPFNASPSITESGTITSFPESRFTMSVTESTHHLSTDLLPSAETIS
 TGTVMPSLSEAMTSFATTGVPRAISGSGSPFSRTESGPGDATLSTIAESLPSSTPVFPSS
 STFTTTDSSTIPALHEITSSSATPYRVDTSLGTESSTTEGRLVMVSTLDTSSQPGRTSSS
 10 PILDTRMTESVELGTVTSAYQVPSLSTRLTRTDGIMEHITKIPNEAAHRTIRPVKGPQT
 STSPASPKGLHTGGTKRMETTTTALKTTTALKTTSRATLTTSVYTPTLGTLTPLNASMQ
 MASTIPTEMMITTPYVFPDVPETTTSSLATSLGAETSTALPRTTPSVFNRESETTASLVSR
 SGAERSPVIQTLDVSSSEPDTTASWVIHPAETIPTVSKTTPNFFHSELDTVSSSTATSHGA
 DVSSAIPNTNISPELDAITPLVTISGTDTSSTFPPTLTKSPHETETRTTWLTHPAETSSTI
 15 PRTIPNFHSHESDATPSIATSPGAETSSAIPIMTVSPGAEDLVTSQVTSSTGTDNRMTIPT
 LTLSPGEPKTIASLVTHPEAQTSAPITSTISPAVSRLVTSMVTSLAAKTSTTNRALTNS
 PGEPAITVSLVTHSAQTSPTVPWTTSIFFHKSDDTTPSMTTSHGAESSAVPTPTVSTEV
 PGVVTPLVTSSRAVISTTIPILTLSPGEPETTPSMATSHGEEASSAIPPTVSPGVPGVV
 TSLVTSSRAVTSTTIPILTFSLGEPETTPSMATSHGTEAGSAVPTVLPEVPGMVTSLVAS
 20 SRAVTSTTLPTLTLSPGEPETTPSMATSHGAEASSTVPTVSPVPGVVTSLVTSSSGVNS
 TSIPTLILSPGELETTTPSMATSHGAEASSAVPTPTVSPGVSGVVTPLVTSSRAVTSTTIP
 ILLTSSSEPETTPSMATSHGVEASSAVLTVSPEVPGMVTFLVTSSRAVTSTTIPTLTISS
 DEPETTTSLVTHSEAKMISAIPTLGVSPTVQGLVTSVLTSSGSETSAFNLTVASSQPET
 IDSWVAHPGTEASSVPTLTVSTGEPFTNISLVTHPAESSSTLPRTTSRFHSSELDTMPS
 25 TVTSPEAESSSAISTTISPGIPGVLTSLVTSSGRDISATFPTVPESPHESEATASWVTHP
 AVTSTTVPRTTFNYSHSEPDTTPSIATSPGAEATSDFPTITVSPDVPMVTSQVTSSTGTD
 TSITIPTLTLSSGEPETTTSFITYSEHTSSAIPTLFVSPDASKMLTSLVISSGTDSTTT
 FPTLTETPYEPETTAIQLIHPAETNTMVPRTTPKFHSHKSDTTLPVAITSPGPEASSAVS
 TTTISPDMSDLVTSVLPSSGTDSTTFPTLSETPYEPETTATWLTHPAETSTTVSGTIPN
 30 FSHRGSDTAPSMVTSVPGVDRSGVFTTTIPPSIPGVVTSQVTSSTADTSTAIPTLTPSPG
 EPETTASSATHPGTQTGFTVPIRTVPSSEPDTMASWVTHPPQTSTPVSRTTSSFSHSSPD
 ATPVMATSPRTEASSAVLTTISPGAPEMVTSQITSSGAATSTTVPTLTHSPGMPETTALL
 STHPRTETSKTFFPASTVFPQVSETTASLTIRPGAETSTALPTQTTSSLFTLLVTGTSRVD
 LSPTASPGVSAKTAPLSTHPGTETSTMIPTSTLSLGLLETTGLLATSSAETSTSTLTLT
 35 VSPAUSGLSSASITTDKPQTVTSWNTETSPSVTSVGPPEFSRTVTGTTIMTLIPSEMPTPF
 KTSHGEGVSPTTILRTTMVEATNLATTGSSPTVAKTTTTFNTLAGSLFTPLTTPGMSTLA
 SESVTSRTSYNHRSWISTTSSYNRRYWPATSTPVTSTFSPGISTSSIPSSTAATVPFMV
 PFTLNFTITNLQYEEDMRHPGSRKFNATERELQGLLKPLFRNSSLEYLYSGCRLASLRPE
 KDSSATAVDAICTHRDPEDLGLDRERLYWELSNLTNGIQELGPYTLDRNSLYVNGFTHR
 40 SSMPTTSTPGTSTVDVGTSGTPSSSPPTAGPLLMPFTLNFTITNLQYEEDMRRTGSRK
 FNTMESVLQGLLKPLFKNTSVGPLYSGCRLTLRPEKDGAATGVDAICTHRLDPKSPGLN
 REQLYWELSKLTNDIEELGPYTLDRNSLYVNGFTHQSSVSTSTPGTSTVDLRTSGTPSS
 LSSPTIMAAGPLLVPFTLNFTITNLQYGEDMGHPGSRKFNTTERTVLQGLLGPIFKNTSVG
 PLYSGCRLTSLRSEKDGAATGVDAICIHHLDPKSPGLNRERLYWELSQLTNGIKELGPYT
 45 LDRNSLYVNGFTHRTSVPTTSTPGTSTVDLGTSGTPFSLPSPATAGPLLVLFTLNFTITN

LKYEEDMHRPGSRKFNTTERTVLQTLVGPMFKNTSVGLLYSGCRLTLLRSEKDGAATGVDA
 ICTHRLDPKSPGVDREQLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHWIPVPTSSTPG
 TSTVDLGSGTPSSLPSPSATSAGPLLVPFTLNFTITNLKYEEDMHCPGSRKFNTTERTVLQ
 5 SLLGPMFKNTSVGPLYSGCRLTLLRSEKDGAATGVDAICTHRLDPKSPGVDREQLYWELS
 QLTNGIKELGPYTLDRNSLYVNGFTHQTSAPNTSTPGTSTVDLGTSGTPSSLPSPSAGP
 LLVPFTLNFTITNLQYEEDMHHPGSRKFNTTERTVLQGLLGPMFKNTSVGLLYSGCRLTLL
 RPEKNGAATGMDAICSHRLDPKSPGLNREQLYWELSQLTHGIKELGPYTLDRNSLYVNGF
 THRSSVAPTSTPGTSTVDLGTSGTPSSLPSPPTTAVPLLVPFTLNFTITNLQYGEDMRHPG
 SRKFNTTERTVLQGLLGPLFKNSSVGPLYSGCRLISLRSEKDGAATGVDAICTHHLNPQSP
 10 GLDREQLYWQLSQMTNGIKELGPYTLDRNSLYVNGFTHRSSGLTTSTPWTSTVDLGTSGT
 PSPVPSPPTTAGPLLVPFTLNFTITNLQYEEDMHRPGSRKFNTERTVLQGLLSPIFKNSSV
 GPLYSGCRLTSLRPEKDGAATGMDAVCLYHPNPKRPGLDREQLYWELSQLTHNITELGPY
 SLDRDSLYVNGFTHQNSVPTTSTPGTSTVYWATTGTPSSFPGHTEPGPLLIPTFNFTIT
 NLHYEENMQHPGSRKFNTTERTVLQGLLKPLFKNTSVGPLYSGCRLTLLRPEKQEAATGVD
 15 TICTHRVDPIGPGLDRERLYWELSQLTNSITELGPYTLDRDSLYVNGFNFWSSVPTTSTP
 GTSTVHLATSGTPSSLPGHTAPVPLLIPTLNFTITNLHYEENMQHPGSRKFNTTERTVLQ
 GLLKPLFKSTSVGPLYSGCRLTLLRPEKHGAATGVDAICTLRDPTGPGGLDRERLYWELS
 QLTNSVTELGPYTLDRDSLYVNGFTHRSSVPTTSTPGTSAVHLETSGTPASLPGHTAPGP
 LLVPFTLNFTITNLQYEEDMRHPGSRKFNTTERTVLQGLLKPLFKSTSVGPLYSGCRLTLL
 20 RPEKGAATGVDICTHRLDPLNPGLDREQLYWELSKLTRGIIELGPYLLDRGSLYVNGF
 THRNFPVITSTPGTSTVHLGTSETPSSLPRIVPGPPLLVPFTLNFTITNLQYEEAMRHPG
 SRKFNTTERTVLQGLLRPLFKNTSIGPLYSSCRLTLLRPEKDKAATRVDICTHHPDPQSP
 GLNREQLYWELSQLTHGITELGPYTLDRDSLYVDGFTHWSPIPTTSTPGTSTIVNLGTSGI
 PPSLPETTATGPLLVPFTLNFTITNLQYEEAMRHPGSRKFNTESVQGLLKPLFKSTSV
 25 GPLYSGCRLTLLRPEKDGVATRVDICTHRPDPKIPGLDRQQLYWELSQLTHSITELGPY
 TLDRDSLYVNGFTQRSSVPTTSTPGTFTVQPETSETPSSLPGTATGPVLLPFTLNFTII
 NLQYEEDMHRPGSRKFNTTERTVLQGLLMPLFKNTSVSSLYSGCRLTLLRPEKDGAATRVD
 AVCTHRPDPKSPGLDRERLYWKLSQLTHGITELGPYTLDRHSLYVNGFTHQSSMTTTRTP
 DTSTMHLATSRTPASLSGPTTASPLLVLFTINFTITNLRYEENMHHPGSRKFNTTERTVLQ
 30 GLLRPVFKNTSVGPLYSGCRLTLLRPKKDGAATKVDAICTYRDPKSPGLDREQLYWELS
 QLTHSITELGPYTLDRDSLYVNGFTQRSSVPTTSTPGTPTVDLGTSGTPVSKPGPSAASP
 LLVLFTLNFTITNLRYEENMQHPGSRKFNTTERTVLQGLLRSLFKSTSVGPLYSGCRLTLL
 RPEKDGTATGVDAICTHHPDPKSPRLDREQLYWELSQLTHNITELGPYALDNDLSLVNGF
 THRSSVSTTSTPGTPTVYLGASKTPASIFGPSAASHLLILFTLNFTITNLRYEENMWPGS
 35 RKFNNTERTVLQGLLRPLFKNTSVGPLYSGCRLTLLRPEKDGEATGVDAICTHRPDPTGPG
 LDREQLYLELSQLTHSITELGPYTLDRDSLYVNGFTHRSSVPTTSTGVVSEEPFTLNFTI
 NNLRYMADMGQPSLKFNITDNVMQHLLSPLPQRSSLGARYTGCRVIALRSVKNGAETRV
 DLLCTYLQPLSGPLPIKQVFHELSQLTHGITRLGPYSLDKDSLYLNGYNEPGPDEPPTT
 PKPATTFPLPPLSEATTAMGYHLKTLTLNFTISNLQYSPDMGKSATFNSTEGVLQHLLRP
 40 LFQKSSMGPFYLGCLISLRPEKDGAATGVDTTCTYHPDPVGPGLDIQQLYWELSQLTHG
 VTQLGFYVLDRLDSLFINGYAPQNLISIRGEYQINFHIVNWNLSNPDPSTSEYITLLRDIQD
 KVTTLYKGSQLDHTRFCLVTNLTMDSVLVTVKALFSSNLDPSLVEQVFLDKTLNASFWH
 LGSTYQLVDIHVTEMESSVYQPTSSSSSTQHLYNFTITNLPYSQDKAQPGTTNYQRNKRN
 IEDALNQLFRNSSIKSYFSDCQVSTFRSVFNRHHTGVDSL CNFSPLARRVDRVAIYEEFL
 45 RMTRNGTQLQNFTLDRSSVLVDGYSPNRNEPLTGNSDLFPWAVILIGLAGLLGLITCLIC

GVLVITRRRKKEGEYNVQQQCPGYYSQSHLDLEDLQ

(SEQ ID NO:4)

- 5 (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823
Yamaguchi, N., *et al.* Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. USA. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. USA. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995)); WO2003101283 (Claim 14); (WO2002102235
10 (Claim 13; Page 287-288); WO2002101075 (Claim 4; Page 308-309); WO200271928 (Page 320-321); WO9410312 (Page 52-57);
Cross-references: MIM:601051; NP_005814.2; NM_005823_1

622 aa

15 MALPTARPLLGSCGTFALGSLLFLLFSLGWVQPSRTLGETGQEAAPLDGVLANPPNISS
LSPRQLLGFPFCAEVSGSLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDLDALPL
DLLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAAALACWGVRSLLSEA
DVRALGGLACDLPGRFVAESAELVLLPRLVSCPGPLDQDQQAARAALQGGGPPYGPSTW
SVSTMDALRGLLPVLGQPIIRSIPQGIVAARQRSSRDPSWRQPRTILRPRFRREVEKT
ACPSGKKAREIDESLI FYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELY
20 PQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLKALLEVNKGHEMSPQVATLIDRFVK
GRGQLDKDTLDTLTAFFPYGFLCSLSPEELSSVPFSSIWAVRPQDLDTCDPRQLDVLYPKA
RLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQ
KLLGPHVEGLKAEERHRPVRDWILRQRQDDLDLGLGLQGGIPNGYLVLDLSMQEALSGT
PCLLGPGPVLTVLALLLASTLA

25

(SEQ ID NO:5)

- (6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no.
30 NM_006424,
J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., *et al.* (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582; WO2004022778 (Claim 2); EP1394274 (Example 11); WO2002102235 (Claim 13; Page 326); EP875569 (Claim 1; Page 17-19); WO200157188 (Claim 20; Page 329); WO2004032842 (Example
35 IV); WO200175177 (Claim 24; Page 139-140);
Cross-references: MIM:604217; NP_006415.1; NM_006424_1

690 aa

MAPWPELGDAQPNPDKYLEGAAGQQPTAPDKSKETNKTDNTEAPVTKIELLPSYSTATLI
 DEPTVEDDPWNLPRTLQDSGIKWSERDTKGKILCFFQIGRLILLGLFLYFFVCSLDILSS
 AFQLVGGKMAGQFFSNSSIMSNPLLGLVIGVLVTVLVQSSSTSTSIIVSMVSSSLLTVRA
 AIPILMGANIGTSITNTIVALMQVGRSEFRRAFAGATVHDFFNWLSVLVLLPVEVATHY
 5 LEIITQLLIVESFHFKNGEDAPDLLKVITKPFTKLIVQLDKKVISQLAMNDEKAKNKSIVK
 IWCKTFTNKTQINVTVPSTANCTSPSLCWTGDIQNWTKMKNVTYKENIAKQHFVNFHLP
 DLAUGTILLILSLLVLCGCLIMIVKILGSLVKGQVATVIKKTINTDFPFPFAWLTGYLAI
 LVGAGMTFIVQSSSVFTSALTPLIGIGVITIERAYPLTLGSGNIGTTTTAILAALASPGNA
 LRSSLQIALCHFFFNISGILLWYPIPFTRLPIRMAGLGNISAKYRWFVAFYLIIFFFLI
 10 PLTVFGLSLAGWRVLVGVGVVVFIIILVLCRLQLQSRCPRVLPKKLQNNWFLPLWMRSL
 KPWDVAVSKFTGCFQMRCCYCCRVCCACCLLCGCPKCCRCCKCCDLEEAQEGQDVPVK
 APETFDNITISREAQGEVPASDSKTECTAL

(SEQ ID NO:6)

15

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b
 Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like),
 transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank
 accession no. AB040878,

20 Nagase T., *et al.* (2000) DNA Res. 7 (2):143-150); WO2004000997 (Claim 1);
 WO2003003984 (Claim 1); WO200206339 (Claim 1; Page 50); WO200188133 (Claim
 1; Page 41-43, 48-58); WO2003054152 (Claim 20); WO2003101400 (Claim 11);
 Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;
 1093 aa

25 MVLGAPLAVSLLPSLTLLVSHLSSSQDVSEPSSEQQLCALSKHPTVAFEDLQPVWSNF
 TYPGARDFSQALALDPSGNQLIVGARNYLFRLSLANVSLLQATEWASSEDTRRSCQSKGKT
 EEECCQNYVRVLIIVAGRKVFMCGTNAFSPMCTSRQVGNLSRTTEKINGVARCPYDPRHNST
 AVISSQGEELYAATVIDFSGRDPAIYRSLGSGPPLRTAQYNSKWLNEPNFVAAYDIGLFAY
 FFLRENAVEHDCGRTVYSRVARVCKNDVGGRFLEDTWTTFMKARLNCNRPGEVPPFYNE
 30 LQSAFHLPEQDLIYGVTNTVNSIAASAVCAFNLSAISQAFNGPFRYQENPRAAWLPAN
 PIPNFQCGTLPETGPNENLTERSLQDAQRLFLMSEAVQPVTPPEPCVTQDSVRFSLVVDL
 VQAKDTLYHVLYIGTESGTLKALSTASRSLHGCYLEELHVLPPGRREPLRSLRILHSAR
 ALFVGLRDGVLRVPLERCAAYRSQGACLGARDPYCGWDGKQQRCTLEDSSNMSLWTQNI
 TACPVRNVTRDGGFGPWSWPQCEHLDGDNSSGSLCRARSCDSFRPRCGGLDCLGPAIHI
 35 ANCSRNGAWTPWSSWALCSTSCGIGFQVRQRSCSNPAPRHGGRICVVGKSREERFCNENTP
 CPVPIFWASWGSWSKCSSNCGGGMQSRRRACENGNSCLGCGVEFKTCNPEGCEVRRNTP
 WTPWLFPVNVTVQGGARQEQRFRFTCRAPLADPHGLQFGRRTTETRTCPADGSGSCDTDALV
 EDLLRSGSTSPHTVSGGWAAGWPSSCSRDCELGFRVRKRTCTNPEPRNGGLPCVGDAAE
 YQDCNPQACPVRGAWSCWTSWSPCSASC GGHYQRTSCTSPAPSPGEDICLGLHTEAL
 40 CATQACPEGWSPWSEWSKCTDDGAQSRSRHCEELLPGSSACAGNSSQSRPCPYSEIPVIL
 PASSMEEATGCAGFNLIHLVATGISCFLGSGLLTLAVYLSQHCQRQSQESTLVHPATPN

HLHYKGGGTPKNEKYTPMEFKTLNKNLIPDDRANFYPLQQTNVYTTTTYPSPLNKH5FR
PEASPGQRCFPNS

(SEQ ID NO:7)

5

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

US2003129192 (Claim 2); US2004044180 (Claim 12); US2004044179 (Claim 11);

US2003096961 (Claim 11); US2003232056 (Example 5); WO2003105758 (Claim 12);

10 US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20);

Cross-references: GI:37182378; AAQ88991.1; AY358628_1

141 aa

MWVLGIAATFCGLFLLPGFALQIQCYQCEEFQLNDCSSPEFIVNCTVNVQDMCQKEVME
QSAGIMYRKSCASSAACLIASAGYQSFCSPGKLNSVCISCCNTPLCNGPRPKKRGSASA

15 LRPGLRTTILFLKLALFSAHC

(SEQ ID NO:8)

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

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

Yanagisawa M., *et al.* Biochem. Biophys. Res. Commun. 178, 656-663, 1991;

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Strausberg R.L., *et al.* Proc. Natl. Acad. Sci. USA. 99, 16899-16903, 2002; Bourgeois C.,

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Hofstra R.M.W., *et al.* Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E.G., *et al.*

30 Cell 79, 1257-1266, 1994; Attie T., *et al.* Hum. Mol. Genet. 4, 2407-2409, 1995;

Auricchio A., *et al.* Hum. Mol. Genet. 5:351-354, 1996; Amiel J., *et al.* Hum. Mol. Genet.

5, 355-357, 1996; Hofstra R.M.W., *et al.* Nat. Genet. 12, 445-447, 1996; Svensson P.J., *et al.* Hum. Genet. 103, 145-148, 1998; Fuchs S., *et al.* Mol. Med. 7, 115-124, 2001;

Pingault V., *et al.* (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1);

35 WO2004048938 (Example 2); WO2004040000 (Claim 151); WO2003087768 (Claim 1);

WO2003016475 (Claim 1); WO2003016475 (Claim 1); WO200261087 (Fig 1);
WO2003016494 (Fig 6); WO2003025138 (Claim 12; Page 144); WO200198351 (Claim
1; Page 124-125); EP522868 (Claim 8; Fig 2); WO200177172 (Claim 1; Page 297-299);
US2003109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34);

5 WO2004001004;

442 aa

MQPPPSLCGRALVALVLACGLSRIWGEERGFPDRATPLLQTAEIMTPPTKTLWPKGSNA
SLARSLAPAEVPGKDRTAGSPPTISPPPCQGPIEIKETFKYINTVVSCLVFLGIIGNS
TLLRIITYKNKCMRNGPNILIASLALGDLHIVIDIPINVKLLAEDWPFGAEMCKLVFFI
10 QKASVGITVLSLCAISIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVSVVLAVPEAIGF
DIITMDYKGSYLRIICLLHPVQKTAFMQFYKTAKDWWLFSFYFCLPLAITAFFYTLMTCEM
LRKKSGMQIALNDHLKQRREVAKTVFCLVLVLFALCWLPLHLRLKLTLYNQNDPNRCEL
LSFLLVLDYIGINMASLNSCINPIALYLVSkrfKNCfkSCLCCWCQSFEKQSLSEKQSC
LKPKANDHGYDNFRSSNKYSSS

15

(SEQ ID NO:9)

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no.
NM_017763);

20 WO2003104275 (Claim 1); WO2004046342 (Example 2); WO2003042661 (Claim 12);
WO2003083074 (Claim 14; Page 61); WO2003018621 (Claim 1); WO2003024392
(Claim 2; Fig 93); WO200166689 (Example 6);

Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

783 aa

25 MSGGHQLQLAALWPWLLMATLQAGFGRTGLVLA AVESERSAEQKAIIRVIPLKMDPTGK
LNLTLLEGVFAGVAEITPAEGKLMQSHPLYLCNASDDDNLEPGFISIVKLESPPRRAPRCL
SLASKARMAGERGASAVLFDITEDRAAAEQ LQQPLGLTWPVVLIWGNDAEKLMEFVYKNQ
KAHVRIELKEPPAWPDYDVWILMTTVGTIFVIIASVLRIRCRPRHSRPDPLQORTAWAI
SQLATRRYQASCRQARGEWPDSGSSCSSAPVCAICLEEFSEGQELRVISCLHEFHRNCVD
30 PWLHQHRTCPLCVFNITEGDSFSQSLGSPRSYQEPGRRLHLIRQHPGHAHYHLPAAYLLG
PSRSAVARPFRPGFPLPSQEPGMGPRHHRFPRAAHPRAPGEQORLAGAHPYAQGWGMSH
LQSTSQHAAACPVPLRRARPPDSSSGSGESYCTERSGYLADGPASDSSSGPCHGSSSDSVV
NCTDISLQGVHGSSSTFCSSLSDFDPLVYCSPKGD PQRVDMQPSVTSRPRSILDSVVPTG
ETQVSSHVHYHRHRHHHYKKRFQWHGRKPGPETGVPQSRPPIPRTPQPEPPSPDQQVTG
35 SNSAAPSGRLSNPQCPRALPEPAGPVDASSICPSTSSLFNLQKSSLSARHPQRKRGGP
SEPTPGSRPQDATVHPACQIFPHYTPSVAYPWSPEAHPLICGPPGLDKRLLPETPGPCYS
NSQFVWLCLTPRQPLEPHPPGEGPSEWSSDTAEGRPCPYPHCQVLSAQPGSEEBLEELCE
QAV

40

(SEQ ID NO:10)

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138,

Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (Claim 1; Fig 1); WO200272596 (Claim 13; Page 54-55); WO200172962 (Claim 1; Fig 4B); WO2003104270 (Claim 11); WO2003104270 (Claim 16); US2004005598 (Claim 22); WO2003042661 (Claim 12); US2003060612 (Claim 12; Fig 10); WO200226822 (Claim 23; Fig 2); WO200216429 (Claim 12; Fig 10);

Cross-references: GI:22655488; AAN04080.1; AF455138_1

490 aa

MESISMMGSPKSLSETVLPNGINGIKDARKVTVGVIGSGDFAKSLTIRLIRCGYHVVIGS
RNPKFASEFFPHVVDVTHHEDALTKTNII FVAIHREHYTSLWDLRHLLVGKILIDVSNM
RINQYPESNAEYLASLFPDSLIVKGFNVVSAWALQLGPKDASRQVYICSNNIQARQQVIE
LARQLNFIPIDLGLSLSAREIENLPLRLFTLWRGPFVVVAISLATFFFLYSFVRDVIHPYA
RNQQSDFYKIPIEIVNKTLPIVAITLLSLVYLAGLLAAAYQLYYGTKYRRPPPWLWTWQ
CRKQLGLLSFFFAMVHVAYSCLPMMRSEERYLFLNMAYQQVHANIENSWNEEEVWRIEMY
ISFGIMSLGLLSLLAVTSIPSVSNALNWREFSFIQSTLGYVALLISTFHVLIYGWKRAFE
EEYYRFYTPPNFVLALVLPISIVILGKIILFLPCISQKLKRIKKGWEKSQFLEEGIGGTIP
HVSPERVTVM

(SEQ ID NO:11)

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636

Xu,X.Z., *et al.* Proc. Natl. Acad. Sci. USA. 98 (19):10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33):30813-30820 (2003)); US2003143557 (Claim 4); WO200040614 (Claim 14; Page 100-103); WO200210382 (Claim 1; Fig 9A); WO2003042661 (Claim 12); WO200230268 (Claim 27; Page 391); US2003219806 (Claim 4); WO200162794 (Claim 14; Fig 1A-D);

Cross-references: MIM:606936; NP_060106.2; NM_017636_1

1214 aa

MVVPEKEQSWIPKIFKKKTCTTFIVDSTDPGGTLCQCGRPRTAHPAVAMEDAFGAADVTV
WSDAHTTEKPTDAYGELDFTGAGRKHSNFLRLSDRTDPAAVYSLVTRTWGFRAPNLVVS
VLGGSGGFVLQTLQDILLRGLVRAAQSTGAWIVTGGLHTGIGRHVGVAVRDHQMASTGG
TKVVAMGVAPWGVVRNRDTLINPKGSFPARYRWRGDPEDGVQFPLDYNYSAFFLVDGTH

GCLGGENRFRRLRESYISQKKTGVGGTGIDIPVLLLLLIDGDEXMLTRIENATQAQLPCLL
VAGSGGAADCLAETLEDTLAPGSGGARQGEARDRIIRRFPPKGDLEVLQAQVERIMTRKEL
LTVYSSSEDGSEEFETIVLKALVKACGSSEASAYLDELRLAVAWNVRVDIAQSELFRGDIQW
5 SHSAGTKAPALKGGAAELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGESEMYLL
SDKATSPSLDAGLGQAPWSDLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMA
RLEPDAAEEAARRKDLAFKFEGMGVDLFGCEYRSSEVRAARLLLRRCPLWG DATCLQLAMQ
ADARAFFAQDGVQSLLTQKWGDMASSTPIWALVLAFFCPPLIYTRLITFRKSEEEPTRE
ELEFDMDSVINGEGPVGTADPAEKTPLGVPRQSGRPGCCGGRCGGRCLRRWFHFWGAPV
10 TIFMGNVVSYLLFLLLSRVLLVDFQPAPPGSLELLLYFWAFTLLCEELRQGLSGGGGSL
ASGGPGPGRHASLSQRLRLYLADSWNQCDLVALTCFLLGVGCRLTPGLYHLGRTVLCIDFM
VFTVRLLLHIFTVKNQQLGPKIVIVSKMMKDVFFFLFGLGVWLVA YGVATEGLLRPRDSDFP
SILRRVFYRPYLQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPPGAQACTCVSQYANWL
VLLLVIIFLLVANILLVNLLIAMFSYTFGKVQGNSDLYWKAQRYRLIREFHSR PALAPFFI
15 VISHLRLLLRQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWESVHKNFLLARARDK
RESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLP
PGGPPPPDLPGSKD

(SEQ ID NO:12)

20 (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth
factor, Genbank accession no. NP_003203 or NM_003212,
Ciccodicola, A., *et al.* EMBO J. 8 (7):1987-1991 (1989), Am. J. Hum. Genet. 49 (3):555-
565 (1991)); US2003224411 (Claim 1); WO2003083041 (Example 1); WO2003034984
25 (Claim 12); WO200288170 (Claim 2; Page 52-53); WO2003024392 (Claim 2; Fig 58);
WO200216413 (Claim 1; Page 94-95, 105); WO200222808 (Claim 2; Fig 1);
US5854399 (Example 2; Col 17-18); US5792616 (Fig 2);
Cross-references: MIM:187395; NP_003203.1; NM_003212_1
188 aa

30 MDCRKMARFSYSVIWIMAIKVFELGLVAGLGHQEFARPSRGYLAFRDDSIWPQEPAIR
PRSSQRVPPMGIQHSKELNRTCCLNGGTCLMGSFCACPPSFYGRNCEHVRKENCGSVPH
DTWLPKKCSLCKCWHGQLRCFPQAFPLPGCDGLVMDEHLVASRTPELPPSARTTTFMLVGI
CLSIQSY

(SEQ ID NO:13)

35 (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or
Hs.73792 Genbank accession no. M26004,
Fujisaku *et al.* (1989) J. Biol. Chem. 264 (4):2118-2125); Weis J.J., *et al.* J.
40 Exp. Med. 167, 1047-1066, 1988; Moore M., *et al.* Proc. Natl. Acad. Sci. USA.

84, 9194-9198, 1987; Barel M., *et al.* Mol. Immunol. 35, 1025-1031, 1998;
Weis J.J., *et al.* Proc. Natl. Acad. Sci. USA. 83, 5639-5643, 1986; Sinha S.K.,
et al. (1993) J. Immunol. 150, 5311-5320; WO2004045520 (Example 4);
US2004005538 (Example 1); WO2003062401 (Claim 9); WO2004045520
5 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Claim 1);
Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

1033 aa

MGAAGLLGVFLALVAPGVLGISCGSPFPILNGRISYSTPIAVGTVIRYSCSGTFRLIGE
KSLLCITKDKVDGTWDPAPKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKT
10 NFSMNGNKSVMWCQANNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVT
YSCESGYLLVGEKIINCLSSGKWSAVPPTCEEARKSLGRFPNGKVKEPPILRVGVTANF
FCDEGYRLQGPPSSRCVIAQGQVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANVSYSI
VTYTCDPDPEEGVNFILIGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQC PHPQILRGR
MVSQKQDRYTYNDTVIFACMPGFTLKGSKQIRCNAQGTWEPSPAPVCEKECQAPPNINLNGQ
15 KEDRHMVRFDPGTSIKYSCNPGYVLVGEESIQTSEGVWTPPVPOCKVAACEATGRQLLT
KPQHGFVRPDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLCKEITCPPPVIYNGAHTG
SSLEDFPYGTTVTYTCNPGPERGVEFSLIGESTIRCTSNDQERGTSWGPAPLCKLSLLAV
QCSHVHIANGYKISGKEAPYFYNDTVTFKCYSGFTLKGSSQIRCKADNTWDPEIPVCEKE
TCQHVRSQSLQELPAGSRVELVNTSCQDGYQLTG HAYQMCQDAENGIWFKKIP LCKVIHCH
20 PPPVIVNGKHTGMMAENFLYGNEVSYECDQGFYLLGEKKLQCRSDSKGHGSGWSGSPQCL
RSPPVTRCPNPEVKHGYKLNKTHSAYSHNDIVYVDCNPGFIMNGSRVIRCHTDNTWVPGV
PTCIKKAFIGCPPPKTPNGNHTGGNIARFSPGMSILYSCDQGYLLVGEALLLCTHEGTW
SQPAPHCKEVNCCSPADMDGIQKGLEPRKMYQYGAVVTLECEDGYMLEGSPQSQCSQSDHQ
WNPFLAVCRSRSLAPVLCGLAAGLILLTFLIVITLYVISKHREERNYTDTSQKEAFHLEA
25 REVYSVDPYNPAS

(SEQ ID NO:14)

(15) CD79b (CD79B, CD79 β , Igb (immunoglobulin-associated beta), B29, Genbank
30 accession no. NM_000626 or 11038674, Proc. Natl. Acad. Sci. USA. (2003) 100
(7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller *et al.* (1992) Eur. J. Immunol. 22
(6):1621-1625); WO2004016225 (claim 2, Fig 140); WO2003087768, US2004101874
(claim 1, page 102); WO2003062401 (claim 9); WO200278524 (Example 2);
US2002150573 (claim 5, page 15); US5644033; WO2003048202 (claim 1, pages 306 and
35 309); WO 99/558658, US6534482 (claim 13, Fig 17A/B); WO200055351 (claim 11,
pages 1145-1146);

Cross-references: MIM:147245; NP_000617.1; NM_000626_1

229 aa

MARLALSPVPSHWMVALLLLLSAEPVPAARSEDYRNPKGSACSRIWQSPRFIARKRGFT
VKMH CYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIY
FCQQKCNNTSEVYQCGTELRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFL
LDKDDSKAGMEEDHTYEGLDIDQATYEDIVTLRTGEVKWSVGEHPGQE

5

(SEQ ID NO:15)

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764,

- 10 Genome Res. 13 (10):2265-2270 (2003), Immunogenetics 54 (2):87-95 (2002), Blood 99 (8):2662-2669 (2002), Proc. Natl. Acad. Sci. USA. 98 (17):9772-9777 (2001), Xu, M.J., *et al.* (2001) Biochem. Biophys. Res. Commun. 280 (3):768-775; WO2004016225 (Claim 2); WO2003077836; WO200138490 (Claim 5; Fig 18D-1-18D-2); WO2003097803 (Claim 12); WO2003089624 (Claim 25);

- 15 Cross-references: MIM:606509; NP_110391.2; NM_030764_1

508 aa

- 20 MLLWSLLVIFDAVTEQADSLTLVAPSSVFEGDSIVLKCQGEQNWKIQKMAYHKDNKELSV
FKKFSDFLIQSAVLSDSGNYFCSTKGQLFLWDKTSNIVKIKVQELFORPVLTAASSFQPIE
GGPVSLKCETRLSPQRLDVQLQFCFFRENQVLGSGWSSSPQLQISAVWSEDTGSYWKAE
TVTHRIRKQSLQSQIHVQRIPISNVSLERAPGGQVTEGQKLILLCSVAGGTGNVTFWSY
REATGTSMGKKTQRLSLSAELEIPAVKESDAGKYCRADNGHVP IQSKVNIIPVRI PVS RP
VLTLSRSPGAQA AVGD LLELHCEALRGSPPILYQFYHEDVT LGNSSAPSGGASFNLSLTA
EHS GNYSCEANGLGAQCSEAVPVVISGPDGYRRDLMTAGVLWGLFGVLGFTGVALLLYA
LFHKISGESSATNEPRGASRPNPQEFTYSSPTPDMEELQPVYVNVGSVDVDVVYSQVWSM
25 QQPESANIRTLLENKDSQVIYSSVKKS

(SEQ ID NO:16)

- (17) HER2 (ErbB2, Genbank accession no. M11730, Coussens L., *et al.* Science (1985)
30 230(4730):1132-1139); Yamamoto T., *et al.* Nature 319, 230-234, 1986; Semba K., *et al.*
Proc. Natl. Acad. Sci. USA. 82, 6497-6501, 1985; Swiercz J.M., *et al.* J. Cell Biol. 165,
869-880, 2004; Kuhns J.J., *et al.* J. Biol. Chem. 274, 36422-36427, 1999; Cho H.-S., *et al.* Nature 421, 756-760, 2003; Ehsani A., *et al.* (1993) Genomics 15, 426-429;
WO2004048938 (Example 2); WO2004027049 (Fig 1D); WO2004009622;
35 WO2003081210; WO2003089904 (Claim 9); WO2003016475 (Claim 1);
US2003118592; WO2003008537 (Claim 1); WO2003055439 (Claim 29; Fig 1A-B);
WO2003025228 (Claim 37; Fig 5C); WO200222636 (Example 13; Page 95-107);

WO200212341 (Claim 68; Fig 7); WO200213847 (Page 71-74); WO200214503 (Page 114-117); WO200153463 (Claim 2; Page 41-46); WO200141787 (Page 15); WO200044899 (Claim 52; Fig 7); WO200020579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7);
5 WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4);
Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761;
AAA35808.1.

1255 aa

10 MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVGQNL
ELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTLFEDNYALAVLDNG
DPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLA
LTLIDTNRSRACHPCSPMCKGSRGWESSEDCQSLTRTVCAAGGCARCKGPLPTDCCHEQC
AAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNIDTFESMPNPEGRYTFGASCVTACP
15 YNYLSTDVGSCTLVCPHNEVTAEDGTQRCCKSKPCARVCYGLGMEHLREVRVTSAN
IQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSL
DLSVFQNLQVIRGRILHNGAYSILTLQGLGISWLGLRSLRELGSLALIHNTLHLCFVHTV
PWDQLFRNPHQALLHTANRPEDECVGEGGLACHQLCARGHCWGPPTQCVNCSQFLRGQEC
VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPFPCVARC
PSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCAPQASPLTSIIISAVVG
20 ILLVVVLGVVFGILIKRRQKKIRKYTMRRLLQETELVEPLTPSGAMPNQAQMRILKETEL
RKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSF
YVSRLLGICLTSTVQLVTQLMPYGCILLDHVRENRLGSGQDLLNWCMTAKGMSYLEDVR
LVHRDLAARNVLVKS PNHVKITDFGLARLLDIDETEHADGGKVPIKWMALSTLRRRFT
HQSDVWSYGVTVWELMTFGAKPYDGIPIAREIPDLLEKGERLPQPPICTIDVYMIMVKCWM
25 IDSECRPRFRELVSFESRMARDPQRFVVIQNEGLGPASPLDSTFYRSLLEDDDMGDLVDA
EEYLVPQQGFFCPDPAPGAGGMVHHRSSSTRSGGGLTLGLEPSEEEAPRSPLAPSEG
AGSDVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYV
NQPDVRPQPPSPREGPLPAARPAATLERPKTLSPGKNGVVKDVFAGGAVENPEYLTPO
GGAAPQPHPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV

30

(SEQ ID NO:17)

(18) NCA (CEACAM6, Genbank accession no. M18728);
Barnett T., et al Genomics 3, 59-66, 1988; Tawaragi Y., et al. Biochem. Biophys. Res.
35 Commun. 150, 89-96, 1988; Strausberg R.L., et al. Proc. Natl. Acad. Sci. USA.
99:16899-16903, 2002; WO2004063709; EP1439393 (Claim 7); WO2004044178
(Example 4); WO2004031238; WO2003042661 (Claim 12); WO200278524 (Example
2); WO200286443 (Claim 27; Page 427); WO200260317 (Claim 2);

Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;
344 aa

MGPPSAPPCLRHPWPKEVLLTASLLTFWNPPTAKLTIESTPFNVAEKGKVVLLAHNLPQ
NRIGYSWYKGERVDGNSLIVGYVIGTQQATPGPAYSGRETIYPNASLLIQNVTONDTGFY
5 TLQVIKSDLVNEEATGQPHVYPELPKPSISSNNSNPVEDKDAVAFTCEPEVQNTTYLWWV
NGQSLPVSRLQLSNGNMILTLLSVKRNDAGSYECEIQNPASANRSDPVTNLNVLYGPDVP
TISPSKANYRPGENLNLSCHAASNPPAQYSWFINGTFQQSTQELFIPNITVNNSGSYMCQ
AHNSATGLNRTTVMITVSGSAPVLSAVATVGITIGVLARVALI

(SEQ ID NO:18)

10

(19) MDP (DPEP1, Genbank accession no. BC017023,
Proc. Natl. Acad. Sci. USA. 99 (26):16899-16903 (2002)); WO2003016475
(Claim 1); WO200264798 (Claim 33; Page 85-87); JP05003790 (Fig 6-8);
WO9946284 (Fig 9);

15 Cross-references: MIM:179780; AAH17023.1; BC017023_1

411 aa

MWSGNWLWPLVAVCTADFFRDEAERIMRDSFVIDGHNDLPWQLLDMFNNRLQDERANLTT
LAGTHTNIPKLRAGFVGGQFWSVYTPCDTQNKDAVRRRTLEQMDVVHRMCRMYPETFLYVT
SSAGIRQAFREGKVASLIGVEGCHSIDSSLGVLRLALYQLGMRYLTLTHSCNTPWADNWLWV
20 DTGDSEPPQSQGLSPFGQRVVKELNRLGVLIDLAHVSVATMKATLQLSRAPVIFSHSSAYS
VCASRRNVDPDDVLRVLVKQTDLSLVMVNFYNNYISCTNKANLSQVADHLDHIKEVAGARAVG
FGGDFDGVPRVPEGLEDVSKYPDLIAELLRRNWTEAEVKGALADNLLRVFEAVEQASNL
QAPEEPIPLDQLGGSCRTHYGYSSGASSLHRHWGLLLASLAPLVLCLSLL

(SEQ ID NO:19)

25

(20) IL20R α (IL20Ra, ZCYTOR7, Genbank accession no. AF184971);
Clark H.F., *et al.* Genome Res. 13, 2265-2270, 2003; Mungall A.J., *et al.*
Nature 425, 805-811, 2003; Blumberg H., *et al.* Cell 104, 9-19, 2001;
30 Dumoutier L., *et al.* J. Immunol. 167, 3545-3549, 2001; Parrish-Novak J., *et al.*
J. Biol. Chem. 277, 47517-47523, 2002; Pletnev S., *et al.* (2003) Biochemistry
42:12617-12624; Sheikh F., *et al.* (2004) J. Immunol. 172, 2006-2010;
EP1394274 (Example 11); US2004005320 (Example 5); WO2003029262
(Page 74-75); WO2003002717 (Claim 2; Page 63); WO200222153 (Page 45-
35 47); US2002042366 (Page 20-21); WO200146261 (Page 57-59);
WO200146232 (Page 63-65); WO9837193 (Claim 1; Page 55-59);

Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

553 aa

MRAPGRPALRPLPLPPLLLLLLAAPWGRAVPCVSGGLPKPANITFLSINMKNVLQWTPPE
GLQGVKVTYTVQYFIYGQKKWLNKSECRNINRTYCDLSAETSDYEHQYYAKVKAIWGTTC
5 SKWAESGRFYFLETQIGPPEVALTTDEKSISVVLTAPEKWKRNPEDLFVSMQQIYSNLK
YNVSVLNTKSNRTWSQCVTNHTLVLTWLEPNTLYCVHVESFVPGPPRAQPSEKQCARTL
KDQSSEFKAKIIFWYVLPISITVFLFSVMGYSIYRYIHVGKEKHPANLILYGNFDFKRF
FVPAEKIVINFITLNISSDKISHQDMSLLGKSSDVSSLNDPQPSGNLRPPQEEEEEVKHL
GYASHLMEIFCDSEENTEGTSFTQQESLSRTIPDKTVIEYEYDVRTTDICAGPEEQELS
10 LQEEVSTQGTLLSQAALAVLGPQTLOYSYTPQLQDLDEPLAQEHTDSEEGPEEPESTTLV
DWDPTGTGRLCIPSLSSFDQDSEGCEPSEGDLGEEGLLSRLYEPPAPDRPPGENETYLMQ
FMEEWGLYVQMN

(SEQ ID NO:20)

15

(21) Brevican (BCAN, BEHAB, Genbank accession no. AF229053)

Gary S.C., *et al.* Gene 256, 139-147, 2000; Clark H.F., *et al.* Genome Res. 13,
2265-2270, 2003; Strausberg R.L., *et al.* Proc. Natl. Acad. Sci. USA. 99,
16899-16903, 2002; US2003186372 (Claim 11); US2003186373 (Claim 11);
20 US2003119131 (Claim 1; Fig 52); US2003119122 (Claim 1; Fig 52);
US2003119126 (Claim 1); US2003119121 (Claim 1; Fig 52); US2003119129
(Claim 1); US2003119130 (Claim 1); US2003119128 (Claim 1; Fig 52);
US2003119125 (Claim 1); WO2003016475 (Claim 1); WO200202634 (Claim
1);

25 911 aa

MAQLFLPLLAALVLAQAPALADVLEGDSSEDRAFRVRIAGDAPLQGVLGGLTIPCHVH
YLRPPPSRRVILGSPRVKWTFLSRGREAEVLVARGVRVKVNEAYRFRVALPAYPASLTDV
SLALSELRPNDSGIYRCEVQHIGDDSSDAVEVKVKGVVFLYREGSARYAFSFGAQEACA
RIGAHIAATPEQLYAAYLGGYEQCDAGWLSAQTVRYPIQTPREACYGDMGFGVRNYGVV
30 DPDDLVDVYCYAEDLNGELFLGDPPEKLTLEEARAYCQERGAEIATGQLYAAWDGGLDH
CSPGWLADGSVRYPIVTPSQRCGGGLPGVKTFLFPNQTGFPPNKHSRFRNVYCFRDSAQPS
AIPEASNPNASNPASDGLEAIVTVTETLEELQLPQEATESESRGAIYSIPIMEDGGGGSST
PEDPAEAPRTLLEFETQSMVPPTGFSEEEGKALEEKEKYEDDEEKEEEEEEEVEDEALW
AWPSELSSPGFEASLPTEPAAQEKSLSQAPARAVLQPGASPLPDGESEASRPFRVHGPPPT
35 ETLPTPRERNLASPSPSTLVEAREVGEATGGPELSCGVPRGESEETGSSEGAPSLLPATRA
PEGTRELEAPSEDNSGRTAPAGTSVQAQPVLPDTSASRGGVAVVPASGDCVPSFCHNGGT
CLEEEEGVRCLCLPGYGGDLCDVGLRFCNPGWDAFQGACYKHFSTRRSWEEAETQCRMYG
AHLASISTPEEQDFINNRYREYQWIGLNDRTIEGDFLWSDGVPLLYENWNPGQPD SYFLS
GENCVVMVWHDDQGWSDVFCNYHLSYTKMGLVSCGPPPELPLAQVFGRPRLRYEVDTVL
40 RYRCREGLAQRNLPLIRCQENGRWEAPQISCVPRRPARALHPEEDPEGRQGRLLGRWKAL

- 5 (22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442)
Chan, J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905
(1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000));
WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576
(Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216
10 (Claim 1; Page 42);

Cross-references: MIM:600997; NP_004433.2; NM_004442_1

987 aa

MALRRLGAALLLLPLLAABETLMDSTTATAELGWMVHPSPGWEEVSGYDENMNTIRTYQ
VCNVFESSQNNWLRTKFIRRRGAHRIHVEMKFSVRDCSSIPSVPGSCKETFNLYYEADF
15 DSATKTFPNWMENPWVKVDIAADESFSQVDLGGRVMKINTEVRSFGPVSRSRGFYLAQD
YGGCMSLIAVRVFYRKCPRIIQNGAIFQETLSGAESTSLVAARGSCIANAEVDVPIKLY
CNGDGEWLVPFIGRCMCKAGFEAVENGTVCRGCPSTFKANQGDEACTHCPINSRTTSEGA
TNCVCRNGYYRADLDPLDMPCTTIPSAQAVISSVNETSLMLEWTPPRDSSGGREDLVYNI
ICKSCGSGRGACTRCGDNVQYAPRQLGLTEPRIYISDLLAHTQYTFEIQAVNGVTDQSPF
20 SPQFASVNITTNAAPSASVIMHQVSRTVDSITLSWSQPDQPNGVILDYELQYYEKELSE
YNATAIKSPTNTVTQGLKAGAIYVFQVRARTVAGYGRYSGKMYFQTMTEAEYQTSIQEK
LPLIIGSSAAGLVFLIAVVVIAIVCNRRRGFERADSEYTDKLQHYTSGHMTPGMKIYIDF
FTYEDPNEAVREFAKEIDISCVKIEQVIGAGEFGEVCSGHLKLPKREIFVAIKTLKSGY
TEKQRRDFLSEASIMGQFDHPNVIHLEGVVTKSTFVMIITEFMENGSLDSFLRQNDGQFT
25 VIQLVGMLRGIAAGMKYLADMNYVHRDLAARNILVNSNLVCKVSDFGLSRFLEDDTSDPT
YTSALGGKIPIRWTAPEAIQYRKFTSASDVWSYGIWMWEVMSYGERPYWDMTNQDVINAI
EQDYRLPPPMDCPSALHQLMLDCWQKDRNHRPKFGQIVNTLDKMIRNPNSLKAMAPLSSG
INLPLLDRTIPDYTSFNTVDEWLEAIKMGQYKESFANAGFTSFDVVSQMMMEDILRVGVT
LAGHQKKILNSIQVMRAQMNQIQSVEV

30

(SEQ ID NO:22)

- (23) ASLG659 (B7h, Genbank accession no. AX092328)
US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3);
35 US2003165504 (Claim 1); US2003124140 (Example 2); US2003065143 (Fig 60);
WO2002102235 (Claim 13; Page 299); US2003091580 (Example 2); WO200210187
(Claim 6; Fig 10); WO200194641 (Claim 12; Fig 7b); WO200202624 (Claim 13; Fig
1A-1B); US2002034749 (Claim 54; Page 45-46); WO200206317 (Example 2; Page 320-
321, Claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587

(Example 1; Fig 1); WO200140269 (Example 3; Pages 190-192); WO200036107
(Example 2; Page 205-207); WO2004053079 (Claim 12); WO2003004989 (Claim 1);
WO200271928 (Page 233-234, 452-453); WO 0116318;

282 aa

5 MASLGQILFWSIIISIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEP
DIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASRLKKNV
QLTDAGTYKCYIITSKGKKNANLEYKTGAFSMPEVNVDYNASSETLRCEAPRWFPQPTVV
WASQVDQGAFSEVSNTSFELNSENVTMKVSVLYNVTINNTYSCMIENDIAKATGDIKV
TESEIKRRSHLQLLNSKASLCVSSFFAISWALLPLSPYMLK

10

(SEQ ID NO:23)

(24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436)
Reiter R.E., *et al.* Proc. Natl. Acad. Sci. USA. 95, 1735-1740, 1998; Gu Z., *et*
15 *al.* Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000)
275(3):783-788; WO2004022709; EP1394274 (Example 11); US2004018553
(Claim 17); WO2003008537 (Claim 1); WO200281646 (Claim 1; Page 164);
WO2003003906 (Claim 10; Page 288); WO200140309 (Example 1; Fig 17);
US2001055751 (Example 1; Fig 1b); WO200032752 (Claim 18; Fig 1);
20 WO9851805 (Claim 17; Page 97); WO9851824 (Claim 10; Page 94);
WO9840403 (Claim 2; Fig 1B);
Accession: O43653; EMBL; AF043498; AAC39607.1.

123 aa

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNECDLQVENCTQLGEQCWTARIRAVGLLT
25 VISKGCSSLNVCDDSDQDYVVGKKNITCCDITLGNASGAHALQPAAILALLPALGLLLWGP
GQL

(SEQ ID NO:24)

30 (25) GEDA (Genbank accession No. AY260763);
AAP14954 lipoma HMGIC fusion-partner-like protein /pid=AAP14954.1 - Homo
sapiens
Species: Homo sapiens (human)
WO2003054152 (Claim 20); WO2003000842 (Claim 1); WO2003023013 (Example 3,
35 Claim 20); US2003194704 (Claim 45);

Cross-references: GI:30102449; AAP14954.1; AY260763_1

236 aa

MPGAAAAAAAAAAMLPAQEAAKLYHTNYVRNSRAIGVLWAIFTICFAIVNVVCFIQPYW
IGDGVDTFPQAGYFGLFHYCIGNGFSRELTCRGSFTDFSTLPSGAFKAASFFIGLSMMLII
5 ACTIICFTLFFFCNTATVYKICAWMQLTSAACLVLGCMIFPDGWDSEVKRMCGEKTDKYT
LGACSVRWAYILAIIGILDALILSFLAFVLGNRQDSLMAEELKAENKVLLSQYSLE

(SEQ ID NO:25)

- 10 (26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, Genbank
accession No. NP_443177.1);
NP_443177 BAFF receptor /pid=NP_443177.1 - Homo sapiens
Thompson,J.S., *et al.* Science 293 (5537), 2108-2111 (2001); WO2004058309;
WO2004011611; WO2003045422 (Example; Page 32-33); WO2003014294 (Claim 35;
15 Fig 6B); WO2003035846 (Claim 70; Page 615-616); WO200294852 (Col 136-137);
WO200238766 (Claim 3; Page 133); WO200224909 (Example 3; Fig 3);
Cross-references: MIM:606269; NP_443177.1; NM_052945_1

184 aa

20 MRRGFRSLRGRDAPAPTPCVPACFDLLVRHCVACGLLRTPRKPAGASSPAPRTALQPP
ESVGAGAGEAALPLPGLLFGAPALLGLALVLALVGLVSWRRRQRRLRGASSAEAPDGD
KDAPEPLDKVIIILSPGISDATAFAWPPPGEDPGTTTPPGHVPVPATELGSTELVTTKTAG
PEQQ

(SEQ ID NO:26)

25

- (27) CD22 (B-cell receptor CD22-B isoform, Genbank accession No. NP-001762.1);
Stamenkovic,I. and Seed,B., Nature 345 (6270), 74-77 (1990); US2003157113;
US2003118592; WO2003062401 (Claim 9); WO2003072036 (Claim 1; Fig 1);
WO200278524 (Example 2);

- 30 Cross-references: MIM:107266; NP_001762.1; NM_001771_1

847 aa

35 MHLGFWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDLESFILFH
NPEYNKNTSKFDGTRLYESTKDGKVPSEQKRQVQLGDKNKNCTLSIHPVHLNDSGQLGLR
MESKTEKWMERIHLNVSERPFPPIQLPPEIQESQEVTLTCLLNFSYGYPIQLQWLLEG
VPMRQAAVTSTSLTIKSVFTRSELKFSPOWSHGKIVTCQLQDADGKFLSNDTVQLNVKH
TPKLEIKVTPSDAIVREGDSVTMTCEVSSSNPEYTTVSWLKGTSCLKKQNTFTLNLREVT
KDQSGKYCCQVSNVGPGRSEEVFLQVQYAPPESTVQILHSPAVEGSQVEFLCMSLANPL
PTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVAENILGTGQRGPGAELDVQYPPK

KVTTVIQNPMPIREGDVTTLSCNYNSSNPSTRYEWKPHGAWEEPVLGVLKIQNVGWDNT
 TIACARCNSWCASWASPVALNVQYAPRDVVRVRIKPLSEIHSGNSVSLQCDFSSSHPKVEVQ
 FFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLRVSM
 SPGDQVMEGKSATLTCESDANPFVSHYTWFDDWNNQSLPHHSQKLRLPEVKVQHSGAYWCQ
 5 GTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSLAILILAICGLKLQRRWKRTQSQQG
 LQENSSGQSFFVRNKKVRRAPLSEGPMSLGCYNPMMEDGISYTTLRFPENNIPTGDAES
 SEMQRPPTCDDTVTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENV
 DYVILKH

(SEQ ID NO:27)

10

(28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation) PROTEIN

15 SEQUENCE Full mpgpggv...dvqlkcp (1..226; 226 aa), pI: 4.84, MW: 25028 TM: 2 [P]
 Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.1;
 WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4,
 pages 13-14); WO9958658 (claim 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha *et*
al. (1992) J. Immunol. 148(5):1526-1531; Mueller *et al.* (1992) Eur. J. Biochem.
 20 22:1621-1625; Hashimoto *et al.* (1994) Immunogenetics 40(4):287-295; Preud'homme *et*
al. (1992) Clin. Exp. Immunol. 90(1):141-146; Yu *et al.* (1992) J. Immunol. 148(2) 633-
 637; Sakaguchi *et al.* (1988) EMBO J. 7(11):3457-3464;

226 aa

25 MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSN
 NANVTWWRVLHGNYTWPFELGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQSCG
 TYLRVRQPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGD
 EYEDENLYEGLNLDDCSMYEDISRGLOQTYQDVGSLNIGDVQLEKP

(SEQ ID NO:28)

30

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia) PROTEIN SEQUENCE Full mnypltl...atstltf (1..372; 372 aa),
 35 pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No.
 NP_001707.1;

WO2004040000; WO2004015426; US2003105292 (Example 2); US6555339 (Example 2); WO200261087 (Fig 1); WO200157188 (Claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7, Fig 5); Dobner *et al.* (1992) Eur. J. Immunol. 22:2795-2799; Barella *et al.* (1995) Biochem. J. 309:773-779;

372 aa

MNYPLTLEMDLENLEDLFWELDRLDNYNDTSLVENHLCPATEGPLMASFKAVFVPVAYSL
IFLLGVIGNVLVLVILERHRQTRSSTETFLFHLAVADLLLVLFPFAVAEGSVGWVLGTF
10 LCKTVIALHKVNFYCSSLLACIAVDRYLAIVHAVHAYRHRLLSIHITCGTIWLVGFLL
ALPEILFAKVSQGHNNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLPMLVMGWCVYG
VVHRLRQAQRPRQKAVRVAILVTSIFFLCWSPYHIVIFLDTLARLKAVDNTCKLNGSL
PVAITMCEFLGLAHCCLNPMPLYTFAGVKFRSDLSRLLTKLGCTGPASLCQLFPSWRRSSL
SESENATSLTTF

15

(SEQ ID NO:29)

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes) PROTEIN SEQUENCE Full

20 msggwvp...vllpqsc (1..273; 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1;

Tonnelle *et al.* (1985) EMBO J. 4(11):2839-2847; Jonsson *et al.* (1989) Immunogenetics 29(6):411-413; Beck *et al.* (1992) J. Mol. Biol. 228:433-441; Strausberg *et al.* (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius *et al.* (1987) J. Biol. Chem.

25 262:8759-8766; Beck *et al.* (1996) J. Mol. Biol. 255:1-13; Naruse *et al.* (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-146); Kasahara *et al.* (1989) Immunogenetics 30(1):66-68; Larhammar *et al.* (1985) J. Biol. Chem. 260(26):14111-14119;

30 273 aa

MSGGWVPVWVALLVNLTRLDSMTQGTDSPEDFVIQAKADCYFTNGTEKVQFVVRFIENL
EEYVRFDSDVGMFVALTKLGQPDABQWNSRLDLLERSRQAVDGVCRHNYRLGAPFTVGRK
VQPEVTVPERTPLLHQHNLHCSVTGFYPGDIKIKWFLNGQEERAGVMSTGPIRNGDWT
PQTVVMLEMTPELGHVYTCLVDHSSLLSPVSVEWRAQSEYSWRKMLSGIAAFLLGLIFLL
35 VGIVIQLRQKGYVRTQMSGNEVSRVLLPQSC

(SEQ ID NO:30)

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability) PROTEIN SEQUENCE Full mgqagck...lephrst (1..422; 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2; Le *et al.* (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman *et al.* (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);

422 aa

MGQAGCKGKGLCLSLFDYKTEKYVIAKNKKVGLLYRLLQASILAYLVVWVFLIKKGYQDVD
TSLQSAVITKVKGVAFNTSDLGQRIWDVADYVIPAQGENVFFVVTNLIVTPNQRQNVCAE
NEGIPDGACSKSDCHAGEAVTAGNGVKTGRCLRRNLARGTCEIFAWCPLETSSRPEEP
FLKEAEDFTIFIKNHIRFPKFNFSKSNVMDVKDRSFLKSCHFGPKNHYPFRGLGSVIRW
AGSDFQDIALEGGVIGINIEWNCDLDKAASECHPHYSFSRLDNKLSKSVSSGYNFRFARY
YRDAAGVEFRTLKAYGIRFDVMVNGKGAFPCDLVLIYLIKREFYRDKKYEEVRGLEDS
SQEAEDRASGLGLSEQLTSGPGLLGMPQQELQEPPEAKRGSSSQKNGSVCPQLLEPHR
ST

(SEQ ID NO:31)

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, Genbank accession No. NP_001773.1; WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-106); Von Hoegen *et al.* (1990) J. Immunol. 144(12):4870-4877; Strausberg *et al.* (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

359 aa

MAEAITYADLRFVKAPLKKSISRLGQDPGADDDGEITYENVQVPAVLGVPSSSLASSVLG
DKAAVKSEQPTASWRAVTSFAVGRILPCRTTCLRYLLGLLLTCLLLGVTAICLGVRYLQ
VSQQLQQTNRVLEVTNSSLRQQLRLKITQLGQSAEDLQGSRRRLAQSQEALQVEQRAHQ
AEGQLQACQADRQKTKETLQSEEQRRALQKLSNMENRLKPFPTCGSADTCCPSGWIMH
QKSCFYISLTSKNWQESQKQCETLSSKLATFSEIYPQSHSYFLNSLLPNGGSGNSYWTG
LSSNKDWKLTDDTQRTQRTYAQSSKCNKVHKTWSWWTLESESCRSSLFPYICEMTAFRFPD

(SEQ ID NO:32)

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis)

5 PROTEIN SEQUENCE Full mafdvsc...rwkyqhi (1..661; 661 aa), pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1; US2002193567; WO9707198 (claim 11, pages 39-42); Miura *et al.* (1996) Genomics 38(3):299-304; Miura *et al.* (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-61); WO200012130 (pages 24-26);

10 661 aa

MAFDVSCFFWVLFSA GCKVITSWDQMCIEKEANKTYNCENLGLSEIPDTLPNTTEFLEF
SFNFLTPTIHNRTFSRLMNLTFDLTRCQINWIHEDTFQSHHQLSTLVLTGNPLIFMAETS
LNGPKSLKHLFLIQTGISNLEFIPVHNLENLESYLGSNHISSIKFPKDFPARNLKVLDF
QNNAIHYISREDMRSLEQAINLSLNFNGNNVKGIELGAFDSTVFQSLNFGGTPNLSVIFN
15 GLQNSTTQSLWLGTTFEDIDDEDISSAMLKGLCEMSVESLNLQEHFSDISSTTFQCFTQL
QELDLTATHLKGLPSGMKGLNLLKKLVLSVNHFDQLCQISAANFPSLTHLYIRGNVKKLH
LGVCLEKLGNLQTLDSLHNDIEASDCSLQLKNLSHLQTLNLSHNEPLGLQSQA FKECP
QLELLDLAFTRLHINAPQSPFQNLHFLQVLNLTTCFLDTSNQHLLAGLPVLRHLNLKGNH
FQDGTITKTNLLQTVGSLEVLILSSCGLLSIDQQA FHS LGKMSHVDLSHNSLTCDSDSL
20 SHLKG IYLNLAANSINIISPRLLPILSQOSTINLSHNPLDCTCSNIHFLTWYKENLHKLE
GSEETTCANPPSLRGVKLS DVKLS CGITAIGIFFLIVFLLLLAILLFFAVKYLLRWKYQH
I

(SEQ ID NO:33)

25

(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation) PROTEIN SEQUENCE Full mlprll...vdyedam (1..429; 429 aa), pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, Genbank accession No. NP_443170.1; WO2003077836; WO200138490 (claim 6, Fig 18E-1-18-E-2); Davis *et al.* (2001) Proc. Natl. Acad. Sci USA 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1); WO2003089624 (claim 7);

429 aa

35 MLPRLLLLICAPLCEPAELFLIASPSHPTGSPVTLTCKMPFLQSSDAQFQFCFFRDTRA
LGPGWSSSPKLQIAAMWKEDTGSYWCEAQT MASKVLRSSRSQINVHRVPVADVSLETQPP
GGQVMEGDRLVLICSVAMGTGDITFLWYKGAVGLNLQSKTQ RSLTAEYEI PSVRESDAEQ

YYCVAENGYGSPSPGLVSITVRIPVSRPILMLRAPRAQAAVEDVLELHCEALRGSPPILY
 WFYHEDITLGSRSAPSGGGASFNLSTLTHSGNYSCEANGLGAQRSEAVTLNFTVPTGA
 RSNHLTSGVIEGLLSTLGPATVALLFCYGLKRRKIGRRSARDPLRSLPSPLPQEFTYLNSP
 TPGQLQPIYENNVVSGDEVYSLAYYNQPEQESVAAETLGTHTMEDKVSLEDIYSRLRKANI
 5 TDVDYEDAM

(SEQ ID NO:34)

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative
 10 immunoreceptor with possible roles in B cell development and lymphomagenesis;
 deregulation of the gene by translocation occurs in some B cell malignancies) PROTEIN
 SEQUENCE Full mllwvil...assaphr (1..977; 977 aa), pI: 6.88 MW: 106468 TM: 1 [P]
 Gene Chromosome: 1q21, Genbank accession No. NP_112571.1;
 WO2003024392 (claim 2, Fig 97); Nakayama *et al.* (2000) Biochem. Biophys. Res.
 15 Commun. 277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2);
 977 aa

MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTCKGFRFYSPQKTKWYHR
 YLGKEILRETPDNILEVQESGEYRCQAQGSPLSSPVHLDFFSSASLILQAPLSVFEGDSV
 LRCAKAEVTLNNTIYKNDNVLAFLNKRDTDFHIFHACLKDNAYRCTGYKESCCPVSSNT
 20 VKIQVQEPFTRFVLRASSFQPIISGNPVTLTCTQSLERSDVLRFRRFRDDQTLGLGWS
 LSPNFQITAMWSKDSGFYCKAATMPHSVISDSRPSWIQVQIPASHPVLTLSPKALNFE
 GTKVTLHCETQEDSLRTLRYFYHEGVPLRHKSVRCERGASISFSLTTENSGNYYCTADNG
 LGAKPSKAVSLSVTPVSHPVNLSSPEDLIFEGAKVTLHCEAQRGSLPILYQFHHEDAA
 LERRSANSAGGVAISFSLTAEHSGNYYCTADNGFGPQRSKAVSLSVTPVSHPVTLSSA
 25 EALTFEAGATVTLHCEVQRGSPQILYQFYHEDMPLWSSSTPSVGRVSFSFSLTEGHSGNYY
 CTADNGFGPQRSEVVSLFVTPVSRPILTLRVPRQAQAVVGDILLELHCEAPRGSPPILYWF
 YHEDVTLGSSSAPSGGEASFNLSTLTHSGNYSCEANGLVAQHSDTISLSVIVPVSRI
 LTFRAPRAQAVVGDILLELHCEALRGSSPILYWPHYHEDVTLGKISAPSGGGASFNLSTTE
 HSGIYSCEADNGPEAQRSEMTLKVAVPVSFVTLRAPGTHAAVGDILLELHCEALRGSP
 30 LILYRFFHEDVTLGNRSSPSGGASNLSTLTHSGNYSCEADNGLGAQRSETVTLTYITGL
 TANRSGPFATGVAGLLSIAGLAAGALLLYCWLRSKAGRKPASDPARSPPDSQSQPTYH
 NVPAAEELQPVYTNANPRGENVVYSEVRIIQEKKKHAVASDPHRLRNKGSPIIYSEVKVA
 STFVSGSLFLASSAPHR

(SEQ ID NO:35)

See also: WO04/045516 (03 Jun 2004); WO03/000113 (03 Jan 2003);
 WO02/016429 (28 Feb 2002); WO02/16581 (28 Feb 2002); WO03/024392 (27 Mar
 2003); WO04/016225 (26 Feb 2004); WO01/40309 (07 Jun 2001), and U.S. Provisional
 40 patent application Serial No. 60/520842 "COMPOSITIONS AND METHODS FOR THE

TREATMENT OF TUMOR OF HEMATOPOIETIC ORIGIN", filed 17 Nov 2003; all of which are incorporated herein by reference in their entirety.

In an embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where the Ligand is an antibody Ab including one that binds at least one of CD30, CD40, CD70, Lewis Y antigen, $w=0$, $y=0$, and D has Formula Ib. Exemplary Conjugates of Formula IIIa include where R^{17} is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa in which D has the structure of Compound 2 in Example 3 and esters thereof. Also included are such Conjugates of Formula IIIa containing about 3 to about 8, in one aspect, about 3 to about 5 Drug moieties D, that is, Conjugates of Formula Ia wherein p is a value in the range about 3-8, for example about 3-5. Conjugates containing combinations of the structural features noted in this paragraph are also contemplated as within the scope of the compounds of the invention.

In another embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where Ligand is an Antibody Ab that binds one of CD30, CD40, CD70, Lewis Y antigen, $w=1$, $y=0$, and D has Formula Ib. Included are such Conjugates of Formula IIIa in which R^{17} is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa in which W is -Val-Cit-, and/or where D has the structure of Compound 2 in Example 3 and esters thereof. Also included are such Conjugates of Formula IIIa containing about 3 to about 8, preferably about 3 to about 5 Drug moieties D, that is, Conjugates of Formula Ia wherein p is a value in the range of about 3-8, preferably about 3-5. Conjugates containing combinations of the structural features noted in this paragraph are also exemplary.

In an embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where the Ligand is an Antibody Ab that binds one of CD30, CD40, CD70, Lewis Y antigen, $w=1$, $y=1$, and D has Formula Ib. Included are Conjugates of Formula IIIa in which R^{17} is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa where: W is -Val-Cit-; Y has Formula X; D has the structure of Compound 2 in Example 3 and esters thereof; p is about 3 to about 8, preferably about 3 to about 5 Drug moieties D. Conjugates containing combinations of the structural features noted in this paragraph are also contemplated within the scope of the compounds of the invention.

A further embodiment is an antibody drug conjugate (ADC), or a pharmaceutically acceptable salt or solvate thereof, wherein Ab is an antibody that binds one of the tumor-associated antigens (1)-(35) noted above (the "TAA Compound").

Another embodiment is the TAA Compound or pharmaceutically acceptable salt or solvate thereof that is in isolated and purified form.

Another embodiment is a method for killing or inhibiting the multiplication of a tumor cell or cancer cell comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to kill or inhibit the multiplication of a tumor cell or cancer cell.

Another embodiment is a method for treating cancer comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to treat cancer, alone or together with an effective amount of an additional anticancer agent.

Another embodiment is a method for treating an autoimmune disease, comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to treat an autoimmune disease.

The antibodies suitable for use in the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

4.5.1 PRODUCTION OF RECOMBINANT ANTIBODIES

Antibodies of the invention can be produced using any method known in the art to be useful for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression.

Recombinant expression of antibodies, or fragment, derivative or analog thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides, *e.g.*, by PCR.

Alternatively, a nucleic acid molecule encoding an antibody can be generated from a suitable source. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody is known, a nucleic acid encoding the antibody can be obtained from a suitable source (*e.g.*, an antibody
5 cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by, *e.g.*, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody that specifically recognizes a particular antigen is not
10 commercially available (or a source for a cDNA library for cloning a nucleic acid encoding such an immunoglobulin), antibodies specific for a particular antigen can be generated by any method known in the art, for example, by immunizing a patient, or suitable animal model such as a rabbit or mouse, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, *e.g.*, as described by Kohler and
15 Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor *et al.* (1983, *Immunology Today* 4:72) or Cole *et al.* (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody can be obtained by screening Fab expression libraries (*e.g.*, as described in Huse *et al.*, 1989, *Science* 246:1275-1281) for clones of Fab fragments that
20 bind the specific antigen or by screening antibody libraries (See, *e.g.*, Clackson *et al.*, 1991, *Nature* 352:624; Hane *et al.*, 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

Once a nucleic acid sequence encoding at least the variable domain of the antibody is obtained, it can be introduced into a vector containing the nucleotide sequence encoding the constant regions of the antibody (see, *e.g.*, International Publication No.
25 WO 86/05807; WO 89/01036; and U.S. Patent No. 5122464). Vectors containing the complete light or heavy chain that allow for the expression of a complete antibody molecule are available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitutions or deletion necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond
30 with an amino acid residue that does not contain a sulfhydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical

mutagenesis and *in vitro* site directed mutagenesis (Hutchinson *et al.*, 1978, *J. Biol. Chem.* 253:6551).

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. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, *e.g.*, humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,694,778; Bird, 1988, *Science* 242:423-42; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward *et al.*, 1989, *Nature* 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra *et al.*, 1988, *Science* 242:1038-1041).

Antibody fragments that recognize specific epitopes can be generated by known techniques. For example, such fragments include, but are not limited to the F(ab')₂ fragments that can be produced by pepsin digestion of the antibody molecule and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragments.

Once a nucleic acid sequence encoding an antibody has been obtained, the vector for the production of the antibody can be produced by recombinant DNA technology using techniques well known in the art. Methods that are well known to those skilled in the art can be used to construct expression vectors containing the antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.* (1990, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel *et al.* (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

An expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody can be transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

The host cells used to express the recombinant antibody can be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant immunoglobulin molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking *et al.*, 198, *Gene* 45:101; Cockett *et al.*, 1990, *BioTechnology* 8:2).

A variety of host-expression vector systems can be utilized to express the immunoglobulin antibodies. Such host-expression systems represent vehicles by which the coding sequences of the antibody can be produced and subsequently purified, but also represent cells that can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody immunoglobulin molecule *in situ*. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing immunoglobulin coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing immunoglobulin coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody being

expressed. For example, when a large quantity of such a protein is to be produced, vectors that direct the expression of high levels of fusion protein products that are readily purified might be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO J.* 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX Vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) or the analogous virus from *Drosophila Melanogaster* is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) results in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts. (*e.g.*, see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of

appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner *et al.*, 1987, *Methods in Enzymol.* 153:51-544).

In addition, a host cell strain can be chosen to modulate the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include, but are not limited to, CHO, VERY, BH, Hela, COS, MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express an antibody can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody. Such engineered cell lines can be particularly useful in screening and evaluation of tumor antigens that interact directly or indirectly with the antibody.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell* 22:817) genes can be employed in tk-, hgpri- or apri- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes:

DHFR, which confers resistance to methotrexate (Wigler *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical*
5 *Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hygromycin (Santerre *et al.*, 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which
10 can be used are described in Ausubel *et al.* (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1).

15 The expression levels of an antibody can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system
20 expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse *et al.*, 1983, *Mol. Cell. Biol.* 3:257).

25 The host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers that enable equal expression of heavy and light chain polypeptides. Alternatively, a single
30 vector can be used to encode both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA.

Once the antibody has been recombinantly expressed, it can be purified using any method known in the art for purification of an antibody, for example, by

chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5 In yet another exemplary embodiment, the antibody is a monoclonal antibody.

In any case, the hybrid antibodies have a dual specificity, preferably with one or more binding sites specific for the hapten of choice or one or more binding sites specific for a target antigen, for example, an antigen associated with a tumor, an autoimmune disease, an infectious organism, or other disease state.

10

4.5.2 PRODUCTION OF ANTIBODIES

The production of antibodies will be illustrated with reference to anti-CD30 antibodies but it will be apparent for those skilled in the art that antibodies to other members of the TNF receptor family can be produced and modified in a similar manner.

15 The use of CD30 for the production of antibodies is exemplary only and not intended to be limiting.

The CD30 antigen to be used for production of antibodies may be, *e.g.*, a soluble form of the extracellular domain of CD30 or a portion thereof, containing the desired epitope. Alternatively, cells expressing CD30 at their cell surface (*e.g.*, L540
20 (Hodgkin's lymphoma derived cell line with a T cell phenotype) and L428 (Hodgkin's lymphoma derived cell line with a B cell phenotype)) can be used to generate antibodies. Other forms of CD30 useful for generating antibodies will be apparent to those skilled in the art.

25 In another exemplary embodiment, the ErbB2 antigen to be used for production of antibodies may be, *e.g.*, a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (*e.g.*, NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line such as SK-BR-3 cells, see Stancovski *et al. Proc. Natl. Acad. Sci. USA* 88:8691-8695 (1991)) can be used to generate antibodies. Other forms of ErbB2
30 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4816567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for

immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

5 The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine,
10 aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

 Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine
15 myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J.*
20 *Immunol.*, 133:3001 (1984); and Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

 Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by
25 immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

 After hybridoma cells are identified that produce antibodies of the desired
30 specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this

purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

5 The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

10 DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückerthun, *Immunol. Revs.*, 130:151-188 (1992).

20 In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

30 The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4816567; and Morrison, *et al.* (1984) Proc. Natl Acad. Sci. USA 81:6851), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) **Humanized antibodies**

A humanized antibody may have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature, 332:323-327 (1988); Verhoeven *et al.*, Science 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993)).

In another embodiment, the antibodies may be humanized with retention of high affinity for the antigen and other favorable biological properties. Humanized

antibodies may be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. 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 recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of the humanized antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

The Examples describe production of an exemplary humanized anti-ErbB2 antibody. The humanized antibody may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). In one embodiment, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H. Another Example describes preparation of purified trastuzumab antibody from the HERCEPTIN® formulation.

(iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in

chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993); and U.S. Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, U.S. Patent Nos. 5565332 and 5573905. As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents Nos. 5567610 and 5229275). Human anti-CD30 antibodies are described in U.S. Patent Application Serial No. 10/338,366.

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods*

24:107-117 (1992); and Brennan *et al.*, Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, Bio/Technology 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent No. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) **Bispecific antibodies**

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the CD30 protein. Alternatively, an anti-CD30 arm may be combined with an arm which binds to a Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the CD30-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD30.

Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, EMBO J., 10:3655-3659 (1991). According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2,

and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a

procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab' -TNB derivatives is then reconverted to the Fab' -thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab' -TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab' -SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.* *J. Immunol.* 147: 60 (1991).

(vii) Other amino acid sequence modifications

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibodies are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antibody that are favored locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a

different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on
5 maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally-occurring residues are divided into groups based on common side-chain properties:

- 10 (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

15 Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further development
20 will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous
25 phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or
30 additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is

subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.*, so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.* J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.* Anti-Cancer Drug Design 3:219-230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent No. 5739277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

(viii) Glycosylation Variants

Antibodies in the ADC of the invention may be glycosylated at conserved positions in their constant regions (Jefferis and Lund, (1997) Chem. Immunol. 65:111-128; Wright and Morrison, (1997) TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd *et al.*, (1996) Mol. Immunol. 32:1311-1318; Wittwe and Howard, (1990) Biochem. 29:4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner, (1996) Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based

upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra *et al.*, (1995) *Nature Med.* 1:237-243). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd *et al.*, (1996) *Mol. Immunol.* 32:1311-1318), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al.* (1999) *Mature Biotech.* 17:176-180).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of the antibody.

5 The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. These methods include, but are not limited to, isolation from a natural source (in the case of naturally-occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

10 The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the amino acid sequence or the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, *e.g.*, antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected. See, *e.g.*, Hse *et al.*, (1997) *J. Biol. Chem.*
15 272:9062-9070. In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in
20 oligosaccharide production (U.S. Patent Nos. 5047335; 5510261; 5278299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, *e.g.*, make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

25 The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing
30 oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo- β -galactosidase), elimination using harsh alkaline environment to release mainly O-linked

structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

4.5.2a SCREENING FOR ANTIBODY-DRUG CONJUGATES (ADC)

5 Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADC) that have potential as prophylactic or therapeutic treatments of diseases or disorders involving overexpression of proteins including Lewis Y, CD30, CD40, and CD70. Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADC) that have potential as prophylactic or
10 therapeutic treatments of diseases or disorders involving overexpression of HER2 (US6632979). Screening for a useful ADC may involve administering candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an
15 inducer of the disease, if applicable. Candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format. The rate at which ADC may be screened for utility for prophylactic or therapeutic treatments of diseases or disorders is limited only by the rate of synthesis or screening methodology, including detecting/measuring/analysis of data.

20 One embodiment is a screening method comprising (a) transplanting cells from a stable renal cell cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the formation of tumors from the transplanted cell line.

Another embodiment is a screening method comprising (a) contacting cells
25 from a stable Hodgkin's disease cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of CD40.

Another embodiment is a screening method comprising (a) contacting cells from a stable Hodgkin's disease cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of
30 the ADC candidate to induce apoptosis is evaluated.

One embodiment is a screening method comprising (a) transplanting cells from a stable cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to

inhibit the formation of tumors from the transplanted cell line. The invention also concerns a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of HER2 comprising (a) contacting cells from a stable breast cancer cell line with a drug candidate and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

Another embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of HER2. In one embodiment the ability of the ADC candidate to block heregulin binding is evaluated. In another embodiment the ability of the ADC candidate to block ligand-stimulated tyrosine phosphorylation is evaluated.

