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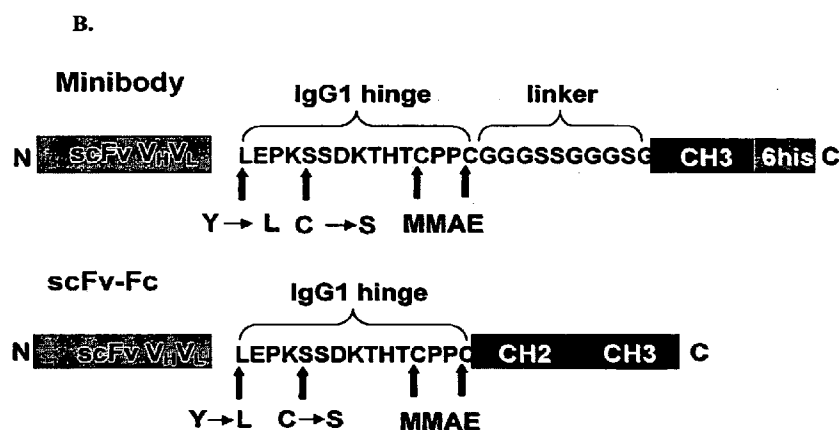
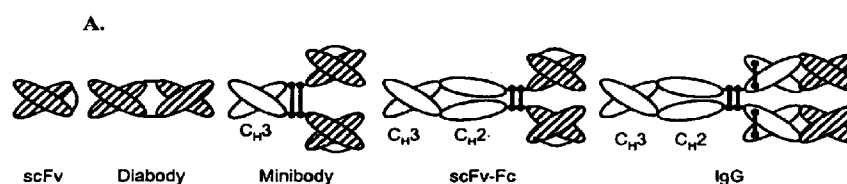
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(54) Title: **ENGINEERED ANTIBODY DRUG CONJUGATES**



(57) **Abstract:** The present invention provides ligand drug conjugates for targeted delivery of drugs. The ligand drug conjugates have potent cytotoxic activity against antigen-specific targets, as compared with intact antibody drug conjugates.

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ENGINEERED ANTIBODY DRUG CONJUGATES

CONTINUITY

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 60/778,472, filed March 02, 2006, and 60/872,348, filed December 01, 2006; the disclosures of which are incorporated by reference herein.

BACKGROUND

[0002] Antibodies are the most rapidly expanding class of therapeutics, including for cancer therapy. Indeed, eight antibodies are now approved for oncologic indications in the United States, including RITUXAN (rituximab), HERCEPTIN (trastuzumab) and CAMPATH (alemtuzumab) for non-Hodgkin's lymphoma, metastatic breast cancer and B cell chronic lymphocytic leukemia, respectively. These new drugs provide significant benefits to some patients, but fall well short of the ultimate goal of curing cancer. This significant progress with antibody therapeutics has revitalized interest in strategies to improve the rates and duration of their antitumor responses.

[0003] Antibody conjugation to cytotoxic drugs is one of the most promising ways to enhance the antitumor activity of antibodies and reduce the systemic toxicity of drugs (reviewed by Lambert, 2005, *Curr. Opin. Pharmacol.* 5:543-549; and Wu and Senter, 2005, *Nat. Biotechnol.* 23:1137-1146). Indeed, antibody conjugates of several different drugs have shown potent antitumor activity in xenograft studies in vivo, including conjugates containing doxorubicin (see, e.g., Trail et al., 1993, *Science* 261:212-215), calicheamicin (see, e.g., Hinman et al., 1993, *Cancer Res.* 53:3336-3342; Hamann et al., 2002, *Bioconjug. Chem.* 13:40-46; Hamann et al., 2002, *Bioconjug. Chem.* 13:47-58.21-23), a maytansinoid (see, e.g., Liu et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:8618-8623; Ross et al., 2002, *Cancer Res.* 62:2546-2553), analogs of CC-106526, a potent taxoid (see, e.g., Ojima et al., 2002, *J. Med. Chem.* 45:5620-5623), and monomethyl auristatin E (MMAE) (see, e.g., Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784; Francisco et al., 2003, *Blood* 102:1458-1465; Mao et al., 2004, *Cancer Res.* 64:781-788;

Bhaskar et al., 2003, *Cancer Res.* 63:6387-6394). Moreover, at least six antibody drug conjugates have progressed into clinical development, including MYLOTARG (gemtuzumab ozogamicin).

[0004] Antibody drug conjugates involve multiple potentially improvable components namely, drug, linker, conjugation chemistry, and antibody. Significant progress has been made in optimizing the chemistry of antibody drug conjugates, whereas in some cases their antibody component remains to be optimized. For example, several innovations have been incorporated into conjugates of the chimeric anti-CD30 antibody, cAC10 IgG, with the drug MMAE (Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784). First, replacing the traditional hydrazone linker with a valine-citrulline dipeptide enhances the plasma stability of IgG conjugates with MMAE, whilst still allowing efficient drug release by proteases within lysosomes of antigen-positive tumor cells. Second, MMAE is coupled to cAC10 IgG through solvent accessible cysteine residues rather than the more widely used alternative, lysines (Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784; Francisco et al., 2003, *Blood* 102:1458-1465). This choice of coupling chemistry reflects that there are far fewer surface cysteines than lysine residues in IgG, enabling greater control over the site and stoichiometry of drug attachment. Moreover, solvent-accessible cysteine residues, unlike lysines, are seldom found within or near the antigen binding loops of IgG. Thus, coupling drug to IgG via cysteine rather than lysine reduces the risk that conjugation will impair antigen binding. These advances in drug and linker design plus advances in conjugation chemistry likely contribute to the significant efficacy of cAC10 IgG-MMAE in SCID mouse xenograft models of anaplastic large cell lymphoma and Hodgkin's disease at doses well below the maximally tolerated dose (Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784; Francisco et al., 2003, *Blood* 102:1458-1465).

[0005] Advances in linker technology notwithstanding, a major challenge for antibody drug conjugates is toxicity due to unwanted release of drug at non-tumor sites. A priori, non-tumor drug release from conjugates may reflect both antigen-dependent and antigen-independent mechanisms. Antigen-dependent toxicity is commonly moot in xenograft studies as the antibodies used bind to human

antigens seldom cross-react with their mouse orthologs. However, in humans, antigen-dependent toxicity of antibody drug conjugates can be significant. Some studies suggest that toxicity can be minimized by judicious selection of a tumor-associated target antigen that has highly restricted distribution on normal tissue.

5 Regarding antigen-independent toxicities, these may reflect release of drug from circulating conjugate and/or catabolism of the entire conjugate. Advances in linker technology have significantly enhanced the plasma stability of conjugates whilst maintaining the ability to release drug efficiently following tumor cell binding and internalization (reviewed by Lambert, 2005, *Curr. Opin. Pharmacol.* 5:543-549;
10 and Wu and Senter, 2005, *Nat. Biotechnol.* 23:1137-1146). In contrast, unwanted drug liberation following conjugate catabolism appears to be the unavoidable ultimate fate of administered IgG drug conjugates. The contribution of such catabolism to antigen-independent toxicity remains to be elucidated.

[0006] IgG, as for other circulating proteins, are subject to micropinocytosis and
15 catabolism by vascular endothelial cells. (See, e.g., Junghans, 1997, *Immunol. Res.* 16: 29-57; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113.) Much of the IgG taken up by these cells is recycled back into circulation via interaction of the IgG Fc region with the salvage receptor, FcRn. This recycling likely accounts for the observation that only ~5 % of circulating IgG is catabolized per day in humans.

20 The liver is another important site of IgG catabolism, and is a common organ for the destruction of IgG glycoforms with terminal galactose or mannose. Drug released following conjugate catabolism may be toxic to the catabolizing tissue and, if circulated, to sensitive tissue that is distant from the site of release.

Undesirable drug release from conjugates catabolized outside of tumors may be
25 exacerbated by the inefficient accumulation of IgG at or within tumors: typically 0.5-50 % of the injected dose per gram of tumor (% ID /g) in mice (see, e.g., Sedalacek et al., *Monoclonal Antibodies in Tumor Therapy*, Vol. 32. Basel, Switzerland: Karger, 1988). Furthermore, the situation is even more challenging in patients where antibody localization rates are substantially lower than in mice (<
30 0.01 % ID/g) (see, e.g., Sedalacek et al., *Antibodies as Carriers of Cytotoxicity*, Vol. 43. Munich, Germany: Karger, 1992).

[0007] Antibody format optimization for antibody drug conjugates has been minimally explored. Antibody format profoundly impacts the in vivo biodistribution and catabolism of unconjugated antibodies and imaging antibodies (see, e.g., Wu. and Yazaki, 2000, *J. Nucl. Med.* 44:268-283; Colcher et al., 1998, *J. Nucl. Med.* 42:225-241; Weir et al., 2002, *Biochem. Soc. Trans.* 30:512-516). The relationship of the antibody format, drug type, linker type and drug loading on antibody drug conjugates has not previously been adequately described. For example, the hydrophilicity or hydrophobicity of a drug moiety might have a more significant impact on antibody fragment drug conjugates than on conjugates with intact antibodies. Also, the absence of Fc receptor sites (e.g., salvage receptors) in antibody fragment drug conjugates might reduce serum half-life, potentially affecting the efficacy of the conjugate against the target cells. In addition, the effect of antibody format on antigen-independent toxicity associated with antibody fragment drug conjugates needs further exploration.

[0008] There remains a need, therefore, for demonstrable antibody formats for antibody drug conjugates that deliver cytotoxic amounts of drugs to antigen-specific targets. These and other limitations are solved by the present invention. (The recitation of any reference in this application is not an admission that the reference is prior art to this application.)

BRIEF SUMMARY

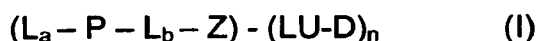
[0009] The present invention provides ligand drug conjugate compounds for targeted delivery of drugs. The ligand drug conjugate compounds have potent cytotoxic activity against antigen-specific targets, as compared with intact antibody drug conjugates.

[0010] The ligand drug conjugate compounds comprise a Ligand unit (L) having two Ligand unit moieties, L_a and L_b , joined by a Peptide (P) linker. Each Ligand unit moiety forms a Ligand Binding unit that binds to an antigen on a target cell or tissue. A Ligand unit moiety can form a Ligand Binding unit by interacting with the other Ligand unit moiety of the same ligand drug conjugate or with a Ligand unit

moiety of another ligand drug conjugate compound. An additional Polypeptide unit (Z) may optionally be linked to either Ligand unit moiety, L_a or L_b .

[0011] One or more Drug units are covalently linked to a Ligand unit moiety, the Peptide unit (P) and/or the optional Polypeptide unit (Z). The Drug unit(s) can be covalently linked directly or via a Linker unit (-LU-).

[0012] In one aspect, a ligand-drug conjugate compound of the following formula is provided:



or a pharmaceutically acceptable salt or solvate thereof;

wherein:

$(L_a - P - L_b - Z)$ is a Ligand unit, wherein:

L_a is a portion of a Ligand Binding unit,

L_b is a portion of a Ligand Binding unit,

-P- is a peptide, and

-Z is an optional polypeptide; and

$(LU-D)$ is a Linker unit-Drug unit moiety, wherein:

LU- is a Linker unit,

-D is a Drug unit having cytostatic or cytotoxic activity against a target cell; and

n is an integer from 1 to about 20;

wherein L_a forms a first Ligand Binding unit with L_b , or L_a forms a first Ligand Binding unit with L_a' and L_b forms a second Ligand Binding unit with L_b' , wherein L_a' and L_b' comprise a second ligand-drug conjugate compound; wherein at least one of the Ligand Binding units binds to a target molecule on a target cell; and wherein each Linker unit-Drug unit moiety is conjugated to an internal cysteine residue of L_a , L_b or P.

[0013] In some embodiments, the cysteine residue is an internal cysteine residue in L_a or L_b . The internal cysteine residue can be an engineered cysteine residue in L_a or L_b .

[0014] In some embodiments, wherein L_a comprises an antibody heavy chain variable region (V_H) and L_b comprises an antibody light chain variable region (V_L), these V_H and V_L regions can form an antigen binding domain. In some embodiments, V_H and V_L each form an antigen binding domain with a V_L or V_H region, respectively, of a second ligand-drug conjugate compound. In some embodiments, the V_H and/or V_L region comprises an engineered cysteine residue.

10 The engineered cysteine residue is typically located distal from the antigen binding face of the antigen binding domain.

[0015] The first or second Ligand Binding unit can bind to any suitable target antigen. In some embodiments, a Ligand Binding unit binds to CA125, CA15-3, CD19-9, Lewis Y antigen, Lewis X antigen, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific antigen, prostate specific membrane antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE-4, anti-transferrin receptor, p97, MUC1-KLH, CEA, gp100, MART1, IL-2 receptor, CD20, CD52, CD33, CD22, human chorionic gonadotropin, CD38, CD40, mucin, P21, MPG, Neu oncogene product, CD2, CD3, CD4, CD8,

20 CD11, CD18, CD19, CD27, CD28, CD29, CD30, CD35, CD40, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD70, CD79, CD90, CD95/Fas, CD134/OX40, CD137/4 1BB, CD152/CTLA 4, PD 1, ICOS, TNF R1, TNFR 2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, or APO 3.

[0016] In some embodiments, P is 1 to 25 amino acids in length. In some embodiments, P is 5 to 15 amino acids in length, or less than 5 amino acids in length. In some embodiments, P is a polyglycine-serine or a polyglycine peptide. P can optionally include at least one cysteine residue. In some embodiments, P includes a cysteine residue.

[0017] In some embodiments, the Linker unit (LU) has the formula:



wherein:

-A- is a Stretcher unit;

a is 0 or 1;

5 each -W- is independently an Amino Acid unit;

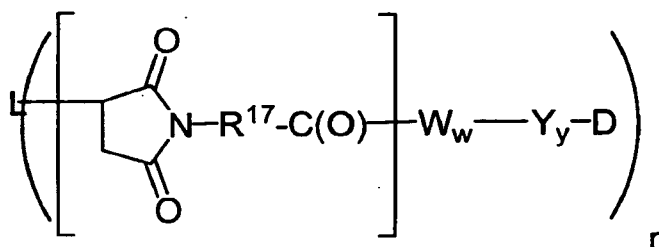
w is independently an integer ranging from 0 to 12;

-Y- is a Spacer unit; and

y is 0, 1 or 2.

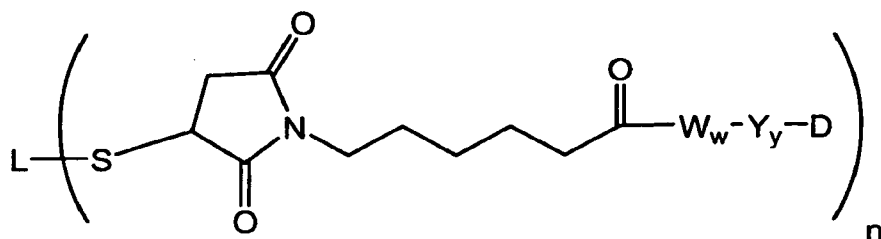
10 **[0018]** In some embodiments, w is an integer ranging from 2 to 12. In some embodiments, w is 2. In some embodiments, W_w is valine citrulline (val cit). In some embodiments, W_w is 5-aminovaleric acid, homo-phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepepotic acid lysine, beta-alanine lysine, glycine serine valine glutamine or isonepepotic acid.

15 **[0019]** In some embodiments, the ligand-drug conjugate compound has the formula:



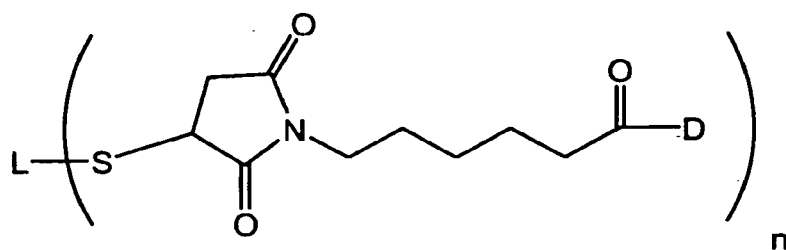
20 wherein R^{17} is selected from -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-, -O-(C₁-C₈ alkyl)-, -arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, and -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging from 1-10.

[0020] In some embodiments, the ligand-drug conjugate compound has the formula:



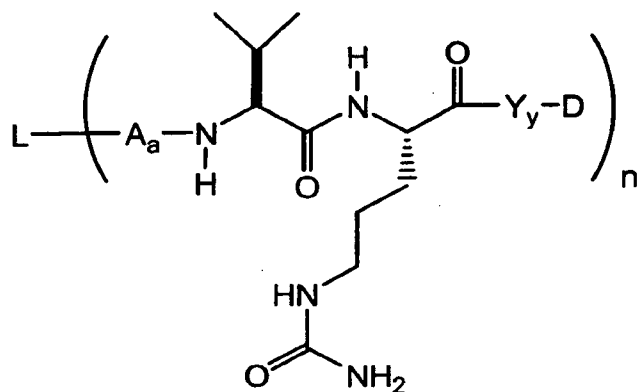
wherein S is a thiol group of L.

5 **[0021]** In some embodiments, the ligand-drug conjugate compound has the formula:

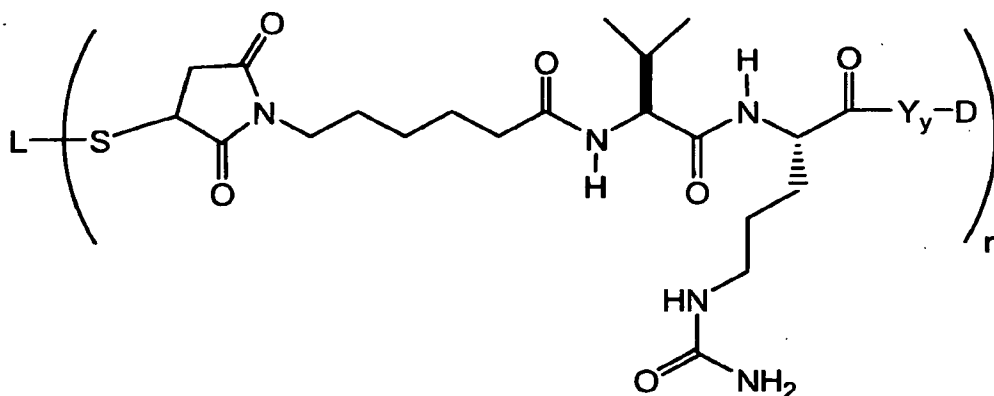


wherein S is a thiol group of L.

10 **[0022]** In some embodiments, the ligand-drug conjugate compound has the formula:

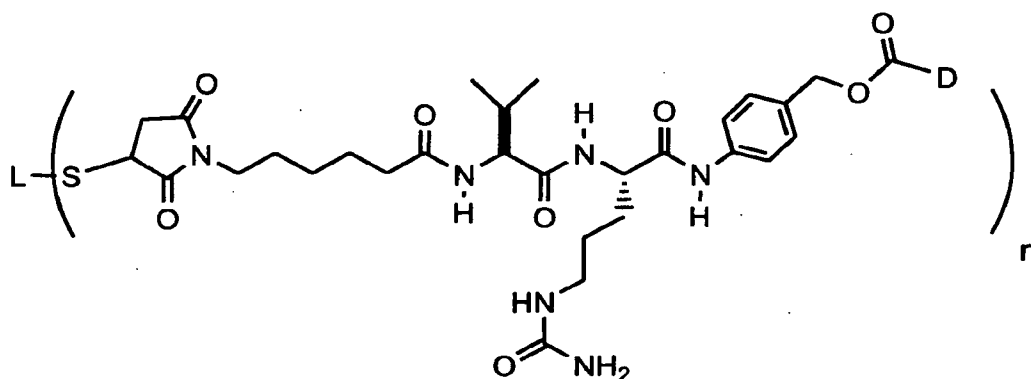


[0023] In some embodiments, the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

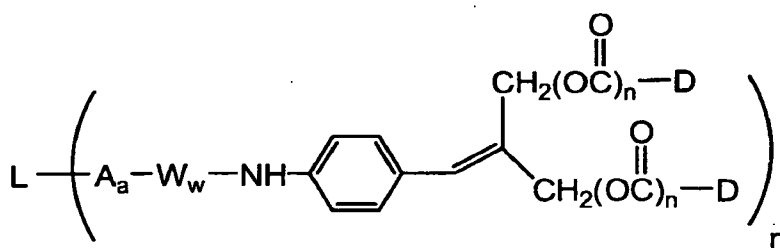
[0024] In some embodiments, the ligand-drug conjugate compound has the formula:



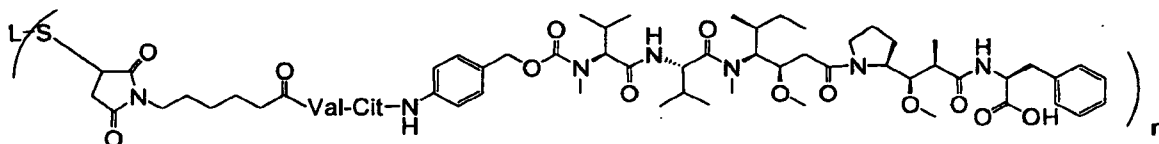
5

wherein S is a thiol group of L.

[0025] In some embodiments, the ligand-drug conjugate compound has the formula:

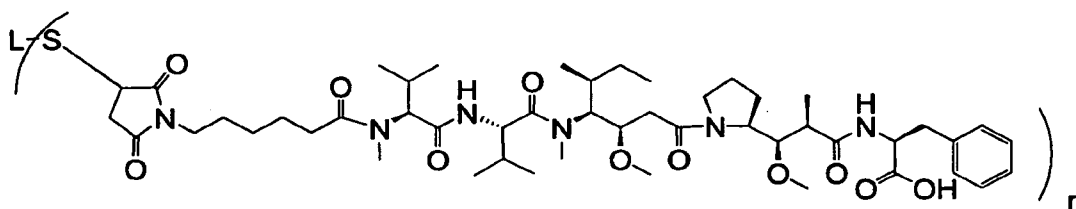


10 **[0026]** In some embodiments, the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

[0027] In some embodiments, the ligand-drug conjugate compound has the formula:

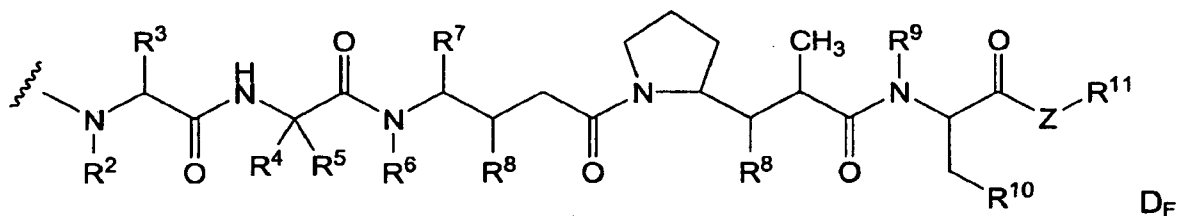


5

wherein S is a thiol group of L.

[0028] The Drug unit (-D) can be, for example, a cytotoxic agent, a cytostatic agent or an immunomodulatory drug. In some embodiments, the Drug unit (-D) is an auristatin.

10 **[0029]** In some embodiments, the Drug unit (-D) has the following Formula D_F:



D_F

wherein, independently at each location:

R² is selected from H and C₁-C₁₀ alkyl;

R³ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ 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_{10} alkyl and C_3 - C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

5 R^6 is selected from H and C_1 - C_{10} alkyl;

R^7 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl- $(C_3$ - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl- $(C_3$ - C_8 heterocycle);

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

R^9 is selected from H and C_1 - C_{10} alkyl;

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

Z is selected from O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_{10} alkyl;

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

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_8 alkyl;

R^{14} is selected from H and C_1 - C_{10} alkyl;

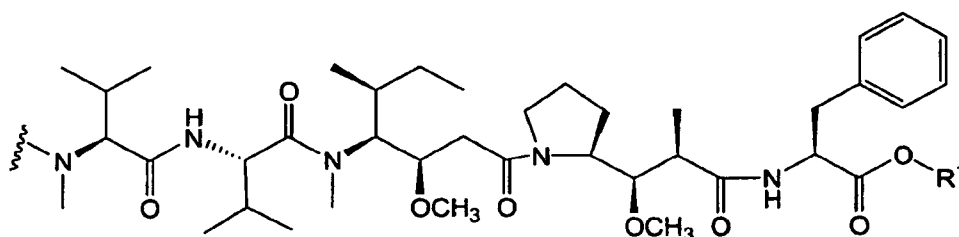
20 each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$ - C_{10} alkyl;

each occurrence of R^{16} is independently H, C_1 - C_{10} 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

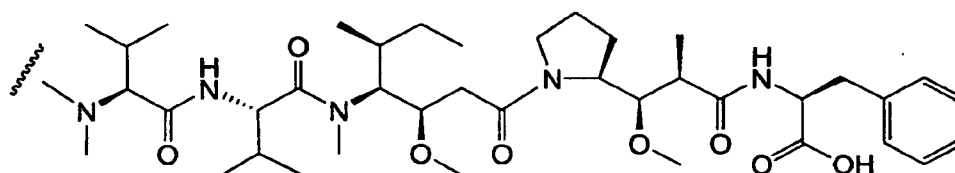
n is an integer ranging from 0 to 6.

25 [0030] For example, the Drug unit can comprise the following formula:

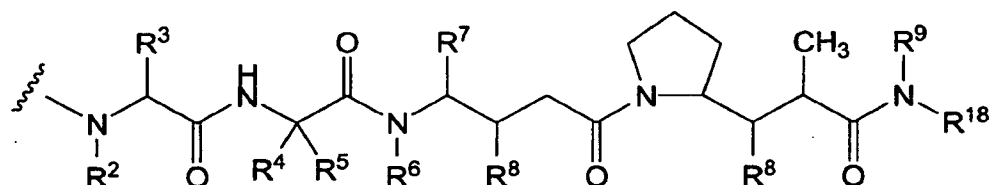


wherein $R^1 = H$ or Me.

[0031] In a specific embodiment, the Drug unit comprises the following formula:



5 **[0032]** In some embodiments, the Drug unit (-D) has the following Formula D_E:



wherein, independently at each location:

R^2 is selected from H and C₁-C₁₀ alkyl;

10 R^3 is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

R^4 is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

15 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, C₁-C₁₀ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1 - C_{10} alkyl;

R^7 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl-(C_3 - C_8 heterocycle);

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

R^9 is selected from H and C_1 - C_{10} alkyl;

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

Z is selected from O, S, NH, or NR^{12} , wherein R^{12} is C_1 - C_{10} alkyl;

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

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_8 alkyl;

R^{14} is selected from H and C_1 - C_{10} alkyl;

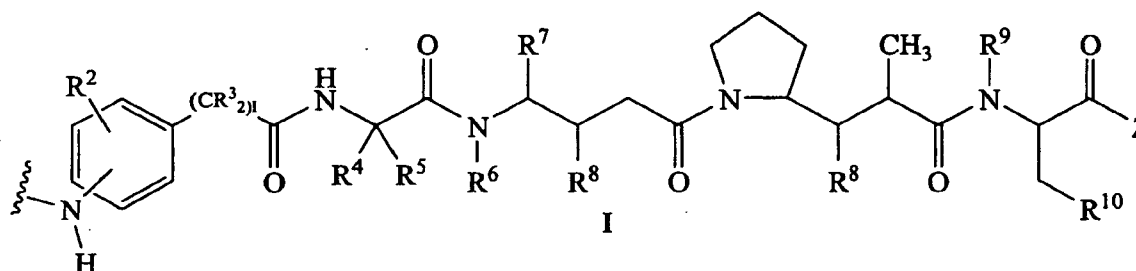
15 each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$ - C_{10} alkyl;

each occurrence of R^{16} is independently H, C_1 - C_{10} alkyl, or $-(CH_2)_n-COOH$;

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

20 n is an integer ranging from 0 to 6.

[0033] In some embodiments, the Drug unit comprises the following formula:



wherein, independently at each location:

R^2 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ alkyl), $-halogen$, $-NO_2$, $-COOH$, and $-C(O)OR^{11}$;

5 each R^3 is selected independently from $-hydrogen$ and $-C_1-C_{10}$ alkyl;

I is an integer ranging from 0-10;

10 R^4 is selected from $-hydrogen$, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, $-aryl$, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- $(C_3-C_8$ carbocycle), $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- $(C_3-C_8$ heterocycle), and 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 $-H$, $-C_1-C_{10}$ 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 $-C_1-C_{10}$ alkyl;

15 R^7 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, $aryl$, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- $(C_3-C_8$ carbocycle), $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- $(C_3-C_8$ heterocycle);

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

20 R^9 is selected from $-H$ and $-C_1-C_{10}$ alkyl;

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

Z is selected from $-O-$, $-S-$, $-NH-$, and $-NR^{12}$ where R^{12} is selected from C_1-C_{10} alkyl and $aryl$; and

R^{11} is selected from $-H$, C_1-C_{10} alkyl, aryl, $-C_3-C_8$ heterocycle, $-(CH_2CH_2O)_r-H$, $-(CH_2CH_2O)_r-CH_3$, and $-(CH_2CH_2O)_r-CH_2CH_2C(O)OH$; wherein r is an integer ranging from 1-10.

[0034] The invention further provides methods of treating cancer, immune
5 disease, infectious disease and other diseases or disorders using a ligand drug conjugate compound of the present invention. The ligand drug conjugate compound(s) can bind to a target cell of a cancer, a tumor or a cell proliferative disorder. In some embodiments, the ligand drug conjugate compound induces cell death, growth inhibition or apoptosis.

10 **[0035]** The cancer can be, for example, breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

[0036] In some embodiments, the method includes administering a ligand-drug conjugate compound as a pharmaceutical composition comprising an effective
15 amount of the ligand-drug conjugate compound, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

[0037] The invention will best be understood by reference to the following detailed description of the preferred embodiment, taken in conjunction with the
20 accompanying drawings. 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

[0038] Figure 1. Antibody Fragment Formats. (A) Shown are antigen-binding
variable domains (V_H and V_L , cross-hatch), constant domains (open) and solvent
25 accessible disulfide bonds ($\bullet \square \bullet$). (B) Structure of cAC10 Minibody and scFv-Fc.

