# MONOMETHYLVALINE COMPOUNDS CAPABLE OF CONJUGATION TO LIGANDS

## CONTINUITY

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

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 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 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 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.

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 $^{111}$In or $^{90}$Y 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, auristain 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 (Kd = 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 (Hotaling 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 x $10^6$ 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:

$$L-\left(-A_a-W_w-Y_y-D\right)_p$$

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,

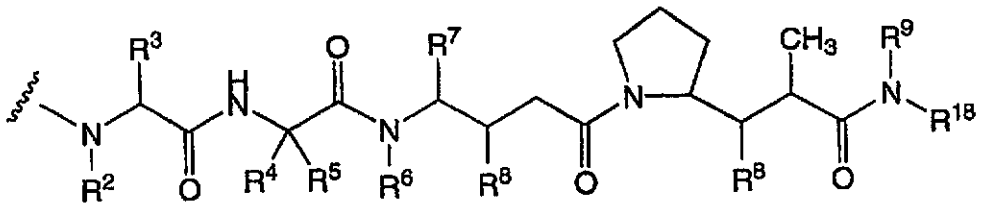
w is an integer ranging from 0 to 12,

-Y- is a Spacer unit, and

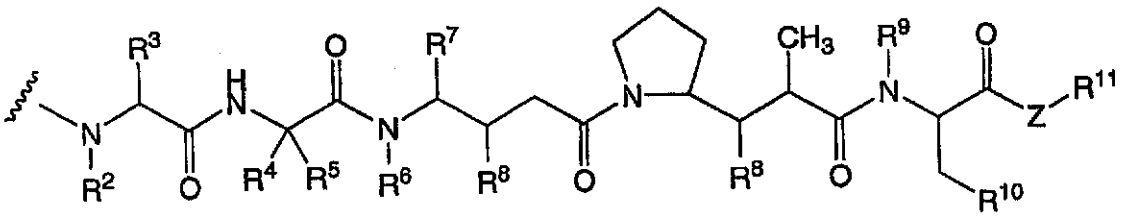
y is 0, 1 or 2;

p ranges from 1 to about 20; and

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



$D_E$



$D_F$

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;

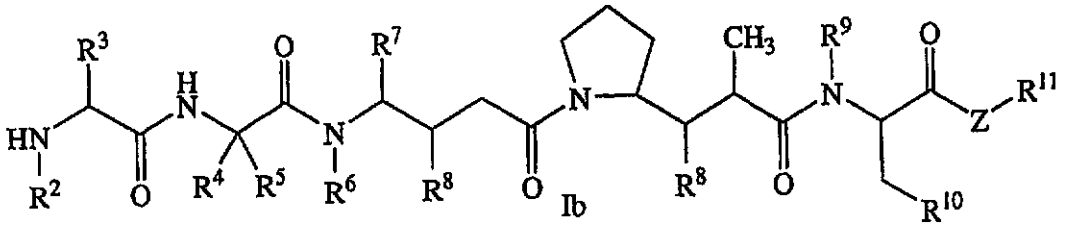
$R^{14}$ is H or $C_1$-$C_8$ alkyl;

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; 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:



**Ib**

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.

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.

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.

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.

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.

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.

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.

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 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.

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 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.

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 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.

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.

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.

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.

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.

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.

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.

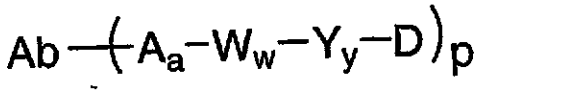
In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

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.

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**:



**Ia'**

or a pharmaceutically acceptable salt or solvate thereof, wherein:

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,

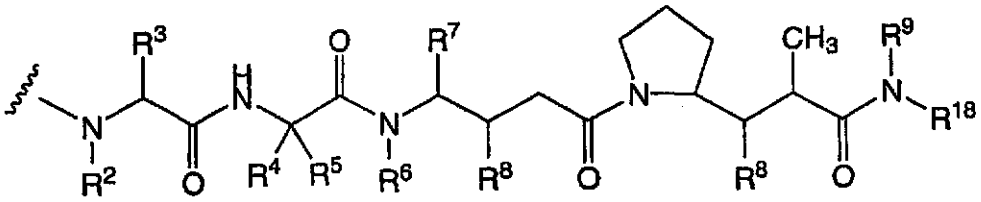
w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

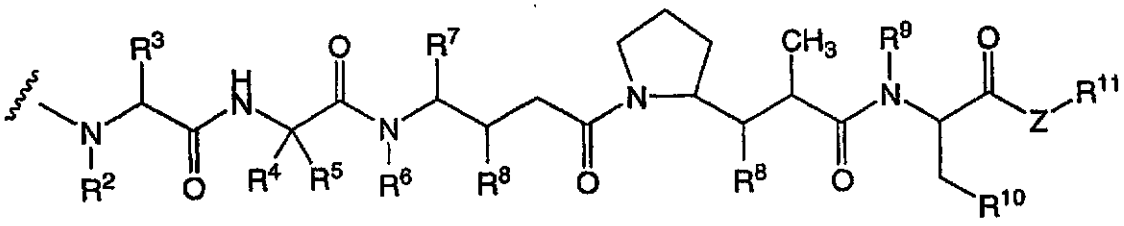
y is 0, 1 or 2,

p ranges from 1 to about 20, and

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



$D_E$



$D_F$

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, Ab is not an antibody which binds to an ErbB receptor or which binds 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, 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 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) IL20Rα (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, CD79α, 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 erythematosis, 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).

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) 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 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-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 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 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.

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.

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.

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.

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.

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.

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.

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.

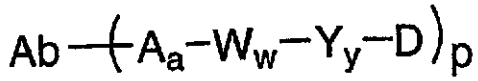
In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

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.

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.

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**:



**Ic**

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);

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

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(28) CD79a (CD79A, CD79α, 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);

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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 erythematosis, 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);

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a is 0 or 1,

each W is independently an Amino Acid unit,

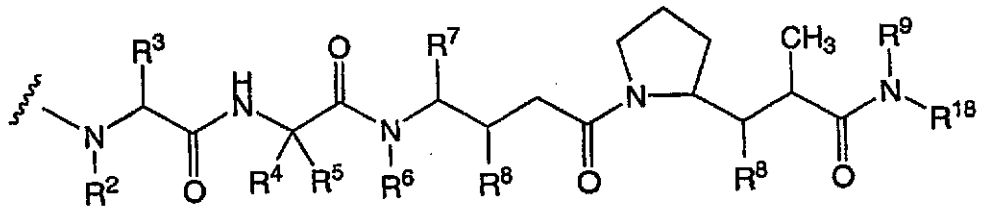
w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

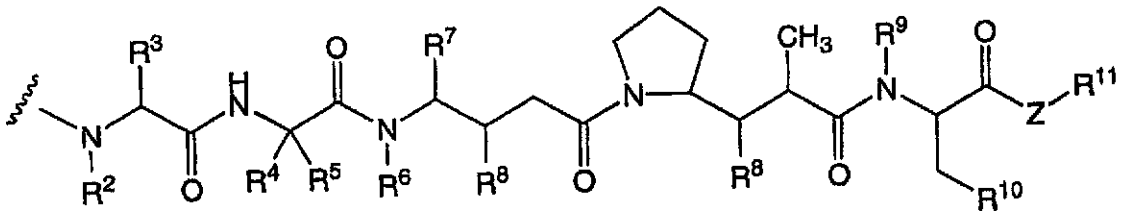
y is 0, 1 or 2,

p ranges from 1 to about 20, and

D is a Drug 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:

$R^2$ 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 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.

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.

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.

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.

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.

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.

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.

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

(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, 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.

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 subcutaneous L2987. The filed 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 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, –o– Trastuzumab-MC-MMAF, 4.1 MMAF/Ab, and –Δ– Trastuzumab-MC-MMAF, 4.8 MMAF/Ab, measured in Relative Fluorescence Units (RLU) versus μg/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, –o– 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, –o– 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, –o– 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 $\mu g/m^2$) and Trastuzumab-MC-vc-PAB-MMAF (555 $\mu g/m^2$). (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 μg/m$^2$) of Trastuzumab-MC-MMAE and 1250 μg/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 μg/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 μg/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± 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± 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± SD) after administration of trastuzumab (H)-MC-MMAF at doses of 2105, 3158, and 4210 μg/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 schwanoma 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-β1, heregulin-β2 and heregulin-β3 (Holmes *et al.*, *Science*, 256:1205-1210 (1992); and U.S. Patent Nno. 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.*, HRGβ1177-244 ).

"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. 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 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 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 (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 in summarized is Table 3 on page 464 of Ravetch and Kinet, (1991) *Annu. Rev. Immunol,* 9:457-92. To assess ADCC 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., Prco. Natl. Acad. 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 Fcγ RIII 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. 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*, 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)).

"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. 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 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, 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).

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.*, 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.

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.

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 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.

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.

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 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, Rosenburg 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 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.

"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, 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 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.

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.

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 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 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.

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.

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.

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.

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.

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, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

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.

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 been 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 x $10^6$ copies/cell. 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).

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 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, $^{60}$C, 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.

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® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide 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®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; 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, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; bisphosphonates, such as clodronate; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)) and anthracyclines such as annamycin, AD 32, alcarubicin, daunorubicin, dexrazoxane, DX-52-1, epirubicin, GPX-100, idarubicin, KRN5500, menogaril, dynemicin, including dynemicin A, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, 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; 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, epitiostanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, 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 formyltransferase inhibitors such as raltitrexed (TOMUDEX$^{RM}$, TDX); inhibitors of dihydropyrimidine dehydrogenase such as eniluracil; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid;

gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 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, Schaumberg, 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; difluorometlhylornithine (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, formestanie, 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 abherant 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, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 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 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, 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 cyotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP 659,439A2, Merck Patent GmbH). 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 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 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 (Gleevac; 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-1α, 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. 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 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 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 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 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, 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 throbocytopenic 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, amylotrophic 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 $C_1$-$C_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -$CH_3$), ethyl (Et, -$CH_2CH_3$), 1-propyl (n-Pr, n-propyl, -$CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, -$CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, -$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, -$CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, -$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, -$C(CH_3)3$), 1-pentyl (n-pentyl, -$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (-$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (-$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (-$C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl (-$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (-$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (-$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (-$CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl (-$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (-$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-

pentyl (-$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (-$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (-$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (-$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (-$C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl (-$CH(CH_3)C(CH_3)3$.

"Alkenyl" is $C_2$-$C_{18}$ 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 (-$CH=CH_2$), allyl (-$CH_2CH=CH_2$), cyclopentenyl (-$C_5H_7$), and 5-hexenyl (-$CH_2CH_2CH_2CH_2CH=CH_2$).

"Alkynyl" is $C_2$-$C_{18}$ 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 (-$C\equiv CH$) and propargyl (-$CH_2C\equiv CH$).

"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 (-$CH_2$-) 1,2-ethyl (-$CH_2CH_2$-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

"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 (-CH=CH-).

"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 (-C≡C-), propargyl (-$CH_2C\equiv C$-), and 4-pentynyl (-$CH_2CH_2CH_2C\equiv CH$-).

"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, $-S^-$, $-NR_2$, $-NR_3$, =NR, $-CX_3$, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, $-NO_2$, $=N_2$, $-N_3$, NC(=O)R, -C(=O)R, $-C(=O)NR_2$, $-SO_3^-$, $-SO_3H$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, -S(=O)R, $-OP(=O)(OR)_2$, $-P(=O)(OR)_2$, $-PO^-_3$, $-PO_3H_2$, -C(=O)R, -C(=O)X, -C(=S)R, $-CO_2R$, $-CO_2^-$, -C(=S)OR, -C(=O)SR, -C(=S)SR, $-C(=O)NR_2$, $-C(=S)NR_2$, $-C(=NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, $C_2$-$C_{18}$ alkyl, $C_6$-$C_{20}$ aryl, $C_3$-$C_{14}$ 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, dihydroypyridyl, 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, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, 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-azetedyl, 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 alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (*e.g.*, polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (*e.g.*, polyethyleneamino, Jeffamine™); 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.

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

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

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or *R* and *S*, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or 1 meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

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-($C_1$-$C_8$ 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.

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-($C_1$-$C_8$ 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.

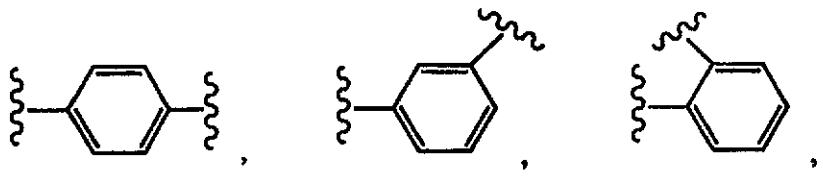
A "$C_3$-$C_8$ carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative $C_3$-$C_8$ carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -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-($C_1$-$C_8$ 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.

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, ocytylene, 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 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 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.

"$C_3-C_8$ 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, -$C_1$-$C_8$ 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)$_2$R', -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.

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-butyldimethyl 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 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 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, 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 diethylphosphorylcyanidate, 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-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 chromatography, ile is isoleucine, lys is lysine, MeCN ($CH_3CN$) is acetonitrile, MeOH is methanol, Mtr is 4-anisyldiphenylmethyl (or 4-methoxytrityl),nor is (*1S, 2R*)-(+)-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.

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) 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, 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 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: 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 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.

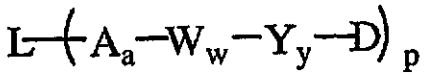
In the context of an infectious disease, the term "treating" includes any or all of: preventing the growth, multiplication or replication of the pathogen that causes the infectious disease and ameliorating one or more symptoms of an infectious disease.

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:



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,

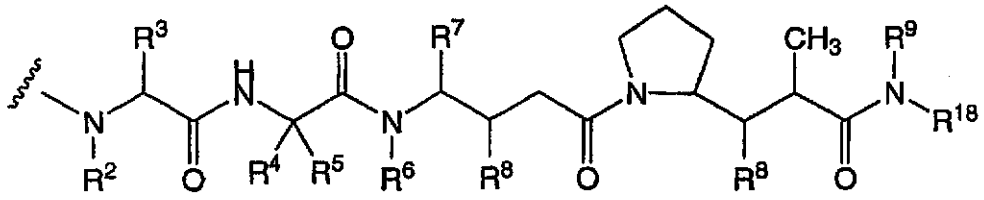
w is an integer ranging from 0 to 12,

-Y- is a Spacer unit, and

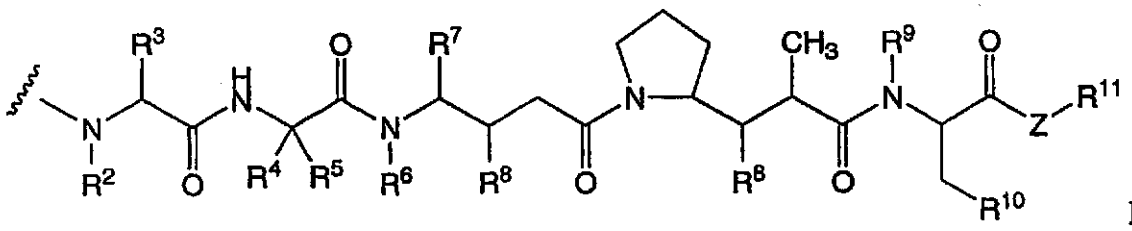
y is 0, 1 or 2;

p ranges from 1 to about 20; and

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



$D_E$



$D_F$

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;

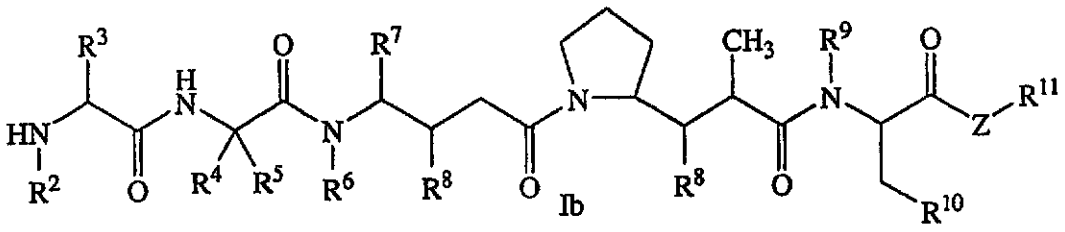
each occurrence of $R^{15}$ is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

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



**Ib**

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.

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

$$Ab-\left(A_a-W_w-Y_y-D\right)_p$$

Formula **Ia'**

or pharmaceutically acceptable salts or solvates thereof.

wherein:

Ab is an antibody,

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

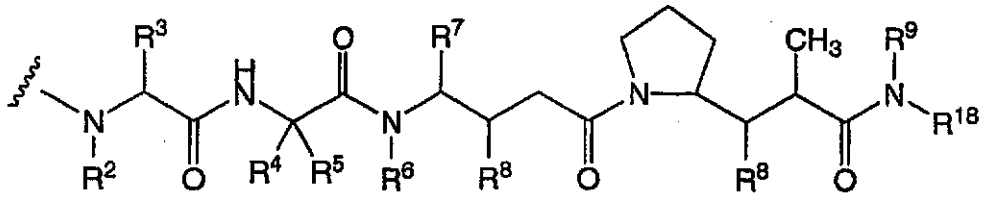
w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

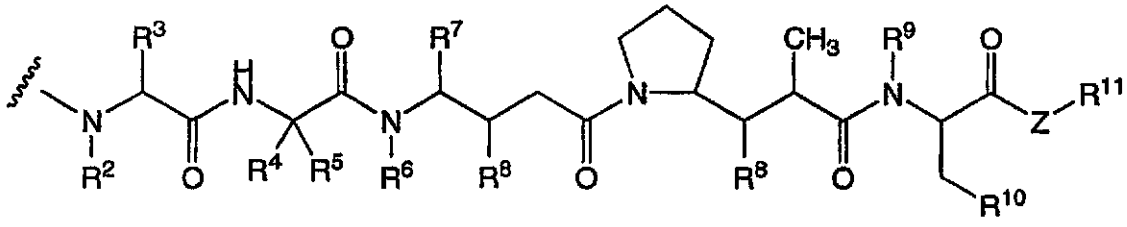
y is 0, 1 or 2,

p ranges from 1 to about 20, and

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



$D_E$



$D_F$

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.

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

(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, 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 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) IL20Rα (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, CD79α, 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 erythematosis, 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, $R^3$, $R^4$ 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 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-.

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

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

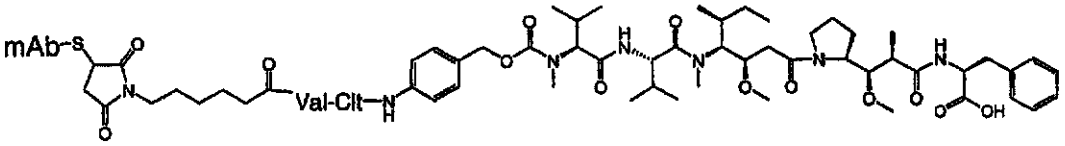
In an exemplary embodiment, when Z is -O-, $R^{11}$ is –H, methyl or t-butyl.

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 $-(CH_2)_n-COOH$.

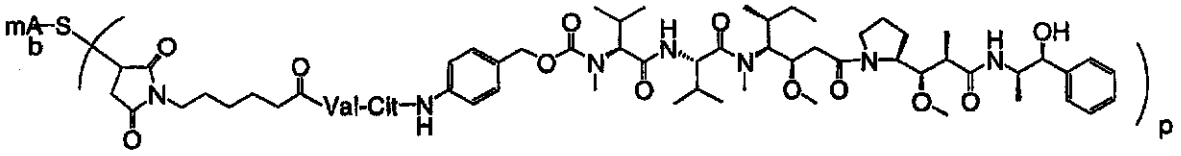
In another embodiment, when Z is -NH, $R^{11}$ is $-CH(R^{15})_2$, wherein $R^{15}$ is $-(CH_2)_n-SO_3H$.

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

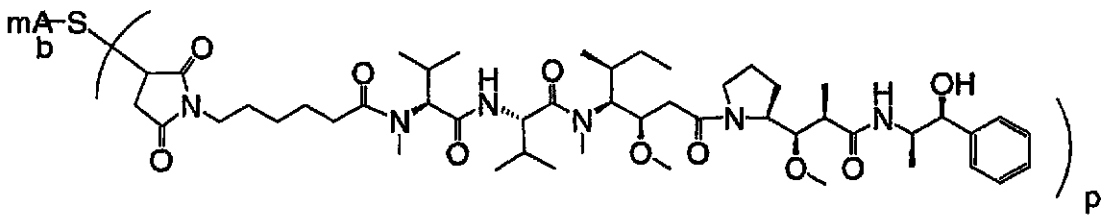
Exemplary embodiments of Formula **Ia** have the following structures:




**L-MC-vc-PAB-MMAF**

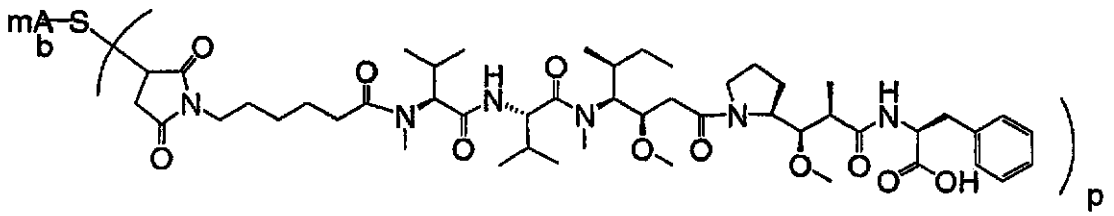



**L-MC-vc-PAB-MMAE**




**L-MC-MMAE**

or



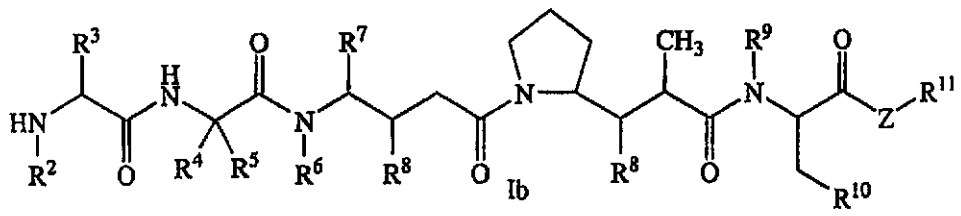
**L-MC-MMAF**

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**):



**Ib**

or a pharmaceutically acceptable salt or solvate 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.

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.

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

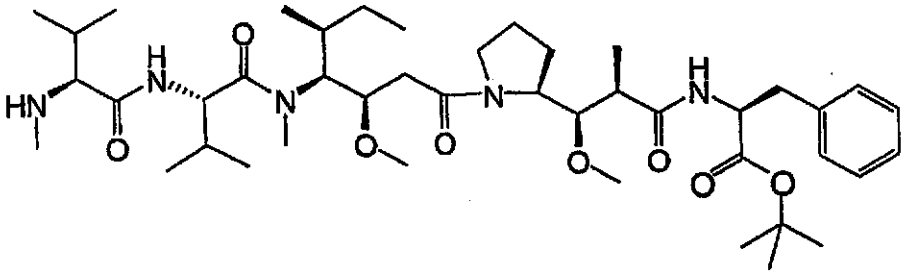
In an exemplary embodiment, $R^{10}$ is -phenyl.

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

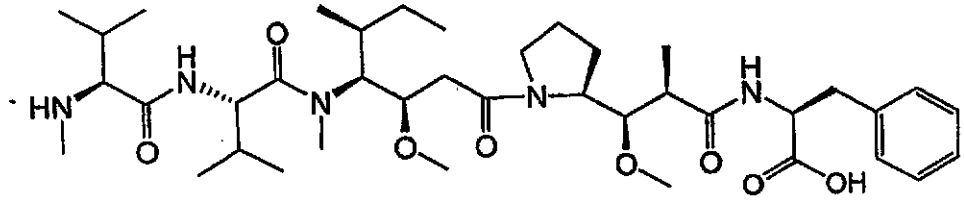
In one embodiment, when Z is -NH, $R^{11}$ is $-CH(R^{15})_2$, wherein $R^{15}$ is $-(CH_2)_n-N(R^{16})_2$, and $R^{16}$ is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

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

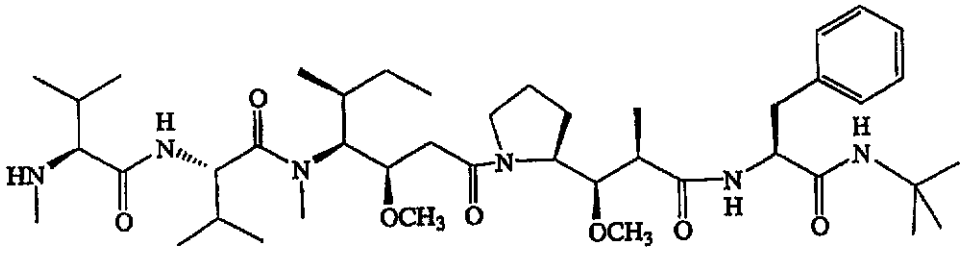
Illustrative Compounds of Formula (**Ib**), each of which may be used as drug moieties (D) in ADC, include compounds having the following structures:



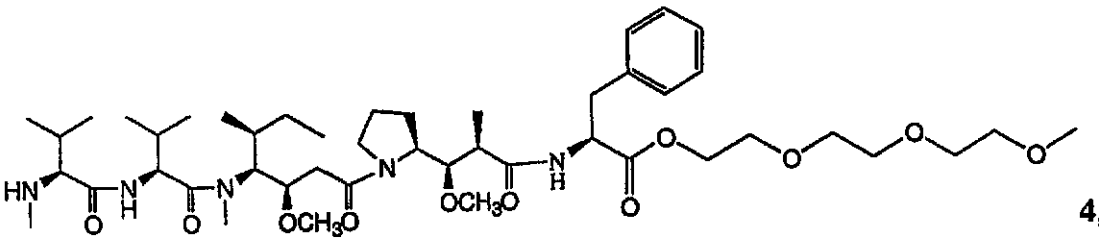
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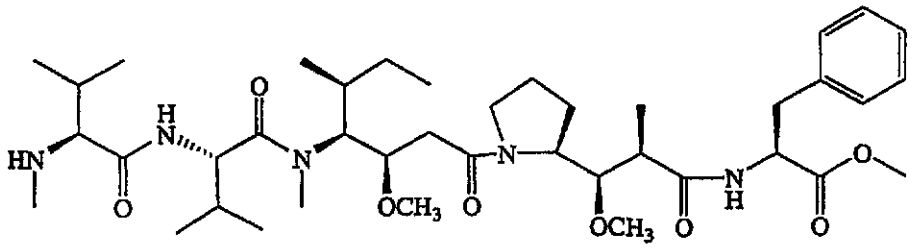
**2,**



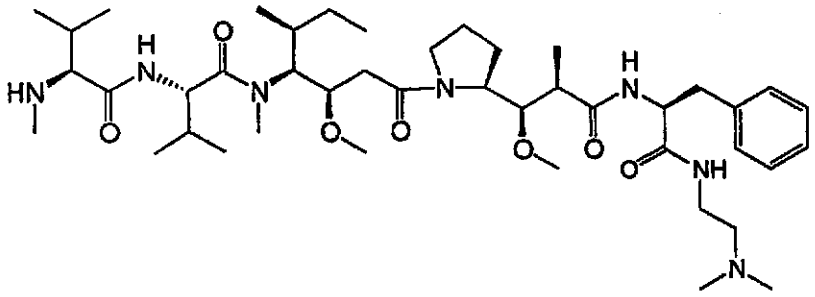
**3,**



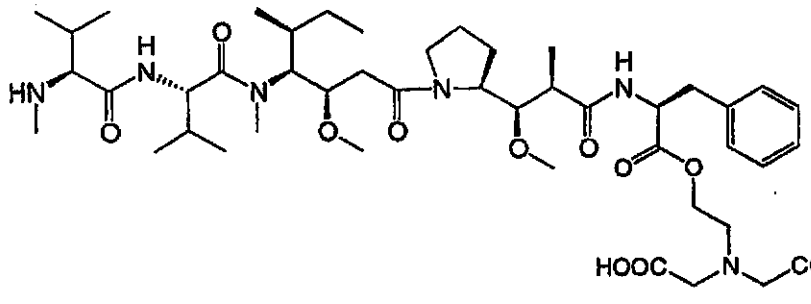
**4,**



5,

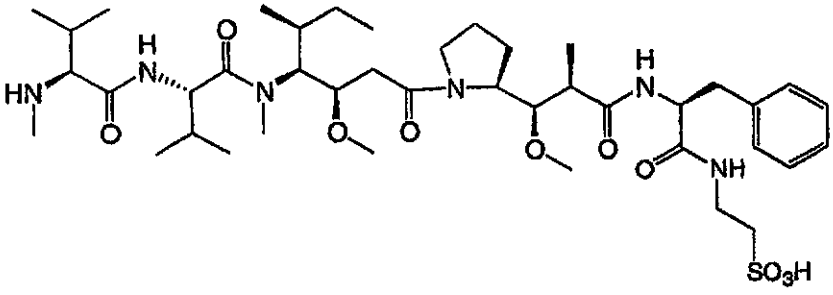


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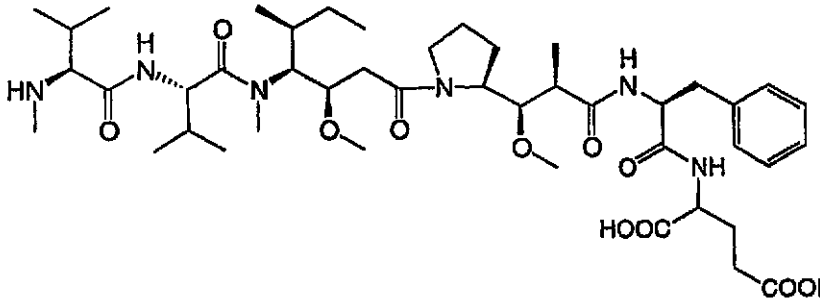


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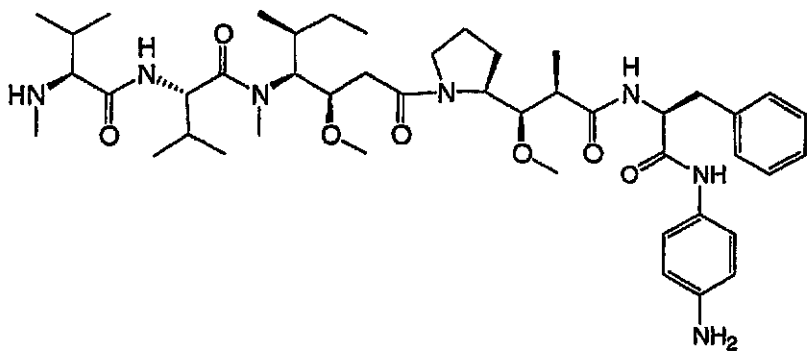
5



8,



9, and



10

and pharmaceutically acceptable salts or solvates thereof.

THE COMPOUNDS OF FORMULA (Ic)

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

$$Ab-\left(A_a-W_w-Y_y-D\right)_p \qquad \text{Ic}$$

comprising an antibody covalently attached to one or more drug units (moieites). 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):

(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, 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 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 (CD79B, CD79β, 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) IL20Rα (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 (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, 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 erythematosis, 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 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,

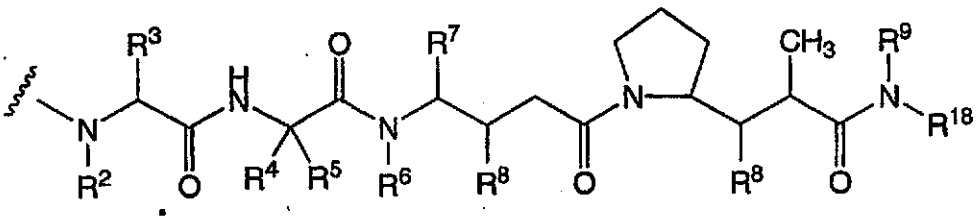
w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

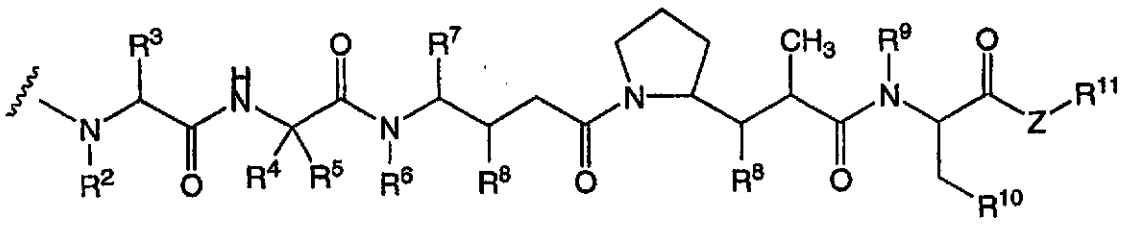
y is 0, 1 or 2,

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:

$R^2$ 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 -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.

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-.

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

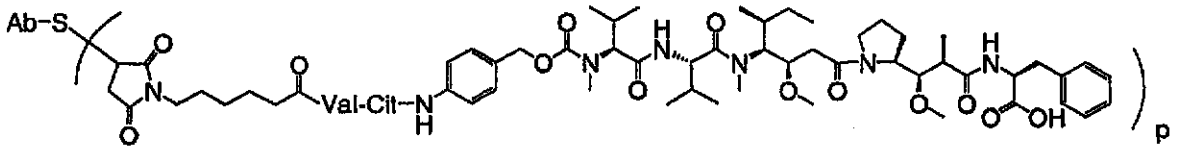
In an exemplary embodiment, $R^{10}$ is -phenyl.