Another embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

Another embodiment is a screening method comprising (a) administering an ADC drug candidate to a transgenic non-human mammal that overexpresses in its mammary gland cells a native human HER2 protein or a fragment thereof, wherein such transgenic mammal has stably integrated into its genome a nucleic acid sequence encoding a native human HER2 protein or a fragment thereof having the biological activity of native human HER2, operably linked to transcriptional regulatory sequences directing its expression to the mammary gland, and develops a mammary tumor not responding or poorly responding to anti-HER2 antibody treatment, or to a non-human mammal bearing a tumor transplanted from said transgenic non-human mammal; and (b) evaluating the effect of the ADC candidate on the target disease or disorder. Without limitations, the disease or disorder may be a HER2-overexpressing cancer, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic and bladder cancer. The cancer preferably is breast cancer which expressed HER2 in at least about 500,000 copies per cell, more preferably at least about 2,000,000 copies per cell. ADC drug candidates may, for example, be evaluated for their ability to induce cell death and/or apoptosis, using assay methods well known in the art and described hereinafter.

In one embodiment, candidate ADC are screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. Administration may be oral, or by suitable injection, depending on the chemical nature of the compound being evaluated. In some cases, it may be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for compounds useful in treating various disorders, the test compounds are added to the cell culture medium at an appropriate time, and the cellular response to the compound is evaluated over time using the appropriate biochemical and/or histological assays. In some cases, it may be appropriate to apply the compound of interest to the culture medium in conjunction with co-factors that would enhance the efficacy of the compound.

Thus, provided herein are assays for identifying ADC which specifically target and bind a target protein, the presence of which is correlated with abnormal cellular function, and in the pathogenesis of cellular proliferation and/or differentiation that is causally related to the development of tumors.

To identify an ADC which blocks ligand activation of an ErbB (*e.g.*, ErbB2) receptor, the ability of the compound to block ErbB ligand binding to cells expressing the ErbB (ErbB2) receptor (*e.g.*, in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells isolated from the transgenic animal overexpressing HER2 and transfected to express another ErbB receptor (with which HER2 forms hetero-oligomer) may be incubated, *i.e.* culturing, with the ADC and then exposed to labeled ErbB ligand. The ability of the compound to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of heregulin (HRG) binding to breast tumor cell lines, overexpressing HER2 and established from the transgenic non-human mammals (*e.g.*, mice) herein, by the candidate ADC may be performed using monolayer cultures on ice in a 24-well-plate format. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ¹²⁵I-labeled rHRGβ1₁₇₇₋₂₂₄ (25,000 cpm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC₅₀ value (cytotoxic activity) may be calculated for the compound of interest.

Alternatively, or additionally, the ability of an ADC to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cell lines established from the transgenic animals herein may be incubated with a test ADC and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal antibody (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S. Patent No. 5766863 is also available for determining ErbB receptor activation and blocking of that activity by the compound.

In one embodiment, one may screen for ADC which inhibit HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described below. For example, a cell line established from a HER2-transgenic animal may be plated in 24-well plates and the compound may be added to each well and incubated for 30 minutes at room temperature; then rHRG $\beta_{1177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for about 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 μ l of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μ l) may be electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 μ g/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r -180,000 may be quantified by reflectance densitometry. An alternate method to evaluate inhibition of receptor phosphorylation is the KIRA (kinase receptor activation) assay of Sadick *et al.* (1998) Jour. of Pharm. and Biomed. Anal. Some of the well established monoclonal antibodies against HER2 that are known to inhibit HRG stimulation of p180 tyrosine phosphorylation can be used as positive control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC_{50} for the compound of interest may be calculated.

One may also assess the growth inhibitory effects of a test ADC on cell lines derived from a HER2-transgenic animal, *e.g.*, essentially as described in Schaefer *et al.* (1997) Oncogene 15:1385-1394. According to this assay, the cells may be treated with a test compound at various concentrations for 4 days and stained with crystal violet or the redox dye Alamar Blue. Incubation with the compound may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4 on

MDA-MB-175 cells (Schaefer *et al.*, supra). In a further embodiment, exogenous HRG will not significantly reverse this inhibition.

To identify growth inhibitory compounds that specifically target an antigen of interest, one may screen for compounds which inhibit the growth of cancer cells overexpressing antigen of interest derived from transgenic animals, the assay described in U.S. Patent No. 5677171 can be performed. According to this assay, cancer cells overexpressing the antigen of interest are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35mm dish) and the test compound is added at various concentrations. After six days, the number of cells, compared to untreated cells is counted using an electronic COULTER™ cell counter. Those compounds which inhibit cell growth by about 20-100% or about 50-100% may be selected as growth inhibitory compounds.

To select for compounds which induce cell death, loss of membrane integrity as indicated by, *e.g.*, PI, trypan blue or 7AAD uptake may be assessed relative to control. The PI uptake assay uses cells isolated from the tumor tissue of interest of a transgenic animal. According to this assay, the cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. The cells are seeded at a density of 3×10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing various concentrations of the compound. The cells are incubated for a 3-day time period. Following each treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM CaCl_2) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing compounds.

In order to select for compounds which induce apoptosis, an annexin binding assay using cells established from the tumor tissue of interest of the transgenic

animal is performed. The cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 µg/ml of the antibody drug conjugate (ADC). Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca²⁺ binding buffer and aliquoted into tubes as discussed above for the cell death assay. Tubes then receive labeled annexin (e.g., annexin V-FITC) (1 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing compounds.

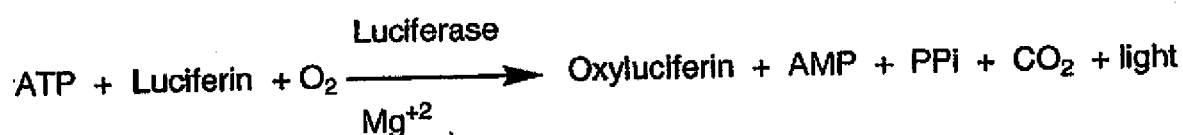
4.5.3 IN VITRO CELL PROLIFERATION ASSAYS

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

The *in vitro* potency of antibody drug conjugates was measured by a cell proliferation assay (Example 18, Figures 7-10). The CellTiter-Glo® Luminescent Cell Viability Assay is a commercially available (Promega Corp., Madison, WI), homogeneous assay method based on the recombinant expression of *Coleoptera* luciferase (U.S. Patent Nos. 5583024; 5674713 and 5700670). This cell proliferation assay determines the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells (Crouch *et al.* (1993) *J. Immunol. Meth.* 160:81-88, U.S. Patent No. 6602677). The CellTiter-Glo® Assay was conducted in 96 well format, making it amenable to automated high-throughput screening (HTS) (Cree *et al.* (1995) *AntiCancer Drugs* 6:398-404). The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent and mixing. The cells may be treated continuously with

ADC, or they may be treated and separated from ADC. Generally, cells treated briefly, *i.e.* 3 hours, showed the same potency effects as continuously treated cells.

The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiter-Glo[®] Assay generates a "glow-type" luminescent signal, produced by the luciferase reaction, which has a half-life generally greater than five hours, depending on cell type and medium used. Viable cells are reflected in relative luminescence units (RLU). The substrate, Beetle Luciferin, is oxidatively decarboxylated by recombinant firefly luciferase with concomitant conversion of ATP to AMP and generation of photons. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch mode processing of multiple plates. This cell proliferation assay can be used with various multiwell formats, *e.g.*, 96 or 384 well format. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is presented as relative light units (RLU), measured over time.



The anti-proliferative effects of antibody drug conjugates were measured by the cell proliferation, *in vitro* cell killing assay above against four different breast tumor cell lines (Figures 7-10). IC₅₀ values were established for SK-BR-3 and BT-474 which are known to over express HER2 receptor protein. Table 2a shows the potency (IC₅₀) measurements of exemplary antibody drug conjugates in the cell proliferation assay against SK-BR-3 cells. Table 2b shows the potency (IC₅₀) measurements of exemplary antibody drug conjugates in the cell proliferation assay against BT-474 cells.

Antibody drug conjugates: Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab; Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab; Trastuzumab-MC-MMAF, 4.1 MMAF/Ab; Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab; and Trastuzumab-MC-vc-PAB-MMAF, 3.7 MMAF/Ab did not inhibit the proliferation of MCF-7 cells (Figure 9).

Antibody drug conjugates: Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAF, 3.7 MMAF/Ab; Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab; Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab; and Trastuzumab-MC-MMAF, 4.1 MMAF/Ab did not inhibit the proliferation of MDA-MB-468 cells (Figure 10).

MCF-7 and MDA-MB-468 cells do not overexpress HER2 receptor protein. The anti-HER2 antibody drug conjugates of the invention therefore show selectivity for inhibition of cells which express HER2.

Table 2a SK-BR-3 cells

Antibody Drug Conjugate H = trastuzumab linked via a cysteine [cys] except where noted	IC ₅₀ (µg ADC/ml)
H-MC-MMAF, 4.1 MMAF/Ab	0.008
H-MC-MMAF, 4.8 MMAF/Ab	0.002
H-MC-vc-PAB-MMAE,	0.007
H-MC-vc-PAB-MMAE	0.015
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.0035 - 0.01
H-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.006 - 0.007
H-MC-vc-PAB-MMAF, 4.8 MMAF/Ab	0.006
H-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab	0.0035
H-MC-MMAF, 4.1 MMAF/Ab	0.0035
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.010
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.007
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.015
H-MC-vc-PAB-MMAF, 3.7 MMAF/Ab.	0.010
H-MC-vc-PAB-MMAE, 7.5 MMAE/Ab	0.0025
H-MC-MMAE, 8.8 MMAE/Ab	0.018
H-MC- MMAE, 4.6 MMAE/Ab	0.05
H-MC-(L)val-(L)cit-PAB-MMAE, 8.7 MMAE/Ab	0.0003
H-MC-(D)val-(D)cit-PAB-MMAE, 8.2 MMAE/Ab	0.02
H-MC-(D)val-(L)cit-PAB-MMAE, 8.4 MMAE/Ab	0.0015
H-MC-(D)val-(L)cit-PAB-MMAE, 3.2 MMAE/Ab	0.003
H-Trastuzumab	0.083
H-vc-MMAE, linked via a lysine [lys]	0.002
H-phe-lys-MMAE, linked via a lysine [lys]	0.0015
4D5-Fc8-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.004
Hg-MC-vc-PAB-MMAF, 4.1 MMAF/Ab	0.01
7C2-MC-vc-PAB-MMAF, 4.0 MMAF/Ab	0.01

4D5 Fab-MC-vc-PAB-MMAF, 1.5 MMAF/Ab	0.02
Anti-TF Fab-MC-vc-PAB-MMAE*	-

Table 2b BT474 cells

Antibody Drug Conjugate H = trastuzumab linked via a cysteine [cys]	IC ₅₀ (µg ADC/ml)
H-MC-MMAF, 4.1 MMAF/Ab	0.008
H-MC-MMAF, 4.8 MMAF/Ab	0.002
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.015
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.02 - 0.05
H-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.01
H-MC-vc-PAB-MMAF, 4.8 MMAF/Ab	0.01
H-MC-vc-PAB-MMAE, 3.3 MMAE/Ab	0.02
H-MC-vc-PAB-MMAF, 3.7 MMAF/Ab.	0.02
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.015
H-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab	0.010
H-MC-MMAF, 4.1 MMAF/Ab	0.00015
H-MC-vc-PAB-MMAE, 7.5 MMAE/Ab	0.0025
H-MC-MMAE, 8.8 MMAE/Ab	0.04
H-MC-MMAE, 4.6 MMAE/Ab	0.07
4D5-Fc8-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.008
Hg-MC-vc-PAB-MMAF, 4.1 MMAF/Ab	0.01
7C2-MC-vc-PAB-MMAF, 4.0 MMAF/Ab	0.015
4D5 Fab-MC-vc-PAB-MMAF, 1.5 MMAF/Ab	0.04
Anti-TF Fab-MC-vc-PAB-MMAE*	-

H = trastuzumab

5 7C2 = anti-HER2 murine antibody which binds a different epitope than
trastuzumab.

Fc8 = mutant that does not bind to FcRn

Hg = "Hingeless" full-length humanized 4D5, with heavy chain hinge
cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated.)

Anti-TF Fab = anti-tissue factor antibody fragment

10 * activity against MDA-MB-468 cells

In a surprising and unexpected discovery, the *in vitro* cell proliferation activity results of the ADC in Tables 2a and 2b show generally that ADC with a low average number of drug moieties per antibody showed efficacy, *e.g.*, $IC_{50} < 0.1 \mu g$ ADC/ml. The results suggest that at least for trastuzumab ADC, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5.

4.5.4 *IN VIVO* PLASMA CLEARANCE AND STABILITY

Pharmacokinetic plasma clearance and stability of ADC were investigated in rats and cynomolgus monkeys. Plasma concentration was measured over time. Table 2c shows pharmacokinetic data of antibody drug conjugates and other dosed samples in rats. Rats are a non-specific model for ErbB receptor antibodies, since the rat is not known to express HER2 receptor proteins.

Table 2c Pharmacokinetics in Rats
H = trastuzumab linked via a cysteine [cys] except where noted
2 mg/kg dose except where noted

Sample dose mg/kg	AUCinf day* $\mu g/mL$	CL mL/day/kg	Cmax $\mu g/mL$	T $\frac{1}{2}$ Term. days	% Conj.
H-MC-vc-PAB-MMAE (Total Ab)	78.6	26.3	39.5	5.80	40.6
H-MC-vc-PAB-MMAE (Conj.)	31.1	64.4	33.2	3.00	
H-MC-vc-PAB-MMAF (Total Ab)	170	12.0	47.9	8.4	50.0
H-MC-vc-PAB-MMAF (Conj.)	83.9	24.0	44.7	4.01	
H-MC-MMAE (Total Ab)	279	18.9	79.6	7.65	33
H-MC-MMAE (Conj.) 5 mg/kg	90.6	62.9	62.9	4.46	
H-MC-MMAF (Total Ab)	299	6.74	49.1	11.6	37
H-MC-MMAF (Conj.)	110	18.26	50.2	4.54	
H-MC-vc-MMAF, wo/PAB, (Total Ab)	306	6.6	78.7	11.9	19.6
H-MC-vc-MMAF, wo/PAB, (Conj.)	59.9	33.4	82.8	2.1	
H-Me-vc-PAB-MMAF (Total Ab)	186	10.8	46.9	8.3	45.3

H-Me-vc-PAB-MMAF (Conj.)	84.0	23.8	49.6	4.3	
H-Me-vc-PAB-MMAE (Total Ab)	135	15.0	44.9	11.2	23.8
H-Me-vc-PAB-MMAE (Conj.)	31.9	63.8	45.2	3.0	
H-MC-vc-MMAF, wo/PAB, (Total Ab)	306	6.6	78.7	11.9	19.6
H-MC-vc-MMAF, wo/PAB, (Conj.)	59.9	33.4	82.8	2.1	
H-MC-(D)val-(L)cit-PAB-MMAE (Total Ab)	107	19.2	30.6	9.6	38.1
H-MC-(D)val-(L)cit-PAB-MMAE (Conj.)	40	50.4	33.7	3.98	
H-MC-(Me)-vc-PAB-MMAE, Total Ab	135.1	15.0	44.9	11.2	23.8
H-MC-(Me)-vc-PAB-MMAE, Conj.	31.9	63.8	45.2	2.96	
H-MC-(D)val-(D)cit-PAB-MMAE, Total Ab	88.2	22.8	33.8	10.5	38.3
H-MC-(D)val-(D)cit-PAB-MMAE, Conj.	33.6	59.8	36.0	4.43	
H-MC-vc-PAB-MMAE, Total Ab	78.6	26.3	39.5	5.8	40.6
H-MC-vc-PAB-MMAE, Conj. H linked to MC by lysine [lys]	31.1	64.4	33.2	3.00	
MMAF 200 µg/kg	0.99	204	280	0.224	-
MMAE 206 µg/kg	3.71	62.6	649	0.743	-
HER F(ab') ₂ -MC-vc-MMAE, Total Ab	9.3	217	34.4	0.35	95
HER F(ab') ₂ -MC-vc-MMAE, Conj.	8.8	227	36.9	0.29	
4D5-H-Fab-MC-vc-MMAF, Total Ab	43.8	46.2	38.5	1.49	68
4D5-H-Fab-MC-vc-MMAF, Conj.	29.9	68.1	34.1	1.12	
4D5-H-Fab-MC-vc-MMAE,	71.5	70.3	108	1.18	59

Total Ab 4D5-H-Fab-MC-vc-MMAE, Conj.	42.2	118.9	114	0.74	
4D5-H-Fab	93.4	53.9	133	1.08	-
H-MC-vc-PAB-MMAF, Total Ab	170	12.03	47.9	8.44	49.5
H-MC-vc-PAB-MMAF, Conj.	83.9	23.96	44.7	4.01	
H-MC-vc-PAB-MMAF- DMAEA, Total Ab	211	9.8	39.8	8.53	34.3
H-MC-vc-PAB-MMAF- DMAEA, Conj.	71.5	28.2	38.8	3.64	
H-MC-vc-PAB-MMAF-TEG, Total Ab	209	9.75	53.2	8.32	29.7
H-MC-vc-PAB-MMAF-TEG, Conj.	63.4	31.8	34.9	4.36	

AUC inf is the area under the plasma concentration-time curve from time of dosing to infinity and is a measure of the total exposure to the measured entity (drug, ADC). CL is defined as the volume of plasma cleared of the measured entity in unit time and is expressed by normalizing to body weight. T1/2 term is the half-life of the drug in the body measured during its elimination phase. The % Conj. term is the relative amount of ADC compared to total antibody detected, by separate ELISA immunoaffinity tests ("Analytical Methods for Biotechnology Products", Ferraiolo et al, p85-98 in Pharmacokinetics of Drugs (1994) P.G. Welling and L.P. Balant, Eds., Handbook of Experimental Pharmacology, Vol. 110, Springer-Verlag. The % Conj. calculation is simply AUCinf of ADC + AUCinf total Ab, and is a general indicator of linker stability, although other factors and mechanisms may be in effect.

Figure 11 shows a graph of a plasma concentration clearance study after administration of the antibody drug conjugates: H-MC-vc-PAB-MMAF-TEG and H-MC-vc-PAB-MMAF to Sprague-Dawley rats. Concentrations of total antibody and ADC were measured over time.

Figure 12 shows a graph of a two stage plasma concentration clearance study where ADC was administered at different dosages and concentrations of total antibody and ADC were measured over time.

IN VIVO EFFICACY

The *in vivo* efficacy of the ADC of the invention was measured by a high expressing HER2 transgenic explant mouse model. An allograft was propagated from the Fo5 mmtv transgenic mouse which does not respond to, or responds poorly to,

- 5 HERCEPTIN® therapy. Subjects were treated once with ADC and monitored over 3-6 weeks to measure the time to tumor doubling, log cell kill, and tumor shrinkage. Follow up dose-response and multi-dose experiments were conducted.

- Tumors arise readily in transgenic mice that express a mutationally activated form of *neu*, the rat homolog of HER2, but the HER2 that is overexpressed in
10 breast cancers is not mutated and tumor formation is much less robust in transgenic mice that overexpress nonmutated HER2 (Webster *et al.* (1994) *Semin. Cancer Biol.* 5:69-76).

- To improve tumor formation with nonmutated HER2, transgenic mice were produced using a HER2 cDNA plasmid in which an upstream ATG was deleted in order to prevent initiation of translation at such upstream ATG codons, which would
15 otherwise reduce the frequency of translation initiation from the downstream authentic initiation codon of HER2 (for example, see Child *et al.* (1999) *J. Biol. Chem.* 274: 24335-24341). Additionally, a chimeric intron was added to the 5' end, which should also enhance the level of expression as reported earlier (Neuberger and Williams (1988) *Nucleic Acids Res.* 16: 6713; Buchman and Berg (1988) *Mol. Cell. Biol.* 8:4395; Brinster
20 *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:836). The chimeric intron was derived from a Promega vector, pCI-neo mammalian expression vector (bp 890-1022). The cDNA 3'-end is flanked by human growth hormone exons 4 and 5, and polyadenylation sequences. Moreover, FVB mice were used because this strain is more susceptible to tumor development. The promoter from MMTV-LTR was used to ensure tissue-specific HER2
25 expression in the mammary gland. Animals were fed the AIN 76A diet in order to increase susceptibility to tumor formation (Rao *et al.* (1997) *Breast Cancer Res. and Treatment* 45:149-158).

Table 2d Tumor measurements in allograft mouse model - MMTV-HER2 Fo5
Mammary Tumor, athymic nude mice
single dose at day 1 (T = 0) except where noted
H = trastuzumab linked via a cysteine [cys] except where noted

5

Sample Drugs per antibody	Dose	Ti	PR	CR	Tumor doubling time (days)	Mean log cell kill
Vehicle					2-5	0
H-MC-vc-PAB-MMAE 8.7 MMAE/Ab	1250 $\mu\text{g}/\text{m}^2$	5/5	4/7	0/7	18	1.5
H-MC-vc-PAB-MMAF 3.8 MMAF/Ab	555 $\mu\text{g}/\text{m}^2$	2/5	2/7	5/7	69	6.6
H-MC(Me)-vc-PAB- MMAF					>50	6.4
H-MC-MMAF 4.8 MMAF/Ab	9.2 mg/kg Ab 550 $\mu\text{g}/\text{m}^2$ at 0, 7, 14 and 21 days	7/7	6/7	0/7	63	9
H-MC-MMAF 4.8 MMAF/Ab	14 mg/kg Ab 840 $\mu\text{g}/\text{m}^2$ at 0, 7, 14 and 21 days	5/5	5/7	2/7	>63	
H-MC-vc-PAB-MMAF 5.9 MMAF/Ab	3.5 mg/kg Ab 300 $\mu\text{g}/\text{m}^2$ at 0, 21, and 42 days	5/6	1/7	3/7	>36	
H-MC-vc-PAB-MMAF 5.9 MMAF/Ab	4.9 mg/kg Ab 425 $\mu\text{g}/\text{m}^2$ at 0, 21, and 42 days	4/7	2/7	5/7	>90	
H-MC-vc-PAB-MMAF 5.9 MMAF/Ab	6.4 mg/kg Ab 550 $\mu\text{g}/\text{m}^2$ at 0, 21, and 42 days	3/6	1/7	6/7	>90	
H-(L)val-(L)cit-MMAE 8.7 MMAE/Ab	10 mg/kg	7/7	1/7	0/7	15.2	1.1
H-MC-MMAE 4.6 MMAE/Ab	10 mg/kg	7/7	0/7	0/7	4	0.1
H-(D)val-(D)cit- MMAE 4.2 MMAE/Ab	10 mg/kg	7/7	0/7	0/7	3	

H-(D)val-(L)cit-MMAE 3.2 MMAE/Ab	13 mg/kg	7/7	0/7	0/7	9	0.6
H-MC(Me)-vc-MMAE 3.0 MMAE/Ab	13 mg/kg	7/7	3/7	0/7	17	1.2
H-(L)val-(D)cit-MMAE 3.5 MMAE/Ab	12 mg/kg	7/7	0/7	0/7	5	0.2
H-vc-MMAE 8.7 MMAE/Ab	10 mg/kg	7/7			17	
H-cys-vc-MMAF 3.8 MMAF/Ab	1 mg/kg	7/7			3	
H-cys-vc-MMAF 3.8 MMAF/Ab	3 mg/kg	7/7			>17	
H-cys-vc-MMAF 3.8 MMAF/Ab	10 mg/kg	4/7	4/7	3/7	>17	
H-MC-vc-MMAF-TEG 4 MMAF/Ab	10 mg/kg	3/6	1/7	6/7	81	7.8
H-MC-vc-MMAF-TEG 4 MMAF/Ab	10 mg/kg q3wk x 3	0/5	0/7	7/7	81	7.9
H-vc-MMAF (lot 1)	10 mg/kg	4/6	2/8	5/8		
H-vc-MMAF (lot 2)	10 mg/kg	7/8	1/8	1/8		
H-MC-MMAF 550 µg/m ²	10 mg/kg	8/8	1/8	0/8	18	
H-(Me)-vc-MMAF	10 mg/kg	3/7	2/8	5/8		
H-vc-MMAE 7.5 MMAE/Ab	3.7 mg/kg at 0, 7, 14, 21, 28 days	6/6	0/7	1/7	17	2.3
H-vc-MMAE 7.5 MMAE/Ab	7.5 mg/kg at 0, 7, 14, 21, 28 days	5/7	3/7	3/7	69	10
anti IL8-vc-MMAE 7.5 MMAE/Ab	7.5 mg/kg at 0, 7, 14, 21, 28 days	7/7	0/7	0/7	5	0.5
anti IL8-vc-MMAE 7.5 MMAE/Ab	3.7 mg/kg at 0, 7, 14, 21, 28 days	6/6	0/7	0/7	3	0.2
H-fk-MMAE 7.5 MMAE/Ab	7.5 mg/kg at 0, 7, 14, 21, 28 days	7/7	1/7	0/7	31	4.4
H-fk-MMAE 7.5 MMAE/Ab	3.7 mg/kg at 0, 7, 14, 21, 28 days	7/7	0/7	0/7	8.3	0.9
anti IL8-fk-MMAE 7.5 MMAE/Ab	7.5 mg/kg at 0, 7, 14, 21, 28 days	7/7	0/7	0/7	6	0.5
anti IL8-fk-MMAE 7.5 MMAE/Ab	3.7 mg/kg at 0, 7, 14, 21, 28 days	7/7	0/7	0/7	3	0.1
Trastuzumab	7.5 mg/kg at	7/7	0/7	0/7	5	0.4

	0, 7, 14, 21, 28 days					
H-vc-MMAE 8.7 MMAE/Ab	10 mg/kg 1250 $\mu\text{g}/\text{m}^2$	6/6	3/6	0/6	15	1.3
H-vc-MMAE	10 mg/kg 1250 $\mu\text{g}/\text{m}^2$ at 0, 7, and 14 days	7/7	5/7		>19	
H-vc-MMAE	3 mg/kg at 0, 7, and 14 days	7/7			8	
H-vc-MMAE	1 mg/kg at 0, 7, and 14 days	7/7			7	
H-vc-MMAF	10 mg/kg	8/8	5/8		>21	
H-vc-MMAF	10 mg/kg at 0, 7, and 14 days	4/7	4/7	3/7	>21	
H-vc-MMAF	3 mg/kg at 0, 7, and 14 days	7/7			6	
H-vc-MMAF	1 mg/kg at 0, 7, and 14 days	8/8			4	
Trastuzumab	10 mg/kg at 0 and 7 days	8/8			3	
Hg-MC-vc-PAB- MMAF 4.1 MMAF/Ab	10 mg/kg at 0 days	6/7	3/8	5/8	56	5.1
Fc8-MC-vc-PAB- MMAF 4.4 MMAF/Ab	10 mg/kg at 0 days	7/7	6/8	0/8	25	2.1
7C2-MC-vc-PAB- MMAF 4 MMAF/Ab	10 mg/kg at 0 days	5/6	6/8	1/8	41	3.7
H-MC-vc-PAB-MMAF 5.9 MMAF/Ab	10 mg/kg at 0 days	3/8	3/8	5/8	62	5.7
2H9-MC-vc-PAB- MMAE		9/9			>14 days	
2H9-MC-vc-PAB- MMAF		9/9			>14 days	
11D10-vc-PAB-MMAE		9/9			>14 days	
11D10-vc-PAB-MMAF		9/9			11 days	

7C2 = anti-HER2 murine antibody which binds a different epitope than trastuzumab.

Fc8 = mutant that does not bind to FcRn

Hg = "Hingeless" full-length humanized 4D5, with heavy chain hinge cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated.)

2H9 = Anti-EphB2R

11D10 = Anti-0772P

5

The term T_i is the number of animals in the study group with tumor at $T = 0$ + total animals in group. The term PR is the number of animals attaining partial remission of tumor + animals with tumor at $T = 0$ in group. The term CR is the number of animals attaining complete remission of tumor + animals with tumor at $T = 0$ in group.

10 The term Log cell kill is the time in days for the tumor volume to double - the time in days for the control tumor volume to double divided by 3.32 X time for tumor volume to double in control animals (dosed with Vehicle). The log-cell-kill calculation takes into account tumor growth delay resulting from treatment and tumor volume doubling time of the control group. Anti-tumor activity of ADC is classified with log-cell-kill values of:

15	++++	≥ 3.4	(highly active)
	+++	$= 2.5-3.4$	
	++	$= 1.7-2.4$	
	+	$= 1.0-1.6$	
	inactive	$= 0$	

20

Figure 13 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with: Vehicle, Trastuzumab-MC-vc-PAB-MMAE ($1250 \mu\text{g}/\text{m}^2$) and Trastuzumab-MC-vc-PAB-MMAF ($555 \mu\text{g}/\text{m}^2$). (H = Trastuzumab). The growth of tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth. Figure 14 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with 10 mg/kg ($660 \mu\text{g}/\text{m}^2$) of Trastuzumab-MC-MMAE and $1250 \mu\text{g}/\text{m}^2$ Trastuzumab-MC-vc-PAB-MMAE. Figure 15 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed with $650 \mu\text{g}/\text{m}^2$ Trastuzumab-MC-MMAF. Table 2d and Figures 13-15 show that the ADC have strong anti-tumor activity in the allograft of a HER2 positive tumor (Fo5) that originally arose in an MMTV-HER2 transgenic mouse. The antibody alone (*e.g.*, Trastuzumab) does not have significant anti-tumor activity in this model (Erickson *et al.* U.S. Patent No. 6632979). As illustrated in Figures 13-15, the

30

growth of the tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth.

In a surprising and unexpected discovery, the *in vivo* anti-tumor activity results of the ADC in Table 2d show generally that ADC with a low average number of drug moieties per antibody showed efficacy, *e.g.*, tumor doubling time > 15 days and mean log cell kill > 1.0. Figure 16 shows that for the antibody drug conjugate, trastuzumab-MC-vc-PAB-MMAF, the mean tumor volume diminished and did not progress where the MMAF:trastuzumab ratio was 2 and 4, whereas tumor progressed at a ratio of 5.9 and 6, but at a rate lower than Vehicle (buffer). The rate of tumor progression in this mouse xenograft model was about the same, *i.e.* 3 days, for Vehicle and trastuzumab. The results suggest that at least for trastuzumab ADC, the optimal ratio of drug moieties per antibody may be less than about 8, and may be about 2 to about 4.

4.5.5 RODENT TOXICITY

Antibody drug conjugates and an ADC-minus control, "Vehicle", were evaluated in an acute toxicity rat model. Toxicity of ADC was investigated by treatment of male and female Sprague-Dawley rats with the ADC and subsequent inspection and analysis of the effects on various organs. Gross observations included changes in body weights and signs of lesions and bleeding. Clinical pathology parameters (serum chemistry and hematology), histopathology, and necropsy were conducted on dosed animals.

It is considered that weight loss, or weight change relative to animals dosed only with Vehicle, in animals after dosing with ADC is a gross and general indicator of systemic or localized toxicity. Figures 17-19 show the effects of various ADC and control (Vehicle) after dosing on rat body weight.

Hepatotoxicity was measured by elevated liver enzymes, increased numbers of mitotic and apoptotic figures and hepatocyte necrosis. Hematolymphoid toxicity was observed by depletion of leukocytes, primarily granulocytes (neutrophils), and/or platelets, and lymphoid organ involvement, *i.e.* atrophy or apoptotic activity. Toxicity was also noted by gastrointestinal tract lesions such as increased numbers of mitotic and apoptotic figures and degenerative enterocolitis.

Enzymes indicative of liver injury that were studied include:

AST (aspartate aminotransferase)

-Localization: cytoplasmic; liver, heart, skeletal muscle, kidney

-Liver:Plasma ratio of 7000:1

-T1/2: 17 hrs

ALT (alanine aminotransferase)

5 -Localization: cytoplasmic; liver, kidney, heart, skeletal muscle

-Liver:Plasma ratio of 3000:1

-T1/2: 42 hrs; diurnal variation

GGT (g-glutamyl transferase)

-Localization: plasma membrane of cells with high secretory or

10 absorptive capacity; liver, kidney, intestine

-Poor predictor of liver injury; commonly elevated in bile duct disorders

The toxicity profiles of trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF were studied in female Sprague-Dawley rats (Example 19). The humanized
15 trastuzumab antibody does not bind appreciably to rat tissue, and any toxicity would be considered non-specific. Variants at dose levels of 840 and 2105 $\mu\text{g}/\text{m}^2$ MMAF were compared to trastuzumab-MC-val-cit-PAB-MMAF at 2105 $\mu\text{g}/\text{m}^2$.

Animals in groups 1, 2, 3, 4, 6, and 7 (Vehicle, 9.94 & 24.90 mg/kg trastuzumab-MC-val-cit-MMAF, 10.69 mg/kg trastuzumab-MC(Me)-val-cit-PAB-MMAF, and 10.17 & 25.50 mg/kg trastuzumab-MC-MMAF, respectively) gained weight
20 during the study. Animals in groups 5 and 8 (26.78 mg/kg trastuzumab-MC(Me)-val-cit-PAB-MMAF and 21.85 mg/kg trastuzumab-MC-val-cit-PAB-MMAF, respectively) lost weight during the study. On Study Day 5, the change in body weights of animals in groups 2, 6 and 7 were not significantly different from group 1 animals. The change in
25 body weights of animals in groups 3, 4, 5 and 8 were statistically different from group 1 animals (Example 19).

Rats treated with trastuzumab-MC-MMAF (groups 6 and 7) were indistinguishable from vehicle-treated control animals at both dose levels; *i.e.* this conjugate showed a superior safety profile in this model. Rats treated with trastuzumab-MC-val-cit-MMAF (without the self-immolative PAB moiety; groups 2 and 3) showed
30 dose-dependent changes typical for MMAF conjugates; the extent of the changes was less compared with a full length MC-val-cit-PAB-MMAF conjugate (group 8). The platelet counts on day 5 were at approximately 30% of baseline values in animals of group 3

(high dose trastuzumab-MC-val-cit-MMAF) compared with 15% in animals of group 8 (high dose trastuzumab-MC-val-cit-PAB-MMAF). Elevation of liver enzymes AST and ALT, of bilirubin and the extent of thrombocytopenia was most evident in animals treated with trastuzumab-MC(Me)-val-cit-PAB-MMAF (groups 4 and 5) in a dose-dependent fashion; animals of group 5 (high dose group) showed on day 5 levels of ALT of approximately 10x the baseline value and platelets were reduced by approximately 90% at the time of necropsy.

Female Sprague Dawley Rats were also dosed at high levels (Example 19, High Dose study: Groups 2, 3, 4) with trastuzumab-MC-MMAF, and Vehicle control (Group 1). Mild toxicity signals were observed, including a dose-dependent elevation of liver enzymes ALT, AST and GGT. On day 5 animals in the highest dose group showed a 2-fold elevation of ALT and a 5-fold elevation of AST; GGT is also elevated (6U/L). Enzyme levels show a trend towards normalization on day 12. There was a mild granulocytosis in all three dose groups on day 5, the platelet count remained essentially unchanged in all animals. Morphological changes were mild; animals treated at the 4210 $\mu\text{g}/\text{m}^2$ dose level (Group 2) showed unremarkable histology of liver, spleen, thymus, intestines and bone marrow. Mildly increased apoptotic and mitotic activity was observed in thymus and liver, respectively in animals treated at the 5500 $\mu\text{g}/\text{m}^2$ dose level (Group 3). The bone marrow was normocellular, but showed evidence of granulocytic hyperplasia, which is consistent with the absolute granulocytosis observed in the peripheral blood counts in these animals. Animals at the highest dose in group 4 showed qualitatively the same features; the mitotic activity in the liver appears somewhat increased compared to animals in Group 3. Also, extramedullary hematopoiesis was seen in spleen and liver.

EphB2R is a type 1 TM tyrosine kinase receptor with close homology between mouse and human, and is over-expressed in colorectal cancer cells. 2H9 is an antibody against EphB2R. The naked antibody has no effect on tumor growth, but 2H9-val-cit-MMAE killed EphB2R expressing cells and showed efficacy in a mouse xenograft model using CXF1103 human colon tumors (Mao et al (2004) Cancer Res. 64:781-788). 2H9 and 7C2 are both mouse IgG1 anti-HER2 antibodies. The toxicity profiles of 2H9-MC-val-cit-PAB-MMAF (3.7 MMAF/Ab), 7C2-MC-val-cit-PAB-MMAF (4 MMAF/Ab), and trastuzumab-MC-val-cit-PAB-MMAF (5.9 MMAF/Ab) were compared. The differences in the structure of each immunoconjugate or the drug portion of the

immunoconjugate may affect the pharmacokinetics and ultimately the safety profile. The humanized trastuzumab antibody does not bind appreciably to rat tissue, and any toxicity would be considered non-specific.

CYNOMOLGUS MONKEY TOXICITY/SAFETY

5 Similar to the rat toxicity/safety study, cynomolgus monkeys were treated with ADC followed by liver enzyme measurements, and inspection and analysis of the effects on various organs. Gross observations included changes in body weights and signs of lesions and bleeding. Clinical pathology parameters (serum chemistry and hematology), histopathology, and necropsy were conducted on dosed animals (Example
10 19).

 The antibody drug conjugate, H-MC-vc-PAB-MMAE (H = trastuzumab linked through cysteine) showed no evidence of liver toxicity at any of the dose levels tested. Peripheral blood granulocytes showed depletion after a single dose of 1100mg/m² with complete recovery 14 days post-dose. The antibody drug conjugate H-MC-vc-PAB-
15 MMAF showed elevation of liver enzymes at 550 (transient) and 880 mg/m² dose level, no evidence of granulocytopenia, and a dose-dependent, transient (groups 2 & 3) decline of platelets.

4.6 SYNTHESIS OF THE COMPOUNDS OF THE INVENTION

 The Exemplary Compounds and Exemplary Conjugates can be made using
20 the synthetic procedures outlined below in Schemes 5-16. As described in more detail below, the Exemplary Compounds or Exemplary Conjugates can be conveniently prepared using a Linker having a reactive site for binding to the Drug and Ligand. In one aspect, a Linker has a reactive site which has an electrophilic group that is reactive to a nucleophilic group present on a Ligand, such as but not limited to an antibody. Useful
25 nucleophilic groups on an antibody include but are not limited to, sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of an antibody is reactive to an electrophilic group on a Linker and forms a covalent bond to a Linker unit. Useful electrophilic groups include, but are not limited to, maleimide and haloacetamide groups. The electrophilic group provides a convenient site for antibody attachment.

30 In another embodiment, a Linker has a reactive site which has a nucleophilic group that is reactive to an electrophilic group present on an antibody.

Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a Linker include, but are not limited to, hydrazide, oxime,
5 amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a Linker.

Carboxylic acid functional groups and chloroformate functional groups are also useful reactive sites for a Linker because they can react with secondary amino groups of a Drug to form an amide linkage. Also useful as a reactive site is a carbonate
10 functional group on a Linker, such as but not limited to p-nitrophenyl carbonate, which can react with an amino group of a Drug, such as but not limited to N-methyl valine, to form a carbamate linkage. Typically, peptide-based Drugs can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (*see*
15 E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry.

The synthesis of an illustrative Stretcher having an electrophilic maleimide group is illustrated below in Schemes 8-9. General synthetic methods useful for the synthesis of a Linker are described in Scheme 10. Scheme 11 shows the construction of a
20 Linker unit having a val-cit group, an electrophilic maleimide group and a PAB self-immolative Spacer group. Scheme 12 depicts the synthesis of a Linker having a phe-lys group, an electrophilic maleimide group, with and without the PAB self-immolative Spacer group. Scheme 13 presents a general outline for the synthesis of a Drug-Linker Compound, while Scheme 14 presents an alternate route for preparing a Drug-Linker
25 Compound. Scheme 15 depicts the synthesis of a branched linker containing a BHMS group. Scheme 16 outlines the attachment of an antibody to a Drug-Linker Compound to form a Drug-Linker-Antibody Conjugate, and Scheme 14 illustrates the synthesis of Drug-Linker-Antibody Conjugates having, for example but not limited to, 2 or 4 drugs per Antibody.

30 As described in more detail below, the Exemplary Conjugates are conveniently prepared using a Linker having two or more Reactive Sites for binding to the Drug and a Ligand. In one aspect, a Linker has a Reactive site which has an electrophilic group that is reactive to a nucleophilic group present on a Ligand, such as an

antibody. Useful nucleophilic groups on an antibody include but are not limited to, sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of an antibody is reactive to an electrophilic group on a Linker and forms a covalent bond to a Linker unit. Useful electrophilic groups include, but are not limited to, maleimide and
5 haloacetamide groups. The electrophilic group provides a convenient site for antibody attachment.

In another embodiment, a Linker has a Reactive site which has a nucleophilic group that is reactive to an electrophilic group present on a Ligand, such as an antibody. Useful electrophilic groups on an antibody include, but are not limited to,
10 aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a Linker include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for
15 attachment to a Linker.

4.6.1 DRUG MOIETY SYNTHESIS

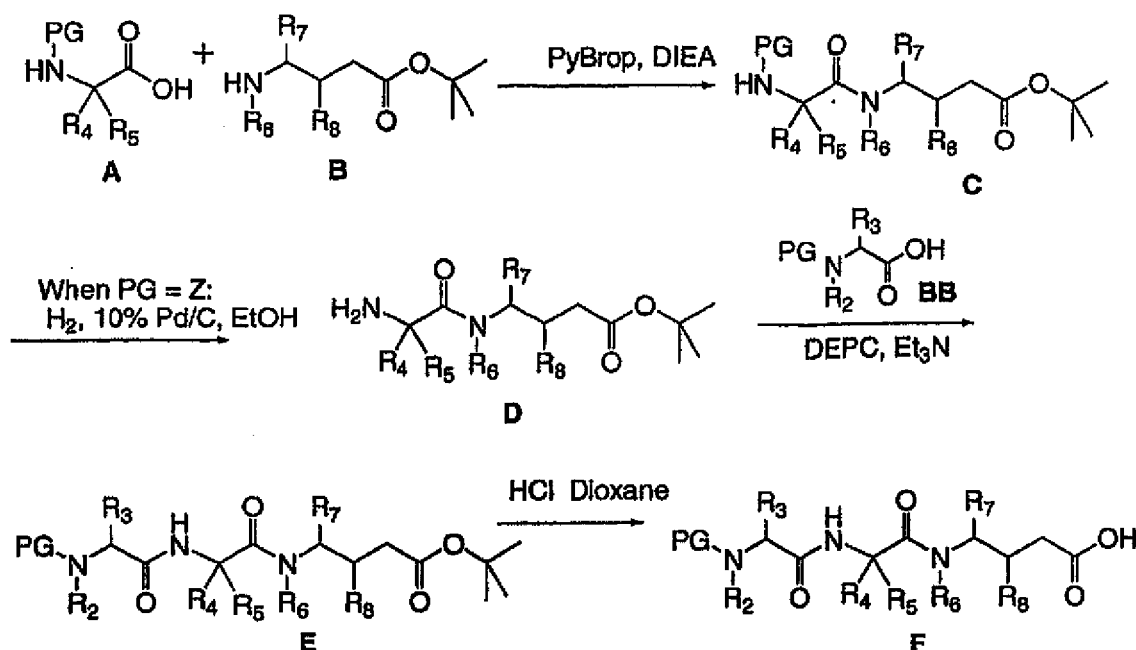
Typically, peptide-based Drugs can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (*see* E. Schröder
20 and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry.

The auristatin/dolastatin drug moieties may be prepared according to the general methods of: U.S. Patent No. 5635483; U.S. Patent No. 5780588; Pettit *et al.* (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit *et al.* (1998) Anti-Cancer Drug Design
25 13:243-277; and Pettit *et al.* (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863.

In one embodiment, a Drug is prepared by combining about a stoichiometric equivalent of a dipeptide and a tripeptide, preferably in a one-pot reaction under suitable condensation conditions. This approach is illustrated in Schemes 5-7,
below.

30 Scheme 5 illustrates the synthesis of an N-terminal tripeptide unit F which is a useful intermediate for the synthesis of the drug compounds of Formula Ib.

Scheme 5



- 5 As illustrated in Scheme 5, a protected amino acid **A** (where PG represents an amine protecting group, R⁴ is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, -O-(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle, alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from H and methyl; or R⁴ and R⁵ join, have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from hydrogen, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached) is coupled to *t*-butyl ester **B** (where R⁶ is selected from -H and -C₁-C₈ alkyl; and R⁷ is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, -O-(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and alkyl-(C₃-C₈ heterocycle)) under suitable coupling conditions, *e.g.*, in the presence of PyBrop and diisopropylethylamine, or using DCC (see, for example, Miyazaki, K. *et. al. Chem. Pharm. Bull.* 1995, 43(10), 1706-1718).

Suitable protecting groups PG, and suitable synthetic methods to protect an amino group with a protecting group are well known in the art. See, *e.g.*, Greene, T.W. and Wuts, P.G.M., *Protective Groups in Organic Synthesis*, 2nd Edition, 1991, John Wiley & Sons. Exemplary protected amino acids **A** are PG-Ile and, particularly, PG-Val, while other suitable protected amino acids include, without limitation: PG-cyclohexylglycine, PG-cyclohexylalanine, PG-aminocyclopropane-1-carboxylic acid, PG-aminoisobutyric acid, PG-phenylalanine, PG-phenylglycine, and PG-*tert*-

butylglycine. Z is an exemplary protecting group. Fmoc is another exemplary protecting group. An exemplary *t*-butyl ester B is dolaisoleuine *t*-butyl ester.

The dipeptide C can be purified, *e.g.*, using chromatography, and subsequently deprotected, *e.g.*, using H₂ and 10% Pd-C in ethanol when PG is benzyloxycarbonyl, or using diethylamine for removal of an Fmoc protecting group. The resulting amine D readily forms a peptide bond with an amino acid BB (wherein R¹ is selected from -H, -C₁-C₈ alkyl and -C₃-C₈ carbocycle; and R² is selected from -H and -C₁-C₈ alkyl; or R¹ and R² join, have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from -H, -C₁-C₈ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the nitrogen atom to which they are attached; and R³ is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, -O-(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and alkyl-(C₃-C₈ heterocycle)). *N,N*-Dialkyl amino acids are exemplary amino acids for BB, such as commercially available *N,N*-dimethyl valine. Other *N,N*-dialkyl amino acids can be prepared by reductive bis -alkylation using known procedures (*see, e.g.*, Bowman, R.E, Stroud, H.H *J. Chem. Soc.*, 1950, 1342-1340). Fmoc-Me-L-Val and Fmoc-Me-L-glycine are two exemplary amino acids BB useful for the synthesis of *N*-monoalkyl derivatives. The amine D and the amino acid BB react to provide the tripeptide E using coupling reagent DEPC with triethylamine as the base. The C-terminus protecting group of E is subsequently deprotected using HCl to provide the tripeptide compound of formula F.

Illustrative DEPC coupling methodology and the PyBrop coupling methodology shown in Scheme 5 are outlined below in General Procedure A and General Procedure B, respectively. Illustrative methodology for the deprotection of a Z-protected amine via catalytic hydrogenation is outlined below in General Procedure C.

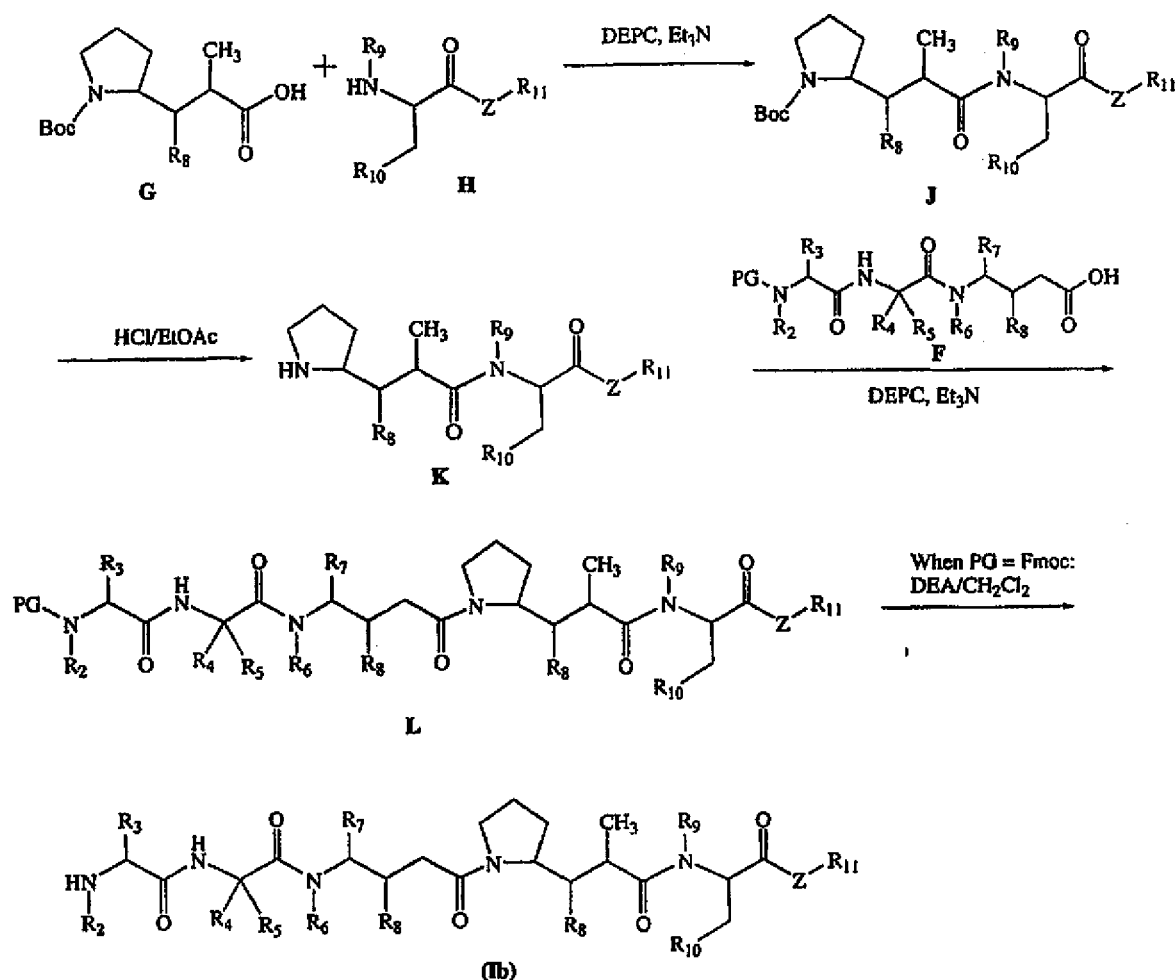
General Procedure A: Peptide synthesis using DEPC. The *N*-protected or *N,N*-disubstituted amino acid or peptide D (1.0 eq.) and an amine BB (1.1 eq.) are diluted with an aprotic organic solvent, such as dichloromethane (0.1 to 0.5 M). An organic base such as triethylamine or diisopropylethylamine (1.5 eq.) is then added, followed by DEPC (1.1 eq.). The resulting solution is stirred, preferably under argon, for up to 12 hours while being monitored by HPLC or TLC. The solvent is removed *in vacuo* at room temperature, and the crude product is purified using, for example, HPLC or flash column chromatography (silica gel column). Relevant fractions are combined and concentrated *in vacuo* to afford tripeptide E which is dried under vacuum overnight.

General procedure B: Peptide synthesis using PyBrop. The amino acid **B** (1.0 eq.), optionally having a carboxyl protecting group, is diluted with an aprotic organic solvent such as dichloromethane or DME to provide a solution of a concentration between 0.5 and 1.0 mM, then diisopropylethylamine (1.5 eq.) is added. Fmoc-, or Z-protected amino acid **A** (1.1 eq.) is added as a solid in one portion, then PyBrop (1.2 eq.) is added to the resulting mixture. The reaction is monitored by TLC or HPLC, followed by a workup procedure similar to that described in General Procedure A.

General procedure C: Z-removal via catalytic hydrogenation. Z-protected amino acid or peptide **C** is diluted with ethanol to provide a solution of a concentration between 0.5 and 1.0 mM in a suitable vessel, such as a thick-walled round bottom flask. 10% palladium on carbon is added (5-10% w/w) and the reaction mixture is placed under a hydrogen atmosphere. Reaction progress is monitored using HPLC and is generally complete within 1-2 h. The reaction mixture is filtered through a pre-washed pad of celite and the celite is again washed with a polar organic solvent, such as methanol after filtration. The eluent solution is concentrated *in vacuo* to afford a residue which is diluted with an organic solvent, preferably toluene. The organic solvent is then removed in *vacuo* to afford the deprotected amine **C**.

Scheme 6 shows a method useful for making a C-terminal dipeptide of formula **K** and a method for coupling the dipeptide of formula **K** with the tripeptide of formula **F** to make drug compounds of Formula **Ib**.

Scheme 6



The dipeptide **K** can be readily prepared by condensation of the modified amino acid Boc-Dolaproine **G** (*see*, for example, Pettit, G.R., *et al. Synthesis*, 1996, 719-725), with an amine of formula **H** using condensing agents well known for peptide chemistry, such as, for example, DEPC in the presence of triethylamine, as shown in Scheme 5.

The dipeptide of formula **K** can then be coupled with a tripeptide of formula **F** using General Procedure D to make the Fmoc-protected drug compounds of formula **L** which can be subsequently deprotected using General Procedure E in order to provide the drug compounds of formula **(Ib)**.

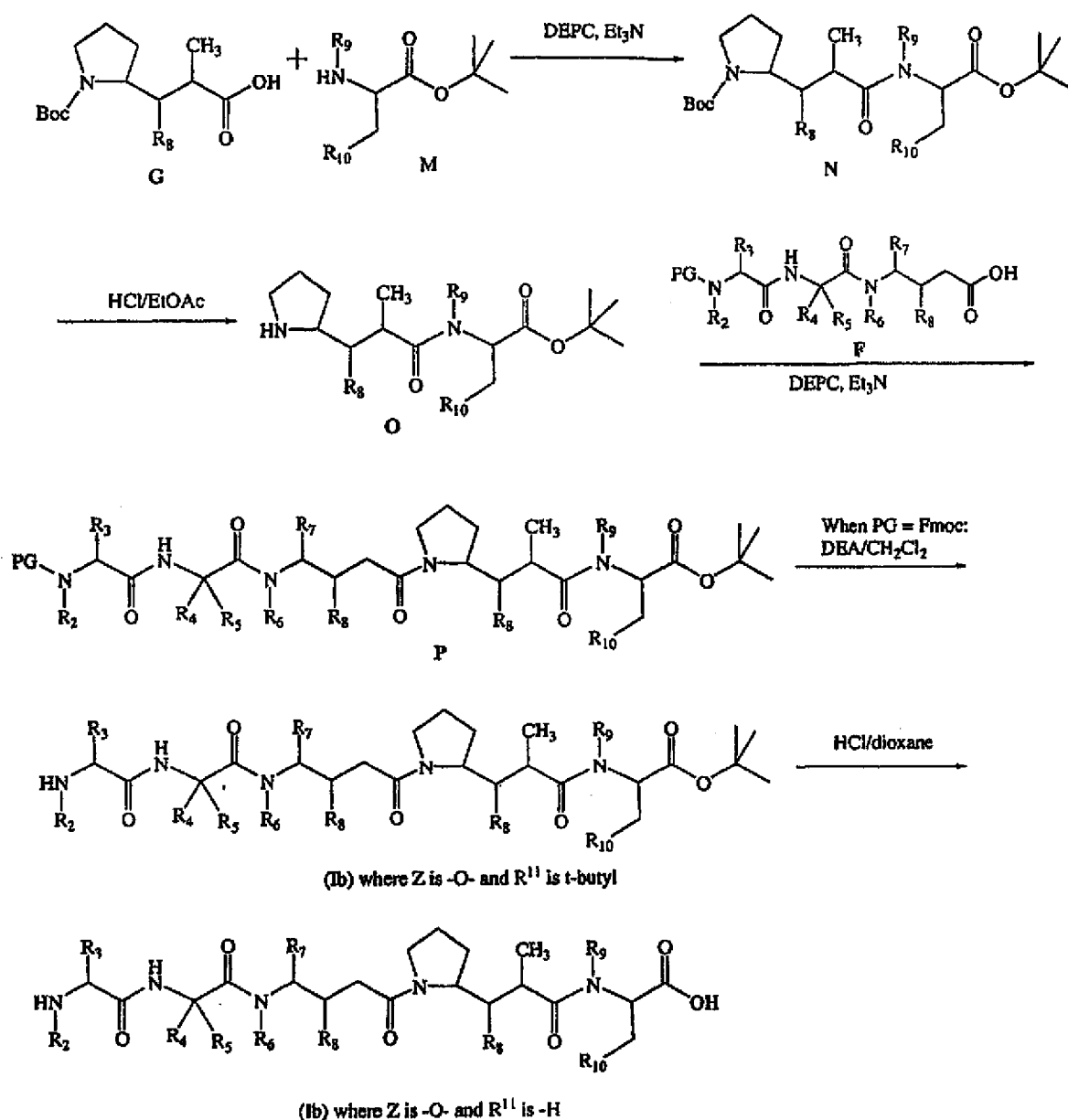
General procedure D: Drug synthesis. A mixture of dipeptide **K** (1.0 eq.) and tripeptide **F** (1 eq.) is diluted with an aprotic organic solvent, such as dichloromethane, to form a 0.1M solution, then a strong acid, such as trifluoroacetic acid (1/2 v/v) is added and the resulting mixture is stirred under a nitrogen atmosphere for two

hours at 0°C. The reaction can be monitored using TLC or, preferably, HPLC. The solvent is removed *in vacuo* and the resulting residue is azeotropically dried twice, preferably using toluene. The resulting residue is dried under high vacuum for 12 h and then diluted with an aprotic organic solvent, such as dichloromethane. An organic base
5 such as triethylamine or diisopropylethylamine (1.5 eq.) is then added, followed by either PyBrop (1.2 eq.) or DEPC (1.2 eq.) depending on the chemical functionality on the residue. The reaction mixture is monitored by either TLC or HPLC and upon completion, the reaction is subjected to a workup procedure similar or identical to that described in General Procedure A.

10 **General procedure E: Fmoc-removal using diethylamine.** An Fmoc-protected Drug L is diluted with an aprotic organic solvent such as dichloromethane and to the resulting solution is added diethylamine (1/2 v/v). Reaction progress is monitored by TLC or HPLC and is typically complete within 2 h. The reaction mixture is concentrated *in vacuo* and the resulting residue is azeotropically dried, preferably using
15 toluene, then dried under high vacuum to afford Drug Ib having a deprotected amino group.

Scheme 7 shows a method useful for making MMAF derivatives of Formula (Ib).

Scheme 7



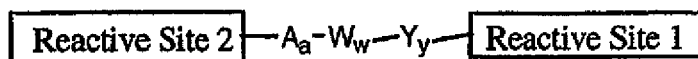
The dipeptide **O** can be readily prepared by condensation of the modified
 5 amino acid Boc-Dolaproine **G** (see, for example, Pettit, G.R., *et al. Synthesis*, 1996, 719-725), with a protected amino acid of formula **M** using condensing agents well known for peptide chemistry, such as, for example, DEPC in the presence of triethylamine, as shown in Schemes 5 and 6.

The dipeptide of formula **O** can then be coupled with a tripeptide of
 10 formula **F** using General Procedure D to make the Fmoc-protected MMAF compounds of formula **P** which can be subsequently deprotected using General Procedure E in order to provide the MMAF drug compounds of formula (**Ib**).

Thus, the above methods are useful for making Drugs that can be used in the present invention.

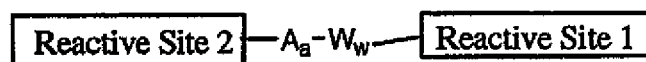
4.6.2 DRUG LINKER SYNTHESIS

5 To prepare a Drug-Linker Compound of the present invention, the Drug is reacted with a reactive site on the Linker. In general, the Linker can have the structure:



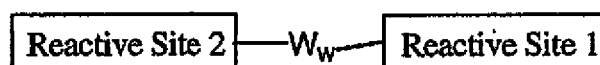
when both a Spacer unit (-Y-) and a Stretcher unit (-A-) are present. Alternately, the Linker can have the structure:

10



when the Spacer unit (-Y-) is absent.

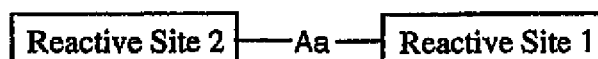
The Linker can also have the structure:



when both the Stretcher unit (-A-) and the Spacer unit (-Y-) are absent.

15

The Linker can also have the structure:



when both the Amino Acid unit (W) and the Spacer Unit (Y) are absent.

20

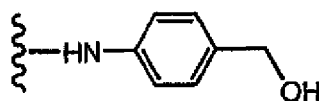
In general, a suitable Linker has an Amino Acid unit linked to an optional Stretcher Unit and an optional Spacer Unit. Reactive Site 1 is present at the terminus of the Spacer and Reactive site 2 is present at the terminus of the Stretcher. If a Spacer unit is not present, then Reactive site 1 is present at the C-terminus of the Amino Acid unit.

25

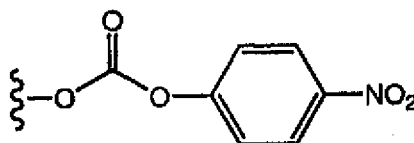
In an exemplary embodiment of the invention, Reactive Site No. 1 is reactive to a nitrogen atom of the Drug, and Reactive Site No. 2 is reactive to a sulfhydryl group on the Ligand. Reactive Sites 1 and 2 can be reactive to different functional groups.

In one aspect of the invention, Reactive Site No. 1 is $\zeta - \text{COOH}$.

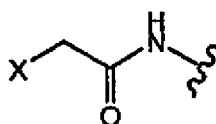
In another aspect of the invention, Reactive Site No. 1 is



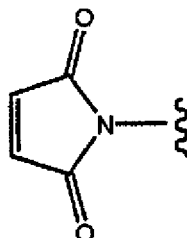
In still another aspect of the invention, Reactive Site No. 1 is a p-nitrophenyl carbonate having the formula



In one aspect of the invention, Reactive Site No. 2 is a thiol-accepting group. Suitable thiol-accepting groups include haloacetamide groups having the formula

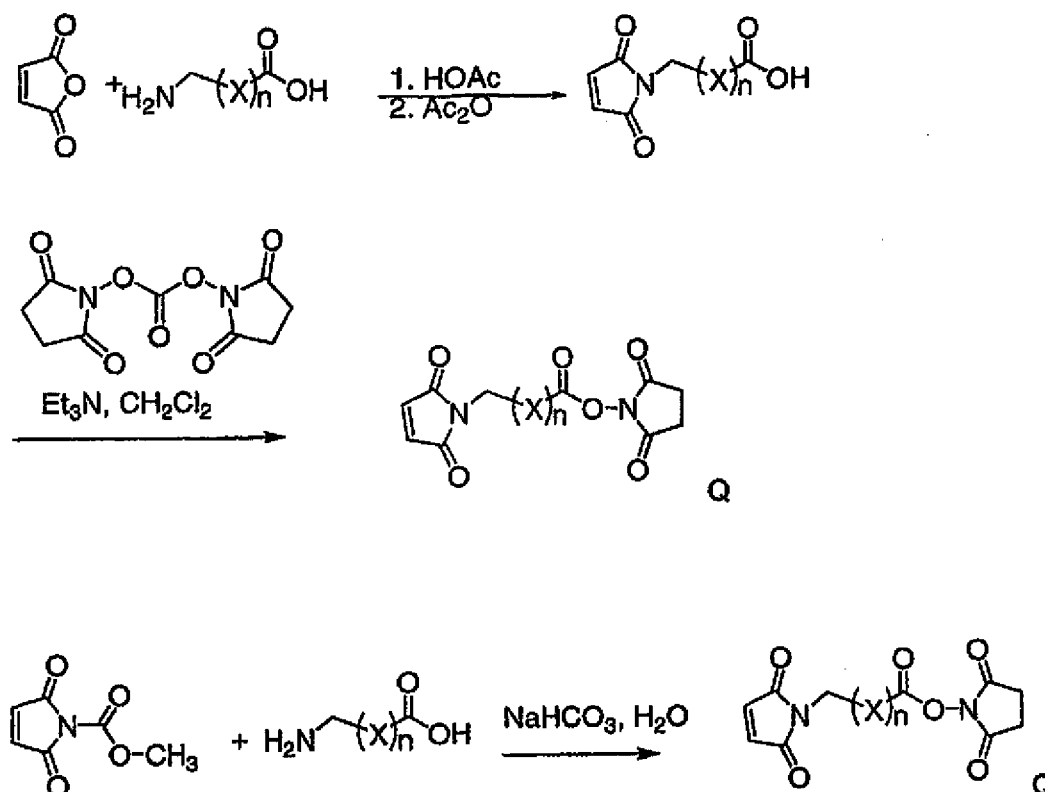


wherein X represents a leaving group, preferably O-mesyl, O-tosyl, -Cl, -Br, or -I; or a maleimide group having the formula



Useful Linkers can be obtained via commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or prepared as summarized in Schemes 8-10 below.

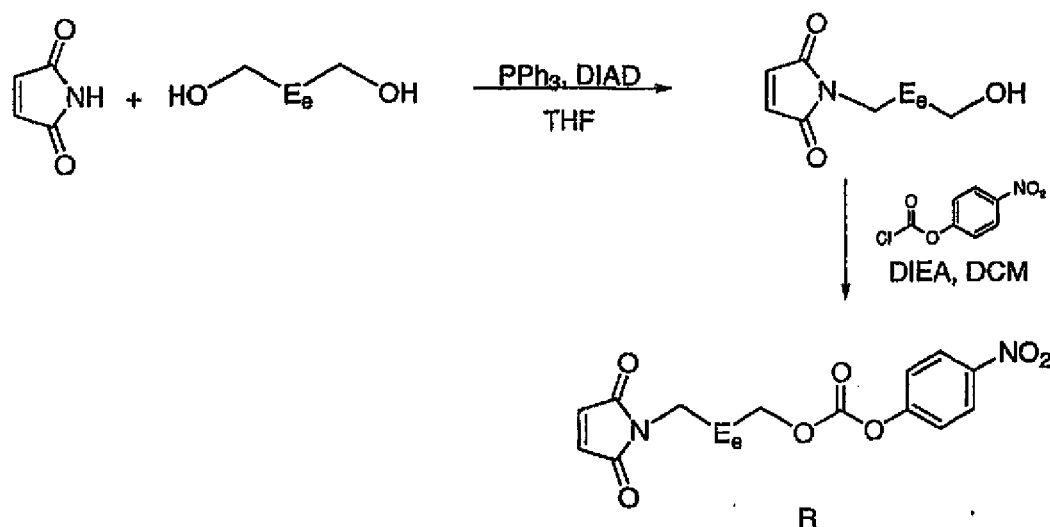
Scheme 8



wherein X is $-\text{CH}_2-$ or $-\text{CH}_2\text{OCH}_2-$; and n is an integer ranging either from 0-10 when X is $-\text{CH}_2-$; or 1-10 when X is $-\text{CH}_2\text{OCH}_2-$.

- 5 The method shown in Scheme 9 combines maleimide with a glycol under Mitsunobu conditions to make a polyethylene glycol maleimide Stretcher (see for example, Walker, M.A. *J. Org. Chem.* 1995, 60, 5352-5), followed by installation of a p-nitrophenyl carbonate Reactive Site group.

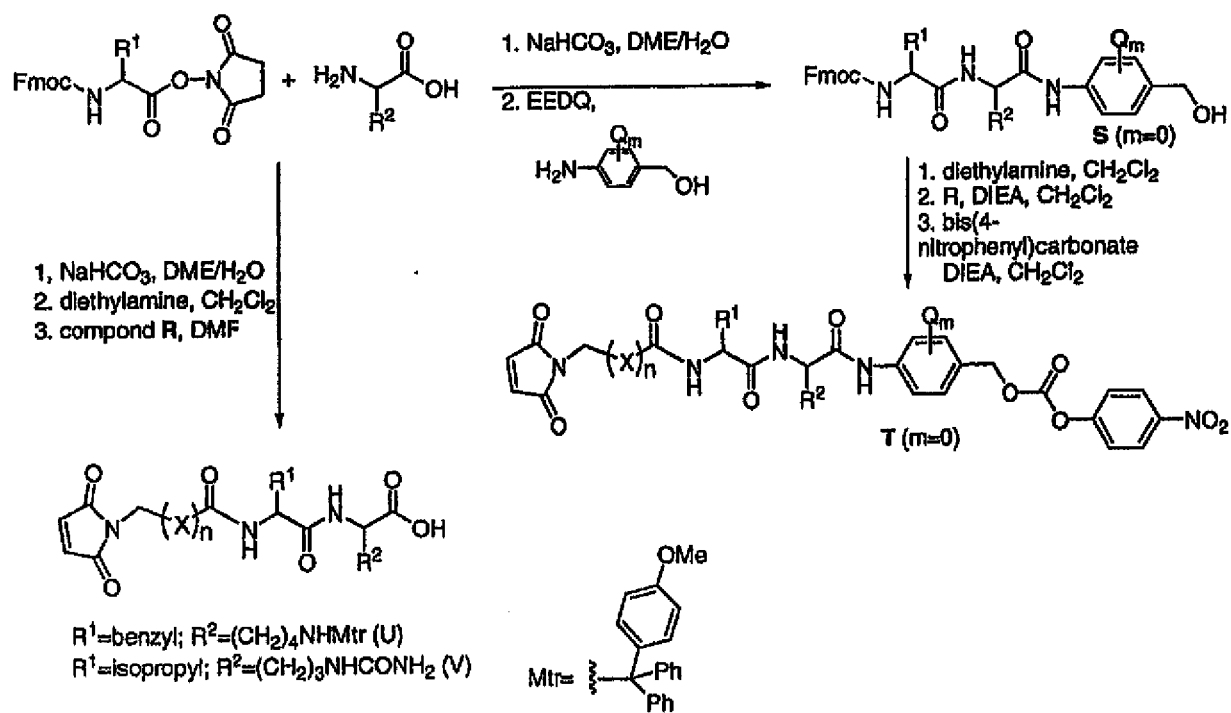
Scheme 9



wherein E is $-\text{CH}_2-$ or $-\text{CH}_2\text{OCH}_2-$; and e is an integer ranging from 0-8;

- Alternatively, PEG-maleimide and PEG-haloacetamide stretchers can be prepared as described by Frisch, *et al.*, *Bioconjugate Chem.* 1996, 7, 180-186.
- 5 Scheme 10 illustrates a general synthesis of an illustrative Linker unit containing a maleimide Stretcher group and optionally a p-aminobenzyl ether self-immolative Spacer.

Scheme 10

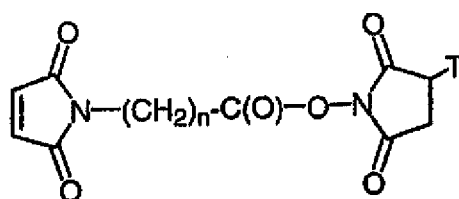


wherein Q is $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, $-\text{halogen}$, $-\text{nitro}$ or $-\text{cyano}$; m is an integer ranging from 0-4; and n is an integer ranging from 0-10.

Useful Stretchers may be incorporated into a Linker using the commercially available intermediates from Molecular Biosciences (Boulder, CO)

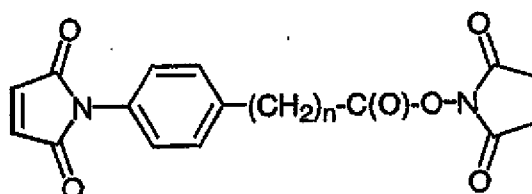
5 described below by utilizing known techniques of organic synthesis.

Stretchers of formula (IIIa) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit as depicted in Schemes 11 and 12:

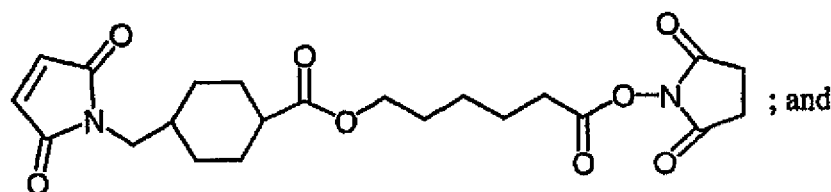
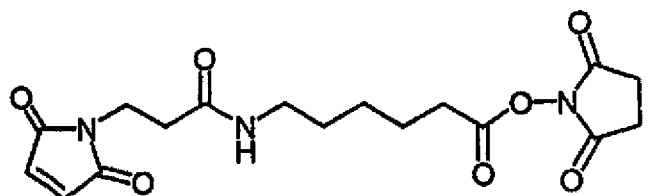
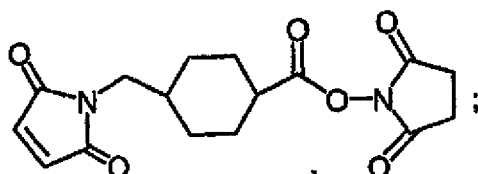


10

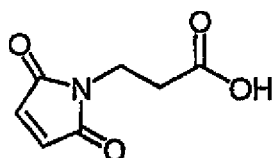
where n is an integer ranging from 1-10 and T is $-\text{H}$ or $-\text{SO}_3\text{Na}$;



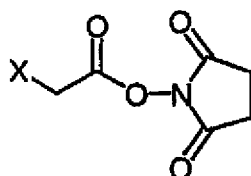
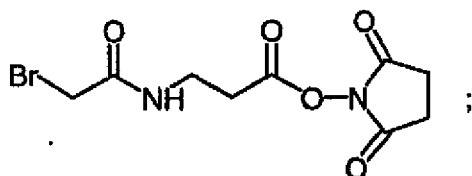
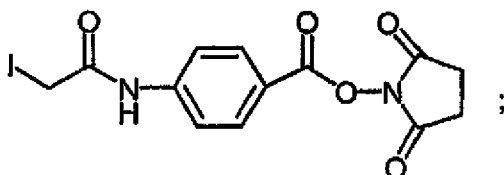
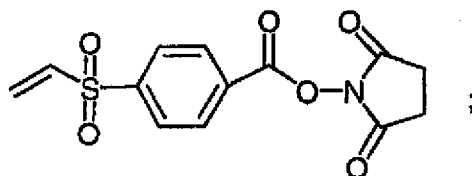
where n is an integer ranging from 0-3;



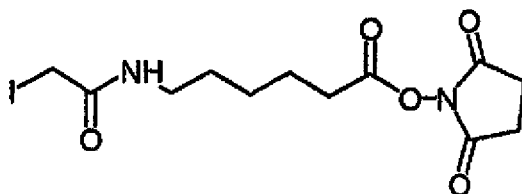
15



Stretcher units of formula (IIIb) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:

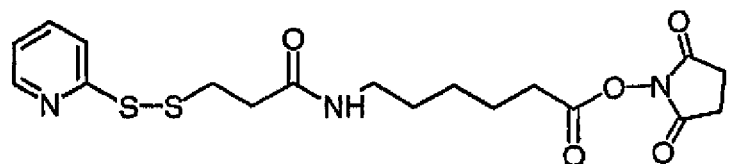
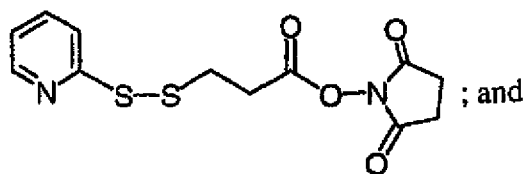


where X is -Br or -I; and

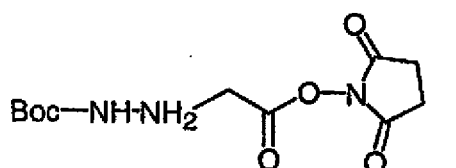
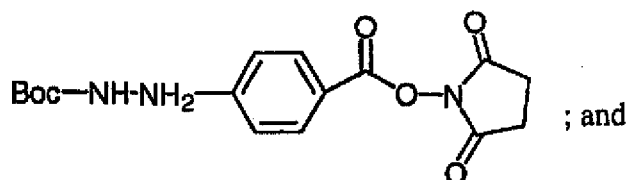


5

Stretcher units of formula (IV) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:



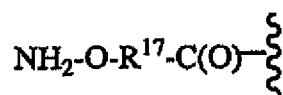
Stretcher units of formula (Va) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:



5

Other useful Stretchers may be synthesized according to known procedures. Aminooxy Stretchers of the formula shown below can be prepared by treating alkyl halides with N-Boc-hydroxylamine according to procedures described in Jones, D.S. *et al.*, *Tetrahedron Letters*, 2000, 41(10), 1531-1533; and Gilon, C. *et al.*, *Tetrahedron*, 1967, 23(11), 4441-4447.

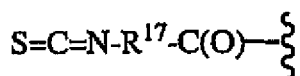
10



wherein -R¹⁷- is selected from -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-, -O-(C₁-C₈ alkyl)-, -arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging from 1-10;

15

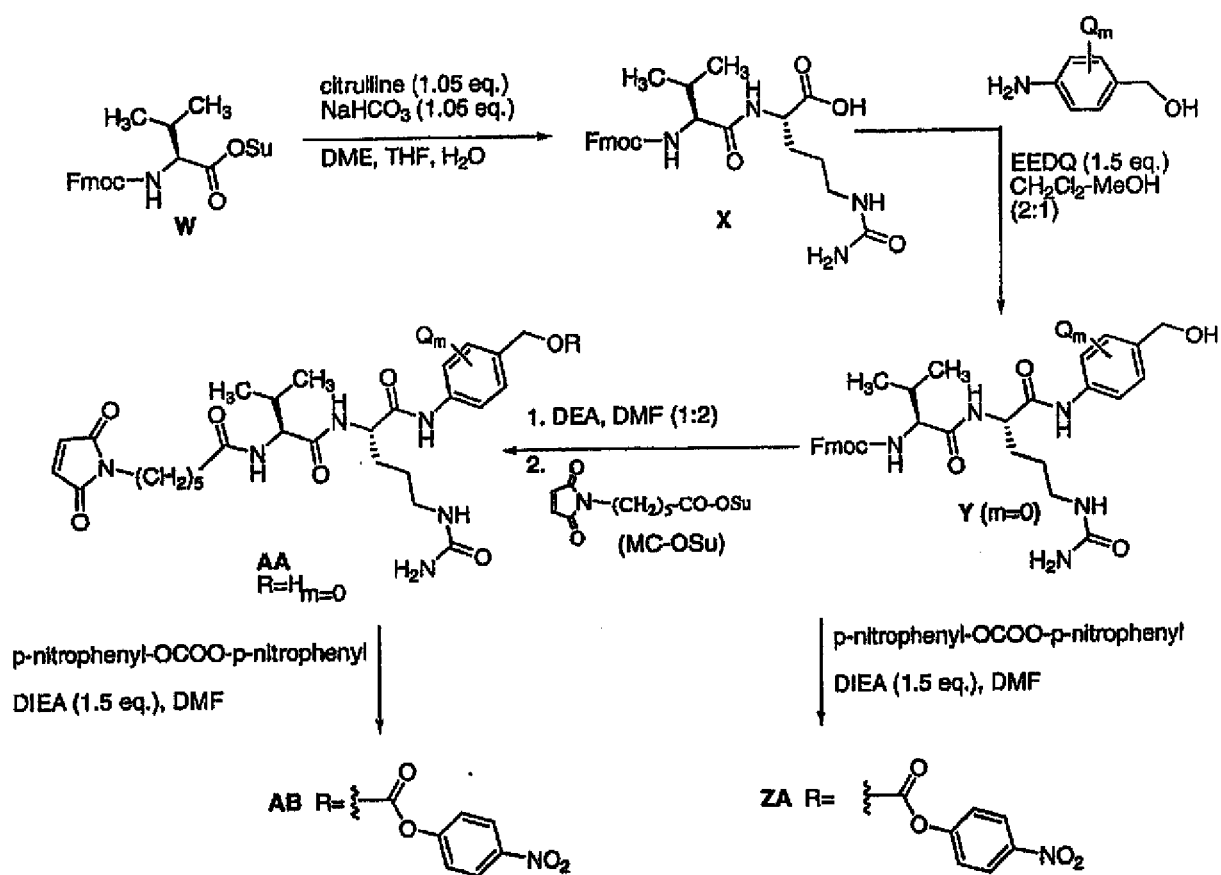
Isothiocyanate Stretchers of the formula shown below may be prepared from isothiocyanatocarboxylic acid chlorides as described in *Angew. Chem.*, 1975, 87(14):517.



wherein $-\text{R}^{17}-$ is as described herein.