[0039] Figure 2. SDS-PAGE analysis of purified cAC10 scFv, diabody, minibody and scFv-Fc fragments.

[0040] Figure 3. Size exclusion chromatographic analysis of purified cAC10 scFv and diabody fragments.

[0041] Figure 4. Competition binding analysis of cAC10 antibody fragments and cAC10 IgG on Karpas 299 cells.

5 **[0042]** Figure 5. Competition binding analysis of cAC10 fragments diabody-HL-cys1 (A), minibody-HL (B) and scFv-Fc-HL (C) and cAC10 IgG together with corresponding MMAE conjugates.

[0043] Figure 6. Cytotoxic activity of cAC10 diabody-HL-cys1-MMAE-4, minibody-HL-MMAE-4, scFv-Fc-MMAE-4 and cAC10 IgG-MMAE-4 against
10 Karpas 299 cells.

[0044] Figure 7. Pharmacokinetics of cAC10 diabody-HL-cys1-MMAE-4, minibody-HL-MMAE-4, scFv-Fc-MMAE-4 and cAC10 IgG-MMAE-4 in Balb-C mice.

[0045] Figure 8. (A) Multidose and single dose efficacy of cAC10 diabody-HL-Cys1-MMAE-4, minibody-HL-MMAE-4, scFv-Fc-MMAE-4 and IgG-MMAE-4 in
15 SCID mice bearing Karpas-299 xenografts. (B) Single dose efficacy of AC10 diabody-HL-Cys1-MMAE-4 and IgG-MMAE-4 in SCID mice bearing Karpas-200 xenografts.

[0046] Figure 9. In vivo antitumor activity of a single administration of AC10
20 diabody MMAF-4 at different doses and in comparison to AC10 IgG MMAF-4 at different doses.

[0047] Figure 10. In vivo antitumor activity of multidose administration of AC10 diabody MMAF-4 at different doses and in comparison to AC10 IgG MMAF-4 at different doses.

25 DETAILED DESCRIPTION

[0048] For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

Definitions

[0049] 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, unless otherwise indicated by context.

[0050] The term "antibody" herein is used in the broadest sense and refers to intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and to antibody fragments that exhibit the desired biological activity. The antibody can be of any type or class (e.g., IgG, IgE, IgM, IgD, and IgA) or sub-class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2).

[0051] An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_{H1} , C_{H2} , C_{H3} , and C_{H4} , as appropriate for the antibody class. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof.

[0052] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. The F_v polypeptide typically further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0053] The term "diabody" refers to a small antibody fragment with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain ($V_H - V_L$ or $V_L - V_H$). By using a linker that is too short to allow pairing between the two domains (V_H and V_L) of 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 0 404 097; WO 93/11161; and Hollinger

et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-6448. The two antigen-binding sites can be the same or different.

[0054] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant

5 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 some

embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, or to greater than 99% by weight,

10 (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 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

15 be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0055] An antibody or antibody fragment "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. As used herein, "specific

20 binding" and "specifically binds" refers to binding to a predetermined antigen.

Typically, the molecule binds with an affinity of at least about $1 \times 10^7 \text{ M}^{-1}$, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen.

25 **[0056]** The term "effective amount" refers to an amount of a drug (e.g., a ligand drug conjugate) 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

30 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 (TDP) and/or determining the response rate (RR).

- 5 **[0057]** The terms "target polypeptide" or "target antigen" refer to a polypeptide expressed by a target cell.

- 10 **[0058]** The term "compound", as in the terms "compound of the formula", "compound of the structure", and the like, refers to and encompasses the chemical compound itself as well as, whether explicitly stated or not, and unless the context makes clear that the following are to be excluded: amorphous and crystalline forms of the compound, including polymorphic forms, where these forms may be part of a mixture or in isolation; free acid and free base forms of the compound, which are typically the forms shown in the structures provided herein; isomers of the compound, which refers to optical isomers, and tautomeric
- 15 isomers, where optical isomers include enantiomers and diastereomers, chiral isomers and non-chiral isomers, and the optical isomers include isolated optical isomers as well as mixtures of optical isomers including racemic and non-racemic mixtures; where an isomer may be in isolated form or in admixture with one or more other isomers; isotopes of the compound, including deuterium- and tritium-
- 20 containing compounds, and including compounds containing radioisotopes, including therapeutically- and diagnostically-effective radioisotopes; multimeric forms of the compound, including dimeric, trimeric, etc. forms; salts of the compound, preferably pharmaceutically acceptable salts, including acid addition salts and base addition salts, including salts having organic counterions and
- 25 inorganic counterions, and including zwitterionic forms, where if a compound is associated with two or more counterions, the two or more counterions may be the same or different; and solvates of the compound, including hemisolvates, monosolvates, disolvates, etc., including organic solvates and inorganic solvates, said inorganic solvates including hydrates; where if a compound is associated with
- 30 two or more solvent molecules, the two or more solvent molecules may be the same or different. In some instances, reference made herein to a compound of

the invention will include an explicit reference to one or more of the above forms, e.g., salts and solvates, however, this reference is for emphasis only, and is not to be construed as excluding other of the above forms as identified above.

[0059] The term "alkyl" refers to a C₁-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms, unless otherwise indicated by context. Examples are methyl (Me, -CH₃), ethyl (Et, -CH₂CH₃), 1-propyl (n-Pr, n-propyl, -CH₂CH₂CH₃), 2-propyl (i-Pr, i-propyl, -CH(CH₃)₂), 1-butyl (n-Bu, n-butyl, -CH₂CH₂CH₂CH₃), 2-methyl-1-propyl (i-Bu, i-butyl, -CH₂CH(CH₃)₂), 2-butyl (s-Bu, s-butyl, -CH(CH₃)CH₂CH₃), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH₃)₃), 1-pentyl (n-pentyl, -CH₂CH₂CH₂CH₂CH₃), 2-pentyl (-CH(CH₃)CH₂CH₂CH₃), 3-pentyl (-CH(CH₂CH₃)₂), 2-methyl-2-butyl (-C(CH₃)₂CH₂CH₃), 3-methyl-2-butyl (-CH(CH₃)CH(CH₃)₂), 3-methyl-1-butyl (-CH₂CH₂CH(CH₃)₂), 2-methyl-1-butyl (-CH₂CH(CH₃)CH₂CH₃), 1-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₃), 2-hexyl (-CH(CH₃)CH₂CH₂CH₂CH₃), 3-hexyl (-CH(CH₂CH₃)(CH₂CH₂CH₃)), 2-methyl-2-pentyl (-C(CH₃)₂CH₂CH₂CH₃), 3-methyl-2-pentyl (-CH(CH₃)CH(CH₃)CH₂CH₃), 4-methyl-2-pentyl (-CH(CH₃)CH₂CH(CH₃)₂), 3-methyl-3-pentyl (-C(CH₃)(CH₂CH₃)₂), 2-methyl-3-pentyl (-CH(CH₂CH₃)CH(CH₃)₂), 2,3-dimethyl-2-butyl (-C(CH₃)₂CH(CH₃)₂), and 3,3-dimethyl-2-butyl (-CH(CH₃)C(CH₃)₃).

[0060] The term "alkenyl" refers to a C₂-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, sp² double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH₂), allyl (-CH₂CH=CH₂), cyclopentenyl (-C₅H₇), and 5-hexenyl (-CH₂CH₂CH₂CH₂CH=CH₂).

[0061] The term "alkynyl" refers to a C₂-C₁₈ 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≡CH) and propargyl (-CH₂C≡CH).

[0062] The term "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 alkylenes include, but are not limited to: methylene ($-\text{CH}_2-$), 1,2-ethyl ($-\text{CH}_2\text{CH}_2-$), 1,3-propyl ($-\text{CH}_2\text{CH}_2\text{CH}_2-$), 1,4-butyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$), and the like.

[0063] The term "alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene ($-\text{CH}=\text{CH}-$).

[0064] The term "alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene ($-\text{C}\equiv\text{C}-$), propargyl ($-\text{CH}_2\text{C}\equiv\text{C}-$), and 4-pentynyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}\equiv\text{CH}-$).

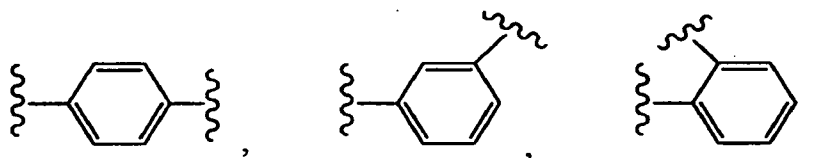
[0065] The term "aryl" refers to a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Some aryl groups are represented in the exemplary structures as "Ar". An aryl group can be unsubstituted or substituted. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, phenyl, naphthalene, anthracene, biphenyl, and the like. An aryl can be substituted with one or more groups including, but not limited to, $-\text{C}_1\text{-C}_{10}$ alkyl, $-\text{O}(\text{C}_1\text{-C}_{10} \text{ alkyl})$, $-\text{aryl}$, $-\text{C}(\text{O})\text{R}'$, $-\text{OC}(\text{O})\text{R}'$, $-\text{C}(\text{O})\text{OR}'$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHR}'$, $-\text{C}(\text{O})\text{N}(\text{R}')_2$, $-\text{NHC}(\text{O})\text{R}'$, $-\text{S}(\text{O})_2\text{R}'$, $-\text{S}(\text{O})\text{R}'$, $-\text{OH}$, $-\text{halogen}$, $-\text{N}_3$, $-\text{NH}_2$, $-\text{NH}(\text{R}')$, $-\text{N}(\text{R}')_2$ and $-\text{CN}$; wherein each R' is independently selected from H, $-\text{C}_1\text{-C}_{10}$ alkyl and aryl.

[0066] The term "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.

[0067] The term "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 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.

[0068] The term "arylene" refers to an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



20

in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ 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, $-C_1-C_{10}$ alkyl and aryl.

25

[0069] The terms "substituted alkyl", "substituted aryl", and "substituted arylalkyl" refer to alkyl, aryl, and arylalkyl, respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents

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

- 10 **[0070]** The terms "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, "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.

- [0071]** Examples of heterocycles include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl,

pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indoliziny, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinoliziny, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β -carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazolinyl, piperazinyl, indoliny, isoindoliny, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazoliny, and isatinoyl.

- 10 **[0072]** By way of example and not limitation, carbon-bonded heterocycles are bonded at the following positions: 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.

- 25 **[0073]** 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, or 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-azetedy, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

- 30 **[0074]** The term "carbocycle" refers to 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.

[0075] The term "C₁-C₁₀ alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms.

Representative "C₁-C₁₀ 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 C₁-C₁₀ alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, isohexyl, 2-methylbutyl, 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, isoheptyl, and isooctyl; and unsaturated C₁-C₁₀ 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-butylnyl, -2-butylnyl, -1-pentylnyl, -2-pentylnyl, -3-methyl-1 butynyl. A C₁-C₁₀ alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -SO₃R', -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; where each R' is independently selected from H, -C₁-C₁₀ alkyl and aryl. In some embodiments, a C₁-C₁₀ alkyl group can be replaced with a C₁-C₈ alkyl group, a C₂-C₈ alkyl group or a C₂-C₁₀ alkyl group.

[0076] A "C₃-C₈ carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C₃-C₈ 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 C₃-C₈ carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C₁-C₁₀ alkyl, -
 5 O-(C₁-C₁₀ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; where each R' is independently selected from H, -C₁-C₁₀ alkyl and aryl.

[0077] A "C₃-C₈ carbocyclo" refers to a C₃-C₈ carbocycle group defined above
 10 wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

[0078] A "C₁-C₁₀ alkylene" is a straight chain, saturated hydrocarbon group of the formula -(CH₂)₁₋₁₀-. Examples of a C₁-C₁₀ alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene and decalene.

[0079] A "C₃-C₈ heterocycle" refers to an aromatic or non-aromatic C₃-C₈ 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 C₃-C₈ heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl,
 20 pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C₃-C₈ heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R',
 25 -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; wherein each R' is independently selected from H, -C₁-C₁₀ alkyl and aryl.

[0080] "C₃-C₈ heterocyclo" refers to a C₃-C₈ heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C₃-C₈ heterocyclo can be unsubstituted or substituted with up to six groups
 30 including, but not limited to, -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -aryl, -C(O)R',

-OC(O)R', -C(O)OR', -C(O)NH₂, -C(O)NHR', -C(O)N(R')₂, -NHC(O)R', -S(O)₂R', -S(O)R', -OH, -halogen, -N₃, -NH₂, -NH(R'), -N(R')₂ and -CN; wherein each R' is independently selected from H, -C₁-C₁₀ alkyl and aryl.

[0081] The phrase "pharmaceutically acceptable salt" refers to a
5 pharmaceutically acceptable organic or inorganic salt of a ligand drug conjugate or linker drug conjugate. The conjugates may contain at least one amino group, and accordingly acid addition salts can be formed with the amino group. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate,
10 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
15 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
20 part of the pharmaceutically acceptable salt can have multiple counterions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

[0082] The phrases "pharmaceutically acceptable solvate" or "solvate" refer to an association of one or more solvent molecules and a ligand drug conjugate or
25 linker drug 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.

[0083] Examples of a "patient" or "subject" include, but are not limited to, a human, rat, mouse, guinea pig, monkey, pig, goat, cow, horse, dog, cat, bird and
30 fowl. In an exemplary embodiment, the patient or subject is a human.

[0084] 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. 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.

[0085] 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.

[0086] 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.

[0087] 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.

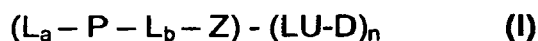
Ligand Drug Conjugates

[0088] The present invention provides ligand drug conjugates for targeted delivery of drugs. The ligand drug conjugates have potent cytotoxic activity against antigen-specific targets as compared with intact antibody drug conjugates.

[0089] The ligand drug conjugates comprise a Ligand unit (L) having Ligand unit moieties, L_a and L_b , joined by a Peptide (-P-) linker. Each Ligand unit moiety forms a Ligand Binding unit that binds to an antigen on a target cell or tissue. The antigen can be, for example, the extracellular domain of a protein, an extracellular matrix molecule or a variant of these. A Ligand unit moiety can form a Ligand Binding unit by interacting with the other Ligand unit moiety of the same ligand drug conjugate or with a Ligand unit moiety of another ligand drug conjugate. A Polypeptide unit (Z) may optionally be linked to the amino-terminal end of Ligand unit moiety L_a and/or to the carboxyl-terminal end of Ligand unit moiety L_b .

[0090] One or more Drug units are covalently linked to a Ligand unit moiety, the Peptide unit (P) and/or the optional Polypeptide unit (Z). The Drug units can be covalently linked directly or via a Linker unit (-LU-). The Drug unit is typically linked via a Linker unit to a cysteine residue in a Ligand unit moiety, L_a and/or L_b , in the Peptide unit (P) and/or in the optional Polypeptide unit (Z).

[0091] In some embodiments, the Ligand Drug conjugate compounds have the following formula:



or a pharmaceutically acceptable salt or solvate thereof;

$(L_a - P - L_b - Z)$ is a Ligand unit, wherein:

L_a is a portion of a Ligand Binding unit,

L_b is a portion of a Ligand Binding unit,

-P- is a peptide, and

-Z is an optional polypeptide; and

$(LU-D)$ is a Linker unit-Drug unit moiety, wherein:

LU- is a Linker unit, and

-D is a Drug unit having cytostatic or cytotoxic activity against a target cell; and

n is an integer from 1 to about 20; and

wherein each Linker unit-Drug unit moiety is conjugated to an internal cysteine residue in L_a , L_b or P.

[0092] In some embodiments, L_a and L_b form a first Ligand Binding unit that binds to a target antigen. In other embodiments, L_a forms a first Ligand Binding unit with L_a' , wherein the first Ligand Binding unit binds to a first target antigen, and L_b forms a second Ligand Binding unit with L_b' , wherein the second Ligand Binding unit binds to a second target antigen. L_a' and L_b' typically comprise a second ligand drug conjugate. The first and second Ligand Binding units can be the same or different. The first and second target antigens can be the same or different.

[0093] In some embodiments, the $L_a - P - L_b - Z$ moiety is single chain antibody, such as an scFv or an (scFv)₂. In some embodiments, a first $L_a - P - L_b - Z$ moiety interacts with one or more additional $L_a - P - L_b - Z$ moieties to form a multimer, such as a diabody, a triabody, a tetrabody, an scFv-Fc, or an scFv-CH3 (a minibody). In some embodiments, Z is a constant region or a constant region domain (e.g., a CH3 domain). In other embodiments, Z is absent.

[0094] In some embodiments, n ranges from 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, n ranges from 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, n is 1, 2, 3, 4, 5 or 6. In some embodiments, n is 2. In some embodiments, n is 4.

[0095] In some embodiments, the Ligand Drug conjugate compounds have the following formula:



or a pharmaceutically acceptable salt or solvate thereof;

wherein:

L_a is a portion of a Ligand Binding unit;

L_b is a portion of a Ligand Binding unit;

-P- is a peptide;

-Z is an optional polypeptide unit; and

$-A_a - W_w - Y_y -$ is a Linker unit (LU), 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,

5 -Y- is a self-immolative spacer unit,

y is 0, 1 or 2;

-D is a Drug unit having cytostatic or cytotoxic activity against a target cell; and

n is an integer from 1 to about 20.

10 **[0096]** In some embodiments, each Linker unit-Drug unit moiety is conjugated to an internal cysteine residue in L_a, L_b or P.

[0097] In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0, 1 or 2. In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0 or 1.

[0098] Each of these units is described in more detail herein.

15

The Ligand Unit

[0099] A Ligand unit (L) includes within its scope any molecule that binds or reactively associates or complexes with a target receptor, antigen or other receptive moiety associated with a given target-cell or cell population. In one aspect, the Ligand unit acts to deliver a Drug unit (infra) to the particular target cell or cell population with which the Ligand unit reacts. Such Ligands include, but are not limited to, multimeric proteins such as, for example, antibody fragments.

[0100] Useful Ligand units can be prepared from monoclonal antibodies. As used herein, monoclonal antibodies (mAbs) are homogeneous populations of antibodies to a particular antigenic determinant (e.g., a cell antigen (such as a cancer or autoimmune cell antigen), a viral antigen, a microbial antigen, a protein, a peptide, a carbohydrate, a chemical, a nucleic acid, or fragments thereof). A monoclonal antibody (mAb) to a target antigen can be prepared by using any technique known in the art. 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 may be cultivated *in vitro* or *in vivo*.

[0101] Useful Ligand units can include functionally active fragments of human monoclonal antibodies, humanized monoclonal antibodies and chimeric monoclonal antibodies that bind to a desired target antigen (e.g., a cancer cell antigen, a viral antigen, or a microbial antigen) or other antibodies bound to a target cell(s) or matrix. In this regard, "functionally active" means that the fragment is able to elicit anti-anti-idiotypic antibodies that recognize the same antigen that the antibody from which the fragment is derived recognized.

[0102] The Ligand unit also can be a functionally active derivative or analog of an antibody that immunospecifically binds to a desired target antigen. 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 BIAcore assay) (see, e.g., Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md; Kabat *et al.*, 1980, *J. Immunology* 125(3):961-969).

[0103] In some embodiments, the Ligand unit is a single chain antibody. Single chain antibodies can be prepared, for example, as described in U.S. Patent No. 4,946,778; Bird, 1988, *Science* 242:423-42; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; Ward *et al.*, 1989, *Nature* 334:544-54; Holliger and Hudson, 2005, *Nat. Biotechnol.* 23:1126-1136; U.S Patent No. 6,545,142; U.S Patent No. 6,492,123; U.S Patent No. 6,248,516; U.S Patent No. 6,010,884; U.S Patent No. 5,837,242; and U.S Patent No. 5,091,513; the disclosures of which are incorporated by reference herein.

[0104] 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. (See, e.g., Cabilly *et al.*, U.S. Patent No. 4,816,567; and Boss *et al.*, U.S. Patent No.

5 4,816,397, 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

10 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. 0 184 187; European Patent Publication No. 0 171 496; European Patent Publication No. 0 173 494; International Publication No. WO
15 86/01533; U.S. Patent No. 4,816,567; European Patent Publication No. 012 023; 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; 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. 5,225,539; 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.

25 **[0105]** Human monoclonal antibodies may be made by any of numerous techniques known in the art (see, e.g., Teng *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80:7308-7312; Kozbor *et al.*, 1983, *Immunology Today* 4:72-79; Olsson *et al.*, 1982, *Meth. Enzymol.* 92:3-16; and U.S. Patent Nos. 5,939,598 and 5,770,429).

30 **[0106]** Completely human antibodies 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 target antigen. 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, e.g., 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. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806; each of which is incorporated herein by reference in its entirety. Other human antibodies can be obtained commercially from, for example, Abgenix, Inc. (now Amgen, Fremont, CA) and Genpharm (now Medarex, San Jose, CA).

[0107] 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, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581; Quan and Carter, 2002, *The rise of monoclonal antibodies as therapeutics*, In *Anti-IgE and Allergic Disease*, Jardieu and Fick Jr., eds., Marcel Dekker, New York, NY, Chapter 20, pp. 427-469).

[0108] In some embodiments, the Ligand Drug conjugate compound is monospecific. In other embodiments, the Ligand Drug conjugate compound is multispecific, such as bi-specific.

[0109] The Ligand unit can be modified, e.g., by the covalent attachment of any type of molecule as long as such covalent attachment permits the Ligand unit to

retain its binding specificity (e.g., antigen binding immunospecificity). For example, the Ligand unit can be modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to another protein, etc.

5 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 Ligand unit can contain one or more unnatural amino acids.

[0110] In specific embodiments, it may be desirable to improve the binding
10 affinity and/or other biological properties of the Ligand unit or Ligand Binding unit. Amino acid sequence variants of the Ligand unit (e.g., the V_H and/or V_L) are prepared by introducing appropriate nucleotide changes into the 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
15 sequences of the Ligand unit (e.g., the V_H and/or V_L). 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 Ligand unit, such as changing the number or position of glycosylation sites.

20 **[0111]** A useful method for identification of certain residues or regions of the Ligand unit (e.g., the V_H and/or V_L) that are favored locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989, *Science* 244:1081-1085). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced
25 by a neutral or negatively charged amino acid (most preferably alanine) 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
30 nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random

mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0112] 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 insertion at the N-terminal methionyl residue or the C terminal residue.

[0113] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the Ligand unit (e.g., the V_H and/or V_L) replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but framework region alterations are also contemplated.

[0114] Substantial modifications in the biological properties of the Ligand unit (e.g., the V_H and/or V_L) 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.

[0115] A particular 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
5 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
10 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
15 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.

[0116] The Ligand unit may be glycosylated at one or more conserved positions
20 in the Ligand unit (see, e.g., Jefferis and Lund, 1997, *Chem. Immunol.* 65:111-128; Wright and Morrison, 1997, *TibTECH* 15:26-32). The oligosaccharide side chains can affect the protein's function (see, e.g., 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
25 the conformation and presented three-dimensional surface of the glycoprotein (see, e.g., Jefferis and Lund, *supra*; Wyss and Wagner, 1996, *Current Opin. Biotech.* 7:409-416). Oligo-saccharides 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
30 moiety 'flips' out of the inter-C_{H2} 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 CMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al.*, 1999, *Nature Biotech.* 17:176-180).

[0117] Glycosylation 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.

[0118] Glycosylation variants of the Ligand unit are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting or adding one or more carbohydrate moieties, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, *etc.*

[0119] Addition of glycosylation sites to the Ligand unit 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 (for O-linked glycosylation

sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration.

[0120] The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. These methods include, but are not limited to, preparation
5 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.

[0121] The glycosylation (including glycosylation pattern) of a Ligand unit may also be altered without altering the amino acid sequence or the underlying
10 nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of Ligand-Drug conjugate compounds as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern can be expected. See, e.g., Hse *et al.*, 1997, *J. Biol. Chem.* 272:9062-9070. In addition to the choice of host cells,
15 factors which affect glycosylation during recombinant production of Ligand units 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
20 production (see, e.g., U.S. Patent Nos. 5,047,335; 5,510,261; and 5,278,299). 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., made defective in processing certain types of polysaccharides. These and similar
25 techniques are well known in the art.

[0122] The glycosylation structure 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
30 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.

5 **[0123]** The Ligand unit can have modifications (e.g., substitutions, deletions or additions) in amino acid residues that interact with Fc receptors. In particular, the Ligand units can have modifications in amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor (see, e.g., International Publication No. WO 97/34631, which is incorporated herein by
10 reference in its entirety).

[0124] Virtually any target protein can be targeted by a Ligand Binding unit, including any target protein which expression is correlated with expression on cells of a cancer, cell proliferative disorder or tumor. Suitable target proteins include human tumor antigens recognized by T cells (see, e.g., Robbins and
15 Kawakami, 1996, *Curr. Opin. Immunol.* 8:628-636, incorporated herein by reference in its entirety), melanocyte lineage proteins, including gp100, MART-1/MelanA, TRP-1 (gp75), tyrosinase; Tumor-specific widely shared antigens, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-1, N-acetylglucosaminyltransferase-V, p15; Tumor-specific mutated antigens, beta-catenin, MUM-1, CDK4;
20 Nonmelanoma antigens for breast, ovarian, cervical and pancreatic carcinoma, HER-2/neu, human papillomavirus-E6, -E7, MUC-1; cancer antigens, such as KS 1/4 pan-carcinoma antigen (see, e.g., Perez and Walker, 1990, *J. Immunol.* 142:3662-3667; Bumal, 1988, *Hybridoma* 7(4):407-415); ovarian carcinoma antigen (CA125) (see, e.g., Yu et al., 1991, *Cancer Res.* 51(2):468-475); prostatic
25 acid phosphate (see, e.g., Tailor et al., 1990, *Nucl. Acids Res.* 18(16):4928); prostate specific antigen (see, e.g., Henttu and Vihko, 1989, *Biochem. Biophys. Res. Comm.* 160(2):903-910; Israeli et al., 1993, *Cancer Res.* 53:227-230); melanoma-associated antigen p97 (see, e.g., Estin et al., 1989, *J. Natl. Cancer Instit.* 81(6):445-446); melanoma antigen gp75 (see, e.g., Vijayasardahl et al.,
30 1990, *J. Exp. Med.* 171(4):1375-1380); high molecular weight melanoma antigen (HMW-MAA) (see, e.g., Natali et al., 1987, *Cancer* 59:55-63; Mittelman et al.,

1990, *J. Clin. Invest.* 86:2136-2144); prostate specific membrane antigen; carcinoembryonic antigen (CEA) (see, e.g., Foon et al., 1994, *Proc. Am. Soc. Clin. Oncol.* 13:294); polymorphic epithelial mucin antigen; human milk fat globule antigen; a colorectal tumor-associated antigen, such as CEA, TAG-72 (see, e.g.,
5 Yokata et al., 1992, *Cancer Res.* 52:3402-3408), CO 17-1A (see, e.g., Ragnhammar et al., 1993, *Int. J. Cancer* 53:751-758); GICA 19-9 (see, e.g., Herlyn et al., 1982, *J. Clin. Immunol.* 2:135), CTA-1 and LEA; Burkitt's lymphoma antigen-38.13; CD19 (see, e.g., Ghetie et al., 1994, *Blood* 83:1329-1336); human B-lymphoma antigen CD20 (see, e.g., Reff et al., 1994, *Blood* 83:435-445); CD33
10 (see, e.g., Sgouros et al., 1993, *J. Nucl. Med.* 34:422-430); melanoma specific antigens such as ganglioside GD2 (see, e.g., Saleh et al., 1993, *J. Immunol.* 151:3390-3398), ganglioside GD3 (see, e.g., Shitara et al., 1993, *Cancer Immunol. Immunother.* 36:373-380), ganglioside GM2 (see, e.g., Livingston et al., 1994, *J. Clin. Oncol.* 12:1036-1044), ganglioside GM3 (see, e.g., Hoon et al.,
15 1993, *Cancer Res.* 53:5244-5250); tumor-specific transplantation type of cell-surface antigen (TSTA) such as virally-induced tumor antigens including T-antigen DNA tumor viruses and envelope antigens of RNA tumor viruses; oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (see, e.g., Hellstrom et al., 1985, *Cancer Res.* 45:2210-2188); differentiation
20 antigen such as human lung carcinoma antigen L6, L20 (see, e.g., Hellstrom et al., 1986, *Cancer Res.* 46:3917-3923); antigens of fibrosarcoma, human leukemia T cell antigen-Gp37 (see, e.g., Bhattacharya-Chatterjee et al., 1988, *J. Immunol.* 141:1398-1403); neoglycoprotein, sphingolipids, breast cancer antigen such as EGFR (epidermal growth factor receptor) or EGFRvIII, HER2 antigen
25 (p185HER2), polymorphic epithelial mucin (PEM) (see, e.g., Hilkens et al., 1992, *Trends in Bio. Chem. Sci.* 17:359); malignant human lymphocyte antigen, APO-1 (see, e.g., Bernhard et al., 1989, *Science* 245:301-304); differentiation antigens (see, e.g., Feizi, 1985, *Nature* 314:53-57) such as I antigen found in fetal erythrocytes, primary endoderm, I antigen found in adult erythrocytes and
30 preimplantation embryos, I(Ma) found in gastric adenocarcinomas, M18, M39 found in breast epithelium, SSEA-1 found in myeloid cells, VEP8, VEP9, Myl, VIM-D5, D156-22 found in colorectal cancer, TRA-1-85 (blood group H), C14 found in

colonic adenocarcinoma, F3 found in lung adenocarcinoma, AH6 found in gastric cancer, Y hapten, Ley found in embryonal carcinoma cells, TL5 (blood group A), EGF receptor found in A431 cells, E1 series (blood group B) found in pancreatic cancer, FC10.2 found in embryonal carcinoma cells, gastric adenocarcinoma antigen, CO-514 (blood group Lea) found in Adenocarcinoma, NS-10 found in adenocarcinomas, CO-43 (blood group Leb), G49 found in EGF receptor of A431 cells, MH2 (blood group AL_{Leb}/Ley) found in colonic adenocarcinoma, 19.9 found in colon cancer, gastric cancer mucins, T5A7 found in myeloid cells, R24 found in melanoma, 4.2, GD3, D1.1, OFA-1, GM2, OFA-2, GD2, and M1:22:25:8 found in embryonal carcinoma cells, and SSEA-3 and SSEA-4.

