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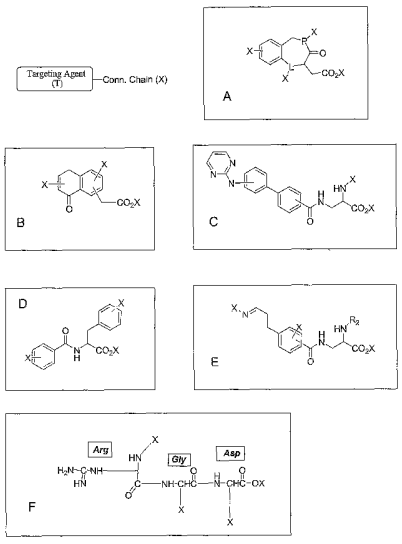
(54) **Title:** NITROGEN CONTAINING INTEGRIN TARGETING COMPOUNDS



(57) **Abstract:** The present invention provides integrin targeting compounds which comprise small molecular weight integrin targeting agent-linker conjugates which are linked to a polymer such as a protein. Various uses of the invention compounds are provided, including methods to prevent or treat cancer or other disease.

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NITROGEN CONTAINING INTEGRIN TARGETING COMPOUNDS

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No.60/463456, filed April 15, 2003, and claims priority to U.S. Provisional Application No.60/507887, filed September 30, 2003, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

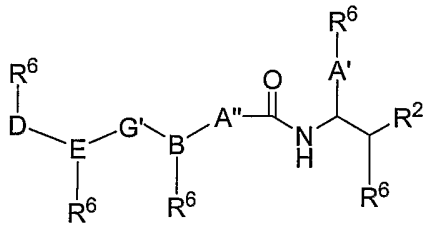
[0002] The invention relates to compounds for targeting biological molecules and methods of making and using the compounds. Conventionally developed pharmaceutical drugs and biological effector molecules are often of limited use in therapy because of high toxicity. Various approaches have been used over the years to improve the therapeutic index of such drugs or effectors. One approach has been to couple a drug or effector to a ligand targeting agent such as an antibody. In this case, the antibody is used to change the distribution of drug or effector such that more of it can localize where it is most needed in vivo. Improved targeting of small molecular weight drugs or effectors has been achieved by complexing the drug or effector with a large molecular weight compound. For example, European Patent EP 217577 discloses that increased half life and targeting by an agent is achieved by forming complexes in vivo between hapten-modified agents and anti-hapten antibodies. Similarly, International Patent Application Publication WO 98/22141 discloses conjugates of therapeutic agents and haptens. The conjugates are administered to a subject and circulate in the blood stream of the subject. Circulating conjugates are recognized and bound by existing antibodies in the subject. Also, Shokat and Schultz (J. Am. Chem. Soc., 1991, 113:1862-1864) have disclosed a process for redirecting the immune response using a process referred to as ligand-mediated immunogenicity. According to this teaching, an invariant antigen is complexed with a specific ligand and administered to a subject. The complexed invariant antigen then binds naturally occurring antibodies present in the subject.

[0003] Integrins are a family of heterodimeric transmembrane glycoprotein complexes that function in cellular adhesion events and signal transduction processes. Integrins, which comprise and alpha and a beta subunit, include numerous types including $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$, $\alpha_8\beta_1$, $\alpha_9\beta_1$, $\alpha_1\beta_1$, $\alpha_6\beta_4$, $\alpha_4\beta_7$, $\alpha_D\beta_2$, $\alpha_D\beta_2$, $\alpha_L\beta_2$, $\alpha_M\beta_2$,$\alpha_v\beta_1$,$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_x\beta_2$, $\alpha_{IIb}\beta_3$, $\alpha_{IELb}\beta_7$. For example, the integrin $\alpha_v\beta_3$ is expressed on numerous cells and has been shown to mediate several biologically relevant processes, including adhesion of osteoclasts to bone matrix, migration of vascular smooth muscle cells, and angiogenesis. Integrin $\alpha_v\beta_3$ antagonists have been used clinically to treat neovascularization in such diseases as rheumatoid arthritis, cancer, and ocular diseases.

[0004] Many integrins bind to adhesion proteins of the extracellular matrix by recongnizing the sequence Arg-Gly-Asp. Platelets contain a large amount of RGD-cell surface receptors of the protein GP II_b/III_a, which is primarily responsible, through interaction with other platelets and with the endothelial surface of injured blood vessels, for the development of coronary artery thrombosis. A variety of RGD peptidomimetics of small molecular weight have been described described in Nicolau, K.C. et al., Design, Synthesis and Biological Evaluation of Nonpeptide Integrin Antagonists, Bioorganic & Medicinal Chemistry 6 (1998) 1185-1208, and in PCT applications WO 96/00730, published January 11, 1996; WO 97/24119, published July 10, 1992; WO 98/14192, published April 9, 1998; W098/30542, published July 16, 1998; W099/15508, published April 1, 1999; W099/05232, published Sept. 16, 1999; WOOO/33838, published June 15, 2000; W097/01540, published Jan. 16, 1997; W099/15170, published April 1, 1999; W099/15178, published April 1, 1999; WOOO/07544, published Feb. 17, 2000; W096/00574, published Jan. 11, 1996; W097/24122, published July 10, 1997; W097/24124, published July 10, 1997; W099/05107, published Feb. 4, 1999; PCT application No. PCT/USOO/24514, filed Sept. 7, 2000; WO 00/35887, published June 22, 2000; US Patent 5,929,120 and W. H. Miller et al., Indentification. and in vivo Efficacy of Small-Molecule Antagonists of Integrin aVO3 (the Vitronectin Receptor), Drug Discovery Today, Vol. 5, Issue 9, Sept. 1, 2000, pp 397.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides integrin targeting compounds with unique specificity and biological properties which are useful in many applications. The integrin targeting compounds of the invention are formed by covalently linking a polymer, e.g. the combining site of the antibody, to an integrin RGD peptidomimetic targeting agent – linker conjugate of formula I, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof:



Formula I

wherein

G' is a covalent bond or $-(CH_2)_a-T-(CH_2)_b-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, and a and b are each independently 0 to 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is $T-(CH_2)_j-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is -$(CH_2)_d$-L'-U wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is attached to R^6 at one of U, L', or -$(CH_2)_d$-;

E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11}, and wherein when U is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group;

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, $(C_{1-8}$ alkyl$)_o$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl$)_o$ amino, (aryl$)_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$S(O)_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -N(R^5) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, $(C_{1-6}$ alkyl$)_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl,

aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, $(aryl)_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, $(aryl)_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

R^6 is, at a first occurrence, a linker moiety having the formula -J-G-K, and is, at a second occurrence R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K is a reactive group; and wherein

if J is an unsubstituted C_{1-21} alkyl and K is $-NH_2$, -SH, $-OS(OH)_3$, or -COOH, G is present;

R^7 is $-C(O)R^8$, $-C(O)-CR'_2-R^9$, $-C(S)R^8$, $-S(O)_o$ OR', $-S(O)_o$ NR'R", -PO(OR'), $-PO(OR')_2$, $-B(OR')_2$, $-NO_2$, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", $-NR'SO_2$ R', -NR'OR', $-OCR'_2$ C(O)OR', $-OCR'_2OC(O)-R'$, $-OCR'_2C$ (O)NR'_2, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', CN, -S(O)$_r$ R', S(O)$_o$ NR'$_2$, -C(O)R'C(O)NR'$_2$ or -CO_2 R';

R^{11} is H, halogen, -OR^{12}, -CN, -NR'R^{12}, -NO_2, -CF_3, -S(O)$_r$-CF_3, -CO_2R', -CONR'$_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-S(O)$_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', -C(O)-NR'$_2$, -C(O)-OR^{13}, -S(O)$_o$-R' or -S(O)$_o$-NR'$_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or -C(O)OR^{13};

R''' is -H, or substituted or unsubstituted C_{1-6} alkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

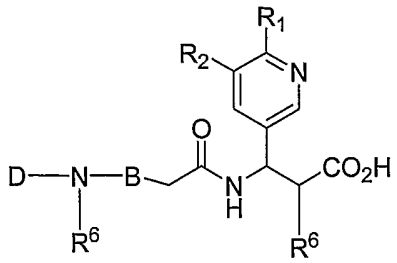
o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

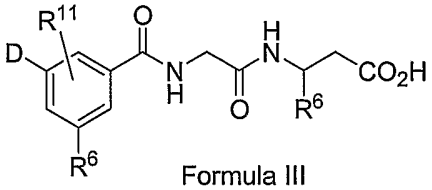
q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

[0006] Preferred embodiments include compounds having formula II and III,



Formula II

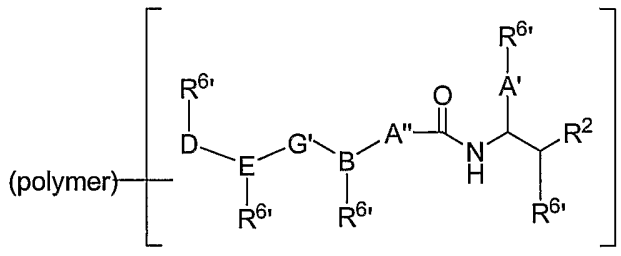


Formula III

wherein

R_1 and R_2 are independently -H, halogen, $-OR^{12}$, -CN, $-NR^8R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R^8$, $-CONR^8{}_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$-, or R_1 and R_2 together form a fused unsubstituted or substituted aryl.

[0007] The integrin targeting compounds of the invention comprise an RGD peptidomimetic integrin targeting agent covalently linked to a polymer such as the combining site of an antibody. In one embodiment, an integrin targeting compound having formula IV, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof is shown:



Formula IV

wherein,

G' is a covalent bond or $-(CH_2)_a-T-(CH_2)_b-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, and a and b are each independently 0 – 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is $T-(CH_2)_j-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is $-(CH_2)_d-L'-U$ wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is attached to $R^{6'}$ at one of U, L', or $-(CH_2)_d-$;

E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11} , and wherein when U is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group;

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino,

amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, $(C_{1-8}$ alkyl$)_o$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl$)_o$ amino, (aryl$)_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$S(O)_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -N(R^5) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, $(C_{1-6}$ alkyl$)_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, $(aryl)_q$ aminocarbonyl, $(aryl\ C_{1-5}\ alkyl)_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, $(C_{1-6}$ alkyl$)_q$ amino C_{1-6} alkyl, $(aryl\ C_{1-6}\ alkyl)_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, $(C_{1-8}$ alkyl$)_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, $(aryl)_q$ aminosulfonylamino, $(aryl\ C_{1-8}\ alkyl)_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

$R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein,

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted

cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K' is an attachment moiety including about 1 to about 20 carbon atoms and is covalently linked to the polymer;

R^7 is $-C(O)R^8$, $-C(O)-CR'_2-R^9$, $-C(S)R^8$, $-S(O)_o$ OR', $-S(O)_o$ NR'R", -PO(OR'), $-PO(OR')_2$, $-B(OR')_2$, $-NO_2$, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", $-NR'SO_2$ R', -NR'OR', $-OCR'_2$ C(O)OR', $-OCR'_2OC(O)-R'$, $-OCR'_2C$ (O)NR'_2, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', CN, $-S(O)_r$ R', $S(O)_o$ NR'_2, $-C(O)R'C(O)NR'_2$ or $-CO_2$ R';

R^{11} is H, halogen, $-OR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R'$, $-CONR'_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', $-C(O)-NR'_2$, $-C(O)-OR^{13}$, $-S(O)_o-R'$ or $-S(O)_o-NR'_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or $-C(O)OR^{13}$;

R''' is -H, or substituted or unsubstituted C_{1-6} alkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

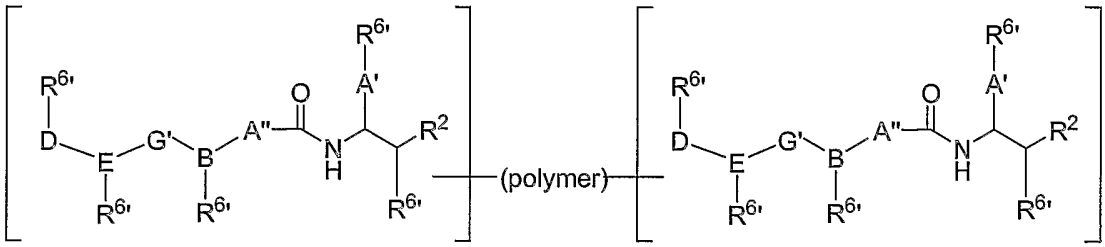
o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

[0008] In another embodiment, the integrin targeting compound includes two RGD peptidomimetic integrin targeting agents covalently linked to a polymer. Thus, there is provided compounds of formula V, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof as shown:



Formula V

wherein,

G' is a covalent bond or $-(CH_2)_a$-T-$(CH_2)_b$- wherein

T is -O-, -N(R''')-, $-S(O)_q$-,-CHR'''-, $-CH_2$-, -C(O)-, $-S(O)_2N(R''')$- , or –C(N)R'''-, and a and b are each independently 0 – 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is T-$(CH_2)_j$- wherein

T is -O-, -N(R''')-, -$S(O)_q$-,-CHR'''-, -CH_2-, -C(O)-, -$S(O)_2N(R''')$- , or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is -$(CH_2)_d$-L'-U and is attached to R^{24} when it is not attached to K', wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is substituted by $R^{6'}$ at one of U, L', or -$(CH_2)_d$-;

E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11}, and wherein when U is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group; and E is attached to R^{24} when it is not attached to K';