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

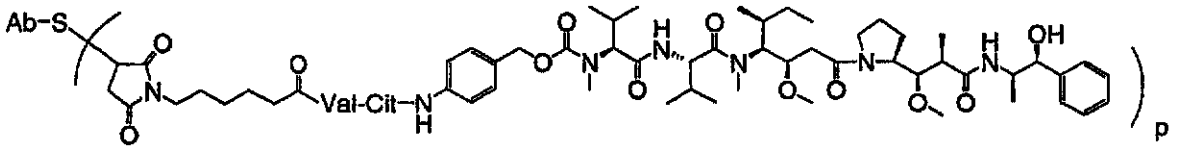
In one embodiment, when Z is -NH, $R^{11}$ is $-CH(R^{15})_2$, wherein $R^{15}$ is $-(CH_2)_n-N(R^{16})_2$, and $R^{16}$ is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

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

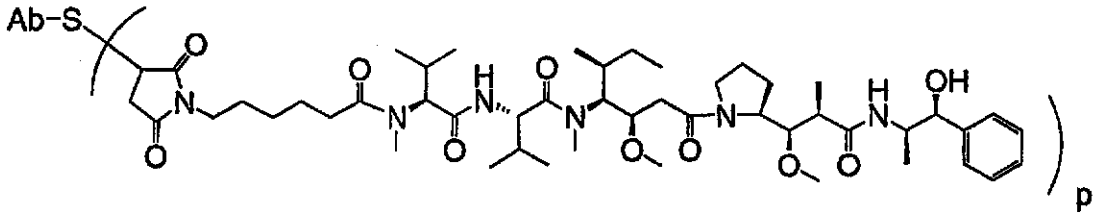
Exemplary embodiments of Formula **Ic** ADC have the following structures:



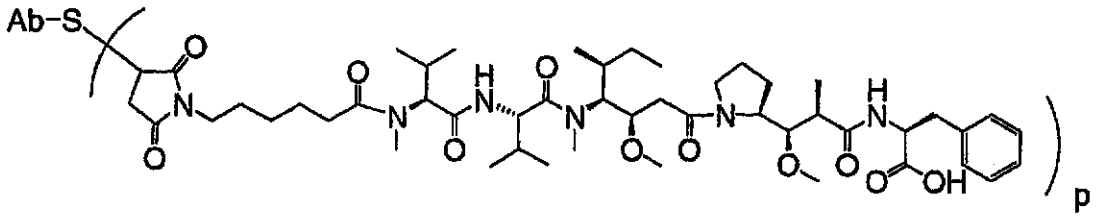

**Ab-MC-vc-PAB-MMAF**




**Ab-MC-vc-PAB-MMAE**




**Ab-MC-MMAE**




**Ab-MC-MMAF**

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; 2004Annual 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:

$$—A_a—W_w—Y_y—$$

wherein:

-A- is a Stretcher unit;

a is 0 or 1;

each -W- is independently an Amino Acid unit;

w is independently an integer ranging from 0 to 12;

-Y- is a Spacer unit; and

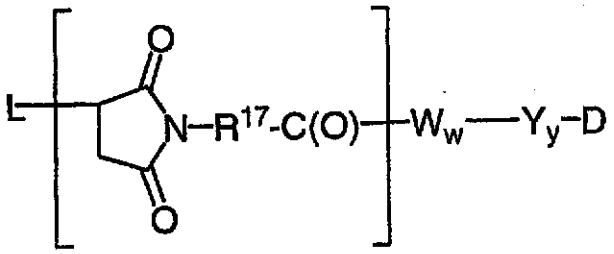
y is 0, 1 or 2.

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

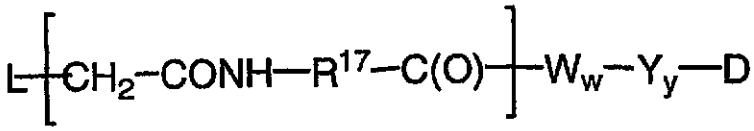
## 4.3.1 THE STRETCHER UNIT

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 $R^{17}$ is selected from -$C_1$-$C_{10}$ alkylene-, -$C_3$-$C_8$ carbocyclo-, -O-($C_1$-$C_8$ alkyl)-, -arylene-, -$C_1$-$C_{10}$ alkylene-arylene-, -arylene-$C_1$-$C_{10}$ alkylene-, -$C_1$-$C_{10}$ alkylene-($C_3$-$C_8$ carbocyclo)-, -($C_3$-$C_8$ carbocyclo)-$C_1$-$C_{10}$ alkylene-, -$C_3$-$C_8$ heterocyclo-, -$C_1$-$C_{10}$ alkylene-($C_3$-$C_8$ heterocyclo)-, -($C_3$-$C_8$ heterocyclo)-$C_1$-$C_{10}$ alkylene-, -$(CH_2CH_2O)_r$-, and -$(CH_2CH_2O)_r$-$CH_2$-; 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).

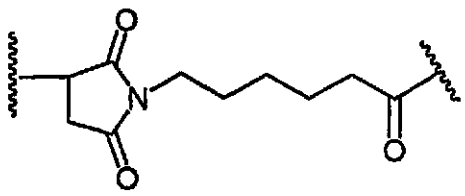


**IIIa**

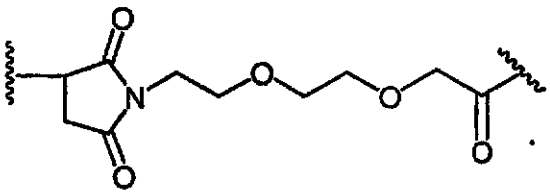


**IIIb**

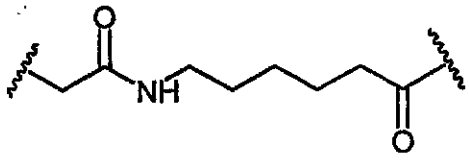
An illustrative Stretcher unit is that of Formula **IIIa** wherein $R^{17}$ is $-(CH_2)_5-$:



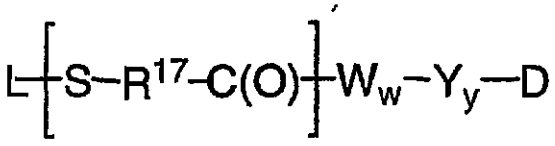
Another illustrative Stretcher unit is that of Formula **IIIa** wherein $R^{17}$ is $-(CH_2CH_2O)_r-CH_2-$; and r is 2:



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



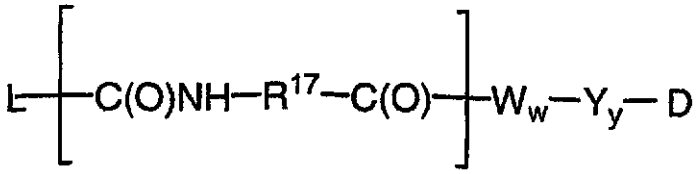
In another embodiment, the Stretcher unit is linked to the Ligand unit via a disulfide bond between a sulfur atom of the Ligand unit and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted within the square brackets of Formula **IV**, wherein $R^{17}$,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



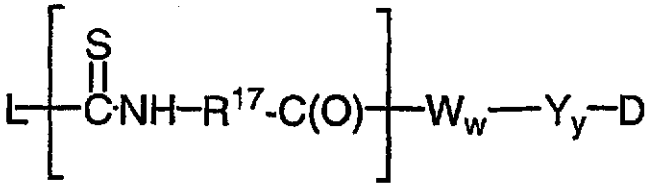
**IV**

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;

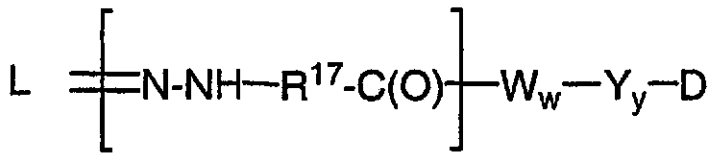


**Va**

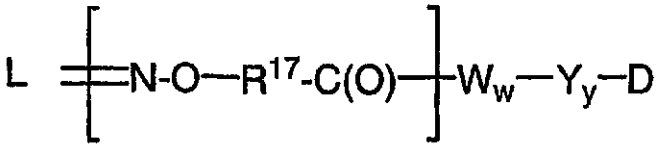


**Vb**

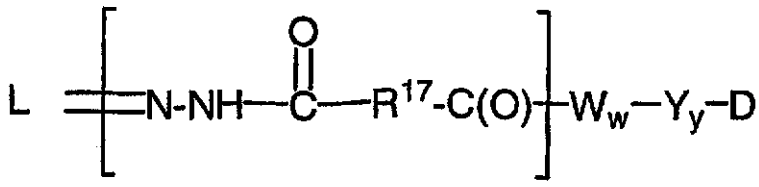
In yet another aspect, the reactive group of the Stretcher contains a reactive site that is reactive to a modified carbohydrate's (-CHO) group that can be present on a Ligand. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting (-CHO) unit of the oxidized carbohydrate can be condensed with a Stretcher that contains a functionality such as a hydrazide, an oxime, 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.



**VIa**



**VIb**

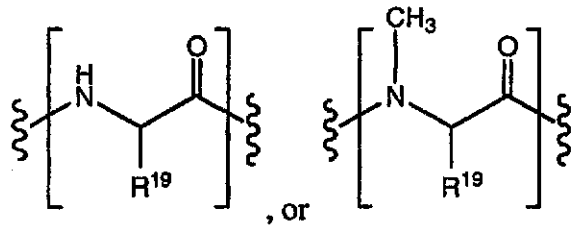


VIc

## 4.3.2 THE AMINO ACID UNIT

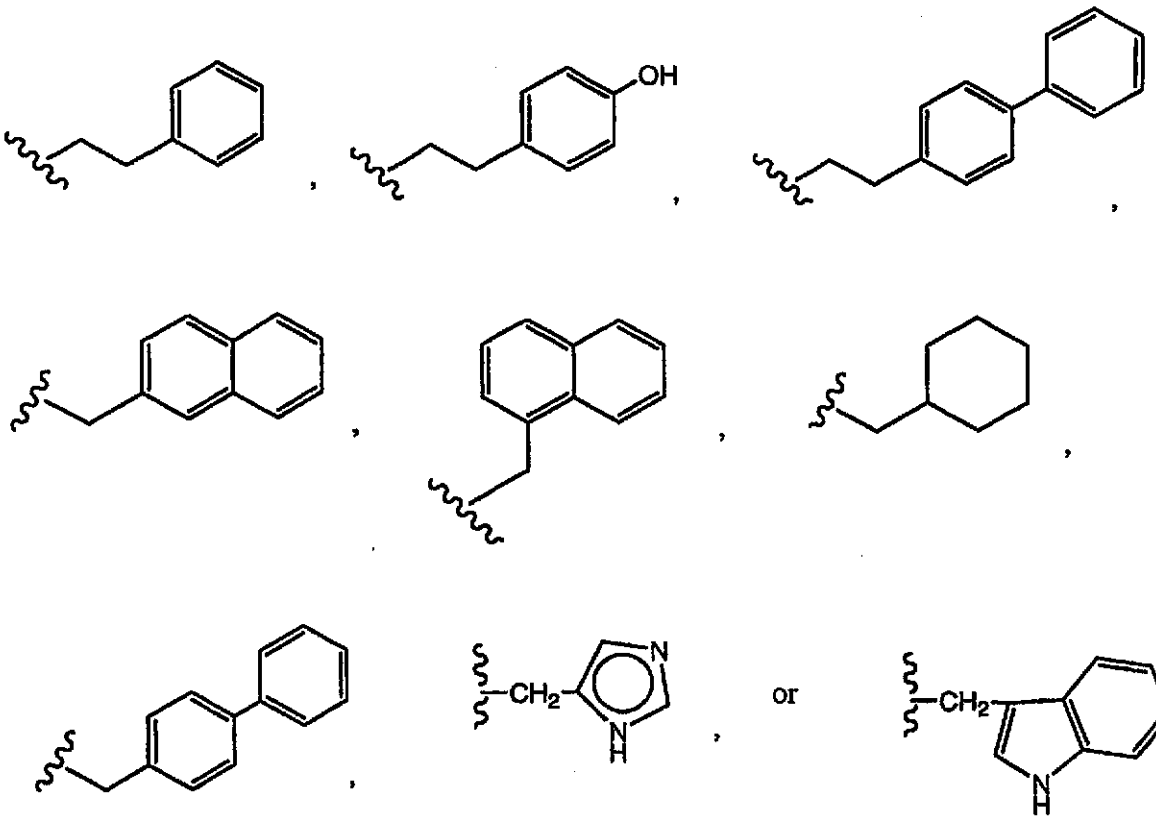
The Amino Acid unit (-W-), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the Drug moiety if the Spacer unit is absent, and links the Ligand unit to the Drug unit if the Stretcher unit and Spacer unit are absent.

$W_w$- 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:



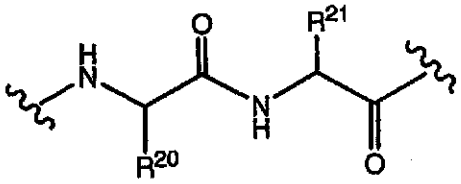
, or

wherein $R^{19}$ is hydrogen, methyl, isopropyl, isobutyl, *sec*-butyl, benzyl, *p*-hydroxybenzyl, $-CH_2OH$, $-CH(OH)CH_3$, $-CH_2CH_2SCH_3$, $-CH_2CONH_2$, $-CH_2COOH$, $-CH_2CH_2CONH_2$, $-CH_2CH_2COOH$, $-(CH_2)_3NHC(=NH)NH_2$, $-(CH_2)_3NH_2$, $-(CH_2)_3NHCOCH_3$, $-(CH_2)_3NHCHO$, $-(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_4NHCOCH_3$, $-(CH_2)_4NHCHO$, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NHCONH_2$, $-CH_2CH_2CH(OH)CH_2NH_2$, 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).

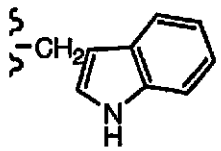
Illustrative $W_w$ units are represented by formulas (VII)-(IX):




**(VII)**

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

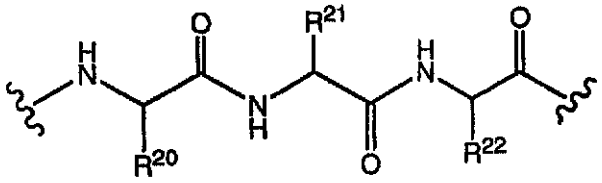
| $R^{20}$ | $R^{21}$ |
|---|---|
| benzyl | $(CH_2)_4NH_2$; |
| methyl | $(CH_2)_4NH_2$; |
| isopropyl | $(CH_2)_4NH_2$; |
| isopropyl | $(CH_2)_3NHCONH_2$; |
| benzyl | $(CH_2)_3NHCONH_2$; |
| isobutyl | $(CH_2)_3NHCONH_2$; |
| *sec*-butyl | $(CH_2)_3NHCONH_2$; |



$(CH_2)_3NHCONH_2$;

benzyl — methyl; and

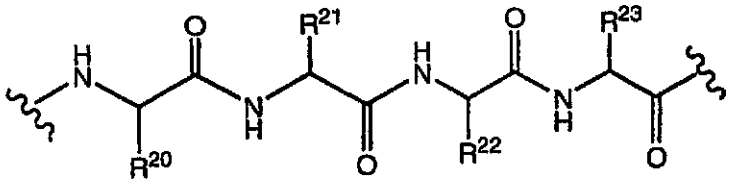
benzyl — $(CH_2)_3NHC(=NH)NH_2$;



(VIII)

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

| $R^{20}$ | $R^{21}$ | $R^{22}$ |
|---|---|---|
| benzyl | benzyl | $(CH_2)_4NH_2$; |
| isopropyl | benzyl | $(CH_2)_4NH_2$; and |
| H | benzyl | $(CH_2)_4NH_2$; |



(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 $-(CH_2)_4NH_2$; $R^{20}$ isopropyl and $R^{21}$ is $-(CH_2)_4NH_2$; $R^{20}$ isopropyl and $R^{21}$ is $-(CH_2)_3NHCONH_2$. Another exemplary Amino Acid unit is a unit of formula (VIII) wherein $R^{20}$ is benzyl, $R^{21}$ is benzyl, and $R^{22}$ is $-(CH_2)_4NH_2$.

Useful $-W_w-$ 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.

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.

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.* fk). 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, isonepecotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepecotic acid.

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

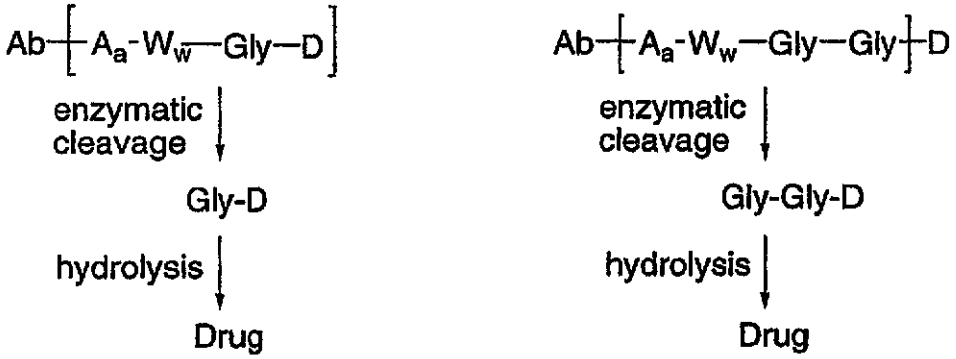
The Spacer unit (-Y-), when present, links an Amino Acid unit to the Drug moiety when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the Drug moiety when the Amino Acid unit is absent. The Spacer unit also links the Drug moiety to the Ligand unit when both the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

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 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-$A_a$-Ww-. 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, $-Y_y-$ is a p-aminobenzyl alcohol (PAB) unit (see Schemes 2 and 3) whose phenylene portion is substituted with $Q_m$ wherein Q is $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), -halogen,- nitro or -cyano; and m is an integer ranging from 0-4.

Scheme 1

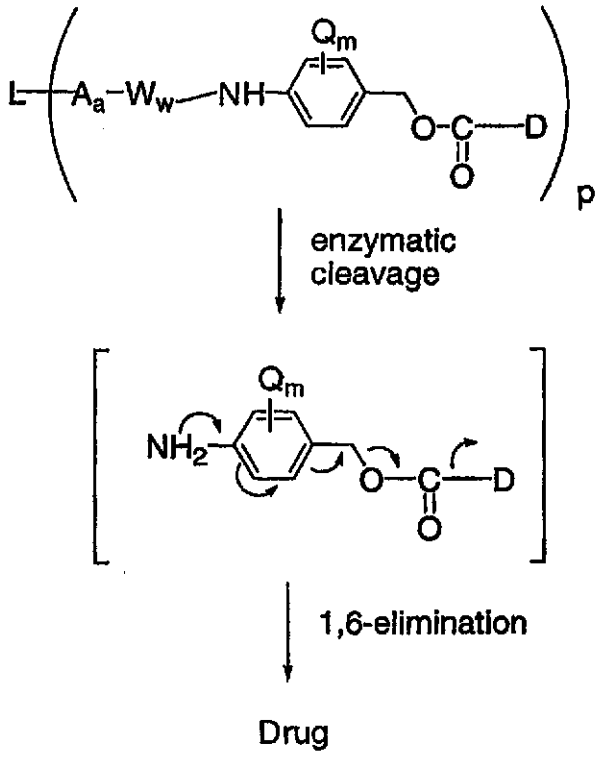



In one embodiment, a non self-immolative Spacer unit (-Y-) is -Gly-Gly-. In another embodiment, a non self-immolative the Spacer unit (-Y-) is -Gly-.

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 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.

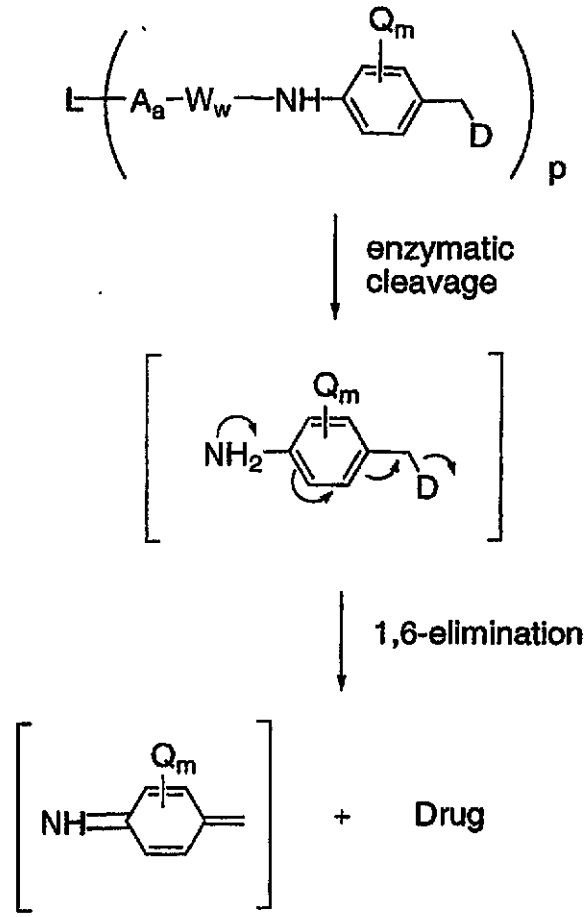
Scheme 2




wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

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

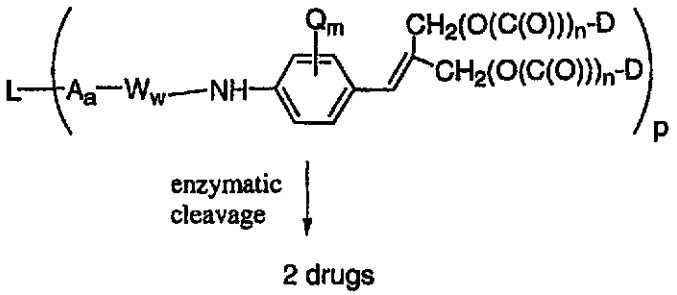



wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen,- nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

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-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 a-position of glycine (Kingsbury, *et al.*, J. Med. Chem., 1984, 27, 1447) 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.

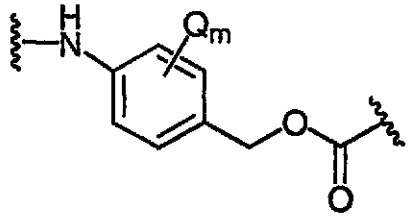
Scheme 4




wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ 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.

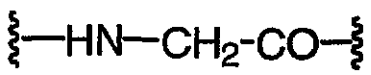
In one embodiment, the -D moieties are the same. In yet another embodiment, the -D moieties are different.

In one aspect, Spacer units (-$Y_y$-) are represented by Formulas (**X**)-(**XII**):



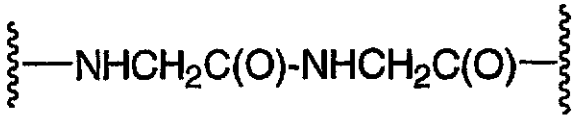

**X**

wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4;



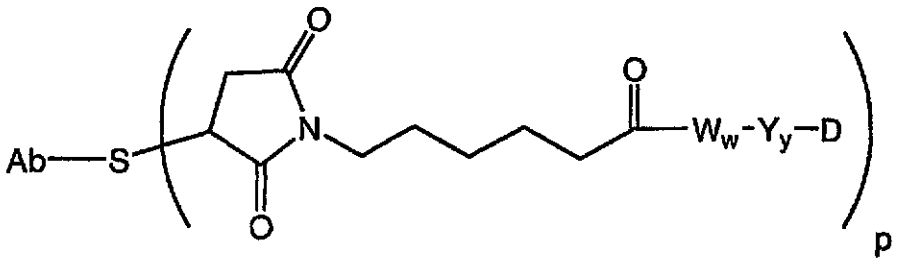

**XI**

and

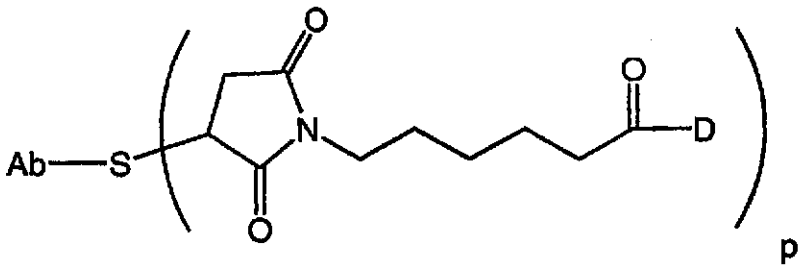



**XII.**

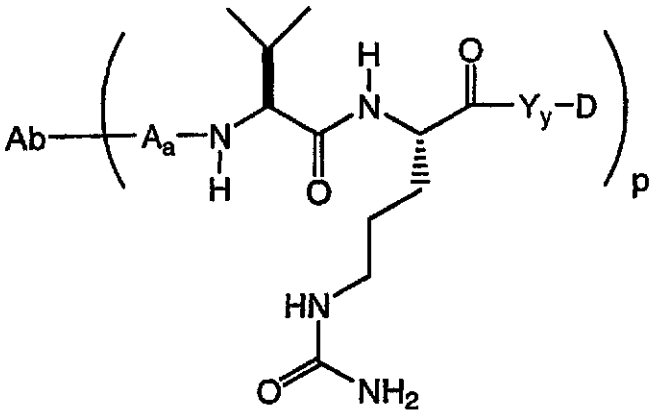
Embodiments of the Formula **Ia' and Ic** antibody-drug conjugate compounds include:



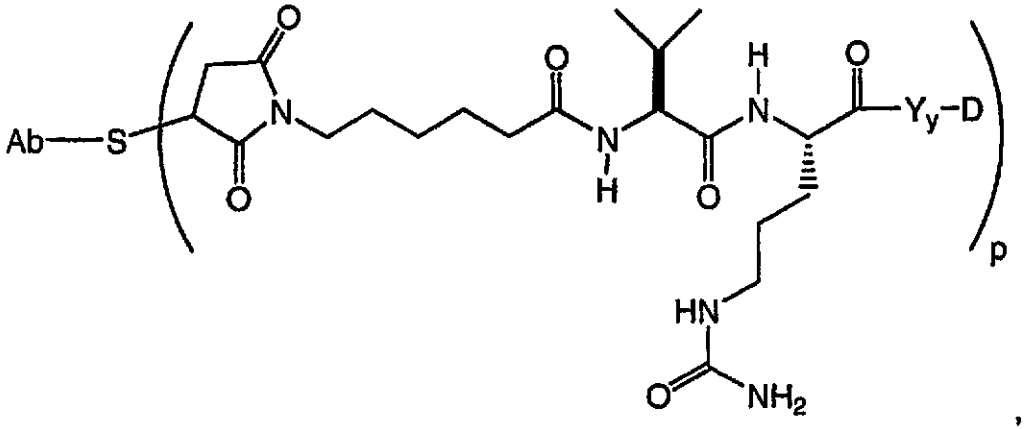
and,



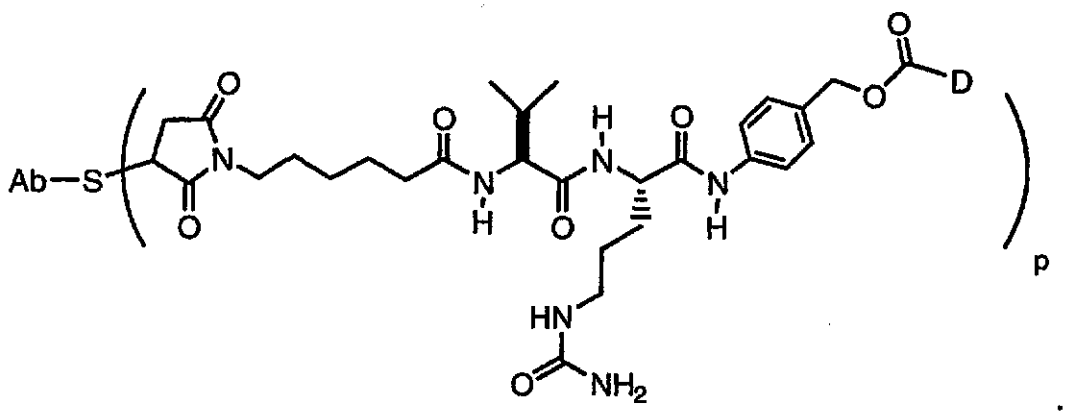
wherein w and y are each 0,



,



, and

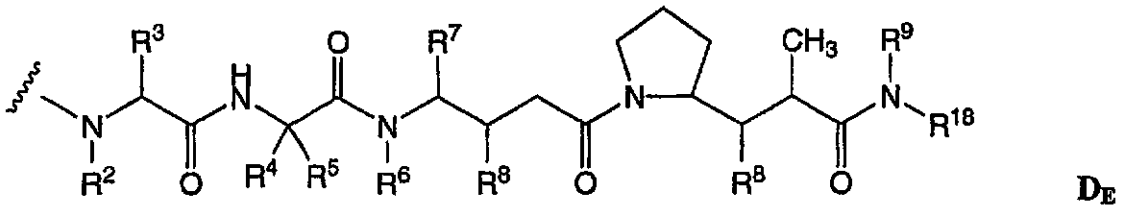


## *4.4 THE DRUG UNIT (MOIETY)*

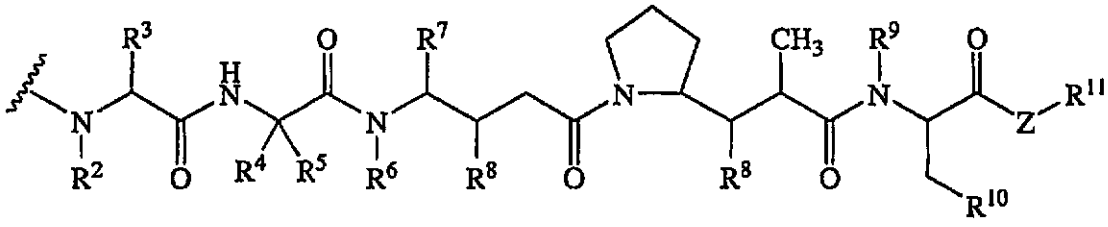
The drug moiety (D) of the antibody drug conjugates (ADC) are of the dolastatin/auristatin type (U.S. Patent Nos. 5635483; 5780588) which have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke *et al.* (2001) Antimicrob. Agents and Chemother. 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 $\mathbf{D_E}$ or $\mathbf{D_F}$:



$\mathbf{D_E}$



$\mathbf{D_F}$

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.

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

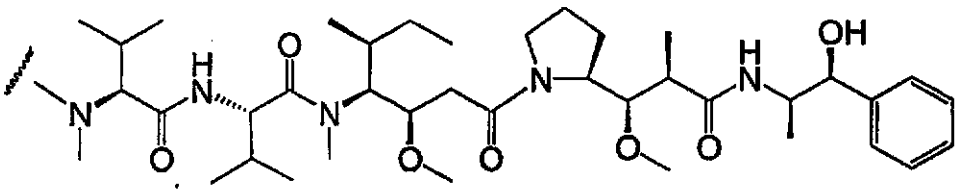
In an exemplary embodiment, $R^{10}$ is -phenyl.