[0125] Ligand Units can be obtained commercially, for example, from commercial companies 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.

[0126] In a specific embodiment, the Ligand unit is derived from a known antibody for the treatment or prevention of cancer. 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 RITUXAN® (rituximab; Genentech) which is a chimeric anti-CD20 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma; OvaRex® (AltaRex Corporation, MA) which is a murine antibody for the treatment of ovarian cancer; Panorex (Glaxo Wellcome, NC) which is a murine IgG_{2a} antibody for the treatment of colorectal cancer; Cetuximab Erbitux® (Imclone Systems Inc., NY) which is an anti-EGFR IgG chimeric antibody for the treatment of epidermal growth factor positive cancers, such as head and neck cancer; Vitaxin®

(MedImmune, Inc., MD) which is a humanized antibody for the treatment of sarcoma; Campath® I/H (Leukosite, MA) which is a humanized IgG₁ antibody for the treatment of chronic lymphocytic leukemia (CLL); lintuzumab (Protein Design Labs, Inc., CA) which is a humanized anti-CD33 IgG antibody for the treatment of acute myeloid leukemia (AML); LymphoCide (Epratuzumab, 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.

[0127] In some embodiments, the Ligand unit is derived from an antibody against the following antigens (where exemplary cancers are indicated in parentheses): 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 membrane 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 *et al.*, 1993, *Science* 261:212-215), BR64 (Trail *et al.*, 1997, *Cancer Research* 57:100-105), mAbs against the CD40 antigen, such as S2C6 mAb (Francisco *et al.*, 2000, *Cancer Res.* 60:3225-3231), mAbs against the CD70 antigen, such as 1F6 mAb and 2F2 mAb (U.S. Patent Publication Nos: 2006-0233794 and 2006-0083736); and mAbs against the CD30 antigen, such as AC10 (Bowen *et al.*, 1993, *J. Immunol.* 151:5896-5906; Wahl *et al.*, 2002 *Cancer Res.* 62(13):3736-42) or MDX-0060 (U.S. Patent Publication No. 2004-0006215). Other internalizing antibodies that bind to tumor associated antigens can be used and have been reviewed (Franke *et al.*, 2000, *Cancer Biother. Radiopharm.* 15:459 76; Murray, 2000, *Semin. Oncol.* 27:64 70; Breitling, F., and Dubel, S., Recombinant Antibodies, John Wiley, and Sons, New York, 1998).

[0128] In some embodiments, the antigen is a tumor-associated polypeptide that is 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.

[0129] In some embodiments, the Ligand unit is derived from an antibody for the treatment or prevention of an autoimmune disease. 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 antibody; anti-ss DNA antibody, anti-cardiolipin antibody IgM, IgG; anti-phospholipid antibody IgM, IgG; anti-SM antibody; anti-mitochondrial antibody; anti-thyroid antibody; anti-microsomal antibody; anti-thyroglobulin antibody; anti-SCL-70 antibody; anti-Jo antibody; anti-U₁RNP antibody; anti-La/SSB antibody; anti-SSA antibody; anti-

SSB antibody; anti-perital cells antibody; anti-histone antibody; anti-RNP antibody; anti-C-ANCA antibody; anti-P-ANCA antibody; anti-centromere antibody; anti-Fibrillarin antibody, and anti-GBM antibody.

[0130] In certain embodiments, the Ligand Binding unit can bind to a receptor or a receptor complex expressed on a target cell. 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.

[0131] In an embodiment, the Ligand binding unit binds to an activated lymphocyte that is associated with an autoimmune disease.

[0132] In another specific embodiment, the Ligand Binding unit binds to (e.g., is immunospecific for) a viral or a microbial antigen and is derived from a monoclonal antibody. 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.

[0133] Antibodies immunospecific for a viral or microbial antigen can be obtained commercially 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.

[0134] In a specific embodiment, the Ligand unit is derived from an antibody useful for the treatment or prevention of viral or microbial infection. 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 IgG₁ antibody useful for the treatment of cytomegalovirus (CMV); and anti-LPS antibodies.

[0135] Other antibodies useful in the treatment of infectious diseases include, but are not limited to, antibodies against the antigens from pathogenic strains of bacteria (e.g., *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenas*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *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*, and *Chlamydia spp.*); pathogenic fungi (e.g., *Coccidioides immitis*, *Aspergillus fumigatus*, *Candida albicans*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*); protozoa (*Entamoeba histolytica*, *Toxoplasma gondii*, *Trichomonas tenax*, *Trichomonas hominis*, *Trichomonas vaginalis*, *Trypanosoma gambiense*,
 5 *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmodium vivax*, *Plasmodium falciparum*, or *Plasmodium malaria*); or Helminths (*Enterobius vermicularis*, *Trichuris trichiura*, *Ascaris lumbricoides*,
 10 *Trichinella spiralis*, *Strongyloides stercoralis*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma haematobium*, and hookworms).

[0136] Other Ligand units for treatment of viral disease include, but are not limited to, Ligand units against antigens of pathogenic viruses, such as for example: Poxviridae, Herpesviridae, Herpes Simplex virus 1, Herpes Simplex
 15 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
 20 Human Immunodeficiency Virus.

Peptide units

[0137] The Peptide unit covalently links the Ligand unit moieties, L_a and L_b . The Peptide unit is typically a flexible linker, permitting association of each Ligand unit
 25 moiety with another Ligand unit moiety. In some embodiments, the Peptide unit is typically around fifteen amino acids in length. In other embodiments, the Peptide unit is around 10-15 amino acids in length. In other embodiments, the Peptide unit is about three to seven amino acids. In other embodiments, the Peptide unit is less than five or less than three amino acids in length.

[0138] Some Peptide units can be a peptide containing glycine or glycine and serine residues. A Peptide unit optionally can include one or more cysteine residues. The cysteine residues are typically solvent accessible for conjugation to a Linker unit or a Drug unit. In some embodiments, the Peptide Unit comprises the amino acid sequence Gly-Gly-Gly-Ser-Cys-Gly-Gly-Gly (SEQ ID NO:1), or Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly (SEQ ID NO:2).

The Polypeptide unit

[0139] A Polypeptide unit (-Z-) can optionally be linked to Ligand unit moiety L_a or L_b. The Polypeptide typically comprises one or more additional functional groups, such as, for example, an antibody hinge region (e.g., an IgG1 hinge region), a constant region domain(s) (e.g., a C_H1, C_H2 and/or C_H3 domain), an affinity tag (e.g., hexahistidine, a FLAG epitope, a myc-epitope or the like). The functional group(s) optionally can be joined by a linker. In some embodiments, the Polypeptide unit is absent. A Polypeptide unit optionally can include one or more cysteine residues. The cysteine residues are typically solvent accessible for conjugation to a Linker unit or a Drug unit.

[0140] In some embodiments, the Polypeptide unit lacks an Fc region, an Fc domain (e.g., a C_H1, C_H2 and/or C_H3 domain), or an Fc receptor binding site.

Linker units

[0141] A "Linker unit" (LU) is a bifunctional compound which can be used to link a Drug unit and a Ligand unit to form Ligand Drug conjugate compounds. In some embodiments, the Linker unit has the formula:



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,

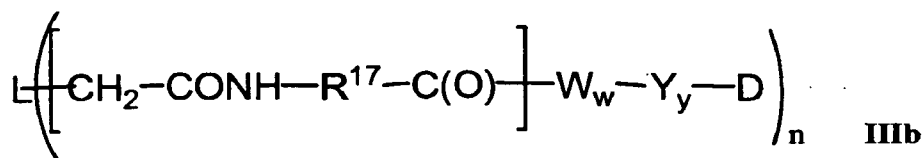
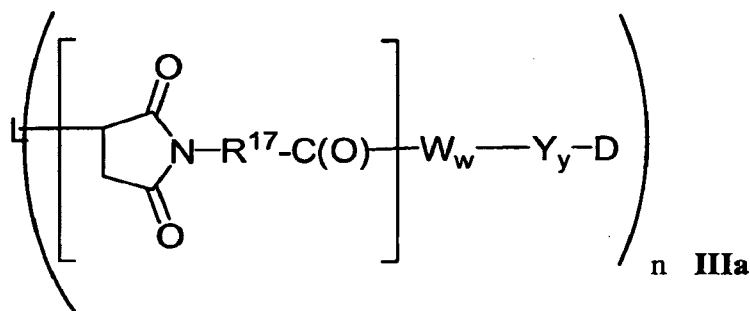
-Y- is a self-immolative Spacer unit, and

y is 0, 1 or 2.

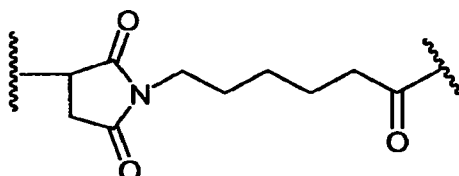
[0142] In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0, 1 or 2. In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0 or 1.

5 The Stretcher Unit

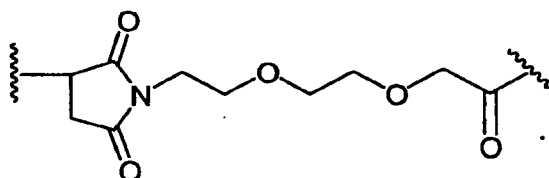
[0143] The Stretcher unit (A), when present, is capable of linking a Ligand unit to an Amino Acid unit (-W-), if present, to a Spacer unit (-Y-), if present; or to a Drug unit (-D). In one embodiment, the Stretcher unit forms a bond with a sulfur atom of the Ligand unit. The sulfur atom can be derived from a sulfhydryl group of a Ligand. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas IIIa and IIIb, wherein L-, -W-, -Y-, -D, w and y are as defined above, and R₁₇ is selected from -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-, -O-(C₁-C₁₀ alkyl)-, -arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, and -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging from 1-10. It is to be understood from all the exemplary embodiments of Formulae I and II, such as III-VI, that even where not denoted expressly, from 1 to 20 drug moieties are linked to a Ligand unit (p = 1-20).



[0144] An illustrative Stretcher unit is that of Formula IIIa wherein R^{17} is $-(CH_2)_5-$:

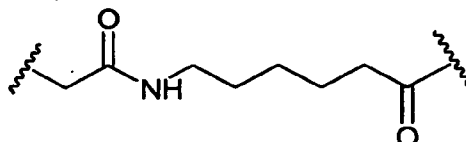


[0145] Another illustrative Stretcher unit is that of Formula IIIa wherein R^{17} is $-(CH_2CH_2O)_r-CH_2-$; and r is 2:



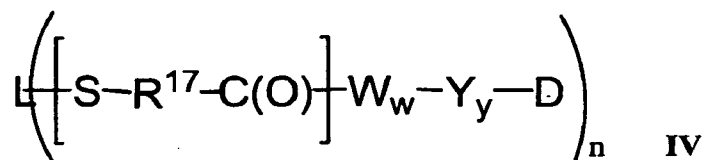
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[0146] Still another illustrative Stretcher unit is that of Formula IIIb wherein R^{17} is $-(CH_2)_5-$:



[0147] 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.

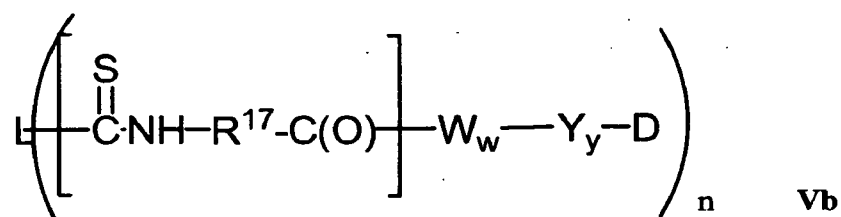
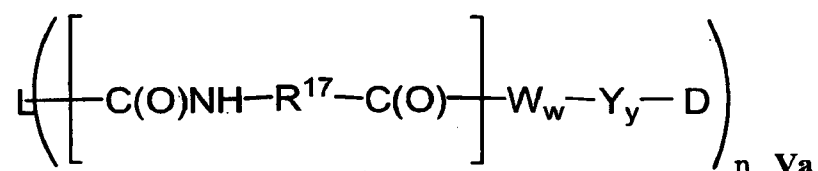
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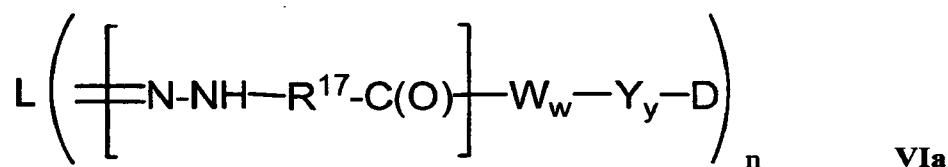
[0148] In yet another embodiment, the reactive group of the Stretcher contains a reactive site that can form a bond with a primary or secondary amino group of a

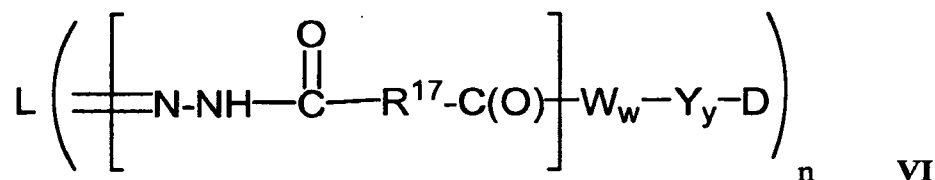
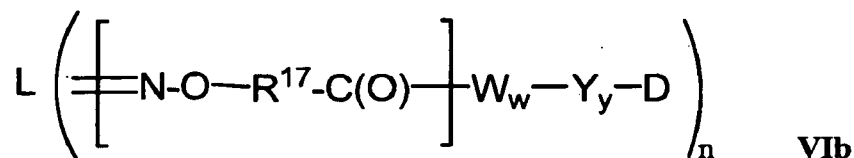
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Ligand unit. Examples of these reactive sites include, but are not limited to, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates 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;



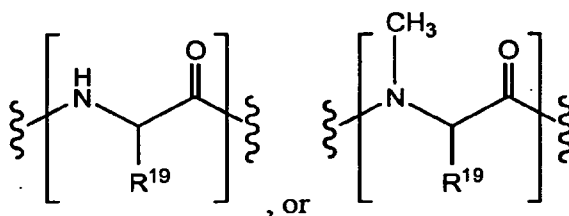
[0149] In some embodiments, the reactive group of the Stretcher contains a reactive site that is reactive to a modified carbohydrate's ($-CHO$) group that can be present on a Ligand unit. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting ($-CHO$) 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 *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.



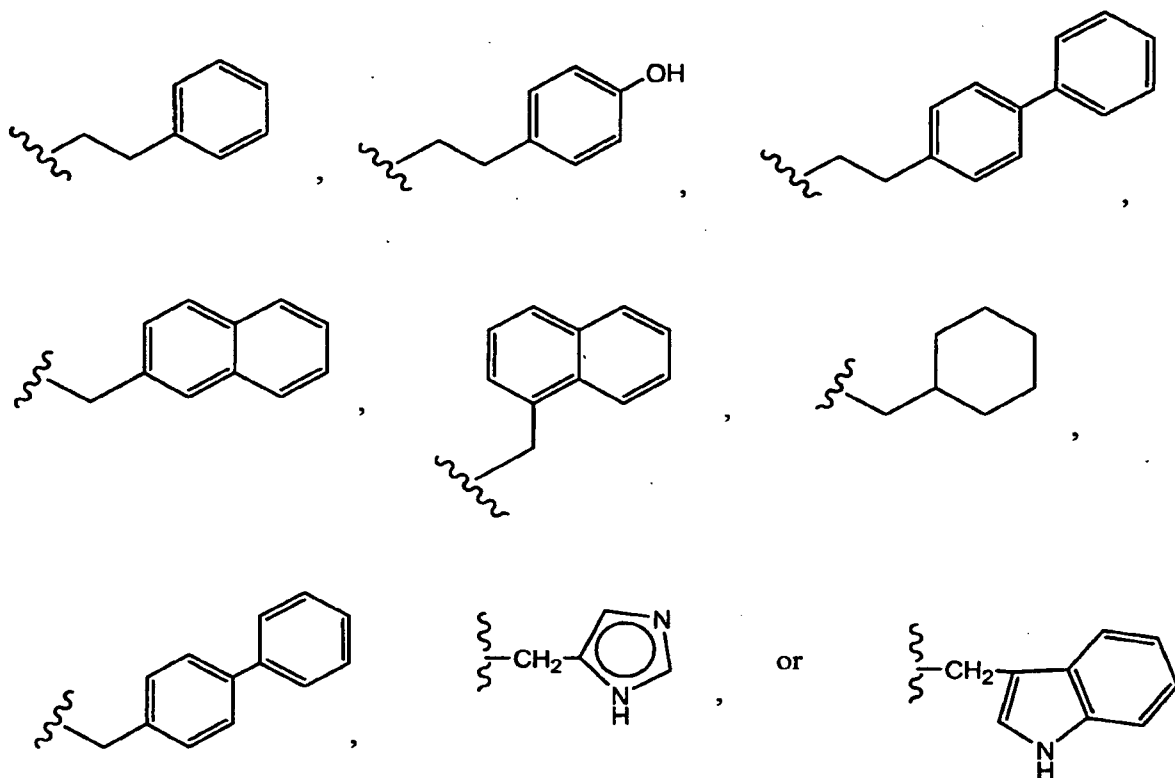


[0150] The Amino Acid unit (-W-), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the Drug moiety if the Spacer unit is absent, and links the Ligand unit to the Drug unit if the Stretcher unit and Spacer unit are absent.

[0151] W_w is a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each -W- unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 to 12:

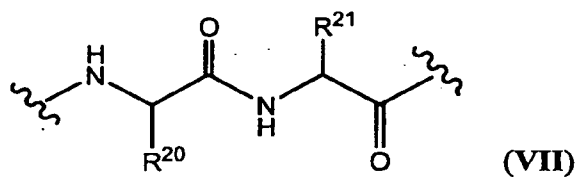


wherein R¹⁹ is hydrogen, methyl, isopropyl, isobutyl, *sec*-butyl, benzyl, *p*-hydroxybenzyl, -CH₂OH, -CH(OH)CH₃, -CH₂CH₂SCH₃, -CH₂CONH₂, -CH₂COOH, -CH₂CH₂CONH₂, -CH₂CH₂COOH, -(CH₂)₃NHC(=NH)NH₂, -(CH₂)₃NH₂, -(CH₂)₃NHCOCH₃, -(CH₂)₃NHCHO, -(CH₂)₄NHC(=NH)NH₂, -(CH₂)₄NH₂, -(CH₂)₄NHCOCH₃, -(CH₂)₄NHCHO, -(CH₂)₃NHCONH₂,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



[0152] In some embodiments, the Amino Acid unit can be enzymatically cleaved by one or more enzymes, including a cancer or tumor-associated protease, to liberate the Drug unit (-D), which in one embodiment is protonated in vivo upon release to provide a Drug (D).

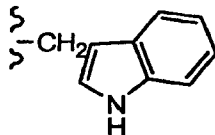
[0153] In certain embodiments, the Amino Acid unit can comprise natural amino acids. In other embodiments, the Amino Acid unit can comprise non-natural amino acids. Illustrative Ww units are represented by formulas (VII)-(IX):

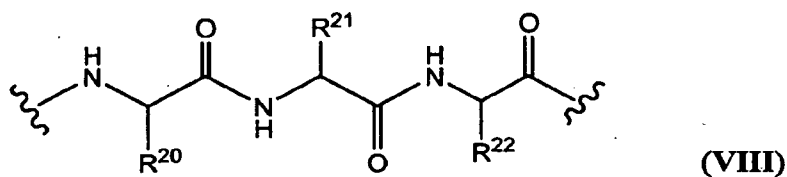


10 wherein R^{20} and R^{21} are as follows:

R^{20}
Benzyl
methyl

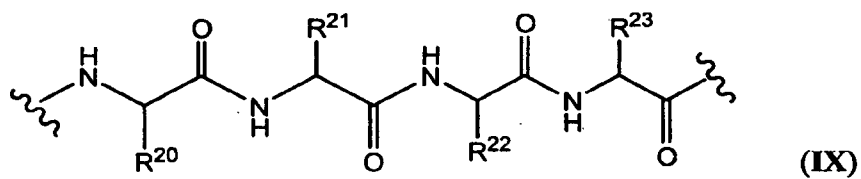
R^{21}
(CH₂)₄NH₂;
(CH₂)₄NH₂;

isopropyl	$(\text{CH}_2)_4\text{NH}_2$;
isopropyl	$(\text{CH}_2)_3\text{NHCONH}_2$;
benzyl	$(\text{CH}_2)_3\text{NHCONH}_2$;
isobutyl	$(\text{CH}_2)_3\text{NHCONH}_2$;
sec-butyl	$(\text{CH}_2)_3\text{NHCONH}_2$;
	$(\text{CH}_2)_3\text{NHCONH}_2$;
benzyl	
benzyl	methyl; and
	$(\text{CH}_2)_3\text{NHC(=NH)NH}_2$;



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

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



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

<u>R^{20}</u>	<u>R^{21}</u>	<u>R^{22}</u>	<u>R^{23}</u>
H	benzyl	isobutyl	H; and
methyl	isobutyl	methyl	isobutyl.

[0154] 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

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

[0155] Useful $-W_w-$ units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease. In one embodiment, a $-W_w-$ unit is that whose cleavage is catalyzed by

10 cathepsin B, C and D, or a plasmin protease.

[0156] 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.

[0157] Each carbon atom to which R^{19} , R^{20} , R^{21} , R^{22} or R^{23} is attached is

15 independently in the (S) or (R) configuration.

[0158] In one aspect of the Amino Acid unit, the Amino Acid unit is valine, citrulline (val cit or vc). In another aspect, the Amino Acid unit is phenylalanine lysine (phe lys or 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-

20 aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepepotic acid lysine, beta-alanine lysine, glycine serine valine glutamine or isonepepotic acid.

The Spacer Unit

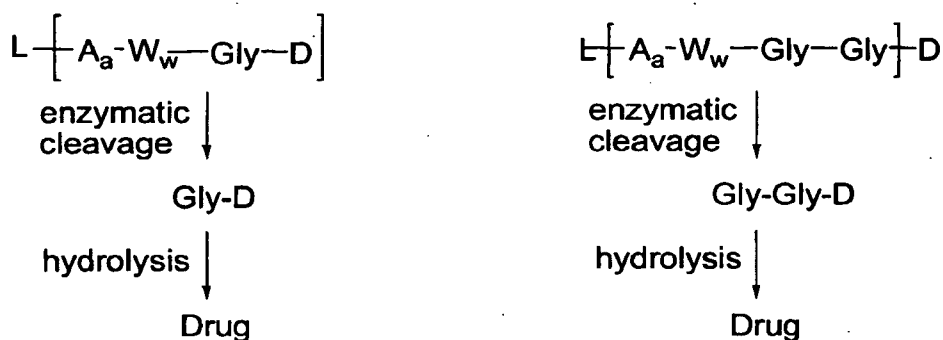
[0159] The Spacer unit (-Y-), when present, links an Amino Acid unit to the Drug unit when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the Drug unit when the Amino Acid unit is absent. The Spacer unit also links the Drug unit to the Ligand unit when both the Amino Acid unit and Stretcher unit are absent.

[0160] Spacer units are of two general types: non self-immolative or 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 Ligand Drug conjugate compound.

- 5 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 a conjugate containing a glycine-glycine Spacer unit or a glycine
- 10 Spacer unit undergoes enzymatic cleavage via an enzyme (e.g., 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-Aa-Ww-. In one embodiment, an independent hydrolysis reaction takes place within the target cell, cleaving the glycine-Drug moiety bond and liberating the Drug.

- [0161]** In another embodiment, -Y- is a p-aminobenzyl alcohol (PAB) unit (see
- 15 Schemes 2 and 3) whose phenylene portion is substituted with Q_m wherein Q is -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

Scheme 1



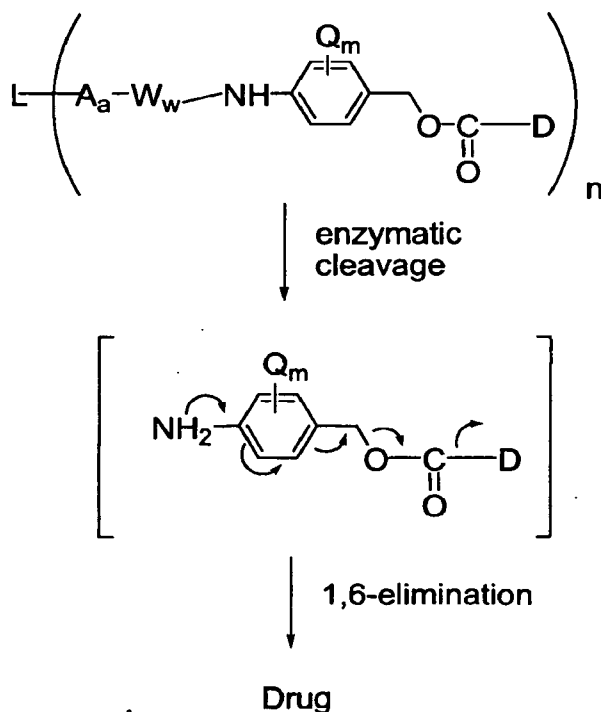
- 20 **[0162]** In some embodiments, a non self-immolative the Spacer unit (-Y-) is -Gly-. In some embodiments, a non self-immolative Spacer unit (-Y-) is -Gly-Gly-.

[0163] In one embodiment, a Drug-Linker conjugate compound is provided in which the Spacer unit is absent ($y=0$), or a pharmaceutically acceptable salt or solvate thereof.

[0164] Alternatively, a conjugate containing a self-immolative Spacer unit can release -D. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved.

[0165] In some embodiments, -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 as described by Toki et al., 2002, *J. Org. Chem.* 67:1866-1872.

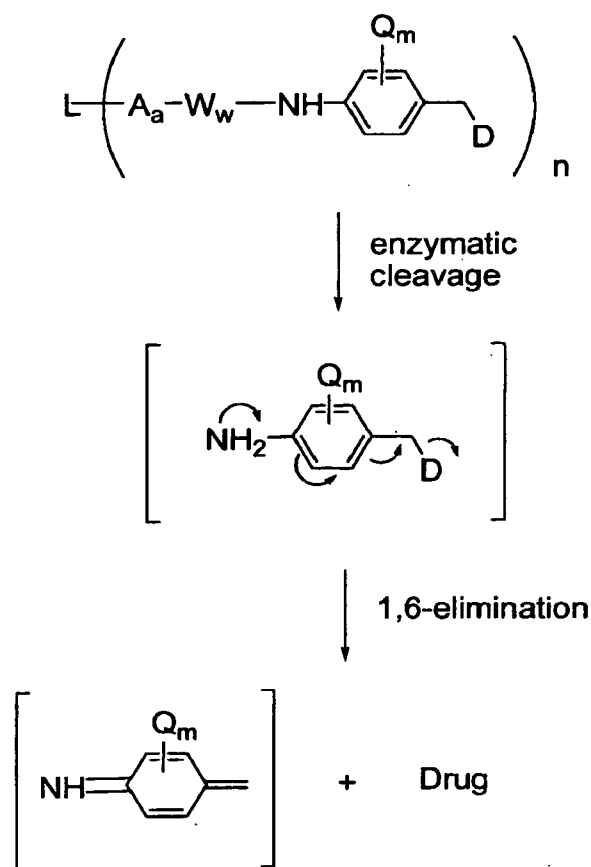
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Scheme 2

In Scheme 2, Q is -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

[0166] 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, wherein D includes the oxygen or nitrogen group is part of the Drug unit.

Scheme 3



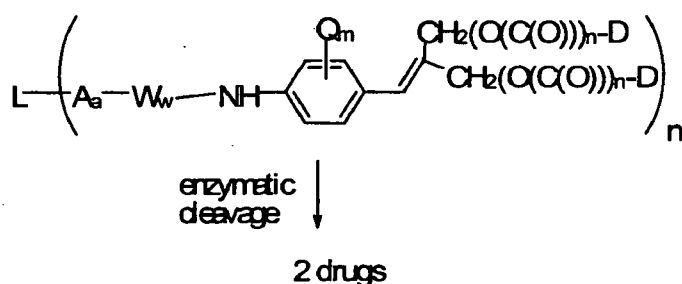
[0167] In Scheme 3, Q is -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

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., 1995, *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al., 1972, *J. Amer. Chem. Soc.* 94:5815) and 2-aminophenylpropionic acid amides (Amsberry et al., 1990, *J. Org. Chem.* 55:5867). Elimination of amine-containing drugs that are substituted at the α -position of glycine (Kingsbury et al., 1984, *J. Med. Chem.* 27:1447) are also examples of self-immolative spacers.

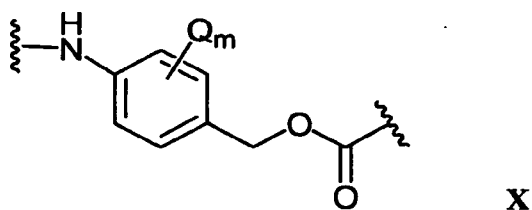
- 10 [0168] In one embodiment, the Spacer unit is a branched bis(hydroxymethyl)-styrene (BHMS) unit as depicted in Scheme 4, which can be used to incorporate and release multiple drugs.

Scheme 4

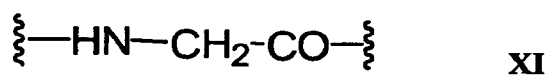


- 15 In Scheme 4, Q is $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20. In some embodiments, the -D moieties are the same. In yet another embodiment, the -D moieties are different.