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, (C_{1-6} alkyl)$_o$ amino, (C_{1-6} alkyl)$_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, (C_{1-8} alkyl)$_o$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl)$_o$ amino, (aryl)$_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ --$S(O)_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -N(R^5) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, (C_{1-6} alkyl)$_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, (aryl)$_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, (aryl)$_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

on each targeting agent, $R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein,

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K' is an attachment moiety including about 1 to about 20 carbon atoms and is covalently linked to the polymer;

R^7 is –C(O)R^8, -C(O)-CR'_2-R^9, -C(S)R^8, -S(O)$_o$ OR', -S(O)$_o$ NR'R'', -PO(OR'), -PO(OR')$_2$, -B(OR')$_2$, -NO_2, or substituted or unsubstituted tetrazole,

substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", -NR'SO_2 R', -NR'OR', -OCR'$_2$ C(O)OR', -OCR'$_2$OC(O)-R', -OCR'$_2$C (O)NR'$_2$, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', CN, -S(O)$_r$ R', S(O)$_o$ NR'$_2$, -C(O)R'C(O)NR'$_2$ or -CO_2 R';

R^{11} is H, halogen, -OR^{12}, -CN, -NR'R^{12}, -NO_2, -CF_3, -S(O)$_r$-CF_3, -CO_2R', -CONR'$_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-S(O)$_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', -C(O)-NR'$_2$, -C(O)-OR^{13}, -S(O)$_o$-R' or S(O)$_o$-NR'$_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or -C(O)OR^{13};

R"' is -H, or substituted or unsubstituted C_{1-6} alkyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

[0009] The polymer in formula V is at least bivalent, comprising at least two binding sites to which two integrin RGD peptidomimetic targeting agent-linker conjugates may be attached. For example, when polymer is an antibody, the antibody is at least bivalent, i.e. comprises at least two combining sites, thus allowing linkage to two integrin RGD peptidomimetic targeting agent-linker conjugates through K' of $R^{6'}$. When polymer is an antibody, it can include whole antibody or unique antibody fragments or any other forms of an antibody as this term is used herein. In a preferred embodiment, the antibody is an aldolase antibody. In a preferred embodiment, the reactive group (K of R^6) is a diketone which covalently binds to an amino side chain in an antibody combining site and forms the covalently linked attachment moiety, K'.

[0010] Also provided are methods of producing integrin targeting compounds of the invention and methods of treating or preventing a disease or condition in an individual wherein the disease or condition involves cells, tissue or fluid that expresses a target molecule. In the method, a therapeutically effective amount of the integrin targeting compound of the invention is administered to the individual. The integrin targeting compounds of the invention also may be labeled and used to image cells or extracellular matrix in an individual wherein the cells or extracellular matrix express the integrin.

[0011] Further provided are methods of antagonizing $\alpha_v\beta_3$ or $\alpha_v\beta_5$ function in vivo comprising comprising administering to the individual an antagonizing amount of the integrin targeting compounds of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1 shows exemplary integrin targeting agents of which Panels A-E are RGD peptidomimetic while Panel F is an RGD peptide. The core structures are from the following: U.S. Patent No. 6,335,330 (Panel A), U.S. Patent No. 5,693,636 (Panel B), U.S. Patent No. 6,040,311 (Panel C), and U.S. Patent No. 6,001,117 (Panel E).

[0013] FIG. 2 shows a general scheme of a targeting agent-linker compound with a non-branched linker (Panel A) with specific embodiments in Panel B (compound **80**), Panel C (PST inhibitor diketo linker; compound **26**), Panel D (compound **81**) and Panel E (folate ligand dikone linker; compound **28**).

[0014] FIG. 3 shows a general scheme of an embodiment of a targeting agent-linker compound with a branched linker and two identical targeting agents (Panel A) with specific embodiments in Panel B (integrin targeting agent diketo linker; compound **29**), and Panel C (integrin targeting agent diketo linker; compound **30**). The branch point is in the connecting chain portion of the linker.

[0015] FIG. 4 shows a general scheme of an embodiment of a targeting agent-linker compound with a branched linker and two different targeting agents (Panel A) with a specific embodiment in Panel B (integrin targeting and folate targeting agent diketo linker; compound **31**). The branch point is in the connecting chain portion of the linker.

[0016] FIG. 5 shows a general scheme of an embodiment of a targeting agent-linker compound with a branched linker and two different targeting agents (Panel A) with a specific embodiment in Panel B (integrin targeting agent diketo linker; compound 32). The branch point is in the recognition group portion of the linker.

[0017] FIG. 6 shows the structure of linker reactive groups. Structures A-C form reversible covalent bonds with reactive nucleophilic group (e.g. lysine or cysteine side chain) in the combining site of an antibody (structure A could form an irreversible covalent bond X is N and if R_1 and R_3 form part of a cyclic structure). R_1 and R_2 and R_3 in structures A-C represent substituents which can be C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof. X is N, C, Si, or any other heteroatom. These substituents may also include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. R_2 and R_3 could be cyclic as exemplified in structures B and C while X could be a heteroatom. Structures D-G form nonreversible covalent bonds with reactive

nucleophilic group (e.g. lysine or cysteine side chain) in the combining site of an antibody. In these structures, R_1 and R_2 represent C, O, N, halide and leaving groups such as mesyl or tosyl.

[0018] FIG. 7 shows various electrophiles suitable for reactive modification with a reactive amino acid side chain of an antibody. Key: (A) acyl beta-lactam; (B) simple diketone; (C) succinimide active ester; (D) maleimide; (E) haloacetamide with linker; (F) haloketone; (G) cyclohexyl diketone; and (H) aldehyde. R refers to other structure that may include a targeting agent, linker or antibody, while X refers to halogen.

[0019] FIG. 8 shows the structure of linker recognition group (Y), situated between the reactive group portion and the connecting chain portion of the linker. Panel A shows the relationship of the recognition group Y within the linker (see FIG. 2). Panels B-D show distance of Y from Z, substituents on the ring and ring member atoms.

[0020] FIG. 9 shows the structure of the linker connecting chain (X), which directly attaches at one end to the targeting agent as shown in Panel A (see FIG. 2). Substituents R_2 to R_4 are C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof, and may include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl as well as a carbocyclic or heterocyclic mono or fused saturated or unsaturated ring structure. Panel B: R_1 is O and R_2 is C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof. In the connecting chain in structures B and C, n, r or m is 1-100. In structures D and E, n is 1, 2, 4, or more preferably is 3.

[0021] FIG. 10 shows Scheme 1, a synthetic scheme for the amine precursor (compound 15) of compound 80, a targeting agent. Key: (a) BBr_3, CH_2Cl_2, –20 °C, 2h; (b) DMF, rt to 80°C, 3h; (c) BnCOCl, sat. aq. $NaHCO_3$, ether; (d) TBDPSiCl, imidazole, DMF, 16h; (e) $Pd(OAc)_2$, $(o\text{-}tol)_3P$, *i*-Pr_2EtN, CH_3CH_2CN, reflux, 3h; (f) 20 % (w/w) Pd-C (10%), H_2, EtOH-AcOH (1:1), 36h; (g) TBAF, THF, rt, 1h; (h) DEAD, PPh_3, THF-benzene (3:1), 16h; (i) 20 % (w/w) Pd-C (10%), cyclohexene-*i*-

PrOH (1:1), 90°C, 12h; (j) i. aq. 2N NaOH, MeOH-THF (1:1), 16h, ii. TFAA, anisole, CH_2Cl_2, 0°C, 2h.

[0022] FIG. 11 shows Scheme 2, a synthetic scheme for making Compound 4, (R = Butoxycarboxyaminohexanoyl-derivative). Key: (a) DMF, rt; (b) EDC, HOBT, DMF; (c) 0.01 M in DMSO, 130 °C; (d) TFAA, anisole, dichloromethane; (e) DMF; (f) EDC, HOBT, DMF; (g) (i) step d, (ii) 2M NaOH, MeOH-THF (1:1).

[0023] FIG. 12 shows Scheme 3, a synthetic scheme for making compounds **80** and **82**.

[0024] FIG. 13 shows Scheme 4, a synthetic scheme for making compounds **83** and **84**. Key: (a) Et_3N, DMF, rt, 16h.

[0025] FIG. 14 shows a scheme for forming a targeting agent-linker compound using a linker with a maleimide-diketone reactive group.

[0026] FIG. 15 shows Scheme 5, a synthetic scheme for making compound **58**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

[0027] FIG. 16 shows Scheme 6, a synthetic scheme for making compound **46**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

[0028] FIG. 17 shows Scheme 7, a synthetic scheme for making compound **86**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

[0029] FIG. 18 shows Scheme 8, a synthetic scheme for making compound **87**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

[0030] FIG. 19 shows Scheme 9, a synthetic scheme for making compound **46**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

[0031] FIG. 20 shows Scheme 10, a synthetic scheme for making compound **88**, an integrin RGD peptidomimetic targeting agent-linker conjugate.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The present invention provides various targeting compounds in which targeting agents and/or biological agents are covalently or noncovalently linked to a polymer, particularly a protein, such as to the combining site of an antibody. When one or more targeting agents are linked, at least one of the targeting agents will be linked so that it can bind its target. This may be achieved by linking the targeting agent in a manner that does not affect its binding specificity for the target and by sufficiently distancing the targeting agent from, e.g., the antibody combining site so that it can bind its target without steric hindrance by the antibody. This may be achieved by using a suitable linker and linking strategy discussed in more detail herein.

[0033] Targeting compounds of the invention include targeting agents that are covalently or non-covalently linked to one or more of a variety of natural polymers comprising protein, carbohydrate or nucleic acid, as well as non-natural water soluble biocompatible polymers. Non-natural polymer

[0034] rs include water-soluble polymers such as polyethylene glycol, polypropylene glycol, and the like that bear or have been modified to bear suitable reactive functional groups such as hydroxy, amino, thiol, carboxy and the like. In some embodiments, polymers may be biodegradable, allowing controlled release of the targeting agent and a biological agent in vivo. Suitable polymers are at least 5kd in molecular size, more preferably at least 10kd, 20kd, 30kd, 40kd, 30kd, 50kd, 60kd, 70kd, 80kd, 90kd, 100kd, 110kd, 120kd, 130kd, 140kd, or 150kd in size. Polymers may include combinations of the above basic components (e.g. block copolymers).

[0035] The targeting compounds of the invention may also comprise a protein, which may include any suitable protein that is known. Preferred proteins are those that

circulate in blood or are present in milk. Thus, targeting agent-linker compounds may be conjugated to monomeric and multimeric proteins, including milk proteins such as lactoferrin and blood proteins such as antibody, serum albumin, transthyretin, α-1 globulins such as α-1 antitrypsin, α-1 glycoprotein, α-fetoprotein, and high density lipoprotein, α-2 globulins such as α-2 macroglobulin, antithrombin III, ceruloplasmin, and haptoglobin, beta-globulins such as low density lipoprotein (LDL) and very low density lipoprotein (VLDL), C3, C-reactive protein, free hemoglobin, plasminogen, and transferrin. The accessible surfaces of serum and milk proteins contain suitably reactive amino acid side chains such as amino (e.g. lysine), thiol (e.g. cysteine), hydroxyl (serine, threonine, and tyrosine), carboxyl (aspartic and glutamic acid) and the like. These side chains can react with linker reactive groups as described herein to form protein conjugates of targeting agent-linker compounds.

[0036] For example, the polymer may be a protein such as human serum albumin. Cys34 is a reactive amino acid side chain on the surface of human serum albumin and may be alkylated or form a disulfide with appropriate linker reactive groups. Methods well known to those of skill in the art may be used to conjugate and detect linkers of the invention with the reactive thiol of Cys34 (see, e.g., Fabisiak JP, et al., Antioxid Redox Signal. 2002 Oct;4(5):855-65; Beck JL, et al., Anal Biochem. 2004 Feb 15;325(2):326-36; Bertucci C, et al., J Pharm Biomed Anal. 1998 Oct;18(1-2):127-36).

[0037] An antibody or an antibody fragment that has a single combining site such as Fab or Fab' antibody fragments can also be used as the polymer of the targeting compounds of the invention. In such cases, the targeting agent may be linked to the combining site of the antibody molecule via the heavy and/or the light chain of the antibody. If an antibody or antibody fragment of a targeting molecule comprises two or more combining sites, at least one of the combining sites can have a covalently linked targeting agent. In some cases, all or most of the combining sites of an antibody can be covalently linked to a targeting agent. If multiple combining sites of an antibody are to be linked to targeting agents, the combining sites may all have the same targeting agent linked thereto or may have different targeting agents linked to the same antibody. It would be readily understood that one could covalently link multiple targeting agents to

a single protein or non-protein polymer. In the case of an antibody, multiple targeting agents may be linked to a single combining site. Such multimeric targeting agents may be heteromultimeric or homomultimeric with respect to the specificity of the targeting agents in the multimer. Targeting agents also can be linked to sites outside of the antibody combining site.

[0038] When a biological agent is not also a targeting agent and an antibody (the polymer) is linked to the biological agent, it is preferred that the antibody retain at least some antigen binding specificity following linkage to one or more biological agents. The antibody compound in which one or more biological agents are linked to the antibody combining site may exhibit biological activity due to a linked biological agent if such agent is biologically active while linked to the antibody. This may be achieved by various strategies such as by linking the antibody combining site to a location on the biological agent that does not affect biological activity. Another strategy is to position the biological agent away from the antibody so that the biological agent can bind to another molecule necessary for activity without steric hindrance by the antibody. Other strategies for obtaining a biological activity of one or more biological agents linked to the antibody combining site are well known to the skilled artisan. In some embodiments, the biological activity of a biological agent may not be realized until the agent is released from the antibody combining site. This may be achieved in some embodiments though the aid of labile linkage as discussed further ahead.