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

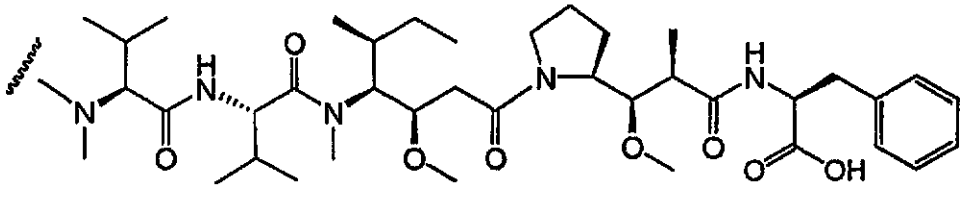
In one embodiment, when Z is -NH, $R^{11}$ is $-CH(R^{15})_2$, wherein $R^{15}$ is $-(CH_2)_n-N(R^{16})_2$, and $R^{16}$ is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

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

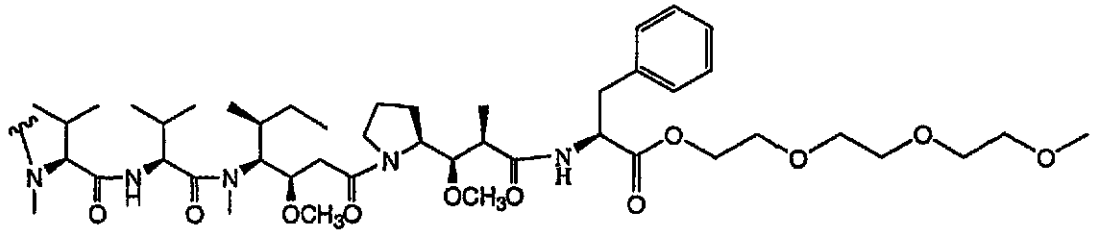
Illustrative Drug units (-D) include the drug units having the following structures:

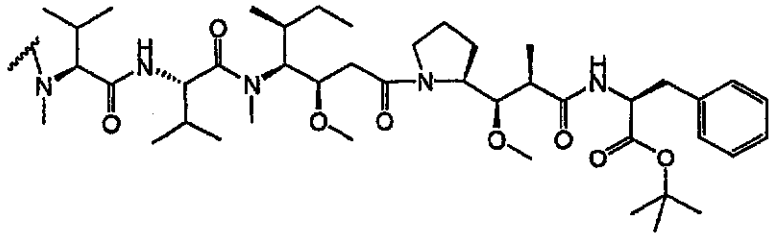


MMAE

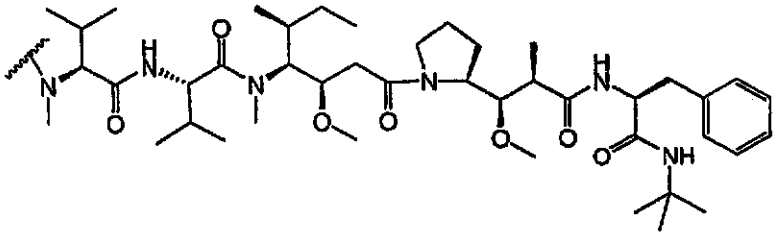


MMAF

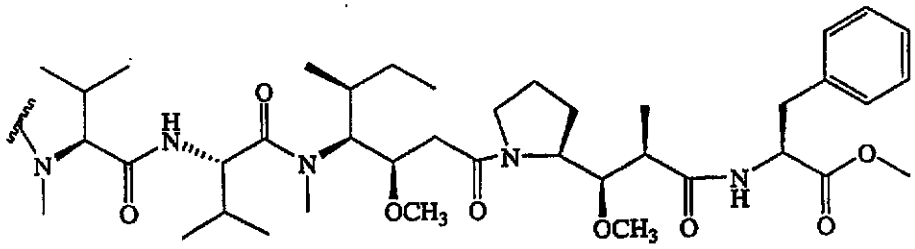




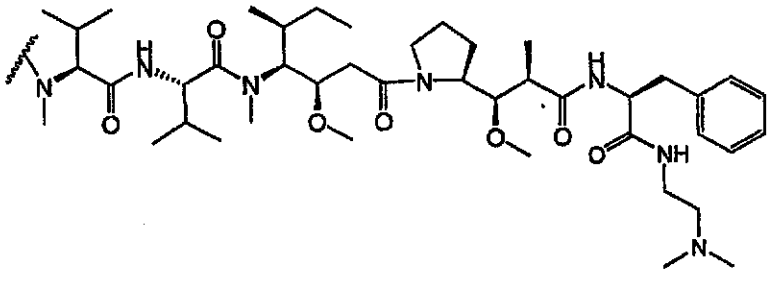
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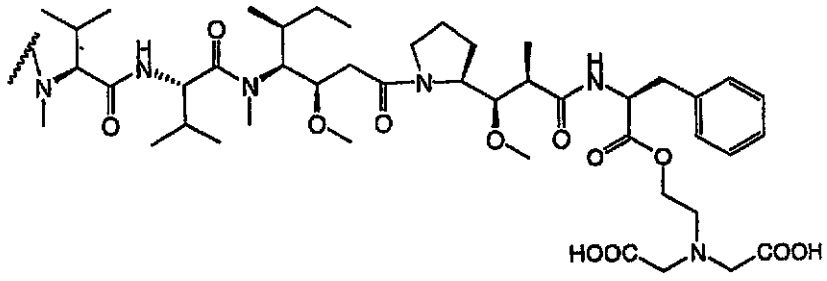
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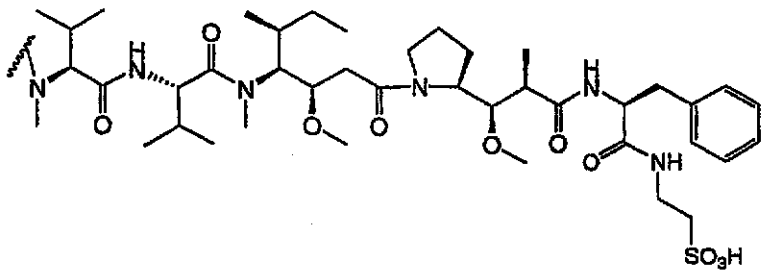
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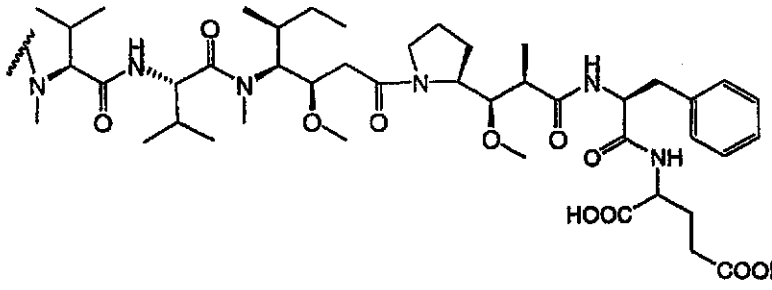


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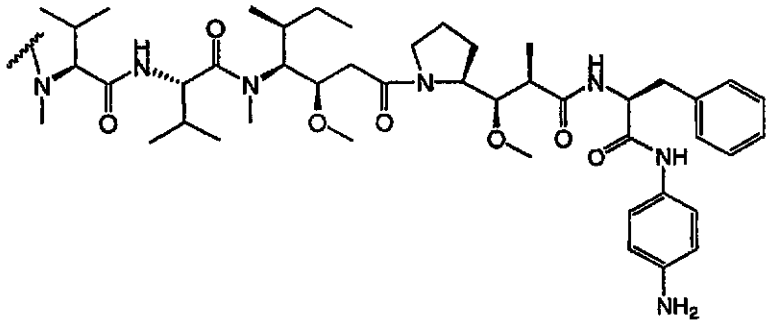


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and pharmaceutically acceptable salts or solvates thereof.

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 $R^{11}$. Without 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 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"), 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-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 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 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 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 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).

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 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 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. 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 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 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, 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.

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 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, "functionally active" means that the fragment, derivative or analog is able to elicit anti-anti-idiotype 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 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 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 $IgG_1$ 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, PA, 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 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-$U_1$RNP; Anti-La/SSB; Anti SSA; Anti-SSB; Anti-Perital Cells Antibody; Anti-Histones; Anti-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 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, 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 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, 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 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.*, 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 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 hepatitis B virus; PROTOVIR (Protein Design Labs, Inc., CA) which is a humanized $IgG_1$ 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 gonorrheae, Neisseria meningitidis, Corynebacterium diphtheriae, Clostridium botulinum, Clostridium perfringens, Clostridium tetani, Hemophilus influenzae, Klebsiella pneumoniae, Klebsiella ozaenas, Klebsiella rhinoscleromotis, Staphylococc aureus, Vibrio colerae, Escherichia coli, Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Bacillus cereus, Edwardsiella tarda, Yersinia enterocolitica, Yersinia pestis, 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 (Entomoeba histolytica, Toxoplasma gondii, Trichomonas tenas, Trichomonas hominis, Trichomonas vaginalis, Tryoanosoma gambiense, Trypanosoma rhodesiense, Trypanosoma cruzi, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria); or Helminiths (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 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

MLLRSAGKLNVGTKKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEED
DSGLPVVTSGCLGLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVD
GPIHHRALLISVTVCSLLLVLIILFCYFRYKRQETRPRYSIGLEQDETYIPPGESLRDLI
EQSQSSGSGSGLPLLVQRTIAKQIQMVKQIGKGRYGEVWMGKWRGEKVAVKVFFTTEEAS
WFRETEIYQTVLMRHENILGFIAADIKGTGSWTQLYLITDYHENGSLYDYLKSTTLDAKS
MLKLAYSSVSGLCHLHTEIFSTQGKPAIAHRDLKSKNILVKKNGTCCIADLGLAVKFISD
TNEVDIPPNTRVGTKRYMPPEVLDESLNRNHFQSYIMADMYSFGLILWEVARRCVSGGIV
EEYQLPYHDLVPSDPSYEDMREIVCIKKLRPSFPNRWSSDECLRQMGKLMTECWAHNPAS
RLTALRVKKTLAKMSESQDIKL

(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);

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);

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

MAGAGPKRRALAAPAAEEKEEAREKMLAAKSADGSAPAGEGEGVTLQRNITLLNGVAIIV
GTIIGSGIFVTPTGVLKEAGSPGLALVVWAACGVFSIVGALCYAELGTTISKSGGDYAYM
LEVYGSLPAFLKLWIELLIIRPSSQYIVALVFATYLLKPLFPTCPVPEEAAKLVACLCVL
LLTAVNCYSVKAATRVQDAFAAAKLLALALIILLGFVQIGKGVVSNLDPNFSFEGTKLDV
GNIVLALYSGLFAYGGWNYLNFVTEEMINPYRNLPLAIIISLPIVTLVYVLTNLAYFTTL
STEQMLSSEAVAVDFGNYHLGVMSWIIPVFVGLSCFGSVNGSLFTSSRLFFVGSREGHLP
SILSMIHPQLLTPVPSLVFTCVMTLLYAFSKDIFSVINFFSFFNWLCVALAIIGMIWLRH
RKPELERPIKVNLALPVFFILACLFLIAVSFWKTPVECGIGFTIILSGLPVYFFGVWWKN
KPKWLLQGIFSTTVLCQKLMQVVPQET

(SEQ ID NO: 2)

(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); 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

Cross-references: MIM:604415; NP_036581.1; NM_012449_1

339 aa

MESRKDITNQEELWKMKPRRNLEEDDYLHKDTGETSMLKRPVLLHLHQTAHADEFDCPSE
LQHTQELFPQWHLPIKIAAIIASLTFLYTLLREVIHPLATSHQQYFYKIPILVINKVLPM
VSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKWMLTRKQFGLLSFFFAVLHAIYSL
SYPMRRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEIYVSLGIVGLAILALLAVTSIPS
VSDSLTWREFHYIQSKLGIVSLLLGTIHALIFAWNKWIDIKQFVWYTPPTFMIAVFLPIV
VLIFKSILFLPCLRKKILKIRHGWEDVTKINKTEICSQL

(SEQ ID NO 3)

(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);

Cross-references: GI:34501467; AAK74120.3; AF361486_1

6995 aa

PVTSLLTPGLVITTDRMGISREPGTSSTSNLSSTSHERLTTLEDTVDTEAMQPSTHTAVT
NVRTSISGHESQSSVLSDSETPKATSPMGTTYTMGETSVSISTSDFFETSRIQIEPTSSL
TSGLRETSSSERISSATEGSTVLSEVPSGATTEVSRTEVISSRGTSMSGPDQFTISPDIS
TEAITRLSTSPIMTESAESAITIETGSPGATSEGTLTLDTSTTTFWSGTHSTASPGFSHS
EMTTLMSRTPGDVPWPSLPSVEEASSVSSSLSSPAMTSTSFFSTLPESISSSPHPVTALL
TLGPVKTTDMLRTSSEPETSSPPNLSSTSAEILATSEVTKDREKIHPSSNTPVVNVGTVI
YKHLSPSSVLADLVTTKPTSPMATTSTLGNTSVSTSTPAFPETMMTQPTSSLTSGLREIS
TSQETSSATERSASLSGMPTGATTKVSRTEALSLGRTSTPGPAQSTISPEISTETITRIS
TPLTTTGSAEMTITPKTGHSGASSQGTFTLDTSSRASWPGTHSAATHRSPHSGMTTPMSR
GPEDVSWPSRPSVEKTSPPSSLVSLSAVTSPSPLYSTPSESSHSSPLRVTSLFTPVMMKT
TDMLDTSLEPVTTSPPSMNITSDESLATSKATMETEAIQLSENTAVTQMGTISARQEFYS
SYPGLPEPSKVTSPVVTSSTIKDIVSTTIPASSEITRIEMESTSTLTPTPRETSTSQEIH
SATKPSTVPYKALTSATIEDSMTQVMSSSRGPSPDQSTMSQDISTEVITRLSTSPIKTES
TEMTITTQTGSPGATSRGTLTLDTSTTFMSGTHSTASQGFSHSQMTALMSRTPGEVPWLS
HPSVEEASSASFSLSSPVMTSSSPVSSTLPDSIHSSSLPVTSLLTSGLVKTTELLGTSSE
PETSSPPNLSSTSAEILATTEVTTDTEKLEMTNVVTSGYTHESPSSVLADSVTTKATSSM
GITYPTGDTNVLTSTPAFSDTSRIQTKSKLSLTPGLMETSISEETSSATEKSTVLSSVPT
GATTEVSRTEAISSSRTSIPGPAQSTMSSDTSMETITRISTPLTRKESTDMAITPKTGPS
GATSQGTFTLDSSSTASWPGTHSATTQRFPRSVVTTPMSRGPEDVSWPSPLSVEKNSPPS
SLVSSSSVTSPSPLYSTPSGSSHSSPVPVTSLFTSIMMKATDMLDASLEPETTSAPNMNI
TSDESLAASKATTETEAIHVFENTAASHVETTSATEELYSSSPGFSEPTKVISPVVTSSS
IRDNMVSTTMPGSSGITRIEIESMSSLTPGLRETRTSQDITSSTETSTVLYKMPSGATPE
VSRTEVMPSSRTSIPGPAQSTMSLDISDEVVTRLSTSPIMTESAEITITTQTGYSLATSQ
VTLPLGTSMTFLSGTHSTMSQGLSHSEMTNLMSRGPESLSWTSPRFVETTRSSSSLTSLP
LTTSLSPVSSTLLDSSPSSPLPVTSLILPGLVKTTEVLDTSSEPKTSSSPNLSSTSVEIP
ATSEIMTDTEKIHPSSNTAVAKVRTSSSVHESHSSVLADSETTITIPSMGITSAVEDTTV

FTSNPAFSETRRIPTEPTFSLTPGFRETSTSEETTSITETSAVLFGVPTSATTEVSMTEI
MSSNRTHIPDSDQSTMSPDIITEVITRLSSSSMMSESTQMTITTQKSSPGATAQSTLTLA
TTTAPLARTHSTVPPRFLHSEMTTLMSRSPENPSWKSSPFVEKTSSSSSLLSLPVTTSPS
VSSTLPQSIPSSSFSVTSLLTPGMVKTTDTSTEPGTSLSPNLSGTSVEILAASEVTTDTE
KIHPSSSMAVTNVGTTSSGHELYSSVSIHSEPSKATYPVGTPSSMAETSISTSMPANFET
TGFEAEPFSHLTSGLRKTNMSLDTSSVTPTNTPSSPGSTHLLQSSKTDFTSSAKTSSPDW
PPASQYTEIPVDIITPFNASPSITESTGITSFPESRFTMSVTESTHHLSTDLLPSAETIS
TGTVMPSLSEAMTSFATTGVPRAISGSGSPFSRTESGPGDATLSTIAESLPSSTPVPFSS
STFTTTDSSTIPALHEITSSSATPYRVDTSLGTESSTTEGRLVMVSTLDTSSQPGRTSSS
PILDTRMTESVELGTVTSAYQVPSLSTRLTRTDGIMEHITKIPNEAAHRGTIRPVKGPQT
STSPASPKGLHTGGTKRMETTTTALKTTTTALKTTSRATLTTSVYTPTLGTLTPLNASMQ
MASTIPTEMMITTPYVFPDVPETTSSLATSLGAETSTALPRTTPSVFNRESETTASLVSR
SGAERSPVIQTLDVSSSEPDTTASWVIHPAETIPTVSKTTPNFFHSELDTVSSTATSHGA
DVSSAIPTNISPSELDALTPLVTISGTDTSTTFPTLTKSPHETETRTTWLTHPAETSSTI
PRTIPNFSHHESDATPSIATSPGAETSSAIPIMTVSPGAEDLVTSQVTSSGTDRNMTIPT
LTLSPGEPKTIASLVTHPEAQTSSAIPTSTISPAVSRLVTSMVTSLAAKTSTTNRALTNS
PGEPATTVSLVTHSAQTSPTVPWTTSIFFHSKSDTTPSMTTSHGAESSSAVPTPTVSTEV
PGVVTPLVTSSRAVISTTIPILTLSPGEPETTPSMATSHGEEASSAIPTPTVSPGVPGVV
TSLVTSSRAVTSTTIPILTFSLGEPETTPSMATSHGTEAGSAVPTVLPEVPGMVTSLVAS
SRAVTSTTLPTLTLSPGEPETTPSMATSHGAEASSTVPTVSPEVPGVVTSLVTSSSGVNS
TSIPTLILSPGELETTPSMATSHGAEASSAVPTPTVSPGVSGVVTPLVTSSRAVTSTTIP
ILTLSSSEPETTPSMATSHGVEASSAVLTVSPEVPGMVTFLVTSSRAVTSTTIPTLTISS
DEPETTTSLVTHSEAKMISAIPTLGVSPTVQGLVTSLVTSSGSETSAFSNLTVASSQPET
IDSWVAHPGTEASSVVPTLTVSTGEPFTNISLVTHPAESSSTLPRTTSRFSHSELDTMPS
TVTSPEAESSSAISTTISPGIPGVLTSLVTSSGRDISATFPTVPESPHESEATASWVTHP
AVTSTTVPRTTPNYSHSEPDTTPSIATSPGAEATSDFPTITVSPDVPDMVTSQVTSSGTD
TSITIPTLTLSSGEPETTTSFITYSETHTSSAIPTLPVSPDASKMLTSLVISSGTDSTTT
FPTLTETPYEPETTAIQLIHPAETNTMVPRTTPKFSHSKSDTTLPVAITSPGPEASSAVS
TTTISPDMSDLVTSLVPSSGTDTSTTFPTLSETPYEPETTATWLTHPAETSTTVSGTIPN
FSHRGSDTAPSMVTSPGVDTRSGVPTTTIPPSIPGVVTSQVTSSATDTSTAIPTLTPSPG
EPETTASSATHPGTQTGFTVPIRTVPSSEPDTMASWVTHPPQTSTPVSRTTSSFSHSSPD
ATPVMATSPRTEASSAVLTTISPGAPEMVTSQITSSGAATSTTVPTLTHSPGMPETTALL
STHPRTETSKTFPASTVFPQVSETTASLTIRPGAETSTALPTQTTSSLFTLLVTGTSRVD
LSPTASPGVSAKTAPLSTHPGTETSTMIPTSTLSLGLLETTGLLATSSSAETSTSTLTLT
VSPAVSGLSSASITTDKPQTVTSWNTETSPSVTSVGPPEFSRTVTGTTMTLIPSEMPTPP
KTSHGEGVSPTTILRTTMVEATNLATTGSSPTVAKTTTTFNTLAGSLFTPLTTPGMSTLA
SESVTSRTSYNHRSWISTTSSYNRRYWTPATSTPVTSTFSPGISTSSIPSSTAATVPFMV
PFTLNFTITNLQYEEDMRHPGSRKFNATERELQGLLKPLFRNSSLEYLYSGCRLASLRPE
KDSSATAVDAICTHRPDPEDLGLDRERLYWELSNLTNGIQELGPYTLDRNSLYVNGFTHR
SSMPTTSTPGTSTVDVGTSGTPSSSPSPTTAGPLLMPFTLNFTITNLQYEEDMRRTGSRK
FNTMESVLQGLLKPLFKNTSVGPLYSGCRLTLLRPEKDGAATGVDAICTHRLDPKSPGLN
REQLYWELSKLTNDIEELGPYTLDRNSLYVNGFTHQSSVSTTSTPGTSTVDLRTSGTPSS
LSSPTIMAAGPLLVPFTLNFTITNLQYGEDMGHPGSRKFNTTERVLQGLLGPIFKNTSVG
PLYSGCRLTSLRSEKDGAATGVDAICIHHLDPKSPGLNRERLYWELSQLTNGIKELGPYT
LDRNSLYVNGFTHRTSVPTTSTPGTSTVDLGTSGTPFSLPSPATAGPLLVLFTLNFTITN

LKYEEDMHRPGSRKFNTTERVLQTLVGPMFKNTSVGLLYSGCRLTLLRSEKDGAATGVDA
ICTHRLDPKSPGVDREQLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHWIPVPTSSTPG
TSTVDLGSGTPSSLPSPTSATAGPLLVPFTLNFTITNLKYEEDMHCPGSRKFNTTERVLQ
SLLGPMFKNTSVGPLYSGCRLTLLRSEKDGAATGVDAICTHRLDPKSPGVDREQLYWELS
QLTNGIKELGPYTLDRNSLYVNGFTHQTSAPNTSTPGTSTVDLGTSGTPSSLPSPTSAGP
LLVPFTLNFTITNLQYEEDMHHPGSRKFNTTERVLQGLLGPMFKNTSVGLLYSGCRLTLL
RPEKNGAATGMDAICSHRLDPKSPGLNREQLYWELSQLTHGIKELGPYTLDRNSLYVNGF
THRSSVAPTSTPGTSTVDLGTSGTPSSLPSPTTAVPLLVPFTLNFTITNLQYGEDMRHPG
SRKFNTTERVLQGLLGPLFKNSSVGPLYSGCRLISLRSEKDGAATGVDAICTHHLNPQSP
GLDREQLYWQLSQMTNGIKELGPYTLDRNSLYVNGFTHRSSGLTTSTPWTSTVDLGTSGT
PSPVPSPTTAGPLLVPFTLNFTITNLQYEEDMHRPGSRKFNATERVLQGLLSPIFKNSSV
GPLYSGCRLTSLRPEKDGAATGMDAVCLYHPNPKRPGLDREQLYWELSQLTHNITELGPY
SLDRDSLYVNGFTHQNSVPTTSTPGTSTVYWATTGTPSSFPGHTEPGPLLIPFTFNFTIT
NLHYEENMQHPGSRKFNTTERVLQGLLKPLFKNTSVGPLYSGCRLTLLRPEKQEAATGVD
TICTHRVDPIGPGLDRERLYWELSQLTNSITELGPYTLDRDSLYVNGFNPWSSVPTTSTP
GTSTVHLATSGTPSSLPGHTAPVPLLIPFTLNFTITNLHYEENMQHPGSRKFNTTERVLQ
GLLKPLFKSTSVGPLYSGCRLTLLRPEKHGAATGVDAICTLRLDPTGPGLDRERLYWELS
QLTNSVTELGPYTLDRDSLYVNGFTHRSSVPTTSIPGTSAVHLETSGTPASLPGHTAPGP
LLVPFTLNFTITNLQYEEDMRHPGSRKFNTTERVLQGLLKPLFKSTSVGPLYSGCRLTLL
RPEKRGAATGVDTICTHRLDPLNPGLDREQLYWELSKLTRGIIELGPYLLDRGSLYVNGF
THRNFVPITSTPGTSTVHLGTSETPSSLPRPIVPGPLLVPFTLNFTITNLQYEEAMRHPG
SRKFNTTERVLQGLLRPLFKNTSIGPLYSSCRLTLLRPEKDKAATRVDAICTHHPDPQSP
GLNREQLYWELSQLTHGITELGPYTLDRDSLYVDGFTHWSPIPTTSTPGTSIVNLGTSGI
PPSLPETTATGPLLVPFTLNFTITNLQYEENMGHPGSRKFNITESVLQGLLKPLFKSTSV
GPLYSGCRLTLLRPEKDGVATRVDAICTHRPDPKIPGLDRQQLYWELSQLTHSITELGPY
TLDRDSLYVNGFTQRSSVPTTSTPGTFTVQPETSETPSSLPGPTATGPVLLPFTLNFTII
NLQYEEDMHRPGSRKFNTTERVLQGLLMPLFKNTSVSSLYSGCRLTLLRPEKDGAATRVD
AVCTHRPDPKSPGLDRERLYWKLSQLTHGITELGPYTLDRHSLYVNGFTHQSSMTTTRTP
DTSTMHLATSRTPASLSGPTTASPLLVLFTINFTITNLRYEENMHHPGSRKFNTTERVLQ
GLLRPVFKNTSVGPLYSGCRLTLLRPKKDGAATKVDAICTYRPDPKSPGLDREQLYWELS
QLTHSITELGPYTLDRDSLYVNGFTQRSSVPTTSIPGTPTVDLGTSGTPVSKPGPSAASP
LLVLFTLNFTITNLRYEENMQHPGSRKFNTTERVLQGLLRSLFKSTSVGPLYSGCRLTLL
RPEKDGTATGVDAICTHHPDPKSPRLDREQLYWELSQLTHNITELGPYALDNDSLFVNGF
THRSSVSTTSTPGTPTVYLGASKTPASIFGPSAASHLLILFTLNFTITNLRYEENMWPGS
RKFNTTERVLQGLLRPLFKNTSVGPLYSGCRLTLLRPEKDGEATGVDAICTHRPDPTGPG
LDREQLYLELSQLTHSITELGPYTLDRDSLYVNGFTHRSSVPTTSTGVVSEEPFTLNFTI
NNLRYMADMGQPGSLKFNITDNVMQHLLSPLFQRSSLGARYTGCRVIALRSVKNGAETRV
DLLCTYLQPLSGPGLPIKQVFHELSQQTHGITRLGPYSLDKDSLYLNGYNEPGPDEPPTT
PKPATTFLPPLSEATTAMGYHLKTLTLNFTISNLQYSPDMGKGSATFNSTEGVLQHLLRP
LFQKSSMGPFYLGCQLISLRPEKDGAATGVDTTCTYHPDPVGPGLDIQQLYWELSQLTHG
VTQLGFYVLDRDSLFINGYAPQNLSIRGEYQINFHIVNWNLSNPDPTSSEYITLLRDIQD
KVTTLYKGSQLHDTFRFCLVTNLTMDSVLVTVKALFSSNLDPSLVEQVFLDKTLNASFHW
LGSTYQLVDIHVTEMESSVYQPTSSSSTQHFYLNFTITNLPYSQDKAQPGTTNYQRNKRN
IEDALNQLFRNSSIKSYFSDCQVSTFRSVPNRHHTGVDSLCNFSPLARRVDRVAIYEEFL
RMTRNGTQLQNFTLDRSSVLVDGYSPNRNEPLTGNSDLPFWAVILIGLAGLLGLITCLIC

GVLVTTRRRKKEGEYNVQQQCPGYYQSHLDLEDLQ

(SEQ ID NO:4)

(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 (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

MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDGVLANPPNISS
LSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDLDALPL
DLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAALACWGVRGSLLSEA
DVRALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTW
SVSTMDALRGLLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELY
PQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGHEMSPQVATLIDRFVK
GRGQLDKDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKA
RLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQ
KLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSMQEALSGT
PCLLGPGPVLTVLALLLASTLA

(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. 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 IV); WO200175177 (Claim 24; Page 139-140);
Cross-references: MIM:604217; NP_006415.1; NM_006424_1
690 aa

MAPWPELGDAQPNPDKYLEGAAGQQPTAPDKSKETNKTDNTEAPVTKIELLPSYSTATLI
DEPTEVDDPWNLPTLQDSGIKWSERDTKGKILCFFQGIGRLILLLGFLYFFVCSLDILSS
AFQLVGGKMAGQFFSNSSIMSNPLLGLVIGVLVTVLVQSSSTSTSIVVSMVSSSLLTVRA
AIPIIMGANIGTSITNTIVALMQVGDRSEFRRAFAGATVHDFFNWLSVLVLLPVEVATHY
LEIITQLIVESFHFKNGEDAPDLLKVITKPFTKLIVQLDKKVISQIAMNDEKAKNKSLVK
IWCKTFTNKTQINVTVPSTANCTSPSLCWTDGIQNWTMKNVTYKENIAKCQHIFVNFHLP
DLAVGTILLILSLLVLCGCLIMIVKILGSVLKGQVATVIKKTINTDFPFPFAWLTGYLAI
LVGAGMTFIVQSSSVFTSALTPLIGIGVITIERAYPLTLGSNIGTTTTAILAALASPGNA
LRSSLQIALCHFFFNISGILLWYPIPFTRLPIRMAKGLGNISAKYRWFAVFYLIIFFFLI
PLTVFGLSLAGWRVLVGVGVPVVFIIILVLCLRLLQSRCPRVLPKKLQNWNFLPLWMRSL
KPWDAVVSKFTGCFQMRCCYCCRVCCRACCLLCGCPKCCRCSKCCEDLEEAQEGQDVPVK
APETFDNITISREAQGEVPASDSKTECTAL

(SEQ ID NO:6)

(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,
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

MVLAGPLAVSLLLPSLTLLVSHLSSSQDVSSEPSSEQQLCALSKHPTVAFEDLQPWVSNF
TYPGARDFSQLALDPSGNQLIVGARNYLFRLSLANVSLLQATEWASSEDTRRSCQSKGKT
EEECQNYVRVLIVAGRKVFMCGTNAFSPMCTSRQVGNLSRTTEKINGVARCPYDPRHNST
AVISSQGELYAATVIDFSGRDPAIYRSLGSGPPLRTAQYNSKWLNEPNFVAAYDIGLFAY
FFLRENAVEHDCGRTVYSRVARVCKNDVGGRFLLEDTWTTFMKARLNCSRPGEVPFYYNE
LQSAFHLPEQDLIYGVFTTNVNSIAASAVCAFNLSAISQAFNGPFRYQENPRAAWLPIAN
PIPNFQCGTLPETGPNENLTERSLQDAQRLFLMSEAVQPVTPEPCVTQDSVRFSHLVVDL
VQAKDTLYHVLYIGTESGTILKALSTASRSLHGCYLEELHVLPPGRREPLRSLRILHSAR
ALFVGLRDGVLRVPLERCAAYRSQGACLGARDPYCGWDGKQQRCSTLEDSSNMSLWTQNI
TACPVRNVTRDGGFGPWSPWQPCEHLDGDNSGSCLCRARSCDSPRPRCGGLDCLGPAIHI
ANCSRNGAWTPWSSWALCSTSCGIGFQVRQRSCSNPAPRHGGRICVGKSREERFCNENTP
CPVPIFWASWGSWSKCSSNCGGGMQSRRRACENGNSCLGCGVEFKTCNPEGCPEVRRNTP
WTPWLPVNVTQGGARQEQRFRFTCRAPLADPHGLQFGRRRTETRTCPADGSGSCDTDALV
EDLLRSGSTSPHTVSGGWAAWGPWSSCSRDCELGFRVRKRTCTNPEPRNGGLPCVGDAAE
YQDCNPQACPVRGAWSCWTSWSPCSASCGGGHYQRTRSCTSPAPSPGEDICLGLHTEEAL
CATQACPEGWSPWSEWSKCTDDGAQSRSRHCEELLPGSSACAGNSSQSRPCPYSEIPVIL
PASSMEEATGCAGFNLIHLVATGISCFLGSGLLTLAVYLSCQHCQRQSQESTLVHPATPN

HLHYKGGGTPKNEKYTPMEFKTLNKNNLIPDDRANFYPLQQTNVYTTTYYPSPLNKHSFR
PEASPGQRCFPNS

(SEQ ID NO:7)

(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); US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20); Cross-references: GI:37182378; AAQ88991.1; AY358628_1

141 aa

MWVLGIAATFCGLFLLPGFALQIQCYQCEEFQLNNDCSSPEFIVNCTVNVQDMCQKEVME
QSAGIMYRKSCASSAACLIASAGYQSFCSPGKLNSVCISCCNTPLCNGPRPKKRGSSASA
LRPGLRTTILFLKLALFSAHC

(SEQ ID NO:8)

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463); Nakamuta M., *et al.* Biochem. Biophys. Res. Commun. 177, 34-39, 1991; Ogawa Y., *et al.* Biochem. Biophys. Res. Commun. 178, 248-255, 1991; Arai H., *et al.* Jpn. Circ. J. 56, 1303-1307, 1992; Arai H., *et al.* J. Biol. Chem. 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., *et al.* Biochem. Biophys. Res. Commun. 178, 656-663, 1991; Elshourbagy N.A., *et al.* J. Biol. Chem. 268, 3873-3879, 1993; Haendler B., *et al.* J. Cardiovasc. Pharmacol. 20, s1-S4, 1992; Tsutsumi M., *et al.* Gene 228, 43-49, 1999; Strausberg R.L., *et al.* Proc. Natl. Acad. Sci. USA. 99, 16899-16903, 2002; Bourgeois C., *et al.* J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., *et al.* Biol. Chem. 272, 21589-21596, 1997; Verheij J.B., *et al.* Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R.M.W., *et al.* Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger *E.G., et al.* Cell 79, 1257-1266, 1994; Attie T., et al, Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., *et al.* Hum. Mol. Genet. 5:351-354, 1996; Amiel J., *et al.* Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R.M.W., *et al.* Nat. Genet. 12, 445-447, 1996; Svensson P.J., *et al.* Hum. Genet. 103, 145-148, 1998; Fuchs S., *et al.* Mol. Med. 7, 115-124, 2001; Pingault V., *et al.* (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1); 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); WO2004001004;

442 aa

MQPPPSLCGRALVALVLACGLSRIWGEERGFPPDRATPLLQTAEIMTPPTKTLWPKGSNA
SLARSLAPAEVPKGDRTAGSPPRTISPPPCQGPIEIKETFKYINTVVSCLVFVLGIIGNS
TLLRIIYKNKCMRNGPNILIASLALGDLLHIVIDIPINVYKLLAEDWPFGAEMCKLVPFI
QKASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVVLAVPEAIGF
DIITMDYKGSYLRICLLHPVQKTAFMQFYKTAKDWWLFSFYFCLPLAITAFFYTLMTCEM
LRKKSGMQIALNDHLKQRREVAKTVFCLVLVFALCWLPLHLSRILKLTLYNQNDPNRCEL
LSFLLVLDYIGINMASLNSCINPIALYLVSKRFKNCFKSCLCCWCQSFEEKQSLEEKQSC
LKFKANDHGYDNFRSSNKYSSS

(SEQ ID NO:9)

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

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

MSGGHQLQLAALWPWLLMATLQAGFGRTGLVLAAAVESERSAEQKAIIRVIPLKMDPTGK
LNLTLEGVFAGVAEITPAEGKLMQSHPLYLCNASDDDNLEPGFISIVKLESPRRAPRPCL
SLASKARMAGERGASAVLFDITEDRAAAEQLQQPLGLTWPVVLIWGNDAEKLMEFVYKNQ
KAHVRIELKEPPAWPDYDVWILMTVVGTIFVIILASVLRIRCRPRHSRPDPLQQRTAWAI
SQLATRRYQASCRQARGEWPDSGSSCSSAPVCAICLEEFSEGQELRVISCLHEFHRNCVD
PWLHQHRTCPLCVFNITEGDSFSQSLGPSRSYQEPGRRLHLIRQHPGHAHYHLPAAYLLG
PSRSAVARPPRPGPFLPSQEPGMGPRHHRFPRAAHPRAPGEQQRLAGAQHPYAQGWGMSH
LQSTSQHPAACPVPLRRARPPDSSGSGESYCTERSGYLADGPASDSSSGPCHGSSSDSVV
NCTDISLQGVHGSSSTFCSSLSSDFDPLVYCSPKGDPQRVDMQPSVTSRPRSLDSVVPTG
ETQVSSHVHYHRHRHHHYKKRFQWHGRKPGPETGVPQSRPPIPRTQPQPEPPSPDQQVTG
SNSAAPSGRLSNPQCPRALPEPAPGPVDASSICPSTSSLFNLQKSSLSARHPQRKRRGGP
SEPTPGSRPQDATVHPACQIFPHYTPSVAYPWSPEAHPLICGPPGLDKRLLPETPGPCYS
NSQPVWLCLTPRQPLEPHPPGEGPSEWSSDTAEGRPCPYPHCQVLSAQPGSEEELEELCE
QAV