[0169] In one aspect, Spacer units (-Y_p-) are represented by Formulas (X)-(XII):

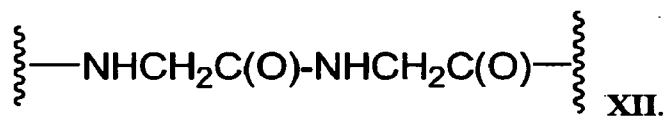


wherein Q is -C₁-C₁₀ alkyl, -O-(C₁-C₁₀ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4;

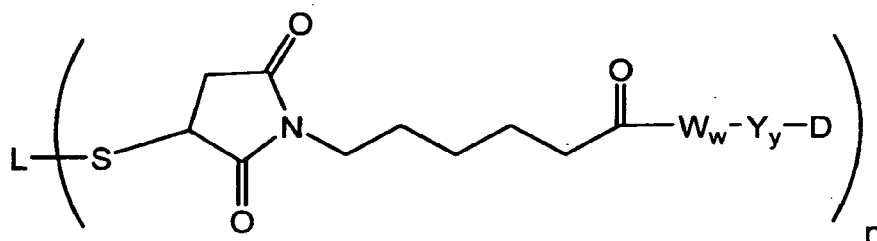


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and

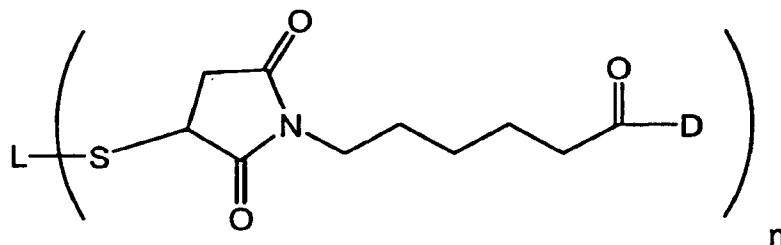


[0170] Embodiments of the Formulae I and II comprising ligand-drug conjugate compounds can include:

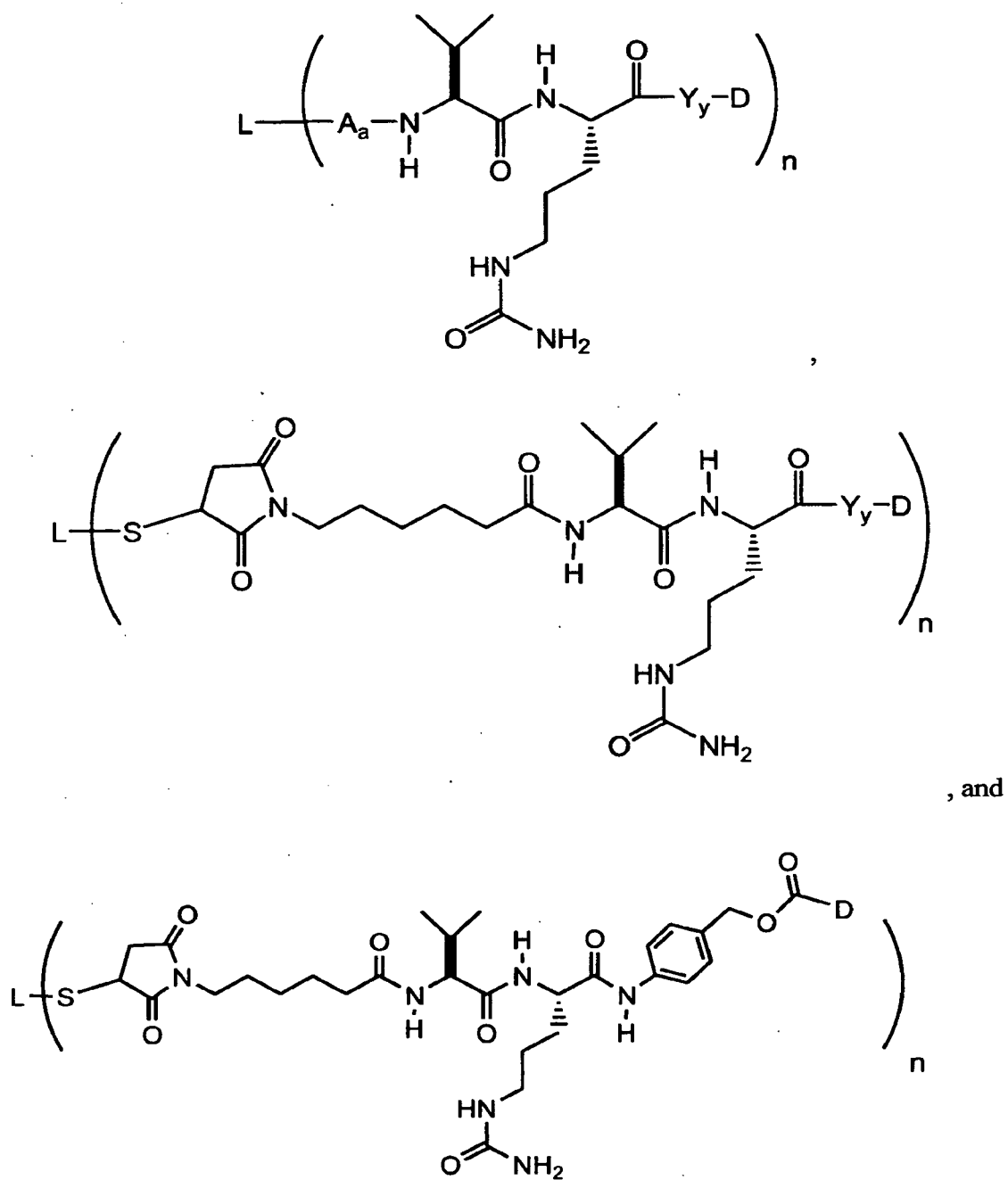


10 wherein w and y are each 0, 1 or 2 and S is a thiol group of L;

and,



wherein w and y are each 0 and S is a thiol group of L;



5

wherein S is a thiol group of L.

The Drug Unit

[0171] The Drug unit or moiety (D) can be any cytotoxic, cytostatic or immunomodulatory drug. D is a Drug unit (moiety) having an atom that can form a bond with the Spacer unit, with the Amino Acid unit, with the Stretcher unit or with the Ligand unit. In some embodiments, the Drug unit D has a nitrogen atom that can form a bond with the Spacer unit. As used herein, the terms "Drug unit" and "Drug moiety" are synonymous and used interchangeably.

[0172] Useful classes of cytotoxic or immunomodulatory agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cis-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like.

[0173] Individual cytotoxic or immunomodulatory agents include, for example, an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, calicheamicin, camptothecin or a camptothecin derivative, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, etoposide, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gemcitabine, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), maytansine, mechlorethamine, melphalan, 6-mercaptapurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, palytoxin, plicamycin, procarbazine, rhizoxin, streptozotocin, tenoposide, 6-thioguanine, thiTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

[0174] In some typical embodiments, suitable cytotoxic agents include, for example, DNA minor groove binders (e.g., enediynes and lexitropsins, a CBI compound; see also U.S. Patent No. 6,130,237), duocarmycins, taxanes (e.g.,

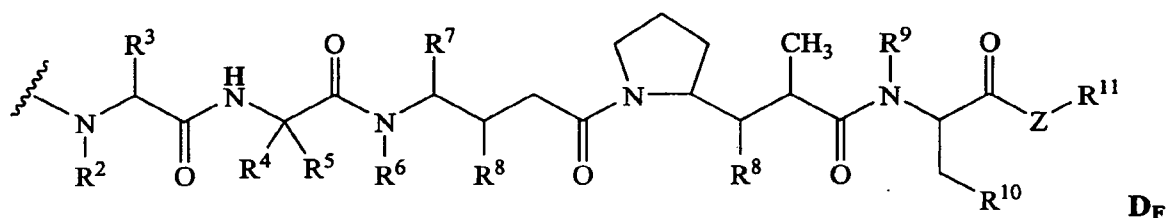
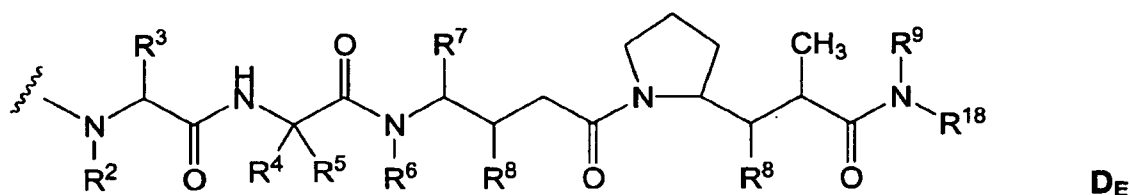
paclitaxel and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemadotin, maytansinoids, discodermolide, eleutherobin, and mitoxantrone.

[0175] In some embodiments, the Drug unit is an anti-tubulin agent. Examples of anti-tubulin agents include, but are not limited to, taxanes (e.g., Taxol® (paclitaxel), Taxotere® (docetaxel)), T67 (Tularik) and vinca alkyls (e.g., vincristine, vinblastine, vindesine, and vinorelbine). Other antitubulin agents include, for example, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine and colchimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermolide, and eleutherobin.

[0176] In certain embodiments, the cytotoxic agent is a maytansinoid, another group of anti-tubulin agents. For example, in specific embodiments, the maytansinoid is maytansine or DM-1 (ImmunoGen, Inc.; see also Chari *et al.*, 1992, Cancer Res. 52:127-131).

[0177] In some embodiments, the Drug is an auristatin, such as auristatin E (a derivative of dolastatin-10) or a derivative thereof. Typically, the auristatin E derivative is, e.g., an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatin derivatives include AFP, MMAF, and MMAE. The synthesis and structure of auristatin derivatives are described in U.S. Patent Application Publication Nos. 2003-0083263, 2005-0238649 and 2005-0009751; International Patent Publication No. WO 04/010957, International Patent Publication No. WO 02/088172, and U.S. Patent Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414.

[0178] In some embodiments, -D is either formula D_E or D_F:



wherein, independently at each location:

- 5 R^2 is selected from H and C₁-C₁₀ alkyl;
- R^3 is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);
- 10 R^4 is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ 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, C₁-C₁₀ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;
- 15 R^6 is selected from H and C₁-C₁₀ alkyl;
- R^7 is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);
- 20 each R^8 is independently selected from H, OH, C₁-C₁₀ alkyl, C₃-C₈ carbocycle and O-(C₁-C₁₀ alkyl);
- R^9 is selected from H and C₁-C₁₀ alkyl;
- R^{10} is selected from aryl and C₃-C₈ heterocycle;
- Z is selected from O, S, NH, and NR¹², wherein R¹² is C₁-C₁₀ 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 1-1000;

R^{13} is C_2 - C_8 alkyl;

5 R^{14} is selected from H and C_1 - C_{10} alkyl;

each occurrence of R^{15} is independently selected from H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, and $-(CH_2)_n-SO_3-C_1-C_{10}$ alkyl;

each occurrence of R^{16} is independently selected from H, C_1 - C_{10} alkyl, and $-(CH_2)_n-COOH$;

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

n is an integer ranging from 0 to 6.

[0179] 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,
15 R^5 is H, and R^7 is sec-butyl.

[0180] In another embodiment, R^2 and R^6 are each methyl, and R^9 is H.

[0181] In still another embodiment, each occurrence of R^8 is $-OCH_3$.

[0182] 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
20 is H.

[0183] In one embodiment, Z is selected from -O- and -NH-.

[0184] In one embodiment, R^{10} is aryl.

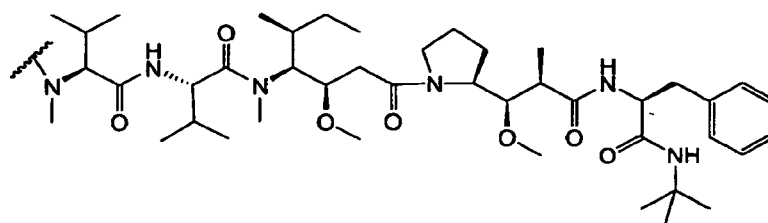
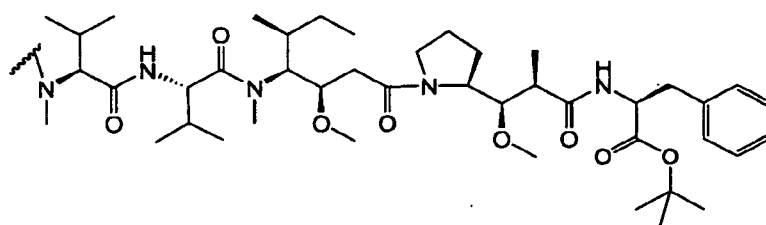
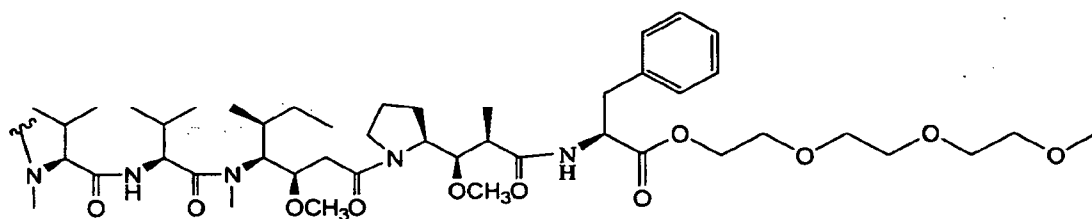
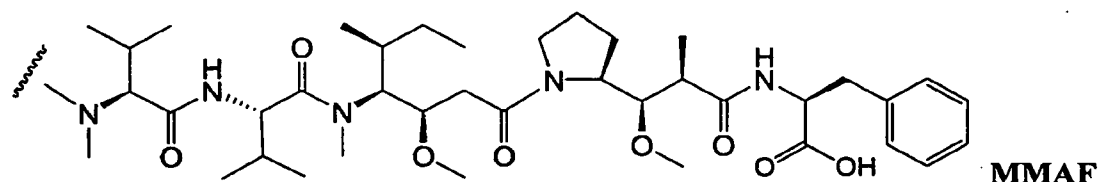
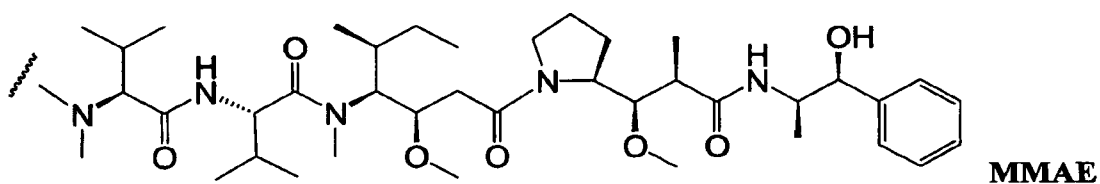
[0185] In an exemplary embodiment, R^{10} is -phenyl.

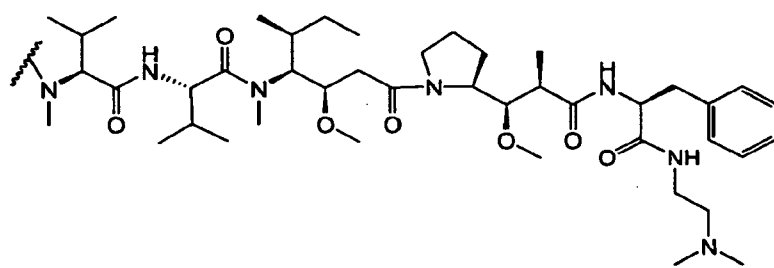
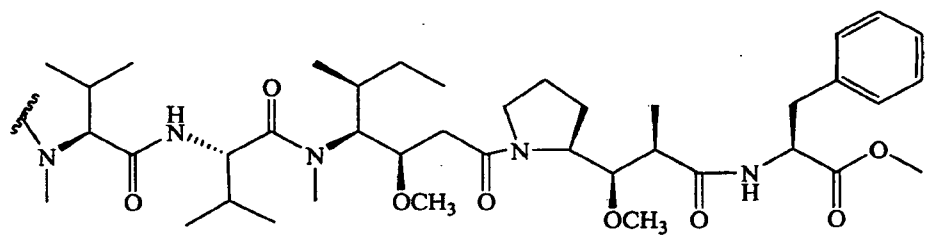
[0186] In an exemplary embodiment, when Z is -O-, R^{11} is H, methyl or t-butyl.

25 [0187] 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 selected from $-C_1-C_{10}$ alkyl or $-(CH_2)_n-COOH$.

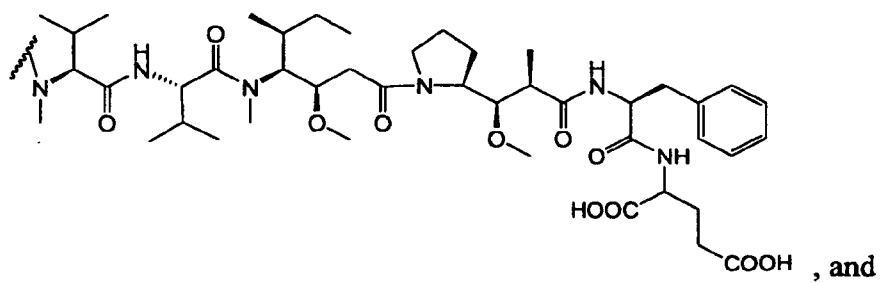
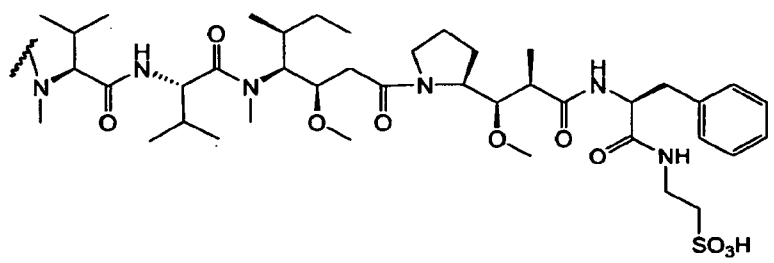
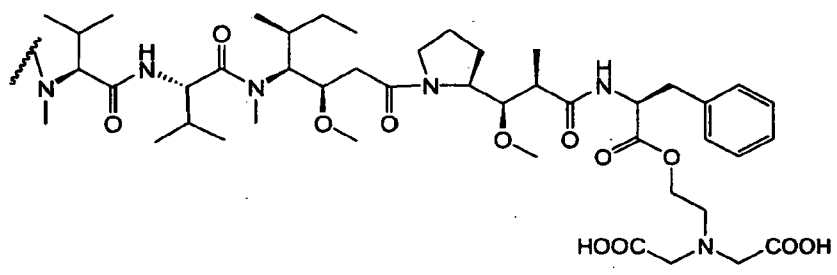
[0188] In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

[0189] Illustrative Drug units (-D) include the drug units having the following structures:

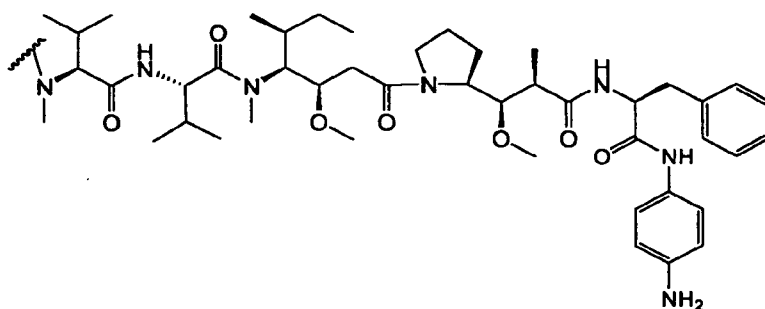




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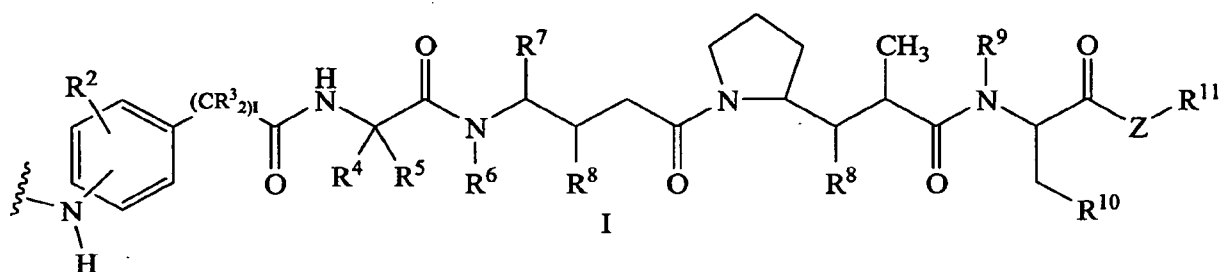
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and pharmaceutically acceptable salts or solvates thereof.

- 5 **[0190]** 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.

- 10 **[0191]** In another aspect, the Drug unit is an amino-benzoic acid derivative of an auristatin of the following formula:



wherein, independently at each location:

R^2 is selected from H, $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ alkyl), -halogen, $-NO_2$, $-COOH$, and $-C(O)OR^{11}$;

- 15 each R^3 is selected independently from -hydrogen and $-C_1-C_{10}$ alkyl;

I is an integer ranging from 0-10;

- 20 R^4 is selected from H, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, -aryl, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- (C_3-C_8) carbocycle, $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- (C_3-C_8) heterocycle, and 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, -C₁-C₁₀ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R⁶ is selected from -H and -C₁-C₁₀ alkyl;

R⁷ is selected from -H, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₁₀ alkyl-aryl, -C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from -H, -OH, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, -O-alkyl-(C₁-C₈ carbocycle) and -O-(C₁-C₁₀ alkyl);

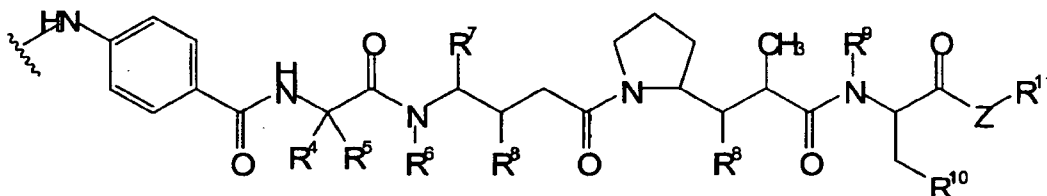
R⁹ is selected from -H and -C₁-C₁₀ alkyl;

R¹⁰ is selected from aryl and -C₃-C₈ heterocycle;

Z is selected from -O-, -S-, -NH-, or -NR¹²- where R¹² is C₁-C₁₀ alkyl or aryl; and

R¹¹ is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_r-H, -(CH₂CH₂O)_r-CH₃, and -(CH₂CH₂O)_r-CH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

[0192] In some embodiments, the Drug unit is of the following formula:



wherein, independently at each location:

R⁴ is selected from H, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, -aryl, -C₁-C₁₀ alkyl-aryl, -C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₁₀ alkyl-(C₃-C₈ heterocycle), and R⁵ is selected from -H and -methyl; or R⁴ and R⁵ jointly have the formula -(CR^aR^b)_n-, wherein R^a and R^b are independently selected from -H, -C₁-C₁₀ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R⁶ is selected from -H and -C₁-C₁₀ alkyl;

R⁷ is selected from -H, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₁₀ alkyl-aryl, -C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from -H, -OH, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, -O-alkyl-(C₁-C₈ carbocycle) and -O-(C₁-C₁₀ alkyl);

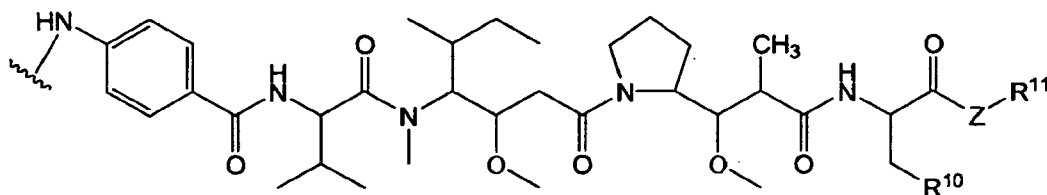
R⁹ is selected from -H and -C₁-C₁₀ alkyl;

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

Z is selected from -O-, -S-, -NH-, and -NR¹²- where R¹² is C₁-C₁₀ alkyl or aryl; and

10 R¹¹ is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_r-H, -(CH₂CH₂O)_r-CH₃, and -(CH₂CH₂O)_r-CH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

[0193] In some embodiments, the Drug unit is of the following formula:



15 wherein, independently at each location:

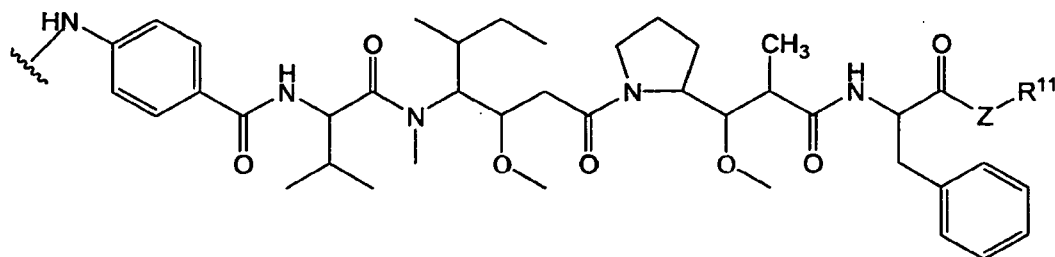
R¹⁰ is selected from an aryl group and -C₃-C₈ heterocycle;

Z is selected from -O-, -S-, -NH-, and -NR¹²- where R¹² is C₁-C₁₀ alkyl or aryl; and

20 R¹¹ is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_rH, -(CH₂CH₂O)_rCH₃, and -(CH₂CH₂O)_rCH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

[0194] In some embodiments, the Drug unit is of the following formula:

le

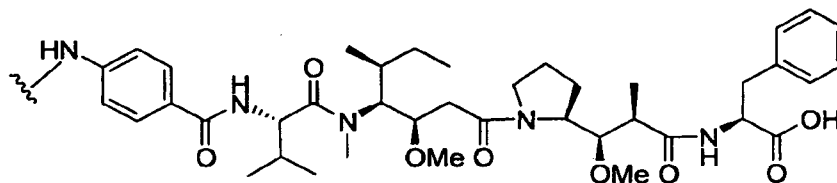


wherein:

Z is selected from -O-, -S-, -NH-, and -NR¹²- where R¹² is C₁-C₁₀ alkyl or aryl; and

5 R¹¹ is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_r-H, -(CH₂CH₂O)_r-CH₃, and -(CH₂CH₂O)_r-CH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

[0195] In some embodiments, the Drug unit is of the following formula:



10 **[0196]** In some embodiments, the Drug is not a radioisotope or is not radiolabeled. In some embodiments, the Drug unit is not TZT-1027. In some embodiments, the Drug unit does not comprise an antibody or antibody fragment.

[0197] In certain embodiments, the Drug is an antimetabolite. The antimetabolite can be, for example, a purine antagonist (e.g., azothioprine or mycophenolate mofetil), a dihydrofolate reductase inhibitor (e.g., methotrexate),
15 acyclovir, gangcyclovir, zidovudine, vidarabine, ribavarin, azidothymidine, cytidine arabinoside, amantadine, dideoxyuridine, iododeoxyuridine, poscarnet, or trifluridine.

[0198] In other embodiments, the Drug is tacrolimus, cyclosporine or rapamycin.
20 In further embodiments, the Drug is aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, bexarotene, bexarotene, calusterone, capecitabine, celecoxib, cladribine, Denileukin diftitox,

dexrazoxane, dromostanolone propionate, epirubicin, estramustine, exemestane, Filgrastim, floxuridine, fludarabine, fulvestrant, gemcitabine, gemtuzumab ozogamicin, goserelin, idarubicin, ifosfamide, imatinib mesylate, Interferon alfa-2a, irinotecan, letrozole, leucovorin, levamisole, meclorethamine or nitrogen mustard, 5 megestrol, mesna, methotrexate, methoxsalen, mitomycin C, mitotane, nandrolone phenpropionate, oprelvekin, oxaliplatin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pentostatin, pipobroman, plicamycin, porfimer sodium, procarbazine, quinacrine, rasburicase, Sargramostim, streptozocin, tamoxifen, temozolomide, teniposide, testolactone, thioguanine, toremifene, 10 tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine and zoledronate.

[0199] In some embodiments, the Drug moiety is an immunomodulatory agent. The immunomodulatory agent can be, for example, gancyclovir, etanercept, tacrolimus, cyclosporine, rapamycin, cyclophosphamide, azathioprine, 15 mycophenolate mofetil or methotrexate. Alternatively, the immunomodulatory agent can be, for example, a glucocorticoid (e.g., cortisol or aldosterone) or a glucocorticoid analogue (e.g., prednisone or dexamethasone).

[0200] In some embodiments, the immunomodulatory agent is an anti-inflammatory agent, such as arylcarboxylic derivatives, pyrazole-containing 20 derivatives, oxicam derivatives and nicotinic acid derivatives. Classes of anti-inflammatory agents include, for example, cyclooxygenase inhibitors, 5-lipoxygenase inhibitors, and leukotriene receptor antagonists.

[0201] Suitable cyclooxygenase inhibitors include meclofenamic acid, mefenamic acid, carprofen, diclofenac, diflunisal, fenbufen, fenoprofen, ibuprofen, 25 indomethacin, ketoprofen, nabumetone, naproxen, sulindac, tenoxicam, tolmetin, and acetylsalicylic acid.

[0202] Suitable lipoxygenase inhibitors include redox inhibitors (e.g., catechol butane derivatives, nordihydroguaiaretic acid (NDGA), masoprocol, phenidone, lanopalen, indazolinones, naphazatrom, benzofuranol, alkylhydroxylamine), and 30 non-redox inhibitors (e.g., hydroxythiazoles, methoxyalkylthiazoles, benzopyrans

and derivatives thereof, methoxytetrahydropyran, boswellic acids and acetylated derivatives of boswellic acids, and quinolinemethoxyphenylacetic acids substituted with cycloalkyl radicals), and precursors of redox inhibitors.