- 5 Scheme 11 shows a method for obtaining of a val-cit dipeptide Linker having a maleimide Stretcher and optionally a p-aminobenzyl self-immolative Spacer.

Scheme 11

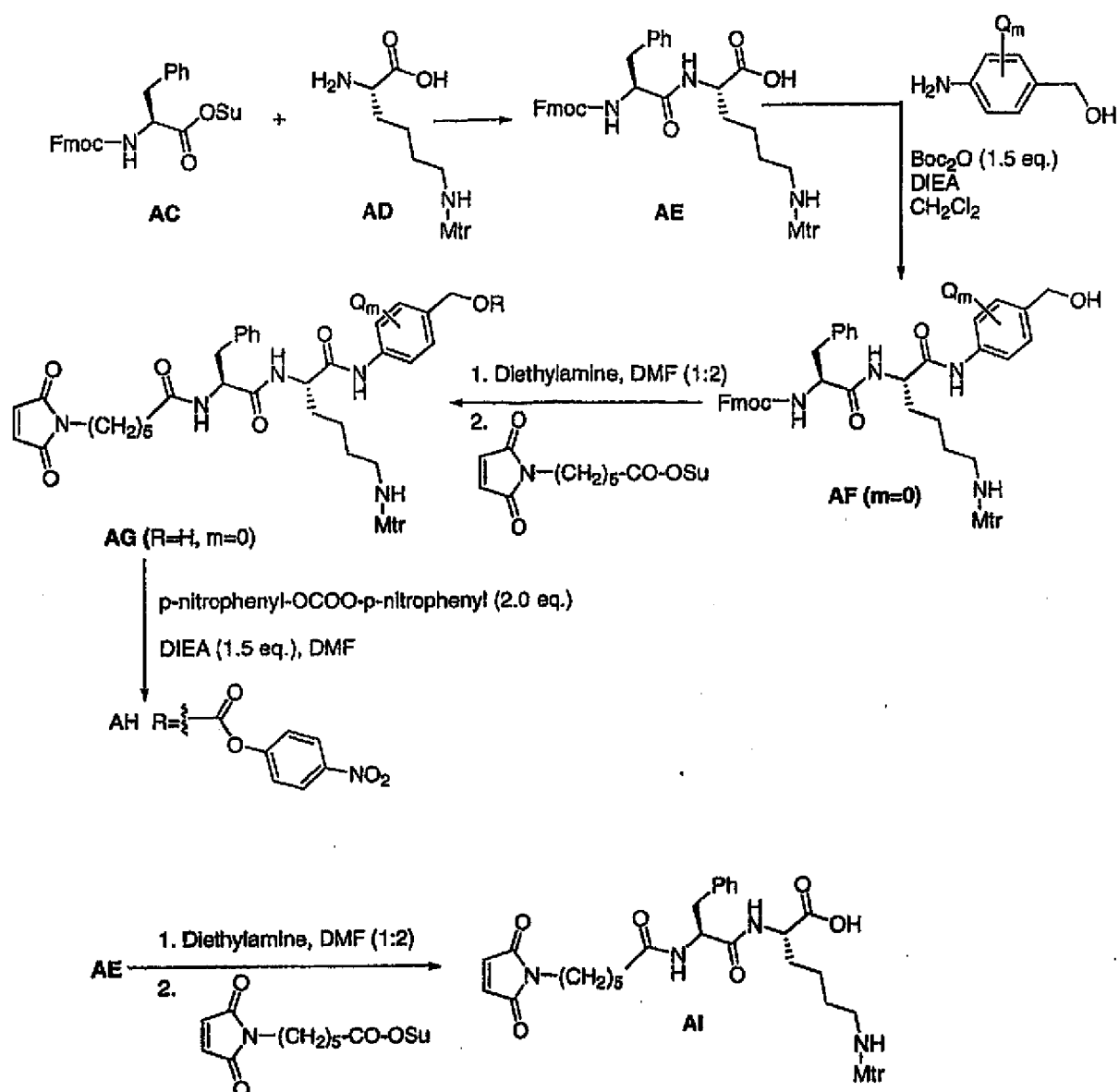


wherein Q is $-\text{C}_1-\text{C}_8$ alkyl, $-\text{O}-(\text{C}_1-\text{C}_8 \text{ alkyl})$, -halogen, -nitro or -cyano;

- 10 and m is an integer ranging from 0-4.

Scheme 12 illustrates the synthesis of a phe-lys(Mtr) dipeptide Linker unit having a maleimide Stretcher unit and a p-aminobenzyl self-immolative Spacer unit. Starting material AD (lys(Mtr)) is commercially available (Bachem, Torrance, CA) or can be prepared according to Dubowchik, *et al. Tetrahedron Letters* (1997) 38:5257-60.

Scheme 12



wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

- 5 As shown in Scheme 13, a Linker can be reacted with an amino group of a Drug Compound of Formula (Ib) to form a Drug-Linker Compound that contains an amide or carbamate group, linking the Drug unit to the Linker unit. When Reactive Site No. 1 is a carboxylic acid group, as in Linker AJ, the coupling reaction can be performed using HATU or PyBrop and an appropriate amine base, resulting in a Drug-Linker
- 10 Compound AK, containing an amide bond between the Drug unit and the Linker unit. When Reactive Site No. 1 is a carbonate, as in Linker AL, the Linker can be coupled to

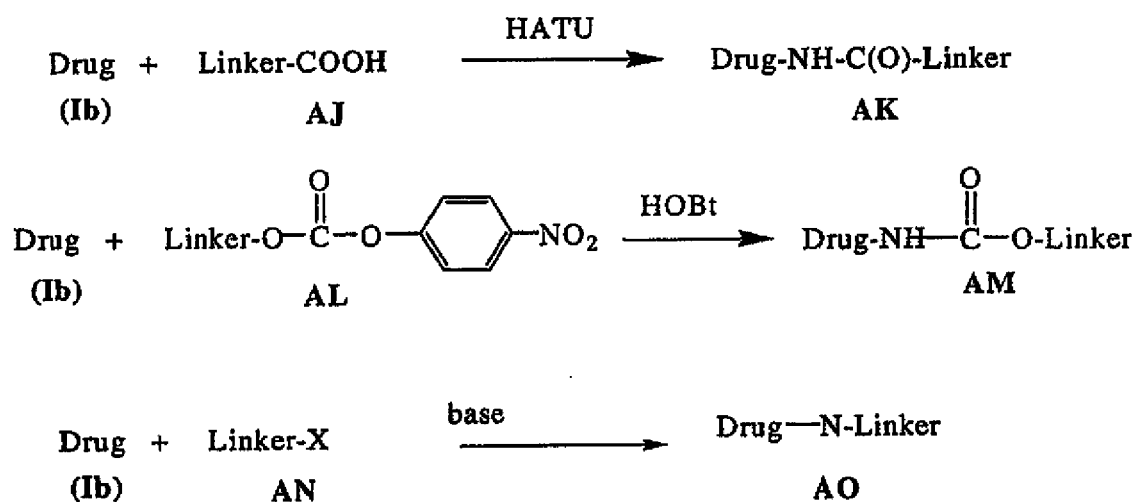
the Drug using HOBt in a mixture of DMF/pyridine to provide a Drug-Linker Compound **AM**, containing a carbamate bond between the Drug unit and the Linker unit

Alternately, when Reactive Site No. 1 is a good leaving group, such as in Linker **AN**, the Linker can be coupled with an amine group of a Drug via a nucleophilic substitution process to provide a Drug-Linker Compound having an amine linkage (**AO**) between the Drug unit and the Linker unit.

Illustrative methods useful for linking a Drug to a Ligand to form a Drug-Linker Compound are depicted in Scheme 13 and are outlined in General Procedures G-H.

10

Scheme 13



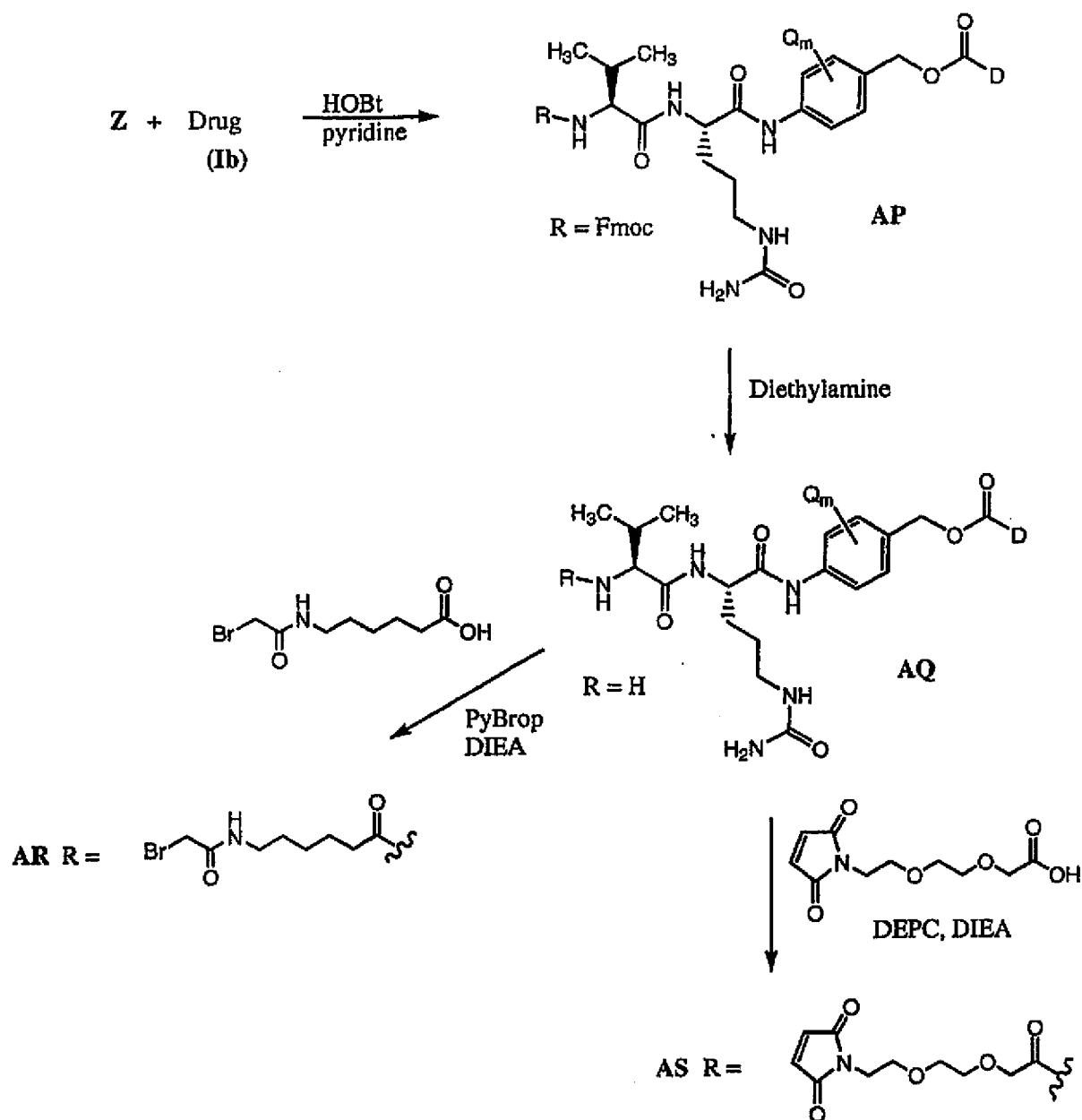
General Procedure G: Amide formation using HATU. A Drug (**Ib**) (1.0 eq.) and an N-protected Linker containing a carboxylic acid Reactive site (1.0 eq.) are diluted with a suitable organic solvent, such as dichloromethane, and the resulting solution is treated with HATU (1.5 eq.) and an organic base, preferably pyridine (1.5 eq.). The reaction mixture is allowed to stir under an inert atmosphere, preferably argon, for 6h, during which time the reaction mixture is monitored using HPLC. The reaction mixture is concentrated and the resulting residue is purified using HPLC to yield the amide of formula **AK**.

Procedure H: Carbamate formation using HOBt. A mixture of a Linker **AL** having a p-nitrophenyl carbonate Reactive site (1.1 eq.) and Drug (**Ib**) (1.0 eq.) are diluted with an aprotic organic solvent, such as DMF, to provide a solution having a concentration of 50-100 mM, and the resulting solution is treated with HOBt

(2.0 eq.) and placed under an inert atmosphere, preferably argon. The reaction mixture is allowed to stir for 15 min, then an organic base, such as pyridine (1/4 v/v), is added and the reaction progress is monitored using HPLC. The Linker is typically consumed within 16 h. The reaction mixture is then concentrated *in vacuo* and the resulting residue is
5 purified using, for example, HPLC to yield the carbamate **AM**.

An alternate method of preparing Drug-Linker Compounds is outlined in Scheme 14. Using the method of Scheme 14, the Drug is attached to a partial Linker unit (**ZA**, for example), which does not have a Stretcher unit attached. This provides intermediate **AP**, which has an Amino Acid unit having an Fmoc-protected N-terminus.
10 The Fmoc group is then removed and the resulting amine intermediate **AQ** is then attached to a Stretcher unit via a coupling reaction catalyzed using PyBrop or DEPC. The construction of Drug-Linker Compounds containing either a bromoacetamide Stretcher **AR** or a PEG maleimide Stretcher **AS** is illustrated in Scheme 14.

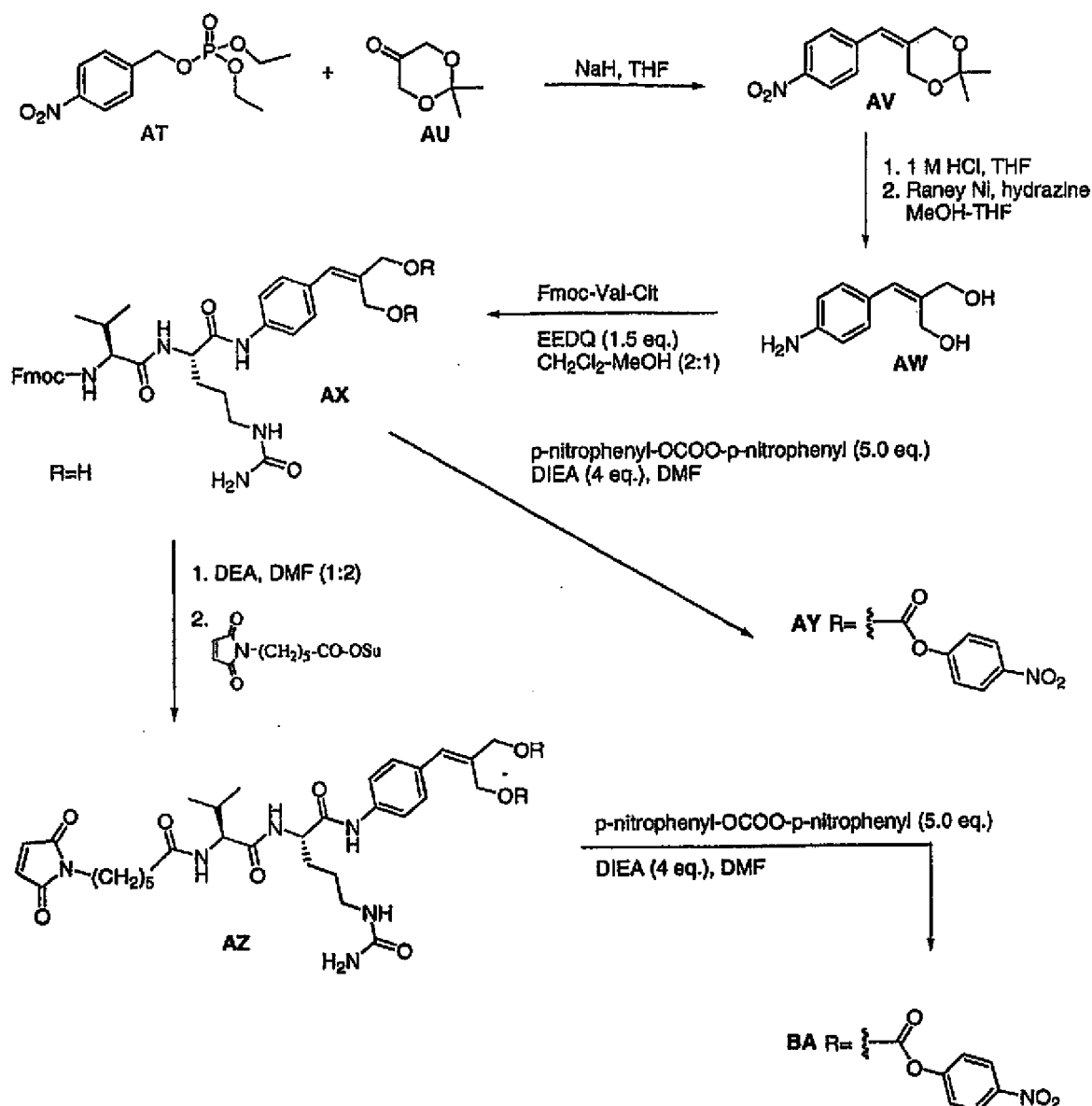
Scheme 14



wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

- 5 Methodology useful for the preparation of a Linker unit containing a branched spacer is shown in Scheme 15.

Scheme 15

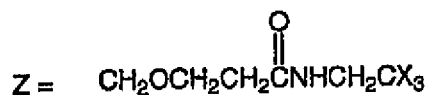
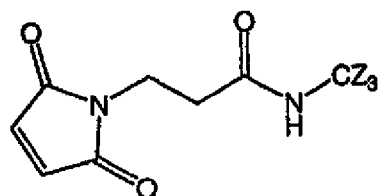
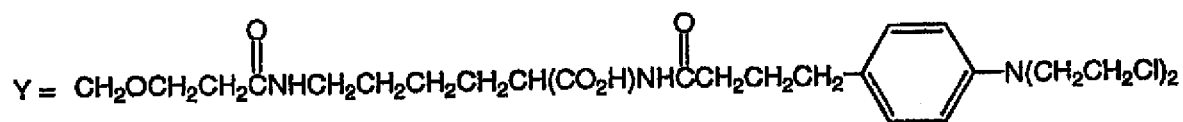
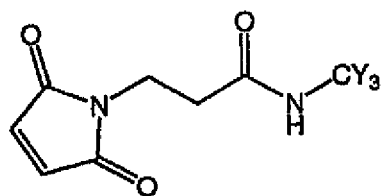
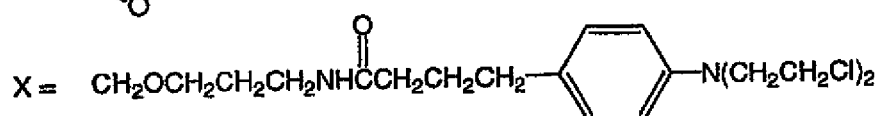
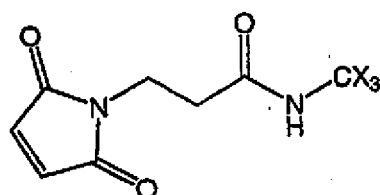


Scheme 15 illustrates the synthesis of a val-cit dipeptide linker having a maleimide Stretcher unit and a bis(4-hydroxymethyl)styrene (BHMS) unit. The synthesis of the BHMS intermediate (AW) has been improved from previous literature procedures (see International Publication No, WO 9813059 to Firestone *et al.*, and Crozet, M.P.; Archaimbault, G.; Vanelle, P.; Nougier, R. *Tetrahedron Lett.* (1985) 26:5133-5134) and utilizes as starting materials, commercially available diethyl (4-nitrobenzyl)phosphonate (AT) and commercially available 2,2-dimethyl-1,3-dioxan-5-one (AU). Linkers AY and BA can be prepared from intermediate AW using the methodology described in Scheme 9.

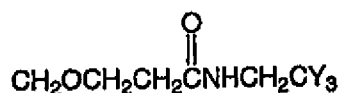
4.6.3 DENDRITIC LINKERS

The linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to a Ligand, such as but not limited to an antibody (Sun *et al.* (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun *et al.* (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, *i.e.* loading, which is related to the potency of the Drug-Linker-Ligand Conjugate. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

The following exemplary embodiments of dendritic linker reagents allow up to nine nucleophilic drug moiety reagents to be conjugated by reaction with the chloroethyl nitrogen mustard functional groups:



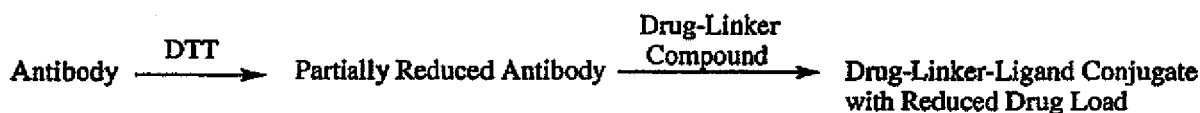
or



4.6.4 CONJUGATION OF DRUG MOIETIES TO ANTIBODIES

Scheme 16 illustrates methodology useful for making Drug-Linker-Ligand conjugates having about 2 to about 4 drugs per antibody. An antibody is treated with a reducing agent, such as dithiothreitol (DTT) to reduce some or all of the cysteine disulfide residues to form highly nucleophilic cysteine thiol groups (-CH₂SH). The partially reduced antibody thus reacts with drug-linker compounds, or linker reagents, with electrophilic functional groups such as maleimide or α -halo carbonyl, according to the conjugation method at page 766 of Klussman, *et al.* (2004), *Bioconjugate Chemistry* 15(4):765-773.

Scheme 16



For example, an antibody, *e.g.*, AC10, dissolved in 500 mM sodium borate and 500 mM sodium chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT).

After incubation at 37 °C for about 30 minutes, the buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm. The reduced antibody dissolved in PBS is chilled on ice. The drug linker, *e.g.*, MC-val-cit-PAB-MMAE in DMSO, dissolved in acetonitrile and water at known concentration, is added to the chilled reduced antibody in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and the ADC, *e.g.*, AC10-MC-vc-PAB-MMAE, is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μ m filters under sterile conditions, and frozen for storage.

A variety of antibody drug conjugates (ADC) were prepared, with a variety of linkers, and the drug moieties, MMAE and MMAF. The following table is an exemplary group of ADC which were prepared following the protocol of Example 27, and characterized by HPLC and drug loading assay.

Target (antigen)	ADC	isolated amount (mg)	drug/Ab ratio
0772P	16E12-MC-vc-PAB-MMAE	1.75	4
0772P	11D10-MC-vc-PAB-MMAE	46.8	4.4
0772P	11D10-MC-vc-PAB-MMAF	54.5	3.8
Brevican	Brevican-MC-MMAF	2	6
Brevican	Brevican-MC-vc-MMAF	2	6
Brevican	Brevican-MC-vc-PAB-MMAF	1.4	6
CD21	CD21-MC-vc-PAB-MMAE	38.1	4.3
CD21	CD21-MC-vc-PAB-MMAF	43	4.1
CRIPTO	11F4-MC-vc-PAB-MMAF	6	4.8
CRIPTO	25G8-MC-vc-PAB-MMAF	7.4	4.7
E16	12G12-MC-vc-PAB-MMAE	2.3	4.6
E16	3B5-MC-vc-PAB-MMAE	2.9	4.6
E16	12B9-MC-vc-PAB-MMAE	1.4	3.8
E16	12B9-MC-vc-PAB-MMAE	5.1	4
E16	12G12-MC-vc-PAB-MMAE	3	4.6
E16	3B5-MC-vc-PAB-MMAE	4.8	4.1
E16	3B5-MC-vc-PAB-MMAF	24.7	4.4
EphB2R	2H9-MC-vc-PAB-MMAE	29.9	7.1
EphB2R	2H9-MC-fk-PAB-MMAE	25	7.5
EphB2R	2H9-MC-vc-PAB-MMAE	175	4.1
EphB2R	2H9-MC-vc-PAB-MMAF	150	3.8

EphB2R	2H9-MC-vc-PAB-MMAF	120	3.7
EphB2R	2H9-MC-vc-PAB-MMAE	10.7	4.4
IL-20Ra	IL20Ra-fk-MMAE	26	6.7
IL-20Ra	IL20Ra-vc-MMAE	27	7.3
EphB2	IL8-MC-vc-PAB-MMAE	251	3.7
MDP	MDP-vc-MMAE	32	
MPF	19C3-vc-MMAE	1.44	6.5
MPF	7D9-vc-MMAE	4.3	3.8
MPF	19C3-vc-MMAE	7.9	3
MPF	7D9-MC-vc-PAB-MMAF	5	4.3
Napi3b	10H1-vc-MMAE	4.5	4.6
Napi3b	4C9-vc-MMAE	3.0	5.4
Napi3b	10H1-vc-MMAE	4.5	4.8
Napi3b	10H1-vc-MMAF	6.5	4
NCA	3E6-MC-fk-PAB-MMAE	49.6	5.4
NCA	3E6-MC-vc-PAB-MMAE	56.2	6.4
PSCA	PSCA-fk-MMAE	51.7	8.9
PSCA	PSCA-vc-MMAE	61.1	8.6
Napi3b	10H1-MC-vc-PAB-MMAE	75	4.2
Napi3b	10H1-MC-vc-PAB-MMAF	95	4.4
Napi3b	10H1-MC-MMAF	92	4
EphB2R	2H9-MC-vc-PAB-MMAE	79	5
EphB2R	2H9-MC-MMAF	92	4.9

0772P	11D10(Fc chimera)-MC-vc-PAB-MMAE	79	4.3
0772P	11D10(Fc chimera)-MC-vc-PAB-MMAF	70	4.5
0772P	11D10(Fc chimera)-MC-MMAF	23	4.5
Brevican	6D2-MC-vc-PAB-MMAF	0.3	4.5
Brevican	6D2-MC-MMAF	0.36	4.5
EphB2R	2H9(Fc chimera)-MC-vc-PAB-MMAE	1983	4.3
E16	12B9-MC-vc-PAB-MMAE	14.1	4.6
E16	12B9-MC-vc-PAB-MMAF	16.4	4.5
E16	12G12-MC-vc-PAB-MMAE	10.5	4.1
E16	12G12-MC-vc-PAB-MMAF	10.2	3.8
E16	3B5-MC-vc-PAB-MMAE	58.6	3.8
E16	3B5-MC-vc-PAB-MMAF	8	3.1
0772P	11D10(Fc chimera)-MC-vc-PAB-MMAE	340	3.9
Steap1	(Steap1-92)-MC-vc-PAB-MMAE	3.5	4
Steap1	(Steap1-92)-MC-vc-PAB-MMAF	4.7	4
Steap1	(Steap1-120)-MC-vc-PAB-MMAE	2	4
Steap1	(Steap1-120)-MC-vc-PAB-MMAF	2.3	4
E16	3B5-MC-vc-PAB-MMAF	52.2	4.5

4.7 COMPOSITIONS AND METHODS OF ADMINISTRATION

In other embodiments, described is a composition including an effective amount of an Exemplary Compound and/or Exemplary Conjugate and a pharmaceutically acceptable carrier or vehicle. For convenience, the Drug units and Drug-Linker

- 5 Compounds can be referred to as Exemplary Compounds, while Drug-Ligand Conjugates and Drug-Linker-Ligand Conjugates can be referred to as Exemplary Conjugates. The compositions are suitable for veterinary or human administration.

- The present compositions can be in any form that allows for the composition to be administered to a patient. For example, the composition can be in the
10 form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral, sublingual, rectal, vaginal, ocular, intra-tumor, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In one aspect, the compositions are administered parenterally. In yet another aspect, the Exemplary
15 Compounds and/or the Exemplary Conjugates or compositions are administered intravenously.

- Pharmaceutical compositions can be formulated so as to allow an Exemplary Compound and/or Exemplary Conjugate to be bioavailable upon administration of the composition to a patient. Compositions can take the form of one or
20 more dosage units, where for example, a tablet can be a single dosage unit, and a container of an Exemplary Compound and/or Exemplary Conjugate in aerosol form can hold a plurality of dosage units.

- Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the
25 optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of animal (*e.g.*, human), the particular form of the Exemplary Compound or Exemplary Conjugate, the manner of administration, and the composition employed.

- The pharmaceutically acceptable carrier or vehicle can be particulate, so
30 that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous or particulate, so as to provide an aerosol composition useful in, *e.g.*, inhalatory administration.

When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring, and a coloring agent.

When the composition is in the form of a capsule, *e.g.*, a gelatin capsule, it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The composition can be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in ampoule, a disposable syringe or a multiple-dose vial

made of glass, plastic or other material. Physiological saline is an exemplary adjuvant. An injectable composition is preferably sterile.

5 The amount of the Exemplary Compound and/or Exemplary Conjugate that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

10 The compositions comprise an effective amount of an Exemplary Compound and/or Exemplary Conjugate such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of an Exemplary Compound and/or Exemplary Conjugate by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1% to about 80% by weight of the composition. In one aspect, oral compositions can comprise from about 4% to about 50% of the Exemplary Compound and/or Exemplary Conjugate by weight of the composition. In yet another aspect, present compositions are prepared so that a parenteral dosage unit contains from about 0.01% to about 2% by weight of the Exemplary Compound and/or Exemplary Conjugate.

20 For intravenous administration, the composition can comprise from about 0.01 to about 100 mg of an Exemplary Compound and/or Exemplary Conjugate per kg of the animal's body weight. In one aspect, the composition can include from about 1 to about 100 mg of an Exemplary Compound and/or Exemplary Conjugate per kg of the animal's body weight. In another aspect, the amount administered will be in the range from about 0.1 to about 25 mg/kg of body weight of the Exemplary Compound and/or Exemplary Conjugate.

Generally, the dosage of an Exemplary Compound and/or Exemplary Conjugate administered to a patient is typically about 0.01 mg/kg to about 2000 mg/kg of the animal's body weight. In one aspect, the dosage administered to a patient is between about 0.01 mg/kg to about 10 mg/kg of the animal's body weight, in another aspect, the dosage administered to a patient is between about 0.1 mg/kg and about 250 mg/kg of the animal's body weight, in yet another aspect, the dosage administered to a patient is between about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, in yet another

aspect the dosage administered is between about 0.1 mg/kg to about 10 mg/kg of the animal's body weight, and in yet another aspect, the dosage administered is between about 1 mg/kg to about 10 mg/kg of the animal's body weight.

The Exemplary Compounds and/or Exemplary Conjugate or compositions
5 can 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.). Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer an Exemplary Compound and/or Exemplary
10 Conjugate or composition. In certain embodiments, more than one Exemplary Compound and/or Exemplary Conjugate or composition is administered to a patient.

In specific embodiments, it can be desirable to administer one or more Exemplary Compounds and/or Exemplary Conjugate or compositions locally to the area in need of treatment. This can be achieved, for example, and not by way of limitation, by
15 local infusion during surgery; topical application, *e.g.*, in conjunction with a wound dressing after surgery; by injection; by means of a catheter; by means of a suppository; or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a
20 cancer, tumor or neoplastic or pre-neoplastic tissue. In another embodiment, administration can be by direct injection at the site (or former site) of a manifestation of an autoimmune disease.

In certain embodiments, it can be desirable to introduce one or more Exemplary Compounds and/or Exemplary Conjugate or compositions into the central
25 nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection can be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a
30 fluorocarbon or synthetic pulmonary surfactant.

In yet another embodiment, the Exemplary Compounds and/or Exemplary Conjugate or compositions can be delivered in a controlled release system, such as but not limited to, a pump or various polymeric materials can be used. In yet another

embodiment, a controlled-release system can be placed in proximity of the target of the Exemplary Compounds and/or Exemplary Conjugate or compositions, *e.g.*, the brain, 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)). Other controlled-release systems discussed in the review by Langer (*Science* 249:1527-1533 (1990)) can be used.

The term "carrier" refers to a diluent, adjuvant or excipient, with which an Exemplary Compound and/or Exemplary Conjugate is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to a patient, the Exemplary Compound and/or Exemplary Conjugate or compositions and pharmaceutically acceptable carriers are sterile. Water is an exemplary carrier when the Exemplary Compounds and/or Exemplary Conjugates are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

In an embodiment, the Exemplary Compounds and/or Exemplary Conjugates are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to animals, particularly human beings. Typically, the carriers or vehicles for intravenous administration are sterile isotonic aqueous buffer solutions. Where necessary, the compositions can also include a

solubilizing agent. Compositions for intravenous administration can optionally comprise a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients 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 an Exemplary Compound and/or Exemplary Conjugate is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the Exemplary Compound and/or Exemplary Conjugate is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be used.

The compositions can be intended for topical administration, in which case the carrier may be in the form of a solution, emulsion, ointment or gel base. If intended for transdermal administration, the composition can be in the form of a transdermal patch or an iontophoresis device. Topical formulations can comprise a concentration of an Exemplary Compound and/or Exemplary Conjugate of from about 0.05% to about 50% w/v (weight per unit volume of composition), in another aspect, from 0.1% to 10% w/v.

The composition can be intended for rectal administration, in the form, *e.g.*, of a suppository which will melt in the rectum and release the Exemplary Compound and/or Exemplary Conjugate.

5 The composition can include various materials that modify the physical form of a solid or liquid dosage unit. For example, the composition can include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and can be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients can be encased in a gelatin capsule.

10 The compositions can consist of gaseous dosage units, *e.g.*, it can be in the form of an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery can be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients.

15 Whether in solid, liquid or gaseous form, the present compositions can include a pharmacological agent used in the treatment of cancer, an autoimmune disease or an infectious disease.

4.8 THERAPEUTIC USES OF THE EXEMPLARY CONJUGATES

20 The Exemplary Compounds and/or Exemplary Conjugates are useful for treating cancer, an autoimmune disease or an infectious disease in a patient.

4.8.1 TREATMENT OF CANCER

25 The Exemplary Compounds and/or Exemplary Conjugates are useful for inhibiting the multiplication of a tumor cell or cancer cell, causing apoptosis in a tumor or cancer cell, or for treating cancer in a patient. The Exemplary Compounds and/or Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of animal cancers. The Drug-Linker-Ligand Conjugates can be used to deliver a Drug or Drug unit to a tumor cell or cancer cell. Without being bound by theory, in one
30 embodiment, the Ligand unit of an Exemplary Conjugate binds to or associates with a cancer-cell or a tumor-cell-associated antigen, and the Exemplary Conjugate can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis. The antigen

can be attached to a tumor cell or cancer cell or can be an extracellular matrix protein associated with the tumor cell or cancer cell. Once inside the cell, one or more specific peptide sequences within the Linker unit are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases, resulting in release of a Drug or a Drug-Linker Compound. The released Drug or Drug-Linker Compound is then free to migrate within the cell and induce cytotoxic or cytostatic activities. In an alternative embodiment, the Drug or Drug unit is cleaved from the Exemplary Conjugate outside the tumor cell or cancer cell, and the Drug or Drug-Linker Compound subsequently penetrates the cell.

In one embodiment, the Ligand unit binds to the tumor cell or cancer cell.

In another embodiment, the Ligand unit binds to a tumor cell or cancer cell antigen which is on the surface of the tumor cell or cancer cell.

In another embodiment, the Ligand unit binds to a tumor cell or cancer cell antigen which is an extracellular matrix protein associated with the tumor cell or cancer cell.

The specificity of the Ligand unit for a particular tumor cell or cancer cell can be important for determining those tumors or cancers that are most effectively treated. For example, Exemplary Conjugates having a BR96 Ligand unit can be useful for treating antigen positive carcinomas including those of the lung, breast, colon, ovaries, and pancreas. Exemplary Conjugates having an Anti-CD30 or an anti-CD40 Ligand unit can be useful for treating hematologic malignancies.

Other particular types of cancers that can be treated with Exemplary Conjugates include, but are not limited to, those disclosed in Table 3.

TABLE 3

Solid tumors, including but not limited to:

fibrosarcoma
myxosarcoma
liposarcoma
chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma

	Ewing's tumor
	leiomyosarcoma
	rhabdomyosarcoma
5	colon cancer
	colorectal cancer
	kidney cancer
	pancreatic cancer
	bone cancer
10	breast cancer
	ovarian cancer
	prostate cancer
	esophageal cancer
	stomach cancer
	oral cancer
15	nasal cancer
	throat cancer
	squamous cell carcinoma
	basal cell carcinoma
20	adenocarcinoma
	sweat gland carcinoma
	sebaceous gland carcinoma
	papillary carcinoma
	papillary adenocarcinomas
25	cystadenocarcinoma
	medullary carcinoma
	bronchogenic carcinoma
	renal cell carcinoma
	hepatoma
30	bile duct carcinoma
	choriocarcinoma
	seminoma
	embryonal carcinoma
	Wilms' tumor
35	cervical cancer
	uterine cancer
	testicular cancer
	small cell lung carcinoma
	bladder carcinoma
40	lung cancer
	epithelial carcinoma
	glioma
	glioblastoma multiforme
	astrocytoma
45	medulloblastoma
	craniopharyngioma
	ependymoma
	pinealoma
	hemangioblastoma
	acoustic neuroma

	oligodendroglioma
	meningioma
	skin cancer
	melanoma
5	neuroblastoma
	retinoblastoma
	blood-borne cancers, including but not limited to:
	acute lymphoblastic leukemia "ALL"
10	acute lymphoblastic B-cell leukemia
	acute lymphoblastic T-cell leukemia
	acute myeloblastic leukemia "AML"
	acute promyelocytic leukemia "APL"
	acute monoblastic leukemia
15	acute erythroleukemic leukemia
	acute megakaryoblastic leukemia
	acute myelomonocytic leukemia
	acute nonlymphocytic leukemia
	acute undifferentiated leukemia
20	chronic myelocytic leukemia "CML"
	chronic lymphocytic leukemia "CLL"
	hairy cell leukemia
	multiple myeloma
	acute and chronic leukemias:
25	lymphoblastic
	myelogenous
	lymphocytic
	myelocytic leukemias
	Lymphomas:
30	Hodgkin's disease
	non-Hodgkin's Lymphoma
	Multiple myeloma
	Waldenström's macroglobulinemia
	Heavy chain disease
	Polycythemia vera
35	The Exemplary Conjugates provide conjugation-specific tumor or cancer targeting, thus reducing general toxicity of these compounds. The Linker units stabilize the Exemplary Conjugates in blood, yet are cleavable by tumor-specific proteases within the cell, liberating a Drug.

4.8.2 MULTI-MODALITY THERAPY FOR CANCER

Cancers, including, but not limited to, a tumor, metastasis, or other disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of an Exemplary Conjugate and/or an Exemplary Compound.

5 In other embodiments, methods for treating or preventing cancer are provided, including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and a chemotherapeutic agent. In one embodiment the chemotherapeutic agent is that with which treatment of the cancer has not been found to be refractory. In another embodiment, the chemotherapeutic agent is that with which the
10 treatment of cancer has been found to be refractory. The Exemplary Conjugates can be administered to a patient that has also undergone surgery as treatment for the cancer.

In one embodiment, the additional method of treatment is radiation therapy.

In a specific embodiment, the Exemplary Conjugate is administered
15 concurrently with the chemotherapeutic agent or with radiation therapy. In another specific embodiment, the chemotherapeutic agent or radiation therapy is administered prior or subsequent to administration of an Exemplary Conjugates, in one aspect at least an hour, five hours, 12 hours, a day, a week, a month, in further aspects several months (e.g., up to three months), prior or subsequent to administration of an Exemplary
20 Conjugate.

A chemotherapeutic agent can be administered over a series of sessions. Any one or a combination of the chemotherapeutic agents listed in Table 4 can be administered. With respect to radiation, any radiation therapy protocol can be used depending upon the type of cancer to be treated. For example, but not by way of
25 limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

30 Additionally, methods of treatment of cancer with an Exemplary Compound and/or Exemplary Conjugate are provided as an alternative to chemotherapy or radiation therapy where the chemotherapy or the radiation therapy has proven or can prove too toxic, e.g., results in unacceptable or unbearable side effects, for the subject

being treated. The animal being treated can, optionally, be treated with another cancer treatment such as surgery, radiation therapy or chemotherapy, depending on which treatment is found to be acceptable or bearable.

The Exemplary Compounds and/or Exemplary Conjugates can also be used in an *in vitro* or *ex vivo* fashion, such as for the treatment of certain cancers, including, but not limited to leukemias and lymphomas, such treatment involving autologous stem cell transplants. This can involve a multi-step process in which the animal's autologous hematopoietic stem cells are harvested and purged of all cancer cells, the animal's remaining bone-marrow cell population is then eradicated via the administration of a high dose of an Exemplary Compound and/or Exemplary Conjugate with or without accompanying high dose radiation therapy, and the stem cell graft is infused back into the animal. Supportive care is then provided while bone marrow function is restored and the animal recovers.

4.8.3 MULTI-DRUG THERAPY FOR CANCER

Methods for treating cancer including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and another therapeutic agent that is an anti-cancer agent are disclosed. Suitable anticancer agents include, but are not limited to, methotrexate, taxol, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, topotecan, nitrogen mustards, cytoxan, etoposide, 5-fluorouracil, BCNU, irinotecan, camptothecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel. In one aspect, the anti-cancer agent includes, but is not limited to, a drug listed in Table 4.

TABLE 4

Alkylating agents	
Nitrogen mustards:	cyclophosphamide ifosfamide trofosfamide chlorambucil melphalan
Nitrosoureas:	carmustine (BCNU) lomustine (CCNU)
Alkylsulphonates	busulfan

	treosulfan
Triazenes:	decabazine
Platinum containing compounds:	cisplatin carboplatin
Plant Alkaloids	
Vinca alkaloids:	vincristine vinblastine vindesine vinorelbine
Taxoids:	paclitaxel docetaxol
DNA Topoisomerase Inhibitors	
Epipodophyllins:	etoposide teniposide topotecan 9-aminocamptothecin camptothecin crisnatol
mitomycins:	mitomycin C
Anti-metabolites	
Anti-folates:	
DHFR inhibitors:	methotrexate trimetrexate
IMP dehydrogenase Inhibitors:	mycophenolic acid tiazofurin ribavirin EICAR
Ribonucleotide reductase Inhibitors:	hydroxyurea deferoxamine
Pyrimidine analogs:	
Uracil analogs	5-Fluorouracil
	floxuridine doxifluridine ratitrexed
Cytosine analogs	cytarabine (ara C) cytosine arabinoside fludarabine
Purine analogs:	mercaptopurine thioguanine
Hormonal therapies:	
Receptor antagonists:	
Anti-estrogen	tamoxifen raloxifene megestrol
LHRH agonists:	goserelin leuprolide acetate
Anti-androgens:	flutamide bicalutamide

Retinoids/Deltoids	
Vitamin D3 analogs:	EB 1089 CB 1093 KH 1060
Photodynamic therapies:	vertoporphin (BPD-MA) phthalocyanine photosensitizer Pc4 demethoxy-hypocrellin A (2BA-2-DMHA)
Cytokines:	Interferon- α Interferon- γ tumor necrosis factor
Others:	Gemcitabine Velcade Revamid Thalamid
Isoprenylation inhibitors:	Lovastatin
Dopaminergic neurotoxins:	1-methyl-4-phenylpyridinium ion
Cell cycle inhibitors:	staurosporine
Actinomycins:	Actinomycin D dactinomycin
Bleomycins:	bleomycin A2 bleomycin B2 peplomycin
Anthracyclines:	daunorubicin Doxorubicin (adriamycin) idarubicin epirubicin pirarubicin zorubicin mitoxantrone
MDR inhibitors:	verapamil
Ca ²⁺ ATPase inhibitors:	thapsigargin

4.8.4 TREATMENT OF AUTOIMMUNE DISEASES

The Exemplary Conjugates are useful for killing or inhibiting the replication of a cell that produces an autoimmune disease or for treating an autoimmune disease. The Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of an autoimmune disease in a patient. The Drug-Linker-Ligand Conjugates can be used to deliver a Drug to a target cell. Without being bound by theory, in one embodiment, the Drug-Linker-Ligand Conjugate associates with an antigen on the surface of a target cell, and the Exemplary Conjugate is then taken up inside a target-cell through receptor-mediated endocytosis. Once inside the cell, one or more specific

peptide sequences within the Linker unit are enzymatically or hydrolytically cleaved, resulting in release of a Drug. The released Drug is then free to migrate in the cytosol and induce cytotoxic or cytostatic activities. In an alternative embodiment, the Drug is cleaved from the Exemplary Conjugate outside the target cell, and the Drug subsequently penetrates the cell.

In one embodiment, the Ligand unit binds to an autoimmune antigen. In one aspect, the antigen is on the surface of a cell involved in an autoimmune condition.

In another embodiment, the Ligand unit binds to an autoimmune antigen which is on the surface of a cell.

In one embodiment, the Ligand binds to activated lymphocytes that are associated with the autoimmune disease state.

In a further embodiment, the Exemplary Conjugates kill or inhibit the multiplication of cells that produce an autoimmune antibody associated with a particular autoimmune disease.

Particular types of autoimmune diseases that can be treated with the Exemplary Conjugates include, but are not limited to, Th2 lymphocyte related disorders (*e.g.*, atopic dermatitis, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, and graft versus host disease); Th1 lymphocyte-related disorders (*e.g.*, rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, and tuberculosis); activated B lymphocyte-related disorders (*e.g.*, systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes); and those disclosed in Table 5.

TABLE 5

25	Active Chronic Hepatitis
	Addison's Disease
	Allergic Alveolitis
	Allergic Reaction
	Allergic Rhinitis
30	Alport's Syndrome
	Anaphlaxis
	Ankylosing Spondylitis
	Anti-phospholipid Syndrome
	Arthritis
35	Ascariasis
	Aspergillosis

	Atopic Allergy
	Atopic Dermatitis
	Atopic Rhinitis
	Behcet's Disease
5	Bird-Fancier's Lung
	Bronchial Asthma
	Caplan's Syndrome
	Cardiomyopathy
	Celiac Disease
10	Chagas' Disease
	Chronic Glomerulonephritis
	Cogan's Syndrome
	Cold Agglutinin Disease
	Congenital Rubella Infection
15	CREST Syndrome
	Crohn's Disease
	Cryoglobulinemia
	Cushing's Syndrome
	Dermatomyositis
20	Discoid Lupus
	Dressler's Syndrome
	Eaton-Lambert Syndrome
	Echovirus Infection
	Encephalomyelitis
25	Endocrine ophthalmopathy
	Epstein-Barr Virus Infection
	Equine Heaves
	Erythematosis
	Evan's Syndrome
30	Felty's Syndrome
	Fibromyalgia
	Fuch's Cyclitis
	Gastric Atrophy
	Gastrointestinal Allergy
35	Giant Cell Arteritis
	Glomerulonephritis
	Goodpasture's Syndrome
	Graft v. Host Disease
	Graves' Disease
40	Guillain-Barre Disease
	Hashimoto's Thyroiditis
	Hemolytic Anemia
	Henoch-Schonlein Purpura
	Idiopathic Adrenal Atrophy
45	Idiopathic Pulmonary Fibrosis
	IgA Nephropathy
	Inflammatory Bowel Diseases
	Insulin-dependent Diabetes Mellitus
	Juvenile Arthritis

	Juvenile Diabetes Mellitus (Type I)
	Lambert-Eaton Syndrome
	Laminitis
	Lichen Planus
5	Lupoid Hepatitis
	Lupus
	Lymphopenia
	Meniere's Disease
	Mixed Connective Tissue Disease
10	Multiple Sclerosis
	Myasthenia Gravis
	Pernicious Anemia
	Polyglandular Syndromes
	Presenile Dementia
15	Primary Agammaglobulinemia
	Primary Biliary Cirrhosis
	Psoriasis
	Psoriatic Arthritis
	Raynauds Phenomenon
20	Recurrent Abortion
	Reiter's Syndrome
	Rheumatic Fever
	Rheumatoid Arthritis
	Sampter's Syndrome
25	Schistosomiasis
	Schmidt's Syndrome
	Scleroderma
	Shulman's Syndrome
	Sjorgen's Syndrome
30	Stiff-Man Syndrome
	Sympathetic Ophthalmia
	Systemic Lupus Erythematosus
	Takayasu's Arteritis
	Temporal Arteritis
35	Thyroiditis
	Thrombocytopenia
	Thyrotoxicosis
	Toxic Epidermal Necrolysis
	Type B Insulin Resistance
40	Type I Diabetes Mellitus
	Ulcerative Colitis
	Uveitis
	Vitiligo
	Waldenstrom's Macroglobulemia
45	Wegener's Granulomatosis

4.8.5 MULTI-DRUG THERAPY OF AUTOIMMUNE DISEASES

Methods for treating an autoimmune disease are also disclosed including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and another therapeutic agent known for the treatment of an autoimmune disease. In one embodiment, the anti-autoimmune disease agent includes, but is not limited to, agents listed in Table 6.

Table 6

	cyclosporine
	cyclosporine A
	mycophenylate mofetil
	sirolimus
	tacrolimus
	enanercept
	prednisone
	azathioprine
	methotrexate cyclophosphamide
	prednisone
	aminocaproic acid
	chloroquine
	hydroxychloroquine
	hydrocortisone
	dexamethasone
	chlorambucil
	DHEA
	danazol
	bromocriptine
	meloxicam
	infliximab

4.8.6 TREATMENT OF INFECTIOUS DISEASES

The Exemplary Conjugates are useful for killing or inhibiting the multiplication of a cell that produces an infectious disease or for treating an infectious disease. The Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of an infectious disease in a patient. The Drug-Linker-Ligand Conjugates can be used to deliver a Drug to a target cell. In one embodiment, the Ligand unit binds to the infectious disease cell.

In one embodiment, the Conjugates kill or inhibit the multiplication of cells that produce a particular infectious disease.

Particular types of infectious diseases that can be treated with the Exemplary Conjugates include, but are not limited to, those disclosed in Table 7.

TABLE 7

Bacterial Diseases:

5	Diphtheria
	Pertussis
	Occult Bacteremia
	Urinary Tract Infection
	Gastroenteritis
10	Cellulitis
	Epiglottitis
	Tracheitis
	Adenoid Hypertrophy
	Retropharyngeal Abscess
15	Impetigo
	Ecthyma
	Pneumonia
	Endocarditis
	Septic Arthritis
20	Pneumococcal
	Peritonitis
	Bacteremia
	Meningitis
	Acute Purulent Meningitis
25	Urethritis
	Cervicitis
	Proctitis
	Pharyngitis
	Salpingitis
30	Epididymitis
	Gonorrhea
	Syphilis
	Listeriosis
	Anthrax
35	Nocardiosis
	Salmonella
	Typhoid Fever
	Dysentery
	Conjunctivitis
40	Sinusitis
	Brucellosis
	Tularemia
	Cholera
	Bubonic Plague
45	Tetanus

	Necrotizing Enteritis
	Actinomycosis
	Mixed Anaerobic Infections
	Syphilis
5	Relapsing Fever
	Leptospirosis
	Lyme Disease
	Rat Bite Fever
	Tuberculosis
10	Lymphadenitis
	Leprosy
	Chlamydia
	Chlamydial Pneumonia
	Trachoma
15	Inclusion Conjunctivitis

Systemic Fungal Diseases:

	Histoplasmosis
	Coccidioidomycosis
	Blastomycosis
20	Sporotrichosis
	Cryptococcosis
	Systemic Candidiasis
	Aspergillosis
	Mucormycosis
25	Mycetoma
	Chromomycosis

Rickettsial Diseases:

	Typhus
	Rocky Mountain Spotted Fever
30	Ehrlichiosis
	Eastern Tick-Borne Rickettsioses
	Rickettsialpox
	Q Fever
	Bartonellosis

35 Parasitic Diseases:

	Malaria
	Babesiosis
	African Sleeping Sickness
	Chagas' Disease
40	Leishmaniasis
	Dum-Dum Fever
	Toxoplasmosis
	Meningoencephalitis
	Keratitis

	Entamebiasis
	Giardiasis
	Cryptosporidiasis
5	Isosporiasis
	Cyclosporiasis
	Microsporidiosis
	Ascariasis
	Whipworm Infection
	Hookworm Infection
10	Threadworm Infection
	Ocular Larva Migrans
	Trichinosis
	Guinea Worm Disease
	Lymphatic Filariasis
15	Loiasis
	River Blindness
	Canine Heartworm Infection
	Schistosomiasis
	Swimmer's Itch
20	Oriental Lung Fluke
	Oriental Liver Fluke
	Fascioliasis
	Fasciolopsiasis
	Opisthorchiasis
25	Tapeworm Infections
	Hydatid Disease
	Alveolar Hydatid Disease

Viral Diseases:

	Measles
30	Subacute sclerosing panencephalitis
	Common Cold
	Mumps
	Rubella
	Roseola
35	Fifth Disease
	Chickenpox
	Respiratory syncytial virus infection
	Croup
	Bronchiolitis
40	Infectious Mononucleosis
	Poliomyelitis
	Herpangina
	Hand-Foot-and-Mouth Disease
	Bornholm Disease
45	Genital Herpes
	Genital Warts
	Aseptic Meningitis

	Myocarditis
	Pericarditis
	Gastroenteritis
	Acquired Immunodeficiency Syndrome (AIDS)
5	Human Immunodeficiency Virus (HIV)
	Reye's Syndrome
	Kawasaki Syndrome
	Influenza
	Bronchitis
10	Viral "Walking" Pneumonia
	Acute Febrile Respiratory Disease
	Acute pharyngoconjunctival fever
	Epidemic keratoconjunctivitis
	Herpes Simplex Virus 1 (HSV-1)
15	Herpes Simplex Virus 2 (HSV-2)
	Shingles
	Cytomegalic Inclusion Disease
	Rabies
	Progressive Multifocal Leukoencephalopathy
20	Kuru
	Fatal Familial Insomnia
	Creutzfeldt-Jakob Disease
	Gerstmann-Straussler-Scheinker Disease
	Tropical Spastic Paraparesis
25	Western Equine Encephalitis
	California Encephalitis
	St. Louis Encephalitis
	Yellow Fever
	Dengue
30	Lymphocytic choriomeningitis
	Lassa Fever
	Hemorrhagic Fever
	Hantavirus Pulmonary Syndrome
	Marburg Virus Infections
35	Ebola Virus Infections
	Smallpox

4.8.7 MULTI-DRUG THERAPY OF INFECTIOUS DISEASES

- Methods for treating an infectious disease are disclosed including
- 40 administering to a patient in need thereof an Exemplary Conjugate and another therapeutic agent that is an anti-infectious disease agent. In one embodiment, the anti-infectious disease agent is, but not limited to, agents listed in Table 8.

TABLE 8

β -Lactam Antibiotics:

5	Penicillin G
	Penicillin V
	Cloxacillin
	Dicloxacillin
	Methicillin
10	Nafcillin
	Oxacillin
	Ampicillin
	Amoxicillin
	Bacampicillin
15	Azlocillin
	Carbenicillin
	Mezlocillin
	Piperacillin
	Ticarcillin

Aminoglycosides:

20	Amikacin
	Gentamicin
	Kanamycin
	Neomycin
	Netilmicin
25	Streptomycin
	Tobramycin

Macrolides:

30	Azithromycin
	Clarithromycin
	Erythromycin
	Lincomycin
	Clindamycin

Tetracyclines:

35	Demeclocycline
	Doxycycline
	Minocycline
	Oxytetracycline
	Tetracycline

Quinolones:

40	Cinoxacin
	Nalidixic Acid

Fluoroquinolones:

	Ciprofloxacin
	Enoxacin
	Grepafloxacin
5	Levofloxacin
	Lomefloxacin
	Norfloxacin
	Ofloxacin
	Sparfloxacin
10	Trovafloxacin

Polypeptides:

	Bacitracin
	Colistin
	Polymyxin B

15 Sulfonamides:

	Sulfisoxazole
	Sulfamethoxazole
	Sulfadiazine
	Sulfamethizole
20	Sulfacetamide

Miscellaneous Antibacterial Agents:

	Trimethoprim
	Sulfamethazole
	Chloramphenicol
25	Vancomycin
	Metronidazole
	Quinupristin
	Dalfopristin
	Rifampin
30	Spectinomycin
	Nitrofurantoin
	Antiviral Agents:

General Antiviral Agents:

	Idoxuradine
35	Vidarabine
	Trifluridine
	Acyclovir
	Famciclovir
	Penciclovir
40	Valacyclovir
	Ganciclovir

5 Foscarnet
Ribavirin
Amantadine
Rimantadine
Cidofovir
Antisense Oligonucleotides
Immunoglobulins
Interferons

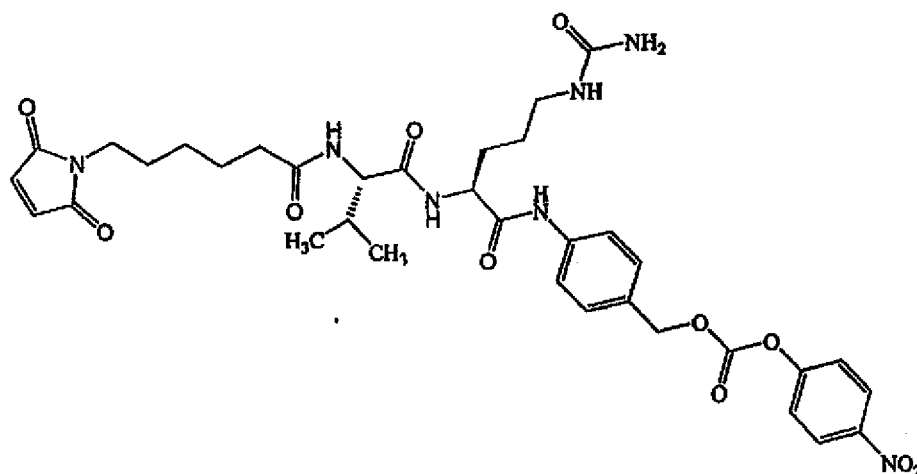
Drugs for HIV infection:

10 Tenofovir
Emtricitabine
Zidovudine
Didanosine
Zalcitabine
15 Stavudine
Lamivudine
Nevirapine
Delavirdine
Saquinavir
20 Ritonavir
Indinavir
Nelfinavir

5. EXAMPLES

25

Example 1 - Preparation of compound AB



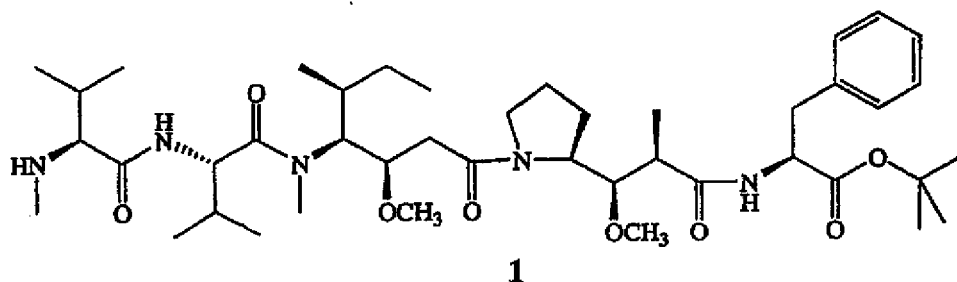
AB

Fmoc-val-cit-PAB-OH (14.61 g, 24.3 mmol, 1.0 eq., U.S. Patent No. 6214345 to Firestone *et al.*) was diluted with DMF (120 mL, 0.2 M) and to this solution was added a diethylamine (60 mL). The reaction was monitored by HPLC and found to be complete in 2 h. The reaction mixture was concentrated and the resulting residue was precipitated using ethyl acetate (ca. 100 mL) under sonication over for 10 min. Ether (200 mL) was added and the precipitate was further sonicated for 5 min. The solution was allowed to stand for 30 min. without stirring and was then filtered and dried under high vacuum to provide Val-cit-PAB-OH, which was used in the next step without further purification. Yield: 8.84 g (96%). Val-cit-PAB-OH (8.0 g, 21 mmol) was diluted with DMF (110 mL) and the resulting solution was treated with MC-OSu (Willner *et al.*, (1993) Bioconjugate Chem. 4:521; 6.5 g, 21 mmol, 1.0 eq.). Reaction was complete according to HPLC after 2 h. The reaction mixture was concentrated and the resulting oil was precipitated using ethyl acetate (50 mL). After sonicating for 15 min, ether (400 mL) was added and the mixture was sonicated further until all large particles were broken up. The solution was then filtered and the solid dried to provide an off-white solid intermediate. Yield: 11.63 g (96%); ES-MS m/z 757.9 [M-H]

Fmoc-val-cit-PAB-OH (14.61 g, 24.3 mmol, 1.0 eq., U.S. Patent No. 6214345 to Firestone *et al.*) was diluted with DMF (120 mL, 0.2 M) and to this solution was added a diethylamine (60 mL). The reaction was monitored by HPLC and found to be complete in 2 h. The reaction mixture was concentrated and the resulting residue was precipitated using ethyl acetate (ca. 100 mL) under sonication over for 10 min. Ether (200 mL) was added and the precipitate was further sonicated for 5 min. The solution was allowed to stand for 30 min. without stirring and was then filtered and dried under high vacuum to provide Val-cit-PAB-OH, which was used in the next step without further purification. Yield: 8.84 g (96%). Val-cit-PAB-OH (8.0 g, 21 mmol) was diluted with DMF (110 mL) and the resulting solution was treated with MC-OSu (Willner *et al.*, (1993) Bioconjugate Chem. 4:521; 6.5 g, 21 mmol, 1.0 eq.). Reaction was complete according to HPLC after 2 h. The reaction mixture was concentrated and the resulting oil was precipitated using ethyl acetate (50 mL). After sonicating for 15 min, ether (400 mL) was added and the mixture was sonicated further until all large particles were broken up. The solution was then filtered and the solid dried to provide an off-white solid intermediate. Yield: 11.63 g (96%); ES-MS m/z 757.9 [M-H].

The off-white solid intermediate (8.0 g, 14.0 mmol) was diluted with DMF (120 mL, 0.12 M) and to the resulting solution was added bis(4-nitrophenyl)carbonate (8.5 g, 28.0 mmol, 2.0 eq.) and DIEA (3.66 mL, 21.0 mmol, 1.5 eq.). The reaction was complete in 1 h according to HPLC. The reaction mixture was concentrated to provide an oil that was precipitated with EtOAc, and then triturated with EtOAc (ca. 25 mL). The solute was further precipitated with ether (ca. 200 mL) and triturated for 15 min. The solid was filtered and dried under high vacuum to provide Compound AB which was 93% pure according to HPLC and used in the next step without further purification. Yield: 9.7 g (94%).

Example 2 - Preparation of compound 1



Phenylalanine *t*-butyl ester HCl salt (868 mg, 3 mmol), *N*-Boc-Dolaproine (668 mg, 1 eq.), DEPC (820 μ L, 1.5 eq.), and DIEA (1.2 mL) were diluted with dichloromethane (3 mL). After 2 hours (h) at room temperature (about 28 degrees Celsius), the reaction mixture was diluted with dichloromethane (20 mL), washed successively with saturated aqueous (aq.) NaHCO_3 (2 x 10 mL), saturated aq. NaCl (2 x 10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography in ethyl acetate. The relevant fractions were combined and concentrated to provide the dipeptide as a white solid: 684 mg (46 %). ES-MS m/z 491.3 $[\text{M}+\text{H}]^+$.

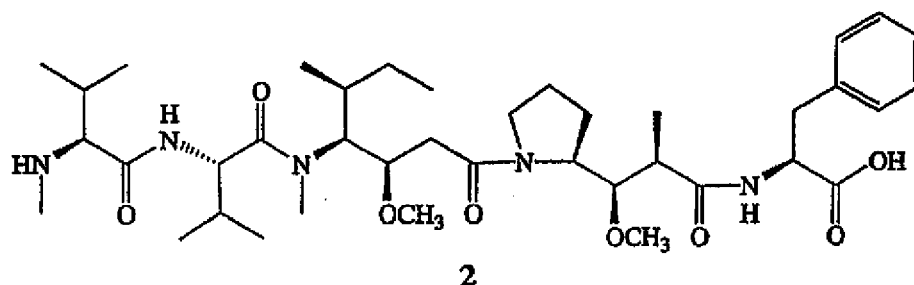
For selective Boc cleavage in the presence of *t*-butyl ester, the above dipeptide (500 mg, 1.28 mmol) was diluted with dioxane (2 mL). 4M HCl/dioxane (960 μ L, 3 eq.) was added, and the reaction mixture was stirred overnight at room temperature. Almost complete Boc deprotection was observed by RP-HPLC with minimal amount of *t*-butyl ester cleavage. The mixture was cooled down on an ice bath, and triethylamine (500 μ L) was added. After 10 min., the mixture was removed from the cooling bath, diluted

with dichloromethane (20 mL), washed successively with saturated aq. NaHCO_3 (2 x 10 mL), saturated aq. NaCl (2 x 10 mL). The organic layer was concentrated to give a yellow foam: 287 mg (57 %). The intermediate was used without further purification.

The tripeptide Fmoc-Meval-val-dil-O-t-Bu (prepared as described in WO 02/088172, entitled "*Pentapeptide Compounds and Uses Related Thereto*"; 0.73 mmol) was treated with TFA (3 mL), dichloromethane (3 mL) for 2 h at room temperature. The mixture was concentrated to dryness, the residue was co-evaporated with toluene (3 x 20 mL), and dried in vacuum overnight. The residue was diluted with dichloromethane (5 mL) and added to the deprotected dipeptide (287 mg, 0.73 mmol), followed by DIEA (550 μL , 4 eq.), DEPC (201 μL , 1.1 eq.). After 2 h at room temperature the reaction mixture was diluted with ethyl acetate (50 mL), washed successively with 10% aq. citric acid (2 x 20 mL), saturated aq. NaHCO_3 (2 x 10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography in ethyl acetate. The relevant fractions were combined and concentrated to provide Fmoc-Meval-val-dil-dap-phe-O-t-Bu as a white solid: 533 mg (71 %). R_f 0.4 (EtOAc). ES-MS m/z 1010.6 $[\text{M}+\text{H}]^+$.

The product (200 mg, 0.2 mmol) was diluted with dichloromethane (3 mL), diethylamine (1 mL). The reaction mixture was stirred overnight at room temperature. Solvents were removed to provide an oil that was purified by flash silica gel chromatography in a step gradient 0-10 % MeOH in dichloromethane to provide Compound 1 as a white solid: 137 mg (87 %). R_f 0.3 (10 % MeOH/ CH_2Cl_2). ES-MS m/z 788.6 $[\text{M}+\text{H}]^+$.

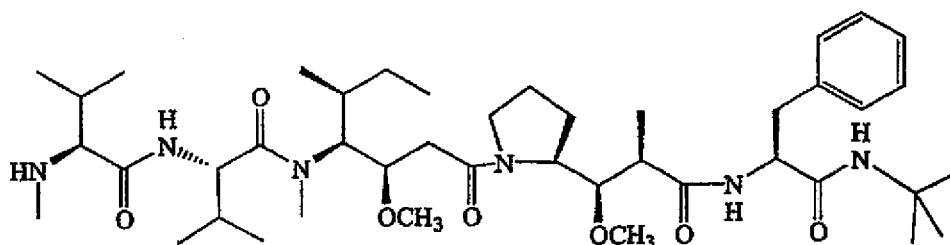
Example 3 - Preparation of compound 2



Compound 2 was prepared from compound 1 (30 mg, 0.038 mmol) by treatment with 4M HCl /dioxane (4 mL) for 7 h at room temperature. The solvent was removed, and the residue was dried in a vacuum overnight to give provide Compound 2

as a hygroscopic white solid: 35 mg (120 % calculated for HCl salt). ES-MS m/z 732.56 $[M+H]^+$.

Example 4 - Preparation of compound 3

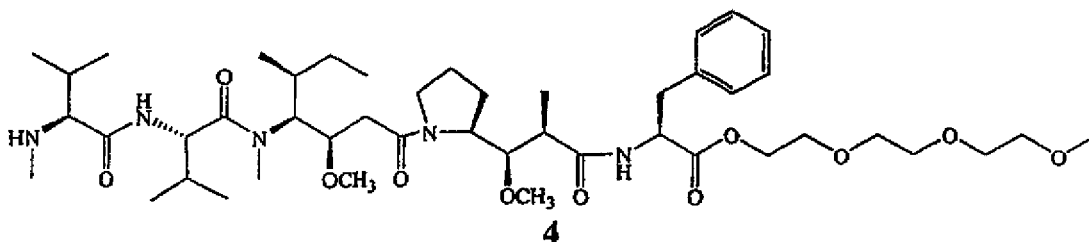


Fmoc-Meal-val-dil-dap-phe-*O*-*t*-Bu (Example 2, 50 mg) was treated with 4M HCl/dioxane (4 ml) for 16 h at room temperature. The solvent was removed, and the residue was dried in vacuum overnight to give 50 mg of a hygroscopic white solid intermediate

The white solid intermediate (20 mg, 0.02 mmol) was diluted with dichloromethane (1 mL); DEPC (5 μ L, 0.03 mmol, 1.5 eq.) was added followed by DIEA (11 μ L, 0.06 mmol, 3 eq.), and *t*-butylamine (3.2 μ L, 0.03 mmol, 1.5 eq.). After 2 h at room temperature, the reaction was found to be uncompleted by RP-HPLC. More DEPC (10 μ L) and *t*-butylamine (5 μ L) were added and the reaction was stirred for additional 4 h. Reaction mixture was diluted with dichloromethane (15 mL), washed successively with water (5 mL), 0.1 M aq. HCl (10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was diluted with dichloromethane and purified via flash chromatography in a step gradient 0–5 % MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide the Fmoc protected intermediate as a white solid: 7.3 mg (36 %). R_f 0.75 (10 % MeOH/ CH_2Cl_2).

Fmoc protected intermediate was diluted with dichloromethane (0.5 mL) and treated with diethylamine (0.5 mL) for 3 h at room temperature. The reaction mixture was concentrated to dryness. The product was isolated by flash silica gel chromatography in a step gradient 0–10 % MeOH in dichloromethane to provide Compound 3 as a white solid: 4 mg (70 %). R_f 0.2 (10 % MeOH/ CH_2Cl_2). ES-MS m/z 787 $[M+H]^+$, 809 $[M+Na]^+$.

Example 5 - Preparation of compound 4



5 Boc-L-Phenylalanine (265 mg, 1 mmol, 1 eq.) and triethyleneglycol monomethyl ether (164 μ L, 1 mmol, 1 eq.) were diluted with dichloromethane (5 mL). Then, DCC (412 mg, 2 mmol, 2 eq.) was added, followed by DMAP (10 mg). The reaction mixture was stirred overnight at room temperature. The precipitate was filtered off. The solvent was removed in a vacuum, the residue was diluted with ethyl acetate, and purified by silica gel flash chromatography in ethyl acetate. The product containing
10 fractions were pulled, concentrated, and dried in vacuum to give a white solid: 377 mg (91 %). R_f 0.5 (EtOAc). ES-MS m/z 434 $[M+Na]^+$.

 Removal of Boc protecting group was performed by treatment of the above material in dioxane (10 mL) with 4M HCl/dioxane (6 mL) for 6 h at room
15 temperature. The solvent was removed in a vacuum, the residue was dried in a vacuum to give a white solid.

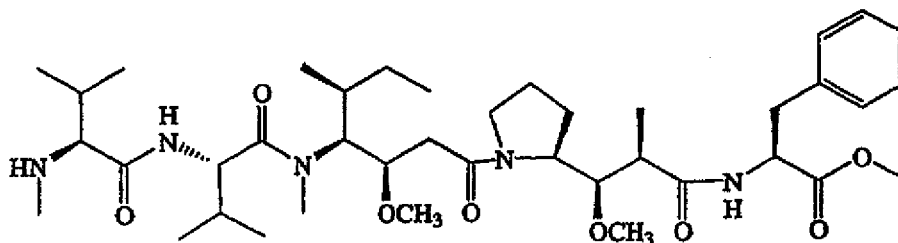
 The HCl salt of Phenylalanine-triethyleneglycol monomethyl ether ester (236 mg, 0.458 mmol, 1eq.) and *N*-Boc-Dolaproine (158 mg, 0.55 mmol, 1.2 eq.) were diluted with dichloromethane (3 mL). DEPC (125 μ L, 1.5 eq.) and added to the mixture
20 followed by DIEA (250 μ L, 3 eq.). After 2 h at room temperature the reaction mixture was diluted with ethyl acetate (30 mL), washed successively with saturated aq. $NaHCO_3$ (2 x 10 mL), 10% aq. citric acid (2 x 10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography on silica gel in ethyl acetate. The
25 relevant fractions were combined and concentrated to provide a white foam intermediate: 131 mg (50 %). R_f 0.25 (EtOAc). ES-MS m/z 581.3 $[M+H]^+$.

 Boc deprotection was done in dichloromethane (2 mL), TFA (0.5 mL) at room temperature for 2 h. Solvent was removed in vacuum, and the residue was co-evaporated with toluene (3 x 25 mL), then dried in vacuum to give 138 mg of dipeptide
30 TFA salt.

Fmoc-Meval-val-dil-OH (Example 2, 147 mg, 0.23 mmol, 1 eq.), and dipeptide TFA salt (138 mg) were diluted with dichloromethane (2 mL). To the mixture DEPC (63 μ L, 1.5 eq.) was added, followed by DIEA (160 μ L, 4 eq.). After 2 h at room temperature the reaction mixture was diluted with dichloromethane (30 mL), washed successively with 10% aq. citric acid (2 x 20 mL), saturated aq. NaCl (20 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in dichloromethane and was purified via flash chromatography on silica gel in a step gradient 0-5 % MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide white foam: 205 mg (81 %). R_f 0.4 (10 % MeOH/ CH_2Cl_2). ES-MS m/z 1100.6 $[\text{M}+\text{H}]^+$, 1122.4 $[\text{M}+\text{Na}]^+$.

Fmoc protecting group was removed by treatment with diethylamine (2 mL) in dichloromethane (6 mL). After 6 h at room temperature solvent was removed in vacuum, product was isolated by flash chromatography on silica gel in a step gradient 0-10 % MeOH in dichloromethane. The relevant fractions were combined and concentrated. After evaporation from dichloromethane/hexane, 1:1, Compound 4 was obtained as a white foam: 133 mg (80 %). R_f 0.15 (10% MeOH/ CH_2Cl_2). ES-MS m/z 878.6 $[\text{M}+\text{H}]^+$.

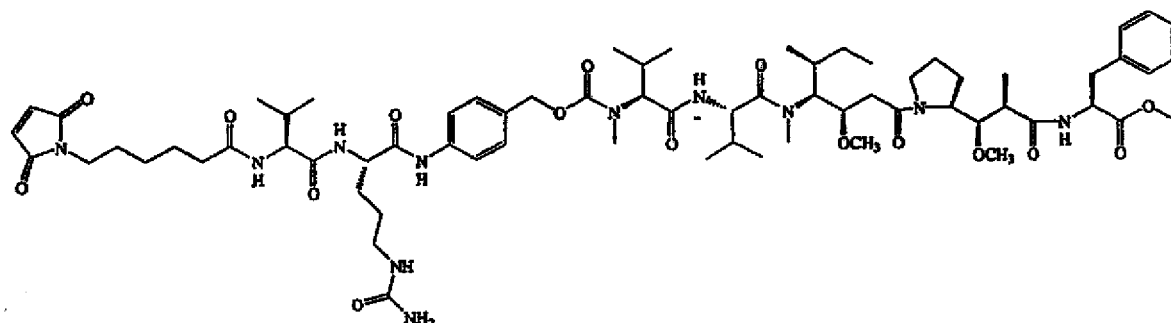
Example 6 - Preparation of compound 5



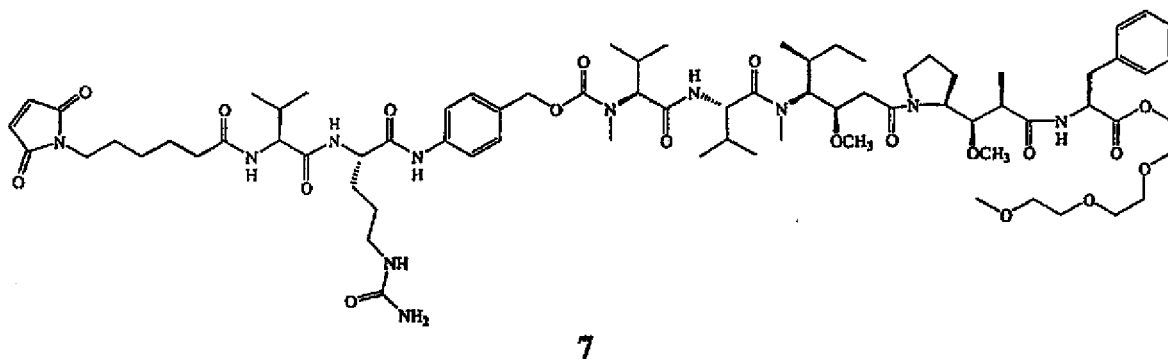
Fmoc-Meval-val-dil-OH (Example 2, 0.50 g, 0.78 mmol) and dap-phe-OMe-HCl (0.3 g, 0.78 mmol, prepared according to Pettit, G.R., *et al. Anti-Cancer Drug Design* 1998, 13, 243-277) were dissolved in CH_2Cl_2 (10 mL) followed by the addition of diisopropylethylamine (0.30 mL, 1.71 mmol, 2.2 eq.). DEPC (0.20 mL, 1.17, 1.5 eq.) was added and the contents stood over Ar. Reaction was complete according to HPLC in 1 h. The mixture was concentrated to an oil and purified by SiO_2 chromatography (300 x 25 mm column) and eluting with 100 % EtOAc. The product was isolated as a white foamy solid. Yield: 0.65 g (87 %). ES-MS m/z 968.35 $[\text{M}+\text{H}]^+$, 991.34 $[\text{M}+\text{Na}]^+$; UV λ_{max} 215, 265 nm.

The Fmoc-protected peptide (0.14 g, 0.14 mmol) in methylene chloride (5 mL) was treated with diethylamine (2 mL) and the contents stood at room temperature for 2 h. The reaction, complete by HPLC, was concentrated to an oil, taken up in 2 mL of DMSO and injected into a preparative-HPLC (C₁₂-RP column, 5 μ , 100 Å, linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100 % in 40 min followed by 20 min at 100 %, at a flow rate of 25 mL/min). Fractions containing the product were evaporated to afford a white powder for the trifluoroacetate salt. Yield: 0.126 g (98 %). *R_f* 0.28 (100 % EtOAc); ES-MS *m/z* 746.59 [M+H]⁺, 768.51 [M+Na]⁺; UV λ_{max} 215 nm.

Example 7 - Preparation of compound 6

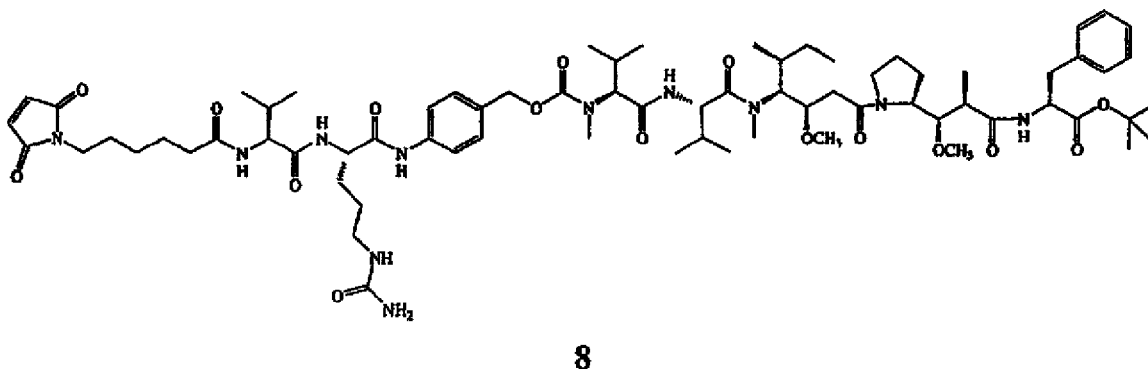


Example 8 - Preparation of compound 7



Compound 4 (133 mg, 0.15 mmol, 1 eq.), Compound AB, (123 mg, 0.167 mmol, 1.1 eq.), and HOBt (4 mg, 0.2 eq.) were diluted with DMF (1.5 mL). After 2 min, pyridine (5 mL) was added and the reaction was monitored using RP-HPLC. The reaction was shown to be complete within 18 h. The reaction mixture was diluted with dichloromethane (20 mL), washed successively with 10 % aq. citric acid (2 x 10 mL), water (10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in dichloromethane and was purified via flash chromatography on silica gel in a step gradient 0-10% MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide Compound 7 as a white foam: 46 mg (21 %). R_f 0.15 (10 % MeOH/ CH_2Cl_2). ES-MS m/z 1476.94 $[\text{M}+\text{H}]^+$.