(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
RNPKFASEFFPHVVDVTHHEDALTKTNIIFVAIHREHYTSLWDLRHLLVGKILIDVSNNM
RINQYPESNAEYLASLFPDSLIVKGFNVVSAWALQLGPKDASRQVYICSNNIQARQQVIE
LARQLNFIPIDLGSLSSAREIENLPLRLFTLWRGPVVVAISLATFFFLYSFVRDVIHPYA
RNQQSDFYKIPIEIVNKTLPIVAITLLSLVYLAGLLAAAYQLYYGTKYRRFPPWLETWLQ
CRKQLGLLSFFFAMVHVAYSLCLPMRRSERYLFLNMAYQQVHANIENSWNEEEVWRIEMY
ISFGIMSLGLLSLLAVTSIPSVSNALNWREFSFIQSTLGYVALLISTFHVLIYGWKRAFE
EEYYRFYTPPNFVLALVLPSIVILGKIILFLPCISQKLKRIKKGWEKSQFLEEGIGGTIP
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

MVVPEKEQSWIPKIFKKKTCTTFIVDSTDPGGTLCQCGRPRTAHPAVAMEDAFGAAVVTV
WDSDAHTTEKPTDAYGELDFTGAGRKHSNFLRLSDRTDPAAVYSLVTRTWGFRAPNLVVS
VLGGSGGPVLQTWLQDLLRRGLVRAAQSTGAWIVTGGLHTGIGRHVGVAVRDHQMASTGG
TKVVAMGVAPWGVVRNRDTLINPKGSFPARYRWRGDPEDGVQFPLDYNYSAFFLVDDGTH

GCLGGENRFRLRLESYISQQKTGVGGTGIDIPVLLLLIDGDEKMLTRIENATQAQLPCLL
VAGSGGAADCLAETLEDTLAPGSGGARQGEARDRIRRFFPKGDLEVLQAQVERIMTRKEL
LTVYSSEDGSEEFETIVLKALVKACGSSEASAYLDELRLAVAWNRVDIAQSELFRGDIQW
RSFHLEASLMDALLNDRPEFVRLLISHGLSLGHFLTPMRLAQLYSAAPSNSLIRNLLDQA
SHSAGTKAPALKGGAAELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGESMYLL
SDKATSPLSLDAGLGQAPWSDLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMA
RLEPDAEEAARRKDLAFKFEGMGVDLFGECYRSSEVRAARLLLRRCPLWGDATCLQLAMQ
ADARAFFAQDGVQSLLTQKWWGDMASTTPIWALVLAFFCPPLIYTRLITFRKSEEEPTRE
ELEFDMDSVINGEGPVGTADPAEKTPLGVPRQSGRPGCCGGRCGGRRCLRRWFHFWGAPV
TIFMGNVVSYLLFLLLFSRVLLVDFQPAPPGSLELLLYFWAFTLLCEELRQGLSGGGGSL
ASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVGCRLTPGLYHLGRTVLCIDFM
VFTVRLLHIFTVNKQLGPKIVIVSKMMKDVFFFLFFLGVWLVAYGVATEGLLRPRDSDFP
SILRRVFYRPYLQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPPGAQAGTCVSQYANWLV
VLLLVIFLLVANILLVNLLIAMFSYTFGKVQGNSDLYWKAQRYRLIREFHSRPALAPPFI
VISHLRLLLRQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWESVHKENFLLARARDK
RESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLP
PGGPPPPDLPGSKD

(SEQ ID NO:12)

(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 (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

MDCRKMARFSYSVIWIMAISKVFELGLVAGLGHQEFARPSRGYLAFRDDSIWPQEEPAIR
PRSSQRVPPMGIQHSKELNRTCCLNGGTCMLGSFCACPPSFYGRNCEHDVRKENCGSVPH
DTWLPKKCSLCKCWHGQLRCFPQAFLPGCDGLVMDEHLVASRTPELPPSARTTTFMLVGI
CLSIQSYY

(SEQ ID NO:13)

(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. 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 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Claim 1); Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

1033 aa

MGAAGLLGVFLALVAPGVLGISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGE
KSLLCITKDKVDGTWDKPAPKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKT
NFSMNGNKSVWCQANNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVT
YSCESGYLLVGEKIINCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANF
FCDEGYRLQGPPSSRCVIAGQGVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANVSYGSI
VTYTCDPDPEEGVNFILIGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQCPHPQILRGR
MVSGQKDRYTYNDTVIFACMFGFTLKGSKQIRCNAQGTWEPSAPVCEKECQAPPNILNGQ
KEDRHMVRFDPGTSIKYSCNPGYVLVGEESIQCTSEGVWTPPVPQCKVAACEATGRQLLT
KPQHQFVRPDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLCKEITCPPPPVIYNGAHTG
SSLEDFPYGTTVTYTCNPGPERGVEFSLIGESTIRCTSNDQERGTWSGPAPLCKLSLLAV
QCSHVHIANGYKISGKEAPYFYNDTVTFKCYSGFTLKGSSQIRCKADNTWDPEIPVCEKE
TCQHVRQSLQELPAGSRVELVNTSCQDGYQLTGHAYQMCQDAENGIWFKKIPLCKVIHCH
PPPVIVNGKHTGMMAENFLYGNEVSYECDQGFYLLGEKKLQCRSDSKGHGSWSGPSPQCL
RSPPVTRCPNPEVKHGYKLNKTHSAYSHNDIVYVDCNPGFIMNGSRVIRCHTDNTWVPGV
PTCIKKAFIGCPPPPKTPNGNHTGGNIARFSPGMSILYSCDQGYLLVGEALLLCTHEGTW
SQPAPHCKEVNCSSPADMDGIQKGLEPRKMYQYGAVVTLECEDGYMLEGSPQSQCQSDHQ
WNPPLAVCRSRSLAPVLCGIAAGLILLTFLIVITLYVISKHRERNYYTDTSQKEAFHLEA
REVYSVDPYNPAS

(SEQ ID NO:14)

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

MARLALSPVPSHWMVALLLLLSAEPVPAARSEDRYRNPKGSACSRIWQSPRFIARKRGFT
VKMHCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIY
FCQQKCNNTSEVYQGCGTELRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFLL
LDKDDSKAGMEEDHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQE

(SEQ ID NO:15)

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

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

508 aa

MLLWSLLVIFDAVTEQADSLTLVAPSSVFEGDSIVLKCQGEQNWKIQKMAYHKDNKELSV
FKKFSDFLIQSAVLSDSGNYFCSTKGQLFLWDKTSNIVKIKVQELFQRPVLTASSFQPIE
GGPVSLKCETRLSPQRLDVQLQFCFFRENQVLGSGWSSSPELQISAVWSEDTGSYWCKAE
TVTHRIRKQSLQSQIHVQRIPISNVSLEIRAPGGQVTEGQKLILLCSVAGGTGNVTFSWY
REATGTSMGKKTQRSLSAELEIPAVKESDAGKYYCRADNGHVPIQSKVVNIPVRIPVSRP
VLTLRSPGAQAAVGDLLELHCEALRGSPPILYQFYHEDVTLGNSSAPSGGGASFNLSLTA
EHSGNYSCEANNGLGAQCSEAVPVSISGPDGYRRDLMTAGVLWGLFGVLGFTGVALLLYA
LFHKISGESSATNEPRGASRPNPQEFTYSSPTPDMEELQPVYVNVGSVDVDVVYSQVWSM
QQPESSANIRTLLENKDSQVIYSSVKKS

(SEQ ID NO:16)

(17) HER2 (ErbB2, Genbank accession no. M11730, Coussens L., *et al.* Science (1985) 230(4730):1132-1139); Yamamoto T., *et al.* Nature 319, 230-234, 1986; Semba K., *et al.* Proc. Natl. Acad. Sci. 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 1I); WO2004009622; 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); WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

1255 aa

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNL
ELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNG
DPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLA
LTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGPLPTDCCHEQC
AAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACP
YNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSAN
IQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLP
DLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTV
PWDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQEC
VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARC
PSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVG
ILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPLTPSGAMPNQAQMRILKETEL
RKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSP
YVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVR
LVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFT
HQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWM
IDSECRPRFRELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDMGDLVDA
EEYLVPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEG
AGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYV
NQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPEYLTPQ
GGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV

(SEQ ID NO:17)

(18) NCA (CEACAM6, Genbank accession no. M18728); Barnett T., etal Genomics 3, 59-66, 1988; Tawaragi Y., *et al.* Biochem. Biophys. Res. Commun. 150, 89-96, 1988; Strausberg R.L., *et al.* Proc. Natl. Acad. Sci. 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

MGPPSAPPCRLHVPWKEVLLTASLLTFWNPPTTAKLTIESTPFNVAEGKEVLLLAHNLPQ
NRIGYSWYKGERVDGNSLIVGYVIGTQQATPGPAYSGRETIYPNASLLIQNVTQNDTGFY
TLQVIKSDLVNEEATGQFHVYPELPKPSISSNNSNPVEDKDAVAFTCEPEVQNTTYLWWV
NGQSLPVSPRLQLSNGNMTLTLLSVKRNDAGSYECEIQNPASANRSDPVTLNVLYGPDVP
TISPSKANYRPGENLNLSCHAASNPPAQYSWFINGTFQQSTQELFIPNITVNNSGSYMCQ
AHNSATGLNRTTVTMITVSGSAPVLSAVATVGITIGVLARVALI

(SEQ ID NO:18)

(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);

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

411 aa

MWSGWWLWPLVAVCTADFFRDEAERIMRDSPVIDGHNDLPWQLLDMFNNRLQDERANLTT
LAGTHTNIPKLRAGFVGGQFWSVYTPCDTQNKDAVRRTLEQMDVVHRMCRMYPETFLYVT
SSAGIRQAFREGKVASLIGVEGGHSIDSSLGVLRALYQLGMRYLTLTHSCNTPWADNWLV
DTGDSEPQSQGLSPFGQRVVKELNRLGVLIDLAHVSVATMKATLQLSRAPVIFSHSSAYS
VCASRRNVPDDVLRLVKQTDSLVMVNFYNNYISCTNKANLSQVADHLDHIKEVAGARAVG
FGGDFDGVPRVPEGLEDVSKYPDLIAELLRRNWTEAEVKGALADNLLRVFEAVEQASNLT
QAPEEEPIPLDQLGGSCRTHYGYSSGASSLHRHWGLLLASLAPLVLCLSLL

(SEQ ID NO:19)

(20) IL20Rα (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;
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-
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
GLQGVKVTYTVQYFIYGQKKWLNKSECRNINRTYCDLSAETSDYEHQYYAKVKAIWGTKC
SKWAESGRFYPFLETQIGPPEVALTTDEKSISVVLTAPEKWKRNPEDLPVSMQQIYSNLK
YNVSVLNTKSNRTWSQCVTNHTLVLTWLEPNTLYCVHVESFVPGPPRRAQPSEKQCARTL
KDQSSEFKAKIIFWYVLPISITVFLFSVMGYSIYRYIHVGKEKHPANLILIYGNEFDKRF
FVPAEKIVINFITLNISDDSKISHQDMSLLGKSSDVSSLNDPQPSGNLRPPQEEEEVKHL
GYASHLMEIFCDSEENTEGTSFTQQESLSRTIPPDKTVIEYEYDVRTTDICAGPEEQELS
LQEEVSTQGTLLESQAALAVLGPQTLQYSYTPQLQDLDPLAQEHTDSEEGPEEEPSTTLV
DWDPQTGRLCIPSLSSFDQDSEGCEPSEGDGLGEEGLLSRLYEEPAPDRPPGENETYLMQ
FMEEWGLYVQMEN

(SEQ ID NO:20)

(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); 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);

911 aa

MAQLFLPLLAALVLAQAPAALADVLEGDSSEDRAFRVRIAGDAPLQGVLGGALTIPCHVH
YLRPPPSRRAVLGSPRVKWTFLSRGREAEVLVARGVRVKVNEAYRFRVALPAYPASLTDV
SLALSELRPNDSGIYRCEVQHGIDDSSDAVEVKVKGVVFLYREGSARYAFSFSGAQEACA
RIGAHIATPEQLYAAYLGGYEQCDAGWLSDQTVRYPIQTPREACYGDMDGFPGVRNYGVV
DPDDLYDVYCYAEDLNGELFLGDPPEKLTLEEARAYCQERGAEIATTGQLYAAWDGGLDH
CSPGWLADGSVRYPIVTPSQRCGGGLPGVKTLFLFPNQTGFPNKHSRFNVYCFRDSAQPS
AIPEASNPASNPASDGLEAIVTVTETLEELQLPQEATESESRGAIYSIPIMEDGGGGSST
PEDPAEAPRTLLEFETQSMVPPTGFSEEEGKALEEEEKYEDEEEKEEEEEEEEVEDEALW
AWPSELSSPGPEASLPTEPAAQEKSLSQAPARAVLQPGASPLPDGESEASRPPRVHGPPT
ETLPTPRERNLASPSPSTLVEAREVGEATGGPELSGVPRGESEETGSSEGAPSLLPATRA
PEGTRELEAPSEDNSGRTAPAGTSVQAQPVLPTDSASRGGVAVVPASGDCVPSPCHNGGT
CLEEEEGVRCLCLPGYGGDLCDVGLRFCNPGWDAFQGACYKHFSTRRSWEEAETQCRMYG
AHLASISTPEEQDFINNRYREYQWIGLNDRTIEGDFLWSDGVPLLYENWNPGQPDSYFLS
GENCVVMVWHDQGQWSDVPCNYHLSYTCKMGLVSCGPPPELPLAQVFGRPRLRYEVDTVL
RYRCREGLAQRNLPLIRCQENGRWEAPQISCVPRRPARALHPEEDPEGRQGRLLGRWKAL

LIPPSSPMPGP

(SEQ ID NO:21)

(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 (Claim 1; Page 42);

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

987 aa

MALRRLGAALLLLPLLAAVEETLMDSTTATAELGWMVHPPSGWEEVSGYDENMNTIRTYQ
VCNVFESSQNNWLRTKFIRRRGAHRIHVEMKFSVRDCSSIPSVPGSCKETFNLYYYEADF
DSATKTFPNWMENPWVKVDTIAADESFSQVDLGGRVMKINTEVRSFGPVSRSGFYLAFQD
YGGCMSLIAVRVFYRKCPRIIQNGAIFQETLSGAESTSLVAARGSCIANAEEVDVPIKLY
CNGDGEWLVPIGRCMCKAGFEAVENGTVCRGCPSGTFKANQGDEACTHCPINSRTTSEGA
TNCVCRNGYYRADLDPLDMPCTTIPSAPQAVISSVNETSLMLEWTPPRDSGGREDLVYNI
ICKSCGSGRGACTRCGDNVQYAPRQLGLTEPRIYISDLLAHTQYTFEIQAVNGVTDQSPF
SPQFASVNITTNQAAPSAVSIMHQVSRTVDSITLSWSQPDQPNGVILDYELQYYEKELSE
YNATAIKSPTNTVTVQGLKAGAIYVFQVRARTVAGYGRYSGKMYFQTMTEAEYQTSIQEK
LPLIIGSSAAGLVFLIAVVVIAIVCNRRRGFERADSEYTDKLQHYTSGHMTPGMKIYIDP
FTYEDPNEAVREFAKEIDISCVKIEQVIGAGEFGEVCSGHLKLPGKREIFVAIKTLKSGY
TEKQRRDFLSEASIMGQFDHPNVIHLEGVVTKSTPVMIITEFMENGSLDSFLRQNDGQFT
VIQLVGMLRGIAAGMKYLADMNYVHRDLAARNILVNSNLVCKVSDFGLSRFLEDDTSDPT
YTSALGGKIPIRWTAPEAIQYRKFTSASDVWSYGIVMWEVMSYGERPYWDMTNQDVINAI
EQDYRLPPPMDCPSALHQLMLDCWQKDRNHRPKFGQIVNTLDKMIRNPNSLKAMAPLSSG
INLPLLDRTIPDYTSFNTVDEWLEAIKMGQYKESFANAGFTSFDVVSQMMMEDILRVGVT
LAGHQKKILNSIQVMRAQMNQIQSVEV

(SEQ ID NO:22)

(23) ASLG659 (B7h, Genbank accession no. AX092328) US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); 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

MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEP
DIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASLRLKNV
QLTDAGTYKCYIITSKGKKNANLEYKTGAFSMPEVNVDYNASSETLRCEAPRWFPQPTVV
WASQVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKV
TESEIKRRSHLQLLNSKASLCVSSFFAISWALLPLSPYLMLK

(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 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); WO9851805 (Claim 17; Page 97); WO9851824 (Claim 10; Page 94); WO9840403 (Claim 2; Fig 1B);

Accession: O43653; EMBL; AF043498; AAC39607.1.

123 aa

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDCLQVENCTQLGEQCWTARIRAVGLLT
VISKGCSLNCVDDSQDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPALGLLLWGP
GQL

(SEQ ID NO:24)

(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, Claim 20); US2003194704 (Claim 45);

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

236 aa

MPGAAAAAAAAAAAMLPAQEAAKLYHTNYVRNSRAIGVLWAIFTICFAIVNVVCFIQPYW
IGDGVDTPQAGYFGLFHYCIGNGFSRELTCRGSFTDFSTLPSGAFKAASFFIGLSMMLII
ACIICFTLFFFCNTATVYKICAWMQLTSAACLVLGCMIFPDGWDSDEVKRMCGEKTDKYT
LGACSVRWAYILAIIGILDALILSFLAFVLGNRQDSLMAEELKAENKVLLSQYSLE

(SEQ ID NO:25)

(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; 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

MRRGPRSLRGRDAPAPTPCVPAECFDLLVRHCVACGLLRTPRPKPAGASSPAPRTALQPQ
ESVGAGAGEAALPLPGLLFGAPALLGLALVLALVLVGLVSWRRRQRRLRGASSAEAPDGD
KDAPEPLDKVIILSPGISDATAPAWPPPGEDPGTTPPGHSVPVPATELGSTELVTTKTAG
PEQQ

(SEQ ID NO:26)

(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);

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

847 aa

MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDLESFILFH
NPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQFLGDKNKNCTLSIHPVHLNDSGQLGLR
MESKTEKWMERIHLNVSERPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPIQLQWLLEG
VPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTCQLQDADGKFLSNDTVQLNVKH
TPKLEIKVTPSDAIVREGDSVTMTCEVSSSNPEYTTVSWLKDGTSLKKQNTFTLNLREVT
KDQSGKYCCQVSNDVGPGRSEEVFLQVQYAPEPSTVQILHSPAVEGSQVEFLCMSLANPL
PTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVAENILGTGQRGPGAELDVQYPPK

KVTTVIQNPMPIREGDTVTLSCNYNSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGWDNT
TIACARCNSWCSWASPVALNVQYAPRDVRVRKIKPLSEIHSGNSVSLQCDFSSSHPKEVQ
FFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLRVSM
SPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLPHHSQKLRLEPVKVQHSGAYWCQ
GTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSCLAILILAICGLKLQRRWKRTQSQQG
LQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDGISYTTLRFPEMNIPRTGDAES
SEMQRPPRTCDDTVTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENV
DYVILKH

(SEQ ID NO:27)

(28) CD79a (CD79A, CD79α, 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 SEQUENCE Full mpggpgv...dvqlekp (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. 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
MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSN
NANVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGNESYQQSCG
TYLRVRQPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGD
EYEDENLYEGLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP

(SEQ ID NO:28)

(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...atslttf (1..372; 372 aa), 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
IFLLGVIGNVLVLVILERHRQTRSSTETFLFHLAVADLLLVFILPFAVAEGSVGWVLGTF
LCKTVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYRHRRLLSIHITCGTIWLVGFLL
ALPEILFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPMLVMGWCYVG
VVHRLRQAQRRPQRQKAVRVAILVTSIFFLCWSPYHIVIFLDTLARLKAVDNTCKLNGSL
PVAITMCEFLGLAHCCLNPMLYTFAGVKFRSDLSRLLTKLGCTGPASLCQLFPSWRRSSL
SESENATSLTTF

(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 mgsgwvp...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. 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;

273 aa

MGSGWVPWVVALLVNLTRLDSSMTQGTDSPEDFVIQAKADCYFTNGTEKVQFVVRFIFNL
EEYVRFDSDVGMFVALTKLGQPDAEQWNSRLDLLERSRQAVDGVCRHNYRLGAPFTVGRK
VQPEVTVYPERTPLLHQHNLLHCSVTGFYPGDIKIKWFLNGQEERAGVMSTGPIRNGDWT
FQTVVMLEMTPELGHVYTCLVDHSSLLSPVSVEWRAQSEYSWRKMLSGIAAFLLGLIFLL
VGIVIQLRAQKGYVRTQMSGNEVSRAVLLPQSC

(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

MGQAGCKGLCLSLFDYKTEKYVIAKNKKVGLLYRLLQASILAYLVVWVFLIKKGYQDVDT
SLQSAVITKVKGVAFTNTSDLGQRIWDVADYVIPAQGENVFFVVTNLIVTPNQRQNVCAE
NEGIPDGACSKDSDCHAGEAVTAGNGVKTGRCLRRENLARGTCEIFAWCPLETSSRPEEP
FLKEAEDFTIFIKNHIRFPKFNFSKSNVMDVKDRSFLKSCHFGPKNHYCPIFRLGSVIRW
AGSDFQDIALEGGVIGINIEWNCDLDKAASECHPHYSFSRLDNKLSKSVSSGYNFRFARY
YRDAAGVEFRTLMKAYGIRFDVMVNGKGAFFCDLVLIYLIKKREFYRDKKYEEVRGLEDS
SQEAEDEASGLGLSEQLTSGPGLLGMPEQQELQEPPEAKRGSSSQKGNGSVCPQLLEPHR
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

MAEAITYADLRFVKAPLKKSISSRLGQDPGADDDGEITYENVQVPAVLGVPSSLASSVLG
DKAAVKSEQPTASWRAVTSPAVGRILPCRTTCLRYLLLGLLLTCLLLGVTAICLGVRYLQ
VSQQLQQTNRVLEVTNSSLRQQLRLKITQLGQSAEDLQGSRRELAQSQEALQVEQRAHQA
AEGQLQACQADRQKTKETLQSEEQQRRALEQKLSNMENRLKPFFTCGSADTCCPSGWIMH
QKSCFYISLTSKNWQESQKQCETLSSKLATFSEIYPQSHSYYFLNSLLPNGGSGNSYWTG
LSSNKDWKLTDDTQRTRTYAQSSKCNKVHKTWSWWTLESESCRSSLPYICEMTAFRFPD

(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) 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);

661 aa

MAFDVSCFFWVVLFSAGCKVITSWDQMCIEKEANKTYNCENLGLSEIPDTLPNTTEFLEF
SFNFLPTIHNRTFSRLMNLTFLDLTRCQINWIHEDTFQSHHQLSTLVLTGNPLIFMAETS
LNGPKSLKHLFLIQTGISNLEFIPVHNLENLESLYLGSNHISSIKFPKDFPARNLKVLDF
QNNAIHYISREDMRSLEQAINLSLNFNGNNVKGIELGAFDSTVFQSLNFGGTPNLSVIFN
GLQNSTTQSLWLGTFEDIDDEDISSAMLKGLCEMSVESLNLQEHRFSDISSTTFQCFTQL
QELDLTATHLKGLPSGMKGLNLLKKLVLSVNHFDQLCQISAANFPSLTHLYIRGNVKKLH
LGVGCLEKLGNLQTLDLSHNDIEASDCCSLQLKNLSHLQTLNLSHNEPLGLQSQAFKECP
QLELLDLAFTRLHINAPQSPFQNLHFLQVLNLTYCFLDTSNQHLLAGLPVLRHLNLKGNH
FQDGTITKTNLLQTVGSLEVLILSSCGLLSIDQQAFHSLGKMSHVDLSHNSLTCDSIDSL
SHLKGIYLNLAANSINIISPRLLPILSQQSTINLSHNPLDCTCSNIHFLTWYKENLHKLE
GSEETTCANPPSLRGVKLSDVKLSCGITAIGIFFLIVFLLLLAILLFFAVKYLLRWKYQH
I

(SEQ ID NO:33)

(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 mlprlll...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

MLPRLLLLICAPLCEPAELFLIASPSHPTEGSPVTLTCKMPFLQSSDAQFQFCFFRDTRA
LGPGWSSSPKLQIAAMWKEDTGSYWCEAQTMASKVLRSRRSQINVHRVPVADVSLETQPP
GGQVMEGDRLVLICSVAMGTGDITFLWYKGAVGLNLQSKTQRSLTAEYEIPSVRESDAEQ

YYCVAENGYGPSPSGLVSITVRIPVSRPILMLRAPRAQAAVEDVLELHCEALRGSPPILY
WFYHEDITLGSRSAPSGGGASFNLSLTEEHSGNYSCEANNGLGAQRSEAVTLNFTVPTGA
RSNHLTSGVIEGLLSTLGPATVALLFCYGLKRKIGRRSARDPLRSLPSPLPQEFTYLNSP
TPGQLQPIYENVNVVSGDEVYSLAYYNQPEQESVAAETLGTHMEDKVSLDIYSRLRKANI
TDVDYEDAM

(SEQ ID NO:34)

(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) 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. Commun. 277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2); 977 aa

MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTCKGFRFYSPQKTKWYHR
YLGKEILRETPDNILEVQESGEYRCQAQGSPLSSPVHLDFSSASLILQAPLSVFEGDSVV
LRCRAKAEVTLNNTIYKNDNVLAFLNKRTDFHIPHACLKDNGAYRCTGYKESCCPVSSNT
VKIQVQEPFTRPVLRASSFQPISGNPVTLTCETQLSLERSDVPLRFRFFRDDQTLGLGWS
LSPNFQITAMWSKDSGFYWCKAATMPHSVISDSPRSWIQVQIPASHPVLTLSPEKALNFE
GTKVTLHCETQEDSLRTLYRFYHEGVPLRHKSVRCERGASISFSLTTENSGNYYCTADNG
LGAKPSKAVSLSVTVPVSHPVLNLSSPEDLIFEGAKVTLHCEAQRGSLPILYQFHHEDAA
LERRSANSAGGVAISFSLTAEHSGNYYCTADNGFGPQRSKAVSLSITVPVSHPVLTLSSA
EALTFEGATVTLHCEVQRGSPQILYQFYHEDMPLWSSSTPSVGRVSFSFSLTEGHSGNYY
CTADNGFGPQRSEVVSLFVTVPVSRPILTLRVPRAQAVVGDLLELHCEAPRGSPPILYWF
YHEDVTLGSSSAPSGGEASFNLSLTAEHSGNYSCEANNGLVAQHSDTISLSVIVPVSRPI
LTFRAPRAQAVVGDLLELHCEALRGSSPILYWFYHEDVTLGKISAPSGGGASFNLSLTTE
HSGIYSCEADNGPEAQRSEMVTLKVAVPVSRPVLTLRAPGTHAAVGDLLELHCEALRGSP
LILYRFFHEDVTLGNRSSPSGGASLNLSLTAEHSGNYSCEADNGLGAQRSETVTLYITGL
TANRSGPFATGVAGGLLSIAGLAAGALLLYCWLSRKAGRKPASDPARSPPDSDSQEPTYH
NVPAWEELQPVYTNANPRGENVVYSEVRIIQEKKKHAVASDPRHLRNKGSPIIYSEVKVA
STPVSGSLFLASSAPHR

(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 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 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 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 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 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. 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 with an amino acid residue that does not contain a sulfhydyl 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, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell* 22:817) genes can be employed in tk-, hgprt- or aprt- 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 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 hygro, which confers resistance to hygromycin (Santerre *et al.*, 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which 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).

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 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).

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 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.

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.

## 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. 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 (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.

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 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 $R^1N=C=NR$, 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)).

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, 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 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. 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 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 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.

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.

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 Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

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.

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); Verhoeyen *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 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:

(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.

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 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 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 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.*, $IgG_1$, $IgG_2$, $IgG_3$, or $IgG_4$) 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 β(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-aceylgalactosamine, 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.

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.

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. 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 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.

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 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)

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 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 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 scrrened 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.

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 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 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. $^{125}$I-labeled rHRG$\beta 1_{177\text{-}224}$ (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 $IC_{50}$ 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 pl80 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 µl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 µl) may be electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 µg/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 interst 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 tissueof 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 $3 \times 10^6$ 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 $Ca^{2+}$ 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.

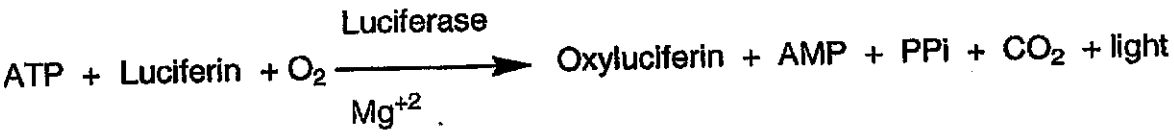
4.5.3 *IN VITRO* CELL PROLIFERATION ASSAYS

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). $IC_{50}$ values were established for SK-BR-3 and BT-474 which are known to over express HER2 receptor protein. Table 2a shows the potency ($IC_{50}$) measurements of exemplary antibody drug conjugates in the cell proliferation assay against SK-BR-3 cells. Table 2b shows the potency ($IC_{50}$) 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<br>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<br>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

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

* 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.*, $IC_{50} < 0.1$ µg 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* µg/mL | CL mL/day/kg | Cmax µg/mL | T ½ 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)<br>H-Me-vc-PAB-MMAE (Conj.) | 135<br>31.9 | 15.0<br>63.8 | 44.9<br>45.2 | 11.2<br>3.0 | 23.8 |
| H-MC-vc-MMAF, wo/PAB, (Total Ab)<br>H-MC-vc-MMAF, wo/PAB, (Conj.) | 306<br>59.9 | 6.6<br>33.4 | 78.7<br>82.8 | 11.9<br>2.1 | 19.6 |
| H-MC-(D)val-(L)cit-PAB-MMAE (Total Ab)<br>H-MC-(D)val-(L)cit-PAB-MMAE (Conj.) | 107<br>40 | 19.2<br>50.4 | 30.6<br>33.7 | 9.6<br>3.98 | 38.1 |
| H-MC-(Me)-vc-PAB-MMAE, Total Ab<br>H-MC-(Me)-vc-PAB-MMAE, Conj. | 135.1<br>31.9 | 15.0<br>63.8 | 44.9<br>45.2 | 11.2<br>2.96 | 23.8 |
| H-MC-(D)val-(D)cit-PAB-MMAE, Total Ab<br>H-MC-(D)val-(D)cit-PAB-MMAE, Conj. | 88.2<br>33.6 | 22.8<br>59.8 | 33.8<br>36.0 | 10.5<br>4.43 | 38.3 |
| H-MC-vc-PAB-MMAE, Total Ab<br>H-MC-vc-PAB-MMAE, Conj.<br>H linked to MC by lysine [lys] | 78.6<br>31.1 | 26.3<br>64.4 | 39.5<br>33.2 | 5.8<br>3.00 | 40.6 |
| MMAF<br>200 µg/kg | 0.99 | 204 | 280 | 0.224 | - |
| MMAE<br>206 µg/kg | 3.71 | 62.6 | 649 | 0.743 | - |
| HER F(ab')$_2$-MC-vc-MMAE, Total Ab<br>HER F(ab')$_2$-MC-vc-MMAE, Conj. | 9.3<br>8.8 | 217<br>227 | 34.4<br>36.9 | 0.35<br>0.29 | 95 |
| 4D5-H-Fab-MC-vc-MMAF, Total Ab<br>4D5-H-Fab-MC-vc-MMAF, Conj. | 43.8<br>29.9 | 46.2<br>68.1 | 38.5<br>34.1 | 1.49<br>1.12 | 68 |
| 4D5-H-Fab-MC-vc-MMAE, | 71.5 | 70.3 | 108 | 1.18 | 59 |

| | | | | | |
|---|---|---|---|---|---|
| Total Ab<br>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<br>H-MC-vc-PAB-MMAF, Conj. | 170<br>83.9 | 12.03<br>23.96 | 47.9<br>44.7 | 8.44<br>4.01 | 49.5 |
| H-MC-vc-PAB-MMAF-DMAEA, Total Ab<br>H-MC-vc-PAB-MMAF-DMAEA, Conj. | 211<br>71.5 | 9.8<br>28.2 | 39.8<br>38.8 | 8.53<br>3.64 | 34.3 |
| H-MC-vc-PAB-MMAF-TEG, Total Ab<br>H-MC-vc-PAB-MMAF-TEG, Conj. | 209<br>63.4 | 9.75<br>31.8 | 53.2<br>34.9 | 8.32<br>4.36 | 29.7 |

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, 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 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 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 *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 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

| Sample<br>Drugs per antibody | Dose | Ti | PR | CR | Tumor doubling time (days) | Mean log cell kill |
|---|---|---|---|---|---|---|
| Vehicle | | | | | 2-5 | 0 |
| H-MC-vc-PAB-MMAE<br>8.7 MMAE/Ab | 1250 μg/m$^2$ | 5/5 | 4/7 | 0/7 | 18 | 1.5 |
| H-MC-vc-PAB-MMAF<br>3.8 MMAF/Ab | 555 μg/m$^2$ | 2/5 | 2/7 | 5/7 | 69 | 6.6 |
| H-MC(Me)-vc-PAB-MMAF | | | | | >50 | 6.4 |
| H-MC-MMAF<br>4.8 MMAF/Ab | 9.2 mg/kg Ab<br>550 μg/m$^2$<br>at 0, 7, 14 and 21 days | 7/7 | 6/7 | 0/7 | 63 | 9 |
| H-MC-MMAF<br>4.8 MMAF/Ab | 14 mg/kg Ab<br>840 μg/m$^2$<br>at 0, 7, 14 and 21 days | 5/5 | 5/7 | 2/7 | >63 | |
| H-MC-vc-PAB-MMAF<br>5.9 MMAF/Ab | 3.5 mg/kg Ab<br>300 μg/m$^2$<br>at 0, 21, and 42 days | 5/6 | 1/7 | 3/7 | >36 | |
| H-MC-vc-PAB-MMAF<br>5.9 MMAF/Ab | 4.9 mg/kg Ab<br>425 μg/m$^2$<br>at 0, 21, and 42 days | 4/7 | 2/7 | 5/7 | >90 | |
| H-MC-vc-PAB-MMAF<br>5.9 MMAF/Ab | 6.4 mg/kg Ab<br>550 μg/m$^2$<br>at 0, 21, and 42 days | 3/6 | 1/7 | 6/7 | >90 | |
| H-(L)val-(L)cit-MMAE<br>8.7 MMAE/Ab | 10 mg/kg | 7/7 | 1/7 | 0/7 | 15.2 | 1.1 |
| H-MC-MMAE<br>4.6 MMAE/Ab | 10 mg/kg | 7/7 | 0/7 | 0/7 | 4 | 0.1 |
| H-(D)val-(D)cit-MMAE<br>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 | 10 mg/kg 550 $\mu g/m^2$ | 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 μg/m$^2$ | 6/6 | 3/6 | 0/6 | 15 | 1.3 |
| H-vc-MMAE | 10 mg/kg 1250 μg/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

The term Ti 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. 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:

| | | |
|---|---|---|
| ++++ | ≥ 3.4 | (highly active) |
| +++ | = 2.5-3.4 | |
| ++ | = 1.7-2.4 | |
| + | = 1.0-1.6 | |
| inactive | = 0 | |

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 μg/m$^2$) and Trastuzumab-MC-vc-PAB-MMAF (555 μg/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 μg/m$^2$) of Trastuzumab-MC-MMAE and 1250 μg/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 μg/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

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 granuloctyes (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)

–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 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 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 ug/m$^2$ MMAF were compared to trastuzumab-MC-val-cit-PAB-MMAF at 2105 ug/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 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 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 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 4210μg/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 5500μg/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 etal (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

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 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/m$^2$ with complete recovery 14 days post-dose. The antibody drug conjugate H-MC-vc-PAB-MMAF showed elevation of liver enzymes at 550 (transient) and 880 mg/m$^2$ 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 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 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.