- [0203]** Other suitable lipoxygenase inhibitors include antioxidants (e.g., phenols, propyl gallate, flavonoids and/or naturally occurring substrates containing flavonoids, hydroxylated derivatives of the flavones, flavonol, dihydroquercetin, luteolin, galangin, orobol, derivatives of chalcone, 4,2',4'-trihydroxychalcone, ortho-aminophenols, N-hydroxyureas, benzofuranols, ebselen and species that increase the activity of the reducing selenoenzymes), iron chelating agents (e.g., hydroxamic acids and derivatives thereof, N-hydroxyureas, 2-benzyl-1-naphthol, catechols, hydroxylamines, carnosol trolox C, catechol, naphthol, sulfasalazine, zyleuton, 5-hydroxyanthranilic acid and 4-(omega-arylalkyl)phenylalkanoic acids), imidazole-containing compounds (e.g., ketoconazole and itraconazole), phenothiazines, and benzopyran derivatives.
- [0204]** Yet other suitable lipoxygenase inhibitors include inhibitors of eicosanoids (e.g., octadecatetraenoic, eicosatetraenoic, docosapentaenoic, eicosahexaenoic and docosahexaenoic acids and esters thereof, PGE1 (prostaglandin E1), PGA2 (prostaglandin A2), viprostol, 15-monohydroxyeicosatetraenoic, 15-monohydroxy-eicosatrienoic and 15-monohydroxyeicosapentaenoic acids, and leukotrienes B5, C5 and D5), compounds interfering with calcium flows, phenothiazines, diphenylbutylamines, verapamil, fuscoidin, curcumin, chlorogenic acid, caffeic acid, 5,8,11,14-eicosatetrayenoic acid (ETYA), hydroxyphenylretinamide, lonapalen, esculin, diethylcarbamazine, phenantrolin, baicalein, proxipromil, thioethers, diallyl sulfide and di-(1-propenyl) sulfide.
- [0205]** Leukotriene receptor antagonists include calcitriol, ontazolast, Bayer Bay-x-1005, Ciba-Geigy CGS-25019C, ebselen, Leo Denmark ETH-615, Lilly LY-293111, Ono ONO-4057, Terumo TMK-688, Boehringer Ingelheim BI-RM-270, Lilly LY 213024, Lilly LY 264086, Lilly LY 292728, Ono ONO-LB457, Pfizer 105696, Perdue Frederick PF 10042, Rhone-Poulenc Rorer RP 66153, SmithKline Beecham SB-201146, SmithKline Beecham SB-201993, SmithKline Beecham SB-209247, Searle SC-53228, Sumitomo SM 15178, American Home

Products WAY 121006, Bayer Bay-o-8276, Warner-Lambert CI-987, Warner-Lambert CI-987BPC-15LY 223982, Lilly LY 233569, Lilly LY-255283, MacroNex MNX-160, Merck and Co. MK-591, Merck and Co. MK-886, Ono ONO-LB-448, Purdue Frederick PF-5901, Rhone-Poulenc Rorer RG 14893, Rhone-Poulenc Rorer RP 66364, Rhone-Poulenc Rorer RP 69698, Shionogi S-2474, Searle SC-41930, Searle SC-50505, Searle SC-51146, Searle SC-52798, SmithKline Beecham SK&F-104493, Leo Denmark SR-2566, Tanabe T-757 and Teijin TEI-1338.

[0206] Methods of determining whether a Drug unit or ligand drug conjugate exerts a cytostatic or a cytotoxic effect on a cell are known. Illustrative examples of such methods for ligand drug conjugate are described infra. Generally, the cytotoxic or cytostatic activity of a ligand drug conjugate can be measured by: exposing mammalian cells expressing a target protein of the ligand drug conjugate 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 can be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the ligand drug conjugate.

[0207] For determining whether a ligand drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μ Ci of ^{3} H-thymidine during the final 8 hours of the 72-hour period. The incorporation of ^{3} H-thymidine into cells of the culture is measured in the presence and absence of the ligand drug conjugate.

[0208] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that a ligand drug conjugate is useful in the treatment of cancers.

[0209] Cell viability can be measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (see, e.g., Page et al., 1993, Intl. J. Oncology 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytotoxicity (Skehan et al., 1990, *J. Natl. Cancer Inst.* 82:1107-12).

[0210] Alternatively, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, *J. Immunol. Methods* 65:55-63).

[0211] Apoptosis can be quantitated by measuring, for example, DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in *Biochemica*, 1999, no. 2, pp. 34-37 (Roche Molecular Biochemicals).

[0212] Apoptosis can also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, *Current Protocols in Immunology* (Coligan et al. eds., 1992, pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[0213] The presence of apoptotic cells can be measured in both the attached and "floating" compartments of the cultures. For example, both compartments can be collected by removing the supernatant, trypsinizing the attached cells,

combining the preparations following a centrifugation wash step (e.g., 10 minutes at 2000 rpm), and detecting apoptosis (e.g., by measuring DNA fragmentation). (See, e.g., Piazza et al., 1995, *Cancer Research* 55:3110-16).

[0214] The effects of Ligand Drug conjugates can be tested or validated in animal models. A number of established animal models of cancers are known to the skilled artisan, any of which can be used to assay the efficacy of a Ligand Drug conjugate. Non-limiting examples of such models are described infra. Moreover, small animal models to examine the in vivo efficacies of Ligand Drug conjugates can be created by implanting human tumor cell lines into appropriate immunodeficient rodent strains, e.g., athymic nude mice or SCID mice.

LIGAND DRUG CONJUGATES

[0215] The Ligand unit (L) has at least one functional group that can form a bond with a functional group of a Linker unit or a Drug unit. Useful functional groups that can be present on a Ligand unit, either naturally, via chemical manipulation or via engineering, include, but are not limited to, sulfhydryl or thiol (-SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl. In some embodiments, a Ligand unit functional group is a sulfhydryl group. The sulfhydryl group is typically a solvent accessible sulfhydryl group, such as a solvent accessible sulfhydryl group on a cysteine residue. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of a Ligand. Sulfhydryl groups also can be generated by reaction of an amino group of a lysine moiety of a Ligand unit using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent.

[0216] In some embodiments, one or more sulfhydryl groups are engineered into a Ligand unit, such as by amino acid substitution or insertion. For example, a sulfhydryl group can be introduced into a Ligand unit moiety L_a and/or L_b, into a Peptide unit (-P-), and/or into a Polypeptide unit (Z). In some embodiments, a sulfhydryl group is introduced by an amino acid substitution of serine or threonine to a cysteine residue. In other embodiments, a sulfhydryl group is introduced by addition of a cysteine residue into a Ligand unit moiety L_a and/or L_b, into a Peptide

unit (-P-), and/or into a Polypeptide unit (Z) (an engineered cysteine residue). In some embodiments, the cysteine residue is an internal cysteine residue, i.e., not located at the N-terminus or C-terminus of the unit or moiety (e.g., a Ligand unit).

[0217] In an exemplary embodiment, a cysteine residue can be engineered into an antibody heavy or light variable region by amino acid substitution or insertion. The amino acid substitution is typically introduced into the framework region and is located distal to the epitope-binding face of the variable region (also referred to as the antigen binding face). For example, the amino acid substitution can be at least 10 angstroms, at least 20 angstroms or at least 25 angstroms from the epitope-binding face or the CDRs. Suitable positions for substitution of a cysteine residue can be determined based on the known or predicted three dimensional structures of antibody variable regions. (See generally Holliger and Hudson, 2005, *Nature BioTechnology* 23(9):1126-1136.) In exemplary embodiments, a serine to cysteine amino acid substitution is introduced at amino acid position 84 of the V_H region and/or position 14 of the V_L region (according to the numbering system of Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th edition, (Bethesda, MD, NIH) 1991).

[0218] In some embodiments, the Drug unit is linked to the Ligand unit (directly or indirectly) via the Peptide unit (P). In a specific embodiment, the Drug unit is linked (directly or indirectly) to a thiol group of the Peptide unit, and Drug units are not attached to the Ligand unit moieties. In some embodiments, one Drug unit is attached to each Ligand unit. In some embodiments, two Drug units are attached to each Ligand unit.

[0219] In some embodiments, the Drug unit is linked to the Ligand unit (directly or indirectly). In a specific embodiment, the Drug unit is linked (directly or indirectly) to a thiol group(s) of a Ligand unit moiety (L_a and/or L_b) and Drug units are not attached to the Peptide unit. In some embodiments, one Drug unit is attached to each Ligand unit. In some embodiments, two Drug units are attached to each Ligand unit.

[0220] In some embodiments, the Drug unit is linked to the Ligand unit (directly or indirectly) via the Polypeptide unit (-Z). For example, the Polypeptide unit can include an immunoglobulin hinge region (e.g., an IgG1 hinge region). A Linker unit can be linked to a cysteine residue in a hinge region.

5 **[0221]** To control the number of Drug units or Linker unit-Drug units attached to a Ligand unit, one or more cysteine residues can be eliminated by amino acid substitution. For example, the number of solvent accessible cysteine residues in an immunoglobulin hinge region can be reduced by amino acid substitution of cysteine to serine residues.

10 **[0222]** In some embodiments, a Ligand unit contains 1, 2, 3, 4, 5 or 6 solvent-accessible cysteine residues. In some embodiments, a Ligand unit preferably contains 2 or 4 solvent-accessible cysteine residues.

[0223] In some embodiments, the ligand drug conjugate compounds exhibit reduced toxicity, as compared to an intact antibody drug conjugate compound that
15 binds to the same target protein. As used herein, toxicity refers to toxicity at a site, cell or tissue other than the target tissue. The toxicity is typically antigen-independent. For example, antigen-independent toxicity can occur in the liver, kidneys, vascular, or other organs or tissues, although such organ or tissue does not express the target antigen or target protein.

20 **[0224]** In some embodiments, the ligand drug conjugate compound exhibits reduced liver toxicity, as compared with an intact antibody drug conjugate. In some embodiments, a ligand drug conjugate compound exhibits reduced toxicity and increased renal clearance, as compared with an intact antibody drug conjugate. For example, a ligand drug conjugate can have a blood serum half-life
25 of less than about 50%, less than about 30%, more preferably less than about 25%, less than about 15, less than about 10%, less than about 5% or less than about 1% of an intact antibody drug conjugate.

[0225] In related embodiments, a ligand drug conjugate can exhibit an increased therapeutic window, as compared with an intact antibody drug conjugate. For
30 example, the therapeutic window can be increased by at least about 50%, at least

about 75%, more preferably at least about 100%, at least about 150% or at least about 200%, as compared with the therapeutic window of an intact antibody drug conjugate. "Therapeutic window" describes the ratio of desired effect to toxic effect. A compound with a narrow therapeutic index (close to 1) exerts its desired effect at a dose close to its toxic dose. A compound with a wide therapeutic index (e.g., greater than 5) exerts its desired effect at a dose substantially below its toxic dose.

[0226] In some embodiments, the ligand drug conjugate exhibits increased accessibility to the target site, as compared with an intact antibody drug conjugate.

For example, the ligand drug conjugate can exhibit increased tumor localization, as compared with an intact antibody drug conjugate. For example, a ligand drug conjugate can exhibit increased tumor localization of at least about 50%, at least about 75%, at least about 100%, at least about 150% or at least about 200%, as compared with an intact antibody drug conjugate. Similarly, a ligand drug conjugate can exhibit increased retention at the target site of at least about 50%, at least about 75%, at least about 100%, at least about 150% or at least about 200%, as compared with an intact antibody drug conjugate, as measured over the same time period. Accessibility and/or retention can be measured, for example, using a labeled ligand drug conjugate and an intact antibody drug conjugate.

COMPOSITIONS AND METHODS OF ADMINISTRATION

[0227] The ligand drug conjugate compounds can be in any form that allows for the compound to be administered to a patient. For example, the compound can be in the form of a liquid or solid. Typical routes of administration include, without limitation, parenteral, topical, oral, 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 compounds are administered intravenously.

[0228] Pharmaceutical compositions can be formulated so as to allow a compound 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 a compound can hold a plurality of dosage units.

[0229] 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 compound, the manner of administration, and the composition employed.

[0230] 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.

[0231] 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.

[0232] As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring, and a coloring agent.

[0233] 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.

[0234] 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.

[0235] The liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, amino acids, 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.

[0236] The amount of the compound 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.

[0237] The compositions comprise an effective amount of a compound such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of a compound 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 compound by weight of the composition. In yet another aspect, parenteral compositions are prepared so that a parenteral dosage unit contains from about 0.01% to about 2% by weight of the compound.

[0238] For intravenous administration, the composition can comprise from about 0.01 to about 100 mg of a compound per kg of the animal's body weight. In one aspect, the composition can include from about 1 to about 100 mg of a compound 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 a compound.

[0239] Generally, the dosage of a compound administered to a patient is typically about 0.01 mg/kg to about 2000 mg/kg of the animal's body weight. In some embodiments, 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 some embodiments, 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 some embodiments, 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 some embodiments, the dosage administered is between about 0.1 mg/kg to about 5 mg/kg or about 0.1 mg/kg to about 10 mg/kg of the animal's body weight. In some embodiments, the dosage administered is between about 1 mg/kg to about 15 mg/kg of the animal's body weight. In some embodiments, the dosage administered is between about 1 mg/kg to about 10 mg/kg of the animal's body weight.

[0240] The compound 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 a compound. In certain embodiments, more than one compounds or composition is administered to a patient.

[0241] In specific embodiments, it can be desirable to administer one or more compounds or compositions locally to the area in need of treatment. This can be achieved, for example, 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.

[0242] In certain embodiments, it can be desirable to introduce one or more compounds 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.

[0243] In yet another embodiment, the compound 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 compound 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 (1990, *Science* 249:1527-1533) can be used.

[0244] The term "carrier" refers to a diluent, adjuvant or excipient, with which a compound 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 compound or compositions and pharmaceutically acceptable carriers are sterile.

Water is an exemplary carrier when the compounds 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.

[0245] 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.

[0246] In an embodiment, the compounds 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 compound 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 compound 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.

[0247] 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.

[0248] 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 a compound 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.

[0249] 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 compound.

[0250] 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.

[0251] Whether in solid or liquid form, the present compositions can include a pharmacological agent used in the treatment of cancer, an autoimmune disease or an infectious disease.

THERAPEUTIC USES OF THE CONJUGATES

[0252] The conjugates are useful for treating cancer, an autoimmune disease, an infectious disease or other disease in a patient. In some embodiments, the conjugates are administered alone. In other embodiments, the conjugates are co-administered with another therapeutic agent. In some embodiments, the conjugates coadministered with standard of care chemotherapeutics.

TREATMENT OF CANCER

[0253] The 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 compounds can be used accordingly in a variety of settings for the treatment of animal cancers. Some exemplary particular types of cancers that can be treated with compounds include, but are not limited to, those disclosed in Table 1

TABLE 1

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

rectal cancer
 colorectal cancer
 kidney cancer
 pancreatic cancer
 5 bone cancer
 breast cancer
 ovarian cancer
 prostate cancer
 penile carcinoma
 10 esophageal cancer
 gastric cancer
 gastrointestinal cancer
 stomach cancer
 peritoneal cancer
 15 hepatic carcinoma
 hepatocellular cancer
 liver cancer
 oral cancer
 nasal cancer
 20 throat cancer
 squamous cell carcinoma (e.g., epithelial)
 basal cell carcinoma
 adenocarcinoma
 sweat gland carcinoma
 25 sebaceous gland carcinoma
 papillary carcinoma
 papillary adenocarcinomas
 cystadenocarcinoma
 medullary carcinoma
 30 bronchogenic carcinoma
 renal cell carcinoma
 hepatoma
 bile duct carcinoma
 choriocarcinoma
 35 seminoma
 embryonal carcinoma
 Wilms' tumor
 cervical cancer
 uterine cancer
 40 endometrial or uterine carcinoma
 vulval cancer
 testicular cancer
 bladder carcinoma
 lung cancer, including small cell lung carcinoma, non-small cell lung
 45 cancer, adenocarcinoma of the lung and squamous
 carcinoma of the lung
 epithelial carcinoma
 glioma

- glioblastoma
glioblastoma multiforme
astrocytoma
medulloblastoma
5 craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
10 oligodendroglioma
meningioma
skin cancer
melanoma
neuroblastoma
15 retinoblastoma
salivary gland carcinoma
thyroid cancer
head cancer
neck cancer
20 anal cancer
- blood-borne cancers, including but not limited to:
- acute lymphoblastic leukemia "ALL"
acute lymphoblastic B-cell leukemia
acute lymphoblastic T-cell leukemia
25 acute myeloblastic leukemia "AML"
acute promyelocytic leukemia "APL"
acute monoblastic leukemia
acute erythroleukemic leukemia
acute megakaryoblastic leukemia
30 acute myelomonocytic leukemia
acute nonlymphocytic leukemia
acute undifferentiated leukemia
chronic myelocytic leukemia "CML"
chronic lymphocytic leukemia "CLL"
35 hairy cell leukemia
multiple myeloma
- acute and chronic leukemias:
- lymphoblastic
myelogenous
40 lymphocytic
myelocytic leukemias
- Lymphomas:

Hodgkin's disease
non-Hodgkin's Lymphoma
Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Polycythemia vera

5

The conjugates provide conjugation-specific tumor or cancer targeting, thus reducing general toxicity of these compounds. The linker stabilizes the conjugates in blood, yet is cleavable by proteases within the cell, liberating the Drug(s).

10 MULTI-MODALITY THERAPY FOR CANCER

[0254] 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 a conjugate according to the present invention.

15 **[0255]** In some embodiments, methods for treating or preventing cancer are provided, including administering to a patient in need thereof an effective amount of a 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
20 that with which the treatment of cancer has been found to be refractory. The conjugates can be administered to a patient that has also undergone surgery as treatment for the cancer.

[0256] In one embodiment, the additional method of treatment is radiation therapy.

25 **[0257]** In a specific embodiment, the 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 a conjugate. In some embodiments, the chemotherapeutic agent or radiation therapy is administered at least an hour, five
30 hours, 12 hours, a day, a week, a month, several months (e.g., up to three months), prior or subsequent to administration of a conjugate.

[0258] A chemotherapeutic agent can be administered over a series of sessions. Any one or a combination of the following chemotherapeutic agents can be administered (see *infra*). With respect to radiation, any radiation therapy protocol can be used depending upon the type of cancer to be treated. For example, but
5 not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

[0259] Additionally, methods of treatment of cancer with a 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 patient being treated can, optionally, be treated with another cancer treatment
15 such as surgery, radiation therapy or chemotherapy, depending on which treatment is found to be acceptable or bearable.

[0260] The 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
20 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 a 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
25 bone marrow function is restored and the animal recovers.

MULTI-DRUG THERAPY FOR CANCER

[0261] Methods for treating cancer including administering to a patient in need thereof an effective amount of a conjugate and another therapeutic agent that is an anti-cancer agent are disclosed.

[0262] Suitable anticancer agents include, but are not limited to, methotrexate, taxol, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, topotecan, nitrogen mustards, cytoxan, etoposide, 5-fluorouracil, BCNU, irinotecan, camptothecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel.

[0263] In some embodiments, the anti-cancer agent includes, but is not limited to, a drug listed in Table 2a.

10

TABLE 2a

	<u>Generic Name</u>	<u>Tradename</u>
	Aldesleukin	Proleukin
	Alemtuzumab	Campath
	alitretinoin	Panretin
15	allopurinol	Zyloprim
	altretamine	Hexalen
	amifostine	Ethyol
	anastrozole	Arimidex
	arsenic trioxide	Trisenox
20	Asparaginase	Elspar
	BCG Live	TICE BCG
	bexarotene capsules	Targretin
	bexarotene gel	Targretin
	bleomycin	Blenoxane
25	busulfan intravenous	Busulfex
	busulfan oral	Myleran
	calusterone	Methosarb
	capecitabine	Xeloda
	carboplatin	Paraplatin
30	carmustine	BCNU, BICNU
	carmustine with Polifeprosan 20 Implant	Gliadel Wafer
	Celecoxib	Celebrex
	chlorambucil	Leukeran
35	cisplatin	Platinol
	cladribine	Leustatin, 2-CdA
	cyclophosphamide	Cytosan
	Cytarabine	Cytosar-U
	cytarabine liposomal	DopoCyt
40	dacarbazine	DTIC-Dome
	dactinomycin, actinomycin D	Cosmegen
	Darbepoetin alfa	Aranesp
	daunorubicin liposomal	DanuoXome
	daunorubicin, daunomycin	Daunorubicin
45	daunorubicin, daunomycin	Cerubidine
	Denileukin diftitox	Ontak

	dexrazoxane	Zinecard
	docetaxel	Taxotere
	doxorubicin	Adriamycin, Rubex
5	doxorubicin	Adriamycin PFS Injection
		intravenous injection
	doxorubicin liposomal	Doxil
	Dromostanolone Propionate	Dromostanolone
	Dromostanolone Propionate	Masterone Injection
10	Elliott's B Solution	Elliott's B Solution
	epirubicin	Ellence
	Epoetin alfa	epogen
	estramustine	Emcyt
	etoposide phosphate	Etopophos
	etoposide, VP-16	Vepesid
15	exemestane	Aromasin
	Filgrastim	Neupogen
	floxuridine (intraarterial) FUDR	
	fludarabine	Fludara
20	fluorouracil, 5-FU	Adrucil
	fulvestrant	Faslodex
	gemcitabine	Gemzar
	gemtuzumab ozogamicin	Mylotarg
	goserelin acetate	Zoladex Implant
	goserelin acetate	Zoladex
25	hydroxyurea	Hydrea
	Ibritumomab Tiuxetan	Zevalin
	idarubicin	Idamycin
	ifosfamide	IFEX
	imatinib mesylate	Gleevec
30	Interferon alfa-2a	Roferon-A
	Interferon alfa-2b	Intron A
	irinotecan	Camptosar
	lenalidomide	Revlimid
	letrozole	Femara
35	leucovorin	Wellcovorin, Leucovorin
	levamisole	Ergamisol
	lomustine, CCNU	CeeBU
	mecllorethamine, nitrogen	Mustargen
	mustard	
40	megestrol acetate	Megace
	melphalan, L-PAM	Alkeran
	melphalan, L-PAM	Alkeran
	mercaptopurine, 6-MP	Purinethol
	mesna	Mesnex
45	methotrexate	Methotrexate
	methoxsalen	Uvadex
	mitomycin C	Mutamycin
	mitomycin C	Mitozytrex
	mitotane	Lysodren
50	mitoxantrone	Novantrone
	nandrolone phenpropionate	Durabolin-50
	Nofetumomab	Verluma
	Oprelvekin	Neumega
	oxaliplatin	Eloxatin
55	paclitaxel	Paxene
	paclitaxel	Taxol
	pamidronate	Aredia

	pegademase	Adagen (Pegademase Bovine)
	Pegaspargase	Oncaspar
	Pegfilgrastim	Neulasta
5	pentostatin	Nipent
	pipobroman	Vercyte
	plicamycin, mithramycin	Mithracin
	porfimer sodium	Photofrin
	procarbazine	Matulane
10	quinacrine	Atabrine
	Rasburicase	Elitek
	Rituximab	Rituxan
	Sargramostim	Prokine
	streptozocin	Zanosar
15	talc	Sclerosol
	tamoxifen	Nolvadex
	tamoxifen	Nolvadex
	temozolomide	Temodar
	teniposide, VM-26	Vumon
20	testolactone	Teslac
	testolactone	Teslac
	thalidomide	Thalidomid
	thioguanine, 6-TG	Thioguanine
	thiotepa	Thioplex
25	topotecan	Hycamtin
	toremifene	Fareston
	Tositumomab	Bexxar
	Trastuzumab	Herceptin
	tretinoin, ATRA	Vesanoid
30	Uracil Mustard	Uracil Mustard Capsules
	valrubicin	Valstar
	vinblastine	Velban
	vincristine	Oncovin
	vinorelbine	Navelbine
35	vinorelbine	Navelbine
	zoledronate	Zometa

[0264] In some embodiments, the anti-cancer agent includes, but is not limited to, a drug listed in Table 2b.

TABLE 2b

Alkylating agents	
Nitrogen mustards:	Cyclophosphamide
	ifosfamide
	trofosfamide
	chlorambucil
	melphalan
Nitrosoureas:	carmustine (BCNU)
	lomustine (CCNU)
Alkylsulphonates	busulfan
	treosulfan
Triazenes:	decarbazine

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

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

TREATMENT OF AUTOIMMUNE DISEASES

[0265] The conjugates are useful for killing or inhibiting the replication of a cell that produces an autoimmune disease or for treating an autoimmune disease.

- The conjugates can be used accordingly in a variety of settings for the treatment of an autoimmune disease in a patient.

[0266] Particular types of autoimmune diseases that can be treated with the 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

5 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
10 3.

TABLE 3

	Active Chronic Hepatitis
	Addison's Disease
15	Allergic Alveolitis
	Allergic Reaction
	Allergic Rhinitis
	Alport's Syndrome
	Anaphlaxis
20	Ankylosing Spondylitis
	Anti-phospholipid Syndrome
	Arthritis
	Ascariasis
	Aspergillosis
25	Atopic Allergy
	Atopic Dermatitis
	Atopic Rhinitis
	Behcet's Disease
	Bird-Fancier's Lung
30	Bronchial Asthma
	Caplan's Syndrome
	Cardiomyopathy
	Celiac Disease
	Chagas' Disease
35	Chronic Glomerulonephritis
	Cogan's Syndrome
	Cold Agglutinin Disease
	Congenital Rubella Infection
	CREST Syndrome
	Crohn's Disease
40	Cryoglobulinemia

	Cushing's Syndrome
	Dermatomyositis
	Discoid Lupus
	Dressler's Syndrome
5	Eaton-Lambert Syndrome
	Echovirus Infection
	Encephalomyelitis
	Endocrine ophthalmopathy
	Epstein-Barr Virus Infection
10	Equine Heaves
	Erythematosis
	Evan's Syndrome
	Felty's Syndrome
	Fibromyalgia
15	Fuch's Cyclitis
	Gastric Atrophy
	Gastrointestinal Allergy
	Giant Cell Arteritis
	Glomerulonephritis
20	Goodpasture's Syndrome
	Graft v. Host Disease
	Graves' Disease
	Guillain-Barre Disease
	Hashimoto's Thyroiditis
25	Hemolytic Anemia
	Henoch-Schonlein Purpura
	Idiopathic Adrenal Atrophy
	Idiopathic Pulmonary Fibrilis
	IgA Nephropathy
30	Inflammatory Bowel Diseases
	Insulin-dependent Diabetes Mellitus
	Juvenile Arthritis
	Juvenile Diabetes Mellitus (Type I)
	Lambert-Eaton Syndrome
35	Laminitis
	Lichen Planus
	Lupoid Hepatitis
	Lupus
	Lymphopenia
40	Meniere's Disease
	Mixed Connective Tissue Disease
	Multiple Sclerosis
	Myasthenia Gravis
	Pernicious Anemia
45	Polyglandular Syndromes
	Presenile Dementia
	Primary Agammaglobulinemia
	Primary Biliary Cirrhosis

	Psoriasis
	Psoriatic Arthritis
	Raynauds Phenomenon
	Recurrent Abortion
5	Reiter's Syndrome
	Rheumatic Fever
	Rheumatoid Arthritis
	Sampter's Syndrome
	Schistosomiasis
10	Schmidt's Syndrome
	Scleroderma
	Shulman's Syndrome
	Sjorgen's Syndrome
	Stiff-Man Syndrome
15	Sympathetic Ophthalmia
	Systemic Lupus Erythematosus
	Takayasu's Arteritis
	Temporal Arteritis
	Thyroiditis
20	Thrombocytopenia
	Thyrotoxicosis
	Toxic Epidermal Necrolysis
	Type B Insulin Resistance
	Type I Diabetes Mellitus
25	Ulcerative Colitis
	Uveitis
	Vitiligo
	Waldenstrom's Macroglobulemia
	Wegener's Granulomatosis
30	

MULTI-DRUG THERAPY OF AUTOIMMUNE DISEASES

[0267] Methods for treating an autoimmune disease are also disclosed including administering to a patient in need thereof an effective amount of a 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 4.

Table 4

	cyclosporine
	cyclosporine A
40	mycophenylate mofetil
	sirolimus

	tacrolimus
	enanercept
	prednisone
	azathioprine
5	methotrexate cyclophosphamide
	prednisone
	aminocaproic acid
	chloroquine
	hydroxychloroquine
10	hydrocortisone
	dexamethasone
	chlorambucil
	DHEA
	danazol
15	bromocriptine
	meloxicam
	infliximab

[0268] The conjugates are useful for killing or inhibiting the multiplication of a cell
20 that produces an infectious disease or for treating an infectious disease.

[0269] In one embodiment, the conjugates kill or inhibit the multiplication of cells that produce a particular infectious disease.

[0270] Particular types of infectious diseases that can be treated with the conjugates include, but are not limited to, those disclosed in Table 5.

25 TABLE 5

Bacterial Diseases:

	Diphtheria
	Pertussis
	Occult Bacteremia
30	Urinary Tract Infection
	Gastroenteritis
	Cellulitis
	Epiglottitis
	Tracheitis
35	Adenoid Hypertrophy
	Retropharyngeal Abscess
	Impetigo
	Ecthyma
	Pneumonia

	Endocarditis
	Septic Arthritis
	Pneumococcal
	Peritonitis
5	Bacteremia
	Meningitis
	Acute Purulent Meningitis
	Urethritis
	Cervicitis
10	Proctitis
	Pharyngitis
	Salpingitis
	Epididymitis
	Gonorrhea
15	Syphilis
	Listeriosis
	Anthrax
	Nocardiosis
	Salmonella
20	Typhoid Fever
	Dysentery
	Conjunctivitis
	Sinusitis
	Brucellosis
25	Tularemia
	Cholera
	Bubonic Plague
	Tetanus
	Necrotizing Enteritis
30	Actinomycosis
	Mixed Anaerobic Infections
	Syphilis
	Relapsing Fever
	Leptospirosis
35	Lyme Disease
	Rat Bite Fever
	Tuberculosis
	Lymphadenitis
	Leprosy
40	Chlamydia
	Chlamydial Pneumonia
	Trachoma
	Inclusion Conjunctivitis
	Systemic Fungal Diseases:
45	Histoplasmosis
	Coccidioidomycosis

5	Blastomycosis Sporotrichosis Cryptococcosis Systemic Candidiasis Aspergillosis Mucormycosis Mycetoma Chromomycosis
	Rickettsial Diseases:
10	Typhus Rocky Mountain Spotted Fever Ehrlichiosis Eastern Tick-Borne Rickettsioses Rickettsialpox
15	Q Fever Bartonellosis
	Parasitic Diseases:
20	Malaria Babesiosis African Sleeping Sickness Chagas' Disease Leishmaniasis Dum-Dum Fever Toxoplasmosis
25	Meningoencephalitis Keratitis Entamebiasis Giardiasis Cryptosporidiasis
30	Isosporiasis Cyclosporiasis Microsporidiosis Ascariasis Whipworm Infection
35	Hookworm Infection Threadworm Infection Ocular Larva Migrans Trichinosis Guinea Worm Disease
40	Lymphatic Filariasis Loiasis River Blindness Canine Heartworm Infection Schistosomiasis

	Swimmer's Itch
	Oriental Lung Fluke
	Oriental Liver Fluke
	Fascioliasis
5	Fasciolopsiasis
	Opisthorchiasis
	Tapeworm Infections
	Hydatid Disease
	Alveolar Hydatid Disease
10	Viral Diseases:
	Measles
	Subacute sclerosing panencephalitis
	Common Cold
	Mumps
15	Rubella
	Roseola
	Fifth Disease
	Chickenpox
	Respiratory syncytial virus infection
20	Croup
	Bronchiolitis
	Infectious Mononucleosis
	Poliomyelitis
	Herpangina
25	Hand-Foot-and-Mouth Disease
	Bornholm Disease
	Genital Herpes
	Genital Warts
	Aseptic Meningitis
30	Myocarditis
	Pericarditis
	Gastroenteritis
	Acquired Immunodeficiency Syndrome (AIDS)
	Human Immunodeficiency Virus (HIV)
35	Reye's Syndrome
	Kawasaki Syndrome
	Influenza
	Bronchitis
	Viral "Walking" Pneumonia
40	Acute Febrile Respiratory Disease
	Acute pharyngoconjunctival fever
	Epidemic keratoconjunctivitis
	Herpes Simplex Virus 1 (HSV-1)
	Herpes Simplex Virus 2 (HSV-2)
45	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

20 MULTI-DRUG THERAPY OF INFECTIOUS DISEASES

[0271] Methods for treating an infectious disease are disclosed including administering to a patient in need thereof a 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 6.