Example 9 - Preparation of MC-Val-Cit-PAB-MMAF t-butyl ester 8

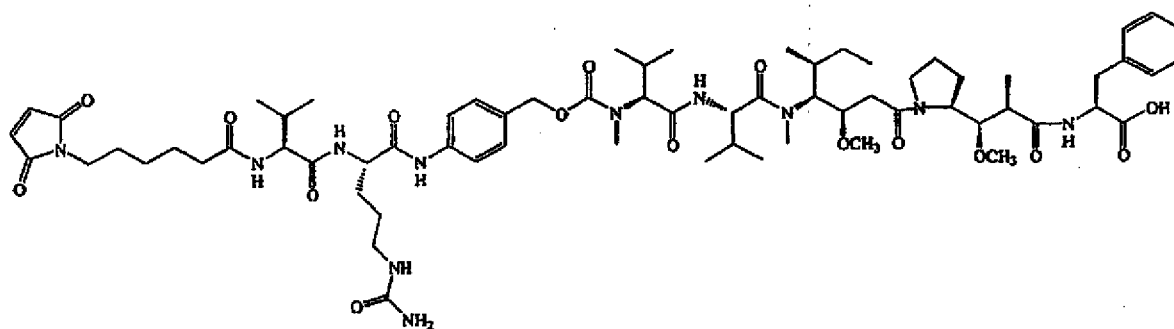


Compound **1** (83 mg, 0.11 mmol), Compound **AB** (85 mg, 0.12 mmol, 1.1 eq.), and HOBT (2.8 mg, 21 μ mol, 0.2 eq.) were taken up in dry DMF (1.5 mL) and pyridine (0.3 mL) while under argon. After 30 h, the reaction was found to be essentially complete by HPLC. The mixture was evaporated, taken up in a minimal amount of

DMSO and purified by prep-HPLC (C₁₂-RP column, 5 μ , 100 Å, linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100 % in 40 min followed by 20 min at 100 %, at a flow rate of 25 mL/min) to provide Compound 8 as a white solid. Yield: 103 mg (71%). ES-MS m/z 1387.06 [M+H]⁺, 1409.04 [M+Na]⁺; UV λ_{max} 205, 248 nm.

5

Example 10 - Preparation of MC-val-cit-PAB-MMAF 9

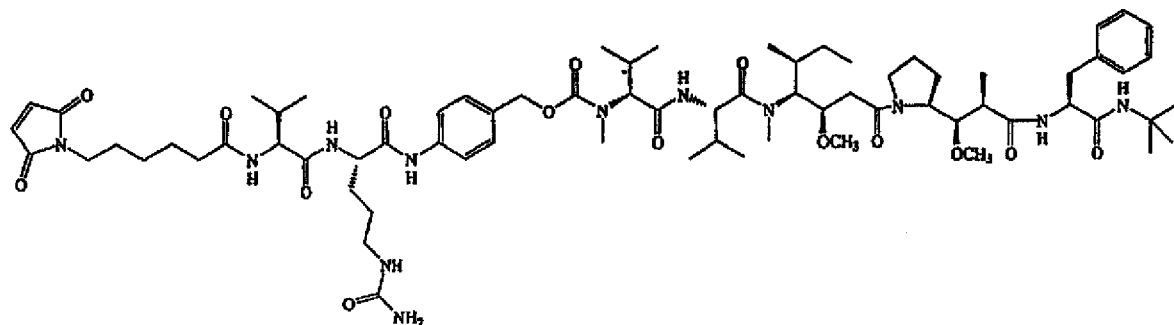


9

10 Compound 8 (45 mg, 32 μ mol) was suspended in methylene chloride (6 mL) followed by the addition of TFA (3 mL). The resulting solution stood for 2 h. The reaction mixture was concentrated *in vacuo* and purified by prep-HPLC (C₁₂-RP column, 5 μ , 100 Å, linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100 % in 40 min followed by 20 min at 100 %, at a flow rate of 25 mL/min). The desired fractions
15 were concentrated to provide maleimidocaproyl-valine-citrulline-p-hydroxymethylaminobenzene-MMAF (MC-val-cit-PAB-MMAF) 9 as an off-white solid. Yield: 11 mg (25%). ES-MS m/z 1330.29 [M+H]⁺, 1352.24 [M+Na]⁺; UV λ_{max} 205, 248 nm.

Example 11 - Preparation of MC-val-cit-PAB-MMAF tert-butyl amide 10

20



10

Compound 3 (217 mg, 0.276 mmol, 1.0 eq.), Compound AB (204 mg, 0.276 mmol, 1.0 eq.), and HOBt (11 mg, 0.0828 mmol, 0.3 eq.) were diluted with pyridine/DMF (6 mL). To this mixture was added DIEA (0.048 mL), and the mixture was stirred ca. 16 hr. Volatile organics were evaporated *in vacuo*. The crude residue was purified by Chromatotron® (radial thin-layer chromatography) with a step gradient (0-5-10% methanol in DCM) to provide MC-val-cit-PAB-MMAF tert-butyl amide 10. Yield: 172 mg (45 %); ES-MS m/z 1386.33 $[M+H]^+$, 1408.36 $[M+Na]^+$; UV λ_{max} 215, 248 nm.

Example 12 - Preparation of AC10-MC-MMAE by conjugation of AC10 and MC-MMAE

AC10, dissolved in 500 mM sodium borate and 500 mM sodium chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT). After incubation at 37 °C for about 30 minutes, the buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm. The reduced antibody dissolved in PBS is chilled on ice.

The drug linker reagent, maleimidocaproyl-monomethyl auristatin E, *i.e.* MC-MMAE, dissolved in DMSO, is diluted in acetonitrile and water at known concentration, and added to the chilled reduced antibody AC10 in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and AC10-MC-MMAE is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μ m filters under sterile conditions, and frozen for storage.

Example 13 - Preparation of AC10-MC-MMAF by conjugation of AC10 and MC-MMAF

AC10-MC-MMAF was prepared by conjugation of AC10 and MC-MMAF following the procedure of Example 12.

Example 14 - Preparation of AC10-MC- val-cit-PAB-MMAE by conjugation of AC10 and MC-val-cit-PAB-MMAE

AC10-MC-val-cit-PAB-MMAE was prepared by conjugation of AC10 and MC-val-cit-PAB-MMAE following the procedure of Example 12.

Example 15 - Preparation of AC10-MC- val-cit-PAB-MMAF by conjugation of AC10 and MC-val-cit-PAB-MMAF (9)

AC10-MC-val-cit-PAB-MMAF was prepared by conjugation of AC10 and MC-val-cit-PAB-MMAF (9) following the procedure of Example 12.

5

Example 16 - Determination of cytotoxicity of selected compounds

Cytotoxic activity of MMAF and Compounds 1-5 was evaluated on the Lewis Y positive cell lines OVCAR-3, H3396 breast carcinoma, L2987 lung carcinoma and LS174t colon carcinoma Lewis Y positive cell lines can be assayed for cytotoxicity.

10 To evaluate the cytotoxicity of Compounds 1-5, cells can be seeded at approximately 5 - 10,000 per well in 150 µl of culture medium then treated with graded doses of Compounds 1-5 in quadruplicates at the initiation of assay. Cytotoxicity assays are usually carried out for 96 hours after addition of test compounds. Fifty µl of resazurin dye may be added to each well during the last 4 to 6 hours of the incubation to assess viable
15 cells at the end of culture. Dye reduction can be determined by fluorescence spectrometry using the excitation and emission wavelengths of 535nm and 590nm, respectively. For analysis, the extent of resazurin reduction by the treated cells can be compared to that of the untreated control cells.

For 1 h exposure assays cells can be pulsed with the drug for 1 h and then
20 washed; the cytotoxic effect can be determined after 96 h of incubation.

EXAMPLE 17 - *in vitro* cytotoxicity data for selected compounds

Table 10 shows cytotoxic effect of cAC10 Conjugates of Compounds 7-
10, assayed as described in General Procedure I on a CD30+ cell line Karpas 299. Data of two separate experiments are presented. The cAC10 conjugates of Compounds 7 and 9
25 were found to be slightly more active than cAC10-val-cit-MMAE.

TABLE 10

Conjugate	IC ₅₀ (ng/mL)
cAC10-val-cit-MMAE	6
cAC10-7	1.0
cAC10-8	15
cAC10-9	0.5
cAC10-10	20

In other experiments, BR96-val-cit-MMAF was at least 250 fold more potent than the free MMAF.

General Procedure I - Cytotoxicity determination. To evaluate the cytotoxicity of Exemplary Conjugates 7-10, cells were seeded at approximately 5 -
5 10,000 per well in 150 µl of culture medium then treated with graded doses of Exemplary Conjugates 7-10 in quadruplicates at the initiation of assay. Cytotoxicity assays were carried out for 96 hours after addition of test compounds. Fifty µl of the resazurin dye was added to each well during the last 4 to 6 hours of the incubation to assess viable cells at the end of culture. Dye reduction was determined by fluorescence spectrometry using the
10 excitation and emission wavelengths of 535nm and 590nm, respectively. For analysis, the extent of resazurin reduction by the treated cells was compared to that of the untreated control cells.

Example 18 - *In vitro* cell proliferation assay

Efficacy of ADC can be measured by a cell proliferation assay employing
15 the following protocol (Promega Corp. Technical Bulletin TB288; Mendoza *et al.* (2002) Cancer Res. 62:5485-5488):

1. An aliquot of 100 µl of cell culture containing about 10^4 cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.
- 20 2. Control wells were prepared containing medium and without cells.
3. ADC was added to the experimental wells and incubated for 3-5 days.
4. The plates were equilibrated to room temperature for approximately 30 minutes.
5. A volume of CellTiter-Glo Reagent equal to the volume of cell culture medium present in each well was added.
- 25 6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.
7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.
8. Luminescence was recorded and reported in graphs as RLU = relative luminescence units.

30 Example19 - Plasma clearance in rat

Plasma clearance pharmacokinetics of antibody drug conjugates and total antibody was studied in Sprague-Dawley rats (Charles River Laboratories, 250-275 gms

each). Animals were dosed by bolus tail vein injection (IV Push). Approximately 300 µl whole blood was collected through jugular cannula, or by tail stick, into lithium/heparin anticoagulant vessels at each timepoint: 0 (predose), 10, and 30 minutes; 1, 2, 4, 8, 24 and 36 hours; and 2, 3, 4, 7, 14, 21, 28 days post dose. Total antibody was measured by
5 ELISA - ECD/GxhuFc-HRP. Antibody drug conjugate was measured by ELISA - MMAE/MMAF/ECD-Bio/SA-HRP.

Example 20 - Plasma clearance in monkey

Plasma clearance pharmacokinetics of antibody drug conjugates and total antibody can be studied in cynomolgus monkeys. Figure 12 shows a two-stage plasma
10 concentration clearance study after administration of H-MC-vc-MMAE to Cynomolgus monkeys at different doses: 0.5, 1.5, 2.5, and 3.0 mg/kg, administered at day 1 and day 21. Concentrations of total antibody and ADC were measured over time. (H = Trastuzumab).

Example 21 - Tumor volume *in vivo* efficacy in transgenic explant mice

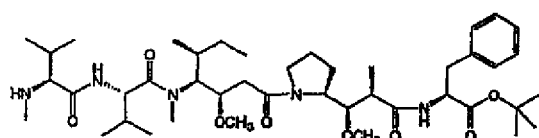
15 Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but FVB female mice are preferred because of their higher susceptibility to tumor formation. FVB males can be used for mating and vasectomized CD.1 studs can be used to stimulate pseudopregnancy. Vasectomized mice can be obtained from any commercial supplier.
20 Founders can be bred with either FVB mice or with 129/BL6 x FVB p53 heterozygous mice. The mice with heterozygosity at p53 allele can be used to potentially increase tumor formation. Some F1 tumors are of mixed strain. Founder tumors can be FVB only.

Animals having tumors (allograft propagated from Fo5 mmtv transgenic
25 mice) can be treated with a single or multiple dose by IV injection of ADC. Tumor volume can be assessed at various time points after injection.

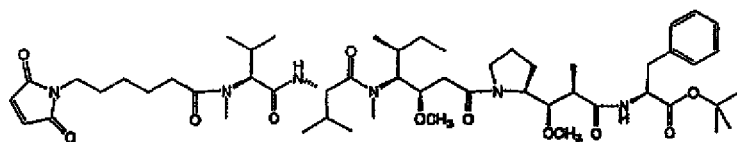
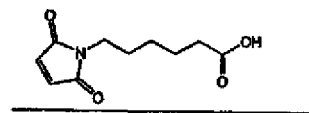
Example 22 - Synthesis of MC-MMAF via t-butyl ester

Synthesis 1:

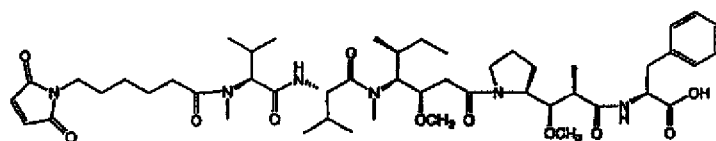
30



MeVal-Val-Dil-Dap-Phe-OtBu, 1001



MC-MeVal-Val-Dil-Dap-Phe-OtBu



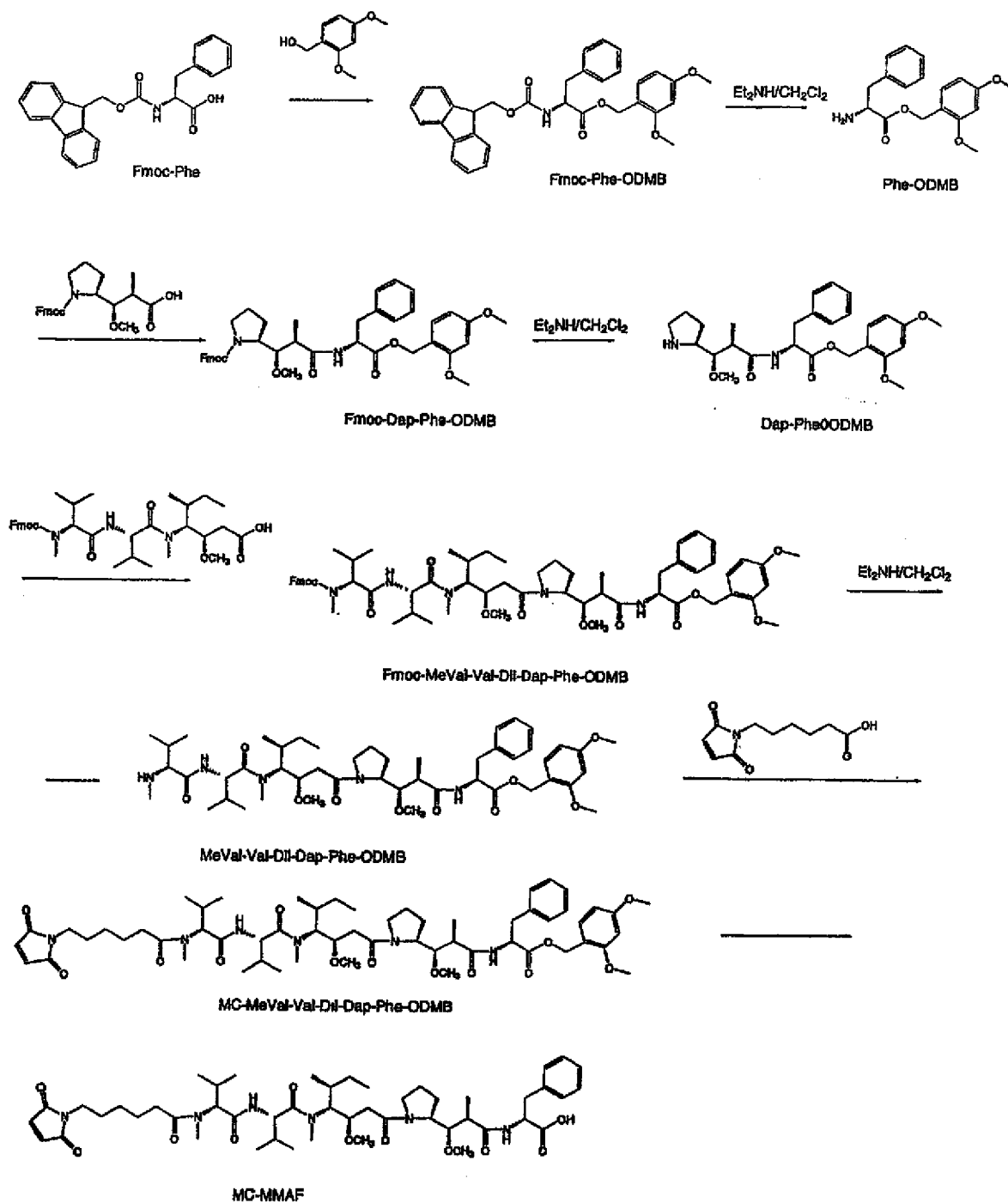
MC-MMAF

MeVal-Val-Dil-Dap-Phe-OtBu (compound 1, 128.6 mg, 0.163 mmol) was suspended in CH_2Cl_2 (0.500 mL). 6-Maleimidocaproic acid (68.9 mg, 0.326 mmol) and 1,3-diisopropylcarbodiimide (0.0505 mL, 0.326 mmol) were added followed by pyridine (0.500 mL). Reaction mixture was allowed to stir for 1.0 hr. HPLC analysis indicated complete consumption of starting compound 1. Volatile organics were evaporated under reduced pressure. Product was isolated via flash column chromatography, using a step gradient from 0 to 5% Methanol in CH_2Cl_2 . A total of 96 mg of pure MC-MeVal-Val-Dil-Dap-Phe-OtBu (12) (60% yield) was recovered. ES-MS m/z 981.26 $[\text{M}+\text{H}]^+$; 1003.47 $[\text{M}+\text{Na}]^+$; 979.65 $[\text{M}-\text{H}]^-$.

MC-MeVal-Val-Dil-Dap-Phe-OtBu (Compound 12, 74 mg, 0.0754 mmol) was suspended in CH_2Cl_2 (2.0 mL) and TFA (1 mL) at room temperature. After 2.5 hr, HPLC analysis indicated complete consumption of starting material. Volatile organics were evaporated under reduced pressure, and the product was isolated via preparatory RP-HPLC, using a Phenomenex C_{12} Synergi Max-RP 80Å Column (250 x 21.20 mm). Eluent: linear gradient 10% to 90% MeCN/0.05% TFA (aq) over 30 minutes, then isocratic 90% MeCN/0.05% TFA (aq) for an additional 20 minutes. ES-MS m/z 925.33 $[\text{M}+\text{H}]^+$; 947.30 $[\text{M}+\text{Na}]^+$; 923.45 $[\text{M}-\text{H}]^-$.

Example 23a - Synthesis of MC-MMAF (11) via dimethoxybenzyl ester

Synthesis 2:



5

Preparation of Fmoc-L-Phenylalanine-2,4-dimethoxybenzyl ester (Fmoc-Phe-ODMB)

A 3-neck, 5-L round-bottom flask was charged with Fmoc-L-Phenylalanine (200 g, 516 mmol Bachem), 2,4-dimethoxybenzyl alcohol (95.4 g, 567 mmol, Aldrich), and CH₂Cl₂ (2.0 L). N,N-dimethylformamide t-butyl acetal (155 mL, 586 mmol, Fluka) was added to the resulting suspension over 20 min under N₂, which resulted in a clear solution. The reaction was then stirred at room temperature overnight, after which time TLC analysis (0.42, Heptane/EtOAc = 2:1) indicated that the reaction was complete. The reaction mixture was concentrated under reduced pressure to give a light yellow oil, which was redissolved in CH₂Cl₂ (200 mL) and purified through a short plug of silica gel (25 cm × 25 cm, CH₂Cl₂) to give a colorless foam (250 g). MeCN (1 L) was added into the resulting foam, which totally dissolved the batch. It was then concentrated to dryness and redissolved in MeCN (1 L) and the resulting suspension was stirred for 1 h, filtered and the filter cake was rinsed with MeCN (2 × 200 mL) to give Fmoc-L-phenylalanine-2,4-dimethoxybenzyl ester as a white solid (113.58 g, 41%, 95.5% AUC by HPLC analysis). Data: HPLC.

Preparation L-Phenylalanine-2,4-dimethoxybenzyl ester (Phe-ODMB)

A 500-mL round-bottom flask was charged with Fmoc-L-phenylalanine-2,4-dimethoxybenzyl ester (26.00g, 48.3 mmol), CH₂Cl₂ (150 mL) and diethylamine (75 mL, Acros). Mixture was stirred at room temperature and the completion monitored by HPLC. After 4h, the mixture was concentrated (bath temp <30 °C). The residue was resuspended in CH₂Cl₂ (200 mL) and concentrated. This was repeated once. To the residue was added MeOH (20 mL), which caused the formation of a gel. This residue was diluted with CH₂Cl₂ (200 mL), concentrated and the cloudy oil left under vacuum overnight. The residue was suspended in CH₂Cl₂ (100 mL), then toluene (120mL) was added. The mixture was concentrated and the residue left under vacuum overnight.

Data: HPLC, ¹H NMR.

Preparation of Fmoc-Dolaproine (Fmoc-Dap)

Boc-Dolaproine (58.8 g, 0.205 mol) was suspended in 4 N HCl in 1,4-dioxane (256 mL, 1.02 mol, Aldrich). After stirring for 1.5 hours, TLC analysis indicated the reaction was complete (10% MeOH/CH₂Cl₂) and the mixture was concentrated to near-dryness. Additional 1,4-dioxane was charged (50 mL) and the mixture was concentrated to dryness and dried under vacuum overnight. The resulting white solid was dissolved in H₂O (400 mL) and transferred to a 3-L, three-neck, round-bottom flask with a mechanical stirrer and temperature probe. N,N-diisopropylethylamine (214.3 mL, 1.23

mol, Acros) was added over one minute, causing an exotherm from 20.5 to 28.2 °C (internal). The mixture was cooled in an ice bath and 1,4-dioxane was added (400 mL). A solution of Fmoc-OSu (89.90 g, 0.267 mol, Advanced ChemTech) in 1,4-dioxane (400 mL) was added from an addition funnel over 15 minutes, maintaining the reaction
5 temperature below 9 °C. The mixture was allowed to warm to room temperature and stir for 19 hours, after which the mixture was concentrated by rotary evaporation to an aqueous slurry (390 g). The suspension was diluted with H₂O (750 mL) and Et₂O (750 mL), causing a copious white precipitate to form. The layers were separated, keeping the solids with the organic layer. The aqueous layer was acidified using conc. HCl (30 mL)
10 and extracted with EtOAc (3 x 500 mL). The combined extracts were dried over MgSO₄, filtered and concentrated to give 59.25 g of a yellow oil A. The Et₂O extract was extracted once with sat. NaHCO₃ (200 mL), keeping the solids with the aqueous layer. The aqueous suspension was acidified using conc. HCl (50 mL) and extracted with Et₂O (50 mL) keeping the solids with the organic layer. The organic layer was filtered and
15 concentrated to give 32.33 g of a yellow oil B. The two oils (A and B) were combined and purified by flash chromatography on silica gel eluting with CH₂Cl₂ (3.5 L), then 3% MeOH/ CH₂Cl₂ (9 L) to give 68.23 g of Fmoc-dolaproine as a white foam (81%, 97.5% purity by HPLC (AUC)).

20 Preparation of Fmoc-Dap-Phe-ODMB

Crude Phe-ODMB (48.3 mmol) was suspended in anhydrous DMF (105 mL, Acros) for 5 minutes and Fmoc-Dap (19.80g, 48.3 mmol) was added. The mixture was cooled in an ice bath and TBTU (17.08 g, 53.20 mmol, Matrix Innovations) was added. N,N-diisopropylethylamine (25.3 mL, 145.0 mmol, Acros) was added via syringe
25 over 3 min. After 1h, the ice bath was removed and the mixture was allowed to warm over 30 min. The mixture was poured into water (1 L) and extracted with ethyl acetate (300 mL). After separation, the aqueous layer was re-extracted with ethyl acetate (2 x 150 mL). The combined organic layers were washed with brine (150 mL), dried (MgSO₄) and filtered (filter paper) to remove the insolubles (inorganics and some dibenzofulvene).
30 After concentration, the residue (41 g) was adsorbed on silica (41 g) and purified by chromatography (22 cm x 8 cm column; 65% Heptane/EtOAc (2.5 L); 33% Heptane/EtOAc (3.8 L), to give 29.4 g of product as a white foam (86%, 92% purity by HPLC).

Data: HPLC, ¹H NMR, TLC (1:1 EtOAc/Heptane R_f = 0.33, red in vanillin stain).

Preparation of Dap-Phe-ODMB

5 A 1-L round bottom flask was charged with Fmoc-Dap-Phe-ODMB (27.66 g), CH₂Cl₂ (122 mL) and diethylamine (61 mL, Acros). The solution was stirred at room temperature and the completion monitored by HPLC. After 7h, the mixture was concentrated (bath temp. <30 °C). The residue was suspended in CH₂Cl₂ (300 mL) and concentrated. This was repeated twice. To the residue was added MeOH (20 mL) and
10 CH₂Cl₂ (300 mL), and the solution was concentrated. The residue was suspended in CH₂Cl₂ (100 mL) and toluene (400mL), concentrated, and the residue left under vacuum overnight to give a cream-like residue.

Data: HPLC, ¹H NMR, MS.

15 Preparation of Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB

Crude Dap-Phe-ODMB (39.1 mmol) was suspended in anhydrous DMF (135 mL, Acros) for 5 minutes and Fmoc-MeVal-Val-Dil-OH (24.94g, 39.1 mmol, see Example 2 for preparation) was added. The mixture was cooled in an ice bath and TBTU (13.81g, 43.0 mmol, Matrix Innovations) was added. N,N-Diisopropylethylamine (20.5
20 mL, 117.3 mmol, Acros) was added via syringe over 2 minutes. After 1 hour, the ice bath was removed and the mixture was allowed to warm over 30 min. The mixture was poured into water (1.5 L) and diluted with ethyl acetate (480 mL). After standing for 15 minutes, the layers were separated and the aqueous layer was extracted with ethyl acetate (300 mL). The combined organic layers were washed with brine (200 mL), dried (MgSO₄) and
25 filtered (filter paper) to remove insolubles (inorganics and some dibenzofulvene). After concentration, the residue (49 g) was scraped from the flask and adsorbed on silica (49 g) and purified by chromatography (15 cm x 10 cm dia column; 2:1 EtOAc/Heptane (3 L), EtOAc (5 L); 250 mL fractions) to give 31.84 g of Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB as a white foam (73%, 93% purity by HPLC (AUC)).

30 Data: HPLC, TLC (2:1 EtOAc/heptane, R_f = 0.21, red in vanillin stain).

Preparation of MeVal-Val-Dil-Dap-Phe-ODMB

A 1-L, round-bottom flask was charged with Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB (28.50 g), CH₂Cl₂ (80 mL) and diethylamine (40 mL). Mixture was stirred at room temperature overnight and then was concentrated under reduced pressure. The residue was adsorbed on silica (30 g) and purified by flash chromatography (15 cm x 8 cm dia column; 2% MeOH/DCM (2 L), 3% MeOH/DCM (1 L), 6% MeOH/DCM (4 L); 250 mL fractions) to give 15.88 g of MeVal-Val-Dil-Dap-Phe-ODMB as a white foam (69%, 96% purity by HPLC (AUC)).

Data: HPLC, TLC (6% MeOH/DCM, R_f = 0.24, red in vanillin stain).

Preparation of MC-MeVal-Val-Dil-Dap-Phe-ODMB

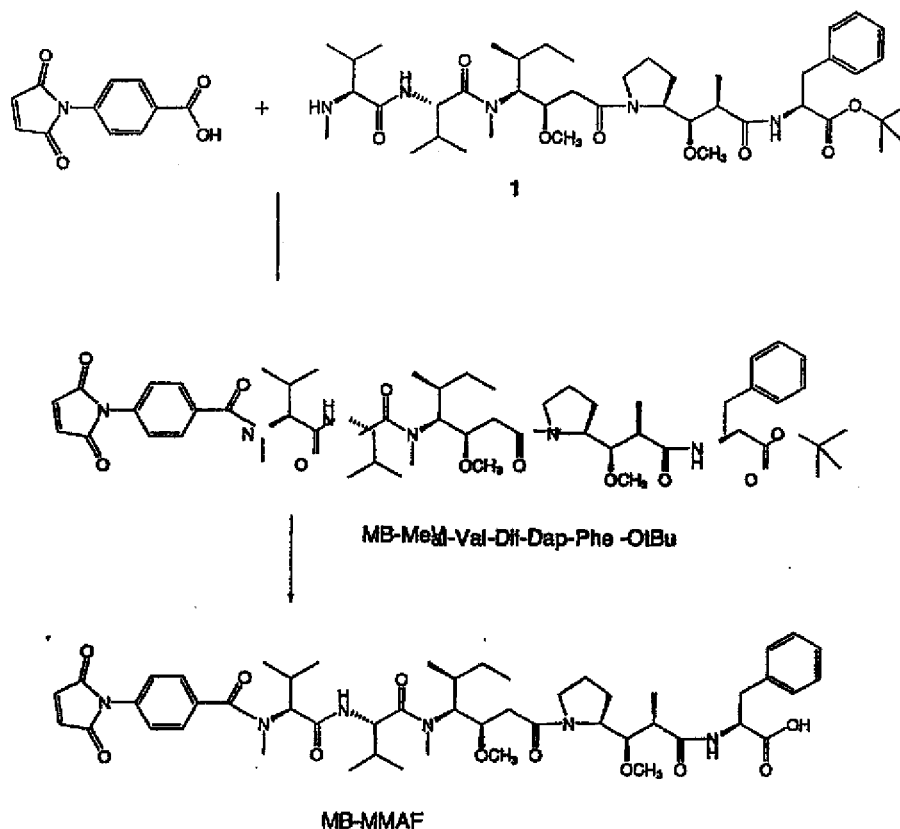
A 50-mL, round-bottom flask was charged with MeVal-Val-Dil-Dap-Phe-ODMB (750 mg, 0.85 mmol), anhydrous DMF (4 mL), maleimidocaproic acid (180 mg, 0.85 mmol), and TBTU (300 mg, 0.93 mmol, Matrix Innovations) at room temperature. N,N-Diisopropylethylamine (450 µL, 2.57 mmol) was added via syringe. After 1.5 hours, the mixture was poured in water (50 mL) and diluted with ethyl acetate (30 mL). NaCl was added to improve the separation. After separation of the layers, the aqueous layer was extracted with ethyl acetate (25 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated. The resulting oil (1 g) was purified by flash chromatography [100 mL silica; 25% Heptane/EtOAc (100 mL), 10% Heptane/EtOAc (200 mL), EtOAc (1.5 L)] to give MC-MeVal-Val-Dil-Dap-Phe-ODMB (13) as a white foam (521 mg, 57%, 94% purity by HPLC(AUC)).

Data: ¹H NMR, HPLC.

Preparation of MC-MeVal-Val-Dil-Dap-Phe-OH (MC-MMAF) (11)

A 50-mL, round-bottom flask was charged with MC-MeVal-Val-Dil-Dap-Phe-ODMB (Compound 13, 428 mg, 0.39 mmol) and dissolved in 2.5% TFA/CH₂Cl₂ (20 mL). The solution turned pink-purple over 2 min. The completion was monitored by HPLC and TLC (6% MeOH/DCM, KMnO₄ stain). After 40 min, three drops of water were added and the cloudy pink-purple mixture was concentrated to give 521 mg of a pink residue. Purification by chromatography (15% IPA/DCM) gave 270 mg of MC-MMAF (73%, 92% purity by HPLC) as a white solid.

Example 23b - Synthesis of analog of mc-MMAF



5

MeVal-Val-Dil-Dap-Phe-OtBu (compound 1, 35 mg, 0.044 mmol) was suspended in DMF (0.250 mL). 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-benzoic acid (11 mg, 0.049 mmol) and HATU (17 mg, 0.044 mmol) were added followed by DIEA (0.031 mL, 0.17 mmol). This reaction mixture was allowed to stir for 2.0 hr. HPLC analysis indicated complete consumption of starting compound 1.

10

Product was isolated via preparatory RP-HPLC, using a Phenomenex C₁₂ Synergi Max-RP 80Å Column (250 x 21.20 mm). Eluent: linear gradient 10% to 80% MeCN/0.05% TFA (aq) over 8 minutes, then isocratic 80% MeCN/0.05% TFA (aq) for an additional 12 minutes. A total of 20 mg of pure product (14) was isolated (0.02 mmol, 46% yield). ES-MS *m/z* 987.85 [M+H]⁺; 1019.41 [M+Na]⁺; 985.54 [M-H]⁻.

15

MB-MeVal-Val-Dil-Dap-Phe-OtBu (Compound 14, 38 mg, 0.0385 mmol) was suspended in CH₂Cl₂ (1 mL) and TFA (1 mL). Mixture was stirred for 2.0 hr, and then volatile organics were evaporated under reduced pressure. Product was purified by

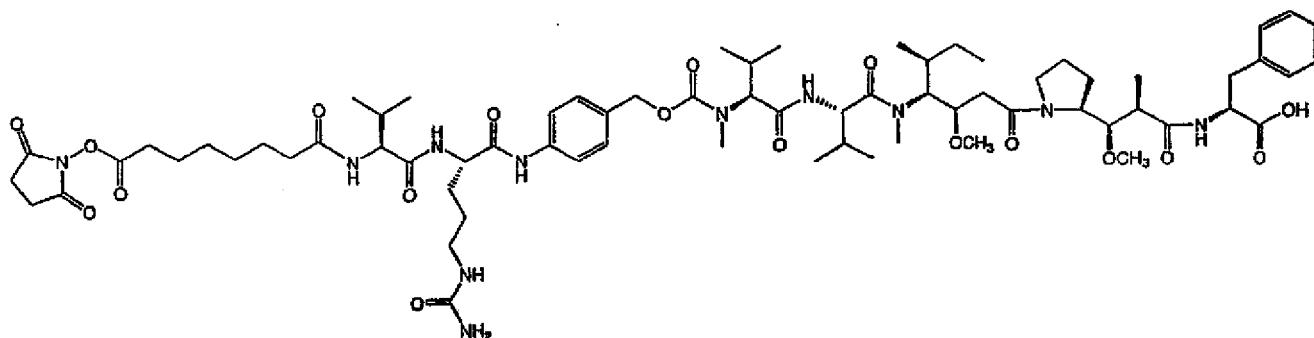
(1.95 g, 7.9 mmol). This suspension was allowed to stir for 125 hr, then the volatile organics were removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel using a 10% MeOH/CH₂Cl₂. Pure Fmoc-MeVal-Cit-PAB-OH (0.55 g, 0.896 mmol, 18.5 % yield) was recovered. ES-MS *m/z* 616.48 [M+H]⁺.

To a suspension of Fmoc-MeVal-Cit-PAB-OH (0.55g, 0.896 mmol) in CH₂Cl₂ (40 mL) was added STRATOSPHERES[™](piperizine-resin-bound) (>5 mmol/g, 150 mg). After being stirred at room temperature for 16 hr the mixture was filtered through celite (pre-washed with MeOH), and concentrated under reduced pressure.

Residue was triturated with diethyl ether and hexanes. Resulting solid material, MeVal-Cit-PAB-OH, was suspended in CH₂Cl₂ (20 mL), and to it was added MC-OSu (0.28 g, 0.896 mmol), DIEA (0.17 mL, 0.99 mmol), and DMF (15 mL). This suspension was stirred for 16 hr, but HPLC analysis of the reaction mixture indicated incomplete reaction, so the suspension was concentrated under reduced pressure to a volume of 6 mL, then a 10% NaHCO₃ (aq) solution was added and the suspension stirred for an additional 16 hr. Solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel using a 0-10% MeOH/CH₂Cl₂ gradient, resulting in 42 mg (0.072 mmol, 8% yield) of MC-MeVal-Cit-PAB-OH.

To a suspension of MC-MeVal-Cit-PAB-OH (2.37 g, 4.04 mmol) and bis(nitrophenyl)carbonate (2.59 g, 8.52 mmol) in CH₂Cl₂ (10 mL) was added DIEA (1.06 mL, 6.06 mmol). This suspension was stirred for 5.5 hr, concentrated under reduced pressure and purified by trituration with diethyl ether. MC-MeVal-Cit-PAB-OCO-pNP (147 mg, 0.196 mmol) was suspended in a 1:5 pyridine/DMF solution (3 mL), and to it was added HOBt (5 mg, 0.039 mmol), DIEA (0.17 mL, 0.978 mmol) and MMAF (compound 2, 150 mg, 0.205 mmol). This reaction mixture was stirred for 16 hr at room temperature, and then purified by preparatory RP-HPLC (x3), using a Phenomenex C₁₂ Synergi Max-RP 80Å Column (250 x 21.20 mm). Eluent: linear gradient 10% to 90% MeCN/0.05% TFA (aq) over 30 minutes, then isocratic 90% MeCN/0.05% TFA (aq) for an additional 20 minutes. MC-MeVal-Cit-PAB-MMAF (16) was obtained as a yellowish solid (24.5 mg, 0.0182, 0.45 % yield). ES-MS *m/z* 1344.95 [M+H]⁺; 1366.94 [M+Na]⁺.

Example 23d - Preparation of succinimide ester of suberyl-Val-Cit-PAB-MMAF (17)



Compound 17

- 5 Compound 1 (300 mg, 0.38 mmol), Fmoc-Val-Cit-PAB-pNP (436 mg, 0.57 mmol, 1.5 eq.) were suspended in anhydrous pyridine, 5 mL. HOBt (10 mg, 0.076 mmol, 0.2 eq.) was added followed by DIEA (199 μ l, 1.14 mmol, 3 eq.). Reaction mixture was sonicated for 10 min, and then stirred overnight at room temperature. Pyridine was removed under reduced pressure, residue was re-suspended in CH_2Cl_2 .
- 10 Mixture was separated by silica gel flash chromatography in a step gradient of MeOH, from 0 to 10%, in CH_2Cl_2 . Product containing fractions were pulled, concentrated, dried in vacuum overnight to give 317 mg (59% yield) of Fmoc-Val-Cit-PAB-MMAF-OtBu. ES-MS m/z 1415.8 $[\text{M}+\text{H}]^+$.
- Fmoc-Val-Cit-PAB-MMAF-OtBu (100 mg) was stirred in 20%
- 15 TFA/ CH_2Cl_2 (10 mL), for 2 hrs. Mixture was diluted with CH_2Cl_2 (50 mL). Organic layer was washed successively with water (2 x 30 mL) and brine (1 x 30 mL). Organic phase was concentrated, loaded onto pad of silica gel in 10% MeOH/ CH_2Cl_2 . Product was eluted with 30% MeOH/ CH_2Cl_2 . After drying in vacuum overnight, Fmoc-Val-Cit-PAB-MMAF was obtained as a white solid, 38 mg, 40% yield. ES-MS m/z 1357.7 $[\text{M}-\text{H}]^-$.
- 20 Fmoc-Val-Cit-PAB-MMAF, 67 mg, was suspended in CH_2Cl_2 (2 mL) diethylamine (2 mL) and DMF (2 mL). Mixture was stirred for 2 hrs at room temperature. Solvent was removed under reduced pressure. Residue was co-evaporated with pyridine (2 mL), then with toluene (2 x 5 mL), dried in vacuum. Val-Cit-PAB-MMAF was obtained as brownish oil, and used without further purification.
- 25 All Val-Cit-PAB-MMAF prepared from 67 mg of Fmoc-Val-Cit-PAB-MMAF, was suspended in pyridine (2 mL), and added to a solution of disuccinimidyl suberate (74 mg, 0.2 mmol, 4 eq.), in pyridine (1 mL). Reaction mixture was stirred at

room temperature. After 3 hrs ether (20 mL) was added. Precipitate was collected, washed with additional amount of ether. Reddish solid was suspended in 30% MeOH/CH₂Cl₂, filtered through a pad of silica gel with 30% MeOH/CH₂Cl₂ as an eluent. Compound 17 was obtained as white solid, 20 mg (29% yield). ES-MS *m/z* 1388.5 [M-H]⁻

Example 24 - In vivo Efficacy of mcMMAF Antibody-Drug Conjugates

Efficacy of cAC10-mcMMAF in Karpas-299 ALCL xenografts: To evaluate the *in vivo* efficacy of cAC10-mcMMAF with an average of 4 drug moieties per antibody (cAC10-mcF4), Karpas-299 human ALCL cells were implanted subcutaneously into immunodeficient C.B-17 SCID mice (5x10⁶ cells per mouse). Tumor volumes were calculated using the formula (0.5xLxW²) where L and W are the longer and shorter of two bidirectional measurements. When the average tumor volume in the study animals reached approximately 100 mm³ (range 48-162) the mice were divided into 3 groups (5 mice per group) and were either left untreated or were given a single intravenous injection through the tail vein of either 1 or 2 mg/kg cAC10-mcF4 (Figure 1). The tumors in the untreated mice grew rapidly to an average volume of >1,000 mm³ within 7 days of the start of therapy. In contrast, all of the cAC10-mcF4 treated tumor showed rapid regression with 3/5 in the 1 mg/kg group and 5/5 in the 2 mg/kg group obtaining complete tumor response. While the tumor in one of the complete responders in the 2 mg/kg group did recur approximately 4 weeks later, there were no detectable tumors in the remaining 4/5 responders in this group and in the 3 complete responders in the 1 mg/kg group at 10 weeks post therapy.

Efficacy of cBR96-mcMMAF in L2987 NSCLC xenografts: cBR96 is a chimeric antibody that recognizes the Le^Y antigen. To evaluate the *in vivo* efficacy of cBR96-mcMMAF with 4 drugs per antibody (cBR96-mcF4) L2987 non-small cell lung cancer (NSCLC) tumor fragments were implanted into athymic nude mice. When the tumors averaged approximately 100 mm³ the mice were divided into 3 groups: untreated and 2 therapy groups. For therapy, as shown in Figure 3a, mice were administered cBR96-mcF4 at either 3 or 10 mg/kg/injection every 4 days for a total of 4 injections (q4dx4). As shown in Figure 3b, mice were administered cBR96-mcF4 or a non-binding control conjugate, cAC10-mcF4, at 10 mg/kg/injection every 4 days for a total of 4

injections (q4dx4). As shown in Figures 3a and 3b, BR96-mcF4 produced pronounced tumor growth delay compared to the controls.

Figure 2 shows an *in vivo*, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous L540CY. For this study there were 4 mice in the untreated group and 10 in each of the treatment groups.

Example 25 – *in vitro* efficacy of MC-MMAF Antibody-Drug Conjugates

Activity of cAC10-antibody-drug conjugates against CD30⁺ cell lines.

Figures 4a and 4b show dose-response curves from a representative experiment where cultures of Karpas 299 (anaplastic large cell lymphoma) and L428 (Hodgkin's Lymphoma) were incubated with serially diluted cAC10-mcMMAF (Figure 4a) or cAC10-vcMMAF (Figure 4b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin [7-hydroxy-3H-phenoxazin-3-one 10-oxide] and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. IC₅₀ values are defined as the concentration where growth is reduced 50% compared with untreated control cultures. Each concentration was tested in quadruplicate.

Activity of cBR96-antibody-drug conjugates against Le^y⁺ cell lines.

Figures 5a and 5b show dose-response curves from a representative experiment where cultures of H3396 (breast carcinoma) and L2987 (non small cell lung carcinoma) were incubated with serially diluted cBR96-mcMMAF (Figure 5a) or -vcMMAF (Figure 5b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. IC₅₀ values are defined as the concentration where growth is reduced 50% compared with untreated control cultures. Each concentration is tested in quadruplicate.

Activity of c1F6-antibody-drug conjugates against CD70⁺ renal cell carcinoma cell lines.

Figures 6a and 6b show dose-response curves from a representative experiment where cultures of Caki-1 and 786-O cells were incubated with serially diluted c1F6-mcMMAF (Figure 6a) or -vcMMAF (Figure 6b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit

procedure. IC₅₀ values are defined as the concentration where growth is reduced 50% compared with untreated control cultures. Each concentration is tested in quadruplicate.

Example 26 - Purification of trastuzumab

One vial containing 440 mg HERCEPTIN® (huMAb4D5-8, rhuMAb
5 HER2, U.S. Patent No. 5821337) antibody) was dissolved in 50 mL MES buffer (25 mM
MES, 50 mM NaCl, pH 5.6) and loaded on a cation exchange column (Sephacrose S, 15
cm x 1.7 cm) that had been equilibrated in the same buffer. The column was then washed
with the same buffer (5 column volumes). Trastuzumab was eluted by raising the NaCl
concentration of the buffer to 200 mM. Fractions containing the antibody were pooled,
10 diluted to 10 mg/mL, and dialyzed into a buffer containing 50 mM potassium phosphate,
50 mM NaCl, 2 mM EDTA, pH 6.5.

Example 27 - Preparation of trastuzumab-MC-MMAE by conjugation of trastuzumab and MC-MMAE

Trastuzumab, dissolved in 500 mM sodium borate and 500 mM sodium
15 chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT). After
incubation at 37 °C for about 30 minutes, the buffer is exchanged by elution over
Sephadex G25 resin and eluted with PBS with 1 mM DTPA. The thiol/Ab value is
checked by determining the reduced antibody concentration from the absorbance at 280
nm of the solution and the thiol concentration by reaction with DTNB (Aldrich,
20 Milwaukee, WI) and determination of the absorbance at 412 nm. The reduced antibody
dissolved in PBS is chilled on ice.

The drug linker reagent, maleimidocaproyl-monomethyl auristatin E
(MMAE), *i.e.* MC-MMAE, dissolved in DMSO, is diluted in acetonitrile and water at
known concentration, and added to the chilled reduced antibody trastuzumab in PBS.
25 After about one hour, an excess of maleimide is added to quench the reaction and cap any
unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal
ultrafiltration and trastuzumab-MC-MMAE is purified and desalted by elution through
G25 resin in PBS, filtered through 0.2 µm filters under sterile conditions, and frozen for
storage.

Example 28 - Preparation of trastuzumab-MC-MMAF by conjugation of trastuzumab and MC-MMAF

Trastuzumab-MC-MMAF was prepared by conjugation of trastuzumab and MC-MMAF following the procedure of Example 27.

5 Example 29 - Preparation of trastuzumab-MC- val-cit-PAB-MMAE by conjugation of trastuzumab and MC-val-cit-PAB-MMAE

Trastuzumab-MC-val-cit-PAB-MMAE was prepared by conjugation of trastuzumab and MC-val-cit-PAB-MMAE following the procedure of Example 27.

10 Example 30 - Preparation of trastuzumab-MC- val-cit-PAB-MMAF by conjugation of trastuzumab and MC-val-cit-PAB-MMAF 9

Trastuzumab-MC-val-cit-PAB-MMAF was prepared by conjugation of trastuzumab and MC-val-cit-PAB-MMAF 9 following the procedure of Example 27.

Example 31 - Rat toxicity

15 The acute toxicity profile of free drugs and ADC was evaluated in adolescent Sprague-Dawley rats (75-125 gms each, Charles River Laboratories (Hollister, CA). Animals were injected on day 1, complete chemistry and hematology profiles were obtained at baseline, day 3 and day 5 and a complete necropsy was performed on day 5. Liver enzyme measurements was done on all animals and routine histology as performed on three random animals for each group for the following tissues: sternum, liver, kidney,
20 thymus, spleen, large and small intestine. The experimental groups were as follows:

Group	Administered	mg/kg	μg MMAF/ m^2	MMAF/ MAb	N/Sex
1	Vehicle	0	0	0	2/F
2	trastuzumab-MC-val-cit-MMAF	9.94	840	4.2	6/F
3	trastuzumab-MC-val-cit-MMAF	24.90	2105	4.2	6/F
4	trastuzumab-MC(Me)-val-cit-PAB-MMAF	10.69	840	3.9	6/F
5	trastuzumab-MC(Me)-val-cit-PAB-MMAF	26.78	2105	3.9	6/F
6	trastuzumab-MC-MMAF	10.17	840	4.1	6/F
7	trastuzumab-MC-MMAF	25.50	2105	4.1	6/F
8	trastuzumab-MC-val-cit-PAB-MMAF	21.85	2105	4.8	6/F

For trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF, the μg MMAF/ m^2 was calculated using 731.5 as the MW of MMAF and 145167 as the MW of Herceptin.

The body surface area was calculated as follows: $\{[(\text{body weight in grams to } 0.667 \text{ power}) \times 11.8]/10000\}$. (Guidance for Industry and Reviewers, 2002).

The dose solutions were administered by a single intravenous bolus tail-vein injection on Study Day 1 at a dose volume of 10 mL/kg. Body weights of the animals were measured pre-dose on Study Day 1 and daily thereafter. Whole blood was collected into EDTA containing tubes for hematology analysis. Whole blood was collected into serum separator tubes for clinical chemistry analysis. Blood samples were collected pre-dose on Study Day -4, Study Day 3 and Study Day 5. Whole blood was also collected into sodium heparin containing tubes at necropsy and the plasma was frozen at -70°C for possible later analysis. The following tissues were collected and placed in neutral buffered formalin at necropsy: liver, kidneys, heart, thymus, spleen, brain, sternum and sections of the GI tract, including stomach, large and small intestine. Sternum, small intestine, large intestine, liver, thymus, spleen and kidney were examined.

Liver associated serum enzyme levels at each timepoint were compared to a range (5th and 95th percentile) from normal female Sprague-Dawley rats. White blood

cell and platelet counts at each timepoint were compared to a range (5th and 95th percentile) from normal female Sprague-Dawley rats.

High dose study in normal female Sprague-Dawley rats:

5	Group 1:	Vehicle
	Group 2:	trastuzumab-MC-MMAF, 52.24mg/kg, 4210 μ g/m ²
	Group 3:	trastuzumab-MC-MMAF, 68.25mg/kg, 5500 μ g/m ²
	Group 4:	trastuzumab-MC-MMAF, 86.00mg/kg, 6930 μ g/m ²

Tissues from 11 animals were submitted for routine histology. These animals had been part of an acute dose-ranging toxicity study using a trastuzumab-MC-MMAF immunoconjugate. Animals were followed for 12 days following dosing.

Example 32 - Cynomolgus Monkey Toxicity/Safety

Three groups of four (2 male, 2 female) naive *Macaca fascicularis* (cynomolgus monkey) were studied for trastuzumab-MC-vc-PAB-MMAE and trastuzumab-MC-vc-PAB-MMAF. Intravenous administration was conducted at days 1 and 22 of the studies.

Sample	Group	Dose
Vehicle	1	day 1
	1M/1F	day 22
H-MC-vc-PAB-MMAE	2	180 μ g/m ² (0.5 mg/kg) at day 1
	2M/2F	1100 μ g/m ² (3.0 mg/kg) at day 22
H-MC-vc-PAB-MMAE	3	550 μ g/m ² (1.5 mg/kg) at day 8
	2M/2F	550 μ g/m ² (1.5 mg/kg) at day 29
H-MC-vc-PAB-MMAE	4	880 μ g/m ² (2.5 mg/kg) at day 15
	2M/2F	880 μ g/m ² (2.5 mg/kg) at day 36

Sample	Group	Dose
Vehicle	1	day 1
	1M/1F	day 22
H-MC-vc-PAB-MMAF	2	180 μ g/m ² (0.5 mg/kg) at day 1
	2M/2F	1100 μ g/m ² (3.0 mg/kg) at day 22

H-MC-vc-PAB-MMAF	3	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 1
	2M/2F	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 22
H-MC-vc-PAB-MMAF	4	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 1
	2M/2F	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 22

H = trastuzumab

Dosing is expressed in surface area of an animal so as to be relevant to other species, *i.e.* dosage at $\mu\text{g}/\text{m}^2$ is independent of species and thus comparable between species. Formulations of ADC contained PBS, 5.4 mM sodium phosphate, 4.2 mM potassium phosphate, 140 mM sodium chloride, pH 6.5.

Blood was collected for hematology analysis predose, and at 5 min., 6 hr, 10 hr, and 1, 3, 5, 7, 14, 21 days after each dose. Erythrocyte (RBC) and platelet (PLT) counts were measured by the light scattering method. Leukocyte (WBC) count was measured by the peroxidase/basophil method. Reticulocyte count was measured by the light scattering method with cationic dye. Cell counts were measured on an Advia 120 apparatus. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in U/L by UV/NADH; IFCC methodology on an Olympus AU400 apparatus, and using Total Ab ELISA - ECD/GxhuFc-HRP. Conj. Ab ELISA -

MMAE/MMAF//ECD-Bio/SA-HRP tests.

Example 33 - Production, Characterization and Humanization of Anti-ErbB2 Monoclonal Antibody 4D5

The murine monoclonal antibody 4D5 which specifically binds the extracellular domain of ErbB2 was produced as described in Fendly *et al.* (1990) Cancer Research 50:1550-1558. Briefly, NIH 3T3/HER2-3₄₀₀ cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak *et al. Proc. Natl. Acad. Sci. (USA)* 84:7158-7163 (1987) were harvested with phosphate buffered saline (PBS) containing 25mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P -labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13. This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes

were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

Epitope mapping and characterization

The ErbB2 epitope bound by monoclonal antibody 4D5 was determined by competitive binding analysis (Fendly *et al. Cancer Research* 50:1550 -1558 (1990)). Cross-blocking studies were done by direct fluorescence on intact cells using the PANDEX™ Screen Machine to quantitate fluorescence. The monoclonal antibody was conjugated with fluorescein isothiocyanate (FITC), using established procedures (Wofsy *et al. Selected Methods in Cellular Immunology*, p. 287, Mishel and Schiigi (eds.) San Francisco: W.J. Freeman Co. (1980)). Confluent monolayers of NIH 3T3/HER2-3₄₀₀ cells were trypsinized, washed once, and resuspended at 1.75×10^6 cell/ml in cold PBS containing 0.5% bovine serum albumin (BSA) and 0.1% NaN₃. A final concentration of 1% latex particles (IDC, Portland, OR) was added to reduce clogging of the PANDEX™ plate membranes. Cells in suspension, 20 µl, and 20 µl of purified monoclonal antibodies (100µg/ml to 0.1 µg/ml) were added to the PANDEX™ plate wells and incubated on ice for 30 minutes. A predetermined dilution of the FITC-labeled monoclonal antibody in 20 µl was added to each well, incubated for 30 minutes, washed, and the fluorescence was quantitated by the PANDEX™. Monoclonal antibodies were considered to share an epitope if each blocked binding of the other by 50% or greater in comparison to an irrelevant monoclonal antibody control. In this experiment, monoclonal antibody 4D5 was assigned epitope I (amino acid residues from about 529 to about 625, inclusive within the ErbB2 extracellular domain).

The growth inhibitory characteristics of monoclonal antibody 4D5 were evaluated using the breast tumor cell line, SK-BR-3 (see Hudziak *et al. (1989) Molec. Cell. Biol.* 9(3):1165-1172). Briefly, SK-BR-3 cells were detached by using 0.25% (vol/vol) trypsin and suspended in complete medium at a density of 4×10^5 cells per ml. Aliquots of 100 µl (4×10^4 cells) were plated into 96-well microdilution plates, the cells were allowed to adhere, and 100 µl of media alone or media containing monoclonal antibody (final concentration 5 µg/ml) was then added. After 72 hours, plates were washed twice with PBS (pH 7.5), stained with crystal violet (0.5% in methanol), and analyzed for relative cell proliferation as described in Sugarman *et al. (1985) Science*

230:943-945. Monoclonal antibody 4D5 inhibited SK-BR-3 relative cell proliferation by about 56%.

Monoclonal antibody 4D5 was also evaluated for its ability to inhibit HRG-stimulated tyrosine phosphorylation of proteins in the M_r 180,000 range from whole-cell lysates of MCF7 cells (Lewis *et al.* (1996) *Cancer Research* 56:1457-1465). MCF7 cells are reported to express all known ErbB receptors, but at relatively low levels. Since ErbB2, ErbB3, and ErbB4 have nearly identical molecular sizes, it is not possible to discern which protein is becoming tyrosine phosphorylated when whole-cell lysates are evaluated by Western blot analysis. However, these cells are ideal for HRG tyrosine phosphorylation assays because under the assay conditions used, in the absence of exogenously added HRG, they exhibit low to undetectable levels of tyrosine phosphorylation proteins in the M_r 180,000 range.

MCF7 cells were plated in 24-well plates and monoclonal antibodies to ErbB2 were added to each well and incubated for 30 minutes at room temperature; then rHRG β 1₁₇₇₋₂₄₄ was added to each well to a final concentration of 0.2 nM, and the incubation was continued for 8 minutes. Media was carefully aspirated from each well, and reactions were stopped by the addition of 100 μ l of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μ l) was electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (4G10, from UBI, used at 1 μ g/ml) immunoblots were developed, and the intensity of the predominant reactive band at $\tilde{M}_r$ 180,000 was quantified by reflectance densitometry, as described previously (Holmes *et al.* (1992) *Science* 256:1205-1210; Sliwkowski *et al. J. Biol. Chem.* 269:14661-14665 (1994)).

Monoclonal antibody 4D5 significantly inhibited the generation of a HRG-induced tyrosine phosphorylation signal at M_r 180,000. In the absence of HRG, but was unable to stimulate tyrosine phosphorylation of proteins in the M_r 180,000 range. Also, this antibody does not cross-react with EGFR (Fendly *et al. Cancer Research* 50:1550-1558 (1990)), ErbB3, or ErbB4. Monoclonal antibody 4D5 was able to block HRG stimulation of tyrosine phosphorylation by ~50%.

The growth inhibitory effect of monoclonal antibody 4D5 on MDA-MB-175 and SK-BR-3 cells in the presence or absence of exogenous rHRG β 1 was assessed (Schaefer *et al. Oncogene* 15:1385-1394 (1997)). ErbB2 levels in MDA-MB-175 cells

are 4-6 times higher than the level found in normal breast epithelial cells and the ErbB2-ErbB4 receptor is constitutively tyrosine phosphorylated in MDA-MB-175 cells.

Monoclonal antibody 4D5 was able to inhibit cell proliferation of MDA-MB-175 cells, both in the presence and absence of exogenous HRG. Inhibition of cell proliferation by

- 5 4D5 is dependent on the ErbB2 expression level (Lewis *et al.* *Cancer Immunol. Immunother.* 37:255-263 (1993)). A maximum inhibition of 66% in SK-BR-3 cells could be detected. However this effect could be overcome by exogenous HRG.

- 10 The murine monoclonal antibody 4D5 was humanized, using a "gene conversion mutagenesis" strategy, as described in U.S. Patent No. 5821337, the entire disclosure of which is hereby expressly incorporated by reference. The humanized monoclonal antibody 4D5 used in the following experiments is designated huMAb4D5-8. This antibody is of IgG1 isotype.

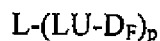
REFERENCES CITED

- 15 The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments that are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

- 20 All references cited herein are incorporated by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

CLAIMS

1. A compound having the formula:



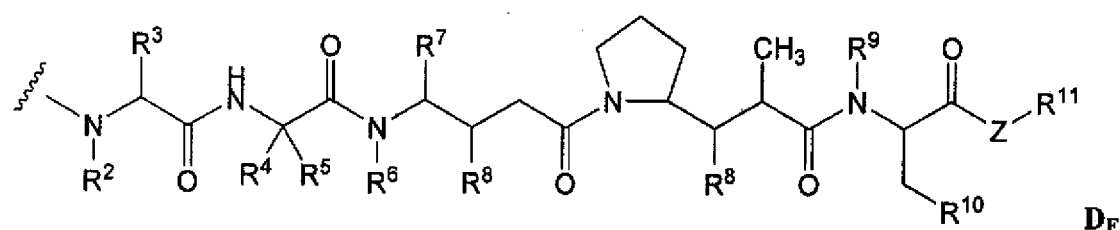
or a pharmaceutically acceptable salt or solvate thereof,

wherein

L- is a Ligand unit selected from the group consisting of a protein, polypeptide and a peptide,

LU is a Linker unit,

D_F has the formula:



or a pharmaceutically acceptable salt of the compound of formula D_F,

wherein the wavy line of D_F indicates a covalent attachment site, and independently at each location:

R² is selected from the group consisting of H and C₁-C₈ alkyl;

R³ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from the group consisting of H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_{n1}- wherein R^a and R^b are independently selected from the group consisting of H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n₁ is selected from the group consisting of 2, 3, 4, 5 and 6;

R⁶ is selected from the group consisting of H and C₁-C₈ alkyl;

R^7 is selected from the group consisting of H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl-(C_3 - C_8 heterocycle);

each R^8 is independently selected from the group consisting of H, OH, C_1 - C_8 alkyl, C_3 - C_8 carbocycle and O-(C_1 - C_8 alkyl);

R^9 is selected from the group consisting of H and C_1 - C_8 alkyl;

R^{10} is selected from the group consisting of aryl and C_3 - C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_8 alkyl;

R^{11} is selected from the group consisting of H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, and $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 11 to 1000;

R^{13} is C_2 - C_8 alkyl;

R^{14} is H or C_1 - C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_{n2}-N(R^{16})_2$, $-(CH_2)_{n2}-SO_3H$, or $-(CH_2)_{n2}-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_{n2}-COOH$; and

$n2$ is an integer ranging from 0 to 6;

p ranges from 1 to about 20;

and the Linker unit can be present or absent.

2. The conjugate of claim 1 having the formula:

$L-(D_F)_p$

or a pharmaceutically acceptable salt or solvate thereof,

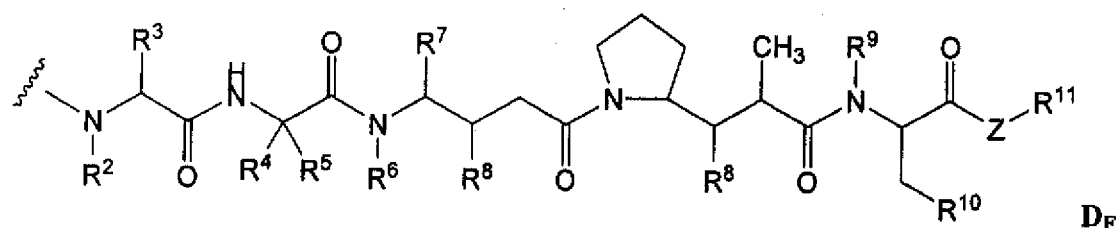
wherein

L- is a Ligand unit, and

p ranges from 1 to about 20.

3. A compound having the formula:
 LU-D_F
 or a pharmaceutically acceptable salt or solvate thereof
 wherein
 -LU- is a Linker unit; and

D_F has the formula:



or a pharmaceutically acceptable salt of the compound of formula D_F,
 wherein the wavy line of D_F indicates a covalent attachment site, and
 independently at each location:

R² is selected from the group consisting of H and C₁-C₈ alkyl;

R³ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from the group consisting of H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_{n1}-
 wherein R^a and R^b are independently selected from the group consisting of H, C₁-C₈ alkyl
 and C₃-C₈ carbocycle and n1 is selected from the group consisting of 2, 3, 4, 5 and 6;

R⁶ is selected from the group consisting of H and C₁-C₈ alkyl;

R⁷ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R^8 is independently selected from the group consisting of H, OH, C_1 - C_8 alkyl, C_3 - C_8 carbocycle and O -(C_1 - C_8 alkyl);

R^9 is selected from the group consisting of H and C_1 - C_8 alkyl;

R^{10} is selected from the group consisting of aryl and C_3 - C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_8 alkyl;

R^{11} is selected from the group consisting of H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, and $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 11 to 1000;

R^{13} is C_2 - C_8 alkyl;

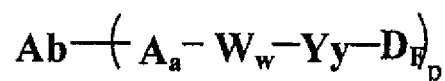
R^{14} is H or C_1 - C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_{n2}-N(R^{16})_2$, $-(CH_2)_{n2}-SO_3H$, or $-(CH_2)_{n2}-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_{n2}-COOH$; and

$n2$ is an integer ranging from 0 to 6.

4. The compound of claim 1, wherein the compound is an antibody-drug conjugate having formula Ia':



Ia'

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody,

$-A_a-W_w-Y_y-$ comprise a Linker unit,

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

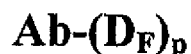
w is an integer ranging from 0 to 12,

Y is a Spacer unit,

y is 0, 1 or 2, and

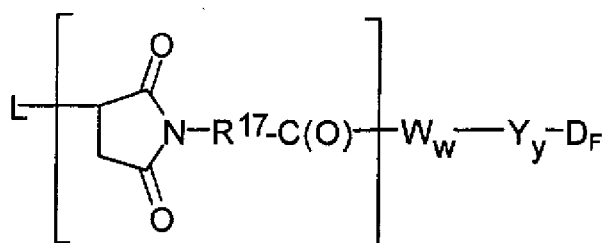
p ranges from 1 to about 20.

5. The antibody-drug conjugate of claim 4 having the formula:



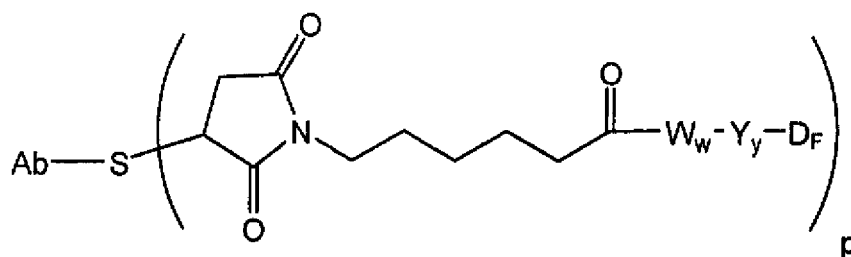
wherein a, w, and y are each 0.

6. The antibody-drug conjugate of claim 4 wherein the antibody is attached to the Linker unit or the drug through a cysteine residue of the antibody.
7. The antibody-drug conjugate of claim 6 wherein p is 2 to 5.
8. The antibody-drug conjugate of claim 4 or 6 having the formula:

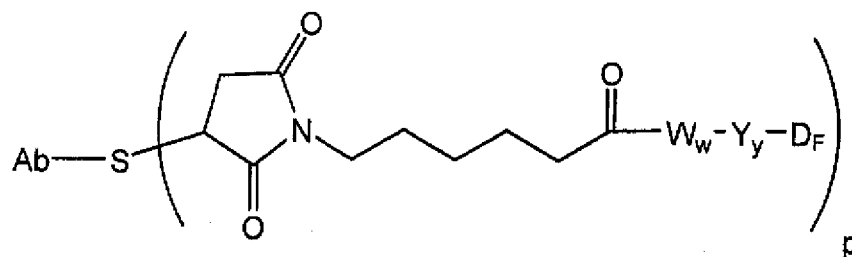


wherein L is an antibody and R^{17} is $\text{C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_3\text{-C}_8$ carbocyclo-, $\text{-O-(C}_1\text{-C}_8\text{ alkyl)-}$, -arylene- , $\text{-C}_1\text{-C}_{10}$ alkylene-arylene-, $\text{-arylene-C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_1\text{-C}_{10}$ alkylene-($\text{C}_3\text{-C}_8$ carbocyclo)-, $\text{-(C}_3\text{-C}_8\text{ carbocyclo)-C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_3\text{-C}_8$ heterocyclo-, $\text{-C}_1\text{-C}_{10}$ alkylene-($\text{C}_3\text{-C}_8$ heterocyclo)-, $\text{-(C}_3\text{-C}_8\text{ heterocyclo)-C}_1\text{-C}_{10}$ alkylene-, $\text{-(CH}_2\text{CH}_2\text{O)}_r\text{-}$, or $\text{-(CH}_2\text{CH}_2\text{O)}_r\text{-CH}_2\text{-}$; and r is an integer ranging from 1 to 10.

9. The antibody-drug conjugate of claim 8 having the formula:

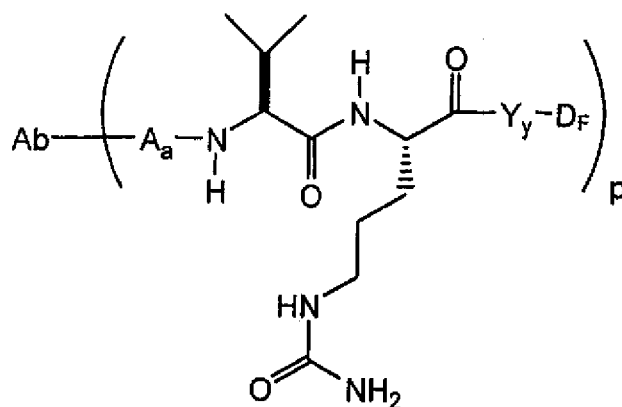


10. The antibody-drug conjugate of claim 8 having the formula:

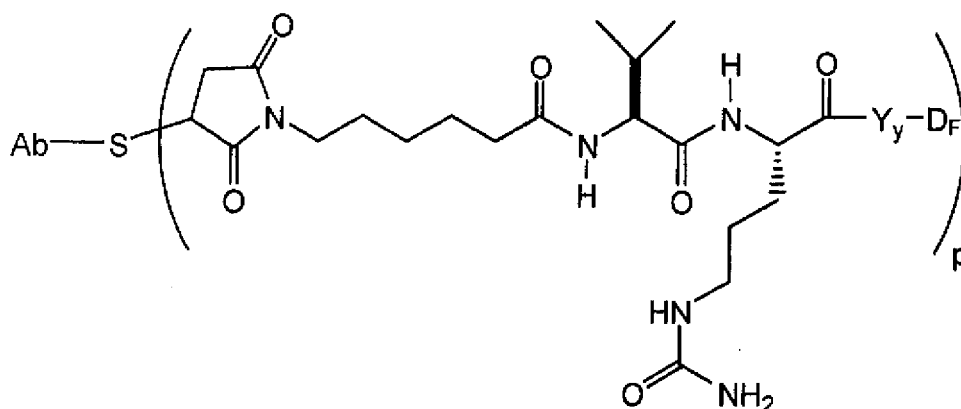


wherein w and y are each 0.

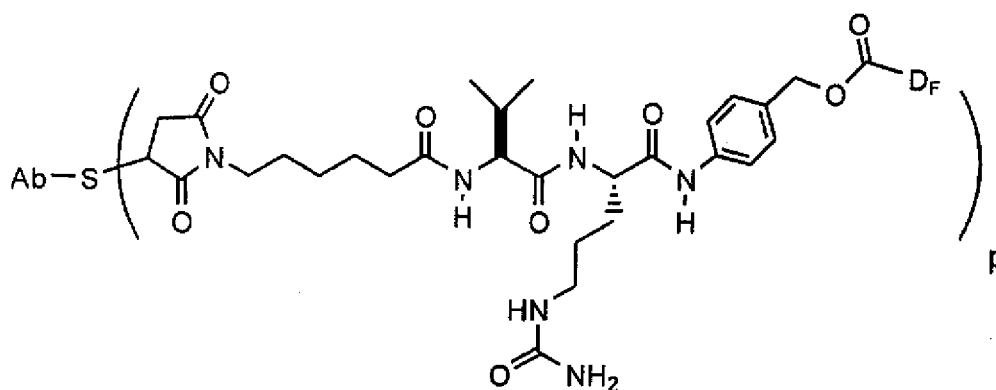
11. The antibody-drug conjugate of claim 4 wherein w is an integer ranging from 2 to 12.
12. The antibody-drug conjugate of claim 11 wherein w is 2.
13. The antibody-drug conjugate of claim 12 wherein W_w is -valine-citrulline-, -phenylalanine-lysine-, or -N-methyl valine-citrulline-.
14. The antibody-drug conjugate of claim 11 wherein W_w is 5-aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepecotic acid lysine, beta-alanine lysine, glycine serine valine glutamine or isonepecotic acid.
15. The antibody-drug conjugate of claim 13 having the formula:



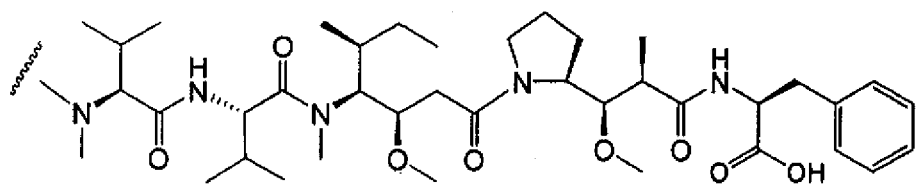
16. The antibody-drug conjugate of claim 13 having the formula:

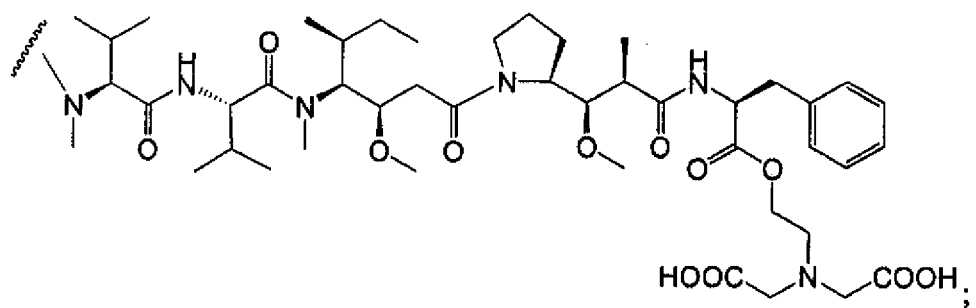
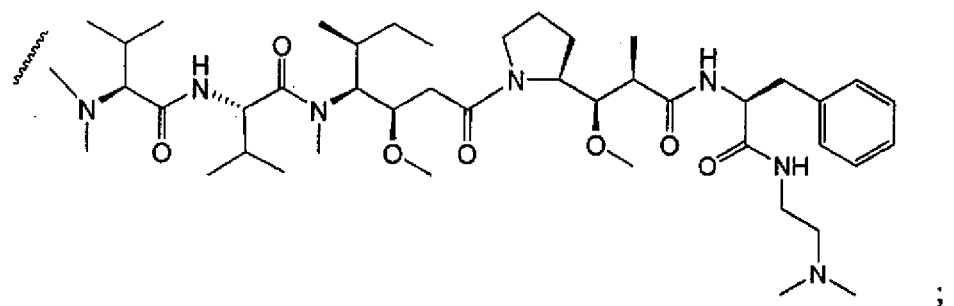
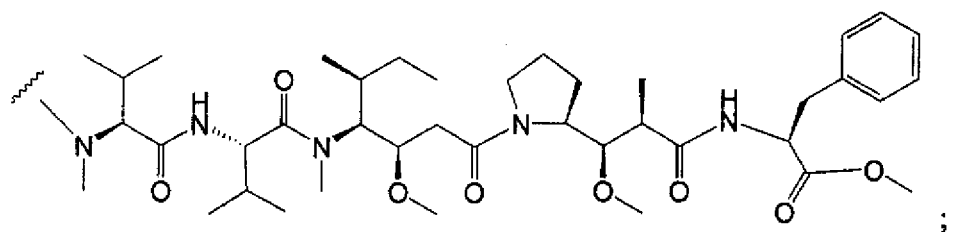
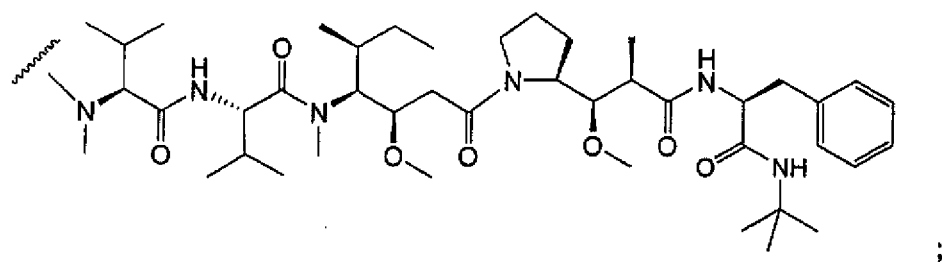
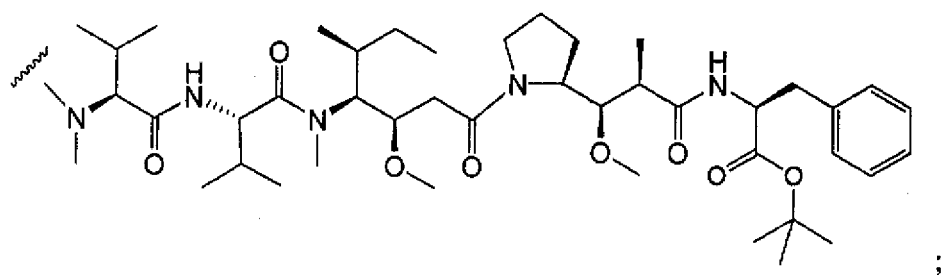


17. The antibody-drug conjugate of claim 13 having the formula:

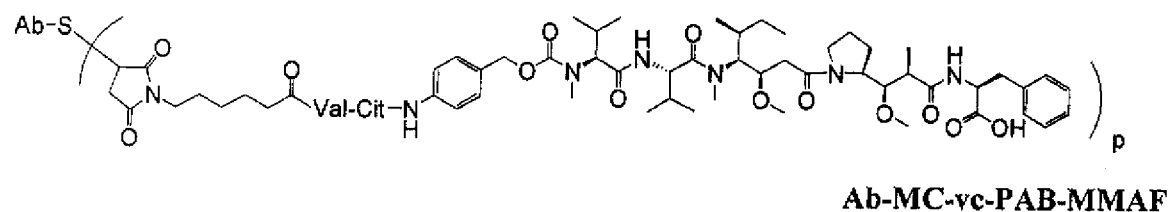


18. The antibody-drug conjugate of any of claims 4-17 wherein D_F has the formula selected from the group consisting of:

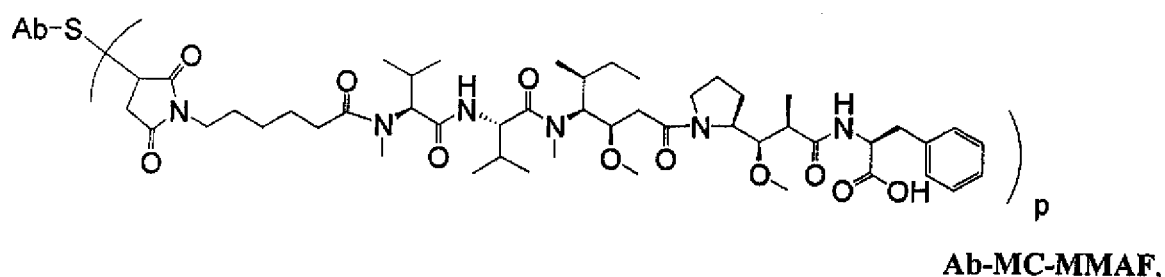




20. The antibody-drug conjugate of claim 4, having the formula:



or



wherein Ab is an antibody, Val is valine, and Cit is citrulline.

21. The antibody-drug conjugate of claim 20 wherein the antibody binds to CD30, CD20, CD33, or the Lewis Y antigen.

22. A pharmaceutical composition comprising an effective amount of the conjugate of any of claims 1, 2 or 4-21, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

23. The pharmaceutical composition of claim 22 further comprising a therapeutically effective amount of chemotherapeutic agent selected from the group consisting of a tubulin-forming inhibitor, a topoisomerase inhibitor, and a DNA binder.

24. The use of the conjugate of any of claims 1-21, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament.

25. The use of the conjugate of any of claims 1-21, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat a cancer.

26. The use of claim 25 for treatment of cancer with an additional anticancer agent, an immunosuppressant agent or an anti-infectious agent.
27. The use of the conjugate of any of claims 1-21, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat an autoimmune disease.
28. The use of the conjugate of any of claims 1-21, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat an infectious disease.
29. A use of the antibody-drug conjugate of any of claims 4-21, or a pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of cancer, wherein the antibody of the antibody-drug conjugate binds to an antigen expressed by a cell of the cancer.
30. The use of claim 29, further comprising an effective amount of an additional anticancer agent, an immunosuppressant agent or an anti-infectious agent.
31. A use of the antibody-drug conjugate of any of claims 4-21, or a pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of an autoimmune disease, wherein the antibody of the antibody-drug conjugate binds to an antigen expressed by a cell of the autoimmune disease.
32. A use of the antibody-drug conjugate of any of claims 4-21, or a pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of an infectious disease, wherein the antibody of the antibody-drug conjugate binds to an antigen expressed by a cell of the infectious disease.
33. The conjugate of claim 1 or 3, wherein the Linker unit (LU) comprises:
-A_a-W_w-Y_y-,
wherein
-A- is a Stretcher unit,
a is 0 or 1,
each -W- is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

-Y- is a Spacer unit, and

y is 0, 1 or 2.