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, 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 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* 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 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 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.

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 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, 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 attachment to a Linker.

4.6.1 DRUG MOIETY SYNTHESIS

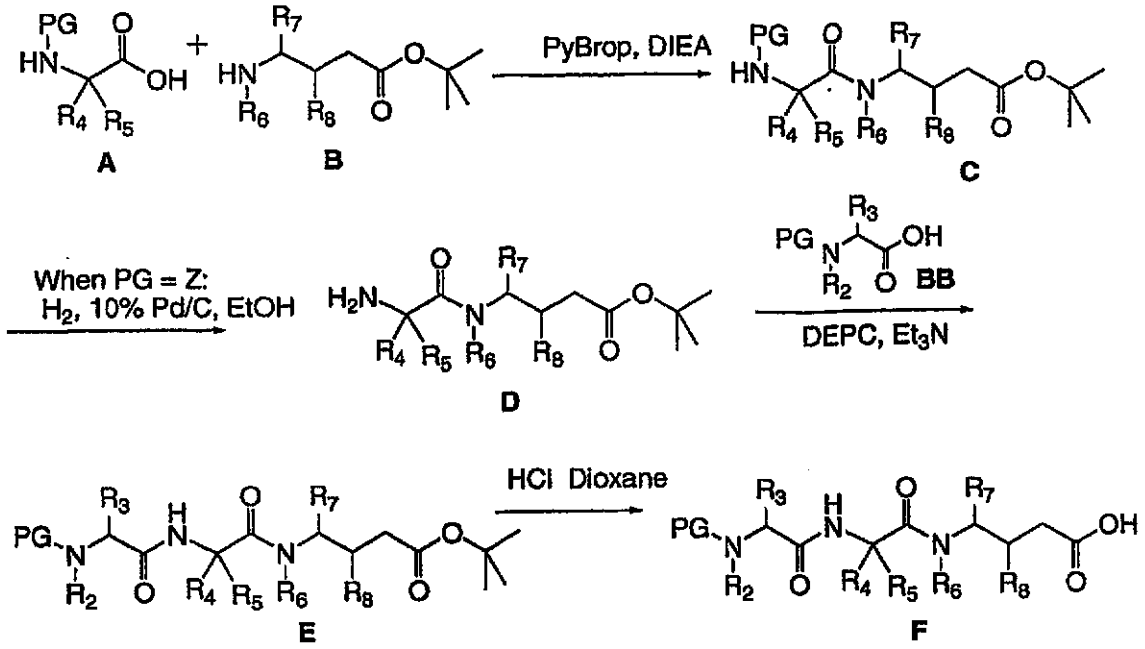
Typically, peptide-based Drugs can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (*see* E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, 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 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.

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




As illustrated in Scheme 5, a protected amino acid **A** (where PG represents an amine protecting group, $R^4$ is selected from hydrogen, $C_1$-$C_8$ alkyl, $C_3$-$C_8$ carbocycle, -O-($C_1$-$C_8$ alkyl), -aryl, alkyl-aryl, alkyl-($C_3$-$C_8$ carbocycle), $C_3$-$C_8$ heterocycle, alkyl-($C_3$-$C_8$ heterocycle) wherein $R^5$ is selected from H and methyl; or $R^4$ and $R^5$ join, have the formula -$(CR^aR^b)_n$- wherein $R^a$ and $R^b$ are independently selected from hydrogen, $C_1$-$C_8$ 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) is coupled to *t*-butyl ester **B** (where $R^6$ is selected from -H and -$C_1$-$C_8$ alkyl; and $R^7$ is selected from hydrogen, $C_1$-$C_8$ alkyl, $C_3$-$C_8$ carbocycle, -O-($C_1$-$C_8$ alkyl), -aryl, alkyl-aryl, alkyl-($C_3$-$C_8$ carbocycle), $C_3$-$C_8$ heterocycle and alkyl-($C_3$-$C_8$ 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 $H_2$ 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 $R^1$ is selected from -H, -$C_1$-$C_8$ alkyl and -$C_3$-$C_8$ carbocycle; and $R^2$ is selected from -H and -$C_1$-$C_8$ alkyl; or $R^1$ and $R^2$ join, 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 nitrogen atom to which they are attached; and $R^3$ is selected from hydrogen, $C_1$-$C_8$ alkyl, $C_3$-$C_8$ carbocycle, -O-($C_1$-$C_8$ alkyl), -aryl, alkyl-aryl, alkyl-($C_3$-$C_8$ carbocycle), $C_3$-$C_8$ heterocycle and alkyl-($C_3$-$C_8$ 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.

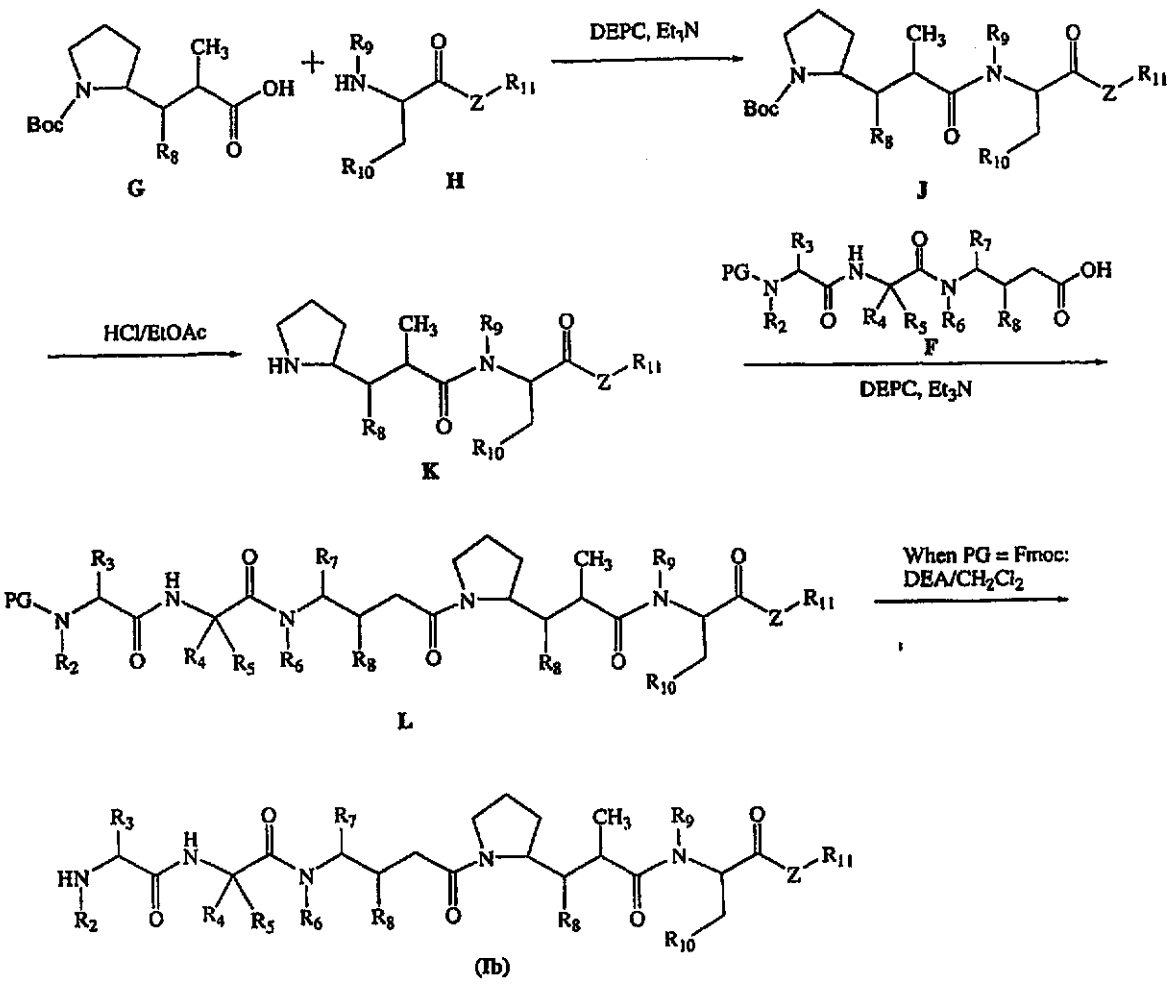
**General Procedure A: Peptide synthesis using DEPC.** The *N*-protected or N, N-disubstituted amino acid or peptide **D** (1.0 eq.) and an amine **BB** (1.1 eq.) are diluted with an aprotic organic solvent, such as dichloromethane (0.1 to 0.5 M). An organic base such as triethylamine or diisopropylethylamine (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)**.

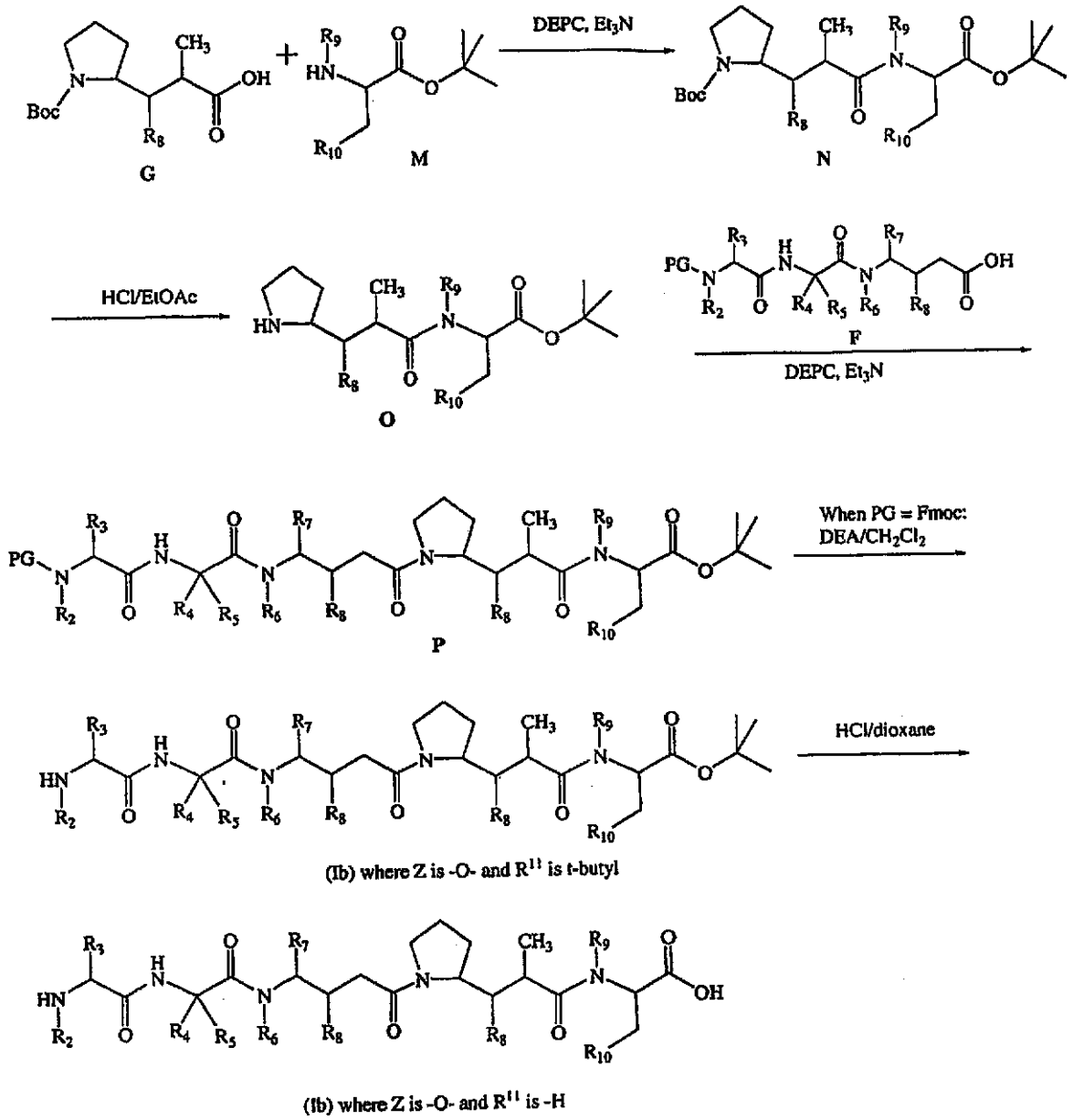
**General procedure D: Drug synthesis.** A mixture of dipeptide **K** (1.0 eq.) and tripeptide **F** (1 eq.) is diluted with an aprotic organic solvent, such as dichloromethane, to form a 0.1M solution, then a strong acid, such as trifluoroacetic acid (1/2 v/v) is added and the resulting mixture is stirred 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 and aprotic organic solvent, such as dichloromethane. An organic base 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.

**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 (½ 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 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



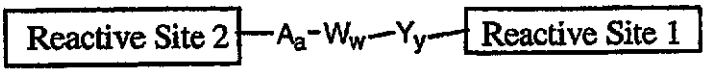

The dipeptide **O** can be readily prepared by condensation of the modified amino acid Boc-Dolaproine **G** (*see*, for example, Pettit, G.R., *et al. Synthesis*, 1996, 719-725), with a protected amino acid of formula **M** using condensing agents well known for peptide chemistry, such as, for example, 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 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**).

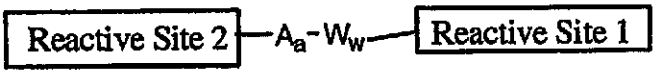
Thus, the above methods are useful for making Drugs that can be used in the present invention.

4.6.2 DRUG LINKER SYNTHESIS

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:

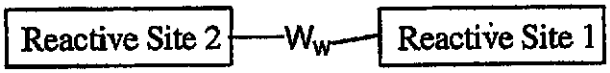



when both a Spacer unit (-Y-) and a Stretcher unit (-A-) are present. Alternately, the Linker can have the structure:



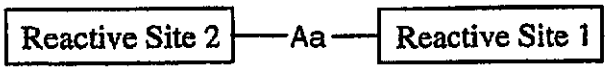

when the Spacer unit (-Y-) is absent.

The Linker can also have the structure:




when both the Stretcher unit (-A-) and the Spacer unit (-Y-) are absent.

The Linker can also have the structure:




when both the Amino Acid unit (W) and the Spacer Unit (Y) are absent.

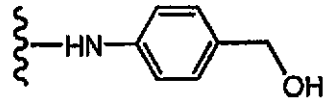
In general, a suitable Linker has an Amino Acid unit linked to an optional Stretcher Unit and an optional Spacer Unit. Reactive Site 1 is present at the terminus of the Spacer and Reactive site 2 is present at the terminus of the Stretcher. If a Spacer unit is not present, then Reactive site 1 is present at the C-terminus of the Amino Acid unit.

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.

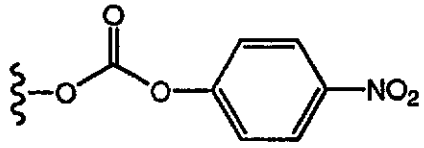
In one aspect of the invention, Reactive Site No. 1 is 

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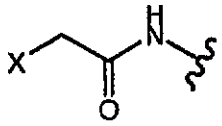
In another aspect of the invention, Reactive Site No. 1 is



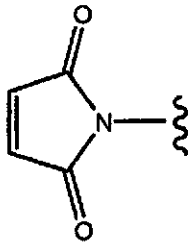
In still another aspect of the invention, Reactive Site No. 1 is a p-nitrophenyl carbonate having the formula



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

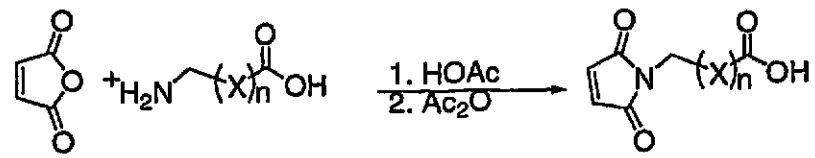


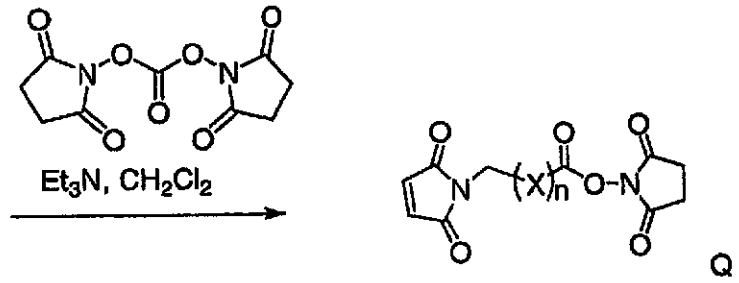
wherein X represents a leaving group, preferably O-mesyl, O-tosyl, -Cl, -Br, or -I; or a maleimide group having the formula

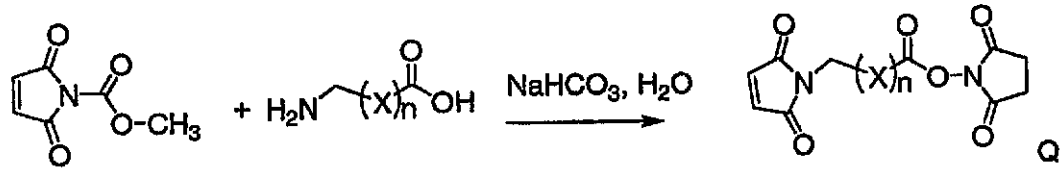


Useful Linkers can be obtained via commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or prepared as summarized in Schemes 8-10 below.

Scheme 8



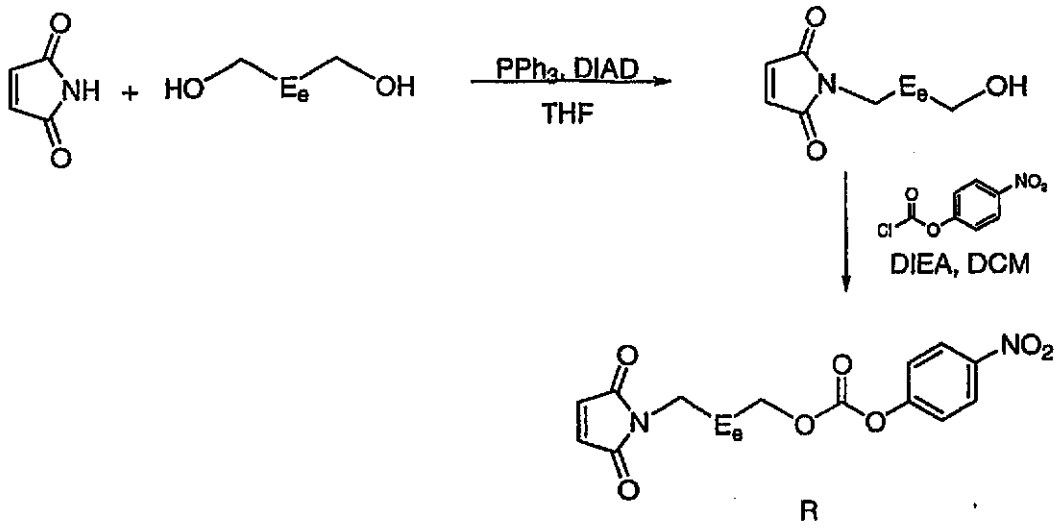




wherein X is $-CH_2-$ or $-CH_2OCH_2-$; and n is an integer ranging either from 0-10 when X is $-CH_2-$ ; or 1-10 when X is $-CH_2OCH_2-$.

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

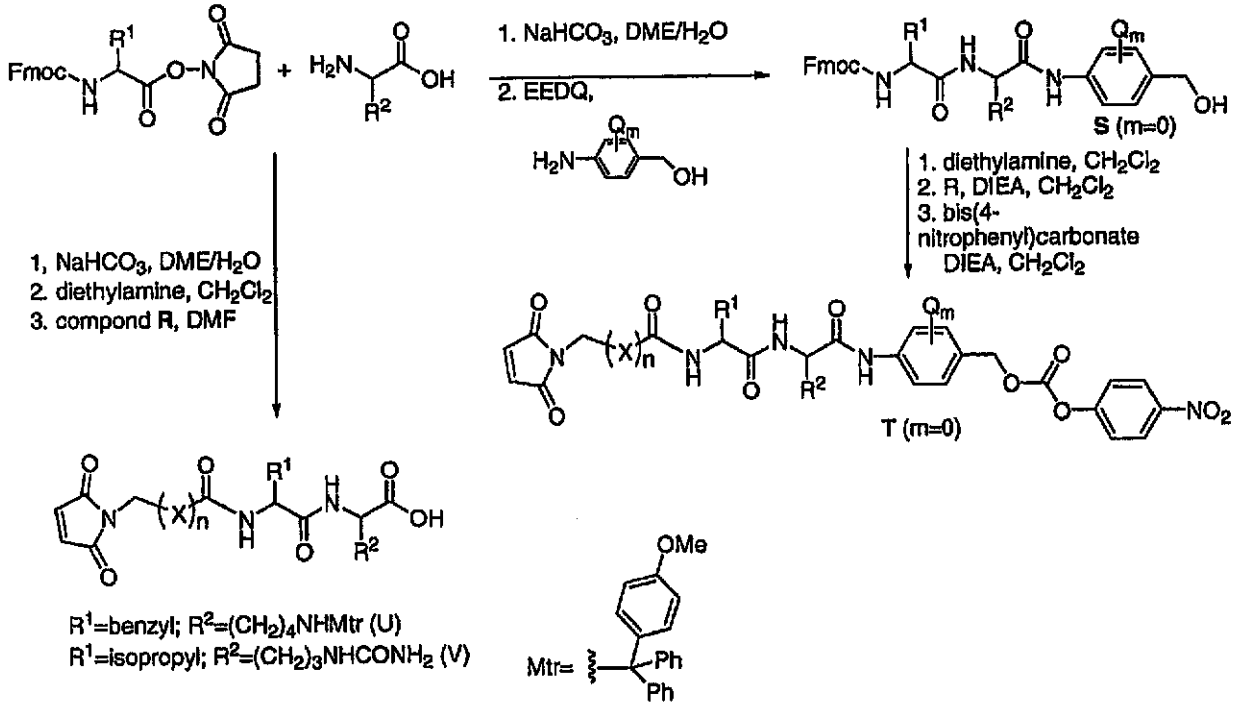



wherein E is $-CH_2-$ or $-CH_2OCH_2-$; and e is an integer ranging from 0-8;

Alternatively, PEG-maleimide and PEG-haloacetamide stretchers can be prepared as described by Frisch, *et al.*, *Bioconjugate Chem.* 1996, 7, 180-186.

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.

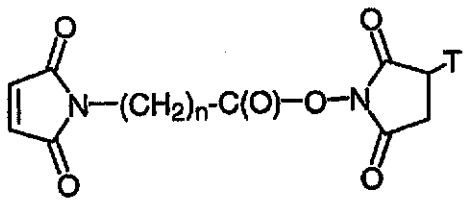
Scheme 10



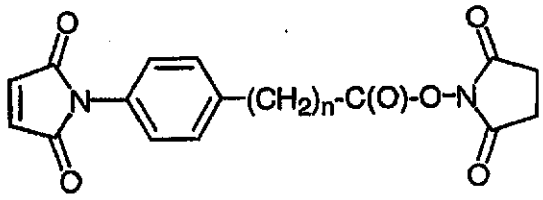
wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen,-nitro or -cyano; m is an integer ranging from 0-4; and n is an integer ranging from 0-10.

Useful Stretchers may be incorporated into a Linker using the commercially available intermediates from Molecular Biosciences (Boulder, CO) described below by utilizing known techniques of organic synthesis.

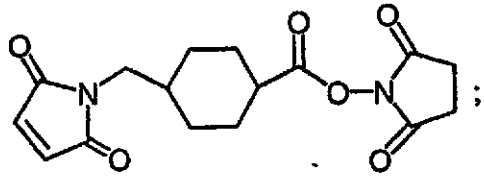
Stretchers of formula (**IIIa**) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit as depicted in Schemes 11 and 12:



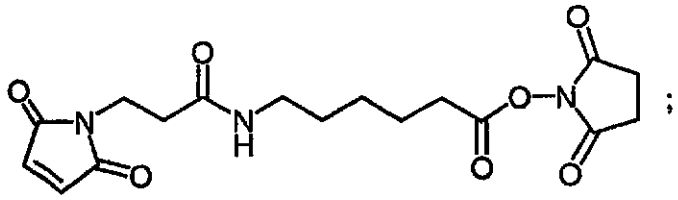
where n is an integer ranging from 1-10 and T is -H or -$SO_3$Na;



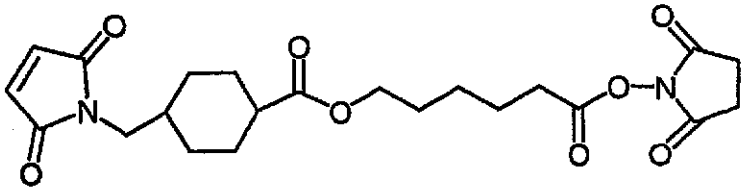
where n is an integer ranging from 0-3;



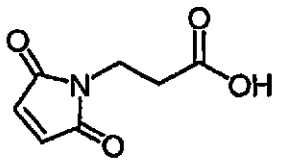
;



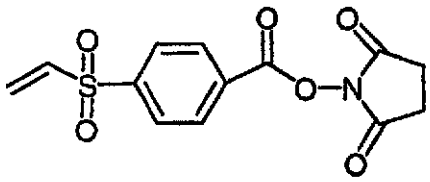
;



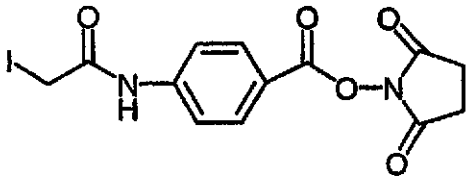
; and



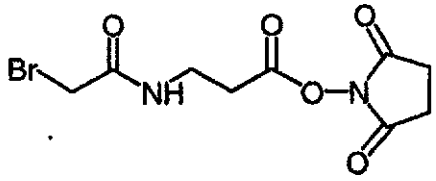
Stretcher units of formula (**IIIb**) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:



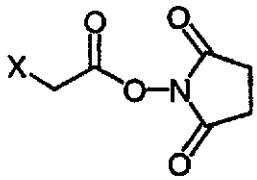
;



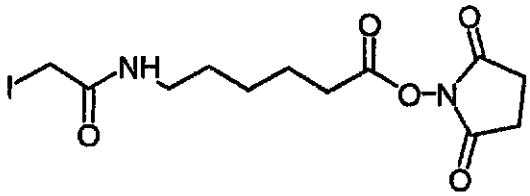
;



;

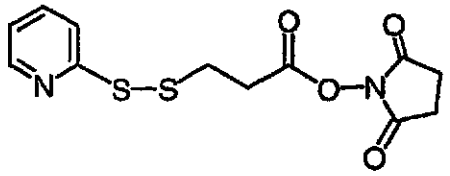


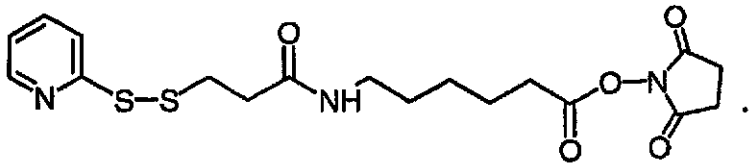
where X is -Br or -I; and



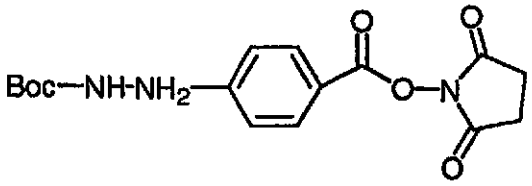
.

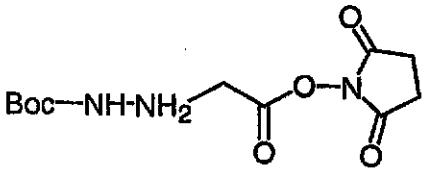
Stretcher units of formula (IV) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:


; and

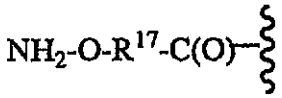

.

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:


; and


.

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.



wherein $-R^{17}-$ is selected from $-C_1-C_{10}$ alkylene-, $-C_3-C_8$ carbocyclo-, $-O-(C_1-C_8$ alkyl)-, -arylene-, $-C_1-C_{10}$ alkylene-arylene-, -arylene-$C_1-C_{10}$ alkylene-, $-C_1-C_{10}$ alkylene-($C_3-C_8$ carbocyclo)-, -($C_3-C_8$ carbocyclo)-$C_1-C_{10}$ alkylene-, $-C_3-C_8$ heterocyclo-, $-C_1-C_{10}$ alkylene-($C_3-C_8$ heterocyclo)-, -($C_3-C_8$ heterocyclo)-$C_1-C_{10}$ alkylene-, $-(CH_2CH_2O)_r-$, $-(CH_2CH_2O)_r-CH_2-$; and r is an integer ranging from 1-10;

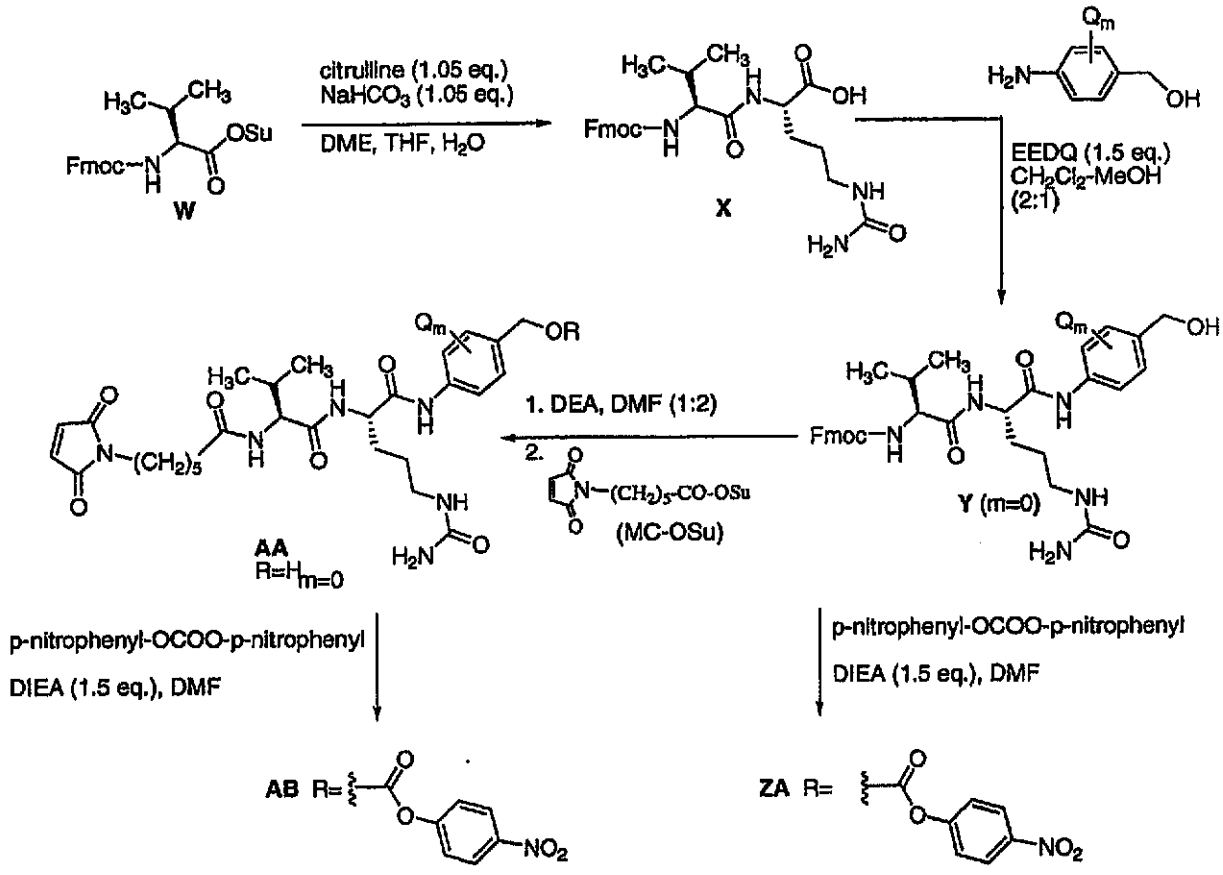
Isothiocyanate Stretchers of the formula shown below may be prepared from isothiocyanatocarboxylic acid chlorides as described in *Angew. Chem.*, 1975, 87(14):517.



wherein $-R^{17}-$ is as described herein.