25 TABLE 6

β -Lactam Antibiotics:

Penicillin G
 Penicillin V
 Cloxacillin
 Dicloxacillin
 Methicillin
 Nafcillin
 Oxacillin
 Ampicillin
 Amoxicillin
 Bacampicillin
 Azlocillin
 Carbenicillin
 Mezlocillin
 Piperacillin

Ticarcillin

Aminoglycosides:

5 Amikacin
 Gentamicin
 Kanamycin
 Neomycin
 Netilmicin
 Streptomycin
 Tobramycin

10 **Macrolides:**

 Azithromycin
 Clarithromycin
 Erythromycin
 Lincomycin
15 Clindamycin

Tetracyclines:

 Demeclocycline
 Doxycycline
 Minocycline
20 Oxytetracycline
 Tetracycline

Quinolones:

 Cinoxacin
 Nalidixic Acid

25 **Fluoroquinolones:**

 Ciprofloxacin
 Enoxacin
 Grepafloxacin
 Levofloxacin
30 Lomefloxacin
 Norfloxacin
 Ofloxacin
 Sparfloxacin
 Trovafoxacin

35 **Polypeptides:**

 Bacitracin

Colistin
Polymyxin B

Sulfonamides:

5 Sulfisoxazole
 Sulfamethoxazole
 Sulfadiazine
 Sulfamethizole
 Sulfacetamide

Miscellaneous Antibacterial Agents:

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

General Antiviral Agents:

 Idoxuradine
 Vidarabine
 Trifluridine
25 Acyclovir
 Famciclovir
 Penciclovir
 Valacyclovir
 Ganciclovir
30 Foscarnet
 Ribavirin
 Amantadine
 Rimantadine
 Cidofovir
35 Antisense Oligonucleotides
 Immunoglobulins
 Interferons

Drugs for HIV infection:

40 Tenofovir
 Emtricitabine

5 Zidovudine
 Didanosine
 Zalcitabine
 Stavudine
 Lamivudine
 Nevirapine
 Delavirdine
 Saquinavir
 10 Ritonavir
 Indinavir
 Nelfinavir

Examples

15 **[0272]** The invention is further described in the following examples, which are not
 intended to limit the scope of the invention. Cell lines described in the following
 examples were maintained in culture according to the conditions specified by the
 American Type Culture Collection (ATCC) or Deutsche Sammlung von
 Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ),
 20 unless otherwise specified. Cell culture reagents were obtained from Invitrogen
 Corp., Carlsbad, CA unless otherwise specified.

Example 1

Transient Mammalian Expression and Purification of AC10 Antibody Fragments

25 **[0273]** Cloning of the genes encoding the variable domains of the monoclonal
 antibody AC10 and the construction of the chimeric AC10 (cAC10) has been
 described previously (Wahl et al., 2002, *Cancer Res.* 62:3736-3742). The
 sequence of the AC10 heavy chain variable region (SEQ ID NO:3) is as follows:

Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Val Val Lys Pro Gly Ala
 1 5 10 15
 30 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 Tyr Ile Thr Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Glu Lys Phe

50 55 60
 Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Phe
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
 5 85 90 95
 Ala Asn Tyr Gly Asn Tyr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Gln
 100 105 110
 Val Thr Val Ser Ala
 115.

10 The sequence of the AC10 light chain variable region (SEQ ID NO:4) is as follows:

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Phe Asp
 20 25 30
 15 Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Lys Val Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 20 65 70 75 80
 Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
 85 90 95
 Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110.

25 **[0274]** These genes were assembled into constructs encoding cAC10 scFv, diabody, minibody and scFv-Fc fragments (Table 8) in the mammalian expression vector pcDNA4mychis (Invitrogen, Carlsbad, CA). The scFv and diabody fragments were created in both possible topologies, namely, V_L-linker-V_H (LH) and V_H-linker-V_L (HL).

30 **[0275]** The diabody and scFv with HL topology had more favorable multimerization behavior than the LH topology (Figure 3). These observations led to the selection of the HL topology (V_H-linker-V_L) for construction of minibody and scFv-Fc fragments (Table 7).

Table 7. Transient Expression of AC10 Antibody Fragments

Construct name	Construct details ^b	Purified yield ^a (μg/mL culture)
scFv-HL	V _H -(G ₄ S) ₃ linker-V _L -H ₆	9.2
scFv-LH	V _L -(G ₄ S) ₃ linker-V _H -H ₆	7.6
Diabody-HL	V _H -G ₃ SG ₄ linker-V _L -H ₆	2.0
Diabody-LH	V _L -G ₃ SG ₄ linker-V _H -H ₆	1.0
Minibody-HL	V _H -(G ₄ S) ₃ linker-V _L -IgG ₁ hinge -G ₄ S linker -C _H 3-H ₆	2.2
scFv-Fc-HL	V _H -(G ₄ S) ₃ linker-V _L -IgG ₁ hinge-C _H 2-C _H 3-H ₆	2.4

^a Constructs were transiently expressed in 293F cells on a 30 mL scale. Proteins were purified from conditioned media by immobilized metal affinity chromatography (IMAC) (for the scFv, diabody, and minibody) or protein A chromatography (for the scFv-Fc). The yield of purified proteins was estimated from their absorbance at 280 nm and their molar extinction coefficients estimated from their sequence.

^b G₄S refers to pentapeptide sequence Gly-Gly-Gly-Gly-Ser (SEQ ID NO:5). (G₄S)₃ refers to a peptide having the sequence Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO:6). G₃SG₄ refers to an octapeptide having the sequence Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly (SEQ ID NO:2). IgG₁ hinge refers to the human IgG₁ hinge sequence. C_H2 and C_H3 refer to the human IgG₁ C_H2 and C_H3 sequences. H₆ refers to a hexahistide peptide.

[0276] For the minibody and scFv-Fc fragments, additional amino acid substitutions were created in the human IgG₁ hinge region. Referring to Figure 1B, the first valine was changed to a leucine residue. In addition, the first (amino terminal) cysteine residue was changed to serine, so the IgG₁ hinge region contained only two cysteine residues.

[0277] A preferred method for coupling cytotoxic drugs to antibodies is via solvent accessible cysteine residues (see, e.g., Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784) as this presents a low risk that conjugation will impair antigen binding. Minibody and scFv-Fc fragments each contain two pairs of such cysteine suitable residues (Figures 1A and 1B). In contrast, diabodies, including those initially constructed for AC10 (Table 7), do not contain suitable cysteine residues. Therefore, molecular modeling was used to design three different variants of AC10 diabody-HL each containing two such cysteine residues per component chain of the diabody thus giving rise to four cysteines per homodimer.

All three diabody cysteine variants were transiently expressed and purified (Table 8).

Table 8. Transient Expression of AC10 Diabody Variants

Construct name	Construct details ^a	Purified yield ^b ($\mu\text{g/mL}$ culture)
Diabody-HL	V_L -G ₃ SG ₄ linker- V_H -H ₆	4.2
Diabody-HL-Cys1	V_H -G ₃ SCG ₃ linker- V_L -H ₆ S84 ^H C	1.3
Diabody-HL-Cys2	V_H -G ₃ SCG ₃ linker- V_L -H ₆ S14 ^L C	2.1
Diabody-HL-Cys3	V_H -G ₃ SG ₄ linker- V_L -H ₆ S84 ^H C:S14 ^L C	0.15

^a Mutations are identified using single letter amino code and residue numbering scheme of Kabat et al. (Sequences of Proteins of Immunological Interest, 5th edition (Bethesda, MD, NIH, 1991). e.g., S84^HC:S14^LC contains Ser to Cys mutations at positions 84 and 14 in V_H and V_L , respectively. G4S refers to pentapeptide sequence Gly-Gly-Gly-Gly-Ser (SEQ ID NO:5). G₃SG₄ refers to an octapeptide having the sequence Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly (SEQ ID NO:2). G₃SCG₃ refers to an octapeptide having the sequence Gly-Gly-Gly-Ser-Cys-Gly-Gly-Gly (SEQ ID NO:1).

^b Diabody constructs were transiently expressed in 293F cells on a 250-300 mL scale and purified from conditioned media by IMAC. The yield of purified diabodies was estimated from their absorbance at 280 nm and their molar extinction coefficients estimated from their sequence.

[0278] Two of the three diabody variants, Cys1 and Cys2 gave workable recovered yields (Table 8). Diabody variant Cys1 was found to have the most favorable biophysical characteristics and was chosen as the lead diabody for these studies.

Example 2

Biochemical Analysis of AC10 Antibody Fragments

[0279] Purified antibody fragments were analyzed by SDS-PAGE (Figure 2). In all cases the electrophoretic mobility of the antibody fragments is slightly less than predicted from the migration of the molecular weight standards. This anomalous mobility has previously been observed for antibody fragments and appears to be an artifact of electrophoresis (see, e.g., Rodrigues et al., 1993, *J. Immunol.* 151:6954-6961). Under non-reducing conditions a single major band was observed for scFv-HL (27 kDa), scFv-LH (27 kDa), diabody-HL (26.5 kDa), diabody-LH (26.5 kDa), and scFv-Fc fragments (110 kDa) (Figure 2A). In contrast, non-reduced minibody gave rise to 2 major bands consistent with the

presence of covalent dimer (86 kDa) and non-covalent dimer or monomer (43 kDa). Under reducing conditions minibody and scFv-Fc gave rise to single bands. The diabody cysteine variants show similar electrophoretic behavior to the parent diabody except that diabody Cys3 apparently forms a disulfide-linked dimer (Figure 2B).

[0280] Referring to Figure 3, size exclusion chromatographic analysis of purified AC10 scFv and diabody fragments was performed. Chromatographs were acquired with 20 μ L 1 mg/mL solution antibody proteins separated on a TSK-GEL G3000SWXL column (5 μ , 7.8 x 300 mm) from Tosoh Biosciences (Montgomeryville, PA) run at 1 mL/min under isocratic conditions with PBS as the mobile phase. Also shown are the peak location for 96 kDa and 48 kDa size standards.

[0281] Size exclusion chromatography (SEC) suggests that scFv-HL and diabody-HL are predominantly the anticipated monomer and dimer, respectively (Figure 3). In contrast, SEC profiles for fragments with the opposite topology, namely, scFv-LH and diabody-LH, suggest more complex behavior and may include oligomerization. Based on these observations, the HL topology (V_H -linker- V_L) was utilized for the construction of minibody and scFv-Fc fragments as well as the diabody Cys variants.

Example 3.

Binding Function of AC10 Antibody Fragments

[0282] The ability of the AC10 antibody fragments to bind the cognate antigen, CD30, was assessed by binding to CD30 positive cells, namely, Karpas 299. Briefly, binding cAC10 IgG (1 μ g/ml) conjugated to Alexa Fluor 488 (Molecular Probes, Eugene, OR) was competed with the recombinant AC10 antibody fragments or unlabeled cAC10 IgG (Figure 4). Diabody-HL, minibody-HL, scFv-Fc-HL (Figure 4A) and diabody Cys variants (Figure 4B) block fluorophore-labeled cAC10 IgG binding to Karpas 299 with efficiencies that approach that of AC10 IgG itself.

Example 4.

Stable CHO Expression and Purification of AC10 Antibody Fragments

[0283] Stable mammalian expression was explored as a possible route to the large quantities (> 100 mg) of antibody fragments required for the construction and characterization of antibody drug conjugates. Cell lines for the stable
 5 expression of AC10 diabody-HL-Cys1, minibody-HL and scFv-Fc-HL were generated by subcloning corresponding coding sequences into the expression vector pDEF38 (Running Deer and Allison, 2004, *Biotechnol. Prog.* 20:880-889) and stably transfecting DG44 CHO cells. These antibody fragments were then purified as described for transiently expressed antibody fragments (supra). The
 10 quality of AC10 diabody-HL-Cys1, minibody HL and scFv-Fc-HL from the CHO cell lines was comparable to those for transiently expressed fragments (supra), as judged by SDS-PAGE and analytical size exclusion chromatography (not shown).

Table 9. Stable Expression of AC10 Antibody Fragments

Construct name	Purification method	Cell line Titer (mg/L)	Purified yield (mg/L culture)
Diabody-HL-cys1	Ultrafiltration, diafiltration and IMAC capture	30	12
Minibody-HL	Ultrafiltration, diafiltration and IMAC capture	17	9
scFv-Fc-HL	Protein A capture	350	126

15 Clones expressing antibody fragments at high titer were cultured in a Wave bioreactor (Wave Biotech, Bridgewater, NJ).

Example 5.

Formulation of the aAC10 antibody fragments.

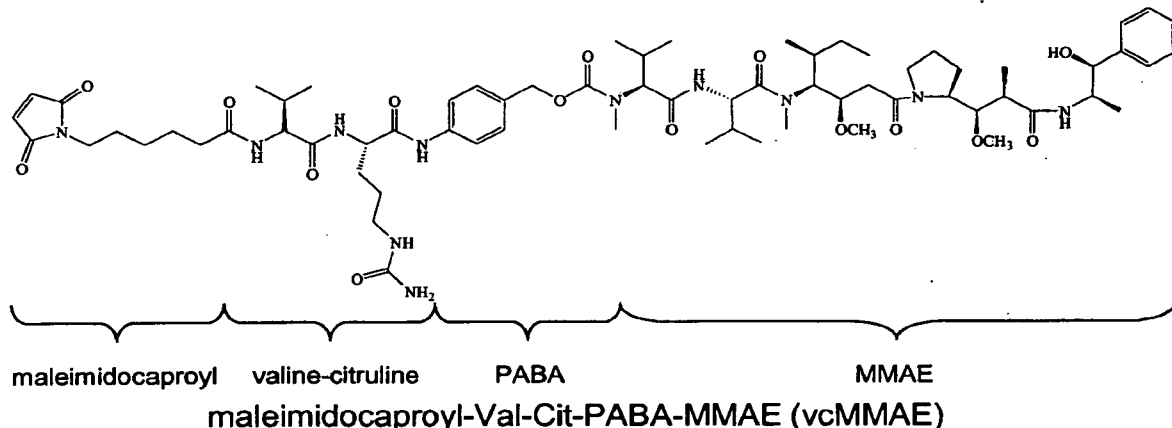
20 **[0284]** The cAC10 diabody, minibody and scFv-Fv were observed to be prone to aggregation and precipitation in phosphate-buffered saline (PBS). To reduce aggregation and precipitation, the diabody, minibody and maxibody were formulated in the following buffer: Buffer A: PBS, 50 mM imidazole, 5% glycerol, 1 mM EDTA, pH 8; and Buffer B: 10 mM Histidine, 150 mM NaCl, 8% trehalose,

0.02% Tween-20, 1mM EDTA, pH 8. The cAC10 fragments were found to be stable in Buffer A.

Example 6.

5 Construction & In Vitro Characterization of AC10 Diabody-HL-Cys1-MMAE-4, Minibody-HL-MMAE-4, scFv-Fc-MMAE-4 and cAC10 IgG-MMAE-4

[0285] Purified AC10 diabody-HL-Cys1, minibody-HL and scFv-Fc-HL were conjugated to MMAE according to standard methods. The conjugation of cAC10 IgG to MMAE by coupling maleimidocaproyl-Val-Cit-PABA-MMAE (vcMMAE) to
 10 free cysteines in cAC10 IgG generated by reduction of solvent accessible disulfide bonds has been described previously (Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784).



Briefly, IgG and antibody fragments were reduced with 10 mM dithiothreitol for 30
 15 min at 37 °C. Excess reducing agent was then removed by gel filtration using a G25 column with a mobile phase of 1 mM DPTA in PBS. The number of free thiols in the antibody protein was determined by titration with 5, 5'-dithiobis(2-nitrobenzoic acid). The reduced antibody proteins were coupled with vcMMAE (1.2 molar equivalents of vcMMAE per thiol group) in 20 % (v/v) acetonitrile for 1 h
 20 at 4 °C. The reactions were quenched with a 2-fold molar excess of free cysteine over vcMMAE. The conjugates were then gel-filtered (G25 column run in PBS) to

remove unincorporated vcMMAE, concentrated by ultrafiltration and then sterile filtered.

[0286] The mean stoichiometry of drug loading was estimated as 3.8 MMAE molecules per each AC10 fragment molecule, i.e., approaching fully loading of the four available cysteine residues. The ability of AC10 fragments and corresponding MMAE conjugates to bind the cognate antigen, CD30, was assessed by binding to CD30 positive cells, namely, Karpas 299. Briefly, binding of cAC10 IgG (1 µg/ml) conjugated to Europium (Perkin Elmer, Boston, MA) was competed separately with conjugated and unconjugated fragment and cAC10 IgG. The binding potencies of minibody-HL and scFv-Fc-HL were indistinguishable from cAC10 IgG (as observed previously) and were not impaired by conjugation with MMAE (Figure 5). Diabody-HL-cys 1 binding to CD30 positive cells was indistinguishable from cAC10 IgG but the binding of the diabody-HL-Cys1-MMAE-4 conjugate was decreased by 3-fold. These results show that conjugation of a drug linker to a diabody does not significantly impair ligand binding.

[0287] The cytotoxic effects of AC10 diabody-HL-Cys1-MMAE-4, minibody-HL-MMAE-4, AC10 scFv-Fc-HL-MMAE-4 and cAC10 IgG-MMAE-4 on the CD30 positive cell line Karpas 299 were determined following long term drug exposure. Detailed procedures for the cytotoxicity assays have been previously described (Doronina et al., 2003, *Nat. Biotechnol.* 21:778-784; Francisco et al., 2003, *Blood* 102:1458-1465). Briefly, Karpas 299 (CD30 positive), L540cy (CD30 positive) and MDA-MB-453 (CD30 negative) cells in RPMI-1640 medium containing 10 % (v/v) bovine fetal serum were plated at 5,000 to 10,000 cells per well. The cells were treated with antibody drug conjugates for 92 h at 37 °C. Resazurin (Sigma-Aldrich, Saint Louis, MO) was added to a final concentration of 50 µM. The cells were incubated for an additional 4 h, and dye reduction then measured using a fluorescent plate reader (excitation at 535 nm, emission at 590 nm).

[0288] The potency of AC10 scFv-Fc-MMAE-4 was equivalent to that of cAC10 IgG-MMAE-4 while the potencies of diabody-HL-cys1-MMAE-4 and minibody-HL-MMAE-4 were reduced by 10-fold and 3-fold, respectively (Figure 6).

Example 7***In Vivo Characterization of AC10 Diabody-HL-Cys1-MMAE-4, Minibody-HL-MMAE-4, scFv-Fc-MMAE-4 and cAC10 IgG-MMAE-4***

[0289] Balb-C mice were dosed with 10 mg/kg of AC10 Diabody-HL-Cys1-MMAE-4, Minibody-HL-MMAE-4, scFv-Fc-MMAE-4 or cAC10 IgG-MMAE-4. Blood samples were collected at 0.02, 0.08, 0.25, 0.92, 1.2, 2, 4 and 7 days post injection for AC10 Diabody-HL-Cys1-MMAE-4 and Minibody-HL-MMAE-4 treated animals, 0.04, 0.25, 1, 4, 7, 10, 14, 21 and 29 days post injection for scFv-Fc-MMAE-4 and 0.04, 0.25, 1, 4, 7, 14, 21 and 28 days post-injection for cAC10 IgG-MMAE-4 and serum was isolated. The concentrations of fragment drug conjugates present in the serum were measured by sandwich ELISA using an anti-idiotypic antibody to capture conjugates and an anti-his antibody reagent conjugated to HRP for detection. Rapid clearance was observed for AC10 Diabody-HL-Cys1-MMAE-4 and Minibody-HL-MMAE-4 (Figure 7). The scFv-Fc-MMAE-4 was cleared with a similar half life to cAC10 IgG-MMAE-4 (Figure 7) reflecting their similar sizes and Fc-mediated recycling.

[0290] The in vivo antitumor activity of AC10 diabody-HL-cys1-MMAE-4, minibody-HL-MMAE-4 and scFv-Fc-MMAE-4 compared to cAC10 IgG-MMAE-4 was assessed in SCID mice with human CD30 positive Karpas-299 subcutaneous xenografts. AC10 diabody-HL-cys1-MMAE-4 and minibody-HL-MMAE-4 were dosed every day at 4 mg/kg for 4 days. scFv-Fc-MMAE-4 and cAC10 IgG-MMAE-4 were administered as single doses at 2 mg/kg and 1 mg/kg, respectively. Complete regressions were observed for 5/5 animals for each of the AC10 diabody-HL-cys1-MMAE-4, minibody-HL-MMAE-4 and scFv-Fc-MMAE-4 treated groups and 4/5 animals treated with cAC10 IgG-MMAE-4. Tumor re-growth subsequently occurred for 2/5 animals in both the diabody-HL-cys1-MMAE-4 and minibody-HL-MMAE-4 treatment groups (Figure 8A).

[0291] In an additional study SCID mice with human CD30 positive Karpas-299 subcutaneous xenografts were treated with a single dose of AC10 diabody-HL-cys1-MMAE-4 at 1.8, 3.6 or 7.2 mg/kg and cAC10 IgG-MMAE-4 at 1 mg/kg.

Complete regressions were achieved for 5/5 mice treated with 3.6 and 7.2 mg/kg and 3/5 mice treated with 1.8 mg/kg (Figure 8B).

Example 8

In Vitro Characterization of Diabody-Auristatin Conjugates

5 [0292] The ability of AC10 diabodies conjugated to MMAE or MMAF were assessed in a competition binding assay using Karpas 299. Briefly, cAC10 IgG1 was labeled with Europium (3 µg/ml; Perkin Elmer, Boston, MA) or AF488 (2 µg/ml; Molecular Probes™; Invitrogen, Carlsbad, CA). The binding of the labeled cAC10 IgG1 to Karpas 299 was competed separately with AC10 diabody MMAE-4, AC10 diabody MMAF-4, cAC10 IgG1-MMAE-4, or IgG1-MMAF-4. The MMAF
10 conjugates (maleimidocaproyl-Val-Cit-PABA-MMAF (vcMMAF)) were prepared as described previously (Doronina et al., 2006, Bioconjugate Chem. 17:114-124). As shown below in Table 10, AC10 diabody-MMAE-4 has an approximate 2-fold decrease in binding when compared with cAC10 IgG1-MMAE-4. In contrast,
15 AC10 diabody-MMAF-4 has similar binding as cAC10 IgG1-MMAF-4 (Table 10).

[0293] The cytotoxic effects of AC10 diabody-MMAE-4 and AC10 diabody-MMAF-4, and cAC10 IgG1-MMAE-4 and cAC10 IgG1-MMAF-4 were determined following long term drug exposure as described above in Example 6. The results
20 are shown below in Table 10. The values represent the mean and stand deviation of at least three experiments ($n \geq 3$). When compared to MMAE conjugated to cAC10 IgG1, AC10 diabody-MMAE-4 showed an approximate 10-fold loss of cytotoxicity in both Karpas 299 and L540cy cell lines. In contrast, the loss of cytotoxic activity of MMAF conjugated to AC10 diabody compared to cAC10 IgG, is approximately 7-fold.

25

Table 10. In Vitro Characterization of Diabody-Auristatin Conjugates

AC10 conjugates	Drugs per conjugate (HPLC)	Competition binding	Cytotoxicity ($n \geq 3$)	
		Karpas 299	Karpas 299	L540cy
		(IC ₅₀ , nM)	(IC ₅₀ , nM)	(IC ₅₀ , nM)
Diabody-E4	3.8	6.6 ± 1.1	0.29 ± 0.03	0.69 ± 0.05
Diabody-F4	3.7	3.8 ± 0.4	0.02 ± 0.01	0.07 ± 0.03
IgG1-E4	4	2.4	0.03 ± 0.01	0.07 ± 0.01
IgG1-F4	4.5	6.4	0.006 ± 0.002	0.01 ± 0.03

Example 9**5 Antitumor Activity of Diabody-Drug Conjugates – Single Dose**

[0294] The dose efficacy of AC10 diabody-MMAF-4 was assessed in an in vivo tumor model of SCID mice with a human CD30+ Karpas 299 subcutaneous xenograft. Eleven days after tumor implant, the mice received a single administration of the AC10 diabody-MMAF-4 or AC10-IgG1-MMAF-4 at the dose indicated below in Table 11. Also shown in Table 11 is the amount of MMAF present at each dose, relative to AC10 diabody-MMAF-4 (0.72 mg/kg) or AC10 IgG1-MMAF-4 (2.0 mg/kg), and the number of mice showing complete tumor regression on day 91.

Table 11. Dosing of MMAF in Diabody-Auristatin Conjugates

	Diabody-F4			IgG1-F4	
Administered Dose (mg/kg)	0.72	2.16	7.2	0.66	2
Relative MMAF dose	1	3	10	0.33	1
Durable responses (day 91; 8 mice per dosage group)	0	7	8	0	1

[0295] A graph plotting mean tumor volume over days 1-60 post tumor implant is shown in Figure 9. Of the five treatment groups, the mice receiving AC10 diabody-MMAF-4 showed both the smallest mean tumor volume at each

measured time point (Figure 9) and the highest number of durable responses on day 91 (Table 11).

Example 10

5 *Antitumor Activity of Diabody-Drug Conjugates - Multidose*

[0296] The dose efficacy of AC10 diabody-MMAF-4 was assessed in an in vivo tumor model of SCID mice with a human CD30+ Karpas 299 subcutaneous xenograft. Starting eleven days after tumor implant, the mice received four administrations of the AC10 diabody-MMAF-4 or AC10-IgG1-MMAF-4, either
10 q1dx4 or q2dx4, by IV at the doses indicated in Figure 10. Also shown in Figure 10 is the amount of MMAF present at each dose (3x - 0.72 mg/kg; 6x - 1.44 mg/kg; or 9x - 2.16 mg/kg), relative to cAC10 IgG1-MMAF-4 (0.66 mg/kg). There is correlation between the tumor growth delay and the cAC10 diabody dose treatment. There did not appear to be an appreciable difference in efficacy for the
15 dose schedules for the diabody- or IgG-drug conjugated treated groups. Comparable efficacy to cAC10-vcMMAF4 (0.66 mg/kg) was achieved with 2.16 mg/kg of diabody-vcMMAF4.

[0297] Referring to Table 12, a comparable molar potency correlation between in vitro cytotoxicity & in vivo efficacy was observed for diabody-vcMMAE4 and
20 diabody-vcMMAF4. Both diabody-vcMMAE4 and -vcMMAF4 showed tumor growth delay with single dose at 3.6 mg/kg and 2.16 mg/kg, respectively, and very similar in vivo efficacy was observed when mice were treated at higher dose. The single dose of diabody-drug conjugate was observed to be as efficacious as the multiple dose of diabody-drug conjugate, and the diabody drug conjugates can be
25 as efficacious as IgG-drug conjugates.

Table 12 – Summary of in vivo anti-tumor activity for diabody drug conjugates

cAC10	Single Dose*	Multidose*
IgG1-vcE4M	1 mg/kg (1x)	n.d.
Diabody4-vcE4	3.6 mg/kg (10x) 7.2 mg/kg (20x)	4mg/kg @ q1dx4 (pilot)
IgG1-vcF4	2 mg/kg (1x)	0.66 mg/kg @ q1dx4 (1x)
Diabody4-vcF4	2.16 mg/kg (3x) 7.2 mg/kg (10x)	2.16 mg/kg @ q1dx4 ~ q4dx4 (9x)

[0298] Deposit of Hybridoma

- 5 **[0299]** The hybridoma secreting native monoclonal antibody AC10 was deposited on April 26, 2005, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassass, Va. 20110-2209, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedures, and assigned accession number PTA-6679.

[0300] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[0301] No license is expressly or implicitly granted to any patent or patent applications referred to or incorporated herein. The discussion above is

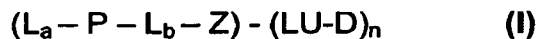
descriptive, illustrative and exemplary and is not to be taken as limiting the scope defined by any appended claims.

[0302] Various references, including patent applications, patents, and scientific publications, are cited herein, the disclosures of each of which is incorporated
5 herein by reference in its entirety.

Claims:

1. A method for treating a target cells comprising administering to a patient in need thereof an effective amount of a ligand-drug conjugate compound of the following formula:

5



or a pharmaceutically acceptable salt or solvate thereof;

wherein:

($L_a - P - L_b - Z$) is a Ligand unit, wherein:

10

L_a is a portion of a Ligand Binding unit,

L_b is a portion of a Ligand Binding unit,

-P- is a peptide, and

-Z is an optional polypeptide; and

(LU-D) is a Linker unit-Drug unit moiety, wherein:

15

LU- is a Linker unit, and

-D is a Drug unit having cytostatic or cytotoxic activity against the target cell; and

n is an integer from 1 to about 6; and

wherein L_a forms a first Ligand Binding unit with L_b , or L_a forms a first

20

Ligand Binding unit with L_a' and L_b forms a second Ligand Binding unit with L_b' , wherein L_a' and L_b' comprise a second ligand-drug conjugate compound; wherein at least one of the Ligand Binding units binds to a target molecule on the target cells; and

wherein at least one Linker unit-Drug unit moiety is conjugated to an

25

internal cysteine residue of L_a , L_b or P; wherein the internal cysteine residue in L_a or L_b is at least 10 angstroms distal from the antigen binding face of the first or second Ligand Binding unit; and

wherein Z does not comprise a hinge region or constant region of an antibody.