[0039] In some embodiments, the native antigen binding specificity of the antibody which exists before covalent linkage will not be substantially modified following covalent linkage. In other words, the antibody compound resulting from covalent linkage of one or more targeting agents or one or more biological agents may bind the same antigens with a similar affinity as it did prior to covalent linkage. In other embodiments, the binding specificity of the antibody before covalent linkage will be substantially modified following covalent linkage. Substantially modified antibody binding specificity resulting from covalent linkage may be due to a substantially reduced ability of the covalently linked antibody to bind to an antigen or a substantially increased ability of the covalently linked antibody to bind to an antigen. In some

embodiments, binding of the antigen binding site to antigen is sufficiently reduced such that the original antigen binding specificity of the antibody is effectively eliminated. In some embodiments, the antigen binding site to antigen is sufficiently reduced such that the original antigen binding specificity of the antibody is effectively eliminated and replaced with that of a targeting agent(s) covalently linked to the antibody combining site. In embodiments where the binding specificity of the antibody is effectively replaced with that of the targeting agent(s), the antibody, after covalent linkage to the targeting agent(s), exhibits an affinity for the target molecule of greater than about 1×10^{-6} moles/liter.

[0040] Although not wishing to be bound by any theory, substantially reduced antibody binding to antigen may result from the targeting agent(s) or biological agent(s) sterically hindering the antigen from contacting the antibody combining site. Alternatively, or in addition, substantially reduced antigen binding may result if the amino acid side chain of the antibody combining site modified by covalent linkage was important for binding to the antigen. Substantially increased antibody binding to an antigen may result when the targeting agent(s) or biological agent(s) do not sterically hinder the antigen from contacting the antibody combining site and amino acid side chain of the antibody combining site modified by covalent linkage was important for binding to the antigen.

[0041] "Targeting agent" or "targeting component" as used herein refers to a moiety that recognizes, binds or adheres to a target moiety of a target molecule located for example in a cell, tissue (e.g. extracellular matrix), fluid, organism, or subset thereof. A targeting agent and its target molecule represent a binding pair of molecules, which interact with each other through any of a variety of molecular forces including, for example, ionic, covalent, hydrophobic, van der Waals, and hydrogen bonding, so that the pair have the property of binding specifically to each other. Specific binding means that the binding pair exhibit binding with each other under conditions where they do not bind to another molecule. Examples of binding pairs are biotin-avidin, hormone-receptor, receptor-ligand, enzyme-substrate, lgG-protein A, antigen-antibody, and the like. The targeting agent and its cognate target molecule exhibit a significant

association for each other. This association may be evaluated by determining an equilibrium association constant (or binding constant) according to methods well known in the art. Affinity is calculated as $K_d = k_{off} / k_{on}$ (k_{off} is the dissociation rate constant, k_{on} is the association rate constant and K_d is the equilibrium constant.

[0042] Affinity can be determined at equilibrium by measuring the fraction bound (r) of labeled ligand at various concentrations (c). The data are graphed using the Scatchard equation: r/c = K(n-r):

where

r = moles of bound ligand/mole of receptor at equilibrium;

c = free ligand concentration at equilibrium;

K = equilibrium association constant; and

n = number of ligand binding sites per receptor molecule

By graphical analysis, r/c is plotted on the Y-axis versus r on the X-axis thus producing a Scatchard plot. The affinity is the negative slope of the line. k_{off} can be determined by competing bound labeled ligand with unlabeled excess ligand (see, e.g., U.S. Pat No. 6,316,409). The affinity of a targeting agent for its target molecule is preferably at least about 1×10^{-6} moles/liter, is more preferably at least about 1×10^{-7} moles/liter, is even more preferably at least about 1×10^{-8} moles/liter, is yet even more preferably at least about 1×10^{-9} moles/liter, and is most preferably at least about 1×10^{-10} moles/liter.

[0043] Targeting agents include, but are not limited to, small molecule organic compounds of 5,000 daltons or less such as drugs, proteins, peptides, peptidomimetics, glycoproteins, proteoglycans, lipids glycolipids, phospholipids, lipopolysaccharide, nucleic acids, proteoglycans, carbohydrates, and the like. Targeting agents may include well known therapeutic compounds including anti-neoplastic agents. Anti-neoplastic targeting agents may include paclitaxel, daunorubicin, doxorubicin, carminomycin, 4'-epiadriamycin, 4-demethoxy-daunomycin, 11 -deoxydaunorubicin, 13-deoxydaunorubicin, adriamycin-14-benzoate, adriamycin-14-octanoate, adriamycin-14-naphthaleneacetate, vinblastine, vincristine, mitomycin C, N-methyl mitomycin C, bleomycin A_2, dideazatetrahydrofolic acid, aminopterin, methotrexate, cholchicine and cisplatin, and the like. Anti-microbial agents include aminoglycosides including

gentamicin, antiviral compounds such as rifampicin, 3'-azido-3'-deoxythymidine (AZT) and acylovir, antifungal agents such as azoles including fluconazole, polyene macrolides such as amphotericin B, and candicidin, anti-parasitic compounds such as antimonials, and the like. Hormone targeting agents include toxins such as diphtheria toxin, cytokines such as CSF, GSF, GMCSF, TNF, erythropoietin, immunomodulators or cytokines such as the interferons or interleukins, a neuropeptide, reproductive hormone such as HGH, FSH, or LH, thyroid hormone, neurotransmitters such as acetylcholine, and hormone receptors such as the estrogen receptor.

[0044] In some preferred embodiments, the targeting agent is not an antibody. In other preferred embodiments, the targeting agent is not a metal chelate. Preferably, the targeting agent is a small molecule as compared with a native immunoglobulin. The targeting agent, including any linking moiety necessary for covalently linking the targeting agent to an amino acid residue of the antibody combining site, preferably is less than about 300 daltons in size, and preferably is less than about 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, 3,000, 3,500, 4,000, 4,500 or even less than 5,000 daltons in size, although larger sizes are possible.

[0045] Suitable targeting agents in targeting compounds of the invention can be a protein or peptide or may comprise amino acids. "Polypeptide", "peptide," and "protein" are used interchangeably to refer to a polymer of amino acid residues. As used herein, these terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid. These terms also apply to naturally occurring amino acid polymers. Amino acids can be in the L or D form as long as the binding function of the peptide is maintained.

[0046] Peptides can be of variable length, but are generally between about 4 and 200 amino acids in length. Peptides may be cyclic, having an intramolecular bond between two non-adjacent amino acids within the peptide, e.g., backbone to backbone, side-chain to backbone and side-chain to side-chain cyclization. Cyclic peptides can be prepared by methods well know in the art. See e.g., U.S. Pat. No. 6,013,625.

[0047] Protein or peptide targeting agents that exhibit binding activity for a target molecule are well known in the art. For example, a targeting agent may be a viral peptide cell fusion inhibitor. This may include the T-20 HIV-1 gp41 fusion inhibitor which targets fusion receptors on HIV infected cells (for T-20, see U.S. Patent Nos. 6,281,331 and 6,015,881 to Kang et al.; Nagashima et al. J. Infectious Diseases 183:1121, 2001; for other HIV inhibitors see U.S. Pat. No. 6,020,459 to Barney and WO 0151673A2 to Jeffs et al), RSV cell fusion inhibitors (see WO 0164013A2 to Antczak and McKimm-Breschkin, Curr. Opin. Invest. Drugs 1:425-427, 2000 (VP-14637)), pneumovirus genus cell fusion inhibitors (see WO 9938508A1 by Nitz et al.), and the like. Targeting agents also include peptide hormones or peptide hormone analogues such as LHRH, bombesin/gastrin releasing peptide, somatastatin (e.g., RC-121 octapeptide), and the like, which may be used to target any of a variety of cancers ovarian, mammary, prostate small cell of the lung, colorectal, gastric, and pancreatic. See, e.g., Schally et al., Eur. J. Endocrinology, 141:1-14, 1999.

[0048] Peptide targeting agents suitable for use in targeting compounds of the invention also may be identified using in vivo targeting of phage libraries that display a random library of peptide sequences (see, e.g., Arap et al., Nature Medicine, 2002 8(2):121-7; Arap et al., Proc. Natl. Acad. Sci. USA 2002 99(3):1527-1531; Trepel et al. Curr. Opin. Chem. Biol. 2002 6(3):399-404).

[0049] In some embodiments, the targeting agent is specific for an integrin. Exemplary integrin targeting agents are integrin antagonists or agonists such as the RGD peptides and peptidomimetics. Integrins are heterodimeric transmembrane glycoprotein complexes that function in cellular adhesion events and signal transduction processes. Integrin $\alpha_v\beta_3$ is expressed on numerous cells and has been shown to mediate several biologically relevant processes, including adhesion of osteoclasts to bone matrix, migration of vascular smooth muscle cells, and angiogenesis. Integrin $\alpha_v\beta_3$ antagonists likely have use in the treatment of several human diseases, including diseases involving neovascularization, such as rheumatoid arthritis, cancer, and ocular diseases.

[0050] Suitable targeting agents for integrins include RGD peptides or peptidomimetics or non-RGD peptides or peptidomimetics. As used herein, reference to "Arg-Gly-Asp peptide" or "RGD peptide" is intended to refer to a peptide having one or more Arg-Gly-Asp containing sequence which may function as a binding site for a receptor of the "Arg-Gly-Asp family of receptors", e.g., an integrin. Integrins, which comprise and alpha and a beta subunit, include numerous types including $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$, $\alpha_8\beta_1$, $\alpha_9\beta_1$, $\alpha_1\beta_1$, $\alpha_6\beta_4$, $\alpha_4\beta_7$, $\alpha_D\beta_2$, $\alpha_D\beta_2$, $\alpha_L\beta_2$, $\alpha_M\beta_2$,$\alpha_v\beta_1$,$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_x\beta_2$, $\alpha_{IIb}\beta_3$, $\alpha_{IELb}\beta_7$, and the like. The sequence RGD is present in several matrix proteins and is the target for cell binding to matrix by integrins. For example, integrin $\alpha_v\beta_3$ or $\alpha_v\beta_5$ binds to an RGD motif in fibrinogen, fibrin, von Willebrand factor, thrombospondin, collagen, fibronectin and osteopontin.

[0051] Platelets contain a large amount of RGD-cell surface receptors of the protein GP II_b/III_a, which is primarily responsible, through interaction with other platelets and with the endothelial surface of injured blood vessels, for the development of coronary artery thrombosis. The term RGD peptide also includes amino acids that are functional equivalents (e.g., RLD or KGD) thereof provided they interact with the same RGD receptor. Peptides containing RGD sequences can be synthesized from amino acids by means well known in the art, using, for example, an automated peptide synthesizer, such as those manufactured by Applied Biosystems, Inc., Foster City, Calif.

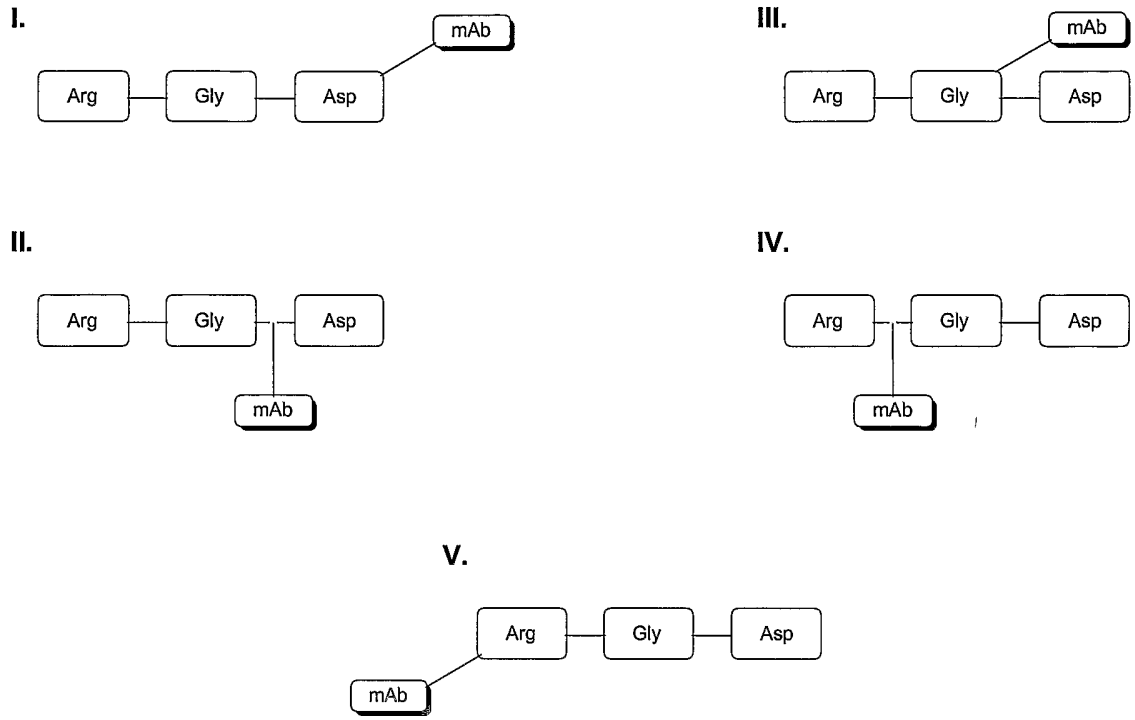
[0052] As used herein, "non-RGD" peptide refers to a peptide that is an antagonist or agonist of integrin binding to its ligand (e.g. fibronectin, vitronectin, laminin, collagen etc.) but does not involve an RGD binding site. Non-RGD integrin peptides are known for $\alpha_v\beta_3$ (see, e.g., U.S. Pat. Nos. 5,767,071 and 5,780,426) as well as for other integrins such as $\alpha_4\beta_1$ (VLA-4), $\alpha_4\beta_7$ (see, e.g., U.S. Pat. Nos. 6,365,619; Chang et al., Bioorganic & Medicinal Chem Lett, 12:159-163 (2002); Lin et al., Bioorganic & Medicinal Chem Lett, 12:133-136 (2002)), and the like.