34. The antibody-drug conjugate of claim 4 or 21 wherein the antibody is an antibody which binds to an ErbB receptor or to one or more of receptors (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type I and type I-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

- (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);
- (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP-001762.1);
- (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, Genbank accession No. NP_002552.2);

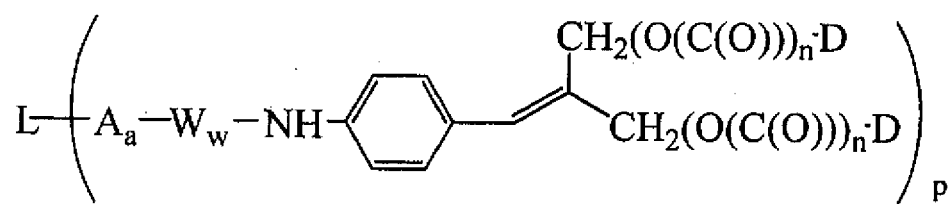
(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, Genbank accession No. NP_005573.1);

(34) FCRH1 (Fc receptor-like protein 1, Genbank accession No. NP_443170.1); and

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2 Genbank accession No. NP_112571.1).

35. A conjugate having the formula:



or a pharmaceutically acceptable salt or solvate thereof,

wherein

L- is a Ligand unit;

-A- is a Stretcher unit;

a is 0 or 1;

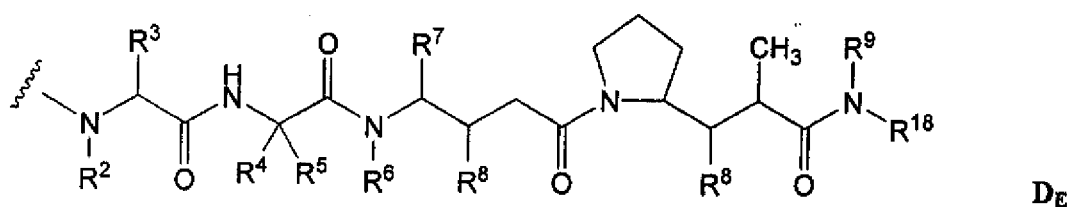
each -W- is independently an Amino Acid unit;

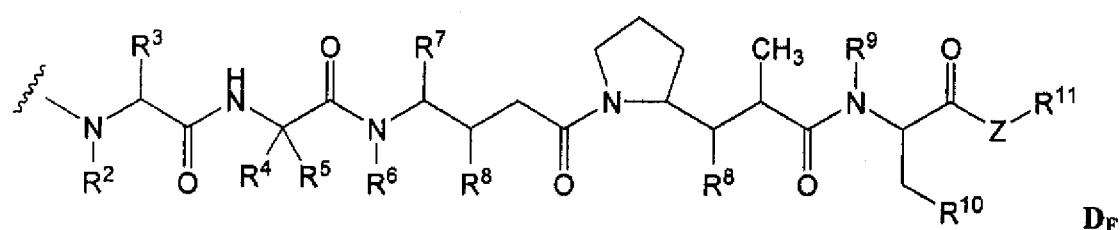
w is an integer ranging from 0 to 12;

each n3 is independently 0 or 1;

p ranges from 1 to about 20; and

each occurrence of D is independently a drug moiety selected from the group consisting of Formulas D_E and D_F:





wherein

at least one D is a drug moiety of Formula **D_F** and independently at each location:

R^2 is selected from the group consisting of H and C_1 - C_8 alkyl;

R^3 is selected from the group consisting of H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl-(C_3 - C_8 heterocycle);

R^4 is selected from the group consisting of H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl-(C_3 - C_8 heterocycle);

R^5 is selected from the group consisting of H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n$ - wherein R^a and R^b are independently selected from the group consisting of H, C_1 - C_8 alkyl and C_3 - C_8 carbocycle and n is selected from the group consisting of 2, 3, 4, 5 and 6;

R^6 is selected from the group consisting of H and C_1 - C_8 alkyl;

R^7 is selected from the group consisting of H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl-(C_3 - C_8 heterocycle);

each R^8 is independently selected from the group consisting of H, OH, C_1 - C_8 alkyl, C_3 - C_8 carbocycle and O-(C_1 - C_8 alkyl);

R^9 is selected from the group consisting of H and C_1 - C_8 alkyl;

R^{10} is selected from the group consisting of aryl and C_3 - C_8 heterocycle;

Z is O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_8 alkyl;

R^{11} is selected from the group consisting of H, C_1 - C_{20} alkyl, aryl, C_3 - C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, and $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1 to 1000;

R^{13} is C_2 - C_8 alkyl;

R^{14} is H or C_1 - C_8 alkyl;

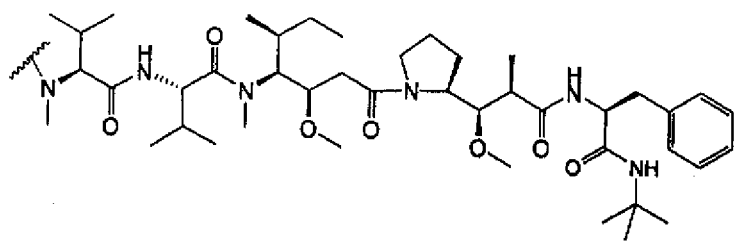
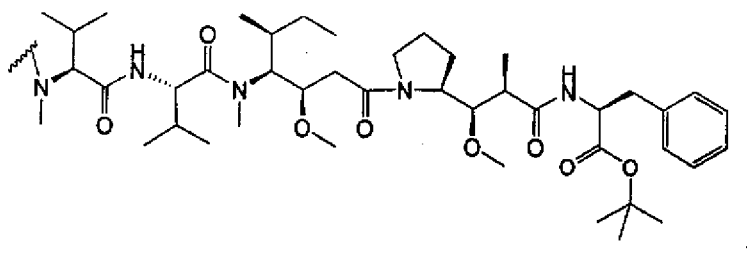
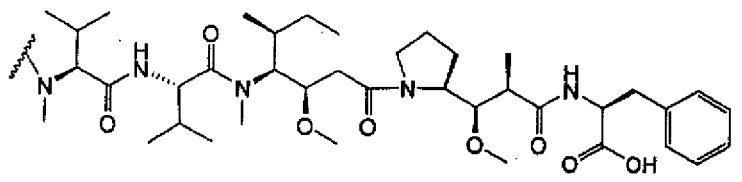
each occurrence of R^{15} is independently H, $COOH$, $-(CH_2)_{n2}-N(R^{16})_2$, $-(CH_2)_{n2}-SO_3H$, or $-(CH_2)_{n2}-SO_3-C_1-C_8$ alkyl;

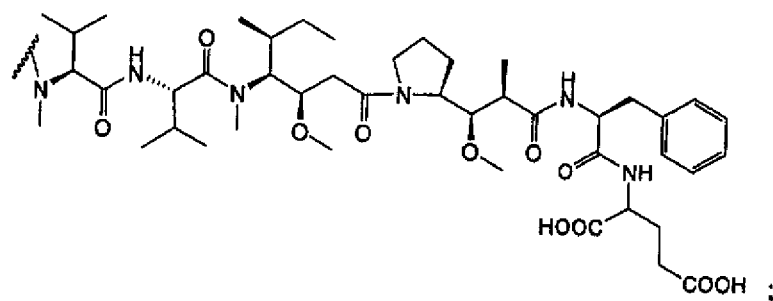
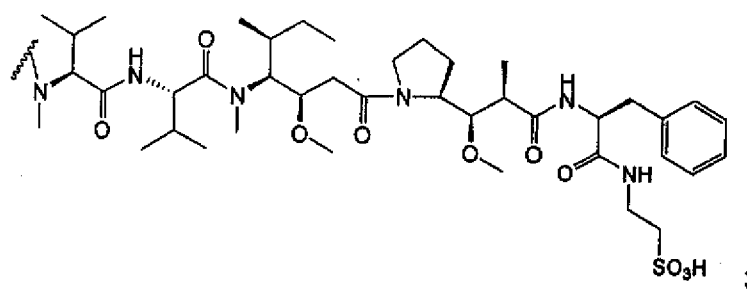
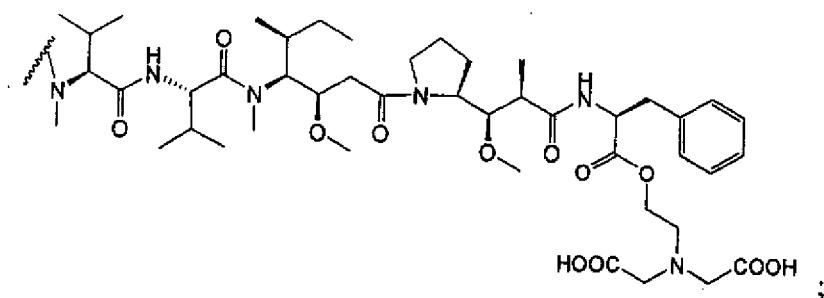
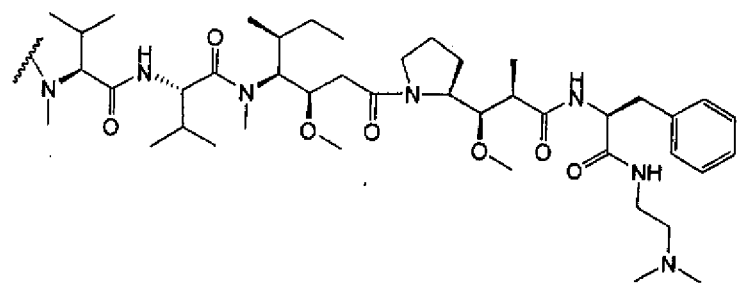
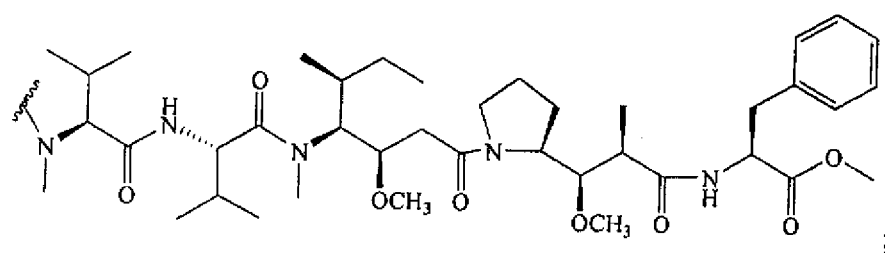
each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_{n2}-COOH$;

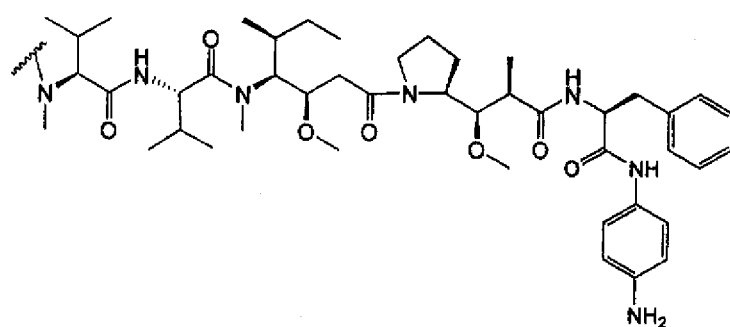
R^{18} is selected from the group consisting of $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and n_2 is an integer ranging from 0 to 6.

36. The conjugate of claim 35 wherein w is an integer ranging from 2 to 12.

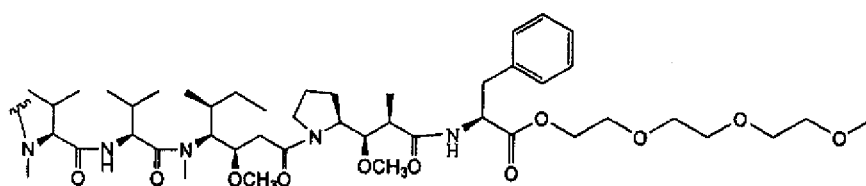
37. The conjugate of claim 35 having the formula D_F selected from the group consisting of:







; and



or a pharmaceutically acceptable salt or solvate thereof.

38. The conjugate of claim 35, wherein the antibody is an antibody which binds to an ErbB receptor or to one or more of receptors (1)-(35):

(1) BMPRI1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTI1b, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

- (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);
- (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);
- (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP-001762.1);

(28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

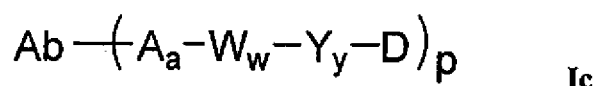
(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, Genbank accession No. NP_005573.1);

(34) FCRH1 (Fc receptor-like protein 1, Genbank accession No. NP_443170.1); and

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated Genbank accession No. NP_112571.1).

39. The conjugate or compound or a pharmaceutically acceptable salt or solvate of the conjugate or compound thereof of any of claims 1-21 and 33-38 that is in isolated and purified form.

40. An antibody-drug conjugate comprising an antibody covalently attached to one or more drug moieties, the antibody-drug conjugate having Formula Ic:



or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to one or more tumor-associated antigens (1)-(35):

(1) BMPRI B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

- (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);
- (6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);
- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type I and type I-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);
- (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);
- (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);
- (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);

- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP-001762.1);
- (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, Genbank accession No. NP_002552.2);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, Genbank accession No. NP_443170.1); and
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, Genbank accession No. NP_112571.1),

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

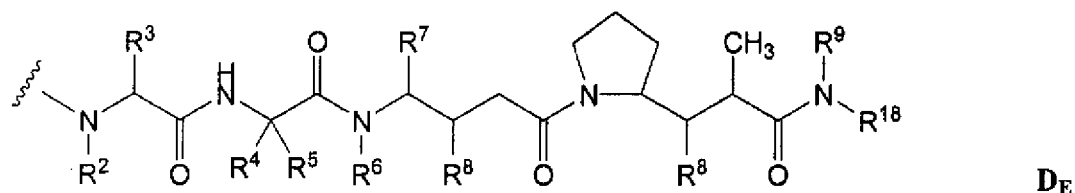
w is an integer ranging from 0 to 12,

Y is a Spacer unit,

y is 0, 1 or 2,

p ranges from 1 to 20, and

D is a drug moiety of Formulae D_E:



wherein the wavy line of D_E indicates the covalent attachment site to A, W, or Y, and independently at each location:

R² is selected from the group consisting of H and C₁-C₈ alkyl;

R³ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from the group consisting of H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from the group consisting of H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n1 is selected from the group consisting of 2, 3, 4, 5 and 6;

R⁶ is selected from the group consisting of H and C₁-C₈ alkyl;

R⁷ is selected from the group consisting of H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from the group consisting of H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O-(C₁-C₈ alkyl);

R⁹ is selected from the group consisting of H and C₁-C₈ alkyl; and

R¹⁸ is selected from the group consisting of -C(R⁸)₂-C(R⁸)₂-aryl, -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ heterocycle), and -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ carbocycle); and

n2 is an integer ranging from 0 to 6.

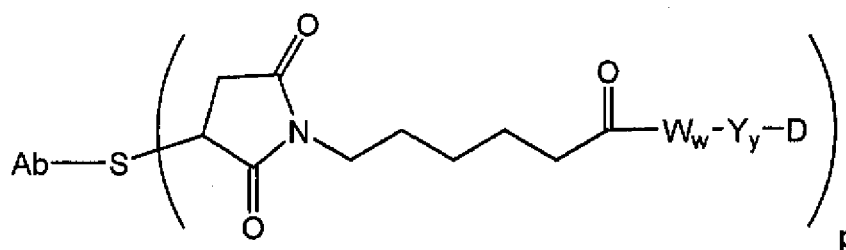
41. The antibody-drug conjugate of claim 40, wherein the antibody is attached to the drug moiety through a cysteine residue of the antibody.

42. The antibody-drug conjugate of claim 41, wherein p is 1 to 4.

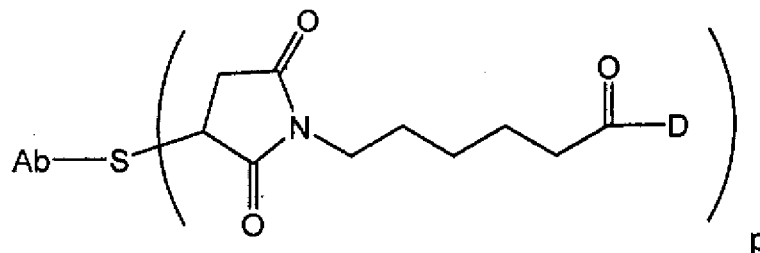
43. The antibody-drug conjugate of claim 40 wherein p is 2 to 8.

44. The antibody-drug conjugate of claim 40 wherein p is 2 to 5.

45. The antibody-drug conjugate of claim 41 having the formula:

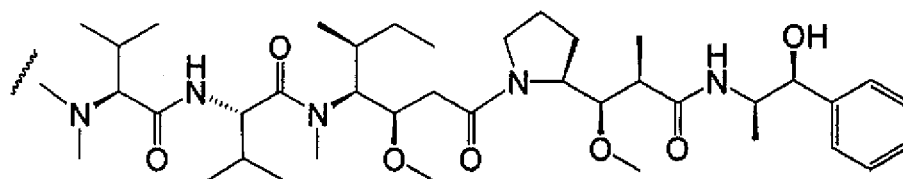


46. The antibody-drug conjugate of claim 45 having the formula:

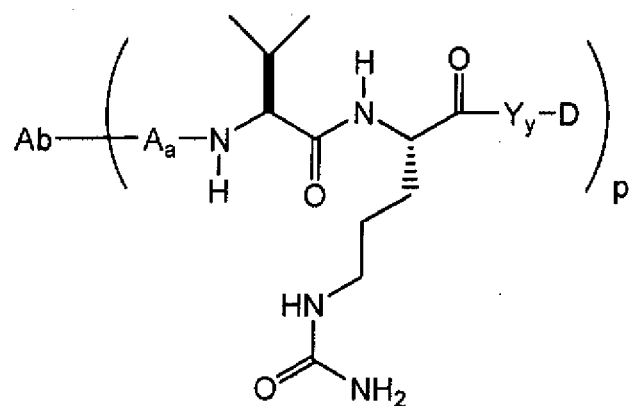


wherein w and y are each 0.

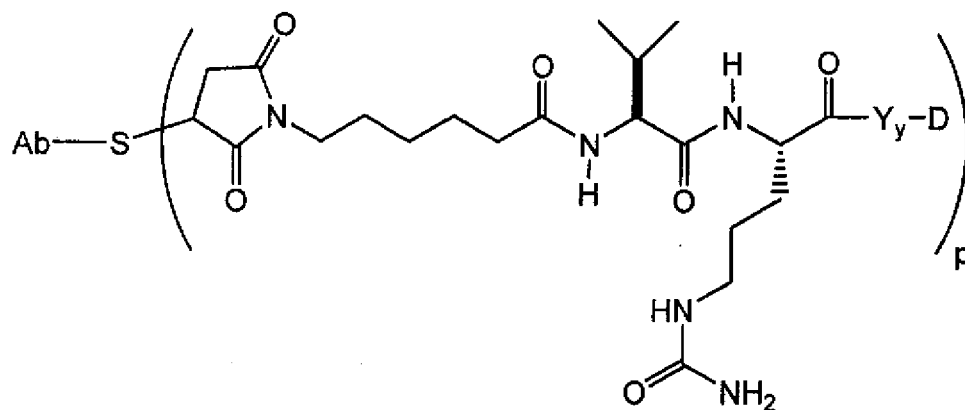
47. The antibody-drug conjugate of claim 45 wherein Formula **D_E** has the formula:



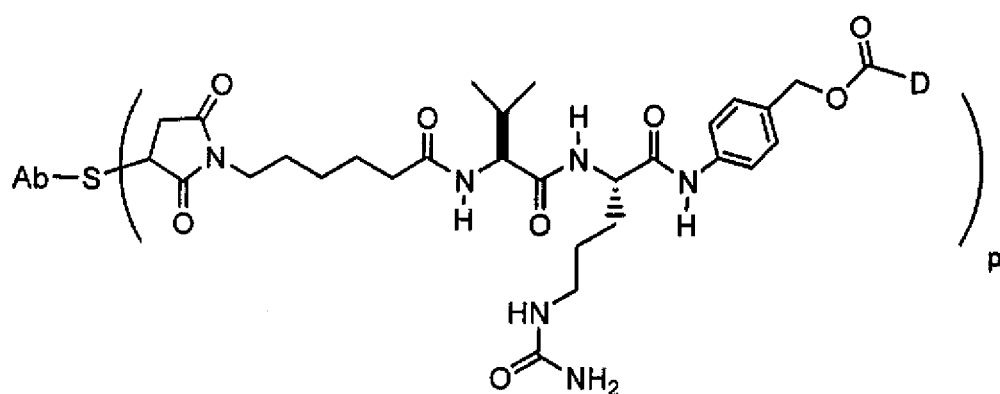
48. The antibody-drug conjugate of claim 40 having the formula:



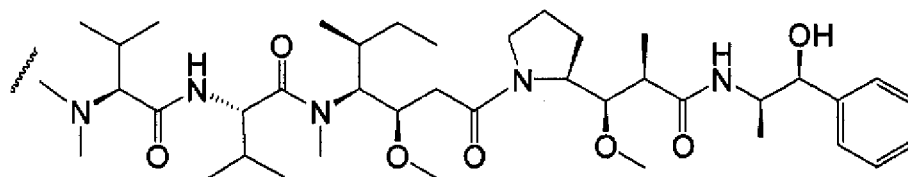
49. The antibody-drug conjugate of claim 48 having the formula:



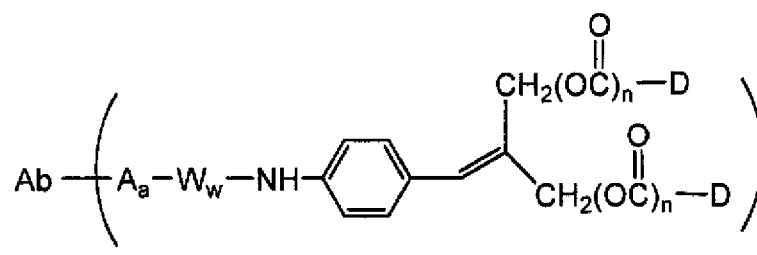
50. The antibody-drug conjugate of claim 49 having the formula:



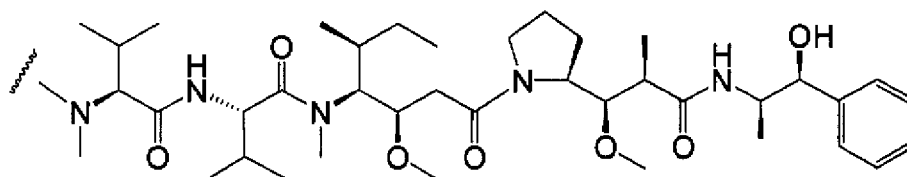
51. The antibody-drug conjugate of claim 49 wherein Formula D_E has the formula:



52. The antibody-drug conjugate of claim 40 having the formula:

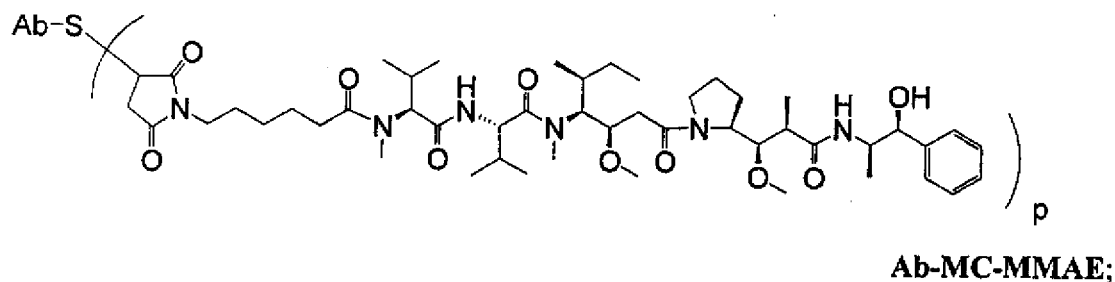
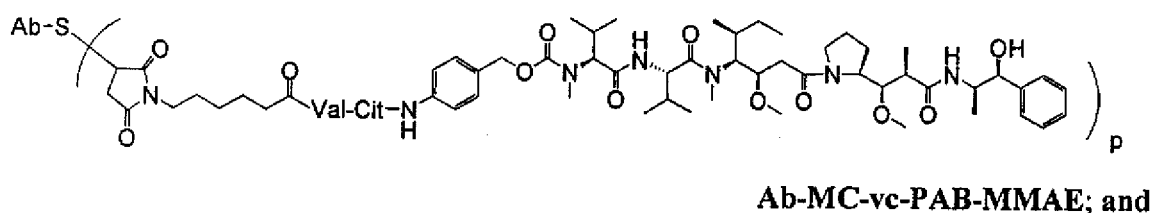


53. The antibody-drug conjugate of claim 40 wherein w is an integer ranging from 2 to 12.
54. The antibody-drug conjugate of claim 40 wherein w is 2.
55. The antibody-drug conjugate of claim 40 wherein W_w is -valine-citrulline-.
56. The antibody-drug conjugate of claim 40 wherein D is of Formulae:



57. The antibody-drug conjugate of claim 40 wherein the antibody specifically binds to a HER2 receptor.
58. The antibody-drug conjugate of claim 57 which specifically binds to the extracellular domain of the HER2 receptor and inhibits growth of tumor cells which overexpress HER2 receptor.

59. The antibody-drug conjugate of claim 40 wherein the antibody is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a chimeric antibody, a humanized antibody, and an antibody fragment.
60. The antibody-drug conjugate of claim 59 wherein the antibody fragment is a Fab fragment.
61. The antibody-drug conjugate of claim 59 wherein the humanized antibody is selected from the group consisting of huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (trastuzumab).
62. The antibody-drug conjugate of claim 61 wherein the antibody is huMAb4D5-8 (trastuzumab).
63. The antibody-drug conjugate of claim 40 selected from the group consisting of formulae:



wherein Val is valine, and Cit is citrulline.

64. The antibody-drug conjugate of claim 63 wherein the antibody is huMAb4D5-8 (trastuzumab).
65. A pharmaceutical composition comprising an effective amount of the antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

66. The pharmaceutical composition of claim 65 further comprising a therapeutically effective amount of chemotherapeutic agent selected from the group consisting of a tubulin-forming inhibitor, a topoisomerase inhibitor, and a DNA binder.
67. A use of the antibody-drug conjugate of claim 40 for the manufacture of a medicament for killing or inhibiting the proliferation of tumor cells or cancer cells.
68. A use of the antibody-drug conjugate of any of claims 40-64 for the manufacture of a medicament for the treatment of a cancer patient with a hyperproliferative disorder.
69. The use of claim 68 wherein the hyperproliferative disorder is cancer selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.
70. The use of claim 68 further comprising an effective amount of an additional agent selected from the group consisting of an anticancer agent, an immunosuppressant agent, and an anti-infectious agent.
71. A use of the antibody-drug conjugate of claim 40 for the manufacture of a medicament for the treatment of an autoimmune disease.
72. A use of the antibody-drug conjugate of claim 40 for the manufacture of a medicament for the treatment of an infectious disease.
73. The use of claim 67 comprising:
 exposing mammalian cells in a cell culture medium to the antibody-drug conjugate of claim 40, and
 measuring a cytotoxic activity of the antibody-drug conjugate,
 whereby proliferation of the cells is inhibited.
74. A use of the antibody-drug conjugate of claim 40 and a pharmaceutically acceptable diluent, carrier or excipient for the manufacture of a medicament for the treatment of cancer.

75. The use of claim 74 wherein the antibody-drug conjugate specifically binds to a receptor encoded by an ErbB2 gene.
76. The use of claim 74 wherein the amount of antibody-drug conjugate administered to the patient is in the range of about 0.1 to about 10 mg/kg of patient weight.
77. The use of claim 74 wherein the antibody-drug conjugate is administered at about three week intervals.
78. The use of claim 74 wherein said antibody-drug conjugate is formulated with a pharmaceutically acceptable parenteral vehicle and administered parenterally.
79. The use of claim 74 wherein said antibody-drug conjugate is formulated in a unit dosage injectable form.
80. The use of claim 74 wherein said antibody-drug conjugate is administered intravenously.
81. The use of claim 74 further comprising the administration of a second antibody which binds a tumor-associated antigen selected from the group consisting of (1)-(35).
82. The use of claim 81 wherein said second antibody is conjugated with a cytotoxic agent.
83. A use of an antibody-drug conjugate of claim 40 which binds specifically to a tumor-associated antigen and a chemotherapeutic agent in the manufacture of a medicament to inhibit the growth of tumor cells that overexpress said tumor-associated antigen.
84. The use of claim 83 wherein said antibody-drug conjugate sensitizes the tumor cells to said chemotherapeutic agent.
85. A use of an effective amount of a combination of an antibody-drug conjugate of claim 40, and a chemotherapeutic agent in the manufacture of a medicament for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor.

86. An assay for detecting cancer cells comprising:
exposing cells to an antibody-drug conjugate of claim 40, and
determining the extent of binding of the antibody-drug conjugate to the cells.
87. The assay of claim 86 wherein the cells are breast tumor cells.
88. The assay of claim 86 wherein the extent of binding is determined by measuring levels of tumor-associated antigen-encoding nucleic acid by fluorescent *in situ* hybridization (FISH).
89. The assay of claim 86 wherein the extent of binding is determined by immunohistochemistry (IHC).
90. An article of manufacture comprising:
an antibody-drug conjugate of claim 40;
a container; and
a package insert or label indicating that the antibody-drug conjugate can be used to treat cancer.
91. The article of manufacture of claim 90 wherein said package insert or label indicates that the antibody-drug conjugate can be used to treat cancer characterized by the overexpression of an ErbB2 receptor.
92. The article of manufacture of claim 90 wherein the cancer is breast cancer.
93. A use of a therapeutically effective amount of an antibody-drug conjugate of claim 40 in the manufacture of a medicament for the treatment of cancer in a mammal, wherein the cancer is characterized by the overexpression of an ErbB2 (HER2) receptor and does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody.
94. The use of claim 93 wherein the mammal is human.
95. The use of an antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament.

96. The use of an antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to kill or inhibit the proliferation of tumor cells or cancer cells.

97. The use of an antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat a hyperproliferative disorder.

98. The use of claim 97 wherein the hyperproliferative disorder is cancer selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

99. The use of claim 97 wherein the medicament further comprises an additional agent selected from an anticancer agent, an immunosuppressant agent, and an anti-infectious agent.

100. The use of an antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat an autoimmune disease.

101. The use of an antibody-drug conjugate of claim 40, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament to treat an infectious disease.

Sequence Listing

<110> Doronina, Svetlana O.
Toki, Brian E.
Senter, Peter D.
Ebens, Allen J.
Polakis, Paul
Sliwkowski, Mark X.
Spencer, Susan D.
Kline, Toni Beth

<120> MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO LIGANDS

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Asp Ser Gly Leu Pro Val Val Thr Ser Gly Cys Leu Gly Leu Glu
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Val	Gln	Arg	Thr	Ile	Ala	Lys	Gln	Ile	Gln	Met	Val	Lys	Gln	Ile		200	205	210
Gly	Lys	Gly	Arg	Tyr	Gly	Glu	Val	Trp	Met	Gly	Lys	Trp	Arg	Gly		215	220	225
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Trp	Phe	Arg	Glu	Thr	Glu	Ile	Tyr	Gln	Thr	Val	Leu	Met	Arg	His		245	250	255
Glu	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ala	Asp	Ile	Lys	Gly	Thr	Gly		260	265	270
Ser	Trp	Thr	Gln	Leu	Tyr	Leu	Ile	Thr	Asp	Tyr	His	Glu	Asn	Gly		275	280	285
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Arg	Asp	Leu	Lys	Ser	Lys	Asn	Ile	Leu	Val	Lys	Lys	Asn	Gly	Thr		335	340	345
Cys	Cys	Ile	Ala	Asp	Leu	Gly	Leu	Ala	Val	Lys	Phe	Ile	Ser	Asp		350	355	360
Thr	Asn	Glu	Val	Asp	Ile	Pro	Pro	Asn	Thr	Arg	Val	Gly	Thr	Lys		365	370	375
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His	Phe	Gln	Ser	Tyr	Ile	Met	Ala	Asp	Met	Tyr	Ser	Phe	Gly	Leu		395	400	405
Ile	Leu	Trp	Glu	Val	Ala	Arg	Arg	Cys	Val	Ser	Gly	Gly	Ile	Val		410	415	420
Glu	Glu	Tyr	Gln	Leu	Pro	Tyr	His	Asp	Leu	Val	Pro	Ser	Asp	Pro		425	430	435
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Pro Ser Phe Pro Asn Arg Trp Ser Ser Asp Glu Cys Leu Arg Gln	455		460		465
Met Gly Lys Leu Met Thr Glu Cys Trp Ala His Asn Pro Ala Ser	470		475		480
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Leu Gln Arg Asn Ile Thr Leu Leu Asn Gly Val Ala Ile Ile Val	50	55	60	
Gly Thr Ile Ile Gly Ser Gly Ile Phe Val Thr Pro Thr Gly Val	65	70	75	
Leu Lys Glu Ala Gly Ser Pro Gly Leu Ala Leu Val Val Trp Ala	80	85	90	
Ala Cys Gly Val Phe Ser Ile Val Gly Ala Leu Cys Tyr Ala Glu	95	100	105	
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Val Phe Ala Thr Tyr Leu Leu Lys Pro Leu Phe Pro Thr Cys Pro	155	160	165	
Val Pro Glu Glu Ala Ala Lys Leu Val Ala Cys Leu Cys Val Leu	170	175	180	
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Val Gln Asp Ala Phe Ala Ala Ala Lys Leu Leu Ala Leu Ala Leu	200	205	210	

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Gly	Asn	Ile	Val	Leu	Ala	Leu	Tyr	Ser	Gly	Leu	Phe	Ala	Tyr	Gly		245	250	255
Gly	Trp	Asn	Tyr	Leu	Asn	Phe	Val	Thr	Glu	Glu	Met	Ile	Asn	Pro		260	265	270
Tyr	Arg	Asn	Leu	Pro	Leu	Ala	Ile	Ile	Ile	Ser	Leu	Pro	Ile	Val		275	280	285
Thr	Leu	Val	Tyr	Val	Leu	Thr	Asn	Leu	Ala	Tyr	Phe	Thr	Thr	Leu		290	295	300
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Phe	Trp	Lys	Thr	Pro	Val	Glu	Cys	Gly	Ile	Gly	Phe	Thr	Ile	Ile		455	460	465
Leu	Ser	Gly	Leu	Pro	Val	Tyr	Phe	Phe	Gly	Val	Trp	Trp	Lys	Asn		470	475	480
Lys	Pro	Lys	Trp	Leu	Leu	Gln	Gly	Ile	Phe	Ser	Thr	Thr	Val	Leu		485	490	495
Cys	Gln	Lys	Leu	Met	Gln	Val	Val	Pro	Gln	Glu	Thr					500	505	

<210> 3
 <211> 339
 <212> PRT
 <213> Homo sapien

<400> 3

Met	Glu	Ser	Arg	Lys	Asp	Ile	Thr	Asn	Gln	Glu	Glu	Leu	Trp	Lys	1	5	10	15
Met	Lys	Pro	Arg	Arg	Asn	Leu	Glu	Glu	Asp	Asp	Tyr	Leu	His	Lys	20	25	30	
Asp	Thr	Gly	Glu	Thr	Ser	Met	Leu	Lys	Arg	Pro	Val	Leu	Leu	His	35	40	45	
Leu	His	Gln	Thr	Ala	His	Ala	Asp	Glu	Phe	Asp	Cys	Pro	Ser	Glu	50	55	60	
Leu	Gln	His	Thr	Gln	Glu	Leu	Phe	Pro	Gln	Trp	His	Leu	Pro	Ile	65	70	75	
Lys	Ile	Ala	Ala	Ile	Ile	Ala	Ser	Leu	Thr	Phe	Leu	Tyr	Thr	Leu	80	85	90	
Leu	Arg	Glu	Val	Ile	His	Pro	Leu	Ala	Thr	Ser	His	Gln	Gln	Tyr	95	100	105	
Phe	Tyr	Lys	Ile	Pro	Ile	Leu	Val	Ile	Asn	Lys	Val	Leu	Pro	Met	110	115	120	
Val	Ser	Ile	Thr	Leu	Leu	Ala	Leu	Val	Tyr	Leu	Pro	Gly	Val	Ile	125	130	135	
Ala	Ala	Ile	Val	Gln	Leu	His	Asn	Gly	Thr	Lys	Tyr	Lys	Lys	Phe	140	145	150	
Pro	His	Trp	Leu	Asp	Lys	Trp	Met	Leu	Thr	Arg	Lys	Gln	Phe	Gly	155	160	165	
Leu	Leu	Ser	Phe	Phe	Phe	Ala	Val	Leu	His	Ala	Ile	Tyr	Ser	Leu	170	175	180	
Ser	Tyr	Pro	Met	Arg	Arg	Ser	Tyr	Arg	Tyr	Lys	Leu	Leu	Asn	Trp	185	190	195	
Ala	Tyr	Gln	Gln	Val	Gln	Gln	Asn	Lys	Glu	Asp	Ala	Trp	Ile	Glu	200	205	210	
His	Asp	Val	Trp	Arg	Met	Glu	Ile	Tyr	Val	Ser	Leu	Gly	Ile	Val	215	220	225	
Gly	Leu	Ala	Ile	Leu	Ala	Leu	Leu	Ala	Val	Thr	Ser	Ile	Pro	Ser	230	235	240	
Val	Ser	Asp	Ser	Leu	Thr	Trp	Arg	Glu	Phe	His	Tyr	Ile	Gln	Ser	245	250	255	
Lys	Leu	Gly	Ile	Val	Ser	Leu	Leu	Leu	Gly	Thr	Ile	His	Ala	Leu	260	265	270	
Ile	Phe	Ala	Trp	Asn	Lys	Trp	Ile	Asp	Ile	Lys	Gln	Phe	Val	Trp				

	275		280		285
Tyr Thr Pro Pro Thr Phe Met Ile Ala Val Phe Leu Pro Ile Val	290		295		300
Val Leu Ile Phe Lys Ser Ile Leu Phe Leu Pro Cys Leu Arg Lys	305		310		315
Lys Ile Leu Lys Ile Arg His Gly Trp Glu Asp Val Thr Lys Ile	320		325		330
Asn Lys Thr Glu Ile Cys Ser Gln Leu	335				

<210> 4
 <211> 6995
 <212> PRT
 <213> Homo sapien

<400> 4
 Pro Val Thr Ser Leu Leu Thr Pro Gly Leu Val Ile Thr Thr Asp
 1 5 10 15
 Arg Met Gly Ile Ser Arg Glu Pro Gly Thr Ser Ser Thr Ser Asn
 20 25 30
 Leu Ser Ser Thr Ser His Glu Arg Leu Thr Thr Leu Glu Asp Thr
 35 40 45
 Val Asp Thr Glu Ala Met Gln Pro Ser Thr His Thr Ala Val Thr
 50 55 60
 Asn Val Arg Thr Ser Ile Ser Gly His Glu Ser Gln Ser Ser Val
 65 70 75
 Leu Ser Asp Ser Glu Thr Pro Lys Ala Thr Ser Pro Met Gly Thr
 80 85 90
 Thr Tyr Thr Met Gly Glu Thr Ser Val Ser Ile Ser Thr Ser Asp
 95 100 105
 Phe Phe Glu Thr Ser Arg Ile Gln Ile Glu Pro Thr Ser Ser Leu
 110 115 120
 Thr Ser Gly Leu Arg Glu Thr Ser Ser Ser Glu Arg Ile Ser Ser
 125 130 135
 Ala Thr Glu Gly Ser Thr Val Leu Ser Glu Val Pro Ser Gly Ala
 140 145 150
 Thr Thr Glu Val Ser Arg Thr Glu Val Ile Ser Ser Arg Gly Thr
 155 160 165
 Ser Met Ser Gly Pro Asp Gln Phe Thr Ile Ser Pro Asp Ile Ser
 170 175 180
 Thr Glu Ala Ile Thr Arg Leu Ser Thr Ser Pro Ile Met Thr Glu
 185 190 195
 Ser Ala Glu Ser Ala Ile Thr Ile Glu Thr Gly Ser Pro Gly Ala
 200 205 210

Thr Ser Glu Gly	Thr Leu Thr Leu Asp	Thr Ser Thr Thr Thr	Phe
215		220	225
Trp Ser Gly Thr	His Ser Thr Ala Ser	Pro Gly Phe Ser His	Ser
230		235	240
Glu Met Thr Thr	Leu Met Ser Arg Thr	Pro Gly Asp Val Pro	Trp
245		250	255
Pro Ser Leu Pro	Ser Val Glu Glu Ala	Ser Ser Val Ser Ser	Ser
260		265	270
Leu Ser Ser Pro	Ala Met Thr Ser Thr	Ser Phe Phe Ser Thr	Leu
275		280	285
Pro Glu Ser Ile	Ser Ser Ser Pro His	Pro Val Thr Ala Leu	Leu
290		295	300
Thr Leu Gly Pro	Val Lys Thr Thr Asp	Met Leu Arg Thr Ser	Ser
305		310	315
Glu Pro Glu Thr	Ser Ser Pro Pro Asn	Leu Ser Ser Thr Ser	Ala
320		325	330
Glu Ile Leu Ala	Thr Ser Glu Val Thr	Lys Asp Arg Glu Lys	Ile
335		340	345
His Pro Ser Ser	Asn Thr Pro Val Val	Asn Val Gly Thr Val	Ile
350		355	360
Tyr Lys His Leu	Ser Pro Ser Ser Val	Leu Ala Asp Leu Val	Thr
365		370	375
Thr Lys Pro Thr	Ser Pro Met Ala Thr	Thr Ser Thr Leu Gly	Asn
380		385	390
Thr Ser Val Ser	Thr Ser Thr Pro Ala	Phe Pro Glu Thr Met	Met
395		400	405
Thr Gln Pro Thr	Ser Ser Leu Thr Ser	Gly Leu Arg Glu Ile	Ser
410		415	420
Thr Ser Gln Glu	Thr Ser Ser Ala Thr	Glu Arg Ser Ala Ser	Leu
425		430	435
Ser Gly Met Pro	Thr Gly Ala Thr Thr	Lys Val Ser Arg Thr	Glu
440		445	450
Ala Leu Ser Leu	Gly Arg Thr Ser Thr	Pro Gly Pro Ala Gln	Ser
455		460	465
Thr Ile Ser Pro	Glu Ile Ser Thr Glu	Thr Ile Thr Arg Ile	Ser
470		475	480
Thr Pro Leu Thr	Thr Thr Gly Ser Ala	Glu Met Thr Ile Thr	Pro
485		490	495
Lys Thr Gly His	Ser Gly Ala Ser Ser	Gln Gly Thr Phe Thr	Leu
500		505	510

Asp Thr Ser Ser Arg Ala Ser Trp Pro Gly Thr His Ser Ala Ala	515	520	525
Thr His Arg Ser Pro His Ser Gly Met Thr Thr Pro Met Ser Arg	530	535	540
Gly Pro Glu Asp Val Ser Trp Pro Ser Arg Pro Ser Val Glu Lys	545	550	555
Thr Ser Pro Pro Ser Ser Leu Val Ser Leu Ser Ala Val Thr Ser	560	565	570
Pro Ser Pro Leu Tyr Ser Thr Pro Ser Glu Ser Ser His Ser Ser	575	580	585
Pro Leu Arg Val Thr Ser Leu Phe Thr Pro Val Met Met Lys Thr	590	595	600
Thr Asp Met Leu Asp Thr Ser Leu Glu Pro Val Thr Thr Ser Pro	605	610	615
Pro Ser Met Asn Ile Thr Ser Asp Glu Ser Leu Ala Thr Ser Lys	620	625	630
Ala Thr Met Glu Thr Glu Ala Ile Gln Leu Ser Glu Asn Thr Ala	635	640	645
Val Thr Gln Met Gly Thr Ile Ser Ala Arg Gln Glu Phe Tyr Ser	650	655	660
Ser Tyr Pro Gly Leu Pro Glu Pro Ser Lys Val Thr Ser Pro Val	665	670	675
Val Thr Ser Ser Thr Ile Lys Asp Ile Val Ser Thr Thr Ile Pro	680	685	690
Ala Ser Ser Glu Ile Thr Arg Ile Glu Met Glu Ser Thr Ser Thr	695	700	705
Leu Thr Pro Thr Pro Arg Glu Thr Ser Thr Ser Gln Glu Ile His	710	715	720
Ser Ala Thr Lys Pro Ser Thr Val Pro Tyr Lys Ala Leu Thr Ser	725	730	735
Ala Thr Ile Glu Asp Ser Met Thr Gln Val Met Ser Ser Ser Arg	740	745	750
Gly Pro Ser Pro Asp Gln Ser Thr Met Ser Gln Asp Ile Ser Thr	755	760	765
Glu Val Ile Thr Arg Leu Ser Thr Ser Pro Ile Lys Thr Glu Ser	770	775	780
Thr Glu Met Thr Ile Thr Thr Gln Thr Gly Ser Pro Gly Ala Thr	785	790	795
Ser Arg Gly Thr Leu Thr Leu Asp Thr Ser Thr Thr Phe Met Ser	800	805	810
Gly Thr His Ser Thr Ala Ser Gln Gly Phe Ser His Ser Gln Met			

815					820					825				
Thr	Ala	Leu	Met	Ser	Arg	Thr	Pro	Gly	Glu	Val	Pro	Trp	Leu	Ser
				830					835					840
His	Pro	Ser	Val	Glu	Glu	Ala	Ser	Ser	Ala	Ser	Phe	Ser	Leu	Ser
				845					850					855
Ser	Pro	Val	Met	Thr	Ser	Ser	Ser	Pro	Val	Ser	Ser	Thr	Leu	Pro
				860					865					870
Asp	Ser	Ile	His	Ser	Ser	Ser	Leu	Pro	Val	Thr	Ser	Leu	Leu	Thr
				875					880					885
Ser	Gly	Leu	Val	Lys	Thr	Thr	Glu	Leu	Leu	Gly	Thr	Ser	Ser	Glu
				890					895					900
Pro	Glu	Thr	Ser	Ser	Pro	Pro	Asn	Leu	Ser	Ser	Thr	Ser	Ala	Glu
				905					910					915
Ile	Leu	Ala	Thr	Thr	Glu	Val	Thr	Thr	Asp	Thr	Glu	Lys	Leu	Glu
				920					925					930
Met	Thr	Asn	Val	Val	Thr	Ser	Gly	Tyr	Thr	His	Glu	Ser	Pro	Ser
				935					940					945
Ser	Val	Leu	Ala	Asp	Ser	Val	Thr	Thr	Lys	Ala	Thr	Ser	Ser	Met
				950					955					960
Gly	Ile	Thr	Tyr	Pro	Thr	Gly	Asp	Thr	Asn	Val	Leu	Thr	Ser	Thr
				965					970					975
Pro	Ala	Phe	Ser	Asp	Thr	Ser	Arg	Ile	Gln	Thr	Lys	Ser	Lys	Leu
				980					985					990
Ser	Leu	Thr	Pro	Gly	Leu	Met	Glu	Thr	Ser	Ile	Ser	Glu	Glu	Thr
				995					1000					1005
Ser	Ser	Ala	Thr	Glu	Lys	Ser	Thr	Val	Leu	Ser	Ser	Val	Pro	Thr
				1010					1015					1020
Gly	Ala	Thr	Thr	Glu	Val	Ser	Arg	Thr	Glu	Ala	Ile	Ser	Ser	Ser
				1025					1030					1035
Arg	Thr	Ser	Ile	Pro	Gly	Pro	Ala	Gln	Ser	Thr	Met	Ser	Ser	Asp
				1040					1045					1050
Thr	Ser	Met	Glu	Thr	Ile	Thr	Arg	Ile	Ser	Thr	Pro	Leu	Thr	Arg
				1055					1060					1065
Lys	Glu	Ser	Thr	Asp	Met	Ala	Ile	Thr	Pro	Lys	Thr	Gly	Pro	Ser
				1070					1075					1080
Gly	Ala	Thr	Ser	Gln	Gly	Thr	Phe	Thr	Leu	Asp	Ser	Ser	Ser	Thr
				1085					1090					1095
Ala	Ser	Trp	Pro	Gly	Thr	His	Ser	Ala	Thr	Thr	Gln	Arg	Phe	Pro
				1100					1105					1110
Arg	Ser	Val	Val	Thr	Thr	Pro	Met	Ser	Arg	Gly	Pro	Glu	Asp	Val
				1115					1120					1125

Ser Trp Pro Ser Pro Leu Ser Val Glu Lys Asn Ser Pro Pro Ser	1130	1135	1140
Ser Leu Val Ser Ser Ser Ser Val Thr Ser Pro Ser Pro Leu Tyr	1145	1150	1155
Ser Thr Pro Ser Gly Ser Ser His Ser Ser Pro Val Pro Val Thr	1160	1165	1170
Ser Leu Phe Thr Ser Ile Met Met Lys Ala Thr Asp Met Leu Asp	1175	1180	1185
Ala Ser Leu Glu Pro Glu Thr Thr Ser Ala Pro Asn Met Asn Ile	1190	1195	1200
Thr Ser Asp Glu Ser Leu Ala Ala Ser Lys Ala Thr Thr Glu Thr	1205	1210	1215
Glu Ala Ile His Val Phe Glu Asn Thr Ala Ala Ser His Val Glu	1220	1225	1230
Thr Thr Ser Ala Thr Glu Glu Leu Tyr Ser Ser Ser Pro Gly Phe	1235	1240	1245
Ser Glu Pro Thr Lys Val Ile Ser Pro Val Val Thr Ser Ser Ser	1250	1255	1260
Ile Arg Asp Asn Met Val Ser Thr Thr Met Pro Gly Ser Ser Gly	1265	1270	1275
Ile Thr Arg Ile Glu Ile Glu Ser Met Ser Ser Leu Thr Pro Gly	1280	1285	1290
Leu Arg Glu Thr Arg Thr Ser Gln Asp Ile Thr Ser Ser Thr Glu	1295	1300	1305
Thr Ser Thr Val Leu Tyr Lys Met Pro Ser Gly Ala Thr Pro Glu	1310	1315	1320
Val Ser Arg Thr Glu Val Met Pro Ser Ser Arg Thr Ser Ile Pro	1325	1330	1335
Gly Pro Ala Gln Ser Thr Met Ser Leu Asp Ile Ser Asp Glu Val	1340	1345	1350
Val Thr Arg Leu Ser Thr Ser Pro Ile Met Thr Glu Ser Ala Glu	1355	1360	1365
Ile Thr Ile Thr Thr Gln Thr Gly Tyr Ser Leu Ala Thr Ser Gln	1370	1375	1380
Val Thr Leu Pro Leu Gly Thr Ser Met Thr Phe Leu Ser Gly Thr	1385	1390	1395
His Ser Thr Met Ser Gln Gly Leu Ser His Ser Glu Met Thr Asn	1400	1405	1410
Leu Met Ser Arg Gly Pro Glu Ser Leu Ser Trp Thr Ser Pro Arg	1415	1420	1425

Phe Val Glu Thr Thr Arg Ser Ser Ser Ser Leu Thr Ser Leu Pro	1430	1435	1440
Leu Thr Thr Ser Leu Ser Pro Val Ser Ser Thr Leu Leu Asp Ser	1445	1450	1455
Ser Pro Ser Ser Pro Leu Pro Val Thr Ser Leu Ile Leu Pro Gly	1460	1465	1470
Leu Val Lys Thr Thr Glu Val Leu Asp Thr Ser Ser Glu Pro Lys	1475	1480	1485
Thr Ser Ser Ser Pro Asn Leu Ser Ser Thr Ser Val Glu Ile Pro	1490	1495	1500
Ala Thr Ser Glu Ile Met Thr Asp Thr Glu Lys Ile His Pro Ser	1505	1510	1515
Ser Asn Thr Ala Val Ala Lys Val Arg Thr Ser Ser Ser Val His	1520	1525	1530
Glu Ser His Ser Ser Val Leu Ala Asp Ser Glu Thr Thr Ile Thr	1535	1540	1545
Ile Pro Ser Met Gly Ile Thr Ser Ala Val Glu Asp Thr Thr Val	1550	1555	1560
Phe Thr Ser Asn Pro Ala Phe Ser Glu Thr Arg Arg Ile Pro Thr	1565	1570	1575
Glu Pro Thr Phe Ser Leu Thr Pro Gly Phe Arg Glu Thr Ser Thr	1580	1585	1590
Ser Glu Glu Thr Thr Ser Ile Thr Glu Thr Ser Ala Val Leu Phe	1595	1600	1605
Gly Val Pro Thr Ser Ala Thr Thr Glu Val Ser Met Thr Glu Ile	1610	1615	1620
Met Ser Ser Asn Arg Thr His Ile Pro Asp Ser Asp Gln Ser Thr	1625	1630	1635
Met Ser Pro Asp Ile Ile Thr Glu Val Ile Thr Arg Leu Ser Ser	1640	1645	1650
Ser Ser Met Met Ser Glu Ser Thr Gln Met Thr Ile Thr Thr Gln	1655	1660	1665
Lys Ser Ser Pro Gly Ala Thr Ala Gln Ser Thr Leu Thr Leu Ala	1670	1675	1680
Thr Thr Thr Ala Pro Leu Ala Arg Thr His Ser Thr Val Pro Pro	1685	1690	1695
Arg Phe Leu His Ser Glu Met Thr Thr Leu Met Ser Arg Ser Pro	1700	1705	1710
Glu Asn Pro Ser Trp Lys Ser Ser Pro Phe Val Glu Lys Thr Ser	1715	1720	1725
Ser Ser Ser Ser Leu Leu Ser Leu Pro Val Thr Thr Ser Pro Ser			

1730	1735	1740
Val Ser Ser Thr Leu Pro Gln Ser Ile Pro Ser Ser Ser Phe Ser		
1745	1750	1755
Val Thr Ser Leu Leu Thr Pro Gly Met Val Lys Thr Thr Asp Thr		
1760	1765	1770
Ser Thr Glu Pro Gly Thr Ser Leu Ser Pro Asn Leu Ser Gly Thr		
1775	1780	1785
Ser Val Glu Ile Leu Ala Ala Ser Glu Val Thr Thr Asp Thr Glu		
1790	1795	1800
Lys Ile His Pro Ser Ser Ser Met Ala Val Thr Asn Val Gly Thr		
1805	1810	1815
Thr Ser Ser Gly His Glu Leu Tyr Ser Ser Val Ser Ile His Ser		
1820	1825	1830
Glu Pro Ser Lys Ala Thr Tyr Pro Val Gly Thr Pro Ser Ser Met		
1835	1840	1845
Ala Glu Thr Ser Ile Ser Thr Ser Met Pro Ala Asn Phe Glu Thr		
1850	1855	1860
Thr Gly Phe Glu Ala Glu Pro Phe Ser His Leu Thr Ser Gly Leu		
1865	1870	1875
Arg Lys Thr Asn Met Ser Leu Asp Thr Ser Ser Val Thr Pro Thr		
1880	1885	1890
Asn Thr Pro Ser Ser Pro Gly Ser Thr His Leu Leu Gln Ser Ser		
1895	1900	1905
Lys Thr Asp Phe Thr Ser Ser Ala Lys Thr Ser Ser Pro Asp Trp		
1910	1915	1920
Pro Pro Ala Ser Gln Tyr Thr Glu Ile Pro Val Asp Ile Ile Thr		
1925	1930	1935
Pro Phe Asn Ala Ser Pro Ser Ile Thr Glu Ser Thr Gly Ile Thr		
1940	1945	1950
Ser Phe Pro Glu Ser Arg Phe Thr Met Ser Val Thr Glu Ser Thr		
1955	1960	1965
His His Leu Ser Thr Asp Leu Leu Pro Ser Ala Glu Thr Ile Ser		
1970	1975	1980
Thr Gly Thr Val Met Pro Ser Leu Ser Glu Ala Met Thr Ser Phe		
1985	1990	1995
Ala Thr Thr Gly Val Pro Arg Ala Ile Ser Gly Ser Gly Ser Pro		
2000	2005	2010
Phe Ser Arg Thr Glu Ser Gly Pro Gly Asp Ala Thr Leu Ser Thr		
2015	2020	2025
Ile Ala Glu Ser Leu Pro Ser Ser Thr Pro Val Pro Phe Ser Ser		
2030	2035	2040

Ser Thr Phe Thr Thr Thr Asp Ser Ser Thr Ile Pro Ala Leu His	2045	2050	2055
Glu Ile Thr Ser Ser Ser Ala Thr Pro Tyr Arg Val Asp Thr Ser	2060	2065	2070
Leu Gly Thr Glu Ser Ser Thr Thr Glu Gly Arg Leu Val Met Val	2075	2080	2085
Ser Thr Leu Asp Thr Ser Ser Gln Pro Gly Arg Thr Ser Ser Ser	2090	2095	2100
Pro Ile Leu Asp Thr Arg Met Thr Glu Ser Val Glu Leu Gly Thr	2105	2110	2115
Val Thr Ser Ala Tyr Gln Val Pro Ser Leu Ser Thr Arg Leu Thr	2120	2125	2130
Arg Thr Asp Gly Ile Met Glu His Ile Thr Lys Ile Pro Asn Glu	2135	2140	2145
Ala Ala His Arg Gly Thr Ile Arg Pro Val Lys Gly Pro Gln Thr	2150	2155	2160
Ser Thr Ser Pro Ala Ser Pro Lys Gly Leu His Thr Gly Gly Thr	2165	2170	2175
Lys Arg Met Glu Thr Thr Thr Thr Ala Leu Lys Thr Thr Thr Thr	2180	2185	2190
Ala Leu Lys Thr Thr Ser Arg Ala Thr Leu Thr Thr Ser Val Tyr	2195	2200	2205
Thr Pro Thr Leu Gly Thr Leu Thr Pro Leu Asn Ala Ser Met Gln	2210	2215	2220
Met Ala Ser Thr Ile Pro Thr Glu Met Met Ile Thr Thr Pro Tyr	2225	2230	2235
Val Phe Pro Asp Val Pro Glu Thr Thr Ser Ser Leu Ala Thr Ser	2240	2245	2250
Leu Gly Ala Glu Thr Ser Thr Ala Leu Pro Arg Thr Thr Pro Ser	2255	2260	2265
Val Phe Asn Arg Glu Ser Glu Thr Thr Ala Ser Leu Val Ser Arg	2270	2275	2280
Ser Gly Ala Glu Arg Ser Pro Val Ile Gln Thr Leu Asp Val Ser	2285	2290	2295
Ser Ser Glu Pro Asp Thr Thr Ala Ser Trp Val Ile His Pro Ala	2300	2305	2310
Glu Thr Ile Pro Thr Val Ser Lys Thr Thr Pro Asn Phe Phe His	2315	2320	2325
Ser Glu Leu Asp Thr Val Ser Ser Thr Ala Thr Ser His Gly Ala	2330	2335	2340

Asp Val Ser Ser Ala Ile Pro Thr Asn Ile Ser Pro Ser Glu Leu	2345	2350	2355
Asp Ala Leu Thr Pro Leu Val Thr Ile Ser Gly Thr Asp Thr Ser	2360	2365	2370
Thr Thr Phe Pro Thr Leu Thr Lys Ser Pro His Glu Thr Glu Thr	2375	2380	2385
Arg Thr Thr Trp Leu Thr His Pro Ala Glu Thr Ser Ser Thr Ile	2390	2395	2400
Pro Arg Thr Ile Pro Asn Phe Ser His His Glu Ser Asp Ala Thr	2405	2410	2415
Pro Ser Ile Ala Thr Ser Pro Gly Ala Glu Thr Ser Ser Ala Ile	2420	2425	2430
Pro Ile Met Thr Val Ser Pro Gly Ala Glu Asp Leu Val Thr Ser	2435	2440	2445
Gln Val Thr Ser Ser Gly Thr Asp Arg Asn Met Thr Ile Pro Thr	2450	2455	2460
Leu Thr Leu Ser Pro Gly Glu Pro Lys Thr Ile Ala Ser Leu Val	2465	2470	2475
Thr His Pro Glu Ala Gln Thr Ser Ser Ala Ile Pro Thr Ser Thr	2480	2485	2490
Ile Ser Pro Ala Val Ser Arg Leu Val Thr Ser Met Val Thr Ser	2495	2500	2505
Leu Ala Ala Lys Thr Ser Thr Thr Asn Arg Ala Leu Thr Asn Ser	2510	2515	2520
Pro Gly Glu Pro Ala Thr Thr Val Ser Leu Val Thr His Ser Ala	2525	2530	2535
Gln Thr Ser Pro Thr Val Pro Trp Thr Thr Ser Ile Phe Phe His	2540	2545	2550
Ser Lys Ser Asp Thr Thr Pro Ser Met Thr Thr Ser His Gly Ala	2555	2560	2565
Glu Ser Ser Ser Ala Val Pro Thr Pro Thr Val Ser Thr Glu Val	2570	2575	2580
Pro Gly Val Val Thr Pro Leu Val Thr Ser Ser Arg Ala Val Ile	2585	2590	2595
Ser Thr Thr Ile Pro Ile Leu Thr Leu Ser Pro Gly Glu Pro Glu	2600	2605	2610
Thr Thr Pro Ser Met Ala Thr Ser His Gly Glu Glu Ala Ser Ser	2615	2620	2625
Ala Ile Pro Thr Pro Thr Val Ser Pro Gly Val Pro Gly Val Val	2630	2635	2640
Thr Ser Leu Val Thr Ser Ser Arg Ala Val Thr Ser Thr Thr Ile			

2645	2650	2655
Pro Ile Leu Thr Phe Ser Leu Gly Glu Pro Glu Thr Thr Pro Ser		
2660	2665	2670
Met Ala Thr Ser His Gly Thr Glu Ala Gly Ser Ala Val Pro Thr		
2675	2680	2685
Val Leu Pro Glu Val Pro Gly Met Val Thr Ser Leu Val Ala Ser		
2690	2695	2700
Ser Arg Ala Val Thr Ser Thr Thr Leu Pro Thr Leu Thr Leu Ser		
2705	2710	2715
Pro Gly Glu Pro Glu Thr Thr Pro Ser Met Ala Thr Ser His Gly		
2720	2725	2730
Ala Glu Ala Ser Ser Thr Val Pro Thr Val Ser Pro Glu Val Pro		
2735	2740	2745
Gly Val Val Thr Ser Leu Val Thr Ser Ser Ser Gly Val Asn Ser		
2750	2755	2760
Thr Ser Ile Pro Thr Leu Ile Leu Ser Pro Gly Glu Leu Glu Thr		
2765	2770	2775
Thr Pro Ser Met Ala Thr Ser His Gly Ala Glu Ala Ser Ser Ala		
2780	2785	2790
Val Pro Thr Pro Thr Val Ser Pro Gly Val Ser Gly Val Val Thr		
2795	2800	2805
Pro Leu Val Thr Ser Ser Arg Ala Val Thr Ser Thr Thr Ile Pro		
2810	2815	2820
Ile Leu Thr Leu Ser Ser Ser Glu Pro Glu Thr Thr Pro Ser Met		
2825	2830	2835
Ala Thr Ser His Gly Val Glu Ala Ser Ser Ala Val Leu Thr Val		
2840	2845	2850
Ser Pro Glu Val Pro Gly Met Val Thr Phe Leu Val Thr Ser Ser		
2855	2860	2865
Arg Ala Val Thr Ser Thr Thr Ile Pro Thr Leu Thr Ile Ser Ser		
2870	2875	2880
Asp Glu Pro Glu Thr Thr Thr Ser Leu Val Thr His Ser Glu Ala		
2885	2890	2895
Lys Met Ile Ser Ala Ile Pro Thr Leu Gly Val Ser Pro Thr Val		
2900	2905	2910
Gln Gly Leu Val Thr Ser Leu Val Thr Ser Ser Gly Ser Glu Thr		
2915	2920	2925
Ser Ala Phe Ser Asn Leu Thr Val Ala Ser Ser Gln Pro Glu Thr		
2930	2935	2940
Ile Asp Ser Trp Val Ala His Pro Gly Thr Glu Ala Ser Ser Val		
2945	2950	2955

Val Pro Thr Leu Thr	Val Ser Thr Gly Glu Pro Phe Thr Asn Ile	
2960	2965	2970
Ser Leu Val Thr His	Pro Ala Glu Ser Ser Ser Thr Leu Pro Arg	
2975	2980	2985
Thr Thr Ser Arg Phe	Ser His Ser Glu Leu Asp Thr Met Pro Ser	
2990	2995	3000
Thr Val Thr Ser Pro	Glu Ala Glu Ser Ser Ser Ala Ile Ser Thr	
3005	3010	3015
Thr Ile Ser Pro Gly	Ile Pro Gly Val Leu Thr Ser Leu Val Thr	
3020	3025	3030
Ser Ser Gly Arg Asp	Ile Ser Ala Thr Phe Pro Thr Val Pro Glu	
3035	3040	3045
Ser Pro His Glu Ser	Glu Ala Thr Ala Ser Trp Val Thr His Pro	
3050	3055	3060
Ala Val Thr Ser Thr	Thr Val Pro Arg Thr Thr Pro Asn Tyr Ser	
3065	3070	3075
His Ser Glu Pro Asp	Thr Thr Pro Ser Ile Ala Thr Ser Pro Gly	
3080	3085	3090
Ala Glu Ala Thr Ser	Asp Phe Pro Thr Ile Thr Val Ser Pro Asp	
3095	3100	3105
Val Pro Asp Met Val	Thr Ser Gln Val Thr Ser Ser Gly Thr Asp	
3110	3115	3120
Thr Ser Ile Thr Ile	Pro Thr Leu Thr Leu Ser Ser Gly Glu Pro	
3125	3130	3135
Glu Thr Thr Thr Ser	Phe Ile Thr Tyr Ser Glu Thr His Thr Ser	
3140	3145	3150
Ser Ala Ile Pro Thr	Leu Pro Val Ser Pro Asp Ala Ser Lys Met	
3155	3160	3165
Leu Thr Ser Leu Val	Ile Ser Ser Gly Thr Asp Ser Thr Thr Thr	
3170	3175	3180
Phe Pro Thr Leu Thr	Glu Thr Pro Tyr Glu Pro Glu Thr Thr Ala	
3185	3190	3195
Ile Gln Leu Ile His	Pro Ala Glu Thr Asn Thr Met Val Pro Arg	
3200	3205	3210
Thr Thr Pro Lys Phe	Ser His Ser Lys Ser Asp Thr Thr Leu Pro	
3215	3220	3225
Val Ala Ile Thr Ser	Pro Gly Pro Glu Ala Ser Ser Ala Val Ser	
3230	3235	3240
Thr Thr Thr Ile Ser	Pro Asp Met Ser Asp Leu Val Thr Ser Leu	
3245	3250	3255

3560	3565	3570
Ser Thr Leu Ser Leu Gly Leu Leu Glu Thr Thr Gly Leu Leu Ala 3575	3580	3585
Thr Ser Ser Ser Ala Glu Thr Ser Thr Ser Thr Leu Thr Leu Thr 3590	3595	3600
Val Ser Pro Ala Val Ser Gly Leu Ser Ser Ala Ser Ile Thr Thr 3605	3610	3615
Asp Lys Pro Gln Thr Val Thr Ser Trp Asn Thr Glu Thr Ser Pro 3620	3625	3630
Ser Val Thr Ser Val Gly Pro Pro Glu Phe Ser Arg Thr Val Thr 3635	3640	3645
Gly Thr Thr Met Thr Leu Ile Pro Ser Glu Met Pro Thr Pro Pro 3650	3655	3660
Lys Thr Ser His Gly Glu Gly Val Ser Pro Thr Thr Ile Leu Arg 3665	3670	3675
Thr Thr Met Val Glu Ala Thr Asn Leu Ala Thr Thr Gly Ser Ser 3680	3685	3690
Pro Thr Val Ala Lys Thr Thr Thr Thr Phe Asn Thr Leu Ala Gly 3695	3700	3705
Ser Leu Phe Thr Pro Leu Thr Thr Pro Gly Met Ser Thr Leu Ala 3710	3715	3720
Ser Glu Ser Val Thr Ser Arg Thr Ser Tyr Asn His Arg Ser Trp 3725	3730	3735
Ile Ser Thr Thr Ser Ser Tyr Asn Arg Arg Tyr Trp Thr Pro Ala 3740	3745	3750
Thr Ser Thr Pro Val Thr Ser Thr Phe Ser Pro Gly Ile Ser Thr 3755	3760	3765
Ser Ser Ile Pro Ser Ser Thr Ala Ala Thr Val Pro Phe Met Val 3770	3775	3780
Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu 3785	3790	3795
Asp Met Arg His Pro Gly Ser Arg Lys Phe Asn Ala Thr Glu Arg 3800	3805	3810
Glu Leu Gln Gly Leu Leu Lys Pro Leu Phe Arg Asn Ser Ser Leu 3815	3820	3825
Glu Tyr Leu Tyr Ser Gly Cys Arg Leu Ala Ser Leu Arg Pro Glu 3830	3835	3840
Lys Asp Ser Ser Ala Thr Ala Val Asp Ala Ile Cys Thr His Arg 3845	3850	3855
Pro Asp Pro Glu Asp Leu Gly Leu Asp Arg Glu Arg Leu Tyr Trp 3860	3865	3870

Glu	Leu	Ser	Asn	Leu	Thr	Asn	Gly	Ile	Gln	Glu	Leu	Gly	Pro	Tyr				3875	3880	3885
Thr	Leu	Asp	Arg	Asn	Ser	Leu	Tyr	Val	Asn	Gly	Phe	Thr	His	Arg				3890	3895	3900
Ser	Ser	Met	Pro	Thr	Thr	Ser	Thr	Pro	Gly	Thr	Ser	Thr	Val	Asp				3905	3910	3915
Val	Gly	Thr	Ser	Gly	Thr	Pro	Ser	Ser	Ser	Pro	Ser	Pro	Thr	Thr				3920	3925	3930
Ala	Gly	Pro	Leu	Leu	Met	Pro	Phe	Thr	Leu	Asn	Phe	Thr	Ile	Thr				3935	3940	3945
Asn	Leu	Gln	Tyr	Glu	Glu	Asp	Met	Arg	Arg	Thr	Gly	Ser	Arg	Lys				3950	3955	3960
Phe	Asn	Thr	Met	Glu	Ser	Val	Leu	Gln	Gly	Leu	Leu	Lys	Pro	Leu				3965	3970	3975
Phe	Lys	Asn	Thr	Ser	Val	Gly	Pro	Leu	Tyr	Ser	Gly	Cys	Arg	Leu				3980	3985	3990
Thr	Leu	Leu	Arg	Pro	Glu	Lys	Asp	Gly	Ala	Ala	Thr	Gly	Val	Asp				3995	4000	4005
Ala	Ile	Cys	Thr	His	Arg	Leu	Asp	Pro	Lys	Ser	Pro	Gly	Leu	Asn				4010	4015	4020
Arg	Glu	Gln	Leu	Tyr	Trp	Glu	Leu	Ser	Lys	Leu	Thr	Asn	Asp	Ile				4025	4030	4035
Glu	Glu	Leu	Gly	Pro	Tyr	Thr	Leu	Asp	Arg	Asn	Ser	Leu	Tyr	Val				4040	4045	4050
Asn	Gly	Phe	Thr	His	Gln	Ser	Ser	Val	Ser	Thr	Thr	Ser	Thr	Pro				4055	4060	4065
Gly	Thr	Ser	Thr	Val	Asp	Leu	Arg	Thr	Ser	Gly	Thr	Pro	Ser	Ser				4070	4075	4080
Leu	Ser	Ser	Pro	Thr	Ile	Met	Ala	Ala	Gly	Pro	Leu	Leu	Val	Pro				4085	4090	4095
Phe	Thr	Leu	Asn	Phe	Thr	Ile	Thr	Asn	Leu	Gln	Tyr	Gly	Glu	Asp				4100	4105	4110
Met	Gly	His	Pro	Gly	Ser	Arg	Lys	Phe	Asn	Thr	Thr	Glu	Arg	Val				4115	4120	4125
Leu	Gln	Gly	Leu	Leu	Gly	Pro	Ile	Phe	Lys	Asn	Thr	Ser	Val	Gly				4130	4135	4140
Pro	Leu	Tyr	Ser	Gly	Cys	Arg	Leu	Thr	Ser	Leu	Arg	Ser	Glu	Lys				4145	4150	4155
Asp	Gly	Ala	Ala	Thr	Gly	Val	Asp	Ala	Ile	Cys	Ile	His	His	Leu				4160	4165	4170

Asp Pro Lys Ser Pro Gly Leu Asn Arg Glu Arg Leu Tyr Trp Glu	4175	4180	4185
Leu Ser Gln Leu Thr Asn Gly Ile Lys Glu Leu Gly Pro Tyr Thr	4190	4195	4200
Leu Asp Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr His Arg Thr	4205	4210	4215
Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Ser Thr Val Asp Leu	4220	4225	4230
Gly Thr Ser Gly Thr Pro Phe Ser Leu Pro Ser Pro Ala Thr Ala	4235	4240	4245
Gly Pro Leu Leu Val Leu Phe Thr Leu Asn Phe Thr Ile Thr Asn	4250	4255	4260
Leu Lys Tyr Glu Glu Asp Met His Arg Pro Gly Ser Arg Lys Phe	4265	4270	4275
Asn Thr Thr Glu Arg Val Leu Gln Thr Leu Val Gly Pro Met Phe	4280	4285	4290
Lys Asn Thr Ser Val Gly Leu Leu Tyr Ser Gly Cys Arg Leu Thr	4295	4300	4305
Leu Leu Arg Ser Glu Lys Asp Gly Ala Ala Thr Gly Val Asp Ala	4310	4315	4320
Ile Cys Thr His Arg Leu Asp Pro Lys Ser Pro Gly Val Asp Arg	4325	4330	4335
Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr Asn Gly Ile Lys	4340	4345	4350
Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr Val Asn	4355	4360	4365
Gly Phe Thr His Trp Ile Pro Val Pro Thr Ser Ser Thr Pro Gly	4370	4375	4380
Thr Ser Thr Val Asp Leu Gly Ser Gly Thr Pro Ser Ser Leu Pro	4385	4390	4395
Ser Pro Thr Ser Ala Thr Ala Gly Pro Leu Leu Val Pro Phe Thr	4400	4405	4410
Leu Asn Phe Thr Ile Thr Asn Leu Lys Tyr Glu Glu Asp Met His	4415	4420	4425
Cys Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln	4430	4435	4440
Ser Leu Leu Gly Pro Met Phe Lys Asn Thr Ser Val Gly Pro Leu	4445	4450	4455
Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Ser Glu Lys Asp Gly	4460	4465	4470
Ala Ala Thr Gly Val Asp Ala Ile Cys Thr His Arg Leu Asp Pro			

4475	4480	4485
Lys Ser Pro Gly Val Asp Arg Glu Gln Leu Tyr Trp Glu Leu Ser		
4490	4495	4500
Gln Leu Thr Asn Gly Ile Lys Glu Leu Gly Pro Tyr Thr Leu Asp		
4505	4510	4515
Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr His Gln Thr Ser Ala		
4520	4525	4530
Pro Asn Thr Ser Thr Pro Gly Thr Ser Thr Val Asp Leu Gly Thr		
4535	4540	4545
Ser Gly Thr Pro Ser Ser Leu Pro Ser Pro Thr Ser Ala Gly Pro		
4550	4555	4560
Leu Leu Val Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln		
4565	4570	4575
Tyr Glu Glu Asp Met His His Pro Gly Ser Arg Lys Phe Asn Thr		
4580	4585	4590
Thr Glu Arg Val Leu Gln Gly Leu Leu Gly Pro Met Phe Lys Asn		
4595	4600	4605
Thr Ser Val Gly Leu Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu		
4610	4615	4620
Arg Pro Glu Lys Asn Gly Ala Ala Thr Gly Met Asp Ala Ile Cys		
4625	4630	4635
Ser His Arg Leu Asp Pro Lys Ser Pro Gly Leu Asn Arg Glu Gln		
4640	4645	4650
Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Gly Ile Lys Glu Leu		
4655	4660	4665
Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr Val Asn Gly Phe		
4670	4675	4680
Thr His Arg Ser Ser Val Ala Pro Thr Ser Thr Pro Gly Thr Ser		
4685	4690	4695
Thr Val Asp Leu Gly Thr Ser Gly Thr Pro Ser Ser Leu Pro Ser		
4700	4705	4710
Pro Thr Thr Ala Val Pro Leu Leu Val Pro Phe Thr Leu Asn Phe		
4715	4720	4725
Thr Ile Thr Asn Leu Gln Tyr Gly Glu Asp Met Arg His Pro Gly		
4730	4735	4740
Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu		
4745	4750	4755
Gly Pro Leu Phe Lys Asn Ser Ser Val Gly Pro Leu Tyr Ser Gly		
4760	4765	4770
Cys Arg Leu Ile Ser Leu Arg Ser Glu Lys Asp Gly Ala Ala Thr		
4775	4780	4785

Gly Val Asp Ala Ile Cys Thr His His Leu Asn Pro Gln Ser Pro	4790	4795	4800
Gly Leu Asp Arg Glu Gln Leu Tyr Trp Gln Leu Ser Gln Met Thr	4805	4810	4815
Asn Gly Ile Lys Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asn Ser	4820	4825	4830
Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser Gly Leu Thr Thr	4835	4840	4845
Ser Thr Pro Trp Thr Ser Thr Val Asp Leu Gly Thr Ser Gly Thr	4850	4855	4860
Pro Ser Pro Val Pro Ser Pro Thr Thr Ala Gly Pro Leu Leu Val	4865	4870	4875
Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu	4880	4885	4890
Asp Met His Arg Pro Gly Ser Arg Lys Phe Asn Ala Thr Glu Arg	4895	4900	4905
Val Leu Gln Gly Leu Leu Ser Pro Ile Phe Lys Asn Ser Ser Val	4910	4915	4920
Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Ser Leu Arg Pro Glu	4925	4930	4935
Lys Asp Gly Ala Ala Thr Gly Met Asp Ala Val Cys Leu Tyr His	4940	4945	4950
Pro Asn Pro Lys Arg Pro Gly Leu Asp Arg Glu Gln Leu Tyr Trp	4955	4960	4965
Glu Leu Ser Gln Leu Thr His Asn Ile Thr Glu Leu Gly Pro Tyr	4970	4975	4980
Ser Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr His Gln	4985	4990	4995
Asn Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Ser Thr Val Tyr	5000	5005	5010
Trp Ala Thr Thr Gly Thr Pro Ser Ser Phe Pro Gly His Thr Glu	5015	5020	5025
Pro Gly Pro Leu Leu Ile Pro Phe Thr Phe Asn Phe Thr Ile Thr	5030	5035	5040
Asn Leu His Tyr Glu Glu Asn Met Gln His Pro Gly Ser Arg Lys	5045	5050	5055
Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Lys Pro Leu	5060	5065	5070
Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu	5075	5080	5085