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.

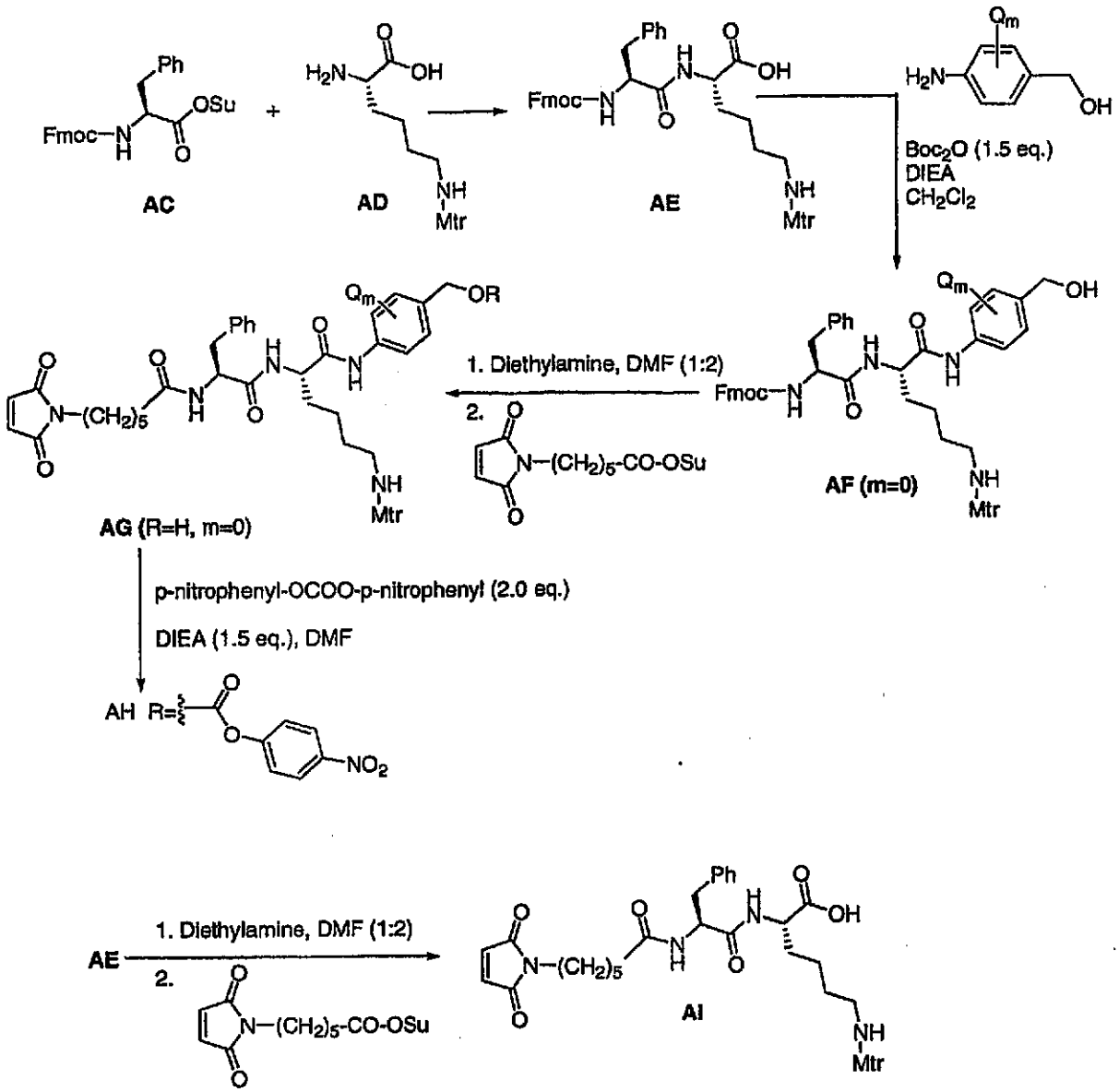
Scheme 11




wherein Q is $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), -halogen, -nitro or -cyano; 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 -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

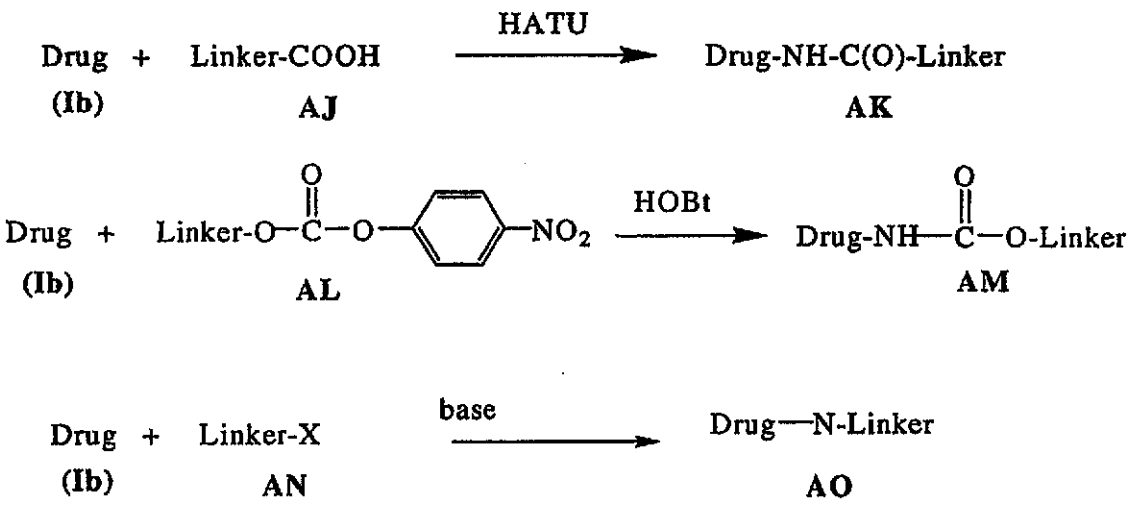
As shown in Scheme 13, a Linker can be reacted with an amino group of a Drug Compound of Formula (Ib) to form a Drug-Linker Compound that contains an amide or carbamate group, linking the Drug unit to the Linker unit. When Reactive Site No. 1 is a carboxylic acid group, as in Linker **AJ**, the coupling reaction can be performed using HATU or PyBrop and an appropriate amine base, resulting in a Drug-Linker 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.

Scheme 13



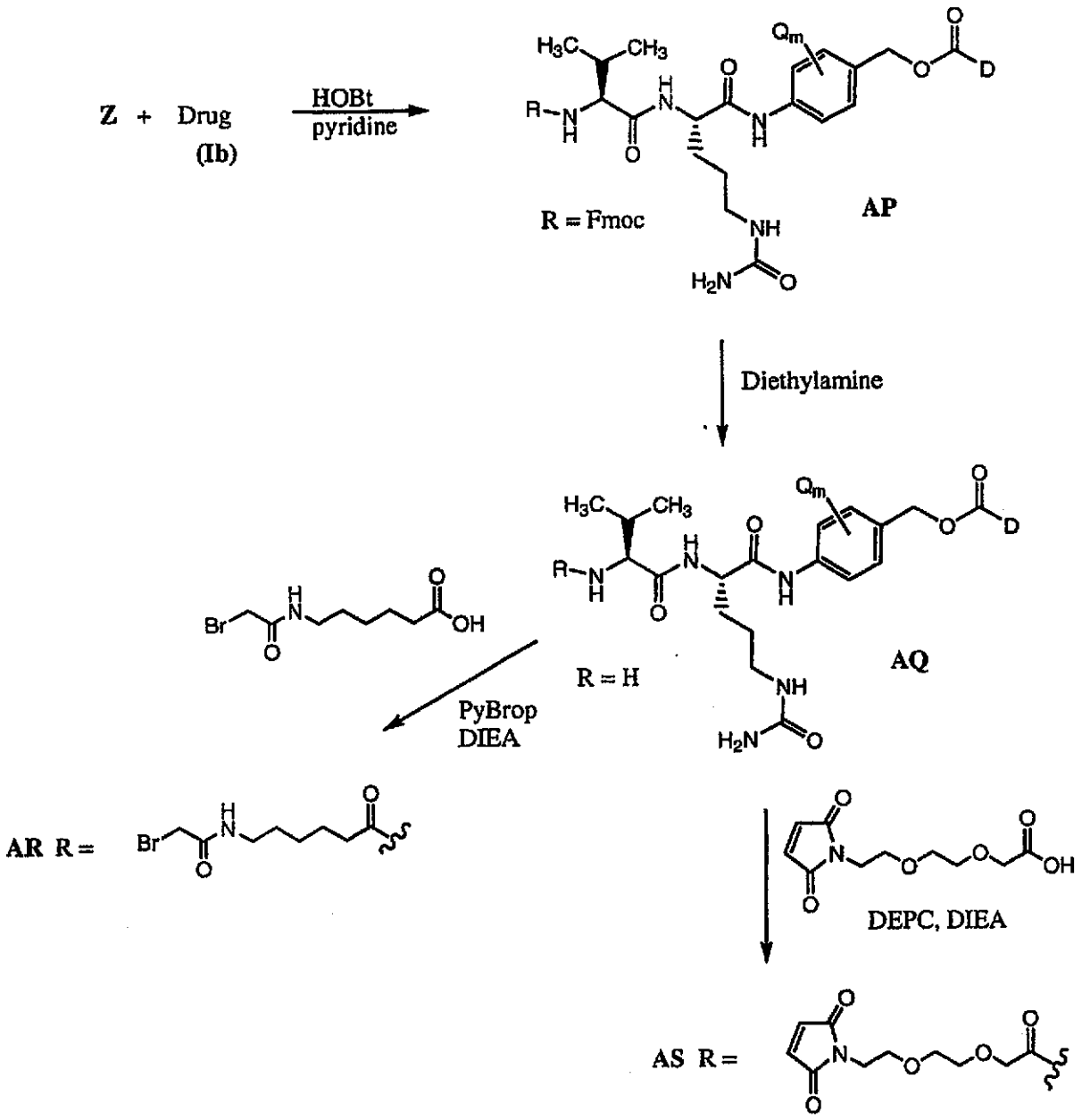

**General Procedure G: Amide formation using HATU.** A Drug (**Ib**) (1.0 eq.) and an N-protected Linker containing a carboxylic acid Reactive site (1.0 eq.) are diluted with a suitable organic solvent, such as dichloromethane, and the resulting solution is treated with HATU (1.5 eq.) and an organic 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 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. 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.

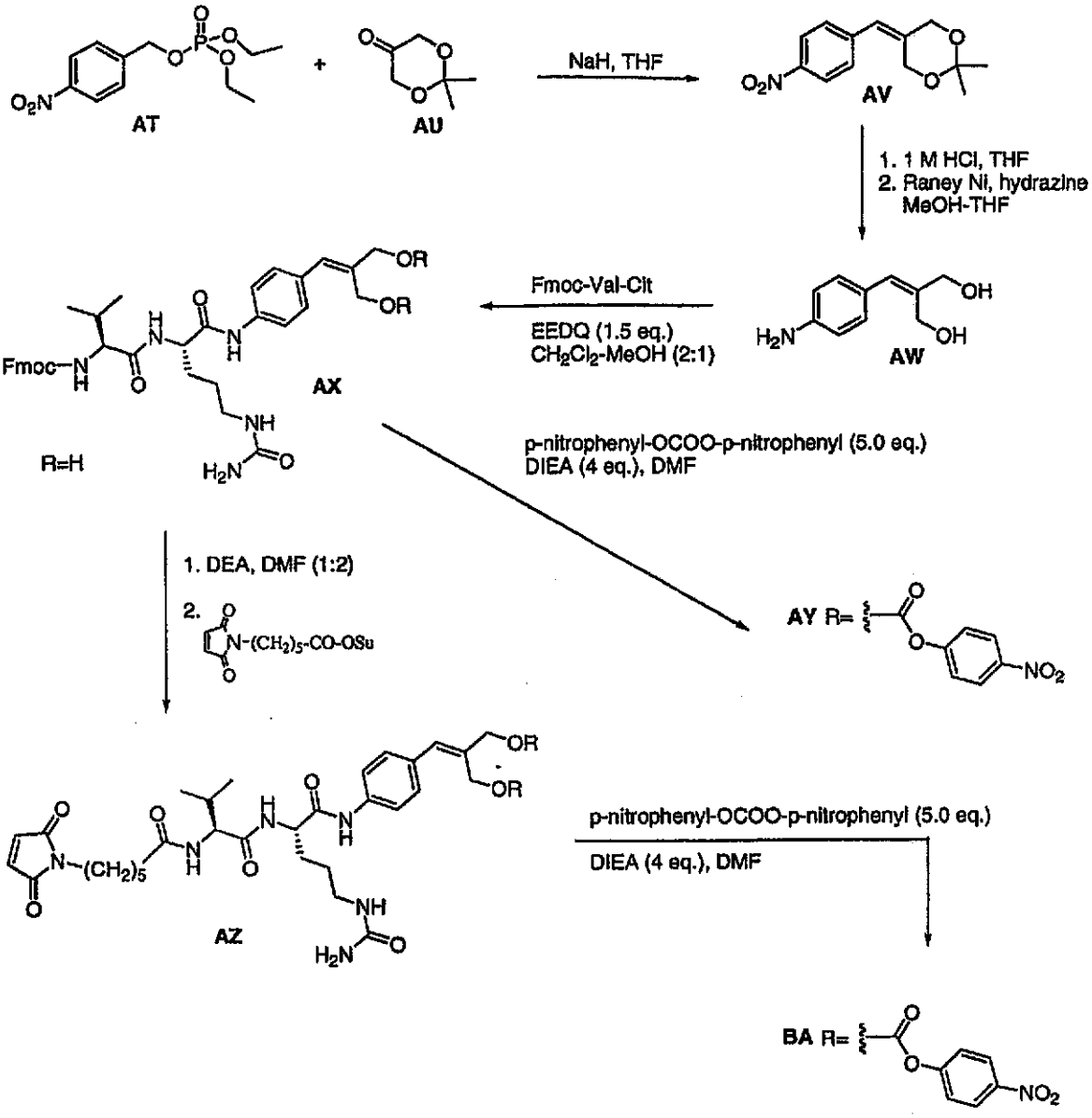
Scheme 14




wherein Q is -$C_1$-$C_8$ alkyl, -O-($C_1$-$C_8$ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

Methodology useful for the preparation of a Linker unit containing a branched spacer is shown in Scheme 15.

Scheme 15

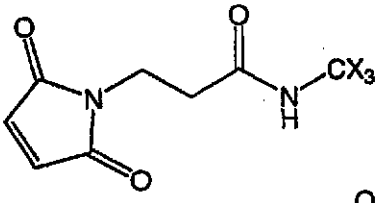


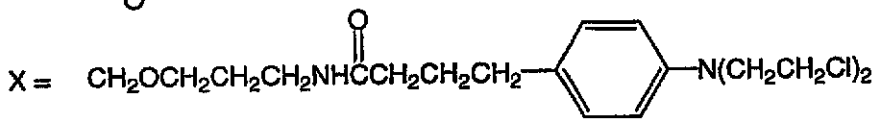

Scheme 15 illustrates the synthesis of a val-cit dipeptide linker having a maleimide Stretcher unit and a bis(4-hydroxymethyl)styrene (BHMS) unit. The synthesis of the BHMS intermediate (**AW**) has been improved from previous literature procedures (see International Publication No, WO 9813059 to Firestone *et al.*, and Crozet, M.P.; Archaimbault, G.; Vanelle, P.; Nouguier, 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.

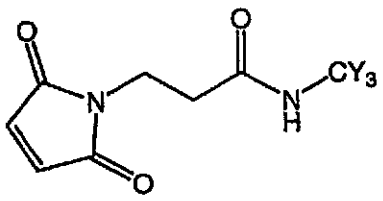
4.6.3 DENDRITIC LINKERS

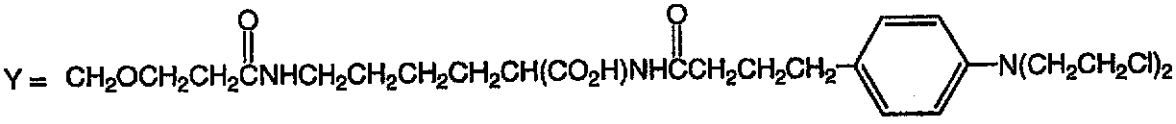
The linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to a Ligand, such as but not limited to an antibody (Sun *et al.* (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun *et al.* (2003) Bioorganic & 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 cytsteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

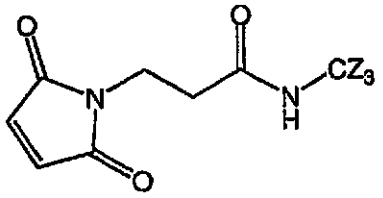
The following exemplary embodiments of dendritic linker reagents allow up to nine nucleophilic drug moiety reagents to be conjugated by reaction with the chloroethyl nitrogen mustard functional groups:

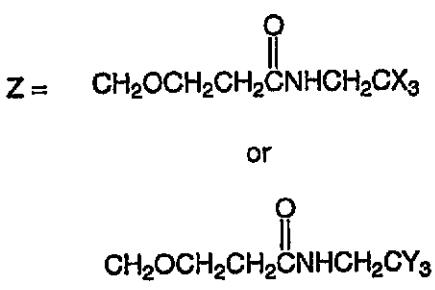








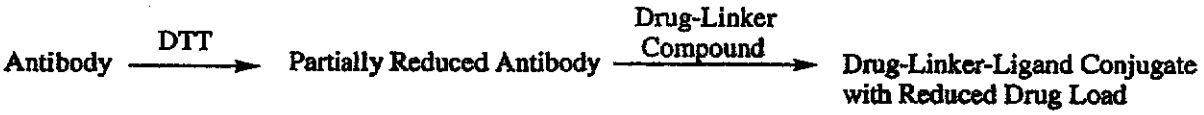




4.6.4 CONJUGATION OF DRUG MOIETIES TO ANTIBODIES

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 ($-CH_2SH$). 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 μm 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 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 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 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 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 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 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 dextrins, 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 digylcerides 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.

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.

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.

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 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 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 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 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 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 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.

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.

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.

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*

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*

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 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
colon cancer
colorectal cancer
kidney cancer
pancreatic cancer
bone cancer
breast cancer
ovarian cancer
prostate cancer
esophogeal cancer
stomach cancer
oral cancer
nasal cancer
throat cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular cancer
small cell lung carcinoma
bladder carcinoma
lung cancer
epithelial carcinoma
glioma
glioblastoma multiforme
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma

oligodendroglioma
meningioma
skin cancer
melanoma
neuroblastoma
retinoblastoma

blood-borne cancers, including but not limited to:

acute lymphoblastic leukemia "ALL"
acute lymphoblastic B-cell leukemia
acute lymphoblastic T-cell leukemia
acute myeloblastic leukemia "AML"
acute promyelocytic leukemia "APL"
acute monoblastic leukemia
acute erythroleukemic leukemia
acute megakaryoblastic leukemia
acute myelomonocytic leukemia
acute nonlymphocyctic leukemia
acute undifferentiated leukemia
chronic myelocytic leukemia "CML"
chronic lymphocytic leukemia "CLL"
hairy cell leukemia
multiple myeloma

acute and chronic leukemias:

lymphoblastic
myelogenous
lymphocytic
myelocytic leukemias

Lymphomas:

Hodgkin's disease
non-Hodgkin's Lymphoma

Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Polycythemia vera

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.

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 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 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 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 limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater that 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.

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, procarbizine, 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<br>ifosfamide<br>trofosfamide<br>chlorambucil<br>melphalan |
| Nitrosoureas: | carmustine (BCNU)<br>lomustine (CCNU) |
| Alkylsulphonates | busulfan |

| | |
|---|---|
| | treosulfan |
| Triazenes: | decarbazine |
| Platinum containing compounds: | cisplatin<br>carboplatin |
| Plant Alkaloids | |
| Vinca alkaloids: | vincristine<br>vinblastine<br>vindesine<br>vinorelbine |
| Taxoids: | paclitaxel<br>docetaxol |
| DNA Topoisomerase Inhibitors | |
| Epipodophyllins: | etoposide<br>teniposide<br>topotecan<br>9-aminocamptothecin<br>camptothecin<br>crisnatol |
| mitomycins: | mitomycin C |
| Anti-metabolites | |
| Anti-folates: | |
| DHFR inhibitors: | methotrexate<br>trimetrexate |
| IMP dehydrogenase Inhibitors: | mycophenolic acid<br>tiazofurin<br>ribavirin<br>EICAR |
| Ribonucleotide reductase Inhibitors: | hydroxyurea<br>deferoxamine |
| Pyrimidine analogs: | |
| Uracil analogs | 5-Fluorouracil |
| | floxuridine<br>doxifluridine<br>ratitrexed |
| Cytosine analogs | cytarabine (ara C)<br>cytosine arabinoside<br>fludarabine |
| Purine analogs: | mercaptopurine<br>thioguanine |
| Hormonal therapies: | |
| Receptor antagonists: | |
| Anti-estrogen | tamoxifen<br>raloxifene<br>megestrol |
| LHRH agonists: | goscrclin<br>leuprolide acetate |
| Anti-androgens: | flutamide<br>bicalutamide |

| | |
|---|---|
| Retinoids/Deltoids | |
| Vitamin D3 analogs: | EB 1089<br>CB 1093<br>KH 1060 |
| Photodynamic therapies: | vertoporfin (BPD-MA)<br>phthalocyanine<br>photosensitizer Pc4<br>demethoxy-hypocrellin A<br>(2BA-2-DMHA) |
| Cytokines: | Interferon- α<br>Interferon- γ<br>tumor necrosis factor |
| Others: | Gemcitabine<br>Velcade<br>Revamid<br>Thalamid |
| Isoprenylation inhibitors: | Lovastatin |
| Dopaminergic neurotoxins: | 1-methyl-4-phenylpyridinium ion |
| Cell cycle inhibitors: | staurosporine |
| Actinomycins: | Actinomycin D |
| | dactinomycin |
| Bleomycins: | bleomycin A2<br>bleomycin B2<br>peplomycin |
| Anthracyclines: | daunorubicin<br>Doxorubicin (adriamycin)<br>idarubicin<br>epirubicin<br>pirarubicin<br>zorubicin<br>mtoxantrone |
| 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. Inone 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

Active Chronic Hepatitis
Addison's Disease
Allergic Alveolitis
Allergic Reaction
Allergic Rhinitis
Alport's Syndrome
Anaphlaxis
Ankylosing Spondylitis
Anti-phosholipid Syndrome
Arthritis
Ascariasis
Aspergillosis

Atopic Allergy
Atropic Dermatitis
Atropic Rhinitis
Behcet's Disease
Bird-Fancier's Lung
Bronchial Asthma
Caplan's Syndrome
Cardiomyopathy
Celiac Disease
Chagas' Disease
Chronic Glomerulonephritis
Cogan's Syndrome
Cold Agglutinin Disease
Congenital Rubella Infection
CREST Syndrome
Crohn's Disease
Cryoglobulinemia
Cushing's Syndrome
Dermatomyositis
Discoid Lupus
Dressler's Syndrome
Eaton-Lambert Syndrome
Echovirus Infection
Encephalomyelitis
Endocrine opthalmopathy
Epstein-Barr Virus Infection
Equine Heaves
Erythematosis
Evan's Syndrome
Felty's Syndrome
Fibromyalgia
Fuch's Cyclitis
Gastric Atrophy
Gastrointestinal Allergy
Giant Cell Arteritis
Glomerulonephritis
Goodpasture's Syndrome
Graft v. Host Disease
Graves' Disease
Guillain-Barre Disease
Hashimoto's Thyroiditis
Hemolytic Anemia
Henoch-Schonlein Purpura
Idiopathic Adrenal Atrophy
Idiopathic Pulmonary Fibritis
IgA Nephropathy
Inflammatory Bowel Diseases
Insulin-dependent Diabetes Mellitus
Juvenile Arthritis

Juvenile Diabetes Mellitus (Type I)
Lambert-Eaton Syndrome
Laminitis
Lichen Planus
Lupoid Hepatitis
Lupus
Lymphopenia
Meniere's Disease
Mixed Connective Tissue Disease
Multiple Sclerosis
Myasthenia Gravis
Pernicious Anemia
Polyglandular Syndromes
Presenile Dementia
Primary Agammaglobulinemia
Primary Biliary Cirrhosis
Psoriasis
Psoriatic Arthritis
Raynauds Phenomenon
Recurrent Abortion
Reiter's Syndrome
Rheumatic Fever
Rheumatoid Arthritis
Sampter's Syndrome
Schistosomiasis
Schmidt's Syndrome
Scleroderma
Shulman's Syndrome
Sjorgen's Syndrome
Stiff-Man Syndrome
Sympathetic Ophthalmia
Systemic Lupus Erythematosis
Takayasu's Arteritis
Temporal Arteritis
Thyroiditis
Thrombocytopenia
Thyrotoxicosis
Toxic Epidermal Necrolysis
Type B Insulin Resistance
Type I Diabetes Mellitus
Ulcerative Colitis
Uveitis
Vitiligo
Waldenstrom's Macroglobulemia
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:

Diphtheria
Pertussis
Occult Bacteremia
Urinary Tract Infection
Gastroenteritis
Cellulitis
Epiglottitis
Tracheitis
Adenoid Hypertrophy
Retropharyngeal Abcess
Impetigo
Ecthyma
Pneumonia
Endocarditis
Septic Arthritis
Pneumococcal
Peritonitis
Bactermia
Meningitis
Acute Purulent Meningitis
Urethritis
Cervicitis
Proctitis
Pharyngitis
Salpingitis
Epididymitis
Gonorrhea
Syphilis
Listeriosis
Anthrax
Nocardiosis
Salmonella
Typhoid Fever
Dysentery
Conjunctivitis
Sinusitis
Brucellosis
Tullaremia
Cholera
Bubonic Plague
Tetanus

Necrotizing Enteritis
Actinomycosis
Mixed Anaerobic Infections
Syphilis
Relapsing Fever
Leptospirosis
Lyme Disease
Rat Bite Fever
Tuberculosis
Lymphadenitis
Leprosy
Chlamydia
Chlamydial Pneumonia
Trachoma
Inclusion Conjunctivitis

Systemic Fungal Diseases:

Histoplamosis
Coccidiodomycosis
Blastomycosis
Sporotrichosis
Cryptococcsis
Systemic Candidiasis
Aspergillosis
Mucormycosis
Mycetoma
Chromomycosis

Rickettsial Diseases:

Typhus
Rocky Mountain Spotted Fever
Ehrlichiosis
Eastern Tick-Borne Rickettsioses
Rickettsialpox
Q Fever
Bartonellosis

Parasitic Diseases:

Malaria
Babesiosis
African Sleeping Sickness
Chagas' Disease
Leishmaniasis
Dum-Dum Fever
Toxoplasmosis
Meningoencephalitis
Keratitis

Entamebiasis
Giardiasis
Cryptosporidiasis
Isosporiasis
Cyclosporiasis
Microsporidiosis
Ascariasis
Whipworm Infection
Hookworm Infection
Threadworm Infection
Ocular Larva Migrans
Trichinosis
Guinea Worm Disease
Lymphatic Filariasis
Loiasis
River Blindness
Canine Heartworm Infection
Schistosomiasis
Swimmer's Itch
Oriental Lung Fluke
Oriental Liver Fluke
Fascioliasis
Fasciolopsiasis
Opisthorchiasis
Tapeworm Infections
Hydatid Disease
Alveolar Hydatid Disease

Viral Diseases:

Measles
Subacute sclerosing panencephalitis
Common Cold
Mumps
Rubella
Roseola
Fifth Disease
Chickenpox
Respiratory syncytial virus infection
Croup
Bronchiolitis
Infectious Mononucleosis
Poliomyelitis
Herpangina
Hand-Foot-and-Mouth Disease
Bornholm Disease
Genital Herpes
Genital Warts
Aseptic Meningitis

Myocarditis
Pericarditis
Gastroenteritis
Acquired Immunodeficiency Syndrome (AIDS)
Human Immunodeficiency Virus (HIV)
Reye's Syndrome
Kawasaki Syndrome
Influenza
Bronchitis
Viral "Walking" Pneumonia
Acute Febrile Respiratory Disease
Acute pharyngoconjunctival fever
Epidemic keratoconjunctivitis
Herpes Simplex Virus 1 (HSV-1)
Herpes Simplex Virus 2 (HSV-2)
Shingles
Cytomegalic Inclusion Disease
Rabies
Progressive Multifocal Leukoencephalopathy
Kuru
Fatal Familial Insomnia
Creutzfeldt-Jakob Disease
Gerstmann-Straussler-Scheinker Disease
Tropical Spastic Paraparesis
Western Equine Encephalitis
California Encephalitis
St. Louis Encephalitis
Yellow Fever
Dengue
Lymphocytic choriomeningitis
Lassa Fever
Hemorrhagic Fever
Hantvirus Pulmonary Syndrome
Marburg Virus Infections
Ebola Virus Infections
Smallpox

4.8.7 MULTI-DRUG THERAPY OF INFECTIOUS DISEASES

Methods for treating an infectious disease are disclosed including 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:

- Penicillin G
- Penicillin V
- Cloxacilliin
- Dicloxacillin
- Methicillin
- Nafcillin
- Oxacillin
- Ampicillin
- Amoxicillin
- Bacampicillin
- Azlocillin
- Carbenicillin
- Mezlocillin
- Piperacillin
- Ticarcillin

Aminoglycosides:

- Amikacin
- Gentamicin
- Kanamycin
- Neomycin
- Netilmicin
- Streptomycin
- Tobramycin

Macrolides:

- Azithromycin
- Clarithromycin
- Erythromycin
- Lincomycin
- Clindamycin

Tetracyclines:

- Demeclocycline
- Doxycycline
- Minocycline
- Oxytetracycline
- Tetracycline

Quinolones:

- Cinoxacin
- Nalidixic Acid

Fluoroquinolones:

Ciprofloxacin
Enoxacin
Grepafloxacin
Levofloxacin
Lomefloxacin
Norfloxacin
Ofloxacin
Sparfloxacin
Trovafloxicin

Polypeptides:

Bacitracin
Colistin
Polymyxin B

Sulfonamides:

Sulfisoxazole
Sulfamethoxazole
Sulfadiazine
Sulfamethizole
Sulfacetamide

Miscellaneous Antibacterial Agents:

Trimethoprim
Sulfamethazole
Chloramphenicol
Vancomycin
Metronidazole
Quinupristin
Dalfopristin
Rifampin
Spectinomycin
Nitrofurantoin
Antiviral Agents:

General Antiviral Agents:

Idoxuradine
Vidarabine
Trifluridine
Acyclovir
Famcicyclovir
Pencicyclovir
Valacyclovir
Gancicyclovir

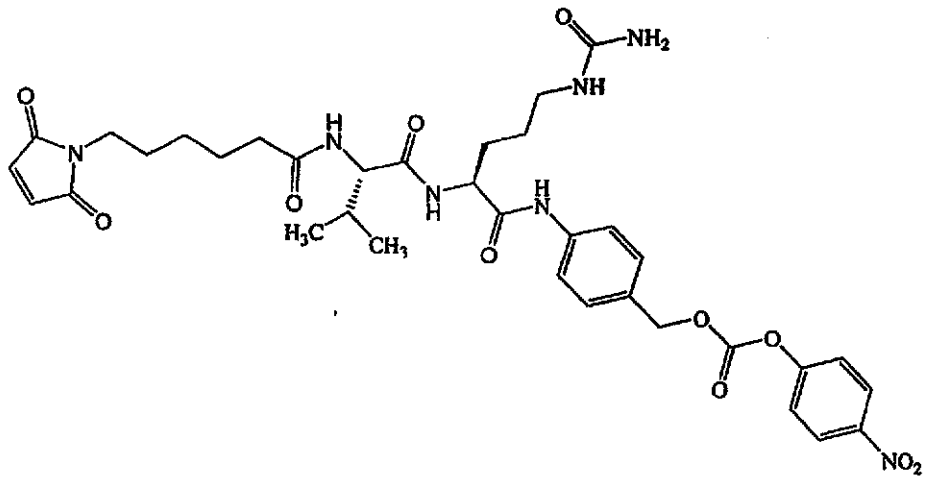
Foscarnet
Ribavirin
Amantadine
Rimantadine
Cidofovir
Antisense Oligonucleotides
Immunoglobulins
Inteferons

Drugs for HIV infection:

Tenofovir
Emtricitabine
Zidovudine
Didanosine
Zalcitabine
Stavudine
Lamivudine
Nevirapine
Delavirdine
Saquinavir
Ritonavir
Indinavir
Nelfinavir

## 5. EXAMPLES

Example 1 - Preparation of compound AB



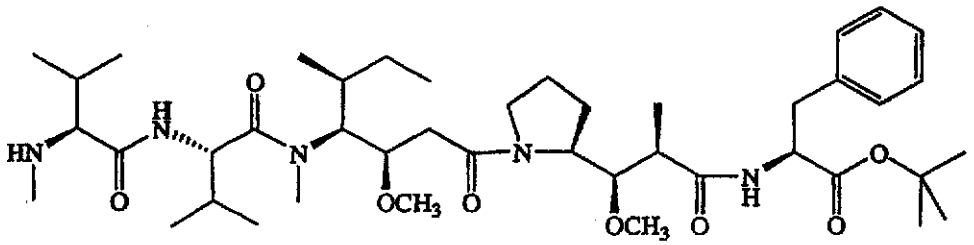
AB

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



1

Phenylalanine *t*-butyl ester HCl salt (868 mg, 3 mmol), *N*-Boc-Dolaproine (668 mg, 1 eq.), DEPC (820 µL, 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 $[M+H]^+$.