[0053] An integrin targeting agent may be a peptidomimetic agonist or antagonist, which preferably is a peptidomimetic agonist or antagonist of an RGD peptide or non-

RGD peptide. As used herein, the term "mimetic" or "peptidomimetic" is an organic compound containing non-peptidic structural elements that are capable of mimicking or antagonizing the biological action(s) of a natural parent peptide. A peptidomimetic of an RGD peptide is an organic molecule that retains similar peptide chain pharmacophore groups of the RGD amino acid sequence but lacks amino acids or peptide bonds in the binding site sequence. Likewise, a peptidomimetic of a non-RGD peptide is an organic molecule that retains similar peptide chain pharmacophore groups of the non-RGD binding site sequence but lacks amino acids or peptide bonds in the binding site sequence. A "pharmacophore" is a particular three-dimensional arrangement of functional groups that are required for a compound to produce a particular response or have a desired activity. The term "RGD peptidomimetic" is intended to refer to a compound that comprises a molecule containing the RGD functional groups supported by an organic/non-peptide structure. An RGD peptidomimetic thus comprises a pharmacophore group representing the arginnine side chain, a pharmacophore group representing the aspartic acid side chain and a spacer between these two pharmcophores representing the function of glycine in the natural RGD peptide. It is understood that an RGD peptidomimetic (or non-RGD peptidomimetic) may be part of a larger molecule that itself includes conventional or modified amino acids linked by peptide bonds.

[0054] The linker of the integrin targeting agent may be placed in any of various locations of the RGD peptidomimetic. Where the polymer is an antibody, the linker may be placed as shown in the scheme below.

RGD Mimetic - Antibody Linking Scheme



[0055] Approach I shows the monoclonal antibody (mAb) attached through the Asp mimic, approach II shows the monoclonal antibody attached to the chemical structure that links the Asp pharmacophore group to the Gly mimic, appraoch III shows the monoclonal antibody attached through the Gly mimic, approach IV shows the monoclonal antibody attached to the chemical structure that links the Gly mimic to the Arg pharmacophore group, while approach V shows the monoclonal antibody attached through the Arg pharmacophore group.

[0056] RGD peptidomimetics are well known in the art, and have been described with respect to integrins such as GPIIb/IIIa, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (See, e.g., Miller et al., J. Med. Chem. 2000, 43:22-26; and International Patent Publications WO 0110867, WO 9915178, WO 9915170, WO 9815278, WO 9814192, WO 0035887, WO 9906049, WO 9724119 and WO 9600730; see also Kumar et al., Cancer Res. 61:2232-2238 (2000)). Many such compounds are specific for more than one integrin. RGD peptidomimetics are generally based on a core or template (also referred to as "fibrinogen receptor antagonist template"), to which are linked by way of spacers to an acidic group at one end and a basic group at the other end of the core. The acidic group is generally a

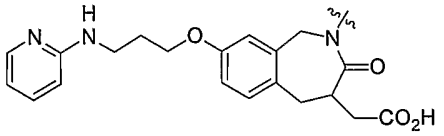
carboxylic acid functionality while the basic group is generally a N-containing moiety such as an amidine or guanidine. Typically, the core structure adds a form of rigid spacing between the acidic moiety and the basic nitrogen moiety, and contains one or more ring structures (e.g., pyridine, indazole, etc.) or amide bonds for this purpose. For a fibrinogen receptor antagonist, generally, about twelve to fifteen, more preferably thirteen or fourteen, intervening covalent bonds are present (via the shortest intramolecular path) between the acidic group of the RGD peptidomimetic and a nitrogen of the basic group. The number of intervening covalent bonds between the acidic and basic moiety is generally shorter, two to five, preferably three or four, for a vitronectin receptor antagonist. The particular core may be chosen to obtain the proper spacing between the acidic moiety of the fibrinogen antagonist template and the nitrogen atom of the pyridine. Generally, a fibrinogen antagonist will have an intramolecular distance of about 16 angstroms (1.6 nm) between the acidic moiety (e.g., the atom which gives up the proton or accepts the electron pair) and the basic moiety (e.g., which accepts a proton or donates an electron pair), while a vitronectin antagonist will have about 14 angstroms (1.4 nm) between the respective acidic and basic centers. Further description for converting from a fibrinogen receptor mimetic to a vitronectin receptor mimetic can be found in U.S. Pat. No. 6,159,964.

[0057] The peptidomimetic RGD core can comprise a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S. The ring system may be unsubstituted or may be substituted on a carbon or nitrogen atom. Preferred core structures with suitable substituents useful for vitronectin binding include monocyclic and bicyclic groups, such as benzazapine described in WO 98/14192, benzdiazapine described in U.S. 6,239,168, and fused tricyclics described in U.S. 6,008,213.

[0058] U.S. Pat. No. 6,159,964 contains an extensive list of references in Table 1 of that document which disclose RGD peptidomimetic cores structures (referred to as fibrinogen templates) which can be used for prepraring RGD peptidomimetics. Preferred vitronectin RGD and fibronectin RGD peptidomimetics are disclosed in U.S. Patent Nos. 6,335,330; 5,977,101; 6,088,213; 6,069,158; 6,191,304; 6,239,138;

6,159,964; 6,117,910; 6,117,866; 6,008,214; 6,127,359; 5,939,412; 5,693,636; 6,403,578; 6,387,895; 6,268,378; 6,218,387; 6,207,663; 6,011,045; 5,990,145; 6,399,620; 6,322,770; 6,017,925; 5,981,546; 5,952,341; 6,413,955; 6,340,679; 6313,119; 6,268,378; 6,211,184; 6,066,648; 5,843,906; 6,251,944; 5,952,381; 5,852,210; 5,811,441; 6,114,328; 5,849,736; 5,446,056; 5,756,441; 6,028,087; 6,037,343; 5,795,893; 5,726,192; 5,741,804; 5,470,849; 6,319,937; 6,172,256; 5,773,644; 6,028,223; 6,232, 308; 6,322,770; 5,760,028.

[0059] Exemplary RGD peptidomimetic integrin targeting agents are shown below as compounds 1, 2, and 3 can be used for preparing an intregrin targeting compound of the present invention. These compounds reflect linking scheme I or II. In the three compounds, the linker is attached as indicated to the nitrogen of the seven membered ring. Other RGD peptidomimetic integrin targeting agents include compound 33, wherein P and L or carbon or nitrogen. The linker may be R_1 or R_2 while the R_3 group includes a basic group such as an –NH group. In some embodiments, the R_3 group is as shown in compounds 1, 2, or 3. In some embodiments, the R_3 group includes a heterocyclic group such a benzimidazole, imidazole, pyridine group, or the like. In some such embodiments, the R_3 group is a alkoxy group, such as a propoxy group or the like, that is substituted with a heterocyclyl group that is substituted with an alkylamine group, such as a methylamino group or the like, whereas in other embodiments, the R_3 group is an alkoxy group, such as a propoxy group or the like, substituted with a heterocyclylamino group, such as with a pyridinylamino group or the like such as a 2-pyridinylamino group. In other embodiments R_3 is a group of formula –C(=O)Rb where Rb is selected from –N(alkyl)-alkyl-heterocyclyl groups such as –N(Me)-CH_2-benzimidazole groups and the like.



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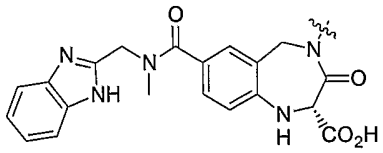

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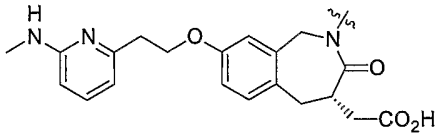

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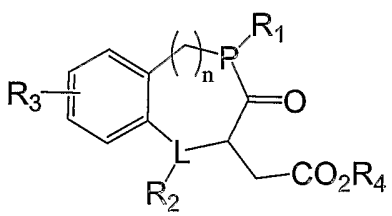
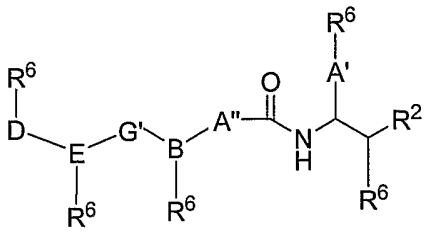


n = 0-5

33

[0060] Referring to 33, the linker may be any of R_1, R_2, R_3, while R_4 may be a linker or a hydrolyzable group such as alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group, and the like. One of skill in the art will readily appreciate that other integrin agonist and antagonist mimetics can also be used in targeting compounds of the present invention. Other exemplary integrin peptidomimetic targeting agents and a peptide targeting agent are shown in FIG. 1.

[0061] An integrin RGD peptidomimetic targeting agent – linker conjugate is provided as shown by formula I,



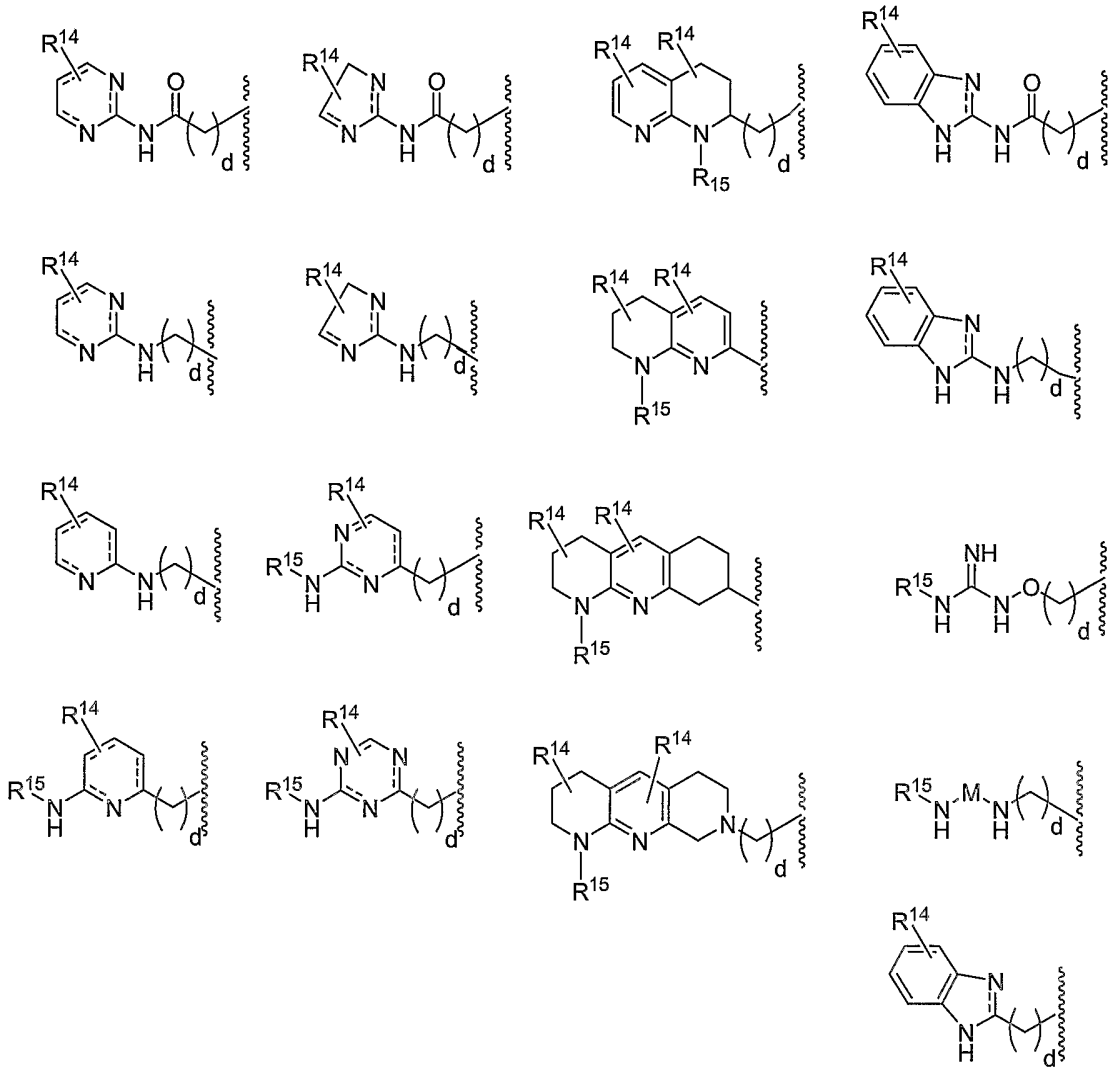
Formula I

[0062] The invention also provides stereoisomers, tautomers, and pharmaceutically acceptable salts of the compound of formula I.

[0063] In compounds having formula I, G' is a covalent bond or $-(CH_2)_a-T-(CH_2)_b-$ wherein T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or -C(N)R'''-, and a and b are each independently 0 – 4, provided that the sum of a and b is not more than 6; In some embodiments, a is 0, and in others b is 0. Preferably, the sum of a and b is 0. R''' is -H, or substituted or unsubstituted C_{1-6} alkyl.