5390	5395	5400
Arg Pro Glu Lys Arg Gly Ala Ala Thr Gly Val Asp Thr Ile Cys		
5405	5410	5415
Thr His Arg Leu Asp Pro Leu Asn Pro Gly Leu Asp Arg Glu Gln		
5420	5425	5430
Leu Tyr Trp Glu Leu Ser Lys Leu Thr Arg Gly Ile Ile Glu Leu		
5435	5440	5445
Gly Pro Tyr Leu Leu Asp Arg Gly Ser Leu Tyr Val Asn Gly Phe		
5450	5455	5460
Thr His Arg Asn Phe Val Pro Ile Thr Ser Thr Pro Gly Thr Ser		
5465	5470	5475
Thr Val His Leu Gly Thr Ser Glu Thr Pro Ser Ser Leu Pro Arg		
5480	5485	5490
Pro Ile Val Pro Gly Pro Leu Leu Val Pro Phe Thr Leu Asn Phe		
5495	5500	5505
Thr Ile Thr Asn Leu Gln Tyr Glu Glu Ala Met Arg His Pro Gly		
5510	5515	5520
Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu		
5525	5530	5535
Arg Pro Leu Phe Lys Asn Thr Ser Ile Gly Pro Leu Tyr Ser Ser		
5540	5545	5550
Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys Asp Lys Ala Ala Thr		
5555	5560	5565
Arg Val Asp Ala Ile Cys Thr His His Pro Asp Pro Gln Ser Pro		
5570	5575	5580
Gly Leu Asn Arg Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr		
5585	5590	5595
His Gly Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser		
5600	5605	5610
Leu Tyr Val Asp Gly Phe Thr His Trp Ser Pro Ile Pro Thr Thr		
5615	5620	5625
Ser Thr Pro Gly Thr Ser Ile Val Asn Leu Gly Thr Ser Gly Ile		
5630	5635	5640
Pro Pro Ser Leu Pro Glu Thr Thr Ala Thr Gly Pro Leu Leu Val		
5645	5650	5655
Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu		
5660	5665	5670
Asn Met Gly His Pro Gly Ser Arg Lys Phe Asn Ile Thr Glu Ser		
5675	5680	5685
Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys Ser Thr Ser Val		
5690	5695	5700

Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu	5705	5710	5715
Lys Asp Gly Val Ala Thr Arg Val Asp Ala Ile Cys Thr His Arg	5720	5725	5730
Pro Asp Pro Lys Ile Pro Gly Leu Asp Arg Gln Gln Leu Tyr Trp	5735	5740	5745
Glu Leu Ser Gln Leu Thr His Ser Ile Thr Glu Leu Gly Pro Tyr	5750	5755	5760
Thr Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr Gln Arg	5765	5770	5775
Ser Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Phe Thr Val Gln	5780	5785	5790
Pro Glu Thr Ser Glu Thr Pro Ser Ser Leu Pro Gly Pro Thr Ala	5795	5800	5805
Thr Gly Pro Val Leu Leu Pro Phe Thr Leu Asn Phe Thr Ile Ile	5810	5815	5820
Asn Leu Gln Tyr Glu Glu Asp Met His Arg Pro Gly Ser Arg Lys	5825	5830	5835
Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Met Pro Leu	5840	5845	5850
Phe Lys Asn Thr Ser Val Ser Ser Leu Tyr Ser Gly Cys Arg Leu	5855	5860	5865
Thr Leu Leu Arg Pro Glu Lys Asp Gly Ala Ala Thr Arg Val Asp	5870	5875	5880
Ala Val Cys Thr His Arg Pro Asp Pro Lys Ser Pro Gly Leu Asp	5885	5890	5895
Arg Glu Arg Leu Tyr Trp Lys Leu Ser Gln Leu Thr His Gly Ile	5900	5905	5910
Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg His Ser Leu Tyr Val	5915	5920	5925
Asn Gly Phe Thr His Gln Ser Ser Met Thr Thr Thr Arg Thr Pro	5930	5935	5940
Asp Thr Ser Thr Met His Leu Ala Thr Ser Arg Thr Pro Ala Ser	5945	5950	5955
Leu Ser Gly Pro Thr Thr Ala Ser Pro Leu Leu Val Leu Phe Thr	5960	5965	5970
Ile Asn Phe Thr Ile Thr Asn Leu Arg Tyr Glu Glu Asn Met His	5975	5980	5985
His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln	5990	5995	6000

Gly Leu Leu Arg Pro Val Phe Lys Asn Thr Ser Val Gly Pro Leu	6005	6010	6015
Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Lys Lys Asp Gly	6020	6025	6030
Ala Ala Thr Lys Val Asp Ala Ile Cys Thr Tyr Arg Pro Asp Pro	6035	6040	6045
Lys Ser Pro Gly Leu Asp Arg Glu Gln Leu Tyr Trp Glu Leu Ser	6050	6055	6060
Gln Leu Thr His Ser Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp	6065	6070	6075
Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr Gln Arg Ser Ser Val	6080	6085	6090
Pro Thr Thr Ser Ile Pro Gly Thr Pro Thr Val Asp Leu Gly Thr	6095	6100	6105
Ser Gly Thr Pro Val Ser Lys Pro Gly Pro Ser Ala Ala Ser Pro	6110	6115	6120
Leu Leu Val Leu Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Arg	6125	6130	6135
Tyr Glu Glu Asn Met Gln His Pro Gly Ser Arg Lys Phe Asn Thr	6140	6145	6150
Thr Glu Arg Val Leu Gln Gly Leu Leu Arg Ser Leu Phe Lys Ser	6155	6160	6165
Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu	6170	6175	6180
Arg Pro Glu Lys Asp Gly Thr Ala Thr Gly Val Asp Ala Ile Cys	6185	6190	6195
Thr His His Pro Asp Pro Lys Ser Pro Arg Leu Asp Arg Glu Gln	6200	6205	6210
Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Asn Ile Thr Glu Leu	6215	6220	6225
Gly Pro Tyr Ala Leu Asp Asn Asp Ser Leu Phe Val Asn Gly Phe	6230	6235	6240
Thr His Arg Ser Ser Val Ser Thr Thr Ser Thr Pro Gly Thr Pro	6245	6250	6255
Thr Val Tyr Leu Gly Ala Ser Lys Thr Pro Ala Ser Ile Phe Gly	6260	6265	6270
Pro Ser Ala Ala Ser His Leu Leu Ile Leu Phe Thr Leu Asn Phe	6275	6280	6285
Thr Ile Thr Asn Leu Arg Tyr Glu Glu Asn Met Trp Pro Gly Ser	6290	6295	6300
Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Arg			

6305	6310	6315
Pro Leu Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys		
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Arg Leu Thr Leu Leu Arg Pro Glu Lys Asp Gly Glu Ala Thr Gly		
6335	6340	6345
Val Asp Ala Ile Cys Thr His Arg Pro Asp Pro Thr Gly Pro Gly		
6350	6355	6360
Leu Asp Arg Glu Gln Leu Tyr Leu Glu Leu Ser Gln Leu Thr His		
6365	6370	6375
Ser Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser Leu		
6380	6385	6390
Tyr Val Asn Gly Phe Thr His Arg Ser Ser Val Pro Thr Thr Ser		
6395	6400	6405
Thr Gly Val Val Ser Glu Glu Pro Phe Thr Leu Asn Phe Thr Ile		
6410	6415	6420
Asn Asn Leu Arg Tyr Met Ala Asp Met Gly Gln Pro Gly Ser Leu		
6425	6430	6435
Lys Phe Asn Ile Thr Asp Asn Val Met Gln His Leu Leu Ser Pro		
6440	6445	6450
Leu Phe Gln Arg Ser Ser Leu Gly Ala Arg Tyr Thr Gly Cys Arg		
6455	6460	6465
Val Ile Ala Leu Arg Ser Val Lys Asn Gly Ala Glu Thr Arg Val		
6470	6475	6480
Asp Leu Leu Cys Thr Tyr Leu Gln Pro Leu Ser Gly Pro Gly Leu		
6485	6490	6495
Pro Ile Lys Gln Val Phe His Glu Leu Ser Gln Gln Thr His Gly		
6500	6505	6510
Ile Thr Arg Leu Gly Pro Tyr Ser Leu Asp Lys Asp Ser Leu Tyr		
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Leu Asn Gly Tyr Asn Glu Pro Gly Pro Asp Glu Pro Pro Thr Thr		
6530	6535	6540
Pro Lys Pro Ala Thr Thr Phe Leu Pro Pro Leu Ser Glu Ala Thr		
6545	6550	6555
Thr Ala Met Gly Tyr His Leu Lys Thr Leu Thr Leu Asn Phe Thr		
6560	6565	6570
Ile Ser Asn Leu Gln Tyr Ser Pro Asp Met Gly Lys Gly Ser Ala		
6575	6580	6585
Thr Phe Asn Ser Thr Glu Gly Val Leu Gln His Leu Leu Arg Pro		
6590	6595	6600
Leu Phe Gln Lys Ser Ser Met Gly Pro Phe Tyr Leu Gly Cys Gln		
6605	6610	6615

Leu	Ile	Ser	Leu	Arg	Pro	Glu	Lys	Asp	Gly	Ala	Ala	Thr	Gly	Val	6620	6625	6630
Asp	Thr	Thr	Cys	Thr	Tyr	His	Pro	Asp	Pro	Val	Gly	Pro	Gly	Leu	6635	6640	6645
Asp	Ile	Gln	Gln	Leu	Tyr	Trp	Glu	Leu	Ser	Gln	Leu	Thr	His	Gly	6650	6655	6660
Val	Thr	Gln	Leu	Gly	Phe	Tyr	Val	Leu	Asp	Arg	Asp	Ser	Leu	Phe	6665	6670	6675
Ile	Asn	Gly	Tyr	Ala	Pro	Gln	Asn	Leu	Ser	Ile	Arg	Gly	Glu	Tyr	6680	6685	6690
Gln	Ile	Asn	Phe	His	Ile	Val	Asn	Trp	Asn	Leu	Ser	Asn	Pro	Asp	6695	6700	6705
Pro	Thr	Ser	Ser	Glu	Tyr	Ile	Thr	Leu	Leu	Arg	Asp	Ile	Gln	Asp	6710	6715	6720
Lys	Val	Thr	Thr	Leu	Tyr	Lys	Gly	Ser	Gln	Leu	His	Asp	Thr	Phe	6725	6730	6735
Arg	Phe	Cys	Leu	Val	Thr	Asn	Leu	Thr	Met	Asp	Ser	Val	Leu	Val	6740	6745	6750
Thr	Val	Lys	Ala	Leu	Phe	Ser	Ser	Asn	Leu	Asp	Pro	Ser	Leu	Val	6755	6760	6765
Glu	Gln	Val	Phe	Leu	Asp	Lys	Thr	Leu	Asn	Ala	Ser	Phe	His	Trp	6770	6775	6780
Leu	Gly	Ser	Thr	Tyr	Gln	Leu	Val	Asp	Ile	His	Val	Thr	Glu	Met	6785	6790	6795
Glu	Ser	Ser	Val	Tyr	Gln	Pro	Thr	Ser	Ser	Ser	Ser	Thr	Gln	His	6800	6805	6810
Phe	Tyr	Leu	Asn	Phe	Thr	Ile	Thr	Asn	Leu	Pro	Tyr	Ser	Gln	Asp	6815	6820	6825
Lys	Ala	Gln	Pro	Gly	Thr	Thr	Asn	Tyr	Gln	Arg	Asn	Lys	Arg	Asn	6830	6835	6840
Ile	Glu	Asp	Ala	Leu	Asn	Gln	Leu	Phe	Arg	Asn	Ser	Ser	Ile	Lys	6845	6850	6855
Ser	Tyr	Phe	Ser	Asp	Cys	Gln	Val	Ser	Thr	Phe	Arg	Ser	Val	Pro	6860	6865	6870
Asn	Arg	His	His	Thr	Gly	Val	Asp	Ser	Leu	Cys	Asn	Phe	Ser	Pro	6875	6880	6885
Leu	Ala	Arg	Arg	Val	Asp	Arg	Val	Ala	Ile	Tyr	Glu	Glu	Phe	Leu	6890	6895	6900
Arg	Met	Thr	Arg	Asn	Gly	Thr	Gln	Leu	Gln	Asn	Phe	Thr	Leu	Asp	6905	6910	6915

Arg Ser Ser Val Leu Val Asp Gly Tyr Ser Pro Asn Arg Asn Glu
 6920 6925 6930
 Pro Leu Thr Gly Asn Ser Asp Leu Pro Phe Trp Ala Val Ile Leu
 6935 6940 6945
 Ile Gly Leu Ala Gly Leu Leu Gly Leu Ile Thr Cys Leu Ile Cys
 6950 6955 6960
 Gly Val Leu Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr
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 Asn Val Gln Gln Gln Cys Pro Gly Tyr Tyr Gln Ser His Leu Asp
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 Leu Glu Asp Leu Gln
 6995

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 Pro Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp
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 Val Gln Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala
 35 40 45
 Ala Pro Leu Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser
 50 55 60
 Leu Ser Pro Arg Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser
 65 70 75
 Gly Leu Ser Thr Glu Arg Val Arg Glu Leu Ala Val Ala Leu Ala
 80 85 90
 Gln Lys Asn Val Lys Leu Ser Thr Glu Gln Leu Arg Cys Leu Ala
 95 100 105
 His Arg Leu Ser Glu Pro Pro Glu Asp Leu Asp Ala Leu Pro Leu
 110 115 120
 Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala Phe Ser Gly Pro Gln
 125 130 135
 Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys Ala Asn Val Asp
 140 145 150
 Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu Leu Pro Ala
 155 160 165
 Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser Glu Ala
 170 175 180
 Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu Pro Gly Arg

185										190					195				
Phe	Val	Ala	Glu	Ser	Ala	Glu	Val	Leu	Leu	Pro	Arg	Leu	Val	Ser					
				200					205					210					
Cys	Pro	Gly	Pro	Leu	Asp	Gln	Asp	Gln	Gln	Glu	Ala	Ala	Arg	Ala					
				215					220					225					
Ala	Leu	Gln	Gly	Gly	Gly	Pro	Pro	Tyr	Gly	Pro	Pro	Ser	Thr	Trp					
				230					235					240					
Ser	Val	Ser	Thr	Met	Asp	Ala	Leu	Arg	Gly	Leu	Leu	Pro	Val	Leu					
				245					250					255					
Gly	Gln	Pro	Ile	Ile	Arg	Ser	Ile	Pro	Gln	Gly	Ile	Val	Ala	Ala					
				260					265					270					
Trp	Arg	Gln	Arg	Ser	Ser	Arg	Asp	Pro	Ser	Trp	Arg	Gln	Pro	Glu					
				275					280					285					
Arg	Thr	Ile	Leu	Arg	Pro	Arg	Phe	Arg	Arg	Glu	Val	Glu	Lys	Thr					
				290					295					300					
Ala	Cys	Pro	Ser	Gly	Lys	Lys	Ala	Arg	Glu	Ile	Asp	Glu	Ser	Leu					
				305					310					315					
Ile	Phe	Tyr	Lys	Lys	Trp	Glu	Leu	Glu	Ala	Cys	Val	Asp	Ala	Ala					
				320					325					330					
Leu	Leu	Ala	Thr	Gln	Met	Asp	Arg	Val	Asn	Ala	Ile	Pro	Phe	Thr					
				335					340					345					
Tyr	Glu	Gln	Leu	Asp	Val	Leu	Lys	His	Lys	Leu	Asp	Glu	Leu	Tyr					
				350					355					360					
Pro	Gln	Gly	Tyr	Pro	Glu	Ser	Val	Ile	Gln	His	Leu	Gly	Tyr	Leu					
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Phe	Leu	Lys	Met	Ser	Pro	Glu	Asp	Ile	Arg	Lys	Trp	Asn	Val	Thr					
				380					385					390					
Ser	Leu	Glu	Thr	Leu	Lys	Ala	Leu	Leu	Glu	Val	Asn	Lys	Gly	His					
				395					400					405					
Glu	Met	Ser	Pro	Gln	Val	Ala	Thr	Leu	Ile	Asp	Arg	Phe	Val	Lys					
				410					415					420					
Gly	Arg	Gly	Gln	Leu	Asp	Lys	Asp	Thr	Leu	Asp	Thr	Leu	Thr	Ala					
				425					430					435					
Phe	Tyr	Pro	Gly	Tyr	Leu	Cys	Ser	Leu	Ser	Pro	Glu	Glu	Leu	Ser					
				440					445					450					
Ser	Val	Pro	Pro	Ser	Ser	Ile	Trp	Ala	Val	Arg	Pro	Gln	Asp	Leu					
				455					460					465					
Asp	Thr	Cys	Asp	Pro	Arg	Gln	Leu	Asp	Val	Leu	Tyr	Pro	Lys	Ala					
				470					475					480					
Arg	Leu	Ala	Phe	Gln	Asn	Met	Asn	Gly	Ser	Glu	Tyr	Phe	Val	Lys					
				485					490					495					

Ile	Gln	Ser	Phe	Leu	Gly	Gly	Ala	Pro	Thr	Glu	Asp	Leu	Lys	Ala	
				500					505					510	
Leu	Ser	Gln	Gln	Asn	Val	Ser	Met	Asp	Leu	Ala	Thr	Phe	Met	Lys	
				515					520					525	
Leu	Arg	Thr	Asp	Ala	Val	Leu	Pro	Leu	Thr	Val	Ala	Glu	Val	Gln	
				530					535					540	
Lys	Leu	Leu	Gly	Pro	His	Val	Glu	Gly	Leu	Lys	Ala	Glu	Glu	Arg	
				545					550					555	
His	Arg	Pro	Val	Arg	Asp	Trp	Ile	Leu	Arg	Gln	Arg	Gln	Asp	Asp	
				560					565					570	
Leu	Asp	Thr	Leu	Gly	Leu	Gly	Leu	Gln	Gly	Gly	Ile	Pro	Asn	Gly	
				575					580					585	
Tyr	Leu	Val	Leu	Asp	Leu	Ser	Met	Gln	Glu	Ala	Leu	Ser	Gly	Thr	
				590					595					600	
Pro	Cys	Leu	Leu	Gly	Pro	Gly	Pro	Val	Leu	Thr	Val	Leu	Ala	Leu	
				605					610					615	
Leu	Leu	Ala	Ser	Thr	Leu	Ala									
				620											

<210> 6
 <211> 690
 <212> PRT
 <213> Homo sapien

<400> 6

Met	Ala	Pro	Trp	Pro	Glu	Leu	Gly	Asp	Ala	Gln	Pro	Asn	Pro	Asp	
1				5					10					15	
Lys	Tyr	Leu	Glu	Gly	Ala	Ala	Gly	Gln	Gln	Pro	Thr	Ala	Pro	Asp	
				20					25					30	
Lys	Ser	Lys	Glu	Thr	Asn	Lys	Thr	Asp	Asn	Thr	Glu	Ala	Pro	Val	
				35					40					45	
Thr	Lys	Ile	Glu	Leu	Leu	Pro	Ser	Tyr	Ser	Thr	Ala	Thr	Leu	Ile	
				50					55					60	
Asp	Glu	Pro	Thr	Glu	Val	Asp	Asp	Pro	Trp	Asn	Leu	Pro	Thr	Leu	
				65					70					75	
Gln	Asp	Ser	Gly	Ile	Lys	Trp	Ser	Glu	Arg	Asp	Thr	Lys	Gly	Lys	
				80					85					90	
Ile	Leu	Cys	Phe	Phe	Gln	Gly	Ile	Gly	Arg	Leu	Ile	Leu	Leu	Leu	
				95					100					105	
Gly	Phe	Leu	Tyr	Phe	Phe	Val	Cys	Ser	Leu	Asp	Ile	Leu	Ser	Ser	
				110					115					120	
Ala	Phe	Gln	Leu	Val	Gly	Gly	Lys	Met	Ala	Gly	Gln	Phe	Phe	Ser	
				125					130					135	

Asn Ser Ser Ile Met Ser Asn Pro Leu Leu Gly Leu Val Ile Gly	140	145	150
Val Leu Val Thr Val Leu Val Gln Ser Ser Ser Thr Ser Thr Ser	155	160	165
Ile Val Val Ser Met Val Ser Ser Ser Leu Leu Thr Val Arg Ala	170	175	180
Ala Ile Pro Ile Ile Met Gly Ala Asn Ile Gly Thr Ser Ile Thr	185	190	195
Asn Thr Ile Val Ala Leu Met Gln Val Gly Asp Arg Ser Glu Phe	200	205	210
Arg Arg Ala Phe Ala Gly Ala Thr Val His Asp Phe Phe Asn Trp	215	220	225
Leu Ser Val Leu Val Leu Leu Pro Val Glu Val Ala Thr His Tyr	230	235	240
Leu Glu Ile Ile Thr Gln Leu Ile Val Glu Ser Phe His Phe Lys	245	250	255
Asn Gly Glu Asp Ala Pro Asp Leu Leu Lys Val Ile Thr Lys Pro	260	265	270
Phe Thr Lys Leu Ile Val Gln Leu Asp Lys Lys Val Ile Ser Gln	275	280	285
Ile Ala Met Asn Asp Glu Lys Ala Lys Asn Lys Ser Leu Val Lys	290	295	300
Ile Trp Cys Lys Thr Phe Thr Asn Lys Thr Gln Ile Asn Val Thr	305	310	315
Val Pro Ser Thr Ala Asn Cys Thr Ser Pro Ser Leu Cys Trp Thr	320	325	330
Asp Gly Ile Gln Asn Trp Thr Met Lys Asn Val Thr Tyr Lys Glu	335	340	345
Asn Ile Ala Lys Cys Gln His Ile Phe Val Asn Phe His Leu Pro	350	355	360
Asp Leu Ala Val Gly Thr Ile Leu Leu Ile Leu Ser Leu Leu Val	365	370	375
Leu Cys Gly Cys Leu Ile Met Ile Val Lys Ile Leu Gly Ser Val	380	385	390
Leu Lys Gly Gln Val Ala Thr Val Ile Lys Lys Thr Ile Asn Thr	395	400	405
Asp Phe Pro Phe Pro Phe Ala Trp Leu Thr Gly Tyr Leu Ala Ile	410	415	420
Leu Val Gly Ala Gly Met Thr Phe Ile Val Gln Ser Ser Ser Val	425	430	435
Phe Thr Ser Ala Leu Thr Pro Leu Ile Gly Ile Gly Val Ile Thr			

440	445	450
Ile Glu Arg Ala Tyr Pro Leu Thr Leu	Gly Ser Asn Ile Gly Thr	
455	460	465
Thr Thr Thr Ala Ile Leu Ala Ala Leu	Ala Ser Pro Gly Asn Ala	
470	475	480
Leu Arg Ser Ser Leu Gln Ile Ala Leu	Cys His Phe Phe Phe Asn	
485	490	495
Ile Ser Gly Ile Leu Leu Trp Tyr Pro	Ile Pro Phe Thr Arg Leu	
500	505	510
Pro Ile Arg Met Ala Lys Gly Leu Gly	Asn Ile Ser Ala Lys Tyr	
515	520	525
Arg Trp Phe Ala Val Phe Tyr Leu Ile	Ile Phe Phe Phe Leu Ile	
530	535	540
Pro Leu Thr Val Phe Gly Leu Ser Leu	Ala Gly Trp Arg Val Leu	
545	550	555
Val Gly Val Gly Val Pro Val Val Phe	Ile Ile Ile Leu Val Leu	
560	565	570
Cys Leu Arg Leu Leu Gln Ser Arg Cys	Pro Arg Val Leu Pro Lys	
575	580	585
Lys Leu Gln Asn Trp Asn Phe Leu Pro	Leu Trp Met Arg Ser Leu	
590	595	600
Lys Pro Trp Asp Ala Val Val Ser Lys	Phe Thr Gly Cys Phe Gln	
605	610	615
Met Arg Cys Cys Tyr Cys Cys Arg Val	Cys Cys Arg Ala Cys Cys	
620	625	630
Leu Leu Cys Gly Cys Pro Lys Cys Cys	Arg Cys Ser Lys Cys Cys	
635	640	645
Glu Asp Leu Glu Glu Ala Gln Glu Gly	Gln Asp Val Pro Val Lys	
650	655	660
Ala Pro Glu Thr Phe Asp Asn Ile Thr	Ile Ser Arg Glu Ala Gln	
665	670	675
Gly Glu Val Pro Ala Ser Asp Ser Lys	Thr Glu Cys Thr Ala Leu	
680	685	690
<210> 7		
<211> 1093		
<212> PRT		
<213> Homo sapien		
<400> 7		
Met Val Leu Ala Gly Pro Leu Ala Val	Ser Leu Leu Leu Pro Ser	
1	5	10 15
Leu Thr Leu Leu Val Ser His Leu Ser	Ser Ser Gln Asp Val Ser	
20	25	30

Ser	Glu	Pro	Ser	Ser	Glu	Gln	Gln	Leu	Cys	Ala	Leu	Ser	Lys	His	35	40	45
Pro	Thr	Val	Ala	Phe	Glu	Asp	Leu	Gln	Pro	Trp	Val	Ser	Asn	Phe	50	55	60
Thr	Tyr	Pro	Gly	Ala	Arg	Asp	Phe	Ser	Gln	Leu	Ala	Leu	Asp	Pro	65	70	75
Ser	Gly	Asn	Gln	Leu	Ile	Val	Gly	Ala	Arg	Asn	Tyr	Leu	Phe	Arg	80	85	90
Leu	Ser	Leu	Ala	Asn	Val	Ser	Leu	Leu	Gln	Ala	Thr	Glu	Trp	Ala	95	100	105
Ser	Ser	Glu	Asp	Thr	Arg	Arg	Ser	Cys	Gln	Ser	Lys	Gly	Lys	Thr	110	115	120
Glu	Glu	Glu	Cys	Gln	Asn	Tyr	Val	Arg	Val	Leu	Ile	Val	Ala	Gly	125	130	135
Arg	Lys	Val	Phe	Met	Cys	Gly	Thr	Asn	Ala	Phe	Ser	Pro	Met	Cys	140	145	150
Thr	Ser	Arg	Gln	Val	Gly	Asn	Leu	Ser	Arg	Thr	Thr	Glu	Lys	Ile	155	160	165
Asn	Gly	Val	Ala	Arg	Cys	Pro	Tyr	Asp	Pro	Arg	His	Asn	Ser	Thr	170	175	180
Ala	Val	Ile	Ser	Ser	Gln	Gly	Glu	Leu	Tyr	Ala	Ala	Thr	Val	Ile	185	190	195
Asp	Phe	Ser	Gly	Arg	Asp	Pro	Ala	Ile	Tyr	Arg	Ser	Leu	Gly	Ser	200	205	210
Gly	Pro	Pro	Leu	Arg	Thr	Ala	Gln	Tyr	Asn	Ser	Lys	Trp	Leu	Asn	215	220	225
Glu	Pro	Asn	Phe	Val	Ala	Ala	Tyr	Asp	Ile	Gly	Leu	Phe	Ala	Tyr	230	235	240
Phe	Phe	Leu	Arg	Glu	Asn	Ala	Val	Glu	His	Asp	Cys	Gly	Arg	Thr	245	250	255
Val	Tyr	Ser	Arg	Val	Ala	Arg	Val	Cys	Lys	Asn	Asp	Val	Gly	Gly	260	265	270
Arg	Phe	Leu	Leu	Glu	Asp	Thr	Trp	Thr	Thr	Phe	Met	Lys	Ala	Arg	275	280	285
Leu	Asn	Cys	Ser	Arg	Pro	Gly	Glu	Val	Pro	Phe	Tyr	Tyr	Asn	Glu	290	295	300
Leu	Gln	Ser	Ala	Phe	His	Leu	Pro	Glu	Gln	Asp	Leu	Ile	Tyr	Gly	305	310	315
Val	Phe	Thr	Thr	Asn	Val	Asn	Ser	Ile	Ala	Ala	Ser	Ala	Val	Cys	320	325	330

Ala Phe Asn Leu Ser Ala Ile Ser Gln Ala Phe Asn Gly Pro Phe	335	340	345
Arg Tyr Gln Glu Asn Pro Arg Ala Ala Trp Leu Pro Ile Ala Asn	350	355	360
Pro Ile Pro Asn Phe Gln Cys Gly Thr Leu Pro Glu Thr Gly Pro	365	370	375
Asn Glu Asn Leu Thr Glu Arg Ser Leu Gln Asp Ala Gln Arg Leu	380	385	390
Phe Leu Met Ser Glu Ala Val Gln Pro Val Thr Pro Glu Pro Cys	395	400	405
Val Thr Gln Asp Ser Val Arg Phe Ser His Leu Val Val Asp Leu	410	415	420
Val Gln Ala Lys Asp Thr Leu Tyr His Val Leu Tyr Ile Gly Thr	425	430	435
Glu Ser Gly Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser Arg Ser	440	445	450
Leu His Gly Cys Tyr Leu Glu Glu Leu His Val Leu Pro Pro Gly	455	460	465
Arg Arg Glu Pro Leu Arg Ser Leu Arg Ile Leu His Ser Ala Arg	470	475	480
Ala Leu Phe Val Gly Leu Arg Asp Gly Val Leu Arg Val Pro Leu	485	490	495
Glu Arg Cys Ala Ala Tyr Arg Ser Gln Gly Ala Cys Leu Gly Ala	500	505	510
Arg Asp Pro Tyr Cys Gly Trp Asp Gly Lys Gln Gln Arg Cys Ser	515	520	525
Thr Leu Glu Asp Ser Ser Asn Met Ser Leu Trp Thr Gln Asn Ile	530	535	540
Thr Ala Cys Pro Val Arg Asn Val Thr Arg Asp Gly Gly Phe Gly	545	550	555
Pro Trp Ser Pro Trp Gln Pro Cys Glu His Leu Asp Gly Asp Asn	560	565	570
Ser Gly Ser Cys Leu Cys Arg Ala Arg Ser Cys Asp Ser Pro Arg	575	580	585
Pro Arg Cys Gly Gly Leu Asp Cys Leu Gly Pro Ala Ile His Ile	590	595	600
Ala Asn Cys Ser Arg Asn Gly Ala Trp Thr Pro Trp Ser Ser Trp	605	610	615
Ala Leu Cys Ser Thr Ser Cys Gly Ile Gly Phe Gln Val Arg Gln	620	625	630
Arg Ser Cys Ser Asn Pro Ala Pro Arg His Gly Gly Arg Ile Cys			

635										640					645				
Val	Gly	Lys	Ser	Arg	Glu	Glu	Arg	Phe	Cys	Asn	Glu	Asn	Thr	Pro					
				650					655					660					
Cys	Pro	Val	Pro	Ile	Phe	Trp	Ala	Ser	Trp	Gly	Ser	Trp	Ser	Lys					
				665					670					675					
Cys	Ser	Ser	Asn	Cys	Gly	Gly	Gly	Met	Gln	Ser	Arg	Arg	Arg	Ala					
				680					685					690					
Cys	Glu	Asn	Gly	Asn	Ser	Cys	Leu	Gly	Cys	Gly	Val	Glu	Phe	Lys					
				695					700					705					
Thr	Cys	Asn	Pro	Glu	Gly	Cys	Pro	Glu	Val	Arg	Arg	Asn	Thr	Pro					
				710					715					720					
Trp	Thr	Pro	Trp	Leu	Pro	Val	Asn	Val	Thr	Gln	Gly	Gly	Ala	Arg					
				725					730					735					
Gln	Glu	Gln	Arg	Phe	Arg	Phe	Thr	Cys	Arg	Ala	Pro	Leu	Ala	Asp					
				740					745					750					
Pro	His	Gly	Leu	Gln	Phe	Gly	Arg	Arg	Arg	Thr	Glu	Thr	Arg	Thr					
				755					760					765					
Cys	Pro	Ala	Asp	Gly	Ser	Gly	Ser	Cys	Asp	Thr	Asp	Ala	Leu	Val					
				770					775					780					
Glu	Asp	Leu	Leu	Arg	Ser	Gly	Ser	Thr	Ser	Pro	His	Thr	Val	Ser					
				785					790					795					
Gly	Gly	Trp	Ala	Ala	Trp	Gly	Pro	Trp	Ser	Ser	Cys	Ser	Arg	Asp					
				800					805					810					
Cys	Glu	Leu	Gly	Phe	Arg	Val	Arg	Lys	Arg	Thr	Cys	Thr	Asn	Pro					
				815					820					825					
Glu	Pro	Arg	Asn	Gly	Gly	Leu	Pro	Cys	Val	Gly	Asp	Ala	Ala	Glu					
				830					835					840					
Tyr	Gln	Asp	Cys	Asn	Pro	Gln	Ala	Cys	Pro	Val	Arg	Gly	Ala	Trp					
				845					850					855					
Ser	Cys	Trp	Thr	Ser	Trp	Ser	Pro	Cys	Ser	Ala	Ser	Cys	Gly	Gly					
				860					865					870					
Gly	His	Tyr	Gln	Arg	Thr	Arg	Ser	Cys	Thr	Ser	Pro	Ala	Pro	Ser					
				875					880					885					
Pro	Gly	Glu	Asp	Ile	Cys	Leu	Gly	Leu	His	Thr	Glu	Glu	Ala	Leu					
				890					895					900					
Cys	Ala	Thr	Gln	Ala	Cys	Pro	Glu	Gly	Trp	Ser	Pro	Trp	Ser	Glu					
				905					910					915					
Trp	Ser	Lys	Cys	Thr	Asp	Asp	Gly	Ala	Gln	Ser	Arg	Ser	Arg	His					
				920					925					930					
Cys	Glu	Glu	Leu	Leu	Pro	Gly	Ser	Ser	Ala	Cys	Ala	Gly	Asn	Ser					
				935					940					945					

Ser	Gln	Ser	Arg	Pro	Cys	Pro	Tyr	Ser	Glu	Ile	Pro	Val	Ile	Leu
				950					955					960
Pro	Ala	Ser	Ser	Met	Glu	Glu	Ala	Thr	Gly	Cys	Ala	Gly	Phe	Asn
				965					970					975
Leu	Ile	His	Leu	Val	Ala	Thr	Gly	Ile	Ser	Cys	Phe	Leu	Gly	Ser
				980					985					990
Gly	Leu	Leu	Thr	Leu	Ala	Val	Tyr	Leu	Ser	Cys	Gln	His	Cys	Gln
				995					1000					1005
Arg	Gln	Ser	Gln	Glu	Ser	Thr	Leu	Val	His	Pro	Ala	Thr	Pro	Asn
				1010					1015					1020
His	Leu	His	Tyr	Lys	Gly	Gly	Gly	Thr	Pro	Lys	Asn	Glu	Lys	Tyr
				1025					1030					1035
Thr	Pro	Met	Glu	Phe	Lys	Thr	Leu	Asn	Lys	Asn	Asn	Leu	Ile	Pro
				1040					1045					1050
Asp	Asp	Arg	Ala	Asn	Phe	Tyr	Pro	Leu	Gln	Gln	Thr	Asn	Val	Tyr
				1055					1060					1065
Thr	Thr	Thr	Tyr	Tyr	Pro	Ser	Pro	Leu	Asn	Lys	His	Ser	Phe	Arg
				1070					1075					1080
Pro	Glu	Ala	Ser	Pro	Gly	Gln	Arg	Cys	Phe	Pro	Asn	Ser		
				1085					1090					

<210> 8
 <211> 141
 <212> PRT
 <213> Homo sapien

<400> 8														
Met	Trp	Val	Leu	Gly	Ile	Ala	Ala	Thr	Phe	Cys	Gly	Leu	Phe	Leu
1				5					10					15
Leu	Pro	Gly	Phe	Ala	Leu	Gln	Ile	Gln	Cys	Tyr	Gln	Cys	Glu	Glu
				20					25					30
Phe	Gln	Leu	Asn	Asn	Asp	Cys	Ser	Ser	Pro	Glu	Phe	Ile	Val	Asn
				35					40					45
Cys	Thr	Val	Asn	Val	Gln	Asp	Met	Cys	Gln	Lys	Glu	Val	Met	Glu
				50					55					60
Gln	Ser	Ala	Gly	Ile	Met	Tyr	Arg	Lys	Ser	Cys	Ala	Ser	Ser	Ala
				65					70					75
Ala	Cys	Leu	Ile	Ala	Ser	Ala	Gly	Tyr	Gln	Ser	Phe	Cys	Ser	Pro
				80					85					90
Gly	Lys	Leu	Asn	Ser	Val	Cys	Ile	Ser	Cys	Cys	Asn	Thr	Pro	Leu
				95					100					105
Cys	Asn	Gly	Pro	Arg	Pro	Lys	Lys	Arg	Gly	Ser	Ser	Ala	Ser	Ala
				110					115					120

Leu Arg Pro Gly Leu Arg Thr Thr Ile Leu Phe Leu Lys Leu Ala
 125 130 135

Leu Phe Ser Ala His Cys
 140

<210> 9
 <211> 442
 <212> PRT
 <213> Homo sapien

<400> 9
 Met Gln Pro Pro Pro Ser Leu Cys Gly Arg Ala Leu Val Ala Leu
 1 5 10 15

Val Leu Ala Cys Gly Leu Ser Arg Ile Trp Gly Glu Glu Arg Gly
 20 25 30

Phe Pro Pro Asp Arg Ala Thr Pro Leu Leu Gln Thr Ala Glu Ile
 35 40 45

Met Thr Pro Pro Thr Lys Thr Leu Trp Pro Lys Gly Ser Asn Ala
 50 55 60

Ser Leu Ala Arg Ser Leu Ala Pro Ala Glu Val Pro Lys Gly Asp
 65 70 75

Arg Thr Ala Gly Ser Pro Pro Arg Thr Ile Ser Pro Pro Pro Cys
 80 85 90

Gln Gly Pro Ile Glu Ile Lys Glu Thr Phe Lys Tyr Ile Asn Thr
 95 100 105

Val Val Ser Cys Leu Val Phe Val Leu Gly Ile Ile Gly Asn Ser
 110 115 120

Thr Leu Leu Arg Ile Ile Tyr Lys Asn Lys Cys Met Arg Asn Gly
 125 130 135

Pro Asn Ile Leu Ile Ala Ser Leu Ala Leu Gly Asp Leu Leu His
 140 145 150

Ile Val Ile Asp Ile Pro Ile Asn Val Tyr Lys Leu Leu Ala Glu
 155 160 165

Asp Trp Pro Phe Gly Ala Glu Met Cys Lys Leu Val Pro Phe Ile
 170 175 180

Gln Lys Ala Ser Val Gly Ile Thr Val Leu Ser Leu Cys Ala Leu
 185 190 195

Ser Ile Asp Arg Tyr Arg Ala Val Ala Ser Trp Ser Arg Ile Lys
 200 205 210

Gly Ile Gly Val Pro Lys Trp Thr Ala Val Glu Ile Val Leu Ile
 215 220 225

Trp Val Val Ser Val Val Leu Ala Val Pro Glu Ala Ile Gly Phe
 230 235 240

Asp Ile Ile Thr Met Asp Tyr Lys Gly Ser Tyr Leu Arg Ile Cys

245	250	255
Leu Leu His Pro Val Gln Lys Thr Ala	Phe Met Gln Phe Tyr Lys	
260	265	270
Thr Ala Lys Asp Trp Trp Leu Phe Ser	Phe Tyr Phe Cys Leu Pro	
275	280	285
Leu Ala Ile Thr Ala Phe Phe Tyr Thr	Leu Met Thr Cys Glu Met	
290	295	300
Leu Arg Lys Lys Ser Gly Met Gln Ile	Ala Leu Asn Asp His Leu	
305	310	315
Lys Gln Arg Arg Glu Val Ala Lys Thr	Val Phe Cys Leu Val Leu	
320	325	330
Val Phe Ala Leu Cys Trp Leu Pro Leu	His Leu Ser Arg Ile Leu	
335	340	345
Lys Leu Thr Leu Tyr Asn Gln Asn Asp	Pro Asn Arg Cys Glu Leu	
350	355	360
Leu Ser Phe Leu Leu Val Leu Asp Tyr	Ile Gly Ile Asn Met Ala	
365	370	375
Ser Leu Asn Ser Cys Ile Asn Pro Ile	Ala Leu Tyr Leu Val Ser	
380	385	390
Lys Arg Phe Lys Asn Cys Phe Lys Ser	Cys Leu Cys Cys Trp Cys	
395	400	405
Gln Ser Phe Glu Glu Lys Gln Ser Leu	Glu Glu Lys Gln Ser Cys	
410	415	420
Leu Lys Phe Lys Ala Asn Asp His Gly	Tyr Asp Asn Phe Arg Ser	
425	430	435
Ser Asn Lys Tyr Ser Ser Ser		
440		

<210> 10
 <211> 783
 <212> PRT
 <213> Homo sapien

<400> 10
 Met Ser Gly Gly His Gln Leu Gln Leu Ala Ala Leu Trp Pro Trp
 1 5 10 15
 Leu Leu Met Ala Thr Leu Gln Ala Gly Phe Gly Arg Thr Gly Leu
 20 25 30
 Val Leu Ala Ala Ala Val Glu Ser Glu Arg Ser Ala Glu Gln Lys
 35 40 45
 Ala Ile Ile Arg Val Ile Pro Leu Lys Met Asp Pro Thr Gly Lys
 50 55 60
 Leu Asn Leu Thr Leu Glu Gly Val Phe Ala Gly Val Ala Glu Ile
 65 70 75

Thr	Pro	Ala	Glu	Gly	Lys	Leu	Met	Gln	Ser	His	Pro	Leu	Tyr	Leu			
				80					85					90			
Cys	Asn	Ala	Ser	Asp	Asp	Asp	Asn	Leu	Glu	Pro	Gly	Phe	Ile	Ser			
				95					100					105			
Ile	Val	Lys	Leu	Glu	Ser	Pro	Arg	Arg	Ala	Pro	Arg	Pro	Cys	Leu			
				110					115					120			
Ser	Leu	Ala	Ser	Lys	Ala	Arg	Met	Ala	Gly	Glu	Arg	Gly	Ala	Ser			
				125					130					135			
Ala	Val	Leu	Phe	Asp	Ile	Thr	Glu	Asp	Arg	Ala	Ala	Ala	Glu	Gln			
				140					145					150			
Leu	Gln	Gln	Pro	Leu	Gly	Leu	Thr	Trp	Pro	Val	Val	Leu	Ile	Trp			
				155					160					165			
Gly	Asn	Asp	Ala	Glu	Lys	Leu	Met	Glu	Phe	Val	Tyr	Lys	Asn	Gln			
				170					175					180			
Lys	Ala	His	Val	Arg	Ile	Glu	Leu	Lys	Glu	Pro	Pro	Ala	Trp	Pro			
				185					190					195			
Asp	Tyr	Asp	Val	Trp	Ile	Leu	Met	Thr	Val	Val	Gly	Thr	Ile	Phe			
				200					205					210			
Val	Ile	Ile	Leu	Ala	Ser	Val	Leu	Arg	Ile	Arg	Cys	Arg	Pro	Arg			
				215					220					225			
His	Ser	Arg	Pro	Asp	Pro	Leu	Gln	Gln	Arg	Thr	Ala	Trp	Ala	Ile			
				230					235					240			
Ser	Gln	Leu	Ala	Thr	Arg	Arg	Tyr	Gln	Ala	Ser	Cys	Arg	Gln	Ala			
				245					250					255			
Arg	Gly	Glu	Trp	Pro	Asp	Ser	Gly	Ser	Ser	Cys	Ser	Ser	Ala	Pro			
				260					265					270			
Val	Cys	Ala	Ile	Cys	Leu	Glu	Glu	Phe	Ser	Glu	Gly	Gln	Glu	Leu			
				275					280					285			
Arg	Val	Ile	Ser	Cys	Leu	His	Glu	Phe	His	Arg	Asn	Cys	Val	Asp			
				290					295					300			
Pro	Trp	Leu	His	Gln	His	Arg	Thr	Cys	Pro	Leu	Cys	Val	Phe	Asn			
				305					310					315			
Ile	Thr	Glu	Gly	Asp	Ser	Phe	Ser	Gln	Ser	Leu	Gly	Pro	Ser	Arg			
				320					325					330			
Ser	Tyr	Gln	Glu	Pro	Gly	Arg	Arg	Leu	His	Leu	Ile	Arg	Gln	His			
				335					340					345			
Pro	Gly	His	Ala	His	Tyr	His	Leu	Pro	Ala	Ala	Tyr	Leu	Leu	Gly			
				350					355					360			
Pro	Ser	Arg	Ser	Ala	Val	Ala	Arg	Pro	Pro	Arg	Pro	Gly	Pro	Phe			
				365					370					375			

Leu	Pro	Ser	Gln	Glu	Pro	Gly	Met	Gly	Pro	Arg	His	His	Arg	Phe	380	385	390
Pro	Arg	Ala	Ala	His	Pro	Arg	Ala	Pro	Gly	Glu	Gln	Gln	Arg	Leu	395	400	405
Ala	Gly	Ala	Gln	His	Pro	Tyr	Ala	Gln	Gly	Trp	Gly	Met	Ser	His	410	415	420
Leu	Gln	Ser	Thr	Ser	Gln	His	Pro	Ala	Ala	Cys	Pro	Val	Pro	Leu	425	430	435
Arg	Arg	Ala	Arg	Pro	Pro	Asp	Ser	Ser	Gly	Ser	Gly	Glu	Ser	Tyr	440	445	450
Cys	Thr	Glu	Arg	Ser	Gly	Tyr	Leu	Ala	Asp	Gly	Pro	Ala	Ser	Asp	455	460	465
Ser	Ser	Ser	Gly	Pro	Cys	His	Gly	Ser	Ser	Ser	Asp	Ser	Val	Val	470	475	480
Asn	Cys	Thr	Asp	Ile	Ser	Leu	Gln	Gly	Val	His	Gly	Ser	Ser	Ser	485	490	495
Thr	Phe	Cys	Ser	Ser	Leu	Ser	Ser	Asp	Phe	Asp	Pro	Leu	Val	Tyr	500	505	510
Cys	Ser	Pro	Lys	Gly	Asp	Pro	Gln	Arg	Val	Asp	Met	Gln	Pro	Ser	515	520	525
Val	Thr	Ser	Arg	Pro	Arg	Ser	Leu	Asp	Ser	Val	Val	Pro	Thr	Gly	530	535	540
Glu	Thr	Gln	Val	Ser	Ser	His	Val	His	Tyr	His	Arg	His	Arg	His	545	550	555
His	His	Tyr	Lys	Lys	Arg	Phe	Gln	Trp	His	Gly	Arg	Lys	Pro	Gly	560	565	570
Pro	Glu	Thr	Gly	Val	Pro	Gln	Ser	Arg	Pro	Pro	Ile	Pro	Arg	Thr	575	580	585
Gln	Pro	Gln	Pro	Glu	Pro	Pro	Ser	Pro	Asp	Gln	Gln	Val	Thr	Gly	590	595	600
Ser	Asn	Ser	Ala	Ala	Pro	Ser	Gly	Arg	Leu	Ser	Asn	Pro	Gln	Cys	605	610	615
Pro	Arg	Ala	Leu	Pro	Glu	Pro	Ala	Pro	Gly	Pro	Val	Asp	Ala	Ser	620	625	630
Ser	Ile	Cys	Pro	Ser	Thr	Ser	Ser	Leu	Phe	Asn	Leu	Gln	Lys	Ser	635	640	645
Ser	Leu	Ser	Ala	Arg	His	Pro	Gln	Arg	Lys	Arg	Arg	Gly	Gly	Pro	650	655	660
Ser	Glu	Pro	Thr	Pro	Gly	Ser	Arg	Pro	Gln	Asp	Ala	Thr	Val	His	665	670	675
Pro	Ala	Cys	Gln	Ile	Phe	Pro	His	Tyr	Thr	Pro	Ser	Val	Ala	Tyr			

680										685					690				
Pro	Trp	Ser	Pro	Glu	Ala	His	Pro	Leu	Ile	Cys	Gly	Pro	Pro	Gly					
				695					700					705					
Leu	Asp	Lys	Arg	Leu	Leu	Pro	Glu	Thr	Pro	Gly	Pro	Cys	Tyr	Ser					
				710					715					720					
Asn	Ser	Gln	Pro	Val	Trp	Leu	Cys	Leu	Thr	Pro	Arg	Gln	Pro	Leu					
				725					730					735					
Glu	Pro	His	Pro	Pro	Gly	Glu	Gly	Pro	Ser	Glu	Trp	Ser	Ser	Asp					
				740					745					750					
Thr	Ala	Glu	Gly	Arg	Pro	Cys	Pro	Tyr	Pro	His	Cys	Gln	Val	Leu					
				755					760					765					
Ser	Ala	Gln	Pro	Gly	Ser	Glu	Glu	Glu	Leu	Glu	Glu	Leu	Cys	Glu					
				770					775					780					

Gln Ala Val

<210> 11
 <211> 490
 <212> PRT
 <213> Homo sapien

<400> 11

Met	Glu	Ser	Ile	Ser	Met	Met	Gly	Ser	Pro	Lys	Ser	Leu	Ser	Glu					
1				5					10					15					
Thr	Val	Leu	Pro	Asn	Gly	Ile	Asn	Gly	Ile	Lys	Asp	Ala	Arg	Lys					
				20					25					30					
Val	Thr	Val	Gly	Val	Ile	Gly	Ser	Gly	Asp	Phe	Ala	Lys	Ser	Leu					
				35					40					45					
Thr	Ile	Arg	Leu	Ile	Arg	Cys	Gly	Tyr	His	Val	Val	Ile	Gly	Ser					
				50					55					60					
Arg	Asn	Pro	Lys	Phe	Ala	Ser	Glu	Phe	Phe	Pro	His	Val	Val	Asp					
				65					70					75					
Val	Thr	His	His	Glu	Asp	Ala	Leu	Thr	Lys	Thr	Asn	Ile	Ile	Phe					
				80					85					90					
Val	Ala	Ile	His	Arg	Glu	His	Tyr	Thr	Ser	Leu	Trp	Asp	Leu	Arg					
				95					100					105					
His	Leu	Leu	Val	Gly	Lys	Ile	Leu	Ile	Asp	Val	Ser	Asn	Asn	Met					
				110					115					120					
Arg	Ile	Asn	Gln	Tyr	Pro	Glu	Ser	Asn	Ala	Glu	Tyr	Leu	Ala	Ser					
				125					130					135					
Leu	Phe	Pro	Asp	Ser	Leu	Ile	Val	Lys	Gly	Phe	Asn	Val	Val	Ser					
				140					145					150					
Ala	Trp	Ala	Leu	Gln	Leu	Gly	Pro	Lys	Asp	Ala	Ser	Arg	Gln	Val					
				155					160					165					

Tyr	Ile	Cys	Ser	Asn	Asn	Ile	Gln	Ala	Arg	Gln	Gln	Val	Ile	Glu	170	175	180
Leu	Ala	Arg	Gln	Leu	Asn	Phe	Ile	Pro	Ile	Asp	Leu	Gly	Ser	Leu	185	190	195
Ser	Ser	Ala	Arg	Glu	Ile	Glu	Asn	Leu	Pro	Leu	Arg	Leu	Phe	Thr	200	205	210
Leu	Trp	Arg	Gly	Pro	Val	Val	Val	Ala	Ile	Ser	Leu	Ala	Thr	Phe	215	220	225
Phe	Phe	Leu	Tyr	Ser	Phe	Val	Arg	Asp	Val	Ile	His	Pro	Tyr	Ala	230	235	240
Arg	Asn	Gln	Gln	Ser	Asp	Phe	Tyr	Lys	Ile	Pro	Ile	Glu	Ile	Val	245	250	255
Asn	Lys	Thr	Leu	Pro	Ile	Val	Ala	Ile	Thr	Leu	Leu	Ser	Leu	Val	260	265	270
Tyr	Leu	Ala	Gly	Leu	Leu	Ala	Ala	Ala	Tyr	Gln	Leu	Tyr	Tyr	Gly	275	280	285
Thr	Lys	Tyr	Arg	Arg	Phe	Pro	Pro	Trp	Leu	Glu	Thr	Trp	Leu	Gln	290	295	300
Cys	Arg	Lys	Gln	Leu	Gly	Leu	Leu	Ser	Phe	Phe	Phe	Ala	Met	Val	305	310	315
His	Val	Ala	Tyr	Ser	Leu	Cys	Leu	Pro	Met	Arg	Arg	Ser	Glu	Arg	320	325	330
Tyr	Leu	Phe	Leu	Asn	Met	Ala	Tyr	Gln	Gln	Val	His	Ala	Asn	Ile	335	340	345
Glu	Asn	Ser	Trp	Asn	Glu	Glu	Glu	Val	Trp	Arg	Ile	Glu	Met	Tyr	350	355	360
Ile	Ser	Phe	Gly	Ile	Met	Ser	Leu	Gly	Leu	Leu	Ser	Leu	Leu	Ala	365	370	375
Val	Thr	Ser	Ile	Pro	Ser	Val	Ser	Asn	Ala	Leu	Asn	Trp	Arg	Glu	380	385	390
Phe	Ser	Phe	Ile	Gln	Ser	Thr	Leu	Gly	Tyr	Val	Ala	Leu	Leu	Ile	395	400	405
Ser	Thr	Phe	His	Val	Leu	Ile	Tyr	Gly	Trp	Lys	Arg	Ala	Phe	Glu	410	415	420
Glu	Glu	Tyr	Tyr	Arg	Phe	Tyr	Thr	Pro	Pro	Asn	Phe	Val	Leu	Ala	425	430	435
Leu	Val	Leu	Pro	Ser	Ile	Val	Ile	Leu	Gly	Lys	Ile	Ile	Leu	Phe	440	445	450
Leu	Pro	Cys	Ile	Ser	Gln	Lys	Leu	Lys	Arg	Ile	Lys	Lys	Gly	Trp	455	460	465

Glu Lys Ser Gln Phe Leu Glu Glu Gly Ile Gly Gly Thr Ile Pro
 470 475 480

His Val Ser Pro Glu Arg Val Thr Val Met
 485 490

<210> 12
 <211> 1214
 <212> PRT
 <213> Homo sapien

<400> 12
 Met Val Val Pro Glu Lys Glu Gln Ser Trp Ile Pro Lys Ile Phe
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 Lys Lys Lys Thr Cys Thr Thr Phe Ile Val Asp Ser Thr Asp Pro
 20 25 30
 Gly Gly Thr Leu Cys Gln Cys Gly Arg Pro Arg Thr Ala His Pro
 35 40 45
 Ala Val Ala Met Glu Asp Ala Phe Gly Ala Ala Val Val Thr Val
 50 55 60
 Trp Asp Ser Asp Ala His Thr Thr Glu Lys Pro Thr Asp Ala Tyr
 65 70 75
 Gly Glu Leu Asp Phe Thr Gly Ala Gly Arg Lys His Ser Asn Phe
 80 85 90
 Leu Arg Leu Ser Asp Arg Thr Asp Pro Ala Ala Val Tyr Ser Leu
 95 100 105
 Val Thr Arg Thr Trp Gly Phe Arg Ala Pro Asn Leu Val Val Ser
 110 115 120
 Val Leu Gly Gly Ser Gly Gly Pro Val Leu Gln Thr Trp Leu Gln
 125 130 135
 Asp Leu Leu Arg Arg Gly Leu Val Arg Ala Ala Gln Ser Thr Gly
 140 145 150
 Ala Trp Ile Val Thr Gly Gly Leu His Thr Gly Ile Gly Arg His
 155 160 165
 Val Gly Val Ala Val Arg Asp His Gln Met Ala Ser Thr Gly Gly
 170 175 180
 Thr Lys Val Val Ala Met Gly Val Ala Pro Trp Gly Val Val Arg
 185 190 195
 Asn Arg Asp Thr Leu Ile Asn Pro Lys Gly Ser Phe Pro Ala Arg
 200 205 210
 Tyr Arg Trp Arg Gly Asp Pro Glu Asp Gly Val Gln Phe Pro Leu
 215 220 225
 Asp Tyr Asn Tyr Ser Ala Phe Phe Leu Val Asp Asp Gly Thr His
 230 235 240
 Gly Cys Leu Gly Gly Glu Asn Arg Phe Arg Leu Arg Leu Glu Ser

245										250				255			
Tyr	Ile	Ser	Gln	Gln	Lys	Thr	Gly	Val	Gly	Gly	Thr	Gly	Ile	Asp			
				260					265					270			
Ile	Pro	Val	Leu	Leu	Leu	Leu	Ile	Asp	Gly	Asp	Glu	Lys	Met	Leu			
				275					280					285			
Thr	Arg	Ile	Glu	Asn	Ala	Thr	Gln	Ala	Gln	Leu	Pro	Cys	Leu	Leu			
				290					295					300			
Val	Ala	Gly	Ser	Gly	Gly	Ala	Ala	Asp	Cys	Leu	Ala	Glu	Thr	Leu			
				305					310					315			
Glu	Asp	Thr	Leu	Ala	Pro	Gly	Ser	Gly	Gly	Ala	Arg	Gln	Gly	Glu			
				320					325					330			
Ala	Arg	Asp	Arg	Ile	Arg	Arg	Phe	Phe	Pro	Lys	Gly	Asp	Leu	Glu			
				335					340					345			
Val	Leu	Gln	Ala	Gln	Val	Glu	Arg	Ile	Met	Thr	Arg	Lys	Glu	Leu			
				350					355					360			
Leu	Thr	Val	Tyr	Ser	Ser	Glu	Asp	Gly	Ser	Glu	Glu	Phe	Glu	Thr			
				365					370					375			
Ile	Val	Leu	Lys	Ala	Leu	Val	Lys	Ala	Cys	Gly	Ser	Ser	Glu	Ala			
				380					385					390			
Ser	Ala	Tyr	Leu	Asp	Glu	Leu	Arg	Leu	Ala	Val	Ala	Trp	Asn	Arg			
				395					400					405			
Val	Asp	Ile	Ala	Gln	Ser	Glu	Leu	Phe	Arg	Gly	Asp	Ile	Gln	Trp			
				410					415					420			
Arg	Ser	Phe	His	Leu	Glu	Ala	Ser	Leu	Met	Asp	Ala	Leu	Leu	Asn			
				425					430					435			
Asp	Arg	Pro	Glu	Phe	Val	Arg	Leu	Leu	Ile	Ser	His	Gly	Leu	Ser			
				440					445					450			
Leu	Gly	His	Phe	Leu	Thr	Pro	Met	Arg	Leu	Ala	Gln	Leu	Tyr	Ser			
				455					460					465			
Ala	Ala	Pro	Ser	Asn	Ser	Leu	Ile	Arg	Asn	Leu	Leu	Asp	Gln	Ala			
				470					475					480			
Ser	His	Ser	Ala	Gly	Thr	Lys	Ala	Pro	Ala	Leu	Lys	Gly	Gly	Ala			
				485					490					495			
Ala	Glu	Leu	Arg	Pro	Pro	Asp	Val	Gly	His	Val	Leu	Arg	Met	Leu			
				500					505					510			
Leu	Gly	Lys	Met	Cys	Ala	Pro	Arg	Tyr	Pro	Ser	Gly	Gly	Ala	Trp			
				515					520					525			
Asp	Pro	His	Pro	Gly	Gln	Gly	Phe	Gly	Glu	Ser	Met	Tyr	Leu	Leu			
				530					535					540			
Ser	Asp	Lys	Ala	Thr	Ser	Pro	Leu	Ser	Leu	Asp	Ala	Gly	Leu	Gly			
				545					550					555			

Gln	Ala	Pro	Trp	Ser	Asp	Leu	Leu	Leu	Trp	Ala	Leu	Leu	Leu	Asn	
				560					565					570	
Arg	Ala	Gln	Met	Ala	Met	Tyr	Phe	Trp	Glu	Met	Gly	Ser	Asn	Ala	
				575					580					585	
Val	Ser	Ser	Ala	Leu	Gly	Ala	Cys	Leu	Leu	Leu	Arg	Val	Met	Ala	
				590					595					600	
Arg	Leu	Glu	Pro	Asp	Ala	Glu	Glu	Ala	Ala	Arg	Arg	Lys	Asp	Leu	
				605					610					615	
Ala	Phe	Lys	Phe	Glu	Gly	Met	Gly	Val	Asp	Leu	Phe	Gly	Glu	Cys	
				620					625					630	
Tyr	Arg	Ser	Ser	Glu	Val	Arg	Ala	Ala	Arg	Leu	Leu	Leu	Arg	Arg	
				635					640					645	
Cys	Pro	Leu	Trp	Gly	Asp	Ala	Thr	Cys	Leu	Gln	Leu	Ala	Met	Gln	
				650					655					660	
Ala	Asp	Ala	Arg	Ala	Phe	Phe	Ala	Gln	Asp	Gly	Val	Gln	Ser	Leu	
				665					670					675	
Leu	Thr	Gln	Lys	Trp	Trp	Gly	Asp	Met	Ala	Ser	Thr	Thr	Pro	Ile	
				680					685					690	
Trp	Ala	Leu	Val	Leu	Ala	Phe	Phe	Cys	Pro	Pro	Leu	Ile	Tyr	Thr	
				695					700					705	
Arg	Leu	Ile	Thr	Phe	Arg	Lys	Ser	Glu	Glu	Glu	Pro	Thr	Arg	Glu	
				710					715					720	
Glu	Leu	Glu	Phe	Asp	Met	Asp	Ser	Val	Ile	Asn	Gly	Glu	Gly	Pro	
				725					730					735	
Val	Gly	Thr	Ala	Asp	Pro	Ala	Glu	Lys	Thr	Pro	Leu	Gly	Val	Pro	
				740					745					750	
Arg	Gln	Ser	Gly	Arg	Pro	Gly	Cys	Cys	Gly	Gly	Arg	Cys	Gly	Gly	
				755					760					765	
Arg	Arg	Cys	Leu	Arg	Arg	Trp	Phe	His	Phe	Trp	Gly	Ala	Pro	Val	
				770					775					780	
Thr	Ile	Phe	Met	Gly	Asn	Val	Val	Ser	Tyr	Leu	Leu	Phe	Leu	Leu	
				785					790					795	
Leu	Phe	Ser	Arg	Val	Leu	Leu	Val	Asp	Phe	Gln	Pro	Ala	Pro	Pro	
				800					805					810	
Gly	Ser	Leu	Glu	Leu	Leu	Leu	Tyr	Phe	Trp	Ala	Phe	Thr	Leu	Leu	
				815					820					825	
Cys	Glu	Glu	Leu	Arg	Gln	Gly	Leu	Ser	Gly	Gly	Gly	Gly	Ser	Leu	
				830					835					840	
Ala	Ser	Gly	Gly	Pro	Gly	Pro	Gly	His	Ala	Ser	Leu	Ser	Gln	Arg	
				845					850					855	

Leu	Arg	Leu	Tyr	Leu	Ala	Asp	Ser	Trp	Asn	Gln	Cys	Asp	Leu	Val	860	865	870
Ala	Leu	Thr	Cys	Phe	Leu	Leu	Gly	Val	Gly	Cys	Arg	Leu	Thr	Pro	875	880	885
Gly	Leu	Tyr	His	Leu	Gly	Arg	Thr	Val	Leu	Cys	Ile	Asp	Phe	Met	890	895	900
Val	Phe	Thr	Val	Arg	Leu	Leu	His	Ile	Phe	Thr	Val	Asn	Lys	Gln	905	910	915
Leu	Gly	Pro	Lys	Ile	Val	Ile	Val	Ser	Lys	Met	Met	Lys	Asp	Val	920	925	930
Phe	Phe	Phe	Leu	Phe	Phe	Leu	Gly	Val	Trp	Leu	Val	Ala	Tyr	Gly	935	940	945
Val	Ala	Thr	Glu	Gly	Leu	Leu	Arg	Pro	Arg	Asp	Ser	Asp	Phe	Pro	950	955	960
Ser	Ile	Leu	Arg	Arg	Val	Phe	Tyr	Arg	Pro	Tyr	Leu	Gln	Ile	Phe	965	970	975
Gly	Gln	Ile	Pro	Gln	Glu	Asp	Met	Asp	Val	Ala	Leu	Met	Glu	His	980	985	990
Ser	Asn	Cys	Ser	Ser	Glu	Pro	Gly	Phe	Trp	Ala	His	Pro	Pro	Gly	995	1000	1005
Ala	Gln	Ala	Gly	Thr	Cys	Val	Ser	Gln	Tyr	Ala	Asn	Trp	Leu	Val	1010	1015	1020
Val	Leu	Leu	Leu	Val	Ile	Phe	Leu	Leu	Val	Ala	Asn	Ile	Leu	Leu	1025	1030	1035
Val	Asn	Leu	Leu	Ile	Ala	Met	Phe	Ser	Tyr	Thr	Phe	Gly	Lys	Val	1040	1045	1050
Gln	Gly	Asn	Ser	Asp	Leu	Tyr	Trp	Lys	Ala	Gln	Arg	Tyr	Arg	Leu	1055	1060	1065
Ile	Arg	Glu	Phe	His	Ser	Arg	Pro	Ala	Leu	Ala	Pro	Pro	Phe	Ile	1070	1075	1080
Val	Ile	Ser	His	Leu	Arg	Leu	Leu	Leu	Arg	Gln	Leu	Cys	Arg	Arg	1085	1090	1095
Pro	Arg	Ser	Pro	Gln	Pro	Ser	Ser	Pro	Ala	Leu	Glu	His	Phe	Arg	1100	1105	1110
Val	Tyr	Leu	Ser	Lys	Glu	Ala	Glu	Arg	Lys	Leu	Leu	Thr	Trp	Glu	1115	1120	1125
Ser	Val	His	Lys	Glu	Asn	Phe	Leu	Leu	Ala	Arg	Ala	Arg	Asp	Lys	1130	1135	1140
Arg	Glu	Ser	Asp	Ser	Glu	Arg	Leu	Lys	Arg	Thr	Ser	Gln	Lys	Val	1145	1150	1155
Asp	Leu	Ala	Leu	Lys	Gln	Leu	Gly	His	Ile	Arg	Glu	Tyr	Glu	Gln			

1160	1165	1170
Arg Leu Lys Val Leu Glu Arg Glu Val Gln Gln Cys Ser Arg Val		
1175	1180	1185
Leu Gly Trp Val Ala Glu Ala Leu Ser Arg Ser Ala Leu Leu Pro		
1190	1195	1200
Pro Gly Gly Pro Pro Pro Pro Asp Leu Pro Gly Ser Lys Asp		
1205	1210	

<210> 13
 <211> 188
 <212> PRT
 <213> Homo sapien

<400> 13	
Met Asp Cys Arg Lys Met Ala Arg Phe Ser Tyr Ser Val Ile Trp	
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Ile Met Ala Ile Ser Lys Val Phe Glu Leu Gly Leu Val Ala Gly	
20 25 30	
Leu Gly His Gln Glu Phe Ala Arg Pro Ser Arg Gly Tyr Leu Ala	
35 40 45	
Phe Arg Asp Asp Ser Ile Trp Pro Gln Glu Glu Pro Ala Ile Arg	
50 55 60	
Pro Arg Ser Ser Gln Arg Val Pro Pro Met Gly Ile Gln His Ser	
65 70 75	
Lys Glu Leu Asn Arg Thr Cys Cys Leu Asn Gly Gly Thr Cys Met	
80 85 90	
Leu Gly Ser Phe Cys Ala Cys Pro Pro Ser Phe Tyr Gly Arg Asn	
95 100 105	
Cys Glu His Asp Val Arg Lys Glu Asn Cys Gly Ser Val Pro His	
110 115 120	
Asp Thr Trp Leu Pro Lys Lys Cys Ser Leu Cys Lys Cys Trp His	
125 130 135	
Gly Gln Leu Arg Cys Phe Pro Gln Ala Phe Leu Pro Gly Cys Asp	
140 145 150	
Gly Leu Val Met Asp Glu His Leu Val Ala Ser Arg Thr Pro Glu	
155 160 165	
Leu Pro Pro Ser Ala Arg Thr Thr Thr Phe Met Leu Val Gly Ile	
170 175 180	
Cys Leu Ser Ile Gln Ser Tyr Tyr	
185	

<210> 14
 <211> 1033
 <212> PRT
 <213> Homo sapien

<400> 14

Met	Gly	Ala	Ala	Gly	Leu	Leu	Gly	Val	Phe	Leu	Ala	Leu	Val	Ala	1	5	10	15
Pro	Gly	Val	Leu	Gly	Ile	Ser	Cys	Gly	Ser	Pro	Pro	Pro	Ile	Leu	20	25	30	
Asn	Gly	Arg	Ile	Ser	Tyr	Tyr	Ser	Thr	Pro	Ile	Ala	Val	Gly	Thr	35	40	45	
Val	Ile	Arg	Tyr	Ser	Cys	Ser	Gly	Thr	Phe	Arg	Leu	Ile	Gly	Glu	50	55	60	
Lys	Ser	Leu	Leu	Cys	Ile	Thr	Lys	Asp	Lys	Val	Asp	Gly	Thr	Trp	65	70	75	
Asp	Lys	Pro	Ala	Pro	Lys	Cys	Glu	Tyr	Phe	Asn	Lys	Tyr	Ser	Ser	80	85	90	
Cys	Pro	Glu	Pro	Ile	Val	Pro	Gly	Gly	Tyr	Lys	Ile	Arg	Gly	Ser	95	100	105	
Thr	Pro	Tyr	Arg	His	Gly	Asp	Ser	Val	Thr	Phe	Ala	Cys	Lys	Thr	110	115	120	
Asn	Phe	Ser	Met	Asn	Gly	Asn	Lys	Ser	Val	Trp	Cys	Gln	Ala	Asn	125	130	135	
Asn	Met	Trp	Gly	Pro	Thr	Arg	Leu	Pro	Thr	Cys	Val	Ser	Val	Phe	140	145	150	
Pro	Leu	Glu	Cys	Pro	Ala	Leu	Pro	Met	Ile	His	Asn	Gly	His	His	155	160	165	
Thr	Ser	Glu	Asn	Val	Gly	Ser	Ile	Ala	Pro	Gly	Leu	Ser	Val	Thr	170	175	180	
Tyr	Ser	Cys	Glu	Ser	Gly	Tyr	Leu	Leu	Val	Gly	Glu	Lys	Ile	Ile	185	190	195	
Asn	Cys	Leu	Ser	Ser	Gly	Lys	Trp	Ser	Ala	Val	Pro	Pro	Thr	Cys	200	205	210	
Glu	Glu	Ala	Arg	Cys	Lys	Ser	Leu	Gly	Arg	Phe	Pro	Asn	Gly	Lys	215	220	225	
Val	Lys	Glu	Pro	Pro	Ile	Leu	Arg	Val	Gly	Val	Thr	Ala	Asn	Phe	230	235	240	
Phe	Cys	Asp	Glu	Gly	Tyr	Arg	Leu	Gln	Gly	Pro	Pro	Ser	Ser	Arg	245	250	255	
Cys	Val	Ile	Ala	Gly	Gln	Gly	Val	Ala	Trp	Thr	Lys	Met	Pro	Val	260	265	270	
Cys	Glu	Glu	Ile	Phe	Cys	Pro	Ser	Pro	Pro	Pro	Ile	Leu	Asn	Gly	275	280	285	
Arg	His	Ile	Gly	Asn	Ser	Leu	Ala	Asn	Val	Ser	Tyr	Gly	Ser	Ile	290	295	300	

Val Thr Tyr Thr Cys Asp Pro Asp Pro Glu Glu Gly Val Asn Phe	305	310	315
Ile Leu Ile Gly Glu Ser Thr Leu Arg Cys Thr Val Asp Ser Gln	320	325	330
Lys Thr Gly Thr Trp Ser Gly Pro Ala Pro Arg Cys Glu Leu Ser	335	340	345
Thr Ser Ala Val Gln Cys Pro His Pro Gln Ile Leu Arg Gly Arg	350	355	360
Met Val Ser Gly Gln Lys Asp Arg Tyr Thr Tyr Asn Asp Thr Val	365	370	375
Ile Phe Ala Cys Met Phe Gly Phe Thr Leu Lys Gly Ser Lys Gln	380	385	390
Ile Arg Cys Asn Ala Gln Gly Thr Trp Glu Pro Ser Ala Pro Val	395	400	405
Cys Glu Lys Glu Cys Gln Ala Pro Pro Asn Ile Leu Asn Gly Gln	410	415	420
Lys Glu Asp Arg His Met Val Arg Phe Asp Pro Gly Thr Ser Ile	425	430	435
Lys Tyr Ser Cys Asn Pro Gly Tyr Val Leu Val Gly Glu Glu Ser	440	445	450
Ile Gln Cys Thr Ser Glu Gly Val Trp Thr Pro Pro Val Pro Gln	455	460	465
Cys Lys Val Ala Ala Cys Glu Ala Thr Gly Arg Gln Leu Leu Thr	470	475	480
Lys Pro Gln His Gln Phe Val Arg Pro Asp Val Asn Ser Ser Cys	485	490	495
Gly Glu Gly Tyr Lys Leu Ser Gly Ser Val Tyr Gln Glu Cys Gln	500	505	510
Gly Thr Ile Pro Trp Phe Met Glu Ile Arg Leu Cys Lys Glu Ile	515	520	525
Thr Cys Pro Pro Pro Pro Val Ile Tyr Asn Gly Ala His Thr Gly	530	535	540
Ser Ser Leu Glu Asp Phe Pro Tyr Gly Thr Thr Val Thr Tyr Thr	545	550	555
Cys Asn Pro Gly Pro Glu Arg Gly Val Glu Phe Ser Leu Ile Gly	560	565	570
Glu Ser Thr Ile Arg Cys Thr Ser Asn Asp Gln Glu Arg Gly Thr	575	580	585
Trp Ser Gly Pro Ala Pro Leu Cys Lys Leu Ser Leu Leu Ala Val	590	595	600
Gln Cys Ser His Val His Ile Ala Asn Gly Tyr Lys Ile Ser Gly			

605										610				615			
Lys	Glu	Ala	Pro	Tyr	Phe	Tyr	Asn	Asp	Thr	Val	Thr	Phe	Lys	Cys			
620										625				630			
Tyr	Ser	Gly	Phe	Thr	Leu	Lys	Gly	Ser	Ser	Gln	Ile	Arg	Cys	Lys			
635										640				645			
Ala	Asp	Asn	Thr	Trp	Asp	Pro	Glu	Ile	Pro	Val	Cys	Glu	Lys	Glu			
650										655				660			
Thr	Cys	Gln	His	Val	Arg	Gln	Ser	Leu	Gln	Glu	Leu	Pro	Ala	Gly			
665										670				675			
Ser	Arg	Val	Glu	Leu	Val	Asn	Thr	Ser	Cys	Gln	Asp	Gly	Tyr	Gln			
680										685				690			
Leu	Thr	Gly	His	Ala	Tyr	Gln	Met	Cys	Gln	Asp	Ala	Glu	Asn	Gly			
695										700				705			
Ile	Trp	Phe	Lys	Lys	Ile	Pro	Leu	Cys	Lys	Val	Ile	His	Cys	His			
710										715				720			
Pro	Pro	Pro	Val	Ile	Val	Asn	Gly	Lys	His	Thr	Gly	Met	Met	Ala			
725										730				735			
Glu	Asn	Phe	Leu	Tyr	Gly	Asn	Glu	Val	Ser	Tyr	Glu	Cys	Asp	Gln			
740										745				750			
Gly	Phe	Tyr	Leu	Leu	Gly	Glu	Lys	Lys	Leu	Gln	Cys	Arg	Ser	Asp			
755										760				765			
Ser	Lys	Gly	His	Gly	Ser	Trp	Ser	Gly	Pro	Ser	Pro	Gln	Cys	Leu			
770										775				780			
Arg	Ser	Pro	Pro	Val	Thr	Arg	Cys	Pro	Asn	Pro	Glu	Val	Lys	His			
785										790				795			
Gly	Tyr	Lys	Leu	Asn	Lys	Thr	His	Ser	Ala	Tyr	Ser	His	Asn	Asp			
800										805				810			
Ile	Val	Tyr	Val	Asp	Cys	Asn	Pro	Gly	Phe	Ile	Met	Asn	Gly	Ser			
815										820				825			
Arg	Val	Ile	Arg	Cys	His	Thr	Asp	Asn	Thr	Trp	Val	Pro	Gly	Val			
830										835				840			
Pro	Thr	Cys	Ile	Lys	Lys	Ala	Phe	Ile	Gly	Cys	Pro	Pro	Pro	Pro			
845										850				855			
Lys	Thr	Pro	Asn	Gly	Asn	His	Thr	Gly	Gly	Asn	Ile	Ala	Arg	Phe			
860										865				870			
Ser	Pro	Gly	Met	Ser	Ile	Leu	Tyr	Ser	Cys	Asp	Gln	Gly	Tyr	Leu			
875										880				885			
Leu	Val	Gly	Glu	Ala	Leu	Leu	Leu	Cys	Thr	His	Glu	Gly	Thr	Trp			
890										895				900			
Ser	Gln	Pro	Ala	Pro	His	Cys	Lys	Glu	Val	Asn	Cys	Ser	Ser	Pro			
905										910				915			

Ala	Asp	Met	Asp	Gly	Ile	Gln	Lys	Gly	Leu	Glu	Pro	Arg	Lys	Met	
				920					925					930	
Tyr	Gln	Tyr	Gly	Ala	Val	Val	Thr	Leu	Glu	Cys	Glu	Asp	Gly	Tyr	
				935					940					945	
Met	Leu	Glu	Gly	Ser	Pro	Gln	Ser	Gln	Cys	Gln	Ser	Asp	His	Gln	
				950					955					960	
Trp	Asn	Pro	Pro	Leu	Ala	Val	Cys	Arg	Ser	Arg	Ser	Leu	Ala	Pro	
				965					970					975	
Val	Leu	Cys	Gly	Ile	Ala	Ala	Gly	Leu	Ile	Leu	Leu	Thr	Phe	Leu	
				980					985					990	
Ile	Val	Ile	Thr	Leu	Tyr	Val	Ile	Ser	Lys	His	Arg	Glu	Arg	Asn	
				995					1000					1005	
Tyr	Tyr	Thr	Asp	Thr	Ser	Gln	Lys	Glu	Ala	Phe	His	Leu	Glu	Ala	
				1010					1015					1020	
Arg	Glu	Val	Tyr	Ser	Val	Asp	Pro	Tyr	Asn	Pro	Ala	Ser			
				1025					1030						

<210> 15
 <211> 229
 <212> PRT
 <213> Homo sapien

<400> 15															
Met	Ala	Arg	Leu	Ala	Leu	Ser	Pro	Val	Pro	Ser	His	Trp	Met	Val	
1				5					10					15	
Ala	Leu	Leu	Leu	Leu	Leu	Ser	Ala	Glu	Pro	Val	Pro	Ala	Ala	Arg	
				20					25					30	
Ser	Glu	Asp	Arg	Tyr	Arg	Asn	Pro	Lys	Gly	Ser	Ala	Cys	Ser	Arg	
				35					40					45	
Ile	Trp	Gln	Ser	Pro	Arg	Phe	Ile	Ala	Arg	Lys	Arg	Gly	Phe	Thr	
				50					55					60	
Val	Lys	Met	His	Cys	Tyr	Met	Asn	Ser	Ala	Ser	Gly	Asn	Val	Ser	
				65					70					75	
Trp	Leu	Trp	Lys	Gln	Glu	Met	Asp	Glu	Asn	Pro	Gln	Gln	Leu	Lys	
				80					85					90	
Leu	Glu	Lys	Gly	Arg	Met	Glu	Glu	Ser	Gln	Asn	Glu	Ser	Leu	Ala	
				95					100					105	
Thr	Leu	Thr	Ile	Gln	Gly	Ile	Arg	Phe	Glu	Asp	Asn	Gly	Ile	Tyr	
				110					115					120	
Phe	Cys	Gln	Gln	Lys	Cys	Asn	Asn	Thr	Ser	Glu	Val	Tyr	Gln	Gly	
				125					130					135	
Cys	Gly	Thr	Glu	Leu	Arg	Val	Met	Gly	Phe	Ser	Thr	Leu	Ala	Gln	
				140					145					150	

Leu Lys Gln Arg Asn Thr Leu Lys Asp Gly Ile Ile Met Ile Gln
 155 160 165
 Thr Leu Leu Ile Ile Leu Phe Ile Ile Val Pro Ile Phe Leu Leu
 170 175 180
 Leu Asp Lys Asp Asp Ser Lys Ala Gly Met Glu Glu Asp His Thr
 185 190 195
 Tyr Glu Gly Leu Asp Ile Asp Gln Thr Ala Thr Tyr Glu Asp Ile
 200 205 210
 Val Thr Leu Arg Thr Gly Glu Val Lys Trp Ser Val Gly Glu His
 215 220 225
 Pro Gly Gln Glu