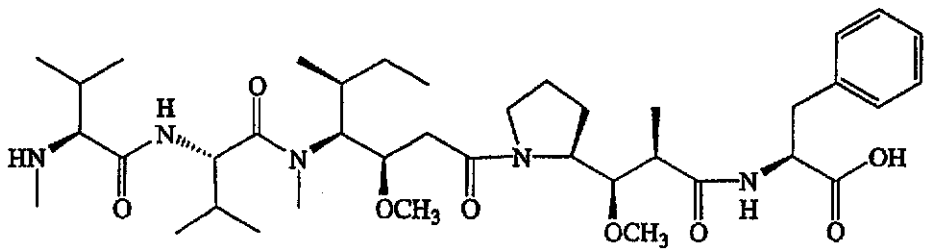
For selective Boc cleavage in the presence of *t*-butyl ester, the above dipeptide (500 mg, 1.28 mmol) was diluted with dioxane (2 mL). 4M HCl/dioxane (960 µL, 3 eq.) was added, and the reaction mixture was stirred overnight at room temperature. Almost complete Boc deprotection was observed by RP-HPLC with minimal amount of *t*-butyl ester cleavage. The mixture was cooled down on an ice bath, and triethylamine (500 µL) 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 $[M+H]^+$.

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 $[M+H]^+$.

Example 3 - Preparation of compound 2

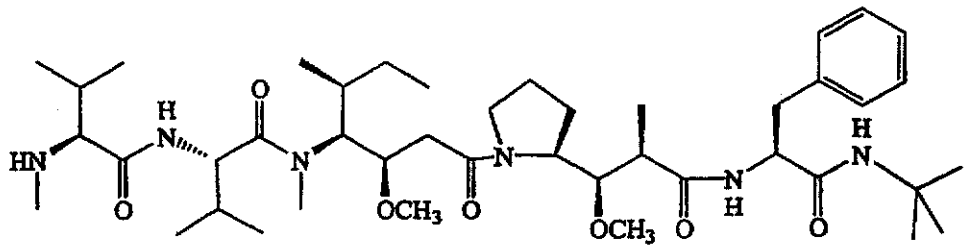


**2**

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 hydroscopic white solid: 35 mg (120 % calculated for HCl salt). ES-MS *m/z* 732.56 $[M+H]^+$.

Example 4 - Preparation of compound **3**



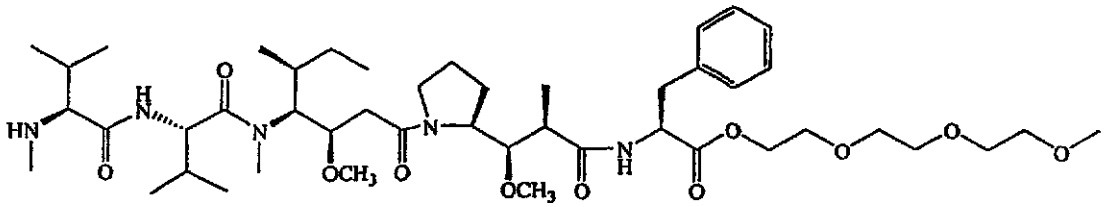
**3**

Fmoc-Meval-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 hydroscopic white solid intermediate

The white solid intermediate (20 mg, 0.02 mmol) was diluted with dichloromethane (1 mL); DEPC (5 µL, 0.03 mmol, 1.5 eq.) was added followed by DIEA (11 µL, 0.06 mmol, 3 eq.), and *t*-butylamine (3.2 µL, 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 µL) and *t*-butylamine (5 µL) 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



4

Boc-L-Phenylalanine (265 mg, 1 mmol, 1 eq.) and triethyleneglycol monomethyl ether (164 µL, 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 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 temperature. The solvent was removed in a vacuum, the residue was dried in a vacuum to give a white solid.

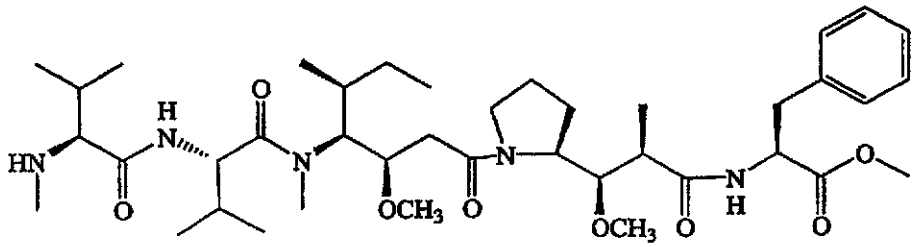
The HCl salt of Phenylalanine-triethyleneglycol monomethyl ether ester (236 mg, 0.458 mmol, 1eq.) and *N*-Boc-Dolaproine (158 mg, 0.55 mmol, 1.2 eq.) were diluted with dichloromethane (3 mL). DEPC (125 µL, 1.5 eq.) and added to the mixture followed by DIEA (250 µL, 3 eq.). After 2 h at room temperature 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 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 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 μL, 1.5 eq.) was added, followed by DIEA (160 μL, 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 $[M+H]^+$, 1122.4 $[M+Na]^+$.

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 $[M+H]^+$.

Example 6 - Preparation of compound **5**

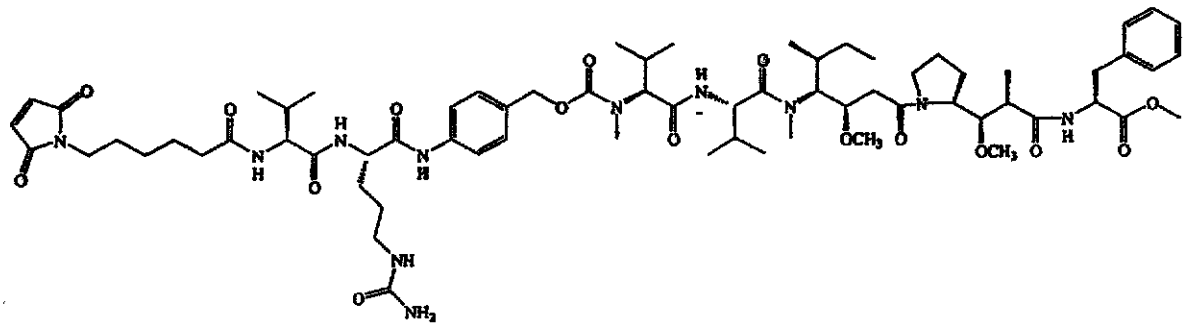


**5**

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 × 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 $[M+H]^+$, 991.34 $[M+Na]^+$; UV $\lambda_{max}$ 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 ($C_{12}$-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 $\lambda_{max}$ 215 nm.

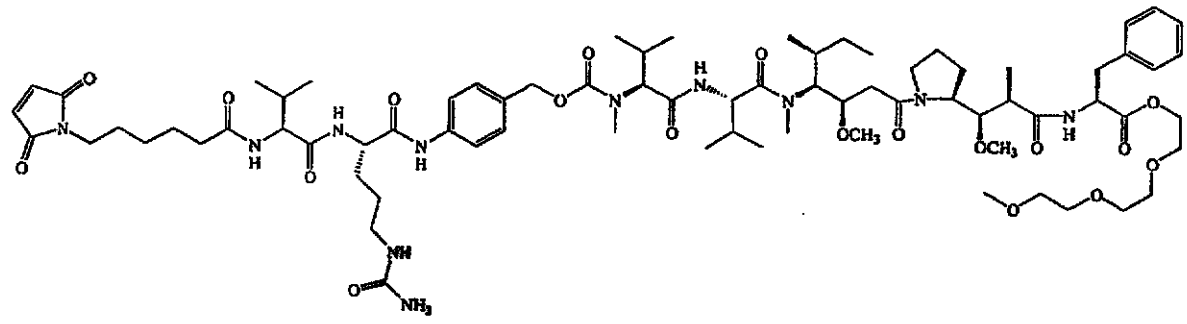
Example 7 - Preparation of compound 6



6

The trifluoroacetate salt of Compound 5 (0.11 g, 0.13 mmol), Compound AB (0.103 g, 0.14 mmol, 1.1 eq.) and HOBt (3.4 mg, 26 μmol, 0.2 eq.) were suspended in DMF/pyridine (2 mL/0.5 mL, respectively). Diisopropylethylamine (22.5 μL, 0.13 mmol, 1.0 eq.) was added and the yellow solution stirred while under argon. After 3 h, an additional 1.0 eq. of DIEA was added. 24 hours later, 0.5 eq. of the activated linker was included in the reaction mixture. After 40 h total, the reaction was complete. The contents were evaporated, taken up in DMSO and injected into a prep-HPLC ($C_{12}$-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 50 mL/min). The desired fractions were evaporated to give the product as a yellow oil. Methylene chloride (ca. 2 mL) and excess ether were added to provide Compound 6 as a white precipitate that was filtered and dried. Yield: 90 mg (52 %). ES-MS *m/z* 1344.32 $[M+H]^+$, 1366.29 $[M+Na]^+$; UV $\lambda_{max}$ 215, 248 nm.

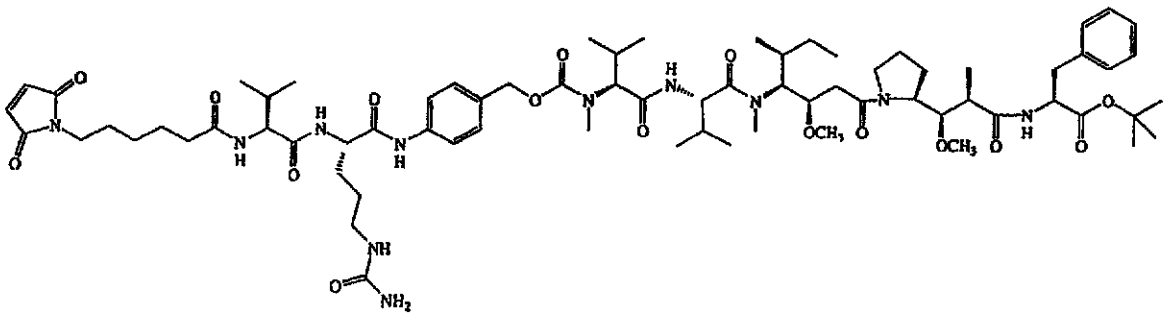
Example 8 - Preparation of compound 7



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 $[M+H]^+$.

Example 9 - Preparation of MC-Val-Cit-PAB-MMAF t-butyl ester 8

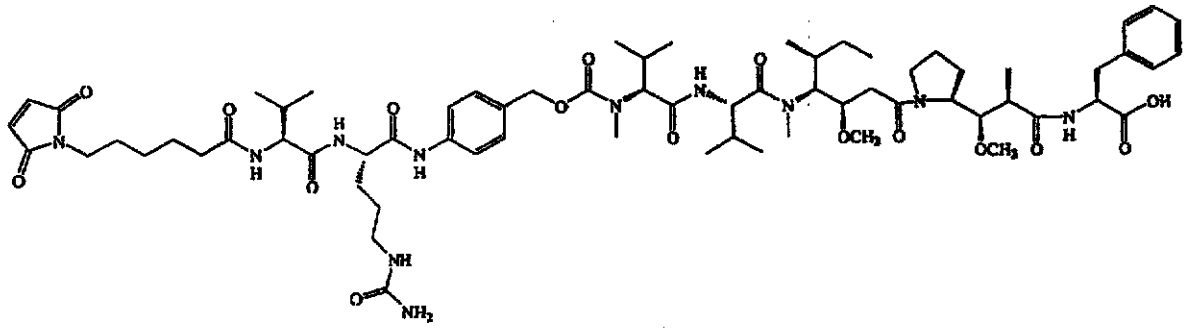


8

Compound 1 (83 mg, 0.11 mmol), Compound AB (85 mg, 0.12 mmol, 1.1 eq.), and HOBt (2.8 mg, 21 µmol, 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 ($C_{12}$-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 $\lambda_{max}$ 205, 248 nm.

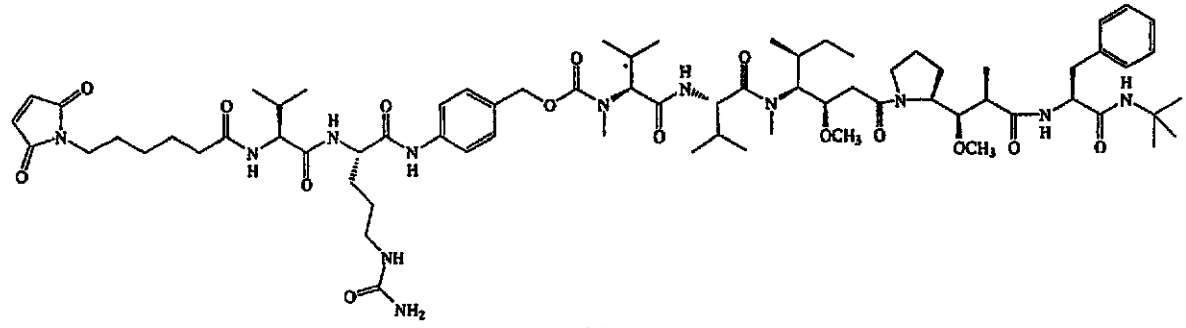
Example 10 - Preparation of MC-val-cit-PAB-MMAF **9**



**9**

Compound **8** (45 mg, 32 μmol) 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 ($C_{12}$-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 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 $\lambda_{max}$ 205, 248 nm.

Example 11 - Preparation of MC-val-cit-PAB-MMAF tert-butyl amide **10**



**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 $\lambda_{max}$ 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 μm 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.

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. 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 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 washed; the cytotoxic effect can be determined after 96 h of incubation.

EXAMPLE 17 - *in vitro* cytotoxicity cata 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 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 - 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 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 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.
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.
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.

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 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 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).

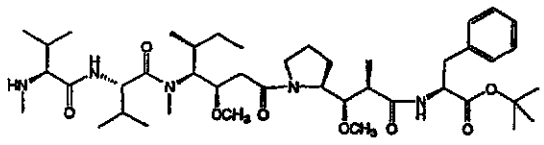
Example 21 - Tumor volume *in vivo* efficacy in transgenic explant mice

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. 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.

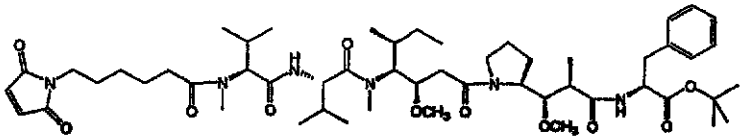
Animals having tumors (allograft propagated from Fo5 mmtv transgenic mice) can be treated with a single or multiple dose by IV injection of ADC. Tumor volume can be assessed at various time points after injection.

Example 22 - Synthesis of MC-MMAF via t-butyl ester

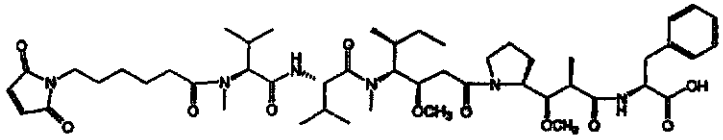
Synthesis 1:



MeVal-Val-Dil-Dap-Phe-OtBu, 1001



MC-MeVal-Val-Dil-Dap-Phe-OtBu



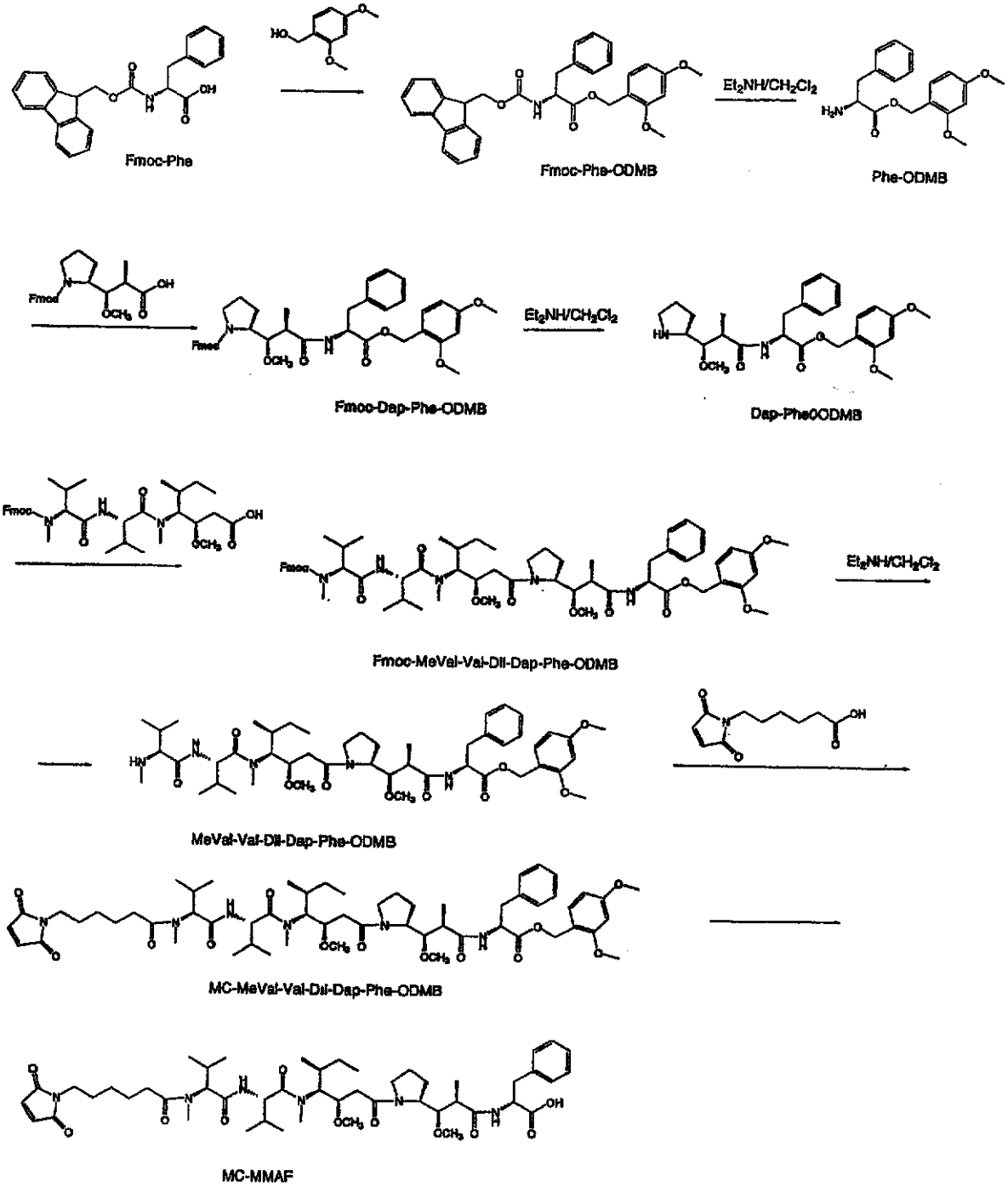
MC-MMAF

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 $[M+H]^+$; 1003.47 $[M+Na]^+$; 979.65 $[M-H]^-$.

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 $[M+H]^+$; 947.30 $[M+Na]^+$; 923.45 $[M-H]^-$.

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 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) 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 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)).

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 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). 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 Rf= 0.33, red in vanillin stain).

Preparation of Dap-Phe-ODMB

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 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.

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 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 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)).

Data: HPLC, TLC (2:1 EtOAc/heptane, Rf = 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 purifed 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, Rf = 0.24, red in vanillin stain).

Preparation of MC-MeVal-Val-Dil-Dap-Phe-ODMB

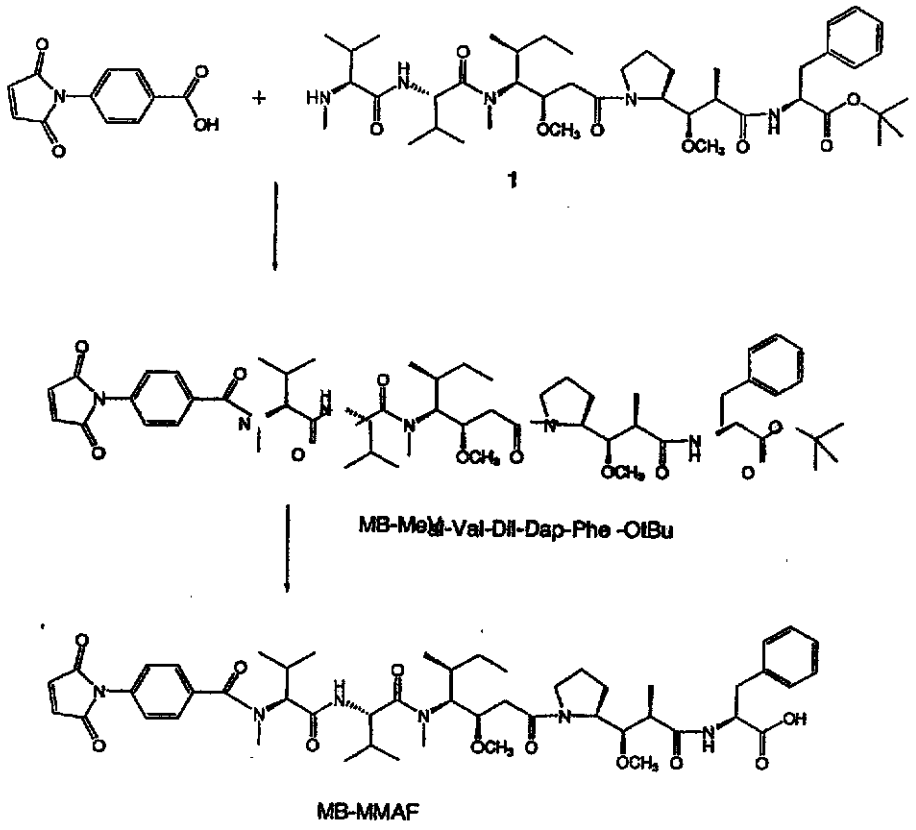
A 50-mL, round-bottom flask was charged with MeVal-Val-Dil-Dap-Phe-ODMB (750 mg, 0.85 mmol), anhydrous DMF (4 mL), maleimidocaproic acid (180 mg, 0.85 mmol), and TBTU (300 mg, 0.93mmol, Matrix Innovations) at room temperature. N,N-Diisopropylethylamine (450 μL, 2.57 mmol) was added via syringe. After 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



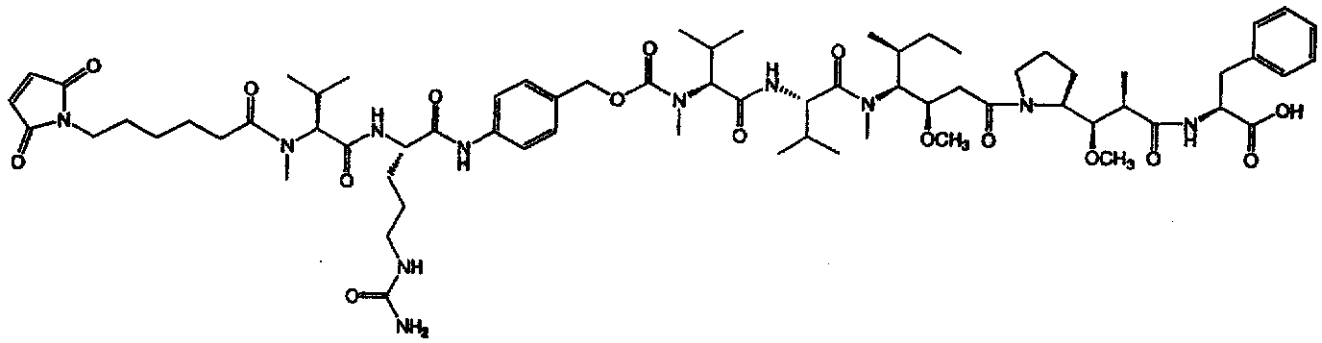

MeVal-Val-Dil-Dap-Phe-OtBu (compound **1**, 35 mg, 0.044 mmol) was suspended in DMF (0.250 mL). 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-benzoic acid (11 mg, 0.049 mmol) and HATU (17 mg, 0.044 mmol) were added followed by DIEA (0.031 mL, 0.17 mmol). This reaction mixture was allowed to stir for 2.0 hr. HPLC analysis indicated complete consumption of starting compound **1**.

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 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 $[M-H]^-$.

MB-MeVal-Val-Dil-Dap-Phe-OtBu (Compound 14, 38 mg, 0.0385 mmol) was suspended in $CH_2Cl_2$ (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

preparatory RP-HPLC, using a Phenomenex $C_{12}$ 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 14.4 mg of MB-MMAF product was isolated (0.015 mmol, 40% yield). ES-MS *m/z* 930.96 $[M+H]^+$ 952.98 $[M+Na]^+$; 929.37 $[M-H]^-$.

**Example 23c - Preparation of MC-MeVal-Cit-PAB-MMAF (16)**



To a room temperature suspension of Fmoc-MeVal-OH (3.03 g, 8.57 mmol) and N,N'-disuccimidyl carbonate (3.29 g, 12.86 mmol) in $CH_2Cl_2$ (80 mL) was added DIEA (4.48 mL, 25.71 mmol). This reaction mixture was allowed to stir for 3.0 hr, and then poured into a separation funnel where the organic mixture was extracted with 0.1 M HCl (aq). The crude organic residue was concentrated under reduced pressure, and the product was isolated by flash column chromatography on silica gel using a 20-100% ethyl acetate/hexanes linear gradient. A total of 2.18 g of pure Fmoc-MeVal-OSu (4.80 mmoles, 56% yield) was recovered.

To a room temperature suspension of Fmoc-MeVal-OSu (2.18 g, 4.84 mmol) in DME (13 mL) and THF (6.5 mL) was added a solution of L-citrulline (0.85 g, 4.84 mmol) and $NaHCO_3$ (0.41 g, 4.84 mmol) in $H_2O$ (13 mL). The suspension was allowed to stir at room temperature for 16 hr, then it was extracted into *tert*-BuOH/$CHCl_3$/$H_2O$, acidified to pH=2-3 with 1 M HCl. The organic phase was separated, dried and concentrated under reduced pressure. The residue was triturated with diethyl ether resulting in 2.01 g of Fmoc-MeVal-Cit-COOH which was used without further purification.

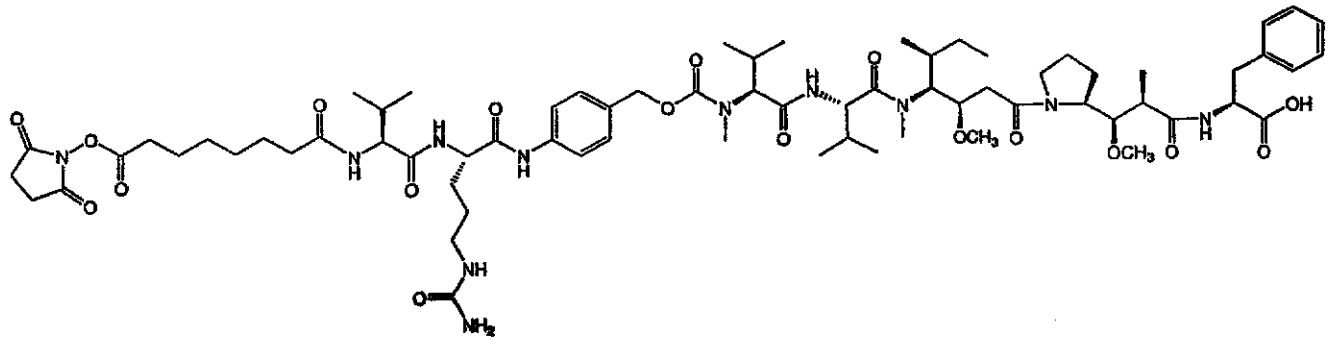
The crude Fmoc-MeVal-Cit-COOH was suspended in 2:1 $CH_2Cl_2$/MeOH (100 mL), and to it was added *p*-aminobenzyl alcohol (0.97 g, 7.9 mmol) and EEDQ

(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/$CH_2Cl_2$. 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 $CH_2Cl_2$ (40 mL) was added STRATOSPHERES$^{tm}$(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 $CH_2Cl_2$ (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% $NaHCO_3$ (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/$CH_2Cl_2$ 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 $CH_2Cl_2$ (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 $C_{12}$ 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. 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**

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 µl, 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 $CH_2Cl_2$. 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 $[M+H]^+$.

Fmoc-Val-Cit-PAB-MMAF-OtBu (100 mg) was stirred in 20% 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 $[M-H]^-$.

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.

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% $MeOH/CH_2Cl_2$, filtered trough a pad of silica gel with 30% $MeOH/CH_2Cl_2$ 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 ($5x10^6$ cells per mouse). Tumor volumes were calculated using the formula ($0.5xLxW^2$) 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 $mm^3$ (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 $mm^3$ 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 $mm^3$ 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 16b 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 μM 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. $IC_{50}$ 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 μM resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. $IC_{50}$ 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 μM resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit

procedure. $IC_{50}$ 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 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 (Sepharose 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, 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 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 (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. 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.

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.

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

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, 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: [{(body weight in grams to 0.667 power) x 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:

| | |
|---|---|
| Group 1: | Vehicle |
| Group 2: | trastuzumab-MC-MMAF, 52.24mg/kg, 4210μg/m$^2$ |
| Group 3: | trastuzumab-MC-MMAF, 68.25mg/kg, 5500μg/m$^2$ |
| Group 4: | trastuzumab-MC-MMAF, 86.00mg/kg, 6930μg/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<br>1M/1F | day 1<br>day 22 |
| H-MC-vc-PAB-MMAE | 2<br>2M/2F | 180 μg/m$^2$ (0.5 mg/kg) at day 1<br>1100 μg/m$^2$ (3.0 mg/kg) at day 22 |
| H-MC-vc-PAB-MMAE | 3<br>2M/2F | 550 μg/m$^2$ (1.5 mg/kg) at day 8<br>550 μg/m$^2$ (1.5 mg/kg) at day 29 |
| H-MC-vc-PAB-MMAE | 4<br>2M/2F | 880 μg/m$^2$ (2.5 mg/kg) at day 15<br>880 μg/m$^2$ (2.5 mg/kg) at day 36 |

| Sample | Group | Dose |
|---|---|---|
| Vehicle | 1<br>1M/1F | day 1<br>day 22 |
| H-MC-vc-PAB-MMAF | 2<br>2M/2F | 180 μg/m$^2$ (0.5 mg/kg) at day 1<br>1100 μg/m$^2$ (3.0 mg/kg) at day 22 |

| | | |
|---|---|---|
| H-MC-vc-PAB-MMAF | 3<br>2M/2F | 550 μg/m$^2$ (1.5 mg/kg) at day 1<br>550 μg/m$^2$ (1.5 mg/kg) at day 22 |
| H-MC-vc-PAB-MMAF | 4<br>2M/2F | 880 μg/m$^2$ (2.5 mg/kg) at day 1<br>880 μg/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 μg/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-$3_{400}$ cells (expressing approximately 1 x $10^5$ 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-$3_{400}$ cells were trypsinized, washed once, and resuspended at $1.75 \times 10^6$ cell/ml in cold PBS containing 0.5% bovine serum albumin (BSA) and 0.1% $NaN_3$. 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 $4 \times 10^5$ cells per ml. Aliquots of 100 µl ($4 \times 10^4$ 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 rHRGβ1$_{177-244}$ 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 µl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 µl) was electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (4G10, from UBI, used at 1 µg/ml) immunoblots were developed, and the intensity of the predominant reactive band at $\bar{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 rHRGβ1 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 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.

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.

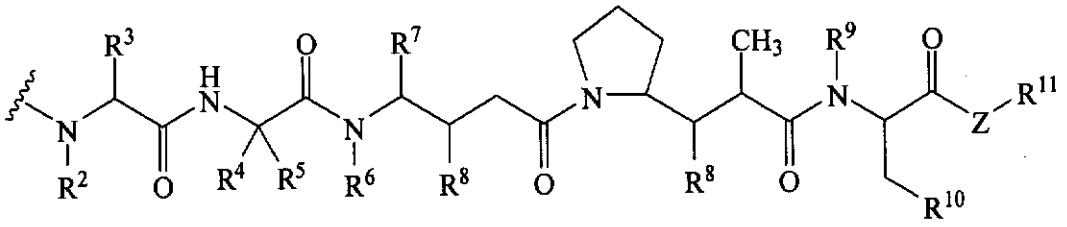
REFERENCES CITED

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.