[0064] In compounds having formula I, D is $-(CH_2)_d-L'-U$ wherein U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents; L' is absent or is -O-, -NH-, or -C(O)-; and d is 0 to 4, provided that the sum of a, b, and d is not more than 8; D is substituted by R^6 at one of U, L', or $-(CH_2)_d-$. Thus, R^6 may be attached at any carbon or heteroatom of D in place of a bond to H. In some embodiments d is 1 to 4, and in others it is 3 or 4. Preferably, d is 4. In some such embodiments of compounds having formula I, U is substituted or unsubstituted heterocyclyl, particularly, a nitrogen-containing heterocyclyl. In other such embodiments, U is substituted or unsubstituted amidine, or substituted or unsubstituted guanidine. In cases where D is amidine or guanidine and E is amidine or guanidine, D and E will be separated by at least one methylene group.

[0065] In some embodiments of compounds having formula I, D is a structure selected from:

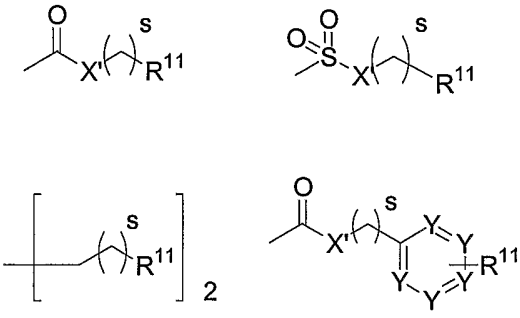


wherein,

M is -C(=O)-, -C(=S)-, -C(=NR'''')-, -C(=N-C(=O)-O-(C_{1-6} alkyl)), or -C(=N-C(=O)-O-(C_{1-6} alkyl)(C_{6-14} aryl));

R^{14} is -H, halogen, -OR^{16}, -SR^{16}, -CN, -$NR^{16}R^{17}$, -NO_2, -CF_3, $CF_3S(O)_r$-, -CO_2R^{16}, -$C(O)R^{16}$, -$C(O)NR^{16}{}_2$, C_{1-10} alkyl optionally substituted by halogen, -$O(CH_2)_{0-1}CF_3$, or $R^{18}S(O)_2O$-;

R^{15} is –H or optionally substituted C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} cycloalkyl, C_{3-8} heterocyclyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} heterocyclyl-C_{1-6} alkyl, aryl C_{1-6} alkyl, or a structure selected from the group consisting of,



wherein

X' is NH, O, or CH_2;

each Y is independently CH or, at up to 3 occurrences, N; and

s is 0 to 5;

R^{16} is, at each occurrence, independently H, C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

R^{17} is, at each occurrence, independently R^{16}, -C(O)R^{16} or -C(O)OR^{16} ;

R^{18} is, at each occurrence, independently C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

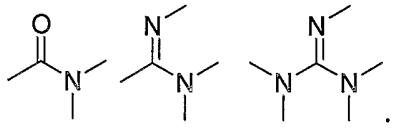
each dashed line is either absent or represents a single bond (as part of a double bond); and

D is substituted by R^6 in place of hydrogen at a CH or NH.

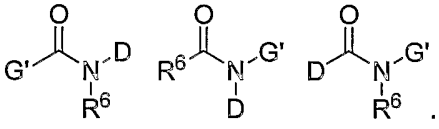
[0066] In other embodiments, D is a structure selected from above, wherein R^{14} is -H, halogen, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} heterocyclyl, C_{3-8} cycloalkyl-C_{1-6} alkyl, C_{3-8} heterocyclyl-C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_q$ amino, $(C_{1-6}$ alkyl$)_q$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy,

hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, $(C_{1-8}$ alkyl$)_q$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, $(C_{1-8}$ alkyl$)_q$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl$)_q$ amino, $(aryl)_q$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino; q is 0,1, or 2; and R^{15} is as defined above.

[0067] In compounds having formula I, E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11}, and wherein when U is amidine or guanidine and E is amidine or guanidine, d is greater than zero. Substituents G', D and R^6 may be attached at any open valence in an E moiety. For example, when E is amide, amidine, or guanidine, G', D, and R^6 may be attached to any atom of the structure having an unfilled valence as indicated by lines in the structures below:



Thus, when E is amide, G', D, and R^6 may be attached to the amide as shown in the following structures:



Any remaining unfilled valences beyond those necessary for C, D, and R^6 are filled by hydrogen or other substituents such as methyl, ethyl, or, where the unfilled valence occurs on a nitrogen, N-protecting groups. For example, when E is carbon, the carbon may bear a hydrogen atom as well as C, D, and R^6. Typically, hydrogen fills any positions on E not occupied by C, D, and R^6.

[0068] In compounds of formula I, R^{11} is H, halogen, $-OR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R'$, $-CONR'_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$-. Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl. Thus, Q-C_{0-6} alkyl refers to an alkyl group having

from 0 to 6 carbons wherein at any position a carbon-hydrogen bond is replaced with a carbon-Q bond. Similarly, Q-C_{1-6} oxoalkyl refers to an oxoalkyl group having from 1 to 6 carbons wherein at any position a carbon-hydrogen bond is replaced with a carbon-Q bond, and so forth.

[0069] In compounds of formula I, R^{12} is R', -C(O)-R', -C(O)-NR'$_2$, -C(O)-OR^{13}, -S(O)$_o$-R' or S(O)$_o$-NR'$_2$. R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl. R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl. R" is R', -C(O)R' or -C(O)OR^{13}.

[0070] In compounds of formula I, R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7. In some embodiments, R^2 is an aspartic acid pharmacophore which is carboxylic acid or an ester thereof or a chemical isostere of carboxylic acid. As used herein, isosteres are molecules or ions of similar size containing the same number of atoms and valence electrons, e.g., O^{2-}, F^-, Ne. In preferred embodiments, R^2 is –COOH.

[0071] In compounds having formula I, R^7 is –C(O)R^8, -C(O)-CR'$_2$-R^9, -C(S)R^8, -S(O)$_o$ OR', -S(O)$_o$ NR'R", -PO(OR'), -PO(OR')$_2$, -B(OR')$_2$, -NO_2, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole. R^8 is -OR', -NR'R", -NR'SO_2 R', -NR'OR', -OCR'$_2$C(O)OR', -OCR'$_2$OC(O)-R', -OCR'$_2$C(O)NR'$_2$, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group. R^9 is -OR', -CN, -S(O)$_r$R', -S(O)$_o$NR'$_2$, -C(O)R'C(O)NR'$_2$, or -CO_2R'.

[0072] "Amino acid" as referred to herein is an amino acid with its carboxyl group optionally protected, wherein the amino acid may be any of the natural or synthetic α or β-amino acids or penicillamine. The unprotected carboxyl group is a free carboxylic acid group. Protecting groups for the carboxyl are esters or amides which are formed, for instance, when the OH of the carboxy group is replaced by R^8. The amino acid also may have its amino group optionally protected. Amino protecting groups are well

known in the art, for instance, when the amino group is substituted by R^{12}. An unprotected amino group is a free NH_2 group.

[0073] In compounds of formula I, R^6 may be a linker moiety that can connect the peptidomimetic targeting agent to a polymer. R^6 typically has the formula -J-G-K, wherein,

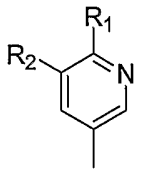
J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K is a reactive group; and wherein

if J is an unsubstituted C_{1-21} alkyl and K is $-NH_2$, -SH, $-OS(OH)_3$, or -COOH, G is present.

[0074] In compounds of formula I, R^6 is, at a first occurrence, the linker moiety, and is at a second occurrence R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21} and is, at a fifth occurrence, R^{24}. R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl. In some embodiments, one or more of R^{19}, R^{20} and R^{21} is

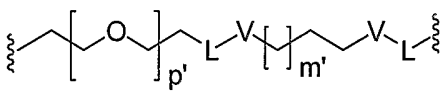


wherein

R_1 and R_2 are independently -H, halogen, $-OR^{12}$, -CN, $-NR^8R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R^8$, $-CONR^8{}_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-,

Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$-, or R_1 and R_2 together form a fused unsubstituted or substituted aryl.

[0075] In some embodiments of compounds having formula I wherein R^6 has the formula -J-G-K, J is substituted. In other embodiments, J includes substituted or unsubstituted cycloalkyl in the backbone; typically the cycloalkyl is cyclohexyl. In other embodiments, J is optionally substituted and has the formula: -$[CH_2\text{-}L]_{n'}$-, wherein n' is 1-100 and L is, at each occurrence, independently -CH_2-, -O-, -NR'-, -SiR'_2-, -$S(O)_{0-2}$-, or a covalent bond. In some such embodiments, L is, at each occurrence, independently -CH_2-, -O-, -NR'-, or a covalent bond. In still other embodiments, J has the formula:

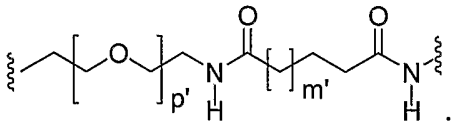


wherein

V is –C(O)-, -CH_2-, or -C(S)-; and

m' and p' are each independently 1 up to about 50, such that the backbone length of J remains about 200 atoms or less.

[0076] The backbone length of J is measured as the shortest chain of atoms directly connecting, for example,E to G. In some such embodiments, the backbone length of J is about 100 atoms or less, and typically 30 atoms or less. In a preferred embodiment, J has the structure:



[0077] In some embodiments of compounds of formula I, J is substituted or unsubstituted and is selected from the group consisting of -R^{22}- $[CH_2\text{-}CH_2\text{-}O]_t$- R^{23}-, -R^{22}-cycloalkyl- R^{23}-, -R^{22}-aryl- R^{23}-, and -R^{22}-heterocyclyl-R^{23}- wherein

R^{22} and R^{23} are independently a covalent bond, -O-, -S-, $-NR^a-$, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

R^a, at each occurrence, is independently hydrogen, substituted or unsubstituted C_{1-10} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-6} alkyl, or substituted or unsubstituted aryl–C_{0-6} alkyl;

t = 0-50;

and the size of R^{22} and R^{23} are such that the backbone length of J remains about 200 atoms or less.

[0078] In some such embodiments,

R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-O-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_u-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_u-NR^a-(CH_2)_v-$, $-(CH_2)_u-O-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-NR^a-(CH_2)_v-$, or $-(CH_2)_u-P(O)(OR^a)-O-(CH_2)_v-$;

R^{23} is -O-, -S-, $-NR^a-$, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

u and v are independently 0-20;

and the values of t, u, and v are such that the backbone length of J remains about 200 atoms or less.

[0079] In other such embodiments, R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-O-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_u-NR^a-(CH_2)_v-$, $-(CH_2)_u-O-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-(CH_2)_v-$, or $-(CH_2)_u-S(O)_{0-2}-NR^a-(CH_2)_v-$. Typically, R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, or $-(CH_2)_u-NR^a-(CH_2)_v$. In still other such embodiments, R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkylene or substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene.

[0080] In certain embodiments of compounds of formula I, J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl in the backbone. In some such emodiments, J has the formula

$$-R^{22}-[CH_2-CH_2-O]_t - R^{23}-$$

wherein

R^{22} is $-(CH_2)_v-$, $-C(O)-(CH_2)_v-$, $-C(O)-O-(CH_2)_v-$, $-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(O)-NR^a-$, $-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(S)-NR^a-$, $-NR^a-(CH_2)_v-$, $-O-(CH_2)_v-$, $-S(O)_{0-2}-(CH_2)_v-$, $-S(O)_{0-2}-NR^a-(CH_2)_v-$, $-(CH_2)_v-S(O)_{0-2}-NR^a-$, or $-P(O)(OR^a)-O-(CH_2)_v-$;

R^a, at each occurrence, is independently -H, substituted or unsubstituted C_{1-8} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl–C_{0-4} alkyl;

R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkyl, substituted or unsubstituted straight or branched chain C_{1-49} heteroalkyl, or substituted or unsubstituted straight or branched chain C_{2-50} alkenyl;

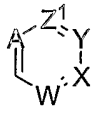
t = 2-50; and

v = 0-20;

and wherein the values of t and v are such that the backbone length of J remains about 200 atoms or less.

[0081] In compounds having formula I wherein R^6 has the formula -J-G-K, the ring structure of G includes saturated, unsaturated, and aromatic carbocyclic rings and saturated, unsaturated, and aromatic heterocyclic rings. The ring structures may be mono-, bi-, or polycyclic, and include fused or unfused rings. Further, the ring structures are optionally substituted with functional groups well known in the art including, but not limited to halogen, oxo, -OH, -CHO, -COOH, $-NO_2$, -CN, $-NH_2$, $-C(O)NH_2$, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, sulfoalkynyl, phosphoalkyl, phosphoalkenyl, and phosphoalkynyl groups.

[0082] In some embodiments of compounds having formula I wherein R^6 has the formula –J-G-K, the ring structure of G has the optionally substituted formula:



or


wherein,

A, W, X, Y, and Z^1 are independently carbon or nitrogen;

Z^2 is carbon, nitrogen, oxygen, or sulfur; and

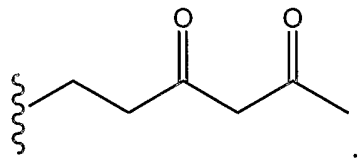
G is attached to J and K independently through a covalent bond or substituted or unsubstituted C_{1-6} alkyl or heteroalkyl; wherein

no more than four of A, W, X, Y, Z^1, or Z^2 are simultaneously nitrogen.