30

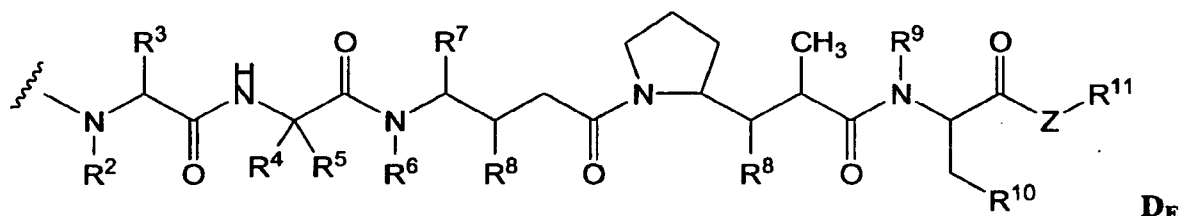
2. The method of claim 1, wherein the cysteine residue is an internal cysteine residue of L_a or L_b .

3. The method of claim 2, wherein the cysteine residue is an engineered cysteine residue of L_a or L_b .

4. The method of claim 1, wherein the Drug unit (-D) is a cytotoxic agent, a cytostatic agent or an immunomodulatory drug.

5. The method of claim 4, wherein the Drug unit (-D) is an auristatin.

6. The method of claim 5, wherein the Drug unit (-D) has the following Formula D_F :



10 wherein, independently at each location:

R^2 is selected from H and C_1 - C_{10} alkyl;

R^3 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl-(C_3 - C_8 heterocycle);

15 R^4 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl-(C_3 - C_8 heterocycle);

R^5 is selected from H and methyl;

20 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_{10} 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_{10} alkyl;

25 R^7 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl-(C_3 - C_8 heterocycle);

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

R^9 is selected from H and C_1 - C_{10} alkyl;

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

5 Z is selected from O, S, NH, and NR^{12} , wherein R^{12} is C_1 - C_{10} 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 1-1000;

R^{13} is C_2 - C_8 alkyl;

10 R^{14} is selected from H and C_1 - C_{10} alkyl;

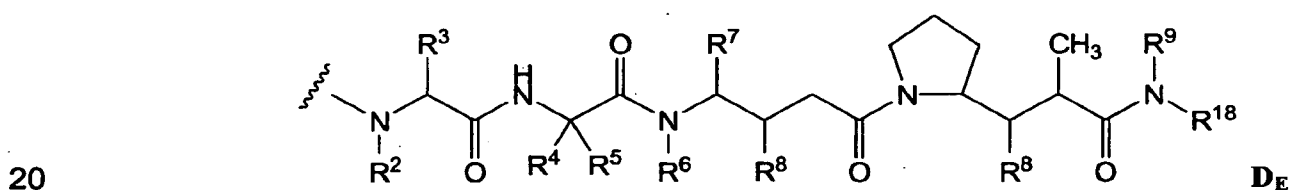
each occurrence of R^{15} is independently selected from H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, and $-(CH_2)_n-SO_3-C_1$ - C_{10} alkyl;

each occurrence of R^{16} is independently selected from H, C_1 - C_{10} alkyl, and $-(CH_2)_n-COOH$;

15 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.

7. The method of claim 5, wherein the Drug unit (-D) has the following Formula D_E :



wherein, independently at each location:

R^2 is selected from H and C_1 - C_{10} alkyl;

25 R^3 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl- $(C_3$ - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl- $(C_3$ - C_8 heterocycle);

R^4 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl- $(C_3$ - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} 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_{10} alkyl and C_3 - C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

5 R^6 is selected from H and C_1 - C_{10} alkyl;

R^7 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl-(C_3 - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl-(C_3 - C_8 heterocycle);

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

R^9 is selected from H and C_1 - C_{10} alkyl;

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

Z is selected from O, S, NH, and NR^{12} , wherein R^{12} is C_1 - C_{10} alkyl;

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

m is an integer ranging from 1-1000;

R^{13} is C_2 - C_{10} alkyl;

R^{14} is selected from H and C_1 - C_{10} alkyl;

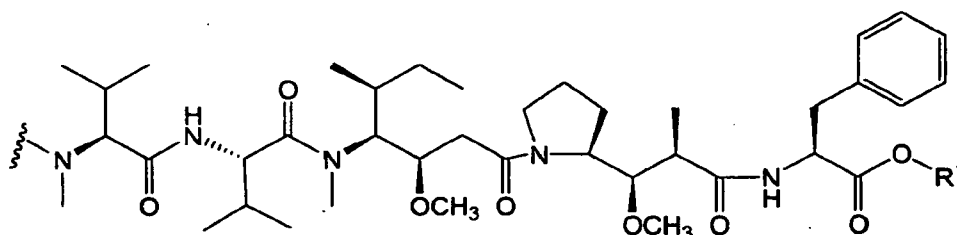
20 each occurrence of R^{15} is independently selected from H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, and $-(CH_2)_n-SO_3-C_1$ - C_{10} alkyl;

each occurrence of R^{16} is independently selected from H, C_1 - C_{10} alkyl, and $-(CH_2)_n-COOH$;

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

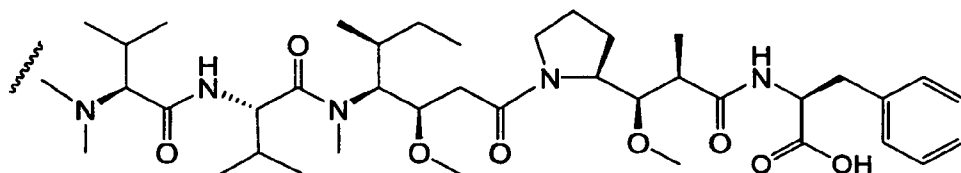
25 n is an integer ranging from 0 to 6.

8. The method of claim 6, wherein the Drug unit comprises the following formula:



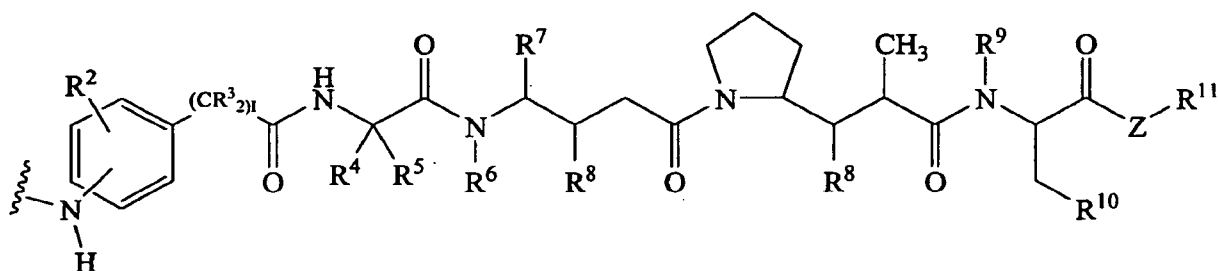
wherein $R^1 = H$ or Me.

9. The method of claim 8, wherein the Drug unit comprises the following formula:



5

10. The method of claim 5, wherein the Drug unit comprises the following formula:



wherein, independently at each location:

R^2 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ alkyl), $-halogen$, $-NO_2$, $-COOH$, and $-C(O)OR^{11}$;

each R^3 is selected independently from $-hydrogen$ and $-C_1-C_{10}$ alkyl;

l is an integer ranging from 0-10;

R^4 is selected from $-hydrogen$, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, $-aryl$, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- (C_3-C_8) carbocycle, $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- (C_3-C_8) heterocycle), and 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 $-H$, $-C_1-C_{10}$ 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 -C₁-C₁₀ alkyl;

R^7 is selected from -H, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₁₀ alkyl-aryl, -C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

5 each R^8 is independently selected from -H, -OH, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, -O-alkyl-(C₁-C₈ carbocycle) and -O-(C₁-C₁₀ alkyl);

R^9 is selected from -H and -C₁-C₁₀ alkyl;

R^{10} is selected from aryl and -C₃-C₈ heterocycle;

10 Z is selected from -O-, -S-, -NH-, and -NR¹²- where R^{12} is C₁-C₁₀ alkyl and aryl; and

R^{11} is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_r-H, -(CH₂CH₂O)_r-CH₃, and -(CH₂CH₂O)_r-CH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

15 11. The method of claim 1, further comprising administering a chemotherapeutic agent to the patient.

12. The method of claim 1, wherein P is 1 to 25 amino acids in length.

13. The method of claim 12, wherein P is 5 to 15 amino acids in length.

20 14. The method of claim 12, wherein P is a polyglycine-serine or a polyglycine peptide.

15. The method of claim 14, wherein P further comprises at least one cysteine residue.

25 16. The method of claim 1, wherein L_a comprises an antibody heavy chain variable region (V_H) and L_b comprises an antibody light chain variable region (V_L), wherein the V_H and V_L each form an antigen binding domain with V_L and V_H regions, respectively, of a second ligand-drug conjugate compound.

17. The method of claim 16, wherein at least one of the V_H or V_L regions comprises an engineered cysteine residue.

18. The method of claim 17, comprising a serine to cysteine amino acid substitution at amino acid position 84 of the V_H region or position 14 of the V_L region.

19. The method of claim 18, wherein the engineered cysteine residue is at least 20 angstroms distal from the antigen binding face of the antigen binding domain.

20. The method of claim 16, wherein the ligand drug conjugate compound exhibits reduced non-specific toxicity, as compared with an intact antibody drug conjugate compound that binds to the same target molecule.

21. The method of claim 1, wherein the first or second Ligand Binding unit binds to CD30, CA125, CA15-3, CA19-9, Lewis Y antigen, Lewis X antigen, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific antigen, prostate specific membrane antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE-4, anti transferrin receptor, p97, MUC1-KLH, CEA, gp100, MART1, IL-2 receptor, CD20, CD52, CD33, CD22, human chorionic gonadotropin, CD38, CD40, mucin, P21, MPG, Neu oncogene product, CD2, CD3, CD4, CD8, CD11, CD18, CD19, CD20, CD27, CD28, CD29, CD33, CD35, CD40, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD70, CD79, CD90, CD95/Fas, CD134/OX40, CD137/4 1BB, CD152/CTLA 4, PD 1, ICOS, TNF R1, TNFR 2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, or APO 3.

22. The method of claim 1, wherein the Linker unit has the formula:



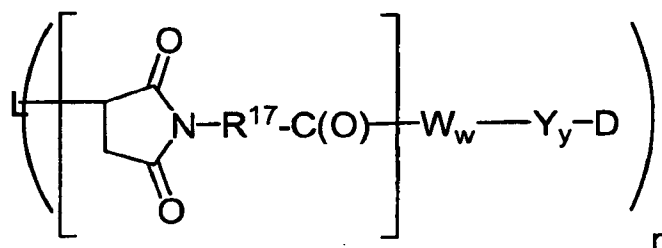
wherein:

-A- is a Stretcher unit;

a is 0 or 1;

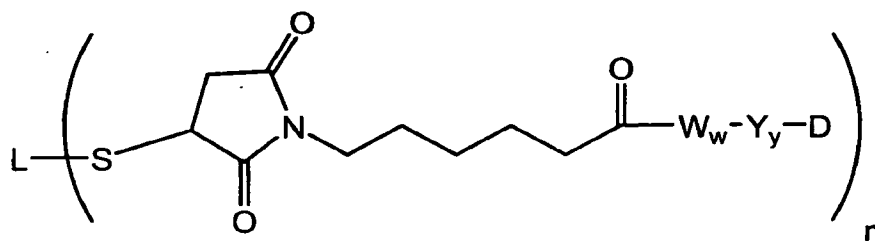
each -W- is independently an Amino Acid unit;
 w is independently an integer ranging from 0 to 12;
 -Y- is a Spacer unit; and
 y is 0, 1 or 2.

- 5 23. The method of claim 1, wherein the ligand-drug conjugate compound has the formula:



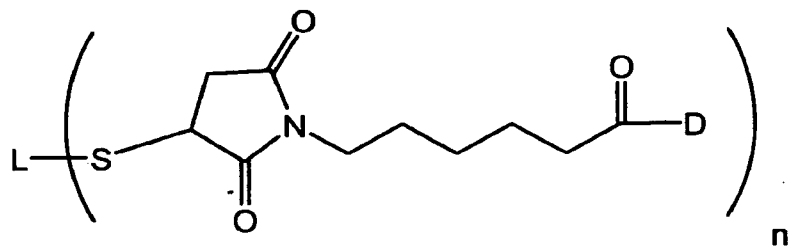
- wherein R¹⁷ is selected from C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-,
 -O-(C₁-C₈ alkyl)-, -arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-,
 10 -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-
 C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-
 C₁₀ alkylene-, -(CH₂CH₂O)_r-, and -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging
 from 1-10.

24. The method of claim 22, wherein the ligand-drug conjugate
 15 compound has the formula:



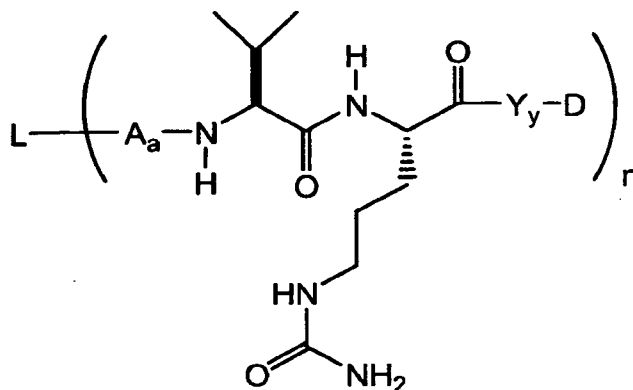
wherein S is a thiol group of L.

25. The method of claim 22, wherein the ligand-drug conjugate
 compound has the formula:



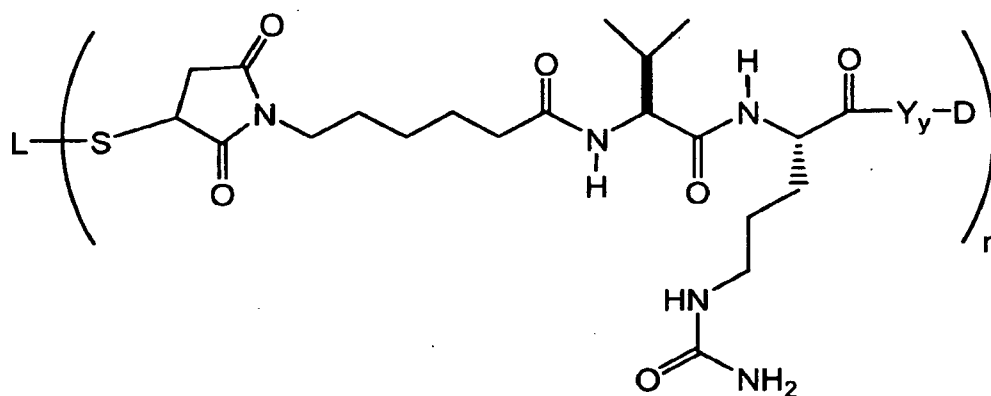
wherein w and y are each 0, and S is a thiol group of L.

26. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



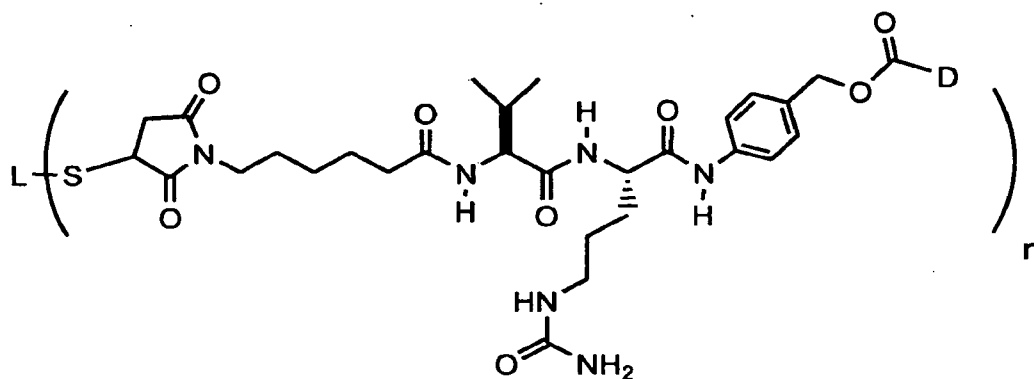
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27. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



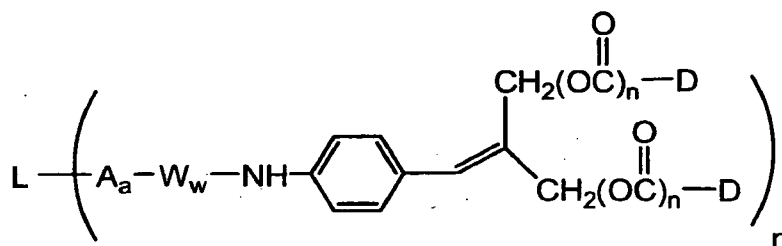
wherein S is a thiol group of L.

28. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

29. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



5

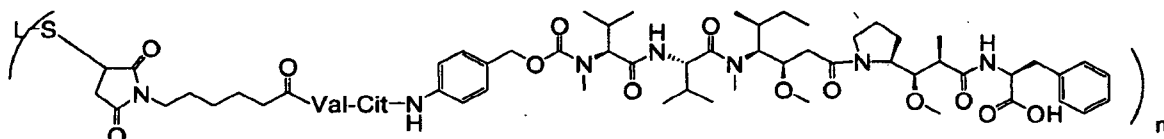
30. The method of claim 22, wherein w is an integer ranging from 2 to 12.

31. The method of claim 22, wherein w is 2.

32. The method of claim 31, wherein W_w is valine citrulline.

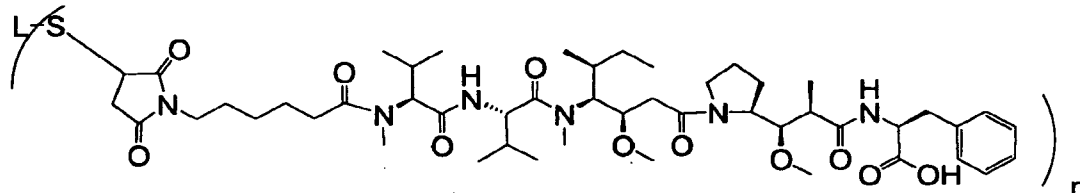
10 33. The method of claim 22, wherein W_w is selected from 5-aminovaleric acid, homo-phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepecotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepecotic acid.

15 34. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

35. The method of claim 22, wherein the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

36. The method of claim 16, wherein L_a comprises the V_H or V_L region of a humanized antibody AC10, BR96, 1F6 or 2F2.

37. The method of claim 16, wherein L_b comprises the V_H or V_L region of a humanized antibody AC10, BR96, 1F6 or 2F2.

38. The method of claim 1, comprising administering the ligand-drug conjugate compound as a pharmaceutical composition comprising an effective amount of the ligand-drug conjugate compound, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

39. The method of claim 1, wherein the target cells are cells of a cancer, a tumor or a cell proliferative disorder.

40. The method of claim 39, wherein the cancer is selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

41. The method of claim 1, wherein the target cells is of an autoimmune disease or an infectious disease.

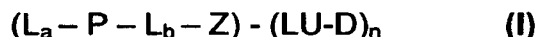
42. The method of claim 16, wherein the antigen binding region is an antigen binding region of a growth inhibitory antibody.

43. The method of claim 1, wherein the ligand-drug conjugate compound induces cell death.

5 44. The method of claim 1, wherein the ligand-drug conjugate compound induces apoptosis.

45. The method of claim 1, wherein the patient is a human.

46. A medicament for the treatment of a cancer, autoimmune disease or infectious disease in a patient in need of such treatment, comprising
10 a ligand-drug conjugate compound of the following formula:



or a pharmaceutically acceptable salt or solvate thereof;

wherein:

15 $(L_a - P - L_b - Z)$ is a Ligand unit, wherein:

L_a is a portion of a Ligand Binding unit,

L_b is a portion of a Ligand Binding unit,

-P- is a peptide, and

-Z is an optional polypeptide; and

20 $(LU-D)$ is a Linker unit-Drug unit moiety, wherein:

LU- is a Linker unit, and

-D is a Drug unit having cytostatic or cytotoxic activity against a target cell of the cancer, autoimmune disease or an infectious disease;

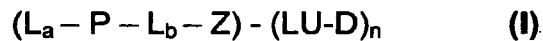
n is an integer from 1 to about 20; and

25 wherein L_a forms a first Ligand Binding unit with L_b , or L_a forms a first Ligand Binding unit with L_a' and L_b forms a second Ligand Binding unit with L_b' , wherein L_a' and L_b' comprise a second ligand-drug conjugate compound; wherein at least one of the Ligand Binding units binds to a target molecule on the target cell; and

wherein each Linker unit-Drug unit moiety is conjugated to an internal cysteine residue in L_a , L_b or P ; wherein the internal cysteine residue in L_a or L_b is at least 10 angstroms distal from the antigen binding face of the first or second Ligand Binding unit; and

5 wherein Z does not comprise a hinge region or constant region domain of an antibody.

47. A ligand-drug conjugate compound of the following formula:



10 or a pharmaceutically acceptable salt or solvate thereof;

wherein:

$(L_a - P - L_b - Z)$ is a Ligand unit, wherein:

L_a is a portion of a Ligand Binding unit,

L_b is a portion of a Ligand Binding unit,

15 $-P-$ is a peptide, and

$-Z$ is an optional polypeptide; and

$(LU-D)$ is a Linker unit-Drug unit moiety, wherein:

$LU-$ is a Linker unit, and

20 $-D$ is a Drug unit having cytostatic or cytotoxic activity against a target cell; and

n is an integer from 1 to about 20; and

wherein L_a forms a first Ligand Binding unit with L_b , or L_a forms a first Ligand Binding unit with L_a' and L_b forms a second Ligand Binding unit with L_b' , wherein L_a' and L_b' comprise a second ligand-drug conjugate compound; wherein
25 at least one of the Ligand Binding units binds to a target molecule on a target cell; and

wherein each Linker unit-Drug unit moiety is conjugated to an internal cysteine residue in L_a , L_b or P .

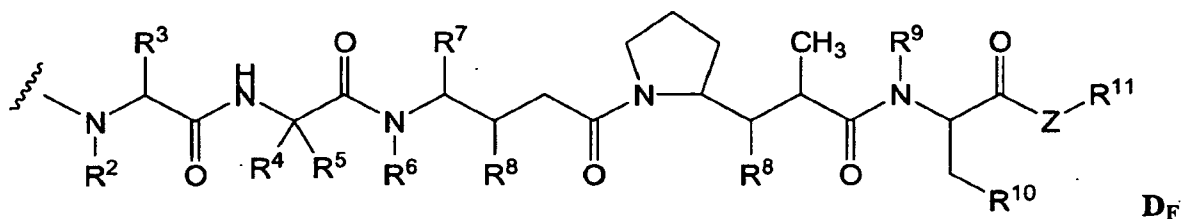
48. The ligand-drug conjugate compound of claim 47, wherein the
30 cysteine residue is an internal cysteine residue of L_a or L_b .

49. The ligand-drug conjugate compound of claim 48, wherein the cysteine residue is an engineered cysteine residue of L_a or L_b.

50. The ligand-drug conjugate compound of claim 47, wherein the Drug unit (-D) is a cytotoxic agent, a cytostatic agent or an immunomodulatory drug.

51. The ligand-drug conjugate compound of claim 50, wherein the Drug unit (-D) is an auristatin.

52. The ligand-drug conjugate compound of claim 51, wherein the Drug unit (-D) has the following Formula D_F:



wherein, independently at each location:

R² is selected from H and C₁-C₁₀ alkyl;

R³ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(C^aR^b)_n-, wherein R^a and R^b are independently selected from H, C₁-C₁₀ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₁₀ alkyl;

R⁷ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);

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

R^9 is selected from H and C_1 - C_{10} alkyl;

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

5 Z is selected from O, S, NH, and NR^{12} , wherein R^{12} is C_1 - C_{10} 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 1-1000;

R^{13} is C_2 - C_8 alkyl;

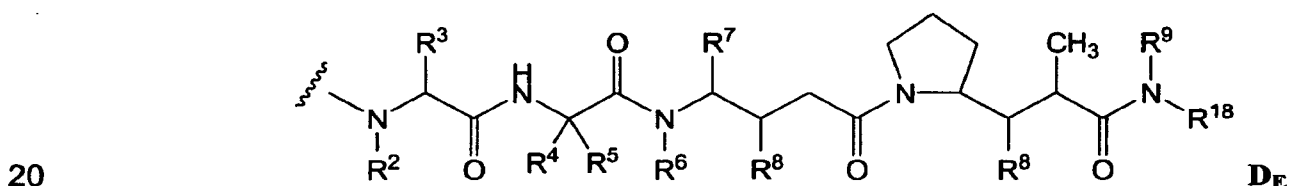
10 R^{14} is selected from H and C_1 - C_{10} alkyl;

each occurrence of R^{15} is independently selected from H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, and $-(CH_2)_n-SO_3-C_1$ - C_{10} alkyl;

each occurrence of R^{16} is independently selected from H, C_1 - C_{10} alkyl, and $-(CH_2)_n-COOH$;

15 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.

53. The ligand-drug conjugate compound of claim 51, wherein the Drug unit (-D) has the following Formula D_E :



wherein, independently at each location:

R^2 is selected from H and C_1 - C_{10} alkyl;

R^3 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl- $(C_3-C_8$ carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl- $(C_3-C_8$ heterocycle);

25 R^4 is selected from H, C_1 - C_{10} alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_{10} alkyl-aryl, C_1 - C_{10} alkyl- $(C_3-C_8$ carbocycle), C_3 - C_8 heterocycle and C_1 - C_{10} alkyl- $(C_3-C_8$ heterocycle);

R^5 is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n-, wherein R^a and R^b are independently selected from H, C₁-C₁₀ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₁₀ alkyl;

5 **R⁷ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₁₀ alkyl-aryl, C₁-C₁₀ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₁₀ alkyl-(C₃-C₈ heterocycle);**

each R⁸ is independently selected from H, OH, C₁-C₁₀ alkyl, C₃-C₈ carbocycle and O-(C₁-C₁₀ alkyl);

10 R^9 is selected from H and C_1 - C_{10} alkyl;

R¹⁰ is selected from aryl and C₃-C₈ heterocycle;

Z is selected from O, S, NH, and NR¹², wherein R¹² is C₁-C₁₀ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle,

$$-(R^{13}O)_m-R^{14}, \text{ and } -(R^{13}O)_m-CH(R^{15})_2;$$

15 m is an integer ranging from 1-1000;

R¹³ is C₂-C₈ alkyl;

R¹⁴ is selected from H and C₁-C₈ alkyl;

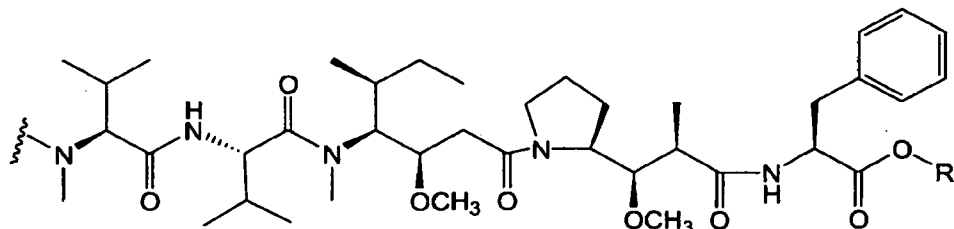
each occurrence of R¹⁵ is independently selected from H, COOH, -(CH₂)_n-N(R¹⁶)₂, -(CH₂)_n-SO₃H, and -(CH₂)_n-SO₃-C₁-C₁₀ alkyl;

20 each occurrence of R¹⁶ is independently selected from H, C₁-C₁₀ alkyl, and -(CH₂)_n-COOH;

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

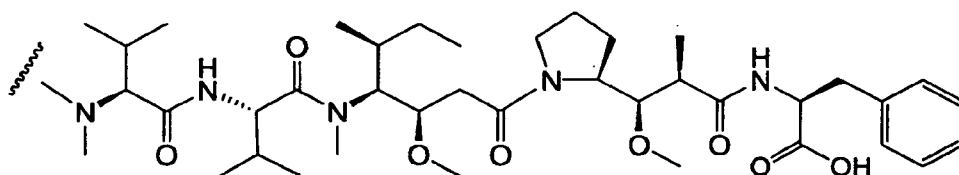
n is an integer ranging from 0 to 6.

25 54. The ligand-drug conjugate compound of claim 52, wherein the
Drug unit comprises the following formula:

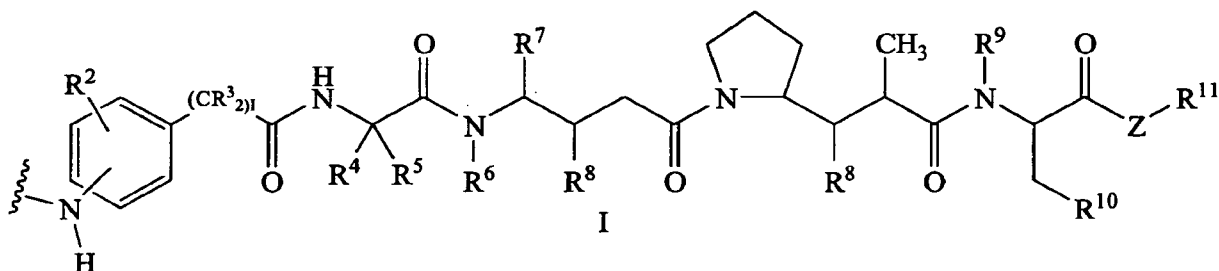


wherein $R^1 = H$ or Me.