<210> 16
 <211> 508
 <212> PRT
 <213> Homo sapien

<400> 16
 Met Leu Leu Trp Ser Leu Leu Val Ile Phe Asp Ala Val Thr Glu
 1 5 10 15
 Gln Ala Asp Ser Leu Thr Leu Val Ala Pro Ser Ser Val Phe Glu
 20 25 30
 Gly Asp Ser Ile Val Leu Lys Cys Gln Gly Glu Gln Asn Trp Lys
 35 40 45
 Ile Gln Lys Met Ala Tyr His Lys Asp Asn Lys Glu Leu Ser Val
 50 55 60
 Phe Lys Lys Phe Ser Asp Phe Leu Ile Gln Ser Ala Val Leu Ser
 65 70 75
 Asp Ser Gly Asn Tyr Phe Cys Ser Thr Lys Gly Gln Leu Phe Leu
 80 85 90
 Trp Asp Lys Thr Ser Asn Ile Val Lys Ile Lys Val Gln Glu Leu
 95 100 105
 Phe Gln Arg Pro Val Leu Thr Ala Ser Ser Phe Gln Pro Ile Glu
 110 115 120
 Gly Gly Pro Val Ser Leu Lys Cys Glu Thr Arg Leu Ser Pro Gln
 125 130 135
 Arg Leu Asp Val Gln Leu Gln Phe Cys Phe Phe Arg Glu Asn Gln
 140 145 150
 Val Leu Gly Ser Gly Trp Ser Ser Ser Pro Glu Leu Gln Ile Ser
 155 160 165
 Ala Val Trp Ser Glu Asp Thr Gly Ser Tyr Trp Cys Lys Ala Glu
 170 175 180
 Thr Val Thr His Arg Ile Arg Lys Gln Ser Leu Gln Ser Gln Ile

185										190				195			
His	Val	Gln	Arg	Ile	Pro	Ile	Ser	Asn	Val	Ser	Leu	Glu	Ile	Arg			
				200					205					210			
Ala	Pro	Gly	Gly	Gln	Val	Thr	Glu	Gly	Gln	Lys	Leu	Ile	Leu	Leu			
				215					220					225			
Cys	Ser	Val	Ala	Gly	Gly	Thr	Gly	Asn	Val	Thr	Phe	Ser	Trp	Tyr			
				230					235					240			
Arg	Glu	Ala	Thr	Gly	Thr	Ser	Met	Gly	Lys	Lys	Thr	Gln	Arg	Ser			
				245					250					255			
Leu	Ser	Ala	Glu	Leu	Glu	Ile	Pro	Ala	Val	Lys	Glu	Ser	Asp	Ala			
				260					265					270			
Gly	Lys	Tyr	Tyr	Cys	Arg	Ala	Asp	Asn	Gly	His	Val	Pro	Ile	Gln			
				275					280					285			
Ser	Lys	Val	Val	Asn	Ile	Pro	Val	Arg	Ile	Pro	Val	Ser	Arg	Pro			
				290					295					300			
Val	Leu	Thr	Leu	Arg	Ser	Pro	Gly	Ala	Gln	Ala	Ala	Val	Gly	Asp			
				305					310					315			
Leu	Leu	Glu	Leu	His	Cys	Glu	Ala	Leu	Arg	Gly	Ser	Pro	Pro	Ile			
				320					325					330			
Leu	Tyr	Gln	Phe	Tyr	His	Glu	Asp	Val	Thr	Leu	Gly	Asn	Ser	Ser			
				335					340					345			
Ala	Pro	Ser	Gly	Gly	Gly	Ala	Ser	Phe	Asn	Leu	Ser	Leu	Thr	Ala			
				350					355					360			
Glu	His	Ser	Gly	Asn	Tyr	Ser	Cys	Glu	Ala	Asn	Asn	Gly	Leu	Gly			
				365					370					375			
Ala	Gln	Cys	Ser	Glu	Ala	Val	Pro	Val	Ser	Ile	Ser	Gly	Pro	Asp			
				380					385					390			
Gly	Tyr	Arg	Arg	Asp	Leu	Met	Thr	Ala	Gly	Val	Leu	Trp	Gly	Leu			
				395					400					405			
Phe	Gly	Val	Leu	Gly	Phe	Thr	Gly	Val	Ala	Leu	Leu	Leu	Tyr	Ala			
				410					415					420			
Leu	Phe	His	Lys	Ile	Ser	Gly	Glu	Ser	Ser	Ala	Thr	Asn	Glu	Pro			
				425					430					435			
Arg	Gly	Ala	Ser	Arg	Pro	Asn	Pro	Gln	Glu	Phe	Thr	Tyr	Ser	Ser			
				440					445					450			
Pro	Thr	Pro	Asp	Met	Glu	Glu	Leu	Gln	Pro	Val	Tyr	Val	Asn	Val			
				455					460					465			
Gly	Ser	Val	Asp	Val	Asp	Val	Val	Tyr	Ser	Gln	Val	Trp	Ser	Met			
				470					475					480			
Gln	Gln	Pro	Glu	Ser	Ser	Ala	Asn	Ile	Arg	Thr	Leu	Leu	Glu	Asn			
				485					490					495			

Lys Asp Ser Gln Val Ile Tyr Ser Ser Val Lys Lys Ser
500 505

<210> 17
<211> 1255
<212> PRT
<213> Homo sapien

<400> 17
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu
1 5 10 15
Leu Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp
20 25 30
Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met
35 40 45
Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu
50 55 60
Glu Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln
65 70 75
Asp Ile Gln Glu Val Gln Gly Tyr Val Leu Ile Ala His Asn Gln
80 85 90
Val Arg Gln Val Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr
95 100 105
Gln Leu Phe Glu Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly
110 115 120
Asp Pro Leu Asn Asn Thr Thr Pro Val Thr Gly Ala Ser Pro Gly
125 130 135
Gly Leu Arg Glu Leu Gln Leu Arg Ser Leu Thr Glu Ile Leu Lys
140 145 150
Gly Gly Val Leu Ile Gln Arg Asn Pro Gln Leu Cys Tyr Gln Asp
155 160 165
Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn Asn Gln Leu Ala
170 175 180
Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys His Pro Cys
185 190 195
Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser Ser Glu
200 205 210
Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys Ala
215 220 225
Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
230 235 240
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys
245 250 255

Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala	260	265	270
Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro	275	280	285
Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro	290	295	300
Tyr Asn Tyr Leu Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys	305	310	315
Pro Leu His Asn Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg	320	325	330
Cys Glu Lys Cys Ser Lys Pro Cys Ala Arg Val Cys Tyr Gly Leu	335	340	345
Gly Met Glu His Leu Arg Glu Val Arg Ala Val Thr Ser Ala Asn	350	355	360
Ile Gln Glu Phe Ala Gly Cys Lys Lys Ile Phe Gly Ser Leu Ala	365	370	375
Phe Leu Pro Glu Ser Phe Asp Gly Asp Pro Ala Ser Asn Thr Ala	380	385	390
Pro Leu Gln Pro Glu Gln Leu Gln Val Phe Glu Thr Leu Glu Glu	395	400	405
Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro Asp Ser Leu Pro	410	415	420
Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg Gly Arg Ile	425	430	435
Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu Gly Ile	440	445	450
Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly Leu	455	460	465
Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val	470	475	480
Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His	485	490	495
Thr Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala	500	505	510
Cys His Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro	515	520	525
Thr Gln Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys	530	535	540
Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val	545	550	555
Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln			

					560						565				570
Asn	Gly	Ser	Val	Thr	Cys	Phe	Gly	Pro	Glu	Ala	Asp	Gln	Cys	Val	
				575					580					585	
Ala	Cys	Ala	His	Tyr	Lys	Asp	Pro	Pro	Phe	Cys	Val	Ala	Arg	Cys	
				590					595					600	
Pro	Ser	Gly	Val	Lys	Pro	Asp	Leu	Ser	Tyr	Met	Pro	Ile	Trp	Lys	
				605					610					615	
Phe	Pro	Asp	Glu	Glu	Gly	Ala	Cys	Gln	Pro	Cys	Pro	Ile	Asn	Cys	
				620					625					630	
Thr	His	Ser	Cys	Val	Asp	Leu	Asp	Asp	Lys	Gly	Cys	Pro	Ala	Glu	
				635					640					645	
Gln	Arg	Ala	Ser	Pro	Leu	Thr	Ser	Ile	Ile	Ser	Ala	Val	Val	Gly	
				650					655					660	
Ile	Leu	Leu	Val	Val	Val	Leu	Gly	Val	Val	Phe	Gly	Ile	Leu	Ile	
				665					670					675	
Lys	Arg	Arg	Gln	Gln	Lys	Ile	Arg	Lys	Tyr	Thr	Met	Arg	Arg	Leu	
				680					685					690	
Leu	Gln	Glu	Thr	Glu	Leu	Val	Glu	Pro	Leu	Thr	Pro	Ser	Gly	Ala	
				695					700					705	
Met	Pro	Asn	Gln	Ala	Gln	Met	Arg	Ile	Leu	Lys	Glu	Thr	Glu	Leu	
				710					715					720	
Arg	Lys	Val	Lys	Val	Leu	Gly	Ser	Gly	Ala	Phe	Gly	Thr	Val	Tyr	
				725					730					735	
Lys	Gly	Ile	Trp	Ile	Pro	Asp	Gly	Glu	Asn	Val	Lys	Ile	Pro	Val	
				740					745					750	
Ala	Ile	Lys	Val	Leu	Arg	Glu	Asn	Thr	Ser	Pro	Lys	Ala	Asn	Lys	
				755					760					765	
Glu	Ile	Leu	Asp	Glu	Ala	Tyr	Val	Met	Ala	Gly	Val	Gly	Ser	Pro	
				770					775					780	
Tyr	Val	Ser	Arg	Leu	Leu	Gly	Ile	Cys	Leu	Thr	Ser	Thr	Val	Gln	
				785					790					795	
Leu	Val	Thr	Gln	Leu	Met	Pro	Tyr	Gly	Cys	Leu	Leu	Asp	His	Val	
				800					805					810	
Arg	Glu	Asn	Arg	Gly	Arg	Leu	Gly	Ser	Gln	Asp	Leu	Leu	Asn	Trp	
				815					820					825	
Cys	Met	Gln	Ile	Ala	Lys	Gly	Met	Ser	Tyr	Leu	Glu	Asp	Val	Arg	
				830					835					840	
Leu	Val	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Val	Leu	Val	Lys	Ser	
				845					850					855	
Pro	Asn	His	Val	Lys	Ile	Thr	Asp	Phe	Gly	Leu	Ala	Arg	Leu	Leu	
				860					865					870	

Asp Ile Asp Glu Thr Glu Tyr His Ala	Asp Gly Gly Lys Val Pro
875	880 885
Ile Lys Trp Met Ala Leu Glu Ser Ile	Leu Arg Arg Arg Phe Thr
890	895 900
His Gln Ser Asp Val Trp Ser Tyr Gly	Val Thr Val Trp Glu Leu
905	910 915
Met Thr Phe Gly Ala Lys Pro Tyr Asp	Gly Ile Pro Ala Arg Glu
920	925 930
Ile Pro Asp Leu Leu Glu Lys Gly Glu	Arg Leu Pro Gln Pro Pro
935	940 945
Ile Cys Thr Ile Asp Val Tyr Met Ile	Met Val Lys Cys Trp Met
950	955 960
Ile Asp Ser Glu Cys Arg Pro Arg Phe	Arg Glu Leu Val Ser Glu
965	970 975
Phe Ser Arg Met Ala Arg Asp Pro Gln	Arg Phe Val Val Ile Gln
980	985 990
Asn Glu Asp Leu Gly Pro Ala Ser Pro	Leu Asp Ser Thr Phe Tyr
995	1000 1005
Arg Ser Leu Leu Glu Asp Asp Asp Met	Gly Asp Leu Val Asp Ala
1010	1015 1020
Glu Glu Tyr Leu Val Pro Gln Gln Gly	Phe Phe Cys Pro Asp Pro
1025	1030 1035
Ala Pro Gly Ala Gly Gly Met Val His	His Arg His Arg Ser Ser
1040	1045 1050
Ser Thr Arg Ser Gly Gly Gly Asp Leu	Thr Leu Gly Leu Glu Pro
1055	1060 1065
Ser Glu Glu Glu Ala Pro Arg Ser Pro	Leu Ala Pro Ser Glu Gly
1070	1075 1080
Ala Gly Ser Asp Val Phe Asp Gly Asp	Leu Gly Met Gly Ala Ala
1085	1090 1095
Lys Gly Leu Gln Ser Leu Pro Thr His	Asp Pro Ser Pro Leu Gln
1100	1105 1110
Arg Tyr Ser Glu Asp Pro Thr Val Pro	Leu Pro Ser Glu Thr Asp
1115	1120 1125
Gly Tyr Val Ala Pro Leu Thr Cys Ser	Pro Gln Pro Glu Tyr Val
1130	1135 1140
Asn Gln Pro Asp Val Arg Pro Gln Pro	Pro Ser Pro Arg Glu Gly
1145	1150 1155
Pro Leu Pro Ala Ala Arg Pro Ala Gly	Ala Thr Leu Glu Arg Pro
1160	1165 1170

Lys	Thr	Leu	Ser	Pro	Gly	Lys	Asn	Gly	Val	Val	Lys	Asp	Val	Phe	
				1175					1180					1185	
Ala	Phe	Gly	Gly	Ala	Val	Glu	Asn	Pro	Glu	Tyr	Leu	Thr	Pro	Gln	
				1190					1195					1200	
Gly	Gly	Ala	Ala	Pro	Gln	Pro	His	Pro	Pro	Pro	Ala	Phe	Ser	Pro	
				1205					1210					1215	
Ala	Phe	Asp	Asn	Leu	Tyr	Tyr	Trp	Asp	Gln	Asp	Pro	Pro	Glu	Arg	
				1220					1225					1230	
Gly	Ala	Pro	Pro	Ser	Thr	Phe	Lys	Gly	Thr	Pro	Thr	Ala	Glu	Asn	
				1235					1240					1245	
Pro	Glu	Tyr	Leu	Gly	Leu	Asp	Val	Pro	Val						
				1250					1255						

<210> 18
 <211> 344
 <212> PRT
 <213> Homo sapien

<400> 18

Met	Gly	Pro	Pro	Ser	Ala	Pro	Pro	Cys	Arg	Leu	His	Val	Pro	Trp	
1				5					10					15	
Lys	Glu	Val	Leu	Leu	Thr	Ala	Ser	Leu	Leu	Thr	Phe	Trp	Asn	Pro	
				20					25					30	
Pro	Thr	Thr	Ala	Lys	Leu	Thr	Ile	Glu	Ser	Thr	Pro	Phe	Asn	Val	
				35					40					45	
Ala	Glu	Gly	Lys	Glu	Val	Leu	Leu	Leu	Ala	His	Asn	Leu	Pro	Gln	
				50					55					60	
Asn	Arg	Ile	Gly	Tyr	Ser	Trp	Tyr	Lys	Gly	Glu	Arg	Val	Asp	Gly	
				65					70					75	
Asn	Ser	Leu	Ile	Val	Gly	Tyr	Val	Ile	Gly	Thr	Gln	Gln	Ala	Thr	
				80					85					90	
Pro	Gly	Pro	Ala	Tyr	Ser	Gly	Arg	Glu	Thr	Ile	Tyr	Pro	Asn	Ala	
				95					100					105	
Ser	Leu	Leu	Ile	Gln	Asn	Val	Thr	Gln	Asn	Asp	Thr	Gly	Phe	Tyr	
				110					115					120	
Thr	Leu	Gln	Val	Ile	Lys	Ser	Asp	Leu	Val	Asn	Glu	Glu	Ala	Thr	
				125					130					135	
Gly	Gln	Phe	His	Val	Tyr	Pro	Glu	Leu	Pro	Lys	Pro	Ser	Ile	Ser	
				140					145					150	
Ser	Asn	Asn	Ser	Asn	Pro	Val	Glu	Asp	Lys	Asp	Ala	Val	Ala	Phe	
				155					160					165	
Thr	Cys	Glu	Pro	Glu	Val	Gln	Asn	Thr	Thr	Tyr	Leu	Trp	Trp	Val	
				170					175					180	
Asn	Gly	Gln	Ser	Leu	Pro	Val	Ser	Pro	Arg	Leu	Gln	Leu	Ser	Asn	

	185	190	195
Gly Asn Met Thr	Leu Thr Leu Leu Ser	Val Lys Arg Asn Asp	Ala
	200	205	210
Gly Ser Tyr Glu	Cys Glu Ile Gln Asn	Pro Ala Ser Ala Asn	Arg
	215	220	225
Ser Asp Pro Val	Thr Leu Asn Val Leu	Tyr Gly Pro Asp Val	Pro
	230	235	240
Thr Ile Ser Pro	Ser Lys Ala Asn Tyr	Arg Pro Gly Glu Asn	Leu
	245	250	255
Asn Leu Ser Cys	His Ala Ala Ser Asn	Pro Pro Ala Gln Tyr	Ser
	260	265	270
Trp Phe Ile Asn	Gly Thr Phe Gln Gln	Ser Thr Gln Glu Leu	Phe
	275	280	285
Ile Pro Asn Ile	Thr Val Asn Asn Ser	Gly Ser Tyr Met Cys	Gln
	290	295	300
Ala His Asn Ser	Ala Thr Gly Leu Asn	Arg Thr Thr Val Thr	Met
	305	310	315
Ile Thr Val Ser	Gly Ser Ala Pro Val	Leu Ser Ala Val Ala	Thr
	320	325	330
Val Gly Ile Thr	Ile Gly Val Leu Ala	Arg Val Ala Leu Ile	
	335	340	

<210> 19
 <211> 411
 <212> PRT
 <213> Homo sapien

<400> 19

Met Trp Ser Gly Trp	Trp Leu Trp Pro	Leu Val Ala Val Cys	Thr
1	5	10	15
Ala Asp Phe Phe Arg	Asp Glu Ala Glu Arg	Ile Met Arg Asp	Ser
	20	25	30
Pro Val Ile Asp Gly	His Asn Asp Leu Pro	Trp Gln Leu Leu Asp	
	35	40	45
Met Phe Asn Asn Arg	Leu Gln Asp Glu Arg	Ala Asn Leu Thr Thr	
	50	55	60
Leu Ala Gly Thr His	Thr Asn Ile Pro Lys	Leu Arg Ala Gly Phe	
	65	70	75
Val Gly Gly Gln Phe	Trp Ser Val Tyr Thr	Pro Cys Asp Thr Gln	
	80	85	90
Asn Lys Asp Ala Val	Arg Arg Thr Leu Glu	Gln Met Asp Val Val	
	95	100	105
His Arg Met Cys Arg	Met Tyr Pro Glu Thr	Phe Leu Tyr Val Thr	
	110	115	120

Ser Ser Ala Gly Ile Arg Gln Ala Phe Arg Glu Gly Lys Val Ala	125	130	135
Ser Leu Ile Gly Val Glu Gly Gly His Ser Ile Asp Ser Ser Leu	140	145	150
Gly Val Leu Arg Ala Leu Tyr Gln Leu Gly Met Arg Tyr Leu Thr	155	160	165
Leu Thr His Ser Cys Asn Thr Pro Trp Ala Asp Asn Trp Leu Val	170	175	180
Asp Thr Gly Asp Ser Glu Pro Gln Ser Gln Gly Leu Ser Pro Phe	185	190	195
Gly Gln Arg Val Val Lys Glu Leu Asn Arg Leu Gly Val Leu Ile	200	205	210
Asp Leu Ala His Val Ser Val Ala Thr Met Lys Ala Thr Leu Gln	215	220	225
Leu Ser Arg Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser	230	235	240
Val Cys Ala Ser Arg Arg Asn Val Pro Asp Asp Val Leu Arg Leu	245	250	255
Val Lys Gln Thr Asp Ser Leu Val Met Val Asn Phe Tyr Asn Asn	260	265	270
Tyr Ile Ser Cys Thr Asn Lys Ala Asn Leu Ser Gln Val Ala Asp	275	280	285
His Leu Asp His Ile Lys Glu Val Ala Gly Ala Arg Ala Val Gly	290	295	300
Phe Gly Gly Asp Phe Asp Gly Val Pro Arg Val Pro Glu Gly Leu	305	310	315
Glu Asp Val Ser Lys Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg	320	325	330
Arg Asn Trp Thr Glu Ala Glu Val Lys Gly Ala Leu Ala Asp Asn	335	340	345
Leu Leu Arg Val Phe Glu Ala Val Glu Gln Ala Ser Asn Leu Thr	350	355	360
Gln Ala Pro Glu Glu Glu Pro Ile Pro Leu Asp Gln Leu Gly Gly	365	370	375
Ser Cys Arg Thr His Tyr Gly Tyr Ser Ser Gly Ala Ser Ser Leu	380	385	390
His Arg His Trp Gly Leu Leu Leu Ala Ser Leu Ala Pro Leu Val	395	400	405
Leu Cys Leu Ser Leu Leu	410		

<400> 20

Tyr Ser Ile Tyr Arg Tyr Ile His Val Gly Lys Glu Lys His Pro

275	280	285
Ala Asn Leu Ile Leu Ile Tyr Gly Asn Glu Phe Asp Lys Arg Phe		
290	295	300
Phe Val Pro Ala Glu Lys Ile Val Ile Asn Phe Ile Thr Leu Asn		
305	310	315
Ile Ser Asp Asp Ser Lys Ile Ser His Gln Asp Met Ser Leu Leu		
320	325	330
Gly Lys Ser Ser Asp Val Ser Ser Leu Asn Asp Pro Gln Pro Ser		
335	340	345
Gly Asn Leu Arg Pro Pro Gln Glu Glu Glu Glu Val Lys His Leu		
350	355	360
Gly Tyr Ala Ser His Leu Met Glu Ile Phe Cys Asp Ser Glu Glu		
365	370	375
Asn Thr Glu Gly Thr Ser Phe Thr Gln Gln Glu Ser Leu Ser Arg		
380	385	390
Thr Ile Pro Pro Asp Lys Thr Val Ile Glu Tyr Glu Tyr Asp Val		
395	400	405
Arg Thr Thr Asp Ile Cys Ala Gly Pro Glu Glu Gln Glu Leu Ser		
410	415	420
Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu Glu Ser Gln		
425	430	435
Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr Ser Tyr		
440	445	450
Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His Thr		
455	460	465
Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val		
470	475	480
Asp Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser		
485	490	495
Ser Phe Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp		
500	505	510
Gly Leu Gly Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro		
515	520	525
Ala Pro Asp Arg Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln		
530	535	540
Phe Met Glu Glu Trp Gly Leu Tyr Val Gln Met Glu Asn		
545	550	

<210> 21
 <211> 911
 <212> PRT
 <213> Homo sapien

<400> 21

Met	Ala	Gln	Leu	Phe	Leu	Pro	Leu	Leu	Ala	Ala	Leu	Val	Leu	Ala	1	5	10	15
Gln	Ala	Pro	Ala	Ala	Leu	Ala	Asp	Val	Leu	Glu	Gly	Asp	Ser	Ser	20	25	30	
Glu	Asp	Arg	Ala	Phe	Arg	Val	Arg	Ile	Ala	Gly	Asp	Ala	Pro	Leu	35	40	45	
Gln	Gly	Val	Leu	Gly	Gly	Ala	Leu	Thr	Ile	Pro	Cys	His	Val	His	50	55	60	
Tyr	Leu	Arg	Pro	Pro	Pro	Ser	Arg	Arg	Ala	Val	Leu	Gly	Ser	Pro	65	70	75	
Arg	Val	Lys	Trp	Thr	Phe	Leu	Ser	Arg	Gly	Arg	Glu	Ala	Glu	Val	80	85	90	
Leu	Val	Ala	Arg	Gly	Val	Arg	Val	Lys	Val	Asn	Glu	Ala	Tyr	Arg	95	100	105	
Phe	Arg	Val	Ala	Leu	Pro	Ala	Tyr	Pro	Ala	Ser	Leu	Thr	Asp	Val	110	115	120	
Ser	Leu	Ala	Leu	Ser	Glu	Leu	Arg	Pro	Asn	Asp	Ser	Gly	Ile	Tyr	125	130	135	
Arg	Cys	Glu	Val	Gln	His	Gly	Ile	Asp	Asp	Ser	Ser	Asp	Ala	Val	140	145	150	
Glu	Val	Lys	Val	Lys	Gly	Val	Val	Phe	Leu	Tyr	Arg	Glu	Gly	Ser	155	160	165	
Ala	Arg	Tyr	Ala	Phe	Ser	Phe	Ser	Gly	Ala	Gln	Glu	Ala	Cys	Ala	170	175	180	
Arg	Ile	Gly	Ala	His	Ile	Ala	Thr	Pro	Glu	Gln	Leu	Tyr	Ala	Ala	185	190	195	
Tyr	Leu	Gly	Gly	Tyr	Glu	Gln	Cys	Asp	Ala	Gly	Trp	Leu	Ser	Asp	200	205	210	
Gln	Thr	Val	Arg	Tyr	Pro	Ile	Gln	Thr	Pro	Arg	Glu	Ala	Cys	Tyr	215	220	225	
Gly	Asp	Met	Asp	Gly	Phe	Pro	Gly	Val	Arg	Asn	Tyr	Gly	Val	Val	230	235	240	
Asp	Pro	Asp	Asp	Leu	Tyr	Asp	Val	Tyr	Cys	Tyr	Ala	Glu	Asp	Leu	245	250	255	
Asn	Gly	Glu	Leu	Phe	Leu	Gly	Asp	Pro	Pro	Glu	Lys	Leu	Thr	Leu	260	265	270	
Glu	Glu	Ala	Arg	Ala	Tyr	Cys	Gln	Glu	Arg	Gly	Ala	Glu	Ile	Ala	275	280	285	
Thr	Thr	Gly	Gln	Leu	Tyr	Ala	Ala	Trp	Asp	Gly	Gly	Leu	Asp	His	290	295	300	

Cys	Ser	Pro	Gly	Trp	Leu	Ala	Asp	Gly	Ser	Val	Arg	Tyr	Pro	Ile	305	310	315
Val	Thr	Pro	Ser	Gln	Arg	Cys	Gly	Gly	Gly	Leu	Pro	Gly	Val	Lys	320	325	330
Thr	Leu	Phe	Leu	Phe	Pro	Asn	Gln	Thr	Gly	Phe	Pro	Asn	Lys	His	335	340	345
Ser	Arg	Phe	Asn	Val	Tyr	Cys	Phe	Arg	Asp	Ser	Ala	Gln	Pro	Ser	350	355	360
Ala	Ile	Pro	Glu	Ala	Ser	Asn	Pro	Ala	Ser	Asn	Pro	Ala	Ser	Asp	365	370	375
Gly	Leu	Glu	Ala	Ile	Val	Thr	Val	Thr	Glu	Thr	Leu	Glu	Glu	Leu	380	385	390
Gln	Leu	Pro	Gln	Glu	Ala	Thr	Glu	Ser	Glu	Ser	Arg	Gly	Ala	Ile	395	400	405
Tyr	Ser	Ile	Pro	Ile	Met	Glu	Asp	Gly	Gly	Gly	Gly	Ser	Ser	Thr	410	415	420
Pro	Glu	Asp	Pro	Ala	Glu	Ala	Pro	Arg	Thr	Leu	Leu	Glu	Phe	Glu	425	430	435
Thr	Gln	Ser	Met	Val	Pro	Pro	Thr	Gly	Phe	Ser	Glu	Glu	Glu	Gly	440	445	450
Lys	Ala	Leu	Glu	Glu	Glu	Glu	Lys	Tyr	Glu	Asp	Glu	Glu	Glu	Lys	455	460	465
Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Val	Glu	Asp	Glu	Ala	Leu	Trp	470	475	480
Ala	Trp	Pro	Ser	Glu	Leu	Ser	Ser	Pro	Gly	Pro	Glu	Ala	Ser	Leu	485	490	495
Pro	Thr	Glu	Pro	Ala	Ala	Gln	Glu	Lys	Ser	Leu	Ser	Gln	Ala	Pro	500	505	510
Ala	Arg	Ala	Val	Leu	Gln	Pro	Gly	Ala	Ser	Pro	Leu	Pro	Asp	Gly	515	520	525
Glu	Ser	Glu	Ala	Ser	Arg	Pro	Pro	Arg	Val	His	Gly	Pro	Pro	Thr	530	535	540
Glu	Thr	Leu	Pro	Thr	Pro	Arg	Glu	Arg	Asn	Leu	Ala	Ser	Pro	Ser	545	550	555
Pro	Ser	Thr	Leu	Val	Glu	Ala	Arg	Glu	Val	Gly	Glu	Ala	Thr	Gly	560	565	570
Gly	Pro	Glu	Leu	Ser	Gly	Val	Pro	Arg	Gly	Glu	Ser	Glu	Glu	Thr	575	580	585
Gly	Ser	Ser	Glu	Gly	Ala	Pro	Ser	Leu	Leu	Pro	Ala	Thr	Arg	Ala	590	595	600
Pro	Glu	Gly	Thr	Arg	Glu	Leu	Glu	Ala	Pro	Ser	Glu	Asp	Asn	Ser			

605					610					615				
Gly	Arg	Thr	Ala	Pro	Ala	Gly	Thr	Ser	Val	Gln	Ala	Gln	Pro	Val
				620					625					630
Leu	Pro	Thr	Asp	Ser	Ala	Ser	Arg	Gly	Gly	Val	Ala	Val	Val	Pro
				635					640					645
Ala	Ser	Gly	Asp	Cys	Val	Pro	Ser	Pro	Cys	His	Asn	Gly	Gly	Thr
				650					655					660
Cys	Leu	Glu	Glu	Glu	Glu	Gly	Val	Arg	Cys	Leu	Cys	Leu	Pro	Gly
				665					670					675
Tyr	Gly	Gly	Asp	Leu	Cys	Asp	Val	Gly	Leu	Arg	Phe	Cys	Asn	Pro
				680					685					690
Gly	Trp	Asp	Ala	Phe	Gln	Gly	Ala	Cys	Tyr	Lys	His	Phe	Ser	Thr
				695					700					705
Arg	Arg	Ser	Trp	Glu	Glu	Ala	Glu	Thr	Gln	Cys	Arg	Met	Tyr	Gly
				710					715					720
Ala	His	Leu	Ala	Ser	Ile	Ser	Thr	Pro	Glu	Glu	Gln	Asp	Phe	Ile
				725					730					735
Asn	Asn	Arg	Tyr	Arg	Glu	Tyr	Gln	Trp	Ile	Gly	Leu	Asn	Asp	Arg
				740					745					750
Thr	Ile	Glu	Gly	Asp	Phe	Leu	Trp	Ser	Asp	Gly	Val	Pro	Leu	Leu
				755					760					765
Tyr	Glu	Asn	Trp	Asn	Pro	Gly	Gln	Pro	Asp	Ser	Tyr	Phe	Leu	Ser
				770					775					780
Gly	Glu	Asn	Cys	Val	Val	Met	Val	Trp	His	Asp	Gln	Gly	Gln	Trp
				785					790					795
Ser	Asp	Val	Pro	Cys	Asn	Tyr	His	Leu	Ser	Tyr	Thr	Cys	Lys	Met
				800					805					810
Gly	Leu	Val	Ser	Cys	Gly	Pro	Pro	Pro	Glu	Leu	Pro	Leu	Ala	Gln
				815					820					825
Val	Phe	Gly	Arg	Pro	Arg	Leu	Arg	Tyr	Glu	Val	Asp	Thr	Val	Leu
				830					835					840
Arg	Tyr	Arg	Cys	Arg	Glu	Gly	Leu	Ala	Gln	Arg	Asn	Leu	Pro	Leu
				845					850					855
Ile	Arg	Cys	Gln	Glu	Asn	Gly	Arg	Trp	Glu	Ala	Pro	Gln	Ile	Ser
				860					865					870
Cys	Val	Pro	Arg	Arg	Pro	Ala	Arg	Ala	Leu	His	Pro	Glu	Glu	Asp
				875					880					885
Pro	Glu	Gly	Arg	Gln	Gly	Arg	Leu	Leu	Gly	Arg	Trp	Lys	Ala	Leu
				890					895					900
Leu	Ile	Pro	Pro	Ser	Ser	Pro	Met	Pro	Gly	Pro				
				905					910					

<210> 22
 <211> 987
 <212> PRT
 <213> Homo sapien

<400> 22
 Met Ala Leu Arg Arg Leu Gly Ala Ala Leu Leu Leu Leu Pro Leu
 1 5 10 15
 Leu Ala Ala Val Glu Glu Thr Leu Met Asp Ser Thr Thr Ala Thr
 20 25 30
 Ala Glu Leu Gly Trp Met Val His Pro Pro Ser Gly Trp Glu Glu
 35 40 45
 Val Ser Gly Tyr Asp Glu Asn Met Asn Thr Ile Arg Thr Tyr Gln
 50 55 60
 Val Cys Asn Val Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr
 65 70 75
 Lys Phe Ile Arg Arg Arg Gly Ala His Arg Ile His Val Glu Met
 80 85 90
 Lys Phe Ser Val Arg Asp Cys Ser Ser Ile Pro Ser Val Pro Gly
 95 100 105
 Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Tyr Glu Ala Asp Phe
 110 115 120
 Asp Ser Ala Thr Lys Thr Phe Pro Asn Trp Met Glu Asn Pro Trp
 125 130 135
 Val Lys Val Asp Thr Ile Ala Ala Asp Glu Ser Phe Ser Gln Val
 140 145 150
 Asp Leu Gly Gly Arg Val Met Lys Ile Asn Thr Glu Val Arg Ser
 155 160 165
 Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu Ala Phe Gln Asp
 170 175 180
 Tyr Gly Gly Cys Met Ser Leu Ile Ala Val Arg Val Phe Tyr Arg
 185 190 195
 Lys Cys Pro Arg Ile Ile Gln Asn Gly Ala Ile Phe Gln Glu Thr
 200 205 210
 Leu Ser Gly Ala Glu Ser Thr Ser Leu Val Ala Ala Arg Gly Ser
 215 220 225
 Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr
 230 235 240
 Cys Asn Gly Asp Gly Glu Trp Leu Val Pro Ile Gly Arg Cys Met
 245 250 255
 Cys Lys Ala Gly Phe Glu Ala Val Glu Asn Gly Thr Val Cys Arg
 260 265 270

Gly Cys Pro Ser	Gly Thr Phe Lys Ala	Asn Gln Gly Asp Glu Ala	275	280	285
Cys Thr His Cys	Pro Ile Asn Ser Arg	Thr Thr Ser Glu Gly Ala	290	295	300
Thr Asn Cys Val	Cys Arg Asn Gly Tyr	Tyr Arg Ala Asp Leu Asp	305	310	315
Pro Leu Asp Met	Pro Cys Thr Thr Ile	Pro Ser Ala Pro Gln Ala	320	325	330
Val Ile Ser Ser	Val Asn Glu Thr Ser	Leu Met Leu Glu Trp Thr	335	340	345
Pro Pro Arg Asp	Ser Gly Gly Arg Glu	Asp Leu Val Tyr Asn Ile	350	355	360
Ile Cys Lys Ser	Cys Gly Ser Gly Arg	Gly Ala Cys Thr Arg Cys	365	370	375
Gly Asp Asn Val	Gln Tyr Ala Pro Arg	Gln Leu Gly Leu Thr Glu	380	385	390
Pro Arg Ile Tyr	Ile Ser Asp Leu Leu	Ala His Thr Gln Tyr Thr	395	400	405
Phe Glu Ile Gln	Ala Val Asn Gly Val	Thr Asp Gln Ser Pro Phe	410	415	420
Ser Pro Gln Phe	Ala Ser Val Asn Ile	Thr Thr Asn Gln Ala Ala	425	430	435
Pro Ser Ala Val	Ser Ile Met His Gln	Val Ser Arg Thr Val Asp	440	445	450
Ser Ile Thr Leu	Ser Trp Ser Gln Pro	Asp Gln Pro Asn Gly Val	455	460	465
Ile Leu Asp Tyr	Glu Leu Gln Tyr Tyr	Glu Lys Glu Leu Ser Glu	470	475	480
Tyr Asn Ala Thr	Ala Ile Lys Ser Pro	Thr Asn Thr Val Thr Val	485	490	495
Gln Gly Leu Lys	Ala Gly Ala Ile Tyr	Val Phe Gln Val Arg Ala	500	505	510
Arg Thr Val Ala	Gly Tyr Gly Arg Tyr	Ser Gly Lys Met Tyr Phe	515	520	525
Gln Thr Met Thr	Glu Ala Glu Tyr Gln	Thr Ser Ile Gln Glu Lys	530	535	540
Leu Pro Leu Ile	Ile Gly Ser Ser Ala	Ala Gly Leu Val Phe Leu	545	550	555
Ile Ala Val Val	Val Ile Ala Ile Val	Cys Asn Arg Arg Arg Gly	560	565	570
Phe Glu Arg Ala	Asp Ser Glu Tyr Thr	Asp Lys Leu Gln His Tyr			

575					580					585				
Thr	Ser	Gly	His	Met	Thr	Pro	Gly	Met	Lys	Ile	Tyr	Ile	Asp	Pro
				590					595					600
Phe	Thr	Tyr	Glu	Asp	Pro	Asn	Glu	Ala	Val	Arg	Glu	Phe	Ala	Lys
				605					610					615
Glu	Ile	Asp	Ile	Ser	Cys	Val	Lys	Ile	Glu	Gln	Val	Ile	Gly	Ala
				620					625					630
Gly	Glu	Phe	Gly	Glu	Val	Cys	Ser	Gly	His	Leu	Lys	Leu	Pro	Gly
				635					640					645
Lys	Arg	Glu	Ile	Phe	Val	Ala	Ile	Lys	Thr	Leu	Lys	Ser	Gly	Tyr
				650					655					660
Thr	Glu	Lys	Gln	Arg	Arg	Asp	Phe	Leu	Ser	Glu	Ala	Ser	Ile	Met
				665					670					675
Gly	Gln	Phe	Asp	His	Pro	Asn	Val	Ile	His	Leu	Glu	Gly	Val	Val
				680					685					690
Thr	Lys	Ser	Thr	Pro	Val	Met	Ile	Ile	Thr	Glu	Phe	Met	Glu	Asn
				695					700					705
Gly	Ser	Leu	Asp	Ser	Phe	Leu	Arg	Gln	Asn	Asp	Gly	Gln	Phe	Thr
				710					715					720
Val	Ile	Gln	Leu	Val	Gly	Met	Leu	Arg	Gly	Ile	Ala	Ala	Gly	Met
				725					730					735
Lys	Tyr	Leu	Ala	Asp	Met	Asn	Tyr	Val	His	Arg	Asp	Leu	Ala	Ala
				740					745					750
Arg	Asn	Ile	Leu	Val	Asn	Ser	Asn	Leu	Val	Cys	Lys	Val	Ser	Asp
				755					760					765
Phe	Gly	Leu	Ser	Arg	Phe	Leu	Glu	Asp	Asp	Thr	Ser	Asp	Pro	Thr
				770					775					780
Tyr	Thr	Ser	Ala	Leu	Gly	Gly	Lys	Ile	Pro	Ile	Arg	Trp	Thr	Ala
				785					790					795
Pro	Glu	Ala	Ile	Gln	Tyr	Arg	Lys	Phe	Thr	Ser	Ala	Ser	Asp	Val
				800					805					810
Trp	Ser	Tyr	Gly	Ile	Val	Met	Trp	Glu	Val	Met	Ser	Tyr	Gly	Glu
				815					820					825
Arg	Pro	Tyr	Trp	Asp	Met	Thr	Asn	Gln	Asp	Val	Ile	Asn	Ala	Ile
				830					835					840
Glu	Gln	Asp	Tyr	Arg	Leu	Pro	Pro	Pro	Met	Asp	Cys	Pro	Ser	Ala
				845					850					855
Leu	His	Gln	Leu	Met	Leu	Asp	Cys	Trp	Gln	Lys	Asp	Arg	Asn	His
				860					865					870
Arg	Pro	Lys	Phe	Gly	Gln	Ile	Val	Asn	Thr	Leu	Asp	Lys	Met	Ile
				875					880					885

Arg Asn Pro Asn Ser Leu Lys Ala Met Ala Pro Leu Ser Ser Gly	890	895	900
Ile Asn Leu Pro Leu Leu Asp Arg Thr Ile Pro Asp Tyr Thr Ser	905	910	915
Phe Asn Thr Val Asp Glu Trp Leu Glu Ala Ile Lys Met Gly Gln	920	925	930
Tyr Lys Glu Ser Phe Ala Asn Ala Gly Phe Thr Ser Phe Asp Val	935	940	945
Val Ser Gln Met Met Met Glu Asp Ile Leu Arg Val Gly Val Thr	950	955	960
Leu Ala Gly His Gln Lys Lys Ile Leu Asn Ser Ile Gln Val Met	965	970	975
Arg Ala Gln Met Asn Gln Ile Gln Ser Val Glu Val	980	985	
<210> 23			
<211> 282			
<212> PRT			
<213> Homo sapien			
<400> 23			
Met Ala Ser Leu Gly Gln Ile Leu Phe Trp Ser Ile Ile Ser Ile	1	5	10 15
Ile Ile Ile Leu Ala Gly Ala Ile Ala Leu Ile Ile Gly Phe Gly	20	25	30
Ile Ser Gly Arg His Ser Ile Thr Val Thr Thr Val Ala Ser Ala	35	40	45
Gly Asn Ile Gly Glu Asp Gly Ile Leu Ser Cys Thr Phe Glu Pro	50	55	60
Asp Ile Lys Leu Ser Asp Ile Val Ile Gln Trp Leu Lys Glu Gly	65	70	75
Val Leu Gly Leu Val His Glu Phe Lys Glu Gly Lys Asp Glu Leu	80	85	90
Ser Glu Gln Asp Glu Met Phe Arg Gly Arg Thr Ala Val Phe Ala	95	100	105
Asp Gln Val Ile Val Gly Asn Ala Ser Leu Arg Leu Lys Asn Val	110	115	120
Gln Leu Thr Asp Ala Gly Thr Tyr Lys Cys Tyr Ile Ile Thr Ser	125	130	135
Lys Gly Lys Lys Asn Ala Asn Leu Glu Tyr Lys Thr Gly Ala Phe	140	145	150
Ser Met Pro Glu Val Asn Val Asp Tyr Asn Ala Ser Ser Glu Thr	155	160	165

Leu Arg Cys Glu Ala Pro Arg Trp Phe Pro Gln Pro Thr Val Val
170 175 180

Trp Ala Ser Gln Val Asp Gln Gly Ala Asn Phe Ser Glu Val Ser
185 190 195

Asn Thr Ser Phe Glu Leu Asn Ser Glu Asn Val Thr Met Lys Val
200 205 210

Val Ser Val Leu Tyr Asn Val Thr Ile Asn Asn Thr Tyr Ser Cys
215 220 225

Met Ile Glu Asn Asp Ile Ala Lys Ala Thr Gly Asp Ile Lys Val
230 235 240

Thr Glu Ser Glu Ile Lys Arg Arg Ser His Leu Gln Leu Leu Asn
245 250 255

Ser Lys Ala Ser Leu Cys Val Ser Ser Phe Phe Ala Ile Ser Trp
260 265 270

Ala Leu Leu Pro Leu Ser Pro Tyr Leu Met Leu Lys
275 280

<210> 24
<211> 123
<212> PRT
<213> Homo sapien

<400> 24
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu
1 5 10 15

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

Ser Asn Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly
35 40 45

Glu Gln Cys Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr
50 55 60

Val Ile Ser Lys Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln
65 70 75

Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp
80 85 90

Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala
95 100 105

Ile Leu Ala Leu Leu Pro Ala Leu Gly Leu Leu Leu Trp Gly Pro
110 115 120

Gly Gln Leu

<210> 25
<211> 236
<212> PRT
<213> Homo sapien

<400> 25

Met	Pro	Gly	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Met
1				5					10						15
Leu	Pro	Ala	Gln	Glu	Ala	Ala	Lys	Leu	Tyr	His	Thr	Asn	Tyr	Val	
			20						25					30	
Arg	Asn	Ser	Arg	Ala	Ile	Gly	Val	Leu	Trp	Ala	Ile	Phe	Thr	Ile	
			35						40					45	
Cys	Phe	Ala	Ile	Val	Asn	Val	Val	Cys	Phe	Ile	Gln	Pro	Tyr	Trp	
			50						55					60	
Ile	Gly	Asp	Gly	Val	Asp	Thr	Pro	Gln	Ala	Gly	Tyr	Phe	Gly	Leu	
			65						70					75	
Phe	His	Tyr	Cys	Ile	Gly	Asn	Gly	Phe	Ser	Arg	Glu	Leu	Thr	Cys	
			80						85					90	
Arg	Gly	Ser	Phe	Thr	Asp	Phe	Ser	Thr	Leu	Pro	Ser	Gly	Ala	Phe	
			95						100					105	
Lys	Ala	Ala	Ser	Phe	Phe	Ile	Gly	Leu	Ser	Met	Met	Leu	Ile	Ile	
			110						115					120	
Ala	Cys	Ile	Ile	Cys	Phe	Thr	Leu	Phe	Phe	Phe	Cys	Asn	Thr	Ala	
			125						130					135	
Thr	Val	Tyr	Lys	Ile	Cys	Ala	Trp	Met	Gln	Leu	Thr	Ser	Ala	Ala	
			140						145					150	
Cys	Leu	Val	Leu	Gly	Cys	Met	Ile	Phe	Pro	Asp	Gly	Trp	Asp	Ser	
			155						160					165	
Asp	Glu	Val	Lys	Arg	Met	Cys	Gly	Glu	Lys	Thr	Asp	Lys	Tyr	Thr	
			170						175					180	
Leu	Gly	Ala	Cys	Ser	Val	Arg	Trp	Ala	Tyr	Ile	Leu	Ala	Ile	Ile	
			185						190					195	
Gly	Ile	Leu	Asp	Ala	Leu	Ile	Leu	Ser	Phe	Leu	Ala	Phe	Val	Leu	
			200						205					210	
Gly	Asn	Arg	Gln	Asp	Ser	Leu	Met	Ala	Glu	Glu	Leu	Lys	Ala	Glu	
			215						220					225	
Asn	Lys	Val	Leu	Leu	Ser	Gln	Tyr	Ser	Leu	Glu					
			230						235						

<210> 26

<211> 184

<212> PRT

<213> Homo sapien

<400> 26

Met	Arg	Arg	Gly	Pro	Arg	Ser	Leu	Arg	Gly	Arg	Asp	Ala	Pro	Ala	
1				5					10					15	
Pro	Thr	Pro	Cys	Val	Pro	Ala	Glu	Cys	Phe	Asp	Leu	Leu	Val	Arg	
			20						25					30	

His	Cys	Val	Ala	Cys	Gly	Leu	Leu	Arg	Thr	Pro	Arg	Pro	Lys	Pro	35	40	45
Ala	Gly	Ala	Ser	Ser	Pro	Ala	Pro	Arg	Thr	Ala	Leu	Gln	Pro	Gln	50	55	60
Glu	Ser	Val	Gly	Ala	Gly	Ala	Gly	Glu	Ala	Ala	Leu	Pro	Leu	Pro	65	70	75
Gly	Leu	Leu	Phe	Gly	Ala	Pro	Ala	Leu	Leu	Gly	Leu	Ala	Leu	Val	80	85	90
Leu	Ala	Leu	Val	Leu	Val	Gly	Leu	Val	Ser	Trp	Arg	Arg	Arg	Gln	95	100	105
Arg	Arg	Leu	Arg	Gly	Ala	Ser	Ser	Ala	Glu	Ala	Pro	Asp	Gly	Asp	110	115	120
Lys	Asp	Ala	Pro	Glu	Pro	Leu	Asp	Lys	Val	Ile	Ile	Leu	Ser	Pro	125	130	135
Gly	Ile	Ser	Asp	Ala	Thr	Ala	Pro	Ala	Trp	Pro	Pro	Pro	Gly	Glu	140	145	150
Asp	Pro	Gly	Thr	Thr	Pro	Pro	Gly	His	Ser	Val	Pro	Val	Pro	Ala	155	160	165
Thr	Glu	Leu	Gly	Ser	Thr	Glu	Leu	Val	Thr	Thr	Lys	Thr	Ala	Gly	170	175	180
Pro Glu Gln Gln																	

<210> 27
 <211> 847
 <212> PRT
 <213> Homo sapien

Met	His	Leu	Leu	Gly	Pro	Trp	Leu	Leu	Leu	Leu	Val	Leu	Glu	Tyr	1	5	10	15
Leu	Ala	Phe	Ser	Asp	Ser	Ser	Lys	Trp	Val	Phe	Glu	His	Pro	Glu	20	25	30	
Thr	Leu	Tyr	Ala	Trp	Glu	Gly	Ala	Cys	Val	Trp	Ile	Pro	Cys	Thr	35	40	45	
Tyr	Arg	Ala	Leu	Asp	Gly	Asp	Leu	Glu	Ser	Phe	Ile	Leu	Phe	His	50	55	60	
Asn	Pro	Glu	Tyr	Asn	Lys	Asn	Thr	Ser	Lys	Phe	Asp	Gly	Thr	Arg	65	70	75	
Leu	Tyr	Glu	Ser	Thr	Lys	Asp	Gly	Lys	Val	Pro	Ser	Glu	Gln	Lys	80	85	90	
Arg	Val	Gln	Phe	Leu	Gly	Asp	Lys	Asn	Lys	Asn	Cys	Thr	Leu	Ser	95	100	105	

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg	110	115	120
Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn	125	130	135
Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu	140	145	150
Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe	155	160	165
Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly	170	175	180
Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile	185	190	195
Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp	200	205	210
Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp	215	220	225
Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His	230	235	240
Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val	245	250	255
Arg Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser	260	265	270
Asn Pro Glu Tyr Thr Thr Val Ser Trp Leu Lys Asp Gly Thr Ser	275	280	285
Leu Lys Lys Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr	290	295	300
Lys Asp Gln Ser Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val	305	310	315
Gly Pro Gly Arg Ser Glu Glu Val Phe Leu Gln Val Gln Tyr Ala	320	325	330
Pro Glu Pro Ser Thr Val Gln Ile Leu His Ser Pro Ala Val Glu	335	340	345
Gly Ser Gln Val Glu Phe Leu Cys Met Ser Leu Ala Asn Pro Leu	350	355	360
Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Met Gln Gly	365	370	375
Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu Pro Trp His	380	385	390
Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Thr Gly	395	400	405
Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro Lys			

				410						415					420
Lys	Val	Thr	Thr	Val	Ile	Gln	Asn	Pro	Met	Pro	Ile	Arg	Glu	Gly	
				425					430					435	
Asp	Thr	Val	Thr	Leu	Ser	Cys	Asn	Tyr	Asn	Ser	Ser	Asn	Pro	Ser	
				440					445					450	
Val	Thr	Arg	Tyr	Glu	Trp	Lys	Pro	His	Gly	Ala	Trp	Glu	Glu	Pro	
				455					460					465	
Ser	Leu	Gly	Val	Leu	Lys	Ile	Gln	Asn	Val	Gly	Trp	Asp	Asn	Thr	
				470					475					480	
Thr	Ile	Ala	Cys	Ala	Arg	Cys	Asn	Ser	Trp	Cys	Ser	Trp	Ala	Ser	
				485					490					495	
Pro	Val	Ala	Leu	Asn	Val	Gln	Tyr	Ala	Pro	Arg	Asp	Val	Arg	Val	
				500					505					510	
Arg	Lys	Ile	Lys	Pro	Leu	Ser	Glu	Ile	His	Ser	Gly	Asn	Ser	Val	
				515					520					525	
Ser	Leu	Gln	Cys	Asp	Phe	Ser	Ser	Ser	His	Pro	Lys	Glu	Val	Gln	
				530					535					540	
Phe	Phe	Trp	Glu	Lys	Asn	Gly	Arg	Leu	Leu	Gly	Lys	Glu	Ser	Gln	
				545					550					555	
Leu	Asn	Phe	Asp	Ser	Ile	Ser	Pro	Glu	Asp	Ala	Gly	Ser	Tyr	Ser	
				560					565					570	
Cys	Trp	Val	Asn	Asn	Ser	Ile	Gly	Gln	Thr	Ala	Ser	Lys	Ala	Trp	
				575					580					585	
Thr	Leu	Glu	Val	Leu	Tyr	Ala	Pro	Arg	Arg	Leu	Arg	Val	Ser	Met	
				590					595					600	
Ser	Pro	Gly	Asp	Gln	Val	Met	Glu	Gly	Lys	Ser	Ala	Thr	Leu	Thr	
				605					610					615	
Cys	Glu	Ser	Asp	Ala	Asn	Pro	Pro	Val	Ser	His	Tyr	Thr	Trp	Phe	
				620					625					630	
Asp	Trp	Asn	Asn	Gln	Ser	Leu	Pro	His	His	Ser	Gln	Lys	Leu	Arg	
				635					640					645	
Leu	Glu	Pro	Val	Lys	Val	Gln	His	Ser	Gly	Ala	Tyr	Trp	Cys	Gln	
				650					655					660	
Gly	Thr	Asn	Ser	Val	Gly	Lys	Gly	Arg	Ser	Pro	Leu	Ser	Thr	Leu	
				665					670					675	
Thr	Val	Tyr	Tyr	Ser	Pro	Glu	Thr	Ile	Gly	Arg	Arg	Val	Ala	Val	
				680					685					690	
Gly	Leu	Gly	Ser	Cys	Leu	Ala	Ile	Leu	Ile	Leu	Ala	Ile	Cys	Gly	
				695					700					705	
Leu	Lys	Leu	Gln	Arg	Arg	Trp	Lys	Arg	Thr	Gln	Ser	Gln	Gln	Gly	
				710					715					720	

Leu	Gln	Glu	Asn	Ser	Ser	Gly	Gln	Ser	Phe	Phe	Val	Arg	Asn	Lys	
				725					730					735	
Lys	Val	Arg	Arg	Ala	Pro	Leu	Ser	Glu	Gly	Pro	His	Ser	Leu	Gly	
				740					745					750	
Cys	Tyr	Asn	Pro	Met	Met	Glu	Asp	Gly	Ile	Ser	Tyr	Thr	Thr	Leu	
				755					760					765	
Arg	Phe	Pro	Glu	Met	Asn	Ile	Pro	Arg	Thr	Gly	Asp	Ala	Glu	Ser	
				770					775					780	
Ser	Glu	Met	Gln	Arg	Pro	Pro	Arg	Thr	Cys	Asp	Asp	Thr	Val	Thr	
				785					790					795	
Tyr	Ser	Ala	Leu	His	Lys	Arg	Gln	Val	Gly	Asp	Tyr	Glu	Asn	Val	
				800					805					810	
Ile	Pro	Asp	Phe	Pro	Glu	Asp	Glu	Gly	Ile	His	Tyr	Ser	Glu	Leu	
				815					820					825	
Ile	Gln	Phe	Gly	Val	Gly	Glu	Arg	Pro	Gln	Ala	Gln	Glu	Asn	Val	
				830					835					840	
Asp	Tyr	Val	Ile	Leu	Lys	His									
				845											

<210> 28
 <211> 226
 <212> PRT
 <213> Homo sapien

<400> 28

Met	Pro	Gly	Gly	Pro	Gly	Val	Leu	Gln	Ala	Leu	Pro	Ala	Thr	Ile	
1				5					10					15	
Phe	Leu	Leu	Phe	Leu	Leu	Ser	Ala	Val	Tyr	Leu	Gly	Pro	Gly	Cys	
				20					25					30	
Gln	Ala	Leu	Trp	Met	His	Lys	Val	Pro	Ala	Ser	Leu	Met	Val	Ser	
				35					40					45	
Leu	Gly	Glu	Asp	Ala	His	Phe	Gln	Cys	Pro	His	Asn	Ser	Ser	Asn	
				50					55					60	
Asn	Ala	Asn	Val	Thr	Trp	Trp	Arg	Val	Leu	His	Gly	Asn	Tyr	Thr	
				65					70					75	
Trp	Pro	Pro	Glu	Phe	Leu	Gly	Pro	Gly	Glu	Asp	Pro	Asn	Gly	Thr	
				80					85					90	
Leu	Ile	Ile	Gln	Asn	Val	Asn	Lys	Ser	His	Gly	Gly	Ile	Tyr	Val	
				95					100					105	
Cys	Arg	Val	Gln	Glu	Gly	Asn	Glu	Ser	Tyr	Gln	Gln	Ser	Cys	Gly	
				110					115					120	
Thr	Tyr	Leu	Arg	Val	Arg	Gln	Pro	Pro	Pro	Arg	Pro	Phe	Leu	Asp	
				125					130					135	

Met	Gly	Glu	Gly	Thr	Lys	Asn	Arg	Ile	Ile	Thr	Ala	Glu	Gly	Ile
				140					145					150
Ile	Leu	Leu	Phe	Cys	Ala	Val	Val	Pro	Gly	Thr	Leu	Leu	Leu	Phe
				155					160					165
Arg	Lys	Arg	Trp	Gln	Asn	Glu	Lys	Leu	Gly	Leu	Asp	Ala	Gly	Asp
				170					175					180
Glu	Tyr	Glu	Asp	Glu	Asn	Leu	Tyr	Glu	Gly	Leu	Asn	Leu	Asp	Asp
				185					190					195
Cys	Ser	Met	Tyr	Glu	Asp	Ile	Ser	Arg	Gly	Leu	Gln	Gly	Thr	Tyr
				200					205					210
Gln	Asp	Val	Gly	Ser	Leu	Asn	Ile	Gly	Asp	Val	Gln	Leu	Glu	Lys
				215					220					225

Pro

<210> 29
 <211> 372
 <212> PRT
 <213> Homo sapien

<400> 29

Met	Asn	Tyr	Pro	Leu	Thr	Leu	Glu	Met	Asp	Leu	Glu	Asn	Leu	Glu
1				5					10					15
Asp	Leu	Phe	Trp	Glu	Leu	Asp	Arg	Leu	Asp	Asn	Tyr	Asn	Asp	Thr
				20					25					30
Ser	Leu	Val	Glu	Asn	His	Leu	Cys	Pro	Ala	Thr	Glu	Gly	Pro	Leu
				35					40					45
Met	Ala	Ser	Phe	Lys	Ala	Val	Phe	Val	Pro	Val	Ala	Tyr	Ser	Leu
				50					55					60
Ile	Phe	Leu	Leu	Gly	Val	Ile	Gly	Asn	Val	Leu	Val	Leu	Val	Ile
				65					70					75
Leu	Glu	Arg	His	Arg	Gln	Thr	Arg	Ser	Ser	Thr	Glu	Thr	Phe	Leu
				80					85					90
Phe	His	Leu	Ala	Val	Ala	Asp	Leu	Leu	Leu	Val	Phe	Ile	Leu	Pro
				95					100					105
Phe	Ala	Val	Ala	Glu	Gly	Ser	Val	Gly	Trp	Val	Leu	Gly	Thr	Phe
				110					115					120
Leu	Cys	Lys	Thr	Val	Ile	Ala	Leu	His	Lys	Val	Asn	Phe	Tyr	Cys
				125					130					135
Ser	Ser	Leu	Leu	Leu	Ala	Cys	Ile	Ala	Val	Asp	Arg	Tyr	Leu	Ala
				140					145					150
Ile	Val	His	Ala	Val	His	Ala	Tyr	Arg	His	Arg	Arg	Leu	Leu	Ser
				155					160					165
Ile	His	Ile	Thr	Cys	Gly	Thr	Ile	Trp	Leu	Val	Gly	Phe	Leu	Leu

	170		175		180
Ala Leu Pro Glu Ile Leu Phe Ala Lys Val Ser Gln Gly His His	185		190		195
Asn Asn Ser Leu Pro Arg Cys Thr Phe Ser Gln Glu Asn Gln Ala	200		205		210
Glu Thr His Ala Trp Phe Thr Ser Arg Phe Leu Tyr His Val Ala	215		220		225
Gly Phe Leu Leu Pro Met Leu Val Met Gly Trp Cys Tyr Val Gly	230		235		240
Val Val His Arg Leu Arg Gln Ala Gln Arg Arg Pro Gln Arg Gln	245		250		255
Lys Ala Val Arg Val Ala Ile Leu Val Thr Ser Ile Phe Phe Leu	260		265		270
Cys Trp Ser Pro Tyr His Ile Val Ile Phe Leu Asp Thr Leu Ala	275		280		285
Arg Leu Lys Ala Val Asp Asn Thr Cys Lys Leu Asn Gly Ser Leu	290		295		300
Pro Val Ala Ile Thr Met Cys Glu Phe Leu Gly Leu Ala His Cys	305		310		315
Cys Leu Asn Pro Met Leu Tyr Thr Phe Ala Gly Val Lys Phe Arg	320		325		330
Ser Asp Leu Ser Arg Leu Leu Thr Lys Leu Gly Cys Thr Gly Pro	335		340		345
Ala Ser Leu Cys Gln Leu Phe Pro Ser Trp Arg Arg Ser Ser Leu	350		355		360
Ser Glu Ser Glu Asn Ala Thr Ser Leu Thr Thr Phe	365		370		

<210> 30
 <211> 273
 <212> PRT
 <213> Homo sapien

<400> 30
Met Gly Ser Gly Trp Val Pro Trp Val Val Ala Leu Leu Val Asn
1 5 10 15
Leu Thr Arg Leu Asp Ser Ser Met Thr Gln Gly Thr Asp Ser Pro
20 25 30
Glu Asp Phe Val Ile Gln Ala Lys Ala Asp Cys Tyr Phe Thr Asn
35 40 45
Gly Thr Glu Lys Val Gln Phe Val Val Arg Phe Ile Phe Asn Leu
50 55 60
Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Met Phe Val Ala
65 70 75

Leu	Thr	Lys	Leu	Gly	Gln	Pro	Asp	Ala	Glu	Gln	Trp	Asn	Ser	Arg	
				80					85					90	
Leu	Asp	Leu	Leu	Glu	Arg	Ser	Arg	Gln	Ala	Val	Asp	Gly	Val	Cys	
				95					100					105	
Arg	His	Asn	Tyr	Arg	Leu	Gly	Ala	Pro	Phe	Thr	Val	Gly	Arg	Lys	
				110					115					120	
Val	Gln	Pro	Glu	Val	Thr	Val	Tyr	Pro	Glu	Arg	Thr	Pro	Leu	Leu	
				125					130					135	
His	Gln	His	Asn	Leu	Leu	His	Cys	Ser	Val	Thr	Gly	Phe	Tyr	Pro	
				140					145					150	
Gly	Asp	Ile	Lys	Ile	Lys	Trp	Phe	Leu	Asn	Gly	Gln	Glu	Glu	Arg	
				155					160					165	
Ala	Gly	Val	Met	Ser	Thr	Gly	Pro	Ile	Arg	Asn	Gly	Asp	Trp	Thr	
				170					175					180	
Phe	Gln	Thr	Val	Val	Met	Leu	Glu	Met	Thr	Pro	Glu	Leu	Gly	His	
				185					190					195	
Val	Tyr	Thr	Cys	Leu	Val	Asp	His	Ser	Ser	Leu	Leu	Ser	Pro	Val	
				200					205					210	
Ser	Val	Glu	Trp	Arg	Ala	Gln	Ser	Glu	Tyr	Ser	Trp	Arg	Lys	Met	
				215					220					225	
Leu	Ser	Gly	Ile	Ala	Ala	Phe	Leu	Leu	Gly	Leu	Ile	Phe	Leu	Leu	
				230					235					240	
Val	Gly	Ile	Val	Ile	Gln	Leu	Arg	Ala	Gln	Lys	Gly	Tyr	Val	Arg	
				245					250					255	
Thr	Gln	Met	Ser	Gly	Asn	Glu	Val	Ser	Arg	Ala	Val	Leu	Leu	Pro	
				260					265					270	

Gln Ser Cys

<210> 31
 <211> 422
 <212> PRT
 <213> Homo sapien

<400> 31
 Met Gly Gln Ala Gly Cys Lys Gly Leu Cys Leu Ser Leu Phe Asp
 1 5 10 15
 Tyr Lys Thr Glu Lys Tyr Val Ile Ala Lys Asn Lys Lys Val Gly
 20 25 30
 Leu Leu Tyr Arg Leu Leu Gln Ala Ser Ile Leu Ala Tyr Leu Val
 35 40 45
 Val Trp Val Phe Leu Ile Lys Lys Gly Tyr Gln Asp Val Asp Thr
 50 55 60

Ser	Leu	Gln	Ser	Ala	Val	Ile	Thr	Lys	Val	Lys	Gly	Val	Ala	Phe	
				65					70					75	
Thr	Asn	Thr	Ser	Asp	Leu	Gly	Gln	Arg	Ile	Trp	Asp	Val	Ala	Asp	
				80					85					90	
Tyr	Val	Ile	Pro	Ala	Gln	Gly	Glu	Asn	Val	Phe	Phe	Val	Val	Thr	
				95					100					105	
Asn	Leu	Ile	Val	Thr	Pro	Asn	Gln	Arg	Gln	Asn	Val	Cys	Ala	Glu	
				110					115					120	
Asn	Glu	Gly	Ile	Pro	Asp	Gly	Ala	Cys	Ser	Lys	Asp	Ser	Asp	Cys	
				125					130					135	
His	Ala	Gly	Glu	Ala	Val	Thr	Ala	Gly	Asn	Gly	Val	Lys	Thr	Gly	
				140					145					150	
Arg	Cys	Leu	Arg	Arg	Glu	Asn	Leu	Ala	Arg	Gly	Thr	Cys	Glu	Ile	
				155					160					165	
Phe	Ala	Trp	Cys	Pro	Leu	Glu	Thr	Ser	Ser	Arg	Pro	Glu	Glu	Pro	
				170					175					180	
Phe	Leu	Lys	Glu	Ala	Glu	Asp	Phe	Thr	Ile	Phe	Ile	Lys	Asn	His	
				185					190					195	
Ile	Arg	Phe	Pro	Lys	Phe	Asn	Phe	Ser	Lys	Ser	Asn	Val	Met	Asp	
				200					205					210	
Val	Lys	Asp	Arg	Ser	Phe	Leu	Lys	Ser	Cys	His	Phe	Gly	Pro	Lys	
				215					220					225	
Asn	His	Tyr	Cys	Pro	Ile	Phe	Arg	Leu	Gly	Ser	Val	Ile	Arg	Trp	
				230					235					240	
Ala	Gly	Ser	Asp	Phe	Gln	Asp	Ile	Ala	Leu	Glu	Gly	Gly	Val	Ile	
				245					250					255	
Gly	Ile	Asn	Ile	Glu	Trp	Asn	Cys	Asp	Leu	Asp	Lys	Ala	Ala	Ser	
				260					265					270	
Glu	Cys	His	Pro	His	Tyr	Ser	Phe	Ser	Arg	Leu	Asp	Asn	Lys	Leu	
				275					280					285	
Ser	Lys	Ser	Val	Ser	Ser	Gly	Tyr	Asn	Phe	Arg	Phe	Ala	Arg	Tyr	
				290					295					300	
Tyr	Arg	Asp	Ala	Ala	Gly	Val	Glu	Phe	Arg	Thr	Leu	Met	Lys	Ala	
				305					310					315	
Tyr	Gly	Ile	Arg	Phe	Asp	Val	Met	Val	Asn	Gly	Lys	Gly	Ala	Phe	
				320					325					330	
Phe	Cys	Asp	Leu	Val	Leu	Ile	Tyr	Leu	Ile	Lys	Lys	Arg	Glu	Phe	
				335					340					345	
Tyr	Arg	Asp	Lys	Lys	Tyr	Glu	Glu	Val	Arg	Gly	Leu	Glu	Asp	Ser	
				350					355					360	
Ser	Gln	Glu	Ala	Glu	Asp	Glu	Ala	Ser	Gly	Leu	Gly	Leu	Ser	Glu	

365	370	375
Gln Leu Thr Ser Gly Pro Gly Leu Leu Gly Met Pro Glu Gln Gln		
380	385	390
Glu Leu Gln Glu Pro Pro Glu Ala Lys Arg Gly Ser Ser Ser Gln		
395	400	405
Lys Gly Asn Gly Ser Val Cys Pro Gln Leu Leu Glu Pro His Arg		
410	415	420
Ser Thr		
<210> 32		
<211> 359		
<212> PRT		
<213> Homo sapien		
<400> 32		
Met Ala Glu Ala Ile Thr Tyr Ala Asp Leu Arg Phe Val Lys Ala		
1	5	10
13		
Pro Leu Lys Lys Ser Ile Ser Ser Arg Leu Gly Gln Asp Pro Gly		
20	25	30
Ala Asp Asp Asp Gly Glu Ile Thr Tyr Glu Asn Val Gln Val Pro		
35	40	45
Ala Val Leu Gly Val Pro Ser Ser Leu Ala Ser Ser Val Leu Gly		
50	55	60
Asp Lys Ala Ala Val Lys Ser Glu Gln Pro Thr Ala Ser Trp Arg		
65	70	75
Ala Val Thr Ser Pro Ala Val Gly Arg Ile Leu Pro Cys Arg Thr		
80	85	90
Thr Cys Leu Arg Tyr Leu Leu Leu Gly Leu Leu Leu Thr Cys Leu		
95	100	105
Leu Leu Gly Val Thr Ala Ile Cys Leu Gly Val Arg Tyr Leu Gln		
110	115	120
Val Ser Gln Gln Leu Gln Gln Thr Asn Arg Val Leu Glu Val Thr		
125	130	135
Asn Ser Ser Leu Arg Gln Gln Leu Arg Leu Lys Ile Thr Gln Leu		
140	145	150
Gly Gln Ser Ala Glu Asp Leu Gln Gly Ser Arg Arg Glu Leu Ala		
155	160	165
Gln Ser Gln Glu Ala Leu Gln Val Glu Gln Arg Ala His Gln Ala		
170	175	180
Ala Glu Gly Gln Leu Gln Ala Cys Gln Ala Asp Arg Gln Lys Thr		
185	190	195
Lys Glu Thr Leu Gln Ser Glu Glu Gln Gln Arg Arg Ala Leu Glu		
200	205	210

Gln	Lys	Leu	Ser	Asn	Met	Glu	Asn	Arg	Leu	Lys	Pro	Phe	Phe	Thr	215	220	225
Cys	Gly	Ser	Ala	Asp	Thr	Cys	Cys	Pro	Ser	Gly	Trp	Ile	Met	His	230	235	240
Gln	Lys	Ser	Cys	Phe	Tyr	Ile	Ser	Leu	Thr	Ser	Lys	Asn	Trp	Gln	245	250	255
Glu	Ser	Gln	Lys	Gln	Cys	Glu	Thr	Leu	Ser	Ser	Lys	Leu	Ala	Thr	260	265	270
Phe	Ser	Glu	Ile	Tyr	Pro	Gln	Ser	His	Ser	Tyr	Tyr	Phe	Leu	Asn	275	280	285
Ser	Leu	Leu	Pro	Asn	Gly	Gly	Ser	Gly	Asn	Ser	Tyr	Trp	Thr	Gly	290	295	300
Leu	Ser	Ser	Asn	Lys	Asp	Trp	Lys	Leu	Thr	Asp	Asp	Thr	Gln	Arg	305	310	315
Thr	Arg	Thr	Tyr	Ala	Gln	Ser	Ser	Lys	Cys	Asn	Lys	Val	His	Lys	320	325	330
Thr	Trp	Ser	Trp	Trp	Thr	Leu	Glu	Ser	Glu	Ser	Cys	Arg	Ser	Ser	335	340	345
Leu	Pro	Tyr	Ile	Cys	Glu	Met	Thr	Ala	Phe	Arg	Phe	Pro	Asp		350	355	

<210> 33
 <211> 661'
 <212> PRT
 <213> Homo sapien

<400> 33																		
Met	Ala	Phe	Asp	Val	Ser	Cys	Phe	Phe	Trp	Val	Val	Leu	Phe	Ser	1	5	10	15
Ala	Gly	Cys	Lys	Val	Ile	Thr	Ser	Trp	Asp	Gln	Met	Cys	Ile	Glu	20	25		30
Lys	Glu	Ala	Asn	Lys	Thr	Tyr	Asn	Cys	Glu	Asn	Leu	Gly	Leu	Ser	35	40		45
Glu	Ile	Pro	Asp	Thr	Leu	Pro	Asn	Thr	Thr	Glu	Phe	Leu	Glu	Phe	50	55		60
Ser	Phe	Asn	Phe	Leu	Pro	Thr	Ile	His	Asn	Arg	Thr	Phe	Ser	Arg	65	70		75
Leu	Met	Asn	Leu	Thr	Phe	Leu	Asp	Leu	Thr	Arg	Cys	Gln	Ile	Asn	80	85		90
Trp	Ile	His	Glu	Asp	Thr	Phe	Gln	Ser	His	His	Gln	Leu	Ser	Thr	95	100		105
Leu	Val	Leu	Thr	Gly	Asn	Pro	Leu	Ile	Phe	Met	Ala	Glu	Thr	Ser	110	115		120

Leu Asn Gly Pro	Lys Ser Leu Lys His	Leu Phe Leu Ile Gln Thr	125	130	135
Gly Ile Ser Asn	Leu Glu Phe Ile Pro	Val His Asn Leu Glu Asn	140	145	150
Leu Glu Ser Leu	Tyr Leu Gly Ser Asn	His Ile Ser Ser Ile Lys	155	160	165
Phe Pro Lys Asp	Phe Pro Ala Arg Asn	Leu Lys Val Leu Asp Phe	170	175	180
Gln Asn Asn Ala	Ile His Tyr Ile Ser	Arg Glu Asp Met Arg Ser	185	190	195
Leu Glu Gln Ala	Ile Asn Leu Ser Leu	Asn Phe Asn Gly Asn Asn	200	205	210
Val Lys Gly Ile	Glu Leu Gly Ala Phe	Asp Ser Thr Val Phe Gln	215	220	225
Ser Leu Asn Phe	Gly Gly Thr Pro Asn	Leu Ser Val Ile Phe Asn	230	235	240
Gly Leu Gln Asn	Ser Thr Thr Gln Ser	Leu Trp Leu Gly Thr Phe	245	250	255
Glu Asp Ile Asp	Asp Glu Asp Ile Ser	Ser Ala Met Leu Lys Gly	260	265	270
Leu Cys Glu Met	Ser Val Glu Ser Leu	Asn Leu Gln Glu His Arg	275	280	285
Phe Ser Asp Ile	Ser Ser Thr Thr Phe	Gln Cys Phe Thr Gln Leu	290	295	300
Gln Glu Leu Asp	Leu Thr Ala Thr His	Leu Lys Gly Leu Pro Ser	305	310	315
Gly Met Lys Gly	Leu Asn Leu Leu Lys	Lys Leu Val Leu Ser Val	320	325	330
Asn His Phe Asp	Gln Leu Cys Gln Ile	Ser Ala Ala Asn Phe Pro	335	340	345
Ser Leu Thr His	Leu Tyr Ile Arg Gly	Asn Val Lys Lys Leu His	350	355	360
Leu Gly Val Gly	Cys Leu Glu Lys Leu	Gly Asn Leu Gln Thr Leu	365	370	375
Asp Leu Ser His	Asn Asp Ile Glu Ala	Ser Asp Cys Cys Ser Leu	380	385	390
Gln Leu Lys Asn	Leu Ser His Leu Gln	Thr Leu Asn Leu Ser His	395	400	405
Asn Glu Pro Leu	Gly Leu Gln Ser Gln	Ala Phe Lys Glu Cys Pro	410	415	420
Gln Leu Glu Leu	Leu Asp Leu Ala Phe	Thr Arg Leu His Ile Asn			

425										430					435				
Ala	Pro	Gln	Ser	Pro	Phe	Gln	Asn	Leu	His	Phe	Leu	Gln	Val	Leu					
				440					445					450					
Asn	Leu	Thr	Tyr	Cys	Phe	Leu	Asp	Thr	Ser	Asn	Gln	His	Leu	Leu					
				455					460					465					
Ala	Gly	Leu	Pro	Val	Leu	Arg	His	Leu	Asn	Leu	Lys	Gly	Asn	His					
				470					475					480					
Phe	Gln	Asp	Gly	Thr	Ile	Thr	Lys	Thr	Asn	Leu	Leu	Gln	Thr	Val					
				485					490					495					
Gly	Ser	Leu	Glu	Val	Leu	Ile	Leu	Ser	Ser	Cys	Gly	Leu	Leu	Ser					
				500					505					510					
Ile	Asp	Gln	Gln	Ala	Phe	His	Ser	Leu	Gly	Lys	Met	Ser	His	Val					
				515					520					525					
Asp	Leu	Ser	His	Asn	Ser	Leu	Thr	Cys	Asp	Ser	Ile	Asp	Ser	Leu					
				530					535					540					
Ser	His	Leu	Lys	Gly	Ile	Tyr	Leu	Asn	Leu	Ala	Ala	Asn	Ser	Ile					
				545					550					555					
Asn	Ile	Ile	Ser	Pro	Arg	Leu	Leu	Pro	Ile	Leu	Ser	Gln	Gln	Ser					
				560					565					570					
Thr	Ile	Asn	Leu	Ser	His	Asn	Pro	Leu	Asp	Cys	Thr	Cys	Ser	Asn					
				575					580					585					
Ile	His	Phe	Leu	Thr	Trp	Tyr	Lys	Glu	Asn	Leu	His	Lys	Leu	Glu					
				590					595					600					
Gly	Ser	Glu	Glu	Thr	Thr	Cys	Ala	Asn	Pro	Pro	Ser	Leu	Arg	Gly					
				605					610					615					
Val	Lys	Leu	Ser	Asp	Val	Lys	Leu	Ser	Cys	Gly	Ile	Thr	Ala	Ile					
				620					625					630					
Gly	Ile	Phe	Phe	Leu	Ile	Val	Phe	Leu	Leu	Leu	Leu	Ala	Ile	Leu					
				635					640					645					
Leu	Phe	Phe	Ala	Val	Lys	Tyr	Leu	Leu	Arg	Trp	Lys	Tyr	Gln	His					
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Ile

<210> 34

<211> 429

<212> PRT

<213> Sarcophaga bullata

<400> 34

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Pro	Ala	Glu	Leu	Phe	Leu	Ile	Ala	Ser	Pro	Ser	His	Pro	Thr	Glu
				20					25					30

Gly Ser Pro Val	Thr	Leu	Thr	Cys	Lys	Met	Pro	Phe	Leu	Gln	Ser	
	35					40					45	
Ser Asp Ala Gln	Phe	Gln	Phe	Cys	Phe	Phe	Arg	Asp	Thr	Arg	Ala	
	50					55					60	
Leu Gly Pro Gly	Trp	Ser	Ser	Ser	Pro	Lys	Leu	Gln	Ile	Ala	Ala	
	65					70					75	
Met Trp Lys Glu	Asp	Thr	Gly	Ser	Tyr	Trp	Cys	Glu	Ala	Gln	Thr	
	80					85					90	
Met Ala Ser Lys	Val	Leu	Arg	Ser	Arg	Arg	Ser	Gln	Ile	Asn	Val	
	95					100					105	
His Arg Val Pro	Val	Ala	Asp	Val	Ser	Leu	Glu	Thr	Gln	Pro	Pro	
	110					115					120	
Gly Gly Gln Val	Met	Glu	Gly	Asp	Arg	Leu	Val	Leu	Ile	Cys	Ser	
	125					130					135	
Val Ala Met Gly	Thr	Gly	Asp	Ile	Thr	Phe	Leu	Trp	Tyr	Lys	Gly	
	140					145					150	
Ala Val Gly Leu	Asn	Leu	Gln	Ser	Lys	Thr	Gln	Arg	Ser	Leu	Thr	
	155					160					165	
Ala Glu Tyr Glu	Ile	Pro	Ser	Val	Arg	Glu	Ser	Asp	Ala	Glu	Gln	
	170					175					180	
Tyr Tyr Cys Val	Ala	Glu	Asn	Gly	Tyr	Gly	Pro	Ser	Pro	Ser	Gly	
	185					190					195	
Leu Val Ser Ile	Thr	Val	Arg	Ile	Pro	Val	Ser	Arg	Pro	Ile	Leu	
	200					205					210	
Met Leu Arg Ala	Pro	Arg	Ala	Gln	Ala	Ala	Val	Glu	Asp	Val	Leu	
	215					220					225	
Glu Leu His Cys	Glu	Ala	Leu	Arg	Gly	Ser	Pro	Pro	Ile	Leu	Tyr	
	230					235					240	
Trp Phe Tyr His	Glu	Asp	Ile	Thr	Leu	Gly	Ser	Arg	Ser	Ala	Pro	
	245					250					255	
Ser Gly Gly Gly	Ala	Ser	Phe	Asn	Leu	Ser	Leu	Thr	Glu	Glu	His	
	260					265					270	
Ser Gly Asn Tyr	Ser	Cys	Glu	Ala	Asn	Asn	Gly	Leu	Gly	Ala	Gln	
	275					280					285	
Arg Ser Glu Ala	Val	Thr	Leu	Asn	Phe	Thr	Val	Pro	Thr	Gly	Ala	
	290					295					300	
Arg Ser Asn His	Leu	Thr	Ser	Gly	Val	Ile	Glu	Gly	Leu	Leu	Ser	
	305					310					315	
Thr Leu Gly Pro	Ala	Thr	Val	Ala	Leu	Leu	Phe	Cys	Tyr	Gly	Leu	
	320					325					330	