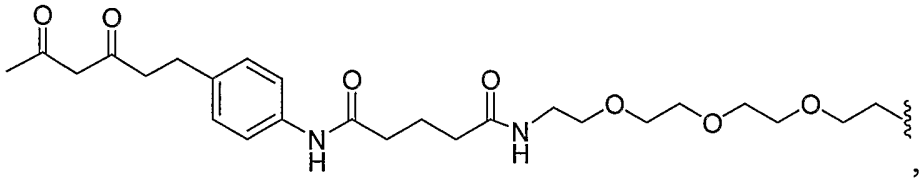
[0083] Any open valences remaining on atoms constituting the ring structure may be filled by hydrogen or other substituents, or by the covalent attachments to J and K. For example, if Z^1 is carbon, its valence may be filled by hydrogen, a substituent such as halogen, a covalent attachment to J, or a covalent attachment to K. In some

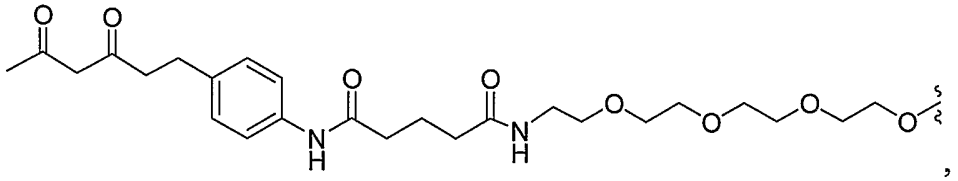
embodiments, A, W, X, Y, and Z^1 are each carbon, while in others, A, X, Y and Z^2 are each carbon. In other embodiments, at least one of A, W, X, Y, or Z^1 is nitrogen, and in still others, Z^2 is oxygen or sulfur. In yet another embodiment, the ring structure of G is unsubstituted. Typically, G is substituted or unsubstituted phenylalkyl and is attached to K at the alkyl.

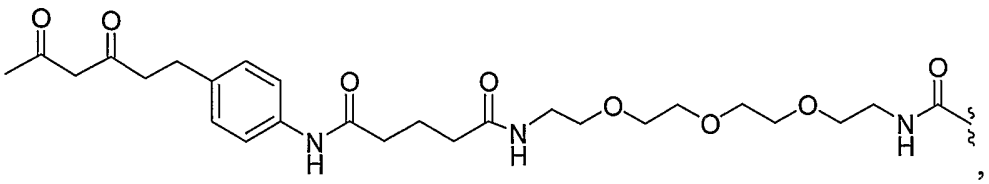
[0084] In compounds having formula I wherein R^6 has the formula –J-G-K, the reactive group K contains a moiety capable of forming a covalent linkage with a polymer such as an antibody. For example, K may be substituted alkyl, substituted cycloalkyl, substituted aryl, substituted arylalkyl, substituted heterocyclyl, or substituted heterocyclylalkyl, wherein at least one substituent is a 1,3-diketone moiety, an acyl beta-lactam, an active ester, an alpha-haloketone, an aldehyde, a maleimide, a lactone, an anhydride, an alpha-haloacetamide, or an epoxide. In some such embodiments, K is substituted alkyl. In other embodiments, the at least one substituent is a 1,3-diketone moiety. In some such embodiments, the 1,3-diketone moiety is separated from G by C_{1-5} alkyl, more preferably by C_{2-4} alkyl, and most preferably by C_2 alkyl. In some embodiments, K is alkyl substituted by a 1,3-diketone moiety and K preferably has the structure:

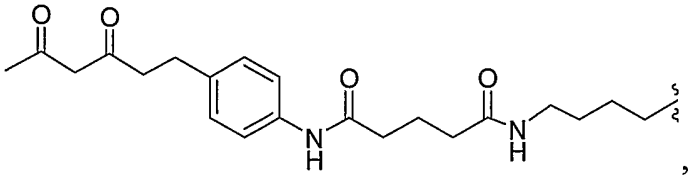


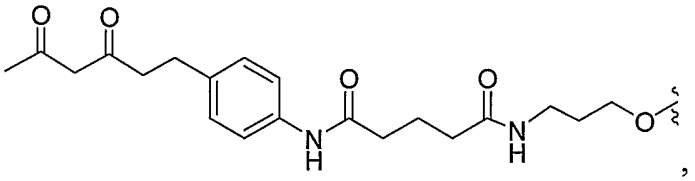
[0085] In certain embodiments of compounds of formula I, R^6 has the structure:

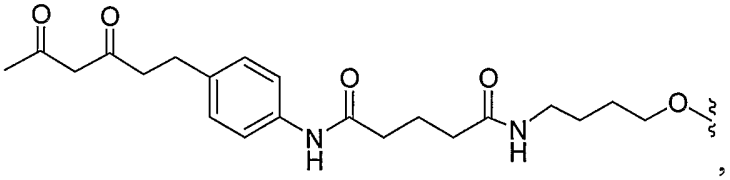


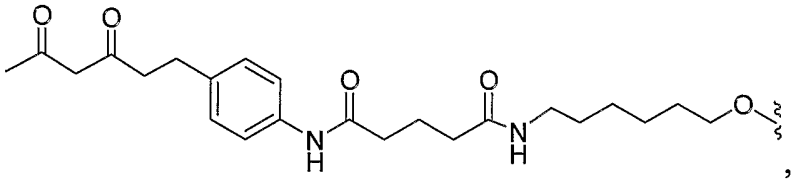


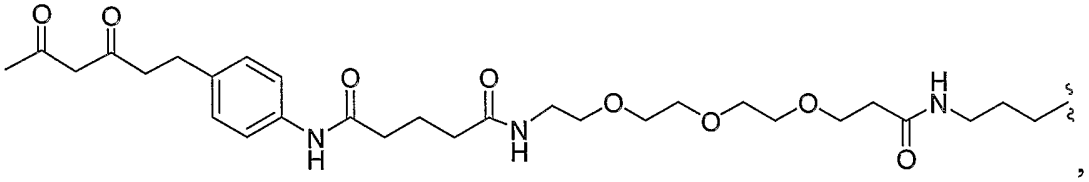


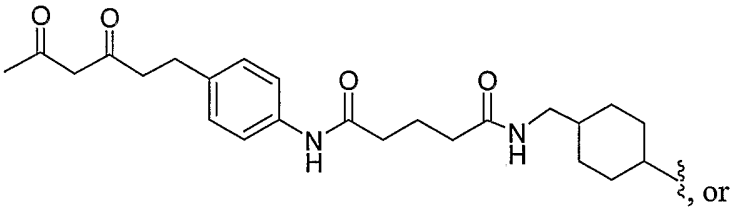


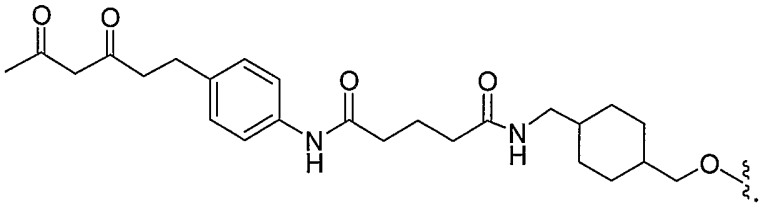




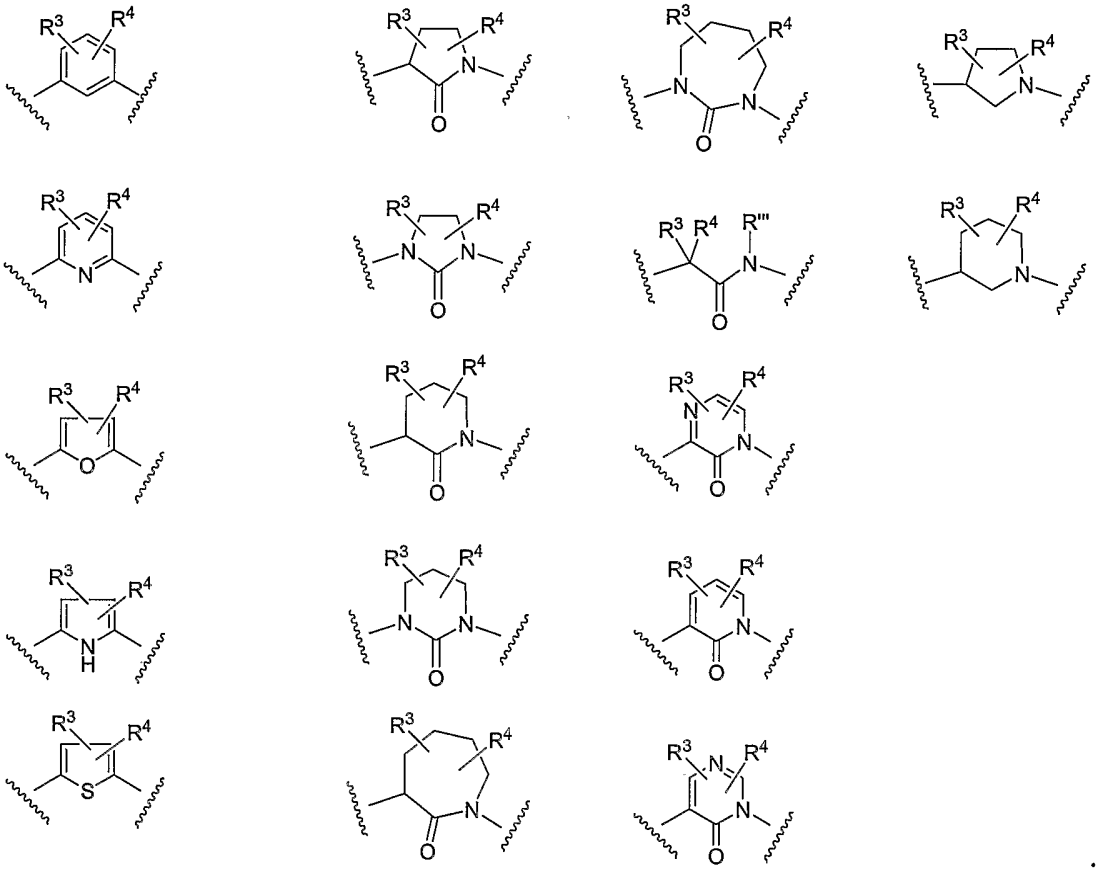




 , or

 .

[0086] In compounds having formula I, B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4. In some embodiments, B is selected from the following structures:



[0087] In compounds having formula I, R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, $(C_{1-8}$ alkyl$)_o$

aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl)$_o$ amino, (aryl)$_o$ amino, aryl C_{1-8} alkylsulfonylamino, or C_{1-8} alkylsulfonylamino.

[0088] In compounds having formula I, R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$-O-$(CH_2)_m$-, aryl-$(CH_2)_n$-S(O)$_q$-$(CH_2)_m$-, aryl-$(CH_2)_n$-C(O)-$(CH_2)_m$-, aryl-$(CH_2)_n$-C(O)-N(R^5)-$(CH_2)_m$-, aryl-$(CH_2)_n$-N(R^5)-C(O)-$(CH_2)_m$-, aryl-$(CH_2)_n$-N(R^5)-$(CH_2)_m$-, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, (C_{1-6} alkyl)$_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

[0089] In compounds having formula I, R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, (aryl)$_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, (aryl)$_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3.

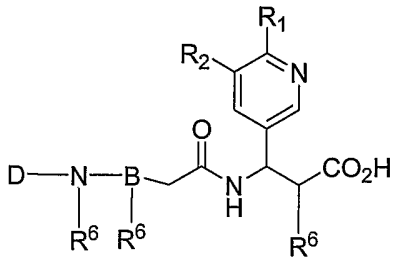
[0090] In compounds having formula I, A'' is T-$(CH_2)_j$- wherein T is -O-, -N(R''')-, -S(O)$_q$-, -CHR''-, -CH_2-, -C(O)-, -$S(O)_2$N(R''')- , or –C(N)(R''')-, or a covalent bond; and j is 0 to 3;

[0091] In compounds having formula I, A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond.

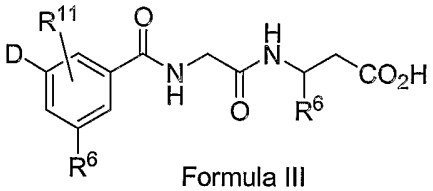
[0092] In compounds having formula I, m at each occurrence is independently 0 to 3; n at each occurrence is independently 1 to 3; o at each occurrence is independently 1

or 2; p at each occurrence is independently 1 to 4; q at each occurrence is independently 0 to 2; and r is 0 to 2.

[0093] Exemplary compound according to formula I are shown below as formulas II and III, wherein D, B, R^6 and R^{11} are as defined above.