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:



wherein the wavy line indicates a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 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 –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;

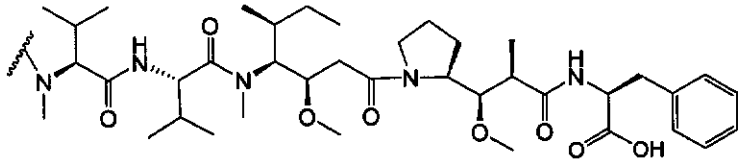
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

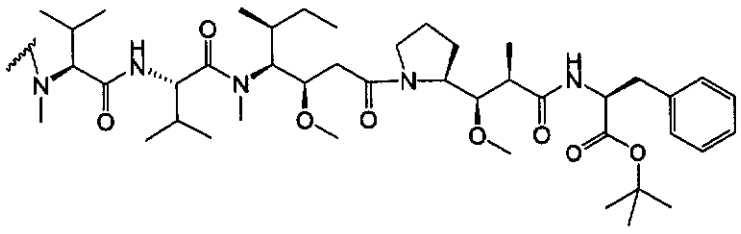
or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 having the formula:



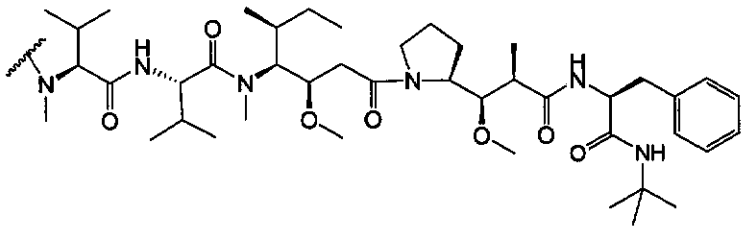
or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 having the formula:



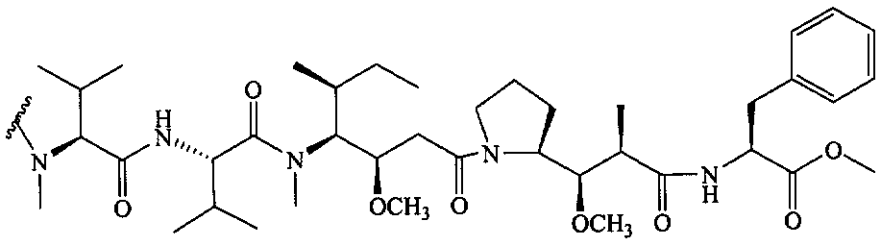
or a pharmaceutically acceptable salt thereof.

4. The compound of claim 1 having the formula:



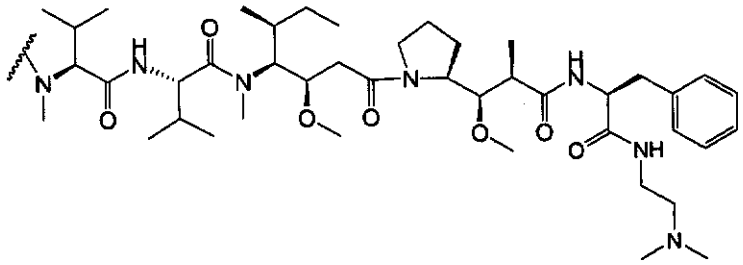
or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 having the formula:



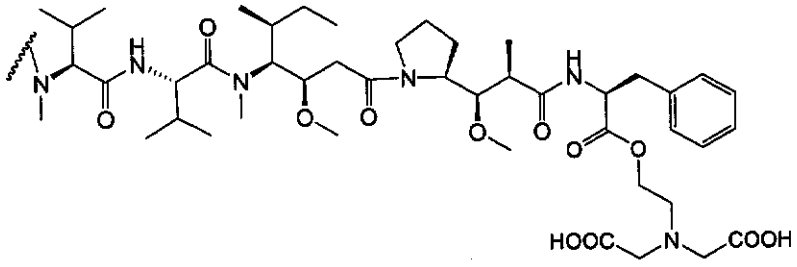
or a pharmaceutically acceptable salt thereof.

6. The compound of claim 1 having the formula:



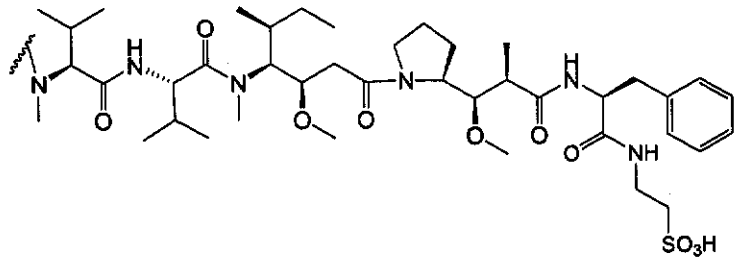
or a pharmaceutically acceptable salt thereof.

7. The compound of claim 1 having the formula:



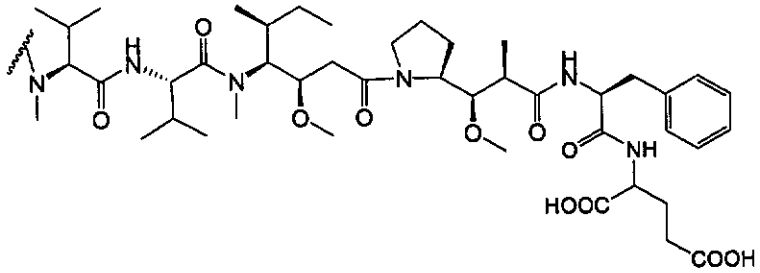
or a pharmaceutically acceptable salt thereof.

8. The compound of claim 1 having the formula:



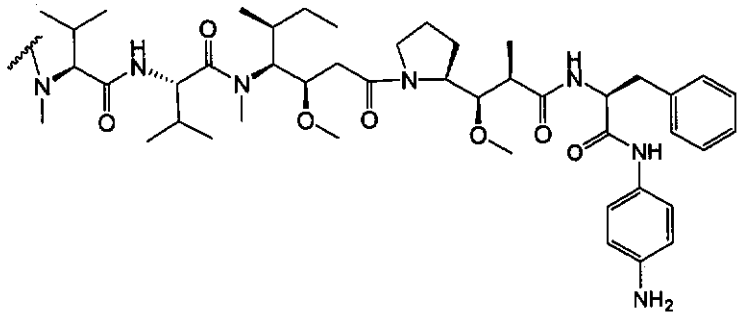
or a pharmaceutically acceptable salt thereof.

9. The compound of claim 1 having the formula:



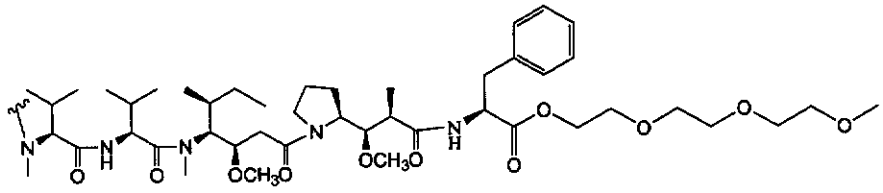
or a pharmaceutically acceptable salt thereof.

10. The compound of claim 1 having the formula:



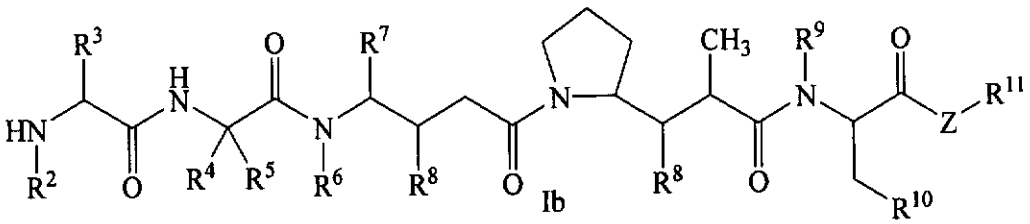
or a pharmaceutically acceptable salt thereof.

11. The compound of claim 1 having the formula:



or a pharmaceutically acceptable salt thereof.

12. A compound having the formula:



Ib

or a pharmaceutically acceptable salt 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 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 –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;

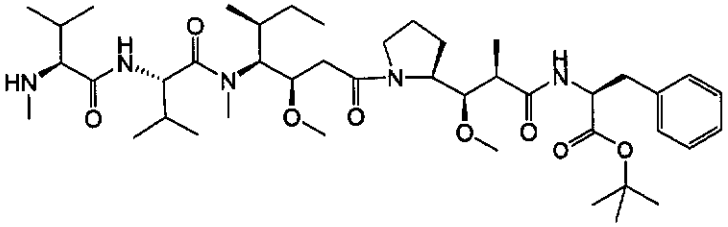
$R^{14}$ is -H or -$C_1$-$C_8$ alkyl;

each occurrence of $R^{15}$ is independently -H, -COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, 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

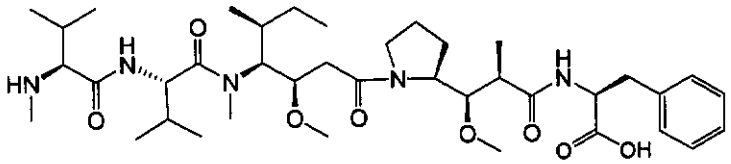
n is an integer ranging from 0 to 6.

13. The compound of claim 12 having the formula:



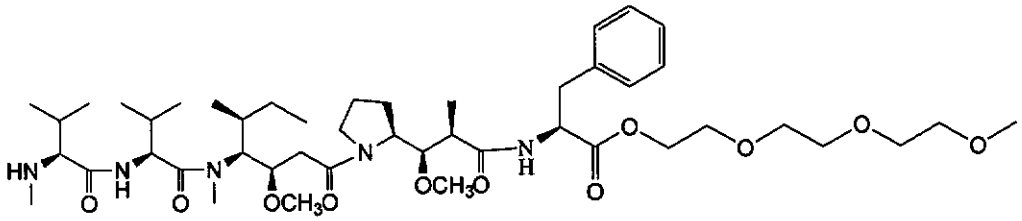
or a pharmaceutically acceptable salt thereof.

14. The compound of claim 12 having the formula:



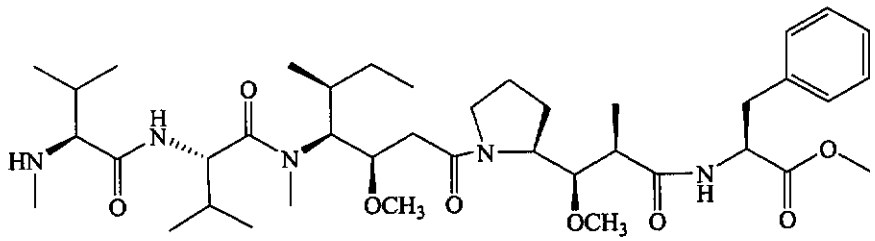
or a pharmaceutically acceptable salt thereof.

15. The compound of claim 12 having the formula:



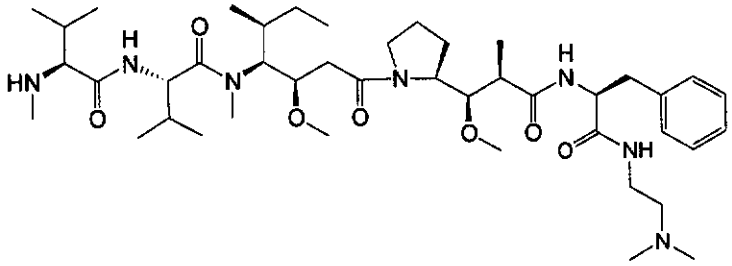
or a pharmaceutically acceptable salt thereof.

16. The compound of claim 12 having the formula:



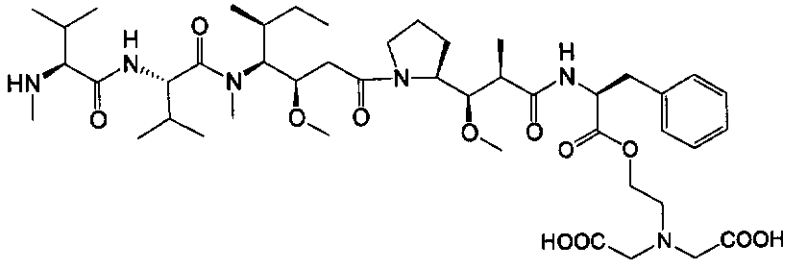
or a pharmaceutically acceptable salt thereof.

17. The compound of claim 12 having the formula:



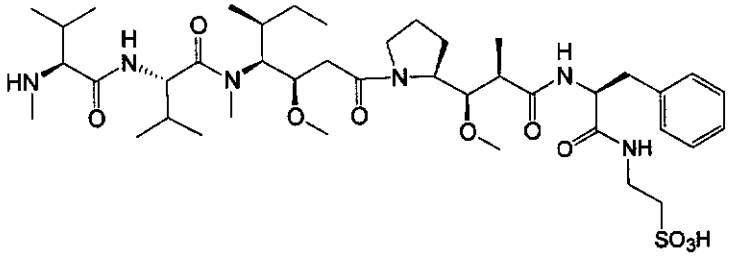
or a pharmaceutically acceptable salt thereof.

18. The compound of claim 12 having the formula:



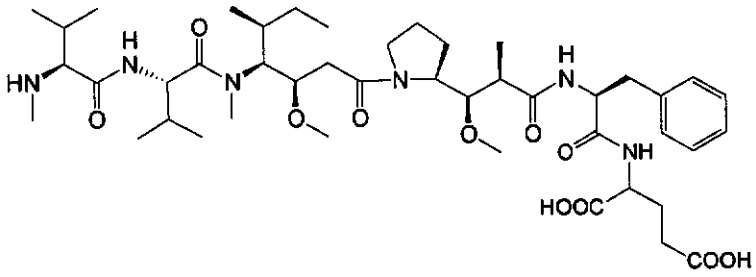
or a pharmaceutically acceptable salt-thereof.

19. The compound of claim 12 having the formula:



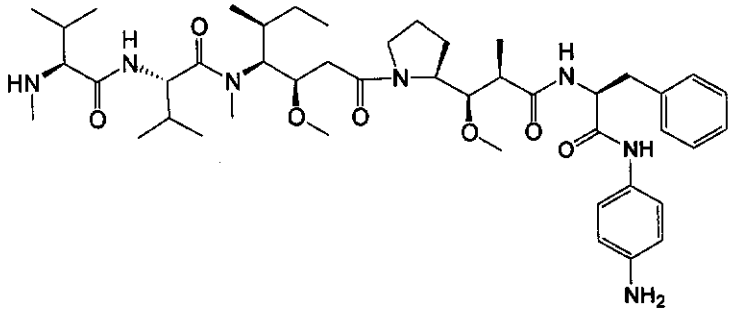
or a pharmaceutically acceptable salt thereof.

20. The compound of claim 12 having the formula:



or a pharmaceutically acceptable salt thereof.

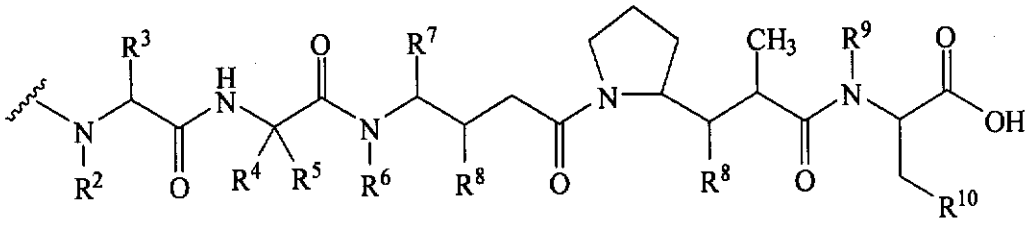
21. The compound of claim 12 having the formula:



or a pharmaceutically acceptable salt thereof.

22. The compound or a pharmaceutically acceptable salt of the compound of any one of claims 12-21 that is in isolated and purified form.

23. The compound having the formula:



wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

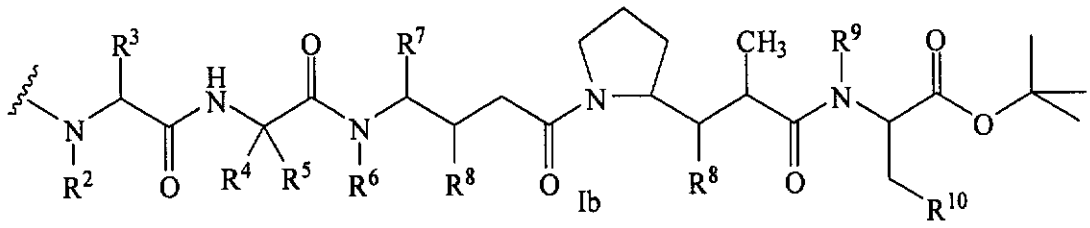
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

24. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, 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

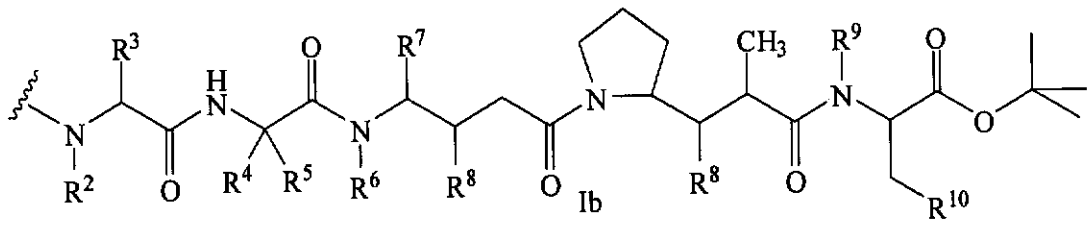
n is an integer ranging from 0 to 6;

or a pharmaceutically acceptable salt thereof.

25. The compound of claim 1 wherein 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.

26. The compound of claim 1 wherein Z is -NH, $R^{11}$ is -$CH(R^{15})_2$, wherein $R^{15}$ is -$(CH_2)_n$-$SO_3H$.

27. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

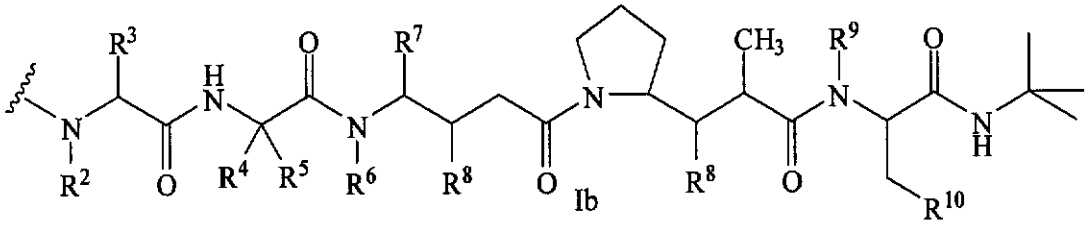
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

28. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

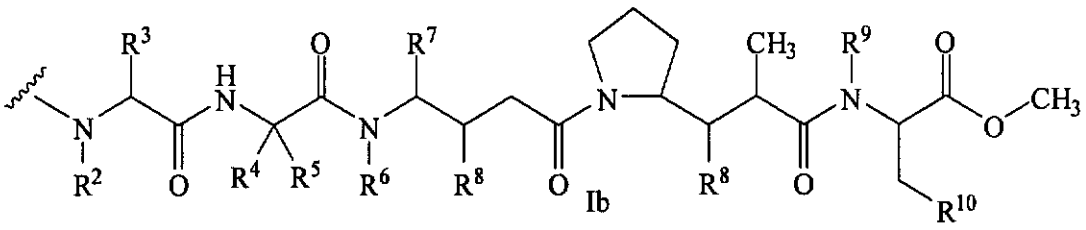
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

29. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

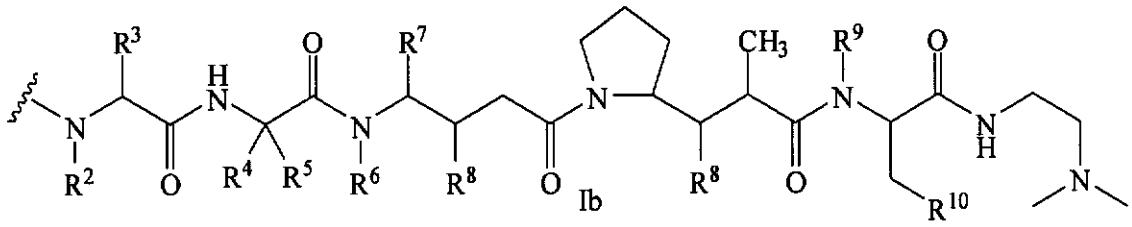
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

30. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

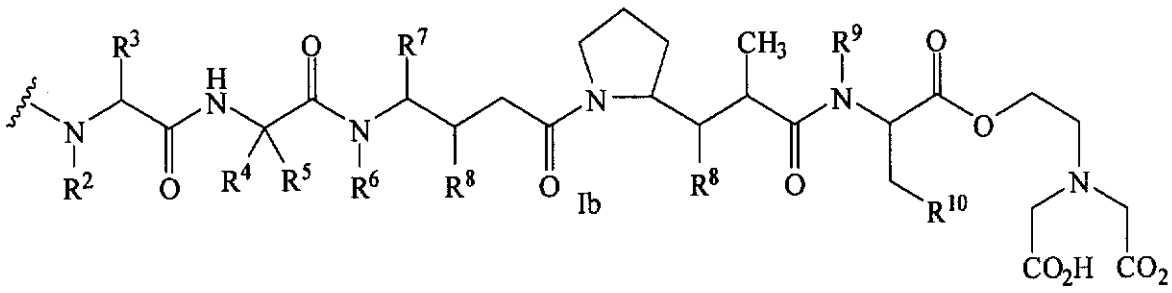
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

31. The compound having the formula:




wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

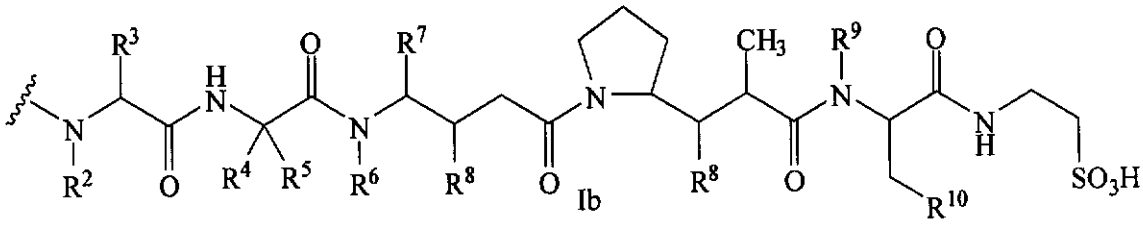
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

32. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

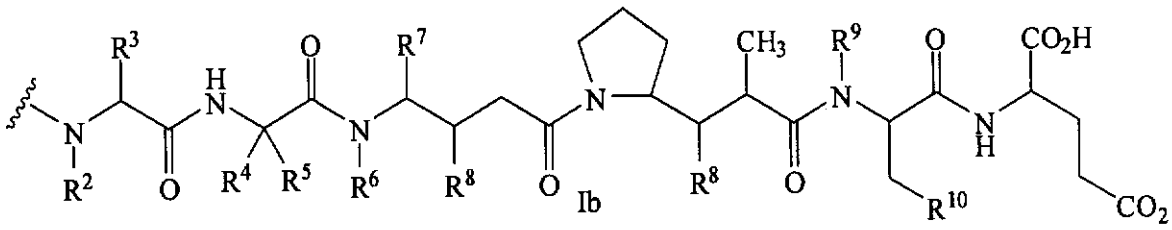
$R^{14}$ is -H or -$C_1$-$C_8$ alkyl;

each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, 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;
or a pharmaceutically acceptable salt thereof.

33. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

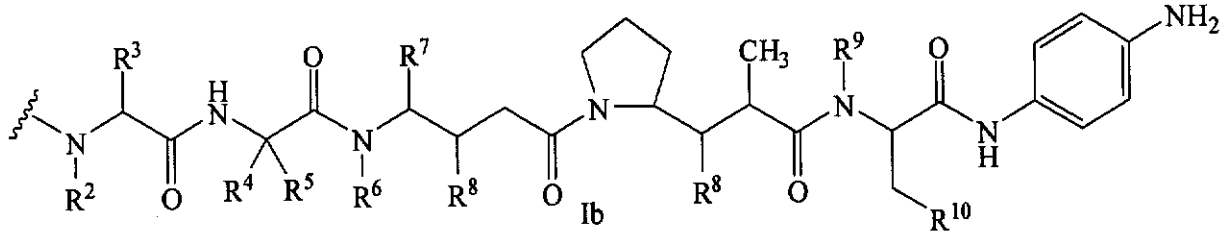
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

34. The compound having the formula:




Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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;

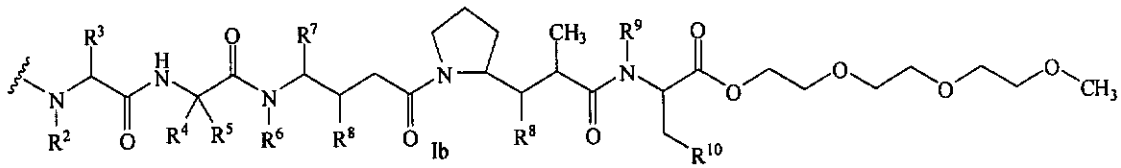
each occurrence of $R^{15}$ is independently -H, -COOH, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, or -$(CH_2)_n$-$SO_3$-$C_1$-$C_8$ alkyl;

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;

or a pharmaceutically acceptable salt thereof.

35. The compound having the formula:



Ib

wherein the wavy line indicates H or a covalent linkage to a Linker Unit or a Ligand unit selected from the group consisting of an antibody, an antibody fragment, a protein, a polypeptide, a peptide, a lectin, a glycoprotein, a vitamin, and a nutrient-transport molecule,

and independently at each location:

$R^2$ 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 $R^5$ join, 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 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 –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, -$(CH_2)_n$-$N(R^{16})_2$, -$(CH_2)_n$-$SO_3H$, 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;

or a pharmaceutically acceptable salt thereof.

36. The compound of claim 1 wherein the Linker Unit 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.

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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| Glu | Asp | Gly | Glu | Ser | Thr | Ala | Pro | Thr | Pro | Arg | Pro | Lys | Val | Leu | |
| | | | | 20 | | | | | 25 | | | | | | 30 |
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| | | | | 95 | | | | | 100 | | | | | | 105 |
| Leu | His | Pro | Thr | Leu | Pro | Pro | Leu | Lys | Asn | Arg | Asp | Phe | Val | Asp | |
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1925 1930 1935

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1940 1945 1950

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1955 1960 1965

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2000 2005 2010

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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

Val Pro Ser Ser Gly Thr Asp Thr Ser Thr Thr Phe Pro Thr Leu
3260 3265 3270

Ser Glu Thr Pro Tyr Glu Pro Glu Thr Thr Ala Thr Trp Leu Thr
3275 3280 3285

His Pro Ala Glu Thr Ser Thr Thr Val Ser Gly Thr Ile Pro Asn
3290 3295 3300

Phe Ser His Arg Gly Ser Asp Thr Ala Pro Ser Met Val Thr Ser
3305 3310 3315

Pro Gly Val Asp Thr Arg Ser Gly Val Pro Thr Thr Thr Ile Pro
3320 3325 3330

Pro Ser Ile Pro Gly Val Val Thr Ser Gln Val Thr Ser Ser Ala
3335 3340 3345

Thr Asp Thr Ser Thr Ala Ile Pro Thr Leu Thr Pro Ser Pro Gly
3350 3355 3360

Glu Pro Glu Thr Thr Ala Ser Ser Ala Thr His Pro Gly Thr Gln
3365 3370 3375

Thr Gly Phe Thr Val Pro Ile Arg Thr Val Pro Ser Ser Glu Pro
3380 3385 3390

Asp Thr Met Ala Ser Trp Val Thr His Pro Pro Gln Thr Ser Thr
3395 3400 3405

Pro Val Ser Arg Thr Thr Ser Ser Phe Ser His Ser Ser Pro Asp
3410 3415 3420

Ala Thr Pro Val Met Ala Thr Ser Pro Arg Thr Glu Ala Ser Ser
3425 3430 3435

Ala Val Leu Thr Thr Ile Ser Pro Gly Ala Pro Glu Met Val Thr
3440 3445 3450

Ser Gln Ile Thr Ser Ser Gly Ala Ala Thr Ser Thr Thr Val Pro
3455 3460 3465

Thr Leu Thr His Ser Pro Gly Met Pro Glu Thr Thr Ala Leu Leu
3470 3475 3480

Ser Thr His Pro Arg Thr Glu Thr Ser Lys Thr Phe Pro Ala Ser
3485 3490 3495

Thr Val Phe Pro Gln Val Ser Glu Thr Thr Ala Ser Leu Thr Ile
3500 3505 3510

Arg Pro Gly Ala Glu Thr Ser Thr Ala Leu Pro Thr Gln Thr Thr
3515 3520 3525

Ser Ser Leu Phe Thr Leu Leu Val Thr Gly Thr Ser Arg Val Asp
3530 3535 3540

Leu Ser Pro Thr Ala Ser Pro Gly Val Ser Ala Lys Thr Ala Pro
3545 3550 3555

Leu Ser Thr His Pro Gly Thr Glu Thr Ser Thr Met Ile Pro Thr

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

Thr Leu Leu Arg Pro Glu Lys Gln Glu Ala Ala Thr Gly Val Asp
5090 5095 5100

Thr Ile Cys Thr His Arg Val Asp Pro Ile Gly Pro Gly Leu Asp
5105 5110 5115

Arg Glu Arg Leu Tyr Trp Glu Leu Ser Gln Leu Thr Asn Ser Ile
5120 5125 5130

Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser Leu Tyr Val
5135 5140 5145

Asn Gly Phe Asn Pro Trp Ser Ser Val Pro Thr Thr Ser Thr Pro
5150 5155 5160

Gly Thr Ser Thr Val His Leu Ala Thr Ser Gly Thr Pro Ser Ser
5165 5170 5175

Leu Pro Gly His Thr Ala Pro Val Pro Leu Leu Ile Pro Phe Thr
5180 5185 5190

Leu Asn Phe Thr Ile Thr Asn Leu His Tyr Glu Glu Asn Met Gln
5195 5200 5205

His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln
5210 5215 5220

Gly Leu Leu Lys Pro Leu Phe Lys Ser Thr Ser Val Gly Pro Leu
5225 5230 5235

Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys His Gly
5240 5245 5250

Ala Ala Thr Gly Val Asp Ala Ile Cys Thr Leu Arg Leu Asp Pro
5255 5260 5265

Thr Gly Pro Gly Leu Asp Arg Glu Arg Leu Tyr Trp Glu Leu Ser
5270 5275 5280

Gln Leu Thr Asn Ser Val Thr Glu Leu Gly Pro Tyr Thr Leu Asp
5285 5290 5295

Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser Val
5300 5305 5310

Pro Thr Thr Ser Ile Pro Gly Thr Ser Ala Val His Leu Glu Thr
5315 5320 5325

Ser Gly Thr Pro Ala Ser Leu Pro Gly His Thr Ala Pro Gly Pro
5330 5335 5340

Leu Leu Val Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln
5345 5350 5355

Tyr Glu Glu Asp Met Arg His Pro Gly Ser Arg Lys Phe Asn Thr
5360 5365 5370

Thr Glu Arg Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys Ser
5375 5380 5385

Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu

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
6320 6325 6330

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
6515 6520 6525

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
6965 6970 6975

Asn Val Gln Gln Gln Cys Pro Gly Tyr Tyr Gln Ser His Leu Asp
6980 6985 6990

Leu Glu Asp Leu Gln
6995

<210> 5
<211> 622
<212> PRT
<213> Homo sapien

<400> 5
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr
1 5 10 15

Pro Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp
20 25 30

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
365 370 375

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
1 5 10 15

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
   1               5                  10                  15

 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

<210> 20
<211> 553
<212> PRT
<213> Homo sapien

<400> 20

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Met Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro
  1               5                  10                  15

Pro Leu Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val
             20                  25                  30

Pro Cys Val Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe
             35                  40                  45

Leu Ser Ile Asn Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu
             50                  55                  60

Gly Leu Gln Gly Val Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile
             65                  70                  75

Tyr Gly Gln Lys Lys Trp Leu Asn Lys Ser Glu Cys Arg Asn Ile
             80                  85                  90

Asn Arg Thr Tyr Cys Asp Leu Ser Ala Glu Thr Ser Asp Tyr Glu
             95                 100                 105

His Gln Tyr Tyr Ala Lys Val Lys Ala Ile Trp Gly Thr Lys Cys
            110                 115                 120

Ser Lys Trp Ala Glu Ser Gly Arg Phe Tyr Pro Phe Leu Glu Thr
            125                 130                 135

Gln Ile Gly Pro Pro Glu Val Ala Leu Thr Thr Asp Glu Lys Ser
            140                 145                 150

Ile Ser Val Val Leu Thr Ala Pro Glu Lys Trp Lys Arg Asn Pro
            155                 160                 165

Glu Asp Leu Pro Val Ser Met Gln Gln Ile Tyr Ser Asn Leu Lys
            170                 175                 180

Tyr Asn Val Ser Val Leu Asn Thr Lys Ser Asn Arg Thr Trp Ser
            185                 190                 195

Gln Cys Val Thr Asn His Thr Leu Val Leu Thr Trp Leu Glu Pro
            200                 205                 210

Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe Val Pro Gly Pro
            215                 220                 225

Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala Arg Thr Leu
            230                 235                 240

Lys Asp Gln Ser Ser Glu Phe Lys Ala Lys Ile Ile Phe Trp Tyr
            245                 250                 255

Val Leu Pro Ile Ser Ile Thr Val Phe Leu Phe Ser Val Met Gly
            260                 265                 270

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

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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
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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 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

<400> 27

```
 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

```
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 15

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
650 655 660

Ile

<210> 34
<211> 429
<212> PRT
<213> Sarcophaga bullata

<400> 34
Met Leu Pro Arg Leu Leu Leu Leu Ile Cys Ala Pro Leu Cys Glu
1 5 10 15

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
335 340 345

Thr Thr Glu Asn Ser Gly Asn Tyr Tyr Cys Thr Ala Asp Asn Gly
350 355 360

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