55. The ligand-drug conjugate compound of claim 54, wherein the Drug unit comprises the following formula:



56. The ligand-drug conjugate compound of claim 51, wherein the Drug unit comprises the following formula:



wherein, independently at each location:

R^2 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-O-(C_1-C_{10}$ alkyl), $-halogen$, $-NO_2$, $-COOH$, and $-C(O)OR^{11}$;

each R^3 is selected independently from $-hydrogen$ and $-C_1-C_{10}$ alkyl;

I is an integer ranging from 0-10;

R^4 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, $-aryl$, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- (C_3-C_8) carbocycle, $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- (C_3-C_8) heterocycle), and 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 $-H$, $-C_1-C_{10}$ 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 $-C_1-C_{10}$ alkyl;

R^7 is selected from $-H$, $-C_1-C_{10}$ alkyl, $-C_3-C_8$ carbocycle, $aryl$, $-C_1-C_{10}$ alkyl-aryl, $-C_1-C_{10}$ alkyl- (C_3-C_8) carbocycle, $-C_3-C_8$ heterocycle and $-C_1-C_{10}$ alkyl- (C_3-C_8) heterocycle);

each R^8 is independently selected from -H, -OH, -C₁-C₁₀ alkyl, -C₃-C₈ carbocycle, -O-alkyl-(C₁-C₈ carbocycle) and -O-(C₁-C₁₀ alkyl);

R^9 is selected from -H and -C₁-C₁₀ alkyl;

R^{10} is selected from aryl and -C₃-C₈ heterocycle;

5 Z is selected from -O-, -S-, -NH-, and -NR¹²- where R^{12} is C₁-C₁₀ alkyl and aryl; and

R^{11} is selected from -H, C₁-C₁₀ alkyl, aryl, -C₃-C₈ heterocycle, -(CH₂CH₂O)_r-H, -(CH₂CH₂O)_r-CH₃, and -(CH₂CH₂O)_r-CH₂CH₂C(O)OH; wherein r is an integer ranging from 1-10.

10 57. The ligand-drug conjugate compound of claim 47, wherein P is 1 to 25 amino acids in length.

58. The ligand-drug conjugate compound of claim 57, wherein P is 5 to 15 amino acids in length.

15 59. The ligand-drug conjugate compound of claim 57, wherein P is a polyglycine-serine or a polyglycine peptide.

60. The ligand-drug conjugate compound of claim 59, wherein P further comprises at least one cysteine residue.

20 61. The ligand-drug conjugate compound of claim 47, wherein L_a comprises an antibody heavy chain variable region (V_H) and L_b comprises an antibody light chain variable region (V_L), wherein V_H and V_L each form an antigen binding domain with V_L and V_H regions, respectively, of a second ligand-drug conjugate compound.

62. The ligand-drug conjugate compound of claim 61, wherein at least one of the V_H or V_L regions comprises an engineered cysteine residue.

25 63. The ligand-drug conjugate compound of claim 62, the engineered cysteine residue is at least 20 angstroms distal from the antigen binding face of the antigen binding domain.

64 The ligand-drug conjugate compound of claim 62, comprising a serine to cysteine amino acid substitution at amino acid position 84 of the V_H region or position 14 of the V_L region.

65. The The ligand-drug conjugate compound of claim 61,
5 wherein the ligand drug conjugate compound exhibits reduced non-specific toxicity, as compared with an intact antibody drug conjugate compound that binds to the same target molecule.

66. The ligand-drug conjugate compound of claim 47, wherein the first or second Ligand Binding unit binds to CD30, CA125, CA15-3, CA19-9, Lewis
10 Y antigen, Lewis X antigen, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific antigen, prostate specific membrane antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE-4, anti transferrin receptor, p97, MUC1-KLH, CEA, gp100, MART1, IL-2 receptor, CD20, CD52, CD33, CD22, human chorionic gonadotropin, CD38,
15 CD40, mucin, P21, MPG, Neu oncogene product, CD2, CD3, CD4, CD8, CD11, CD18, CD19, CD27, CD28, CD29, CD35, CD40, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD70, CD79, CD90, CD95/Fas, CD134/OX40, CD137/4 1BB, CD152/CTLA 4, PD 1, ICOS, TNF R1, TNFR 2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, or APO 3.

20 67. The ligand-drug conjugate compound of claim 47, wherein the

Linker unit has the formula: $\text{---}A_a\text{---}W_w\text{---}Y_y\text{---}$

wherein:

-A- is a Stretcher unit;

a is 0 or 1;

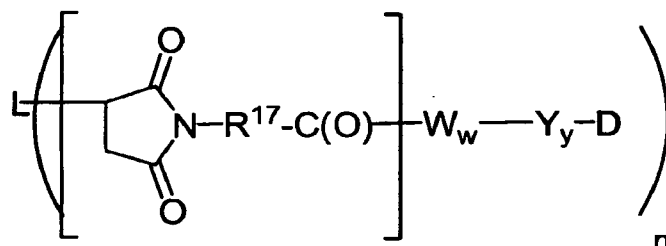
25 each -W- is independently an Amino Acid unit;

w is independently an integer ranging from 0 to 12;

-Y- is a Spacer unit; and

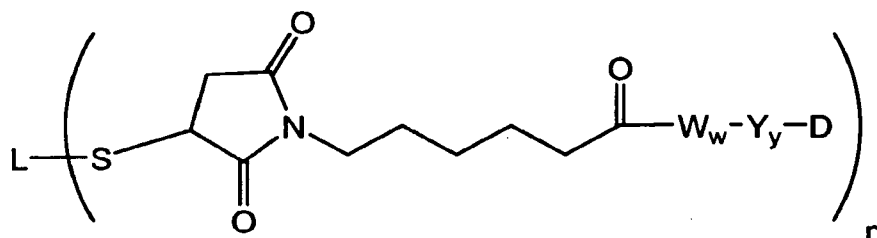
y is 0, 1 or 2.

68. The ligand-drug conjugate compound of claim 67, wherein the ligand-drug conjugate compound has the formula:



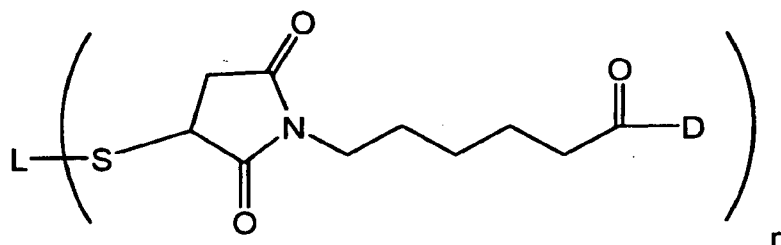
wherein R^{17} is $\text{C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_3\text{-C}_8$ carbocyclo-, $\text{-O-(C}_1\text{-C}_{10}$ alkyl)-, arylene-, $\text{-C}_1\text{-C}_{10}$ alkylene-arylene-, arylene- $\text{-C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_1\text{-C}_{10}$ alkylene-($\text{-C}_3\text{-C}_8$ carbocyclo)-, ($\text{-C}_3\text{-C}_8$ carbocyclo)- $\text{-C}_1\text{-C}_{10}$ alkylene-, $\text{-C}_3\text{-C}_8$ heterocyclo-, $\text{-C}_1\text{-C}_{10}$ alkylene-($\text{-C}_3\text{-C}_8$ heterocyclo)-, ($\text{-C}_3\text{-C}_8$ heterocyclo)- $\text{-C}_1\text{-C}_{10}$ alkylene-, $\text{-(CH}_2\text{CH}_2\text{O)}_r$ -, and $\text{-(CH}_2\text{CH}_2\text{O)}_r\text{-CH}_2$ -; and r is an integer ranging from 1-10.

69. The ligand-drug conjugate compound of claim 67, having the formula:



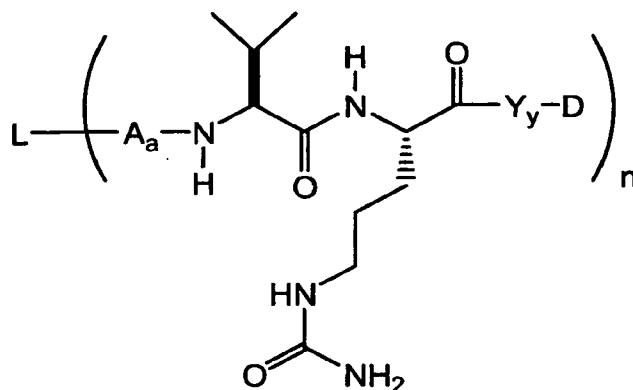
wherein S is a thiol group of L .

70. The ligand-drug conjugate compound of claim 67, having the formula:

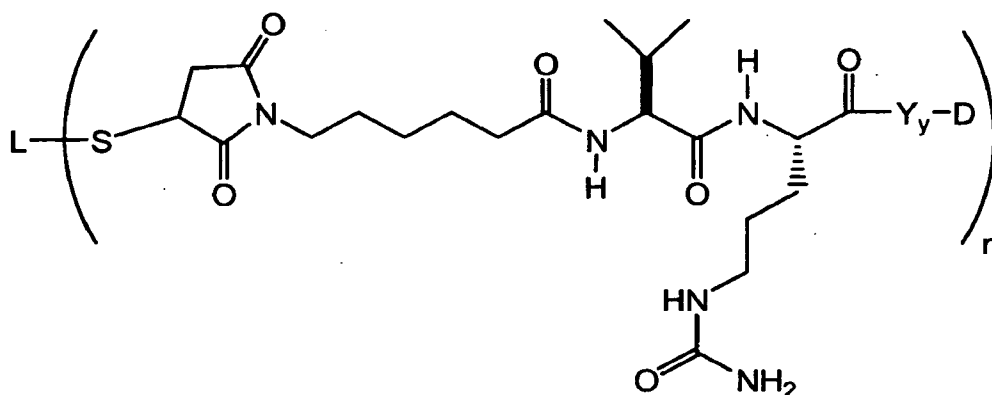


wherein w and y are each 0 and S is a thiol group of L.

71. The ligand-drug conjugate compound of claim 67, having the formula:

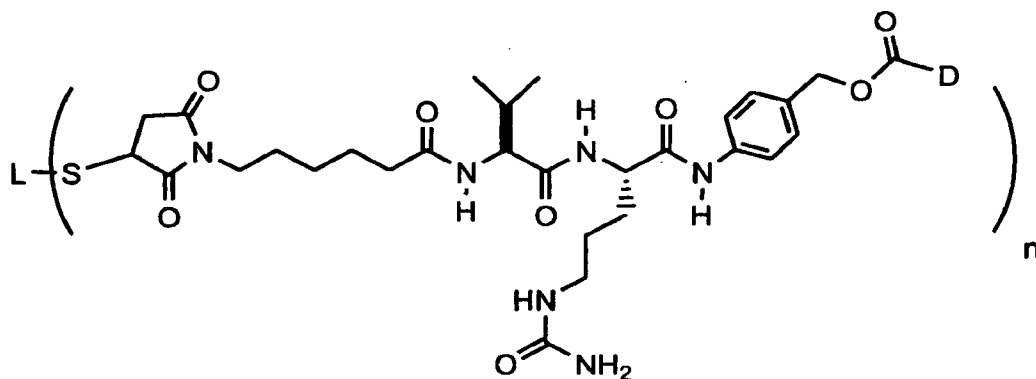


5 72. The ligand-drug conjugate compound of claim 67, having the
formula:



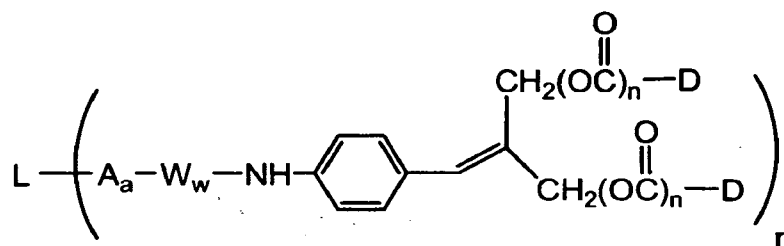
wherein S is a thiol group of L.

73. The ligand-drug conjugate compound of claim 67, having the
10 formula:



wherein S is a thiol group of L.

74. The ligand-drug conjugate compound of claim 67, having the formula:



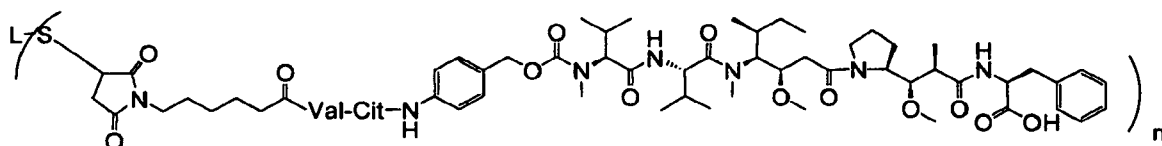
75. The ligand-drug conjugate compound of claim 67, wherein w is an integer ranging from 2 to 12.

76. The ligand-drug conjugate compound of claim 67, wherein w is 2.

77. The ligand-drug conjugate compound of claim 76, wherein W_w is valine citrulline.

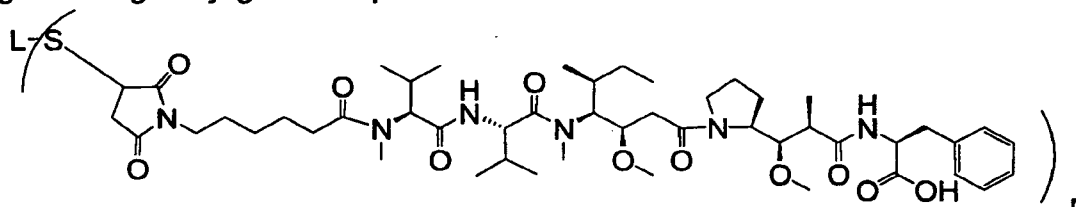
78. The ligand-drug conjugate compound of claim 67, wherein W_w is 5-aminovaleric acid, homo-phenylalanine lysine, tetraisoquinoline-carboxylate lysine, cyclohexylalanine lysine, isonepepotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepepotic acid.

79. The ligand-drug conjugate compound of claim 67, wherein the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

5 80. The ligand-drug conjugate compound of claim 67, wherein the ligand-drug conjugate compound has the formula:



wherein S is a thiol group of L.

81. The ligand-drug conjugate compound of claim 61, wherein L_a
10 comprises the V_H or V_L region of humanized antibody AC10, BR96, 1F6 or 2F2.

82. The ligand-drug conjugate compound of claim 61, wherein L_b
comprises the V_H or V_L region of humanized antibody AC10, BR96, 1F6 or 2F2.

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Figure 1

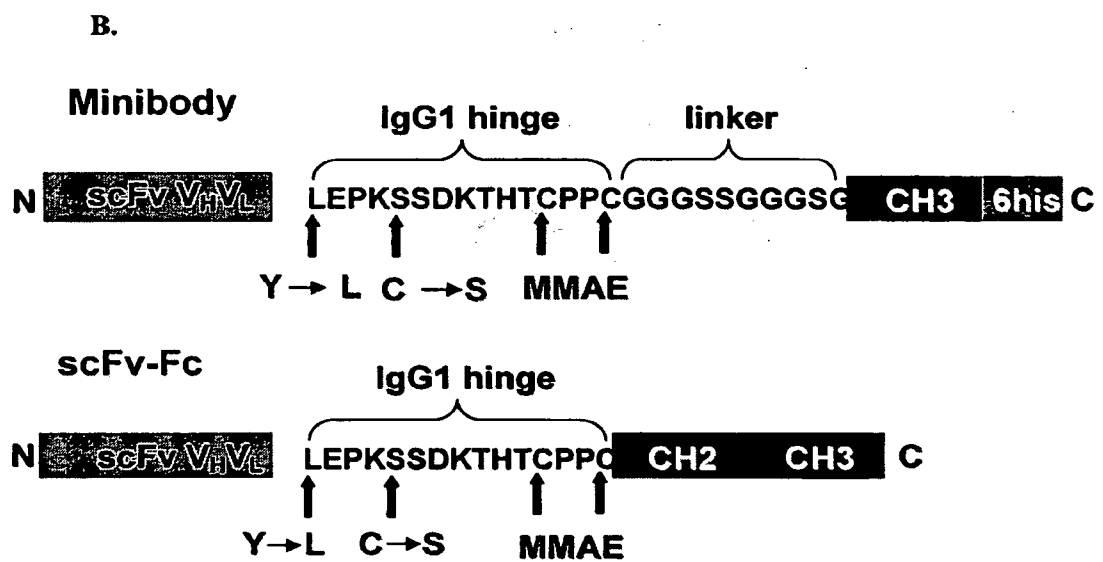
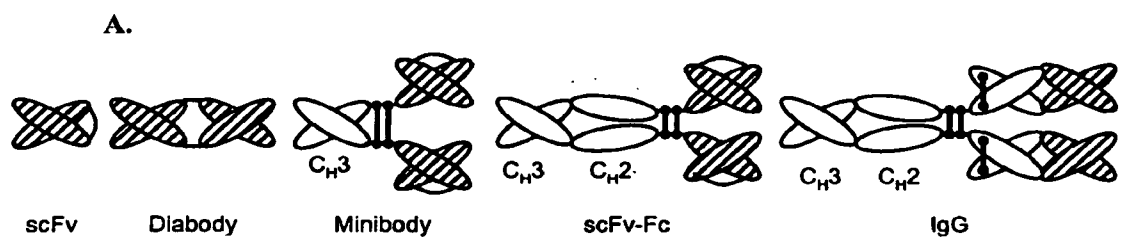


Figure 2

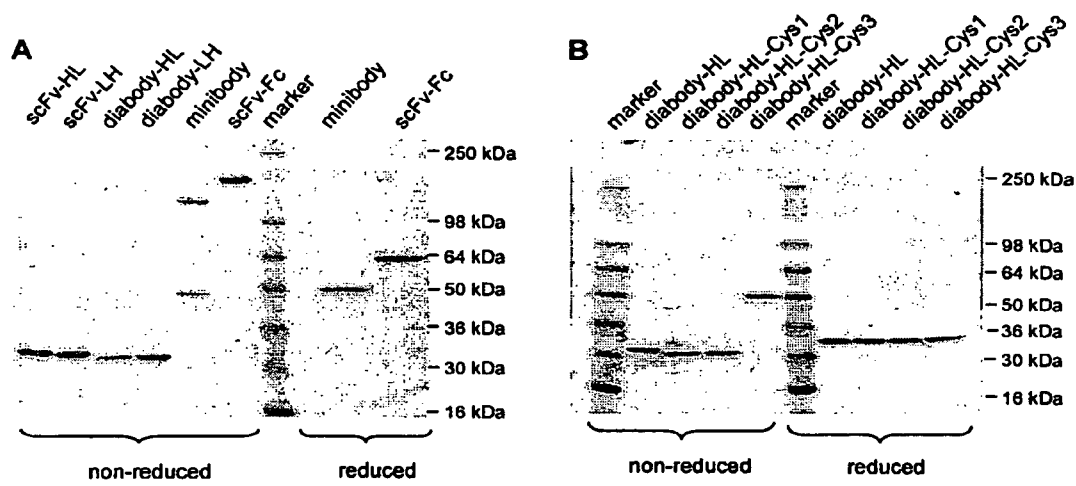
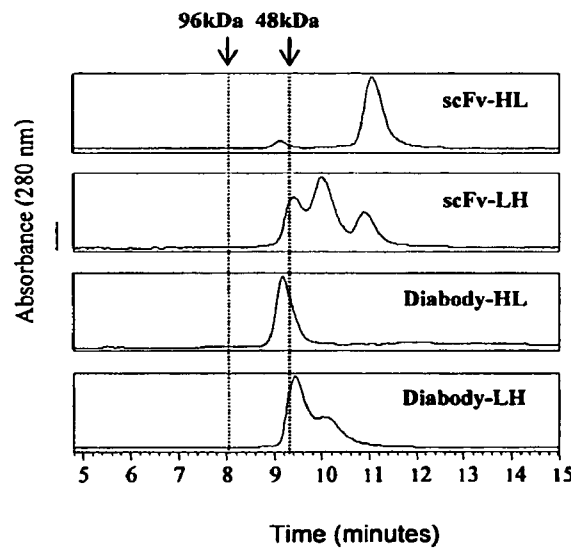


Figure 3



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Figure 4

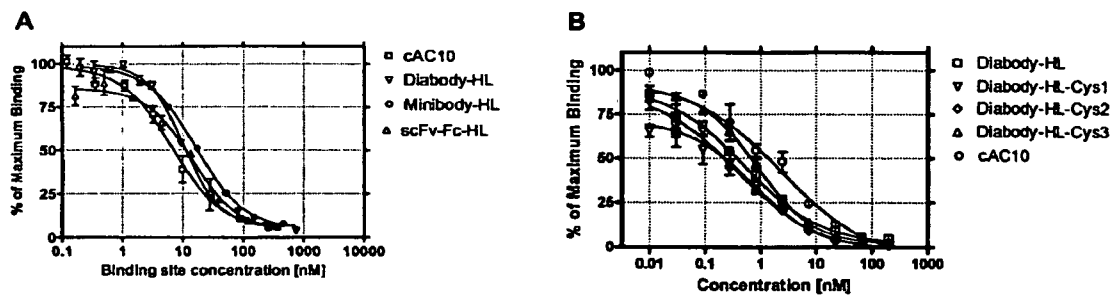
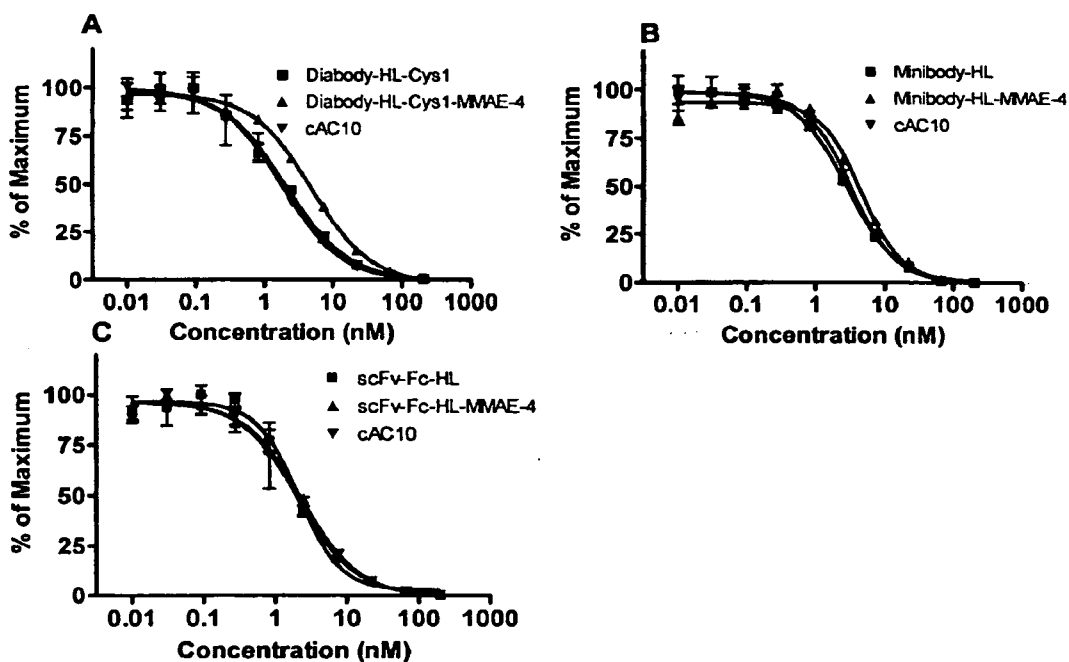


Figure 5



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Figure 6

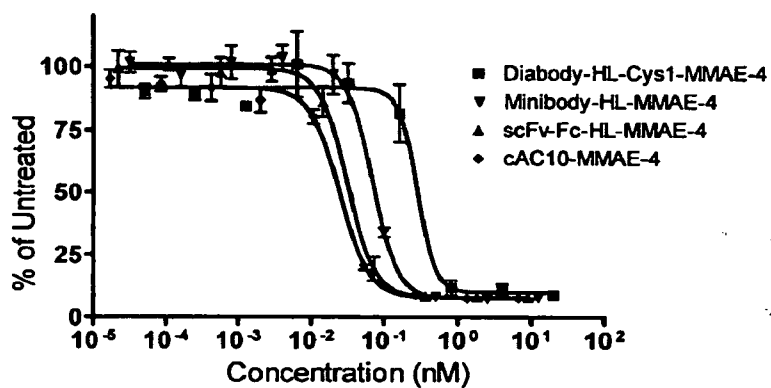
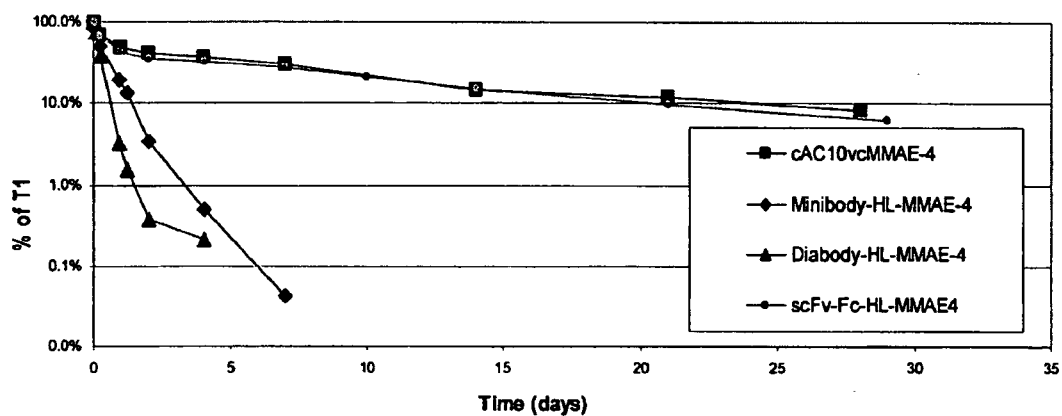


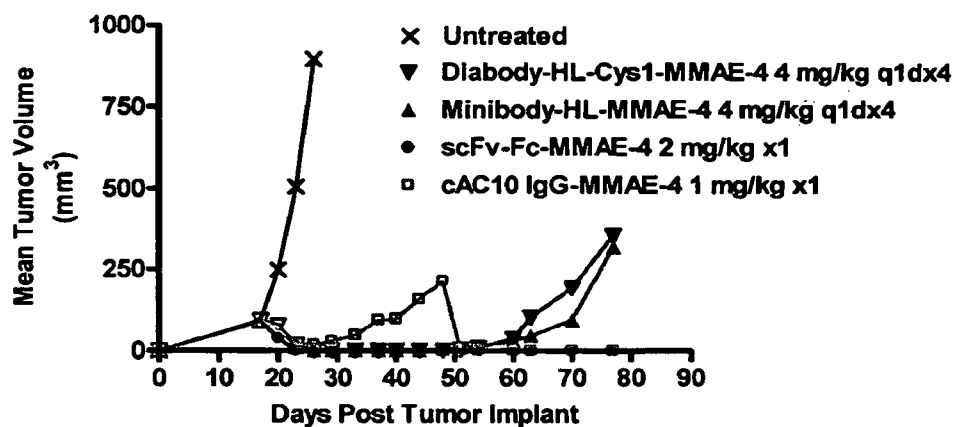
Figure 7



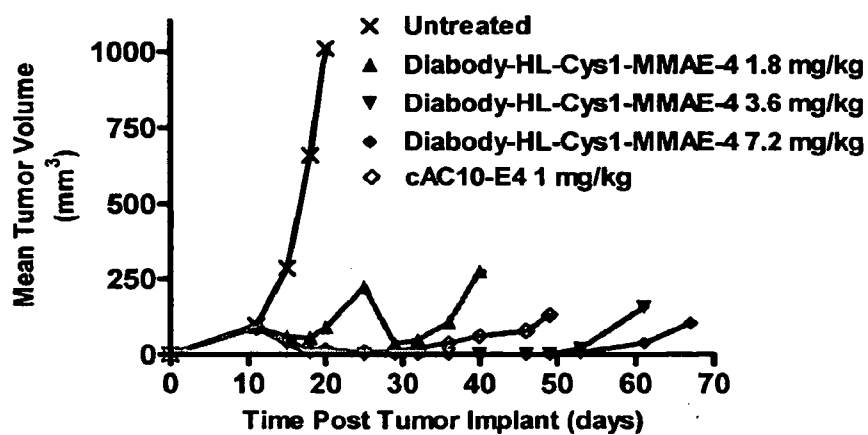
Sheet 5 of 6

Figure 8

A.



B.



Sheet 6 of 6

Figure 9

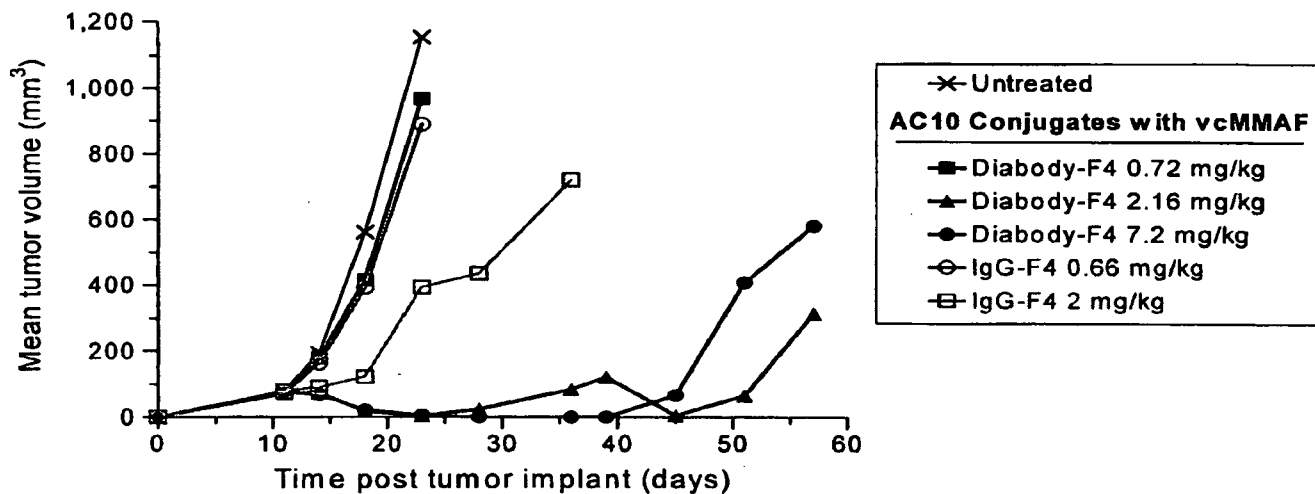


Figure 10