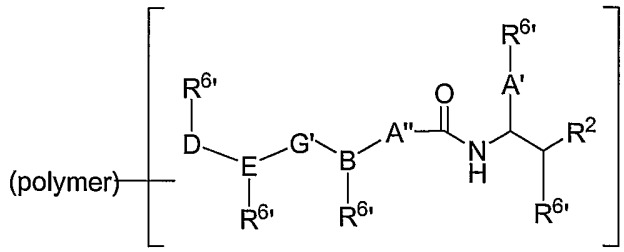


Formula II



Formula III

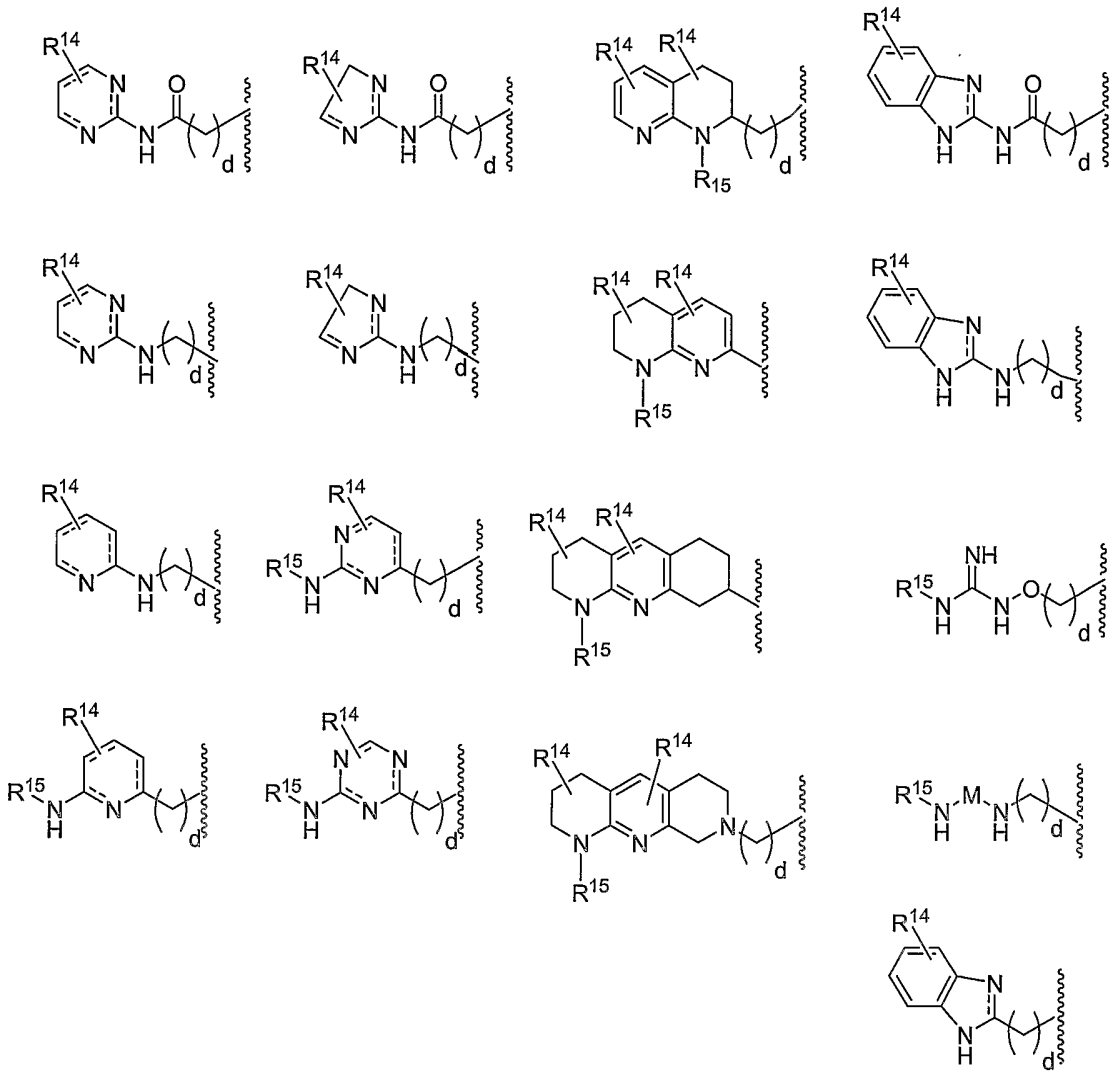
[0094] Also provided is an integrin targeting compound comprising an RGD peptidomimetic integrin targeting agent covalently linked to a polymer, e.g., a protein such as the combining site of an antibody. In a preferred embodiment, the integrin RGD peptidomimetic targeting compound is as shown by formula IV, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof:



Formula IV

[0095] In compounds having formula IV, symbols B, G', E, A', A'', and R^2 are as defined in formula I. In formula IV, D is $-(CH_2)_d$-L'-U wherein U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents; L' is absent or is -O-, -NH-, or -C(O)-; d is 0 to 4, provided that the sum of a, b, and d is not more than 8- and D is substituted by $R^{6'}$ at one of U, L', or $-(CH_2)_d$-. Thus, $R^{6'}$ may be attached at any carbon or heteroatom of D in place of a bond to H. In some embodiments d is 1 to 4, and in others it is 3 or 4. Preferably, d is 4. In some such embodiments of compounds having formula I, U is substituted or unsubstituted heterocyclyl, particularly, a nitrogen-containing heterocyclyl. In other such embodiments, U is substituted or unsubstituted amidine, or substituted or unsubstituted guanidine.

[0096] In some embodiments of compounds having formula IV, D is a structure selected from:

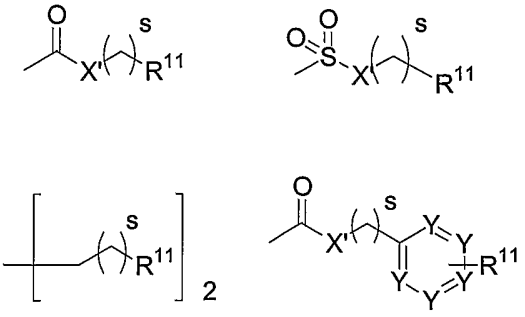


wherein,

M is -C(=O)-, -C(=S)-, -C(=NR$^{'''}$)-, -C(=N-C(=O)-O-(C_{1-6} alkyl)), or -C(=N-C(=O)-O-(C_{1-6} alkyl)(C_{6-14} aryl));

R^{14} is -H, halogen, -OR^{16}, -SR^{16}, -CN, -$NR^{16}R^{17}$, -NO_2, -CF_3, $CF_3S(O)_r$-, -CO_2R^{16}, -C(O)R^{16}, -C(O)NR^{16}_2, C_{1-10} alkyl optionally substituted by halogen, -$O(CH_2)_{0-1}CF_3$, or $R^{18}S(O)_2O$-;

R^{15} is –H or optionally substituted C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} cycloalkyl, C_{3-8} heterocyclyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} heterocyclyl-C_{1-6} alkyl, aryl C_{1-6} alkyl, or a structure selected from the group consisting of,



wherein

X' is NH, O, or CH_2;

each Y is independently CH or, at up to 3 occurrences, N; and

s is 0 to 5;

R^{16} is, at each occurrence, independently H, C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

R^{17} is, at each occurrence, independently R^{16}, -C(O)R^{16} or -C(O)OR^{16};

R^{18} is, at each occurrence, independently C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

each dashed line is absent or represents a single bond (as part of a double bond); and

D is substituted by $R^{6'}$ in place of hydrogen at a CH or NH.

[0097] In formula IV, $R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}. J and G are as defined for compounds of formula I. K' is an attachment moiety including about 1 to about 20 carbon atoms and is covalently linked to the polymer. R^{19}, R^{20}, R^{21} and R^{24} are as defined in formula I. Polymer is as defined herein.

In some embodiments of compounds having formula IV, J is an optionally substituted linear or branched connecting chain having from about 1 to about

200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl in the backbone; -J-G-K'- is attached to D or E; and polymer is an antibody comprising one or more combining sites wherein K' is covalently linked to one of the one or more combining sites. In some such embodiments, J has the formula

$$-R^{22}-[CH_2-CH_2-O]_t - R^{23}-$$

wherein

R^{22} is $-(CH_2)_v-$, $-C(O)-(CH_2)_v-$, $-C(O)-O-(CH_2)_v-$, $-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(O)-NR^a-$, $-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(S)-NR^a-$, $-NR^a-(CH_2)_v-$, $-O-(CH_2)_v-$, $-S(O)_{0-2}-(CH_2)_v-$, $-S(O)_{0-2}-NR^a-(CH_2)_v-$, $-(CH_2)_v-S(O)_{0-2}-NR^a-$, or $-P(O)(OR^a)-O-(CH_2)_v-$;

R^a, at each occurrence, is independently -H, substituted or unsubstituted C_{1-8} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl–C_{0-4} alkyl;

R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkyl, substituted or unsubstituted straight or branched chain C_{1-49} heteroalkyl, or substituted or unsubstituted straight or branched chain C_{2-50} alkenyl;

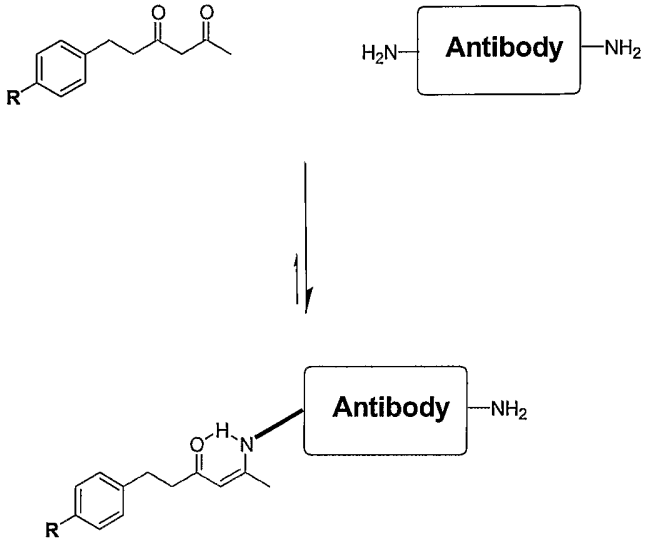
t = 2-50; and

v = 0-20;

and wherein the values of t and v are such that the backbone length of J remains about 200 atoms or less.

[0098] In compounds having formula IV, the attachment moiety K' is covalently linked to a polymer. For example, K' may be linked to an antibody at the antibody combining site. This is shown below for the case where the linker has a diketone moiety as the reactive group (see formula I) and linkage occurs with the side chain amino group of a lysine residue in the antibody combining site. The antibody is shown

schematically as bivalent with a reactive amino acid side chain for each combining site indicated.



[0099] Thus, in compounds of formula IV, typically the attachment moiety is substituted or unsubstituted straight or branched chain C_{1-20} alkyl or C_{2-20} alkenyl, or substituted or unsubstituted straight or branched chain C_{1-20} heteroalkyl or C_{2-20} heteroalkenyl. In some embodiments the attachment moiety is substituted or unsubstituted straight or branched chain C_{1-20} alkyl or C_{2-20} alkenyl, and typically, the attachment moiety is substituted or unsubstituted straight chain C_{3-10} alkyl or C_{3-10} alkenyl.

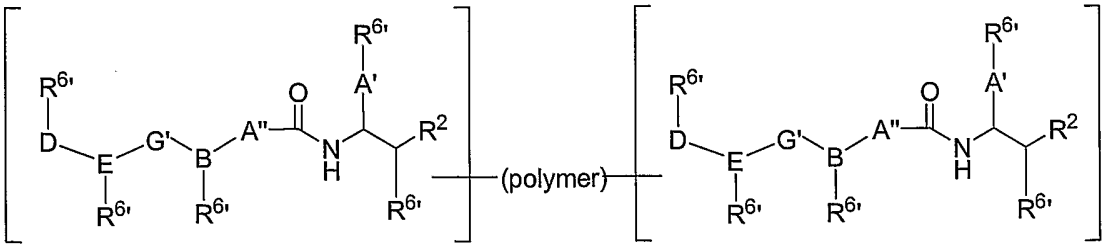
[00100] Alternatively, the linker may have an amine or hydrazide as the reactive group and the polymer may be engineered to have a diketone moiety. If the polymer is a protein such as human serum albumin, any appropriate surface accessible residue may be engineered to have a diketone moiety. Similarly if the polymer is an antibody, and the antibody combining site is to be linked, the combining site may be engineered to have a diketone moiety. An unnatural diketone-containing amino acid may be readily incorporated into an antibody or other protein using techniques well known in the art; proteins containing unnatural amino acids have been produced in yeast and bacteria See Chin et al., *Science*, 301: 964-966 (2003); Wang et al., *Science*, 292: 498 (2001); Chin et al., *J. Am. Chem. Soc.*, 124: 9026 (2002); Chin et al., *Chem. Bio. Chem.*, 3:

1135 (2002); Chin et al., *Proc. Natl. Acad. Sci. U.S.A.*, 99: 11020 (2002); Wang and Schultz, *Chem. Comm.*, 2002: 1 (2002); Zhang et al., *Angew. Chem. Int. Ed.*, 41: 2840 (2002); Wang, *Proc. Natl. Acad. Sci. U.S.A.*, 100: 56 (2003); Schultz et al. U.S. Patent Publication No. 20030082575.

[00101] Thus, for example, to insert an unnatural amino acid containing a diketone moiety into the yeast *Saccharomyces cerevisiae* requires the addition of new components to the protein biosynthetic machinery including a unique codon, tRNA, and aminoacyl-tRNA synthetase (aa RS). For example, the amber suppressor tyrosyl-tRNA synthetase (TyrRS)-$tRNA_{CUA}$ pair from *Ecoli* may be used as reported for eukaryotes in Chin et al. (2003). The amber codon is used to code for the unnatural amino acid of interest. Libraries of mutant TyrRS and $tRNA_{CUA}$ may then be produced and selected for those aaRS-$tRNA_{CUA}$ pairs in which the TyrRS charges the $tRNA_{CUA}$ with the unnatural amino acid of interest, e.g., the diketone-contining amino acid. Subsequently, mutant antibodies incorporating the diketone-containing amino acid may be produced by cloning and expressing a gene containing the amber codon at one or more antibody combining sites.

[00102] In some embodiments of compounds of Formula IV, the polymer is a protein, such as serum albumin. In another embodiment, the protein is full length, such as a full length antibody. In still other embodiments, the protein may be a fragment. In the case of an antibody, the fragment of a full length antibody is Fab, Fab' $F(ab')_2$, Fv or sFv. In other embodiments, the antibody is a human antibody, humanized antibody or chimeric human antibody. In still other embodiments, the antibody is a catalytic antibody.