Lys Arg Lys Ile Gly Arg Arg Ser Ala Arg Asp Pro Leu Arg Ser
325 340 345

Leu Pro Ser Pro Leu Pro Gln Glu Phe Thr Tyr Leu Asn Ser Pro
350 355 360

Thr Pro Gly Gln Leu Gln Pro Ile Tyr Glu Asn Val Asn Val Val
365 370 375

Ser Gly Asp Glu Val Tyr Ser Leu Ala Tyr Tyr Asn Gln Pro Glu
380 385 390

Gln Glu Ser Val Ala Ala Glu Thr Leu Gly Thr His Met Glu Asp
395 400 405

Lys Val Ser Leu Asp Ile Tyr Ser Arg Leu Arg Lys Ala Asn Ile
410 415 420

Thr Asp Val Asp Tyr Glu Asp Ala Met
425

<210> 35

<211> 977

<212> PRT

<213> Homo sapien

<400> 35

Met Leu Leu Trp Val Ile Leu Leu Val Leu Ala Pro Val Ser Gly
1 5 10 15

Gln Phe Ala Arg Thr Pro Arg Pro Ile Ile Phe Leu Gln Pro Pro
20 25 30

Trp Thr Thr Val Phe Gln Gly Glu Arg Val Thr Leu Thr Cys Lys
35 40 45

Gly Phe Arg Phe Tyr Ser Pro Gln Lys Thr Lys Trp Tyr His Arg
50 55 60

Tyr Leu Gly Lys Glu Ile Leu Arg Glu Thr Pro Asp Asn Ile Leu
65 70 75

Glu Val Gln Glu Ser Gly Glu Tyr Arg Cys Gln Ala Gln Gly Ser
80 85 90

Pro Leu Ser Ser Pro Val His Leu Asp Phe Ser Ser Ala Ser Leu
95 100 105

Ile Leu Gln Ala Pro Leu Ser Val Phe Glu Gly Asp Ser Val Val
110 115 120

Leu Arg Cys Arg Ala Lys Ala Glu Val Thr Leu Asn Asn Thr Ile
125 130 135

Tyr Lys Asn Asp Asn Val Leu Ala Phe Leu Asn Lys Arg Thr Asp
140 145 150

Phe His Ile Pro His Ala Cys Leu Lys Asp Asn Gly Ala Tyr Arg
155 160 165

Cys Thr Gly Tyr Lys Glu Ser Cys Cys Pro Val Ser Ser Asn Thr

170										175					180				
Val	Lys	Ile	Gln	Val	Gln	Glu	Pro	Phe	Thr	Arg	Pro	Val	Leu	Arg					
				185					190					195					
Ala	Ser	Ser	Phe	Gln	Pro	Ile	Ser	Gly	Asn	Pro	Val	Thr	Leu	Thr					
				200					205					210					
Cys	Glu	Thr	Gln	Leu	Ser	Leu	Glu	Arg	Ser	Asp	Val	Pro	Leu	Arg					
				215					220					225					
Phe	Arg	Phe	Phe	Arg	Asp	Asp	Gln	Thr	Leu	Gly	Leu	Gly	Trp	Ser					
				230					235					240					
Leu	Ser	Pro	Asn	Phe	Gln	Ile	Thr	Ala	Met	Trp	Ser	Lys	Asp	Ser					
				245					250					255					
Gly	Phe	Tyr	Trp	Cys	Lys	Ala	Ala	Thr	Met	Pro	His	Ser	Val	Ile					
				260					265					270					
Ser	Asp	Ser	Pro	Arg	Ser	Trp	Ile	Gln	Val	Gln	Ile	Pro	Ala	Ser					
				275					280					285					
His	Pro	Val	Leu	Thr	Leu	Ser	Pro	Glu	Lys	Ala	Leu	Asn	Phe	Glu					
				290					295					300					
Gly	Thr	Lys	Val	Thr	Leu	His	Cys	Glu	Thr	Gln	Glu	Asp	Ser	Leu					
				305					310					315					
Arg	Thr	Leu	Tyr	Arg	Phe	Tyr	His	Glu	Gly	Val	Pro	Leu	Arg	His					
				320					325					330					
Lys	Ser	Val	Arg	Cys	Glu	Arg	Gly	Ala	Ser	Ile	Ser	Phe	Ser	Leu					
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Thr	Thr	Glu	Asn	Ser	Gly	Asn	Tyr	Tyr	Cys	Thr	Ala	Asp	Asn	Gly					
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Leu	Gly	Ala	Lys	Pro	Ser	Lys	Ala	Val	Ser	Leu	Ser	Val	Thr	Val					
				365					370					375					
Pro	Val	Ser	His	Pro	Val	Leu	Asn	Leu	Ser	Ser	Pro	Glu	Asp	Leu					
				380					385					390					
Ile	Phe	Glu	Gly	Ala	Lys	Val	Thr	Leu	His	Cys	Glu	Ala	Gln	Arg					
				395					400					405					
Gly	Ser	Leu	Pro	Ile	Leu	Tyr	Gln	Phe	His	His	Glu	Asp	Ala	Ala					
				410					415					420					
Leu	Glu	Arg	Arg	Ser	Ala	Asn	Ser	Ala	Gly	Gly	Val	Ala	Ile	Ser					
				425					430					435					
Phe	Ser	Leu	Thr	Ala	Glu	His	Ser	Gly	Asn	Tyr	Tyr	Cys	Thr	Ala					
				440					445					450					
Asp	Asn	Gly	Phe	Gly	Pro	Gln	Arg	Ser	Lys	Ala	Val	Ser	Leu	Ser					
				455					460					465					
Ile	Thr	Val	Pro	Val	Ser	His	Pro	Val	Leu	Thr	Leu	Ser	Ser	Ala					
				470					475					480					

Glu	Ala	Leu	Thr	Phe	Glu	Gly	Ala	Thr	Val	Thr	Leu	His	Cys	Glu	485	490	495
Val	Gln	Arg	Gly	Ser	Pro	Gln	Ile	Leu	Tyr	Gln	Phe	Tyr	His	Glu	500	505	510
Asp	Met	Pro	Leu	Trp	Ser	Ser	Ser	Thr	Pro	Ser	Val	Gly	Arg	Val	515	520	525
Ser	Phe	Ser	Phe	Ser	Leu	Thr	Glu	Gly	His	Ser	Gly	Asn	Tyr	Tyr	530	535	540
Cys	Thr	Ala	Asp	Asn	Gly	Phe	Gly	Pro	Gln	Arg	Ser	Glu	Val	Val	545	550	555
Ser	Leu	Phe	Val	Thr	Val	Pro	Val	Ser	Arg	Pro	Ile	Leu	Thr	Leu	560	565	570
Arg	Val	Pro	Arg	Ala	Gln	Ala	Val	Val	Gly	Asp	Leu	Leu	Glu	Leu	575	580	585
His	Cys	Glu	Ala	Pro	Arg	Gly	Ser	Pro	Pro	Ile	Leu	Tyr	Trp	Phe	590	595	600
Tyr	His	Glu	Asp	Val	Thr	Leu	Gly	Ser	Ser	Ser	Ala	Pro	Ser	Gly	605	610	615
Gly	Glu	Ala	Ser	Phe	Asn	Leu	Ser	Leu	Thr	Ala	Glu	His	Ser	Gly	620	625	630
Asn	Tyr	Ser	Cys	Glu	Ala	Asn	Asn	Gly	Leu	Val	Ala	Gln	His	Ser	635	640	645
Asp	Thr	Ile	Ser	Leu	Ser	Val	Ile	Val	Pro	Val	Ser	Arg	Pro	Ile	650	655	660
Leu	Thr	Phe	Arg	Ala	Pro	Arg	Ala	Gln	Ala	Val	Val	Gly	Asp	Leu	665	670	675
Leu	Glu	Leu	His	Cys	Glu	Ala	Leu	Arg	Gly	Ser	Ser	Pro	Ile	Leu	680	685	690
Tyr	Trp	Phe	Tyr	His	Glu	Asp	Val	Thr	Leu	Gly	Lys	Ile	Ser	Ala	695	700	705
Pro	Ser	Gly	Gly	Gly	Ala	Ser	Phe	Asn	Leu	Ser	Leu	Thr	Thr	Glu	710	715	720
His	Ser	Gly	Ile	Tyr	Ser	Cys	Glu	Ala	Asp	Asn	Gly	Pro	Glu	Ala	725	730	735
Gln	Arg	Ser	Glu	Met	Val	Thr	Leu	Lys	Val	Ala	Val	Pro	Val	Ser	740	745	750
Arg	Pro	Val	Leu	Thr	Leu	Arg	Ala	Pro	Gly	Thr	His	Ala	Ala	Val	755	760	765
Gly	Asp	Leu	Leu	Glu	Leu	His	Cys	Glu	Ala	Leu	Arg	Gly	Ser	Pro	770	775	780

Leu Ile Leu Tyr Arg Phe Phe His Glu Asp Val Thr Leu Gly Asn	785	790	795
Arg Ser Ser Pro Ser Gly Gly Ala Ser Leu Asn Leu Ser Leu Thr	800	805	810
Ala Glu His Ser Gly Asn Tyr Ser Cys Glu Ala Asp Asn Gly Leu	815	820	825
Gly Ala Gln Arg Ser Glu Thr Val Thr Leu Tyr Ile Thr Gly Leu	830	835	840
Thr Ala Asn Arg Ser Gly Pro Phe Ala Thr Gly Val Ala Gly Gly	845	850	855
Leu Leu Ser Ile Ala Gly Leu Ala Ala Gly Ala Leu Leu Leu Tyr	860	865	870
Cys Trp Leu Ser Arg Lys Ala Gly Arg Lys Pro Ala Ser Asp Pro	875	880	885
Ala Arg Ser Pro Pro Asp Ser Asp Ser Gln Glu Pro Thr Tyr His	890	895	900
Asn Val Pro Ala Trp Glu Glu Leu Gln Pro Val Tyr Thr Asn Ala	905	910	915
Asn Pro Arg Gly Glu Asn Val Val Tyr Ser Glu Val Arg Ile Ile	920	925	930
Gln Glu Lys Lys Lys His Ala Val Ala Ser Asp Pro Arg His Leu	935	940	945
Arg Asn Lys Gly Ser Pro Ile Ile Tyr Ser Glu Val Lys Val Ala	950	955	960
Ser Thr Pro Val Ser Gly Ser Leu Phe Leu Ala Ser Ser Ala Pro	965	970	975
His Arg			

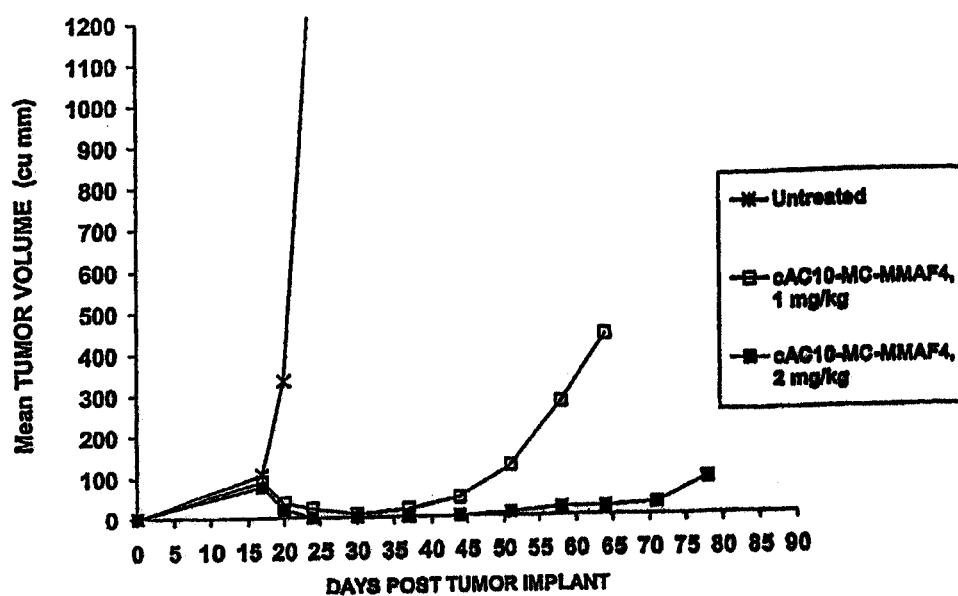


FIGURE 1

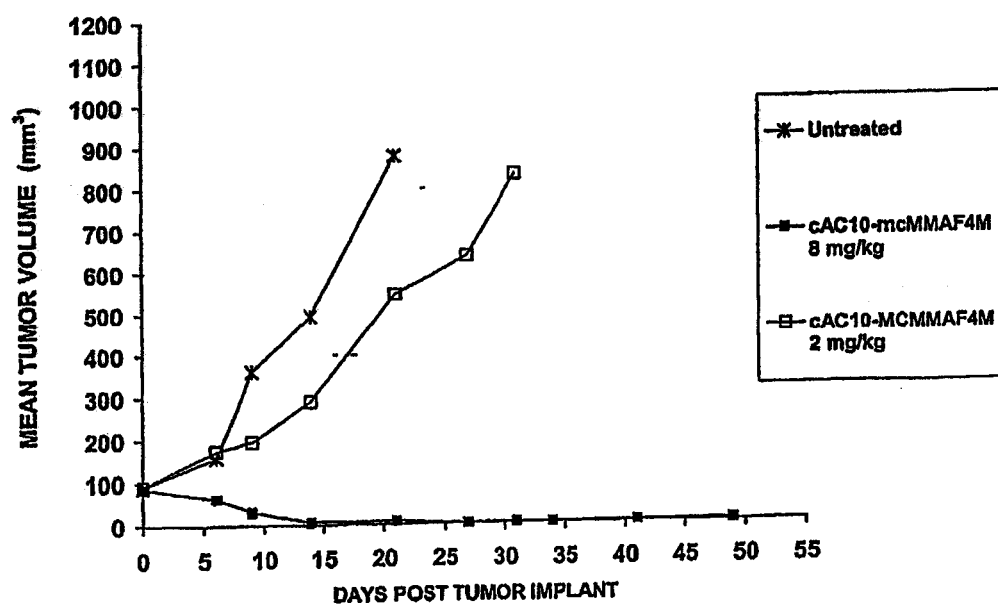


FIGURE 2

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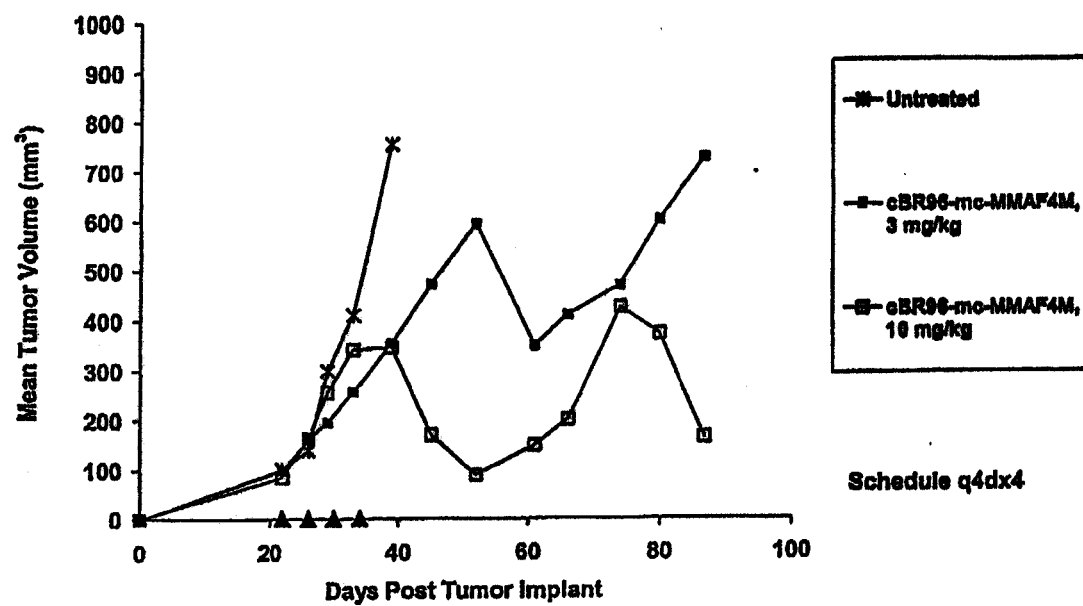


FIGURE 3a

Efficacy of mAb-mc-MMAF in L2987 Lung Carcinoma

L2987-AO1

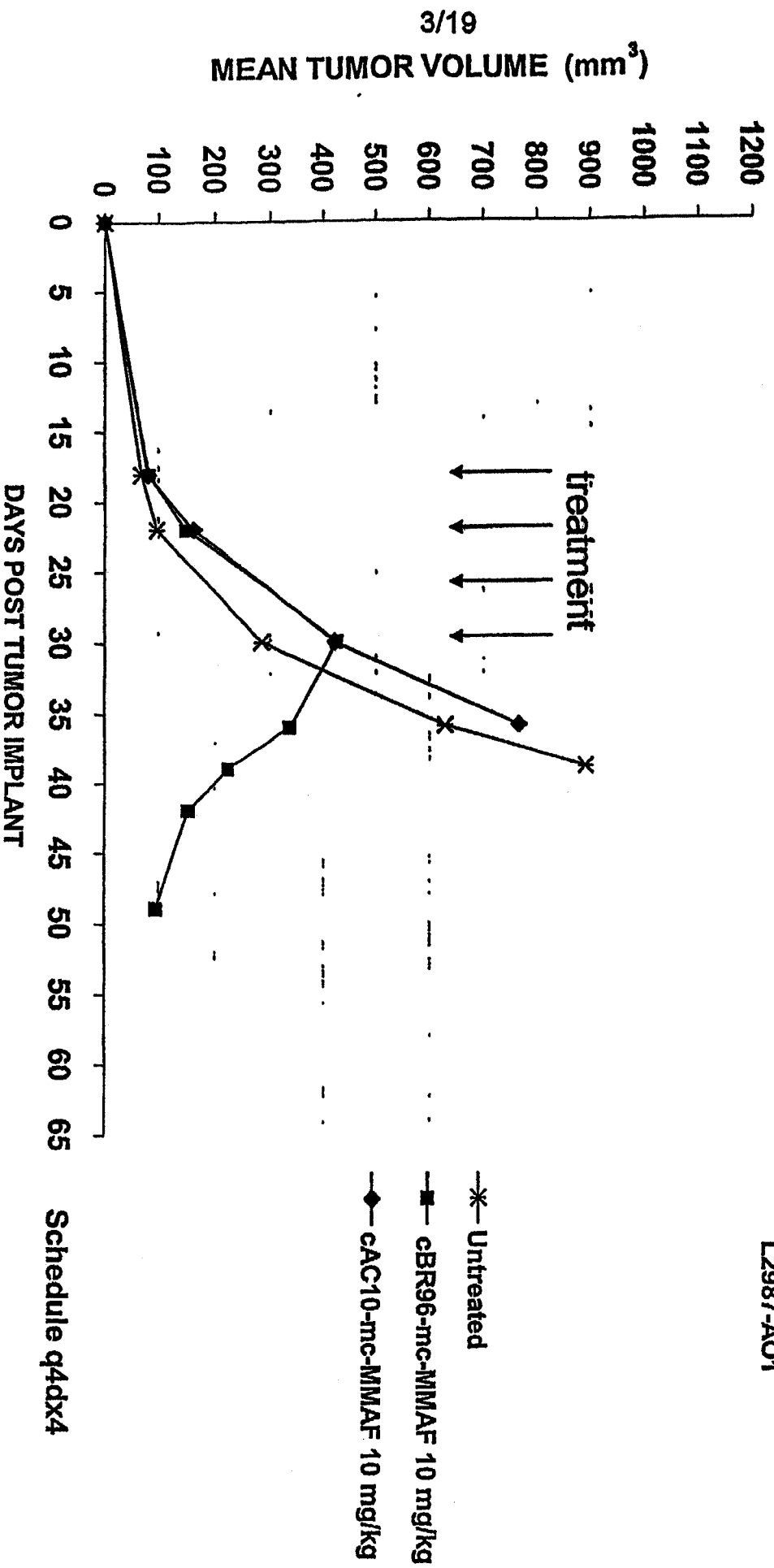


Figure 3b

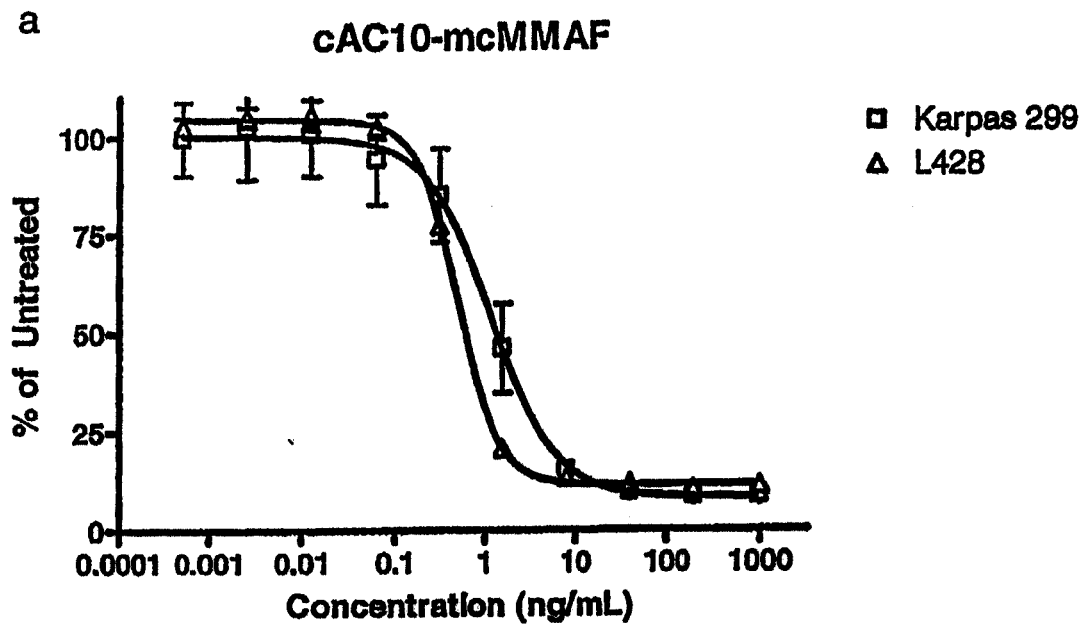


FIGURE 4a

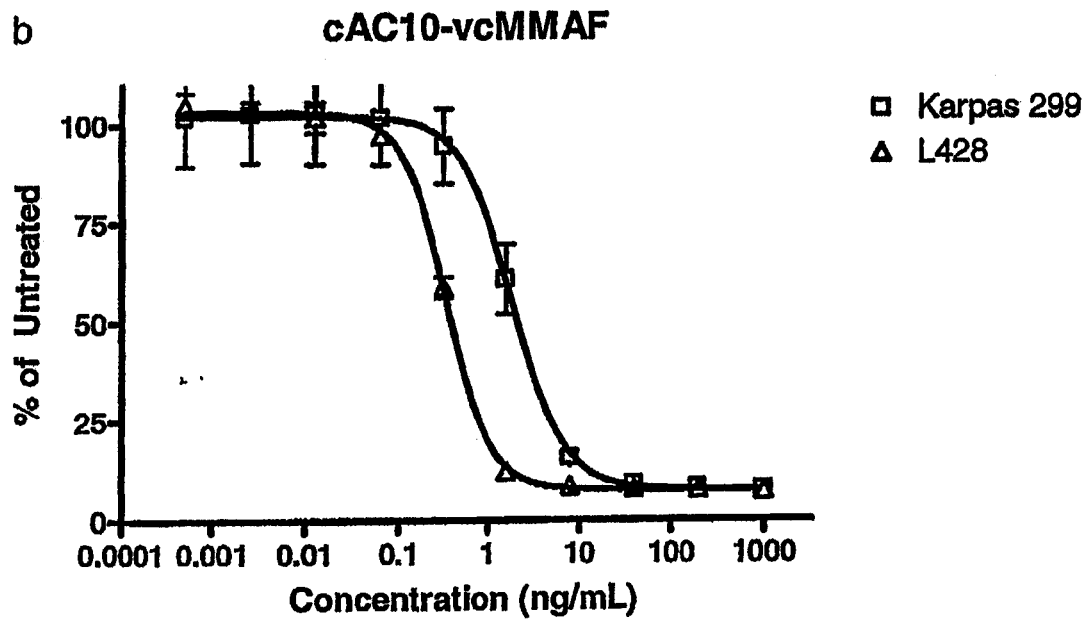


FIGURE 4b

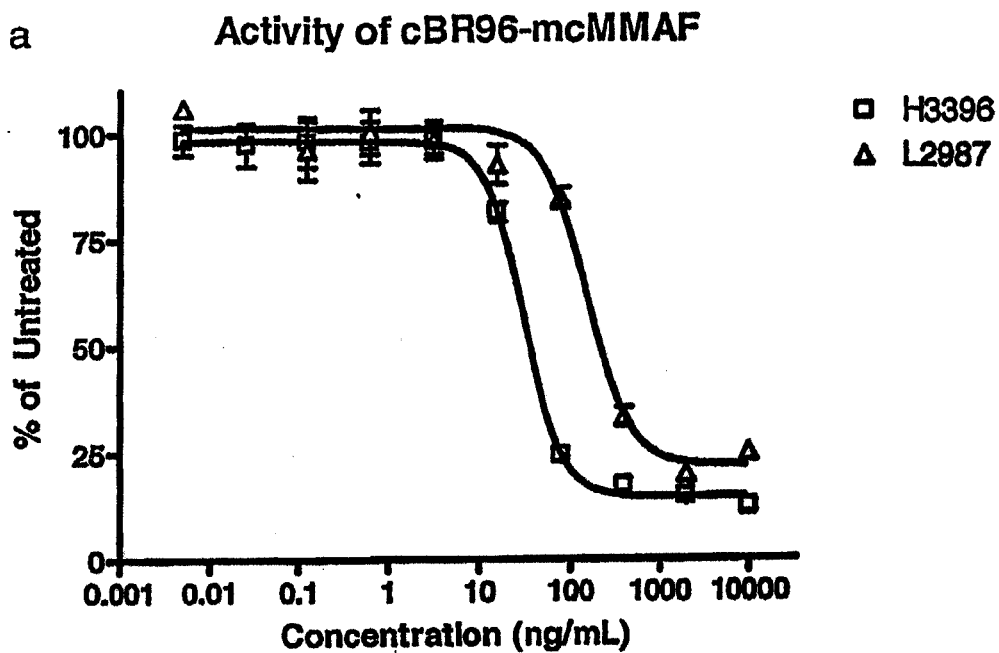


FIGURE 5a

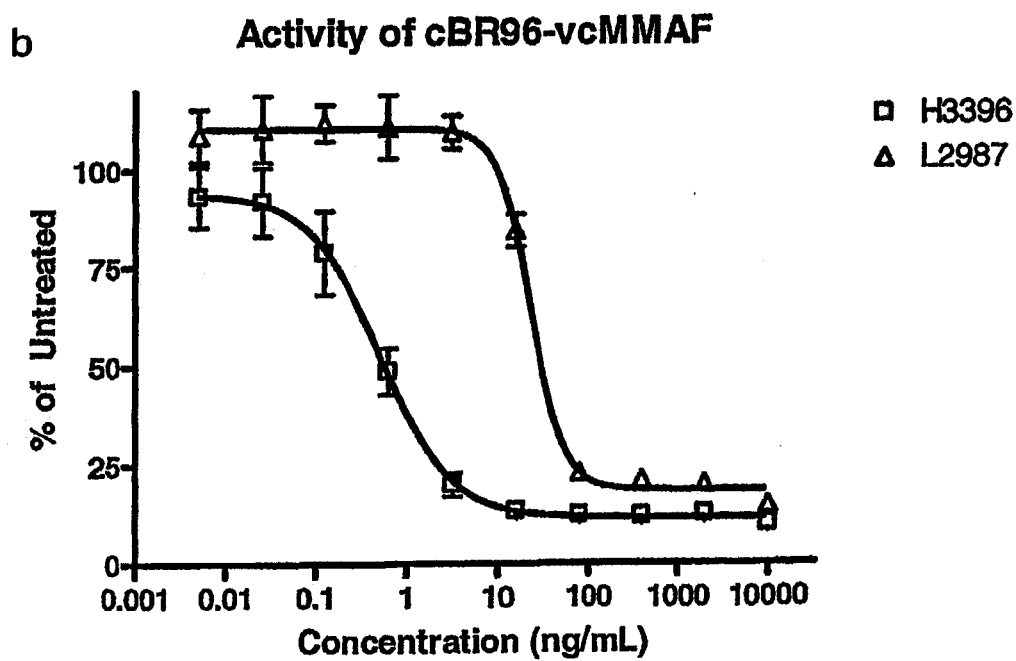


FIGURE 5b

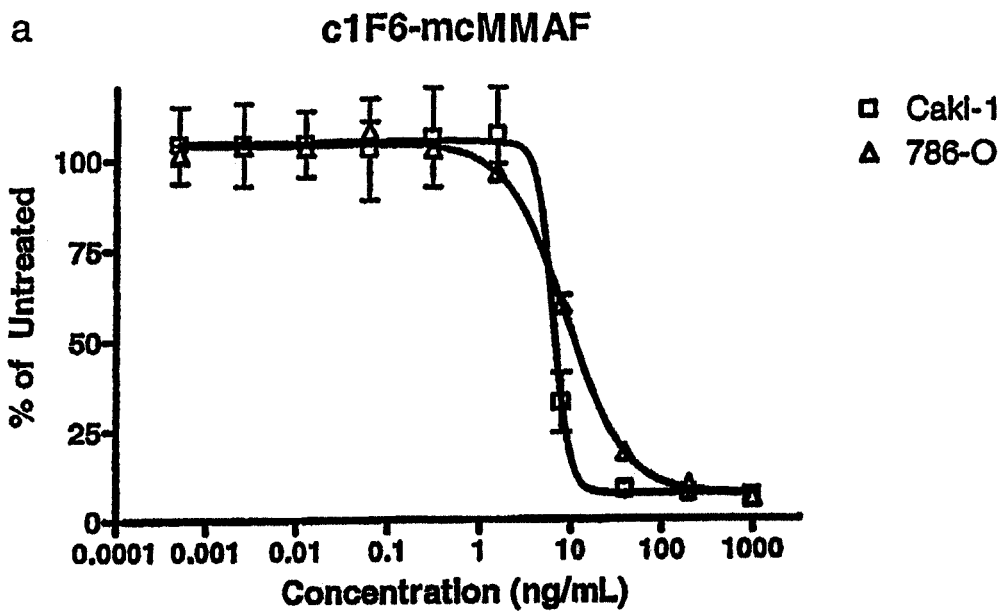


FIGURE 6a

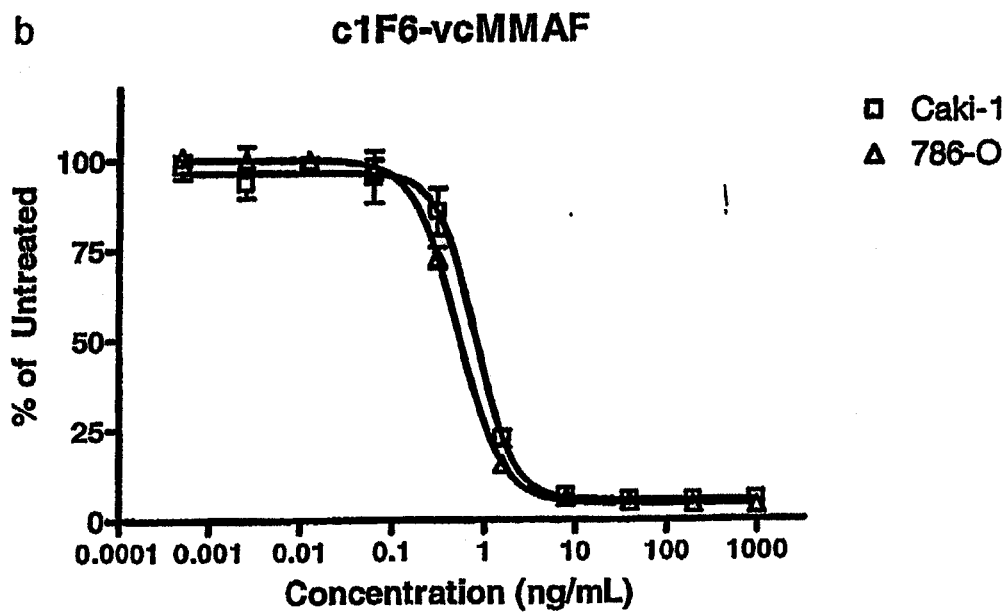


FIGURE 6b

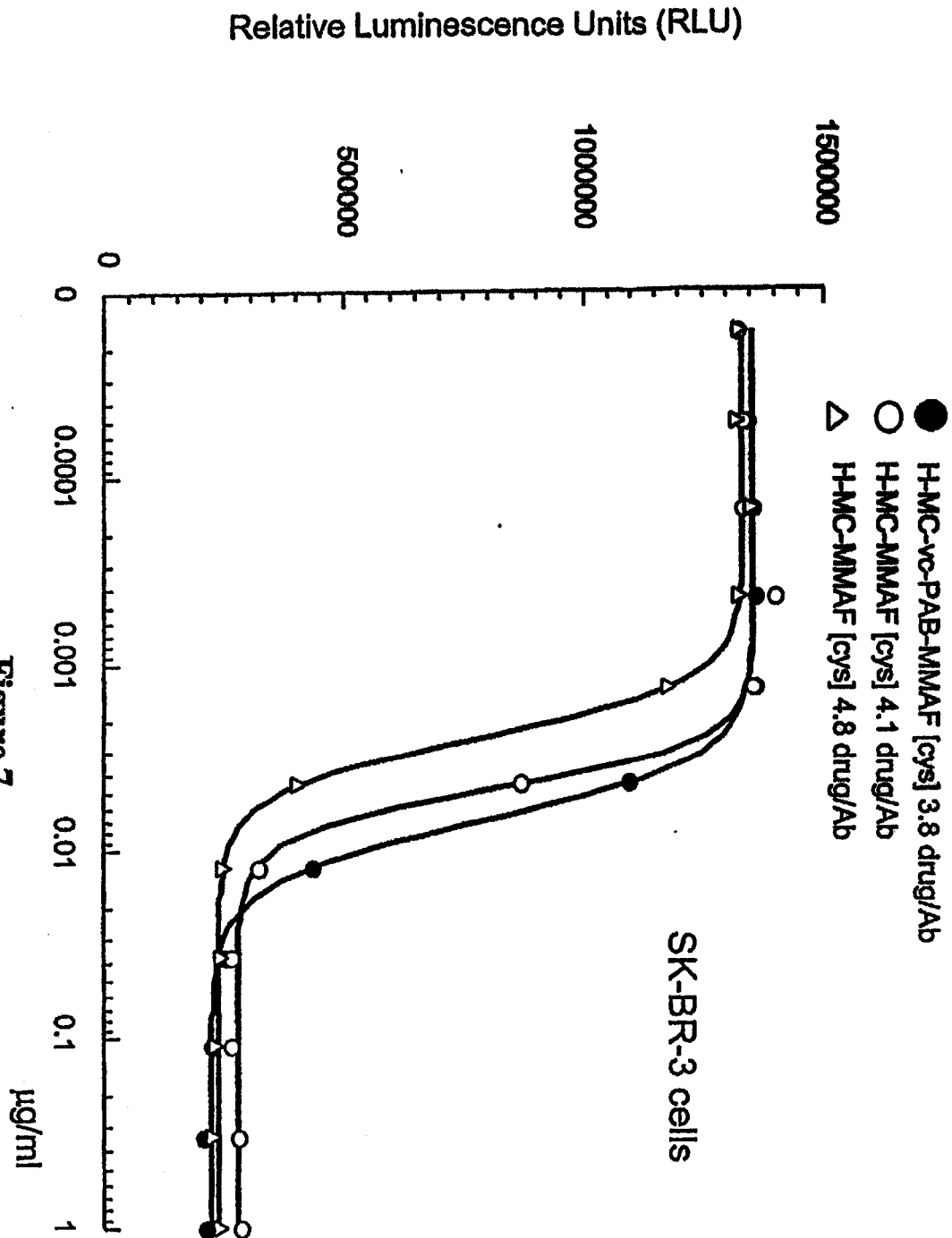
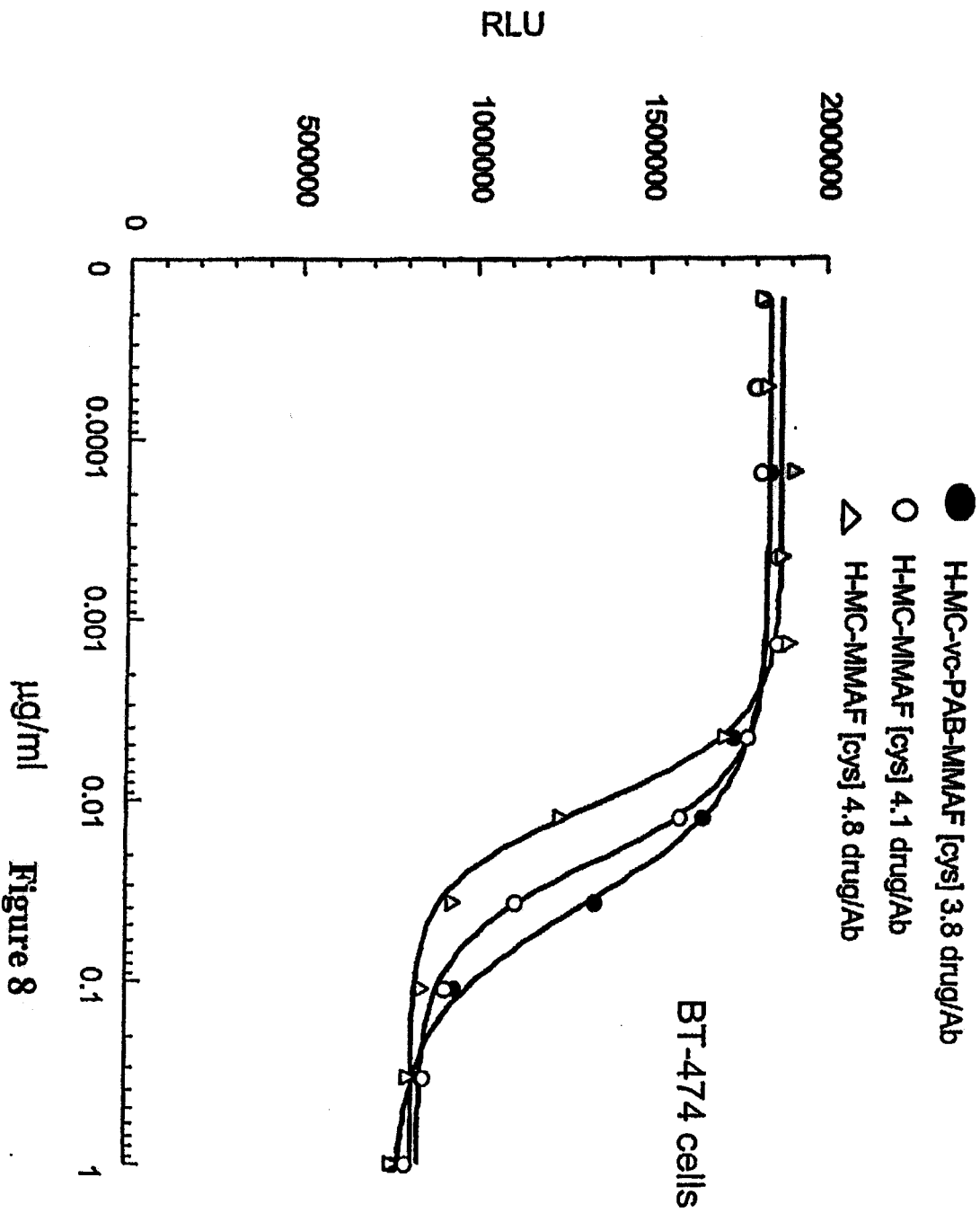


Figure 7



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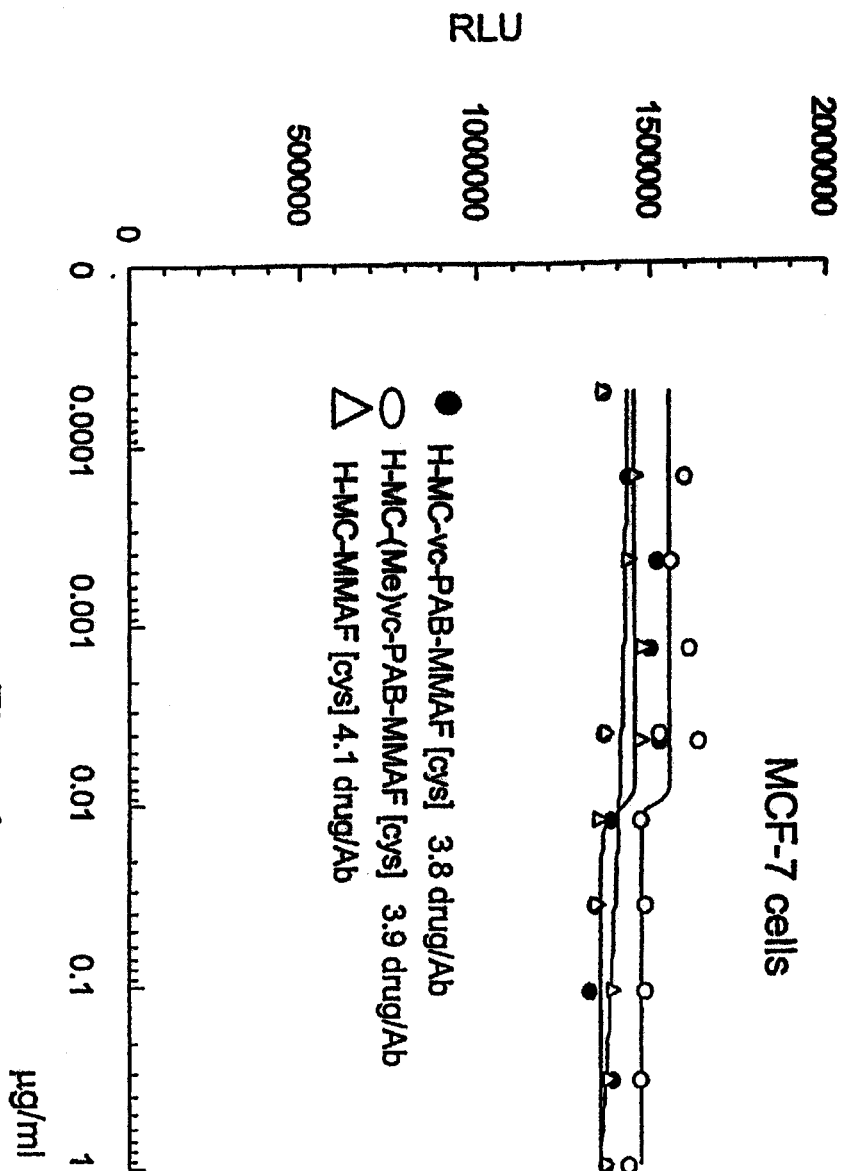


Figure 9 .

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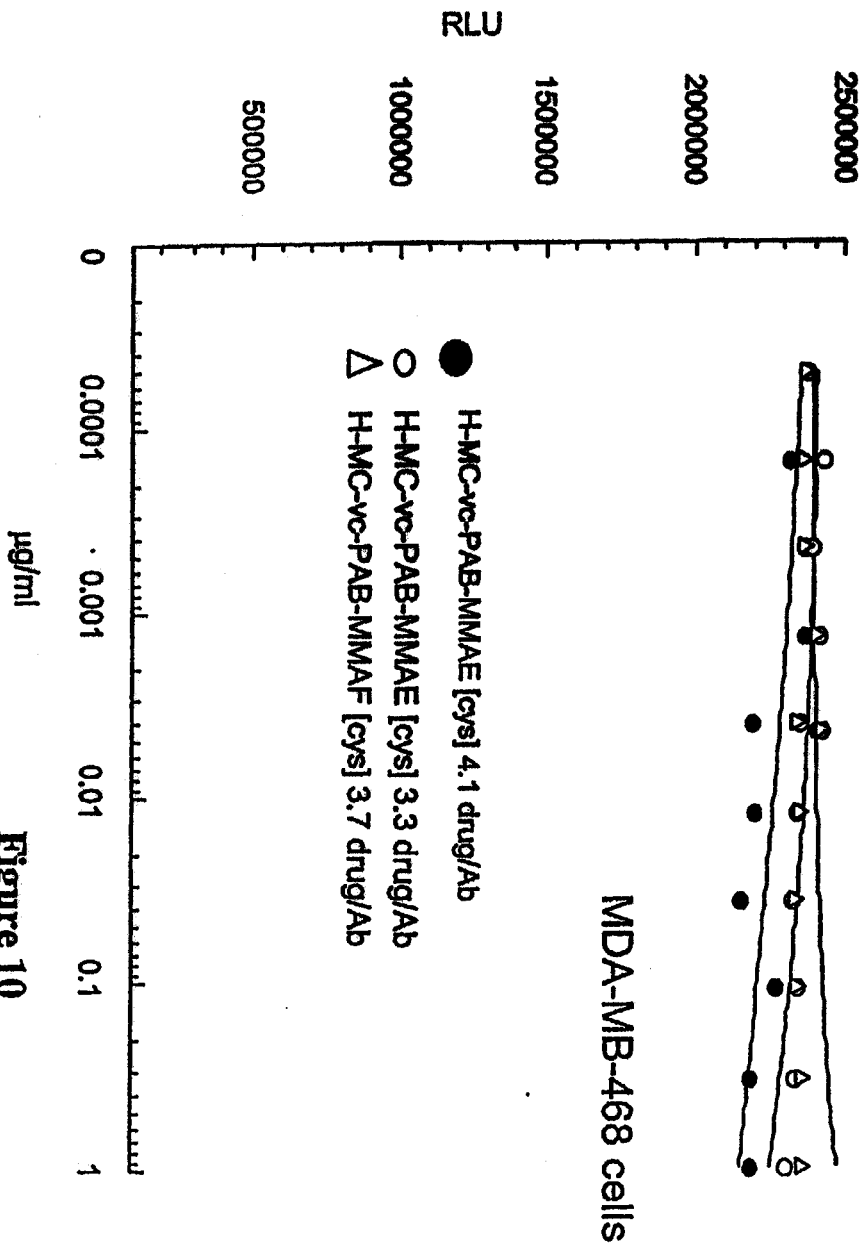


Figure 10

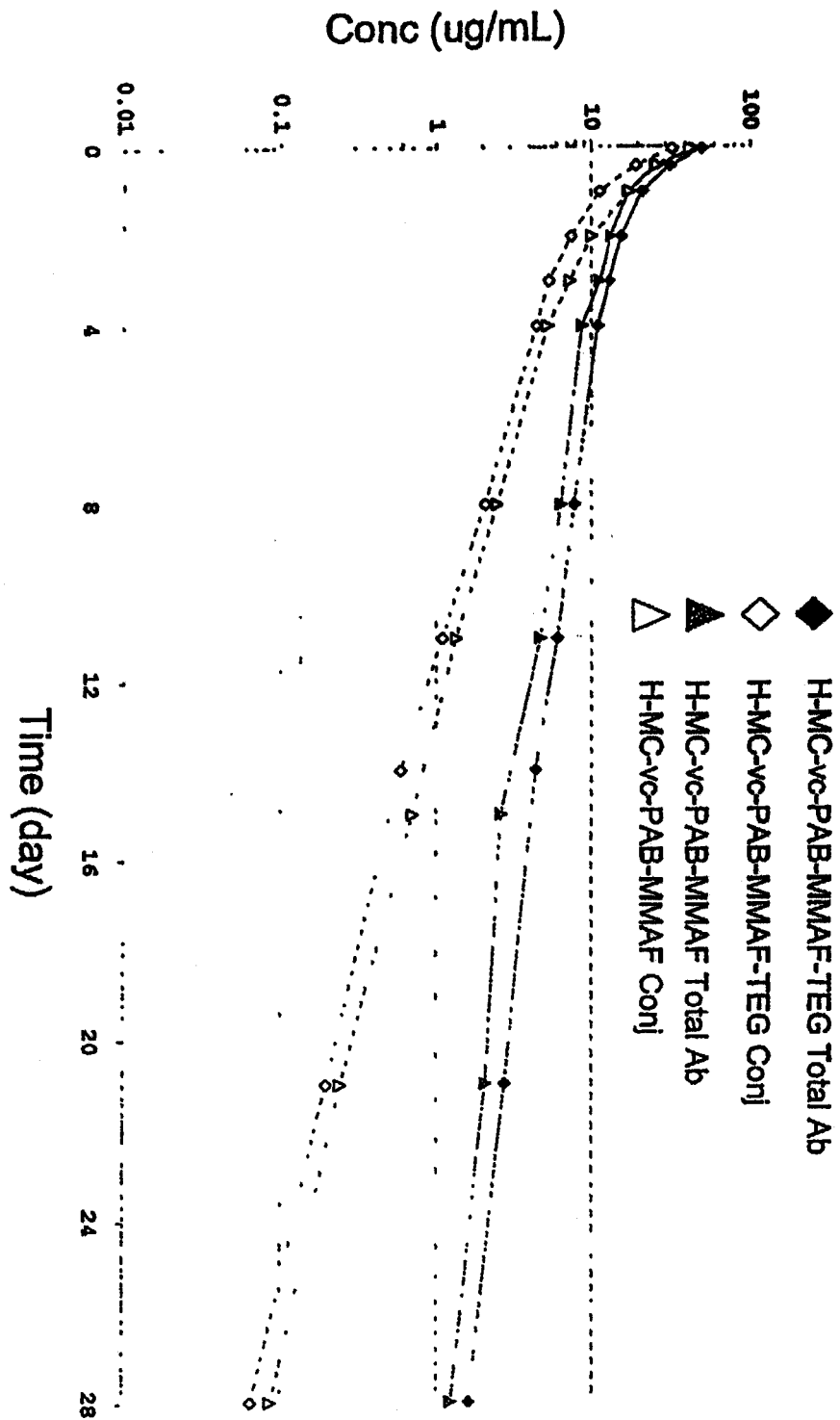


Figure 11

H-MC-vc-PAB-MMAE in Cynomolgus monkeys

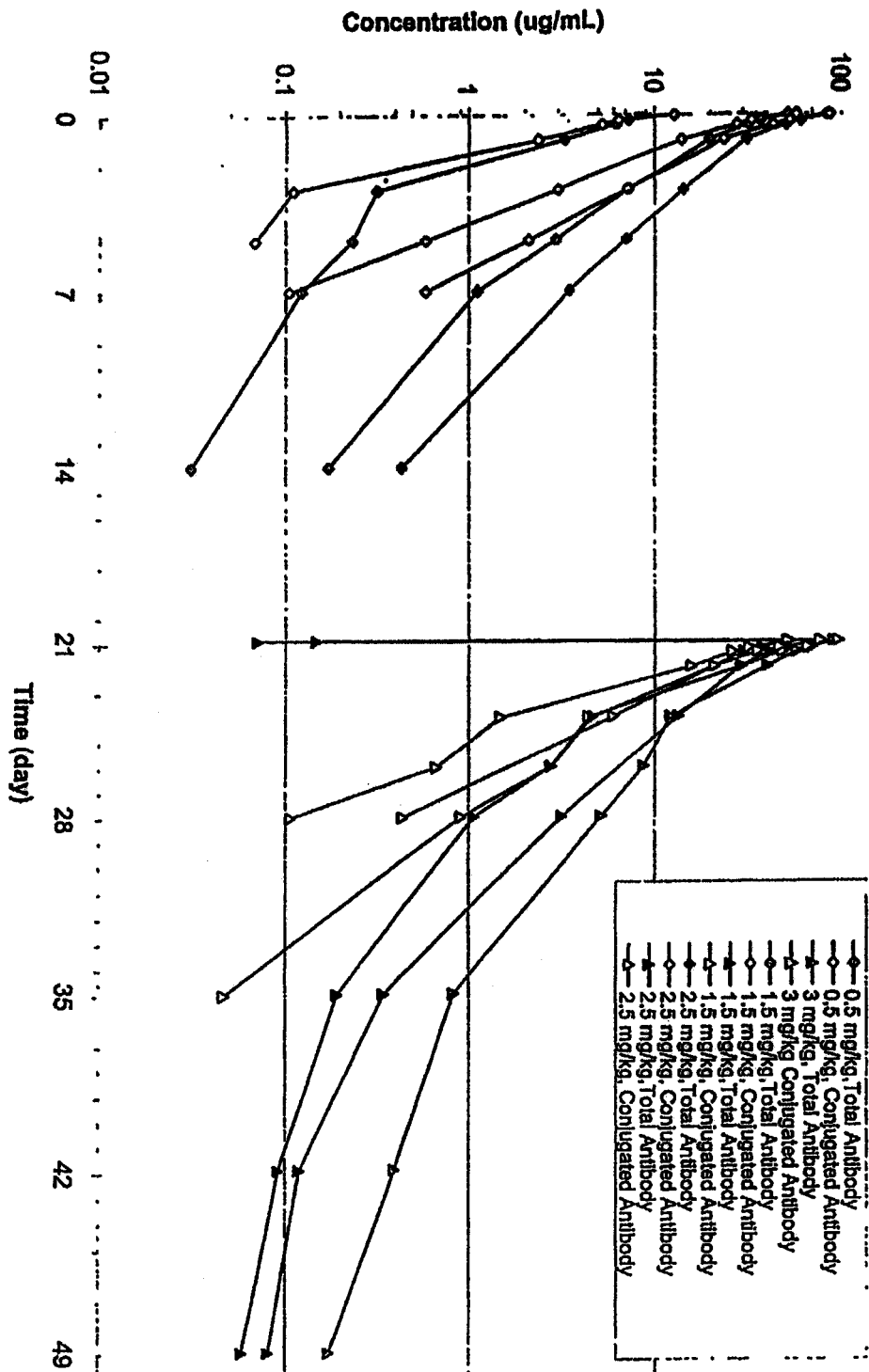


Figure 12

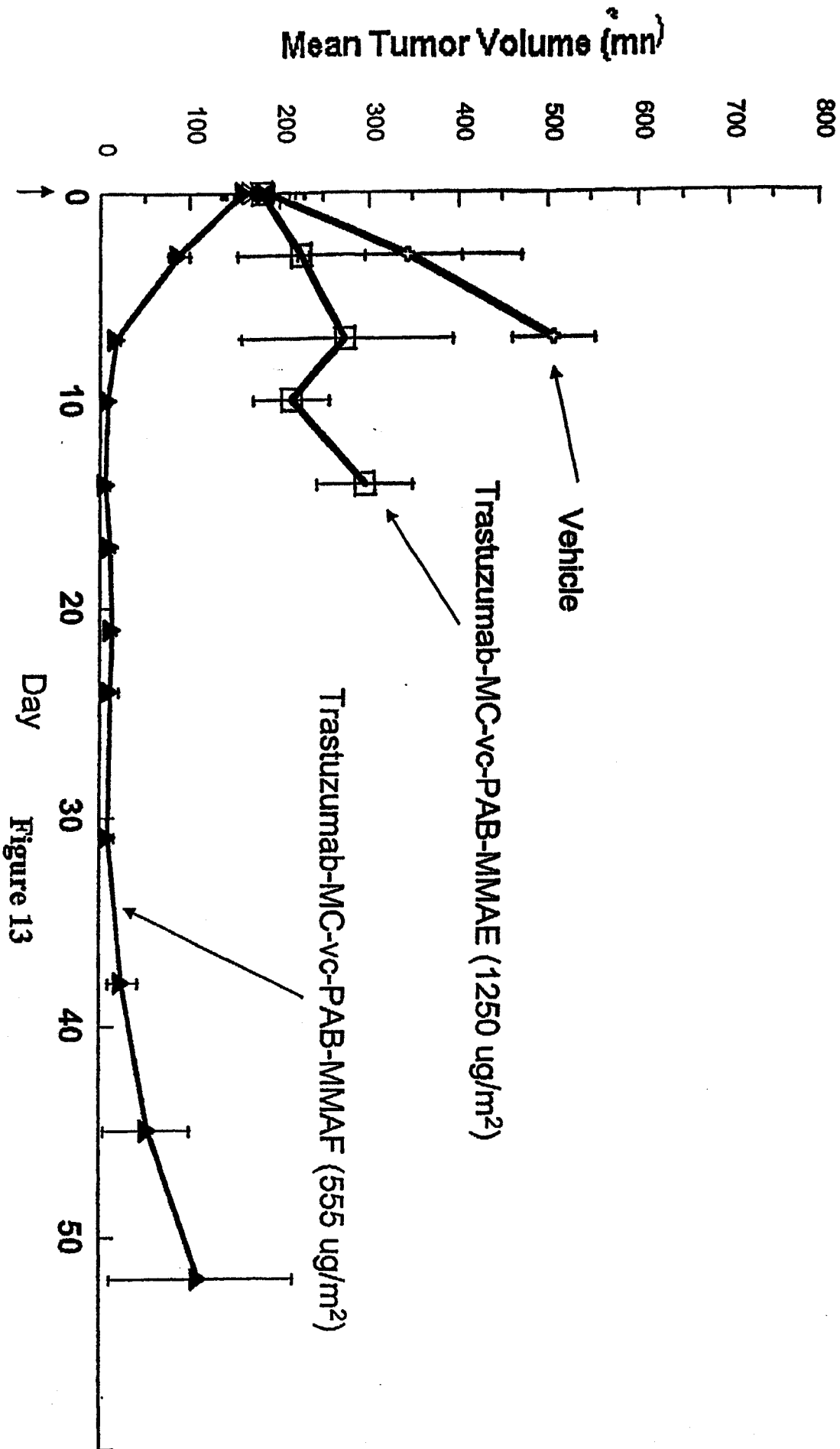


Figure 13

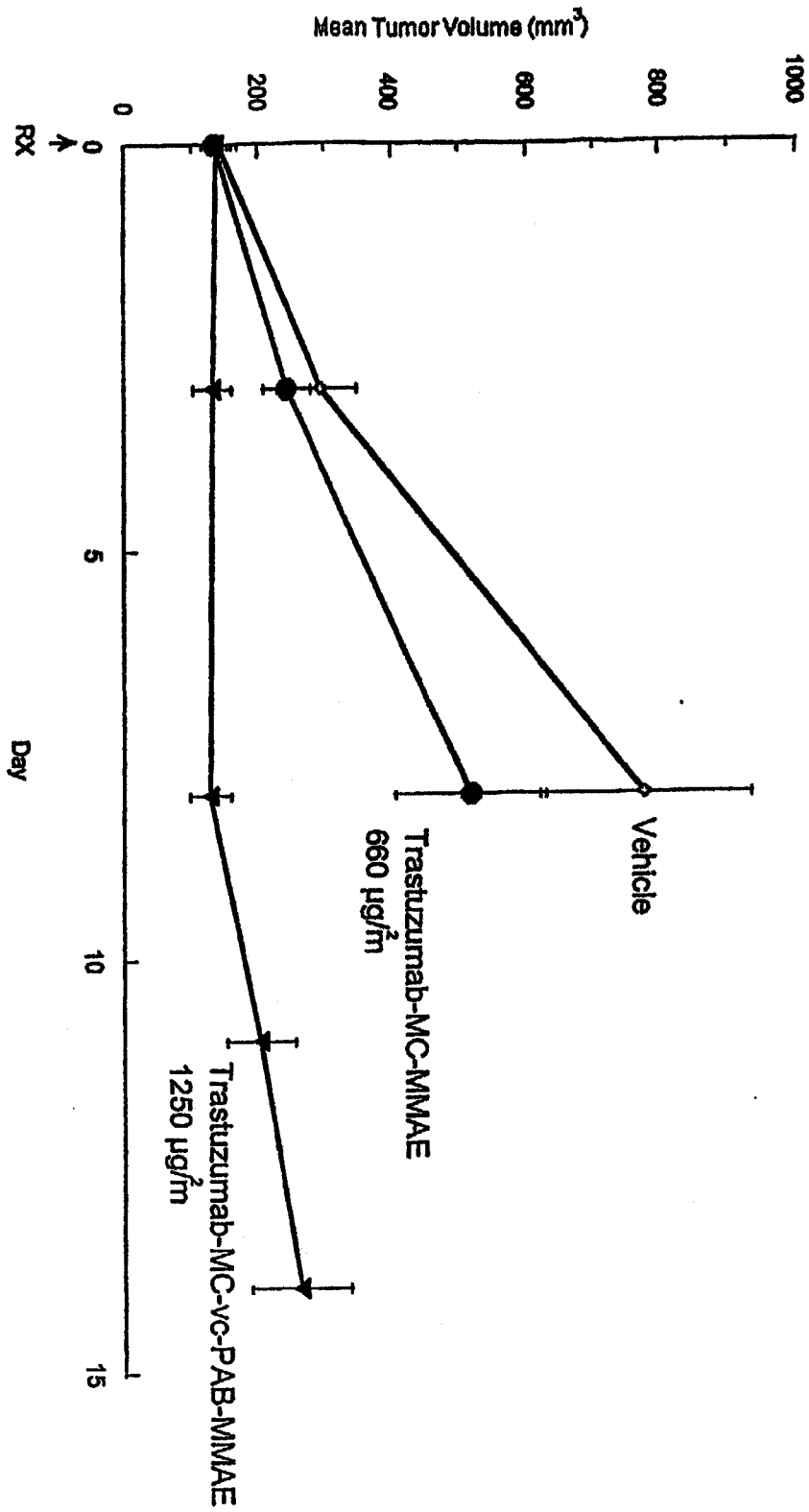


Figure 14

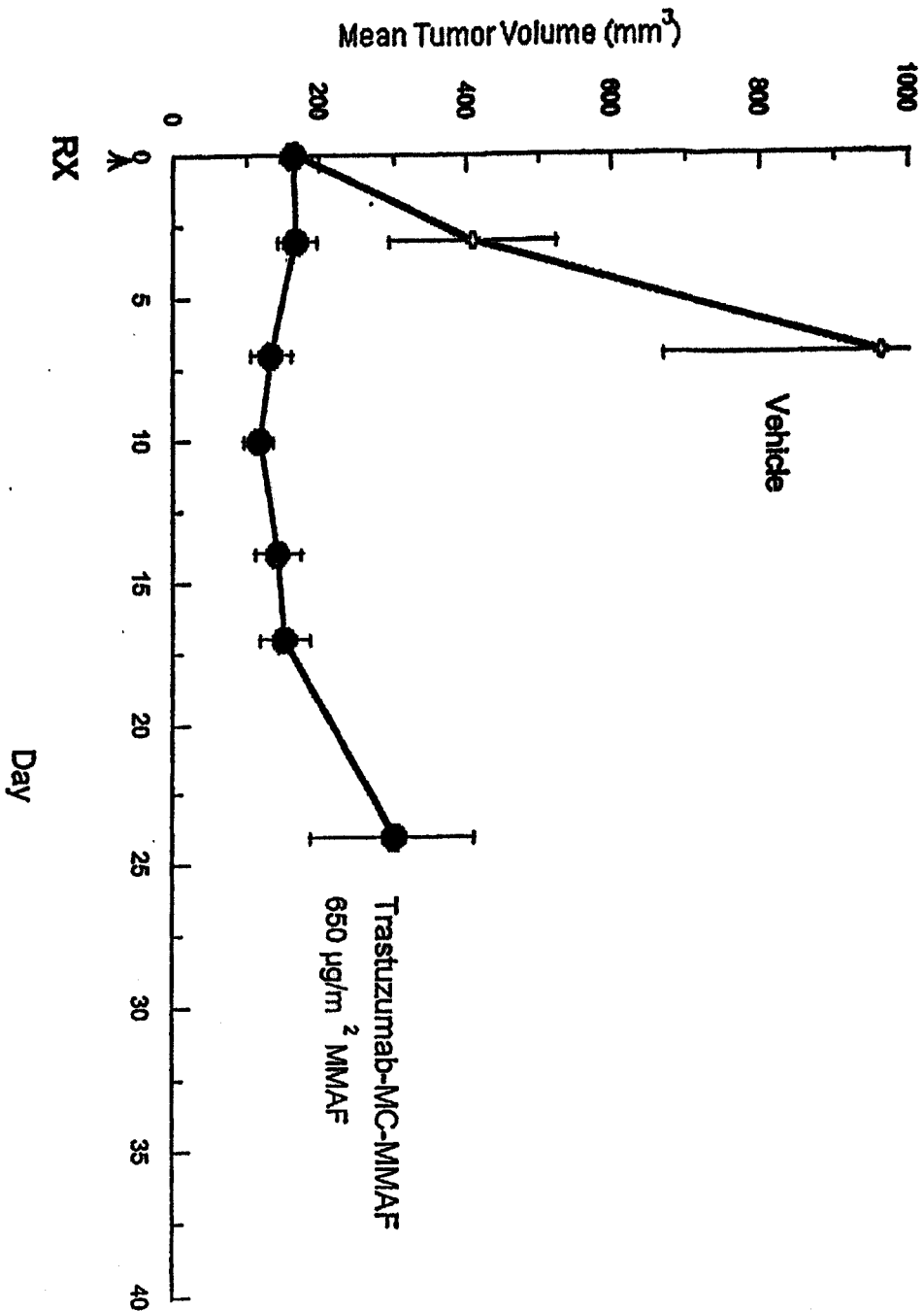


Figure 15

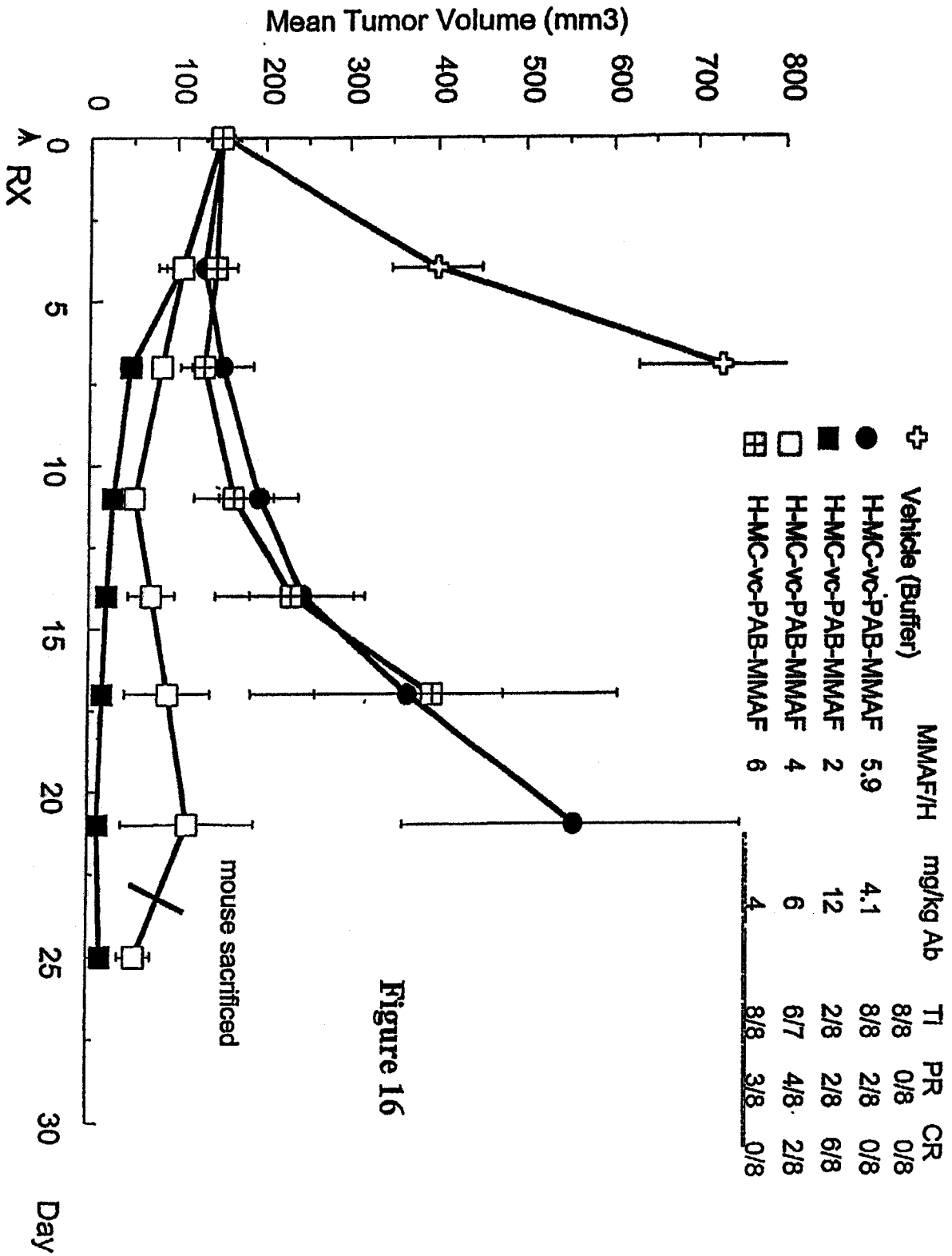


Figure 16

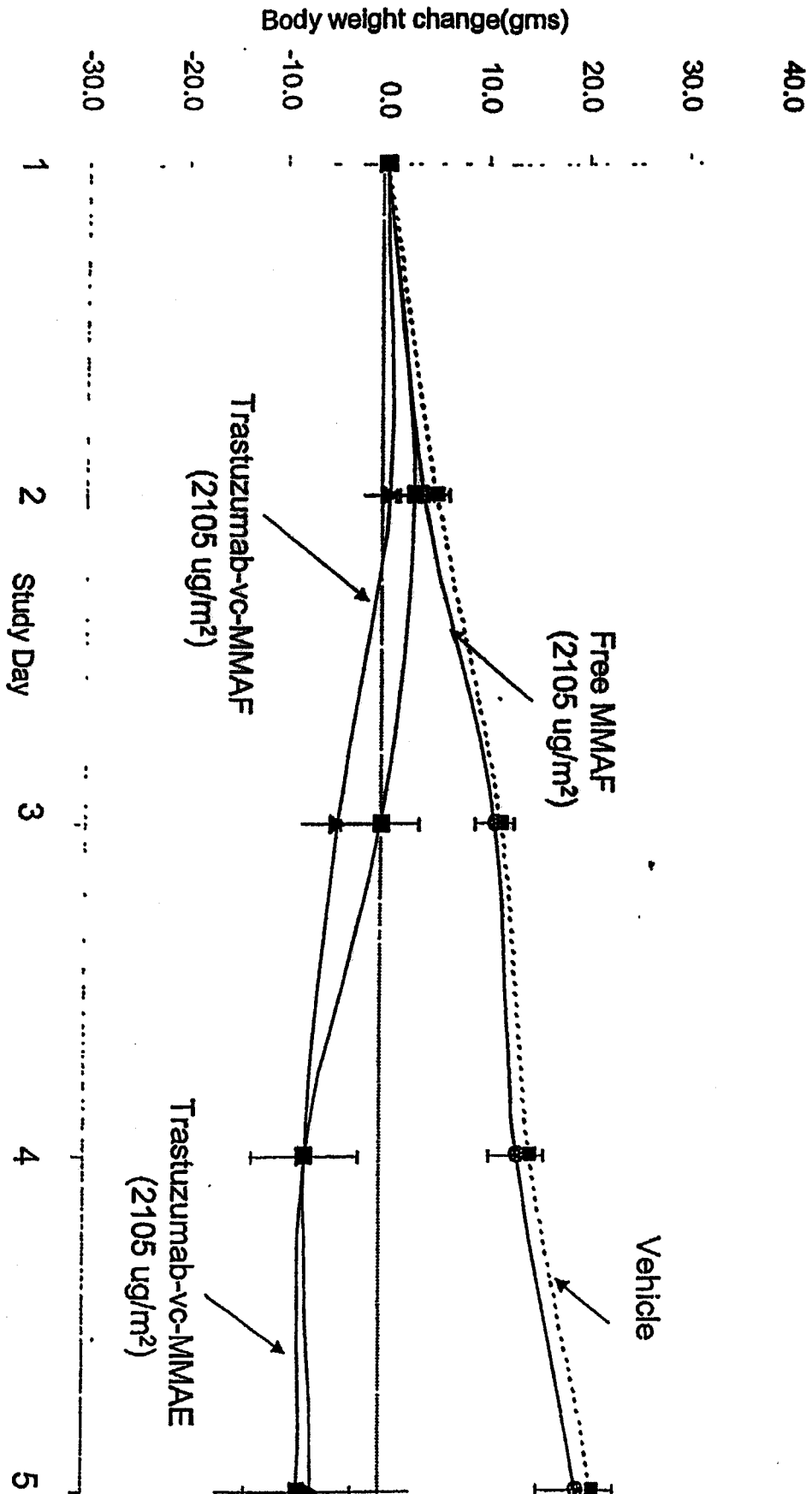


Figure 17

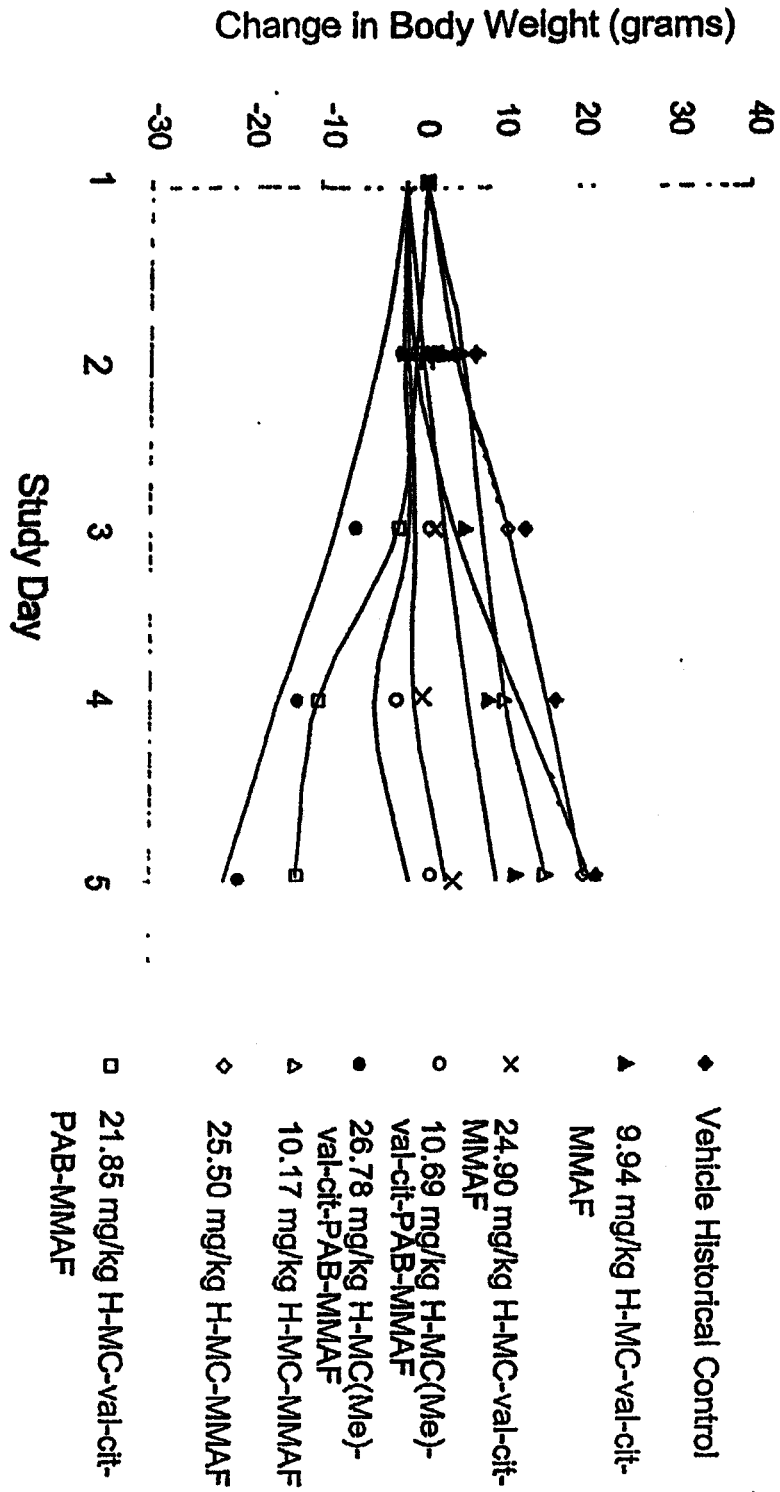


Figure 18

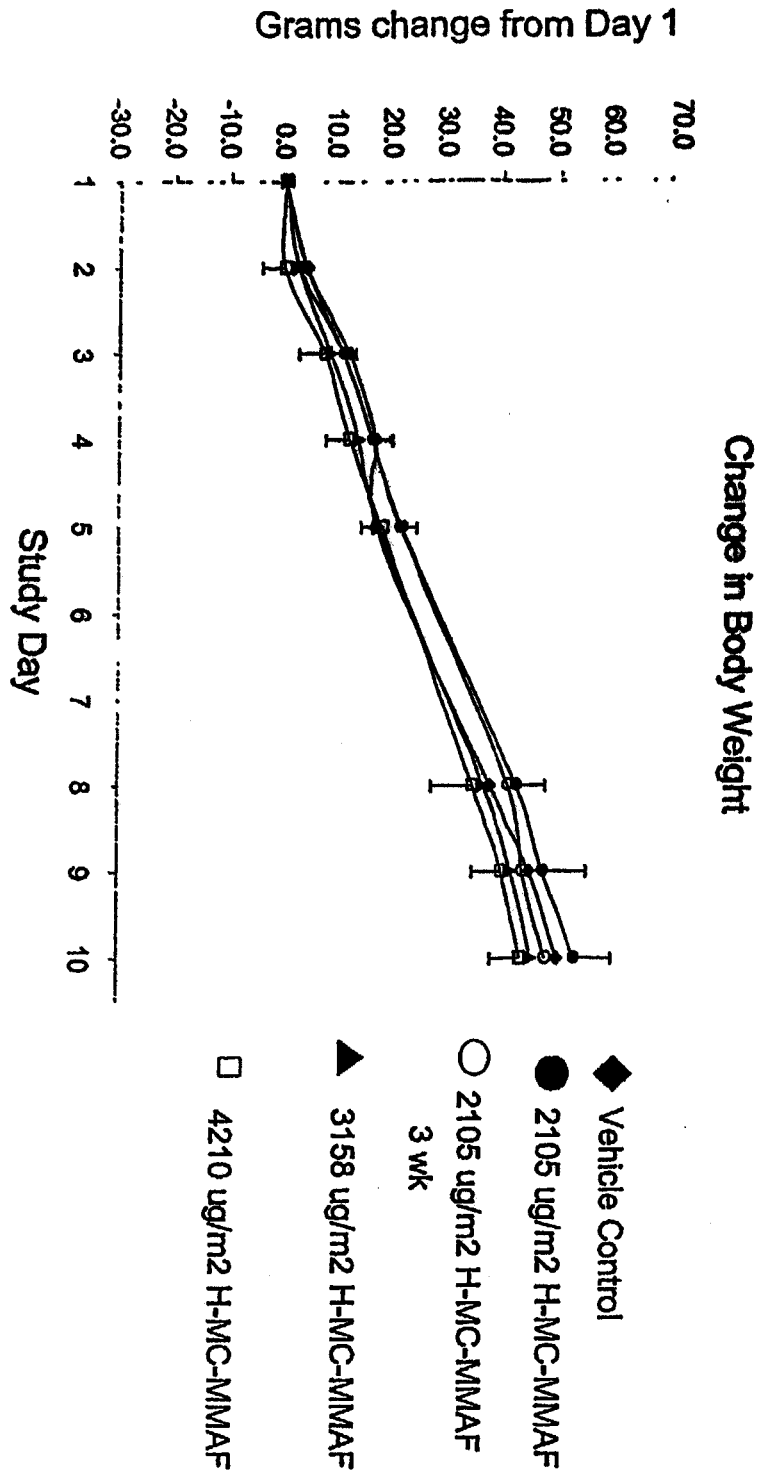


Figure 19