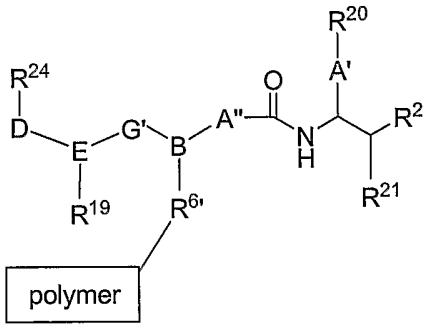
[00103] When the polymer is bivalent, such as a bivalent antibody, it may have one integrin RGD peptidomimetic attached to each combining site. An exemplary such compound is shown below as formula V.



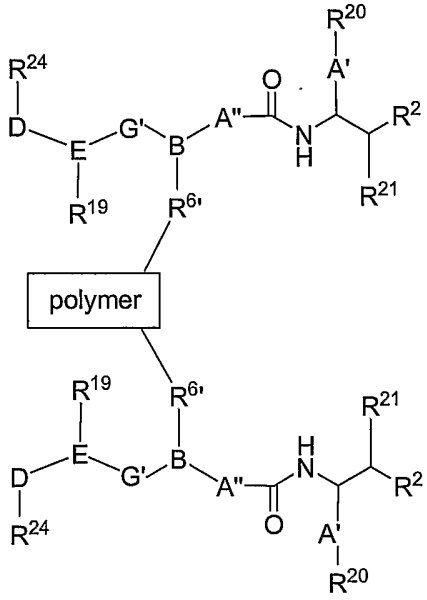
Formula V

[00104] In formula V, symbols B, D, E, G', A', A"', and R^2 are as defined in formula IV. On each targeting agent (which may be the same or different), $R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}. J, G, K', R^{19}, R^{20}, R^{21}, and R^{24} are as defined in formula IV. Polymer is as defined herein. In some embodiments, the polymer is a protein such as serum albumin or an antibody. When the polymer is an antibody it comprises two combining sites wherein each combining site is covalently linked to one attachment moiety K'.

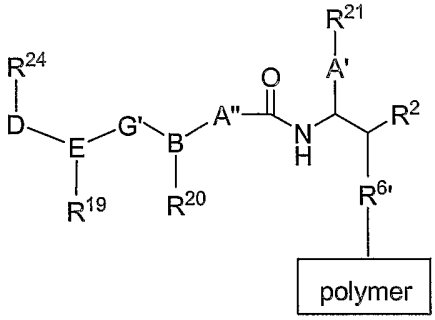
[00105] Examples showing where a polymer can be linked through $R^{6'}$ in both monovalent and bivalent forms are shown as formulas VI to XV below. (For monovalent forms, symbols B, G', D, E, K', A', A'', R^2, R^{19} R^{20}, R^{21}, and R^{24} are as in formula IV; for bivalent forms, symbols B, G', D, E, $R^{6'}$, A', A'', R^2, R^{19} R^{20}, R^{21}, and R^{24} are as in formula V.)



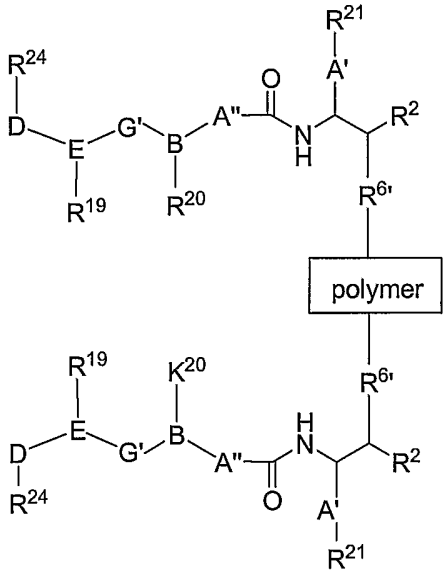
Formula VI



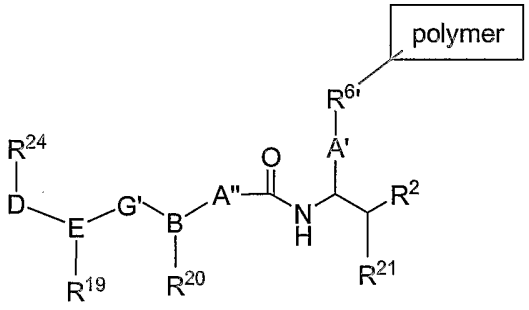
Formula VII



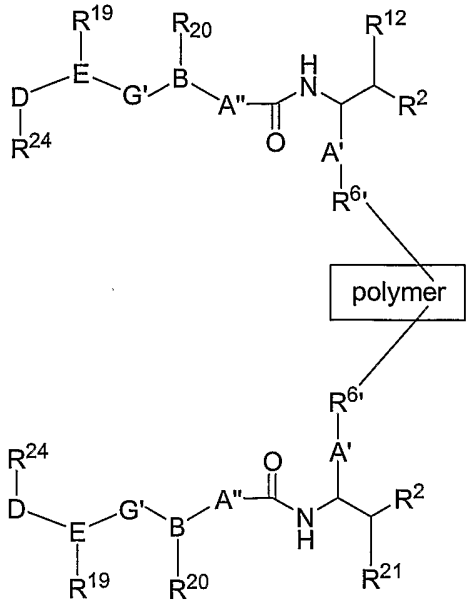
Formula VIII



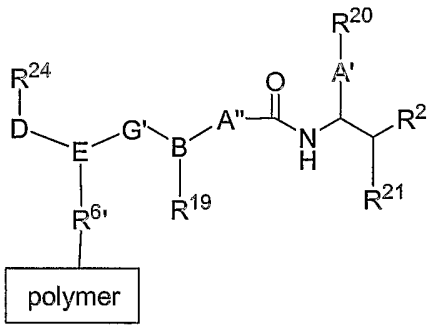
Formula IX



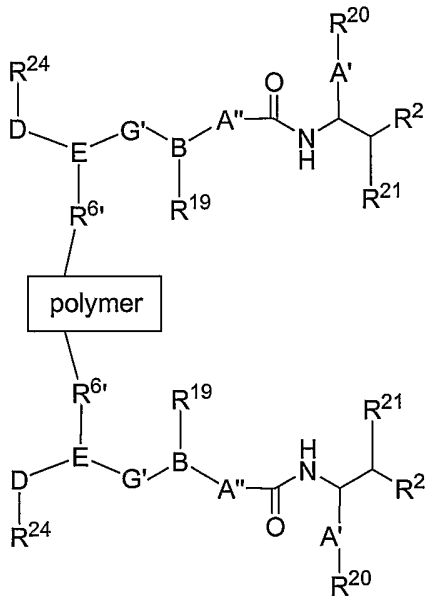
Formula X



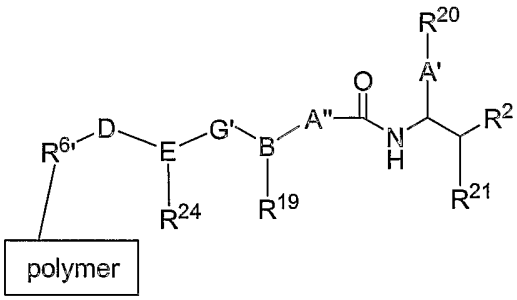
Formula XI



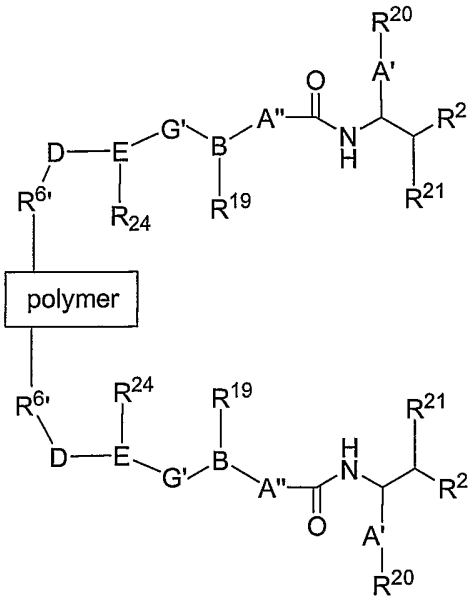
Formula XII



Formula XIII

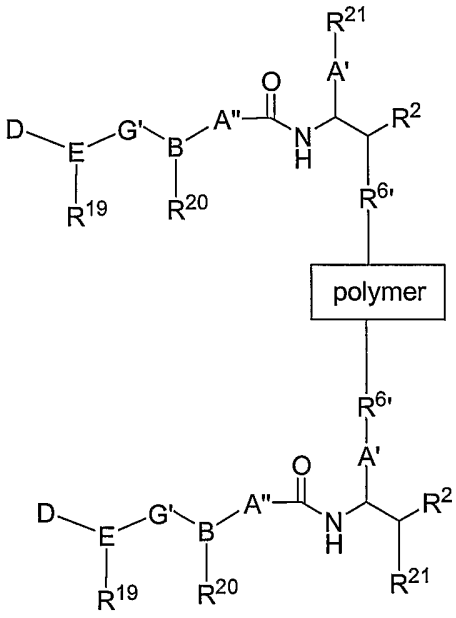


Formula XIV



Formula XV

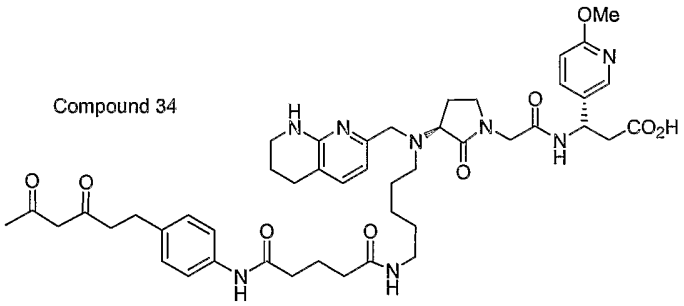
[00106] It should be understood that there is no requirement that the two RGD peptidomimetic compounds that are linked to different binding sites of a polymer, e.g., a protein such as a multivalent antibody, be identical. Thus, one may link different RGD peptidomimetics to the same polymer. Also, when different RGD peptidomimetic compounds are linked to the same antibody, they may be linked through $R^{6'}$ at a different locations in the compound. An exemplary such compound is shown below as formula XVI.



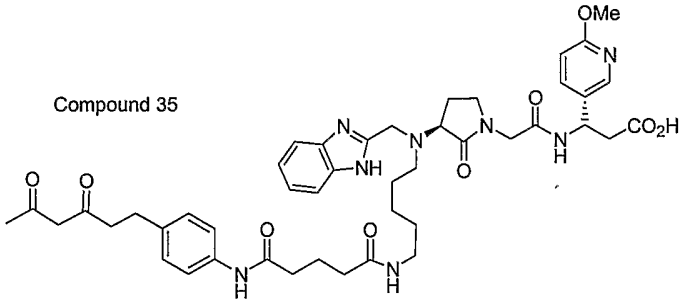
Formula XVI

[00107] Exemplary RGD peptidomimetics in accordance with formula I are shown below as compounds 34-56 and 60-62.

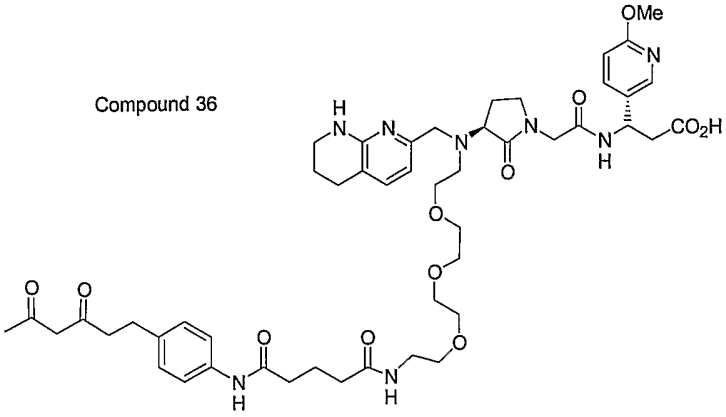
Compound 34



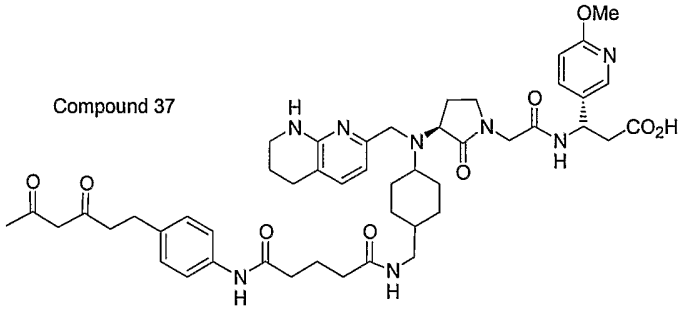
Compound 35



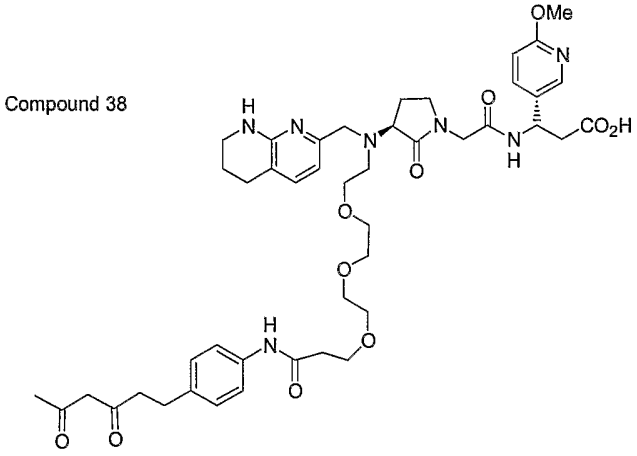
Compound 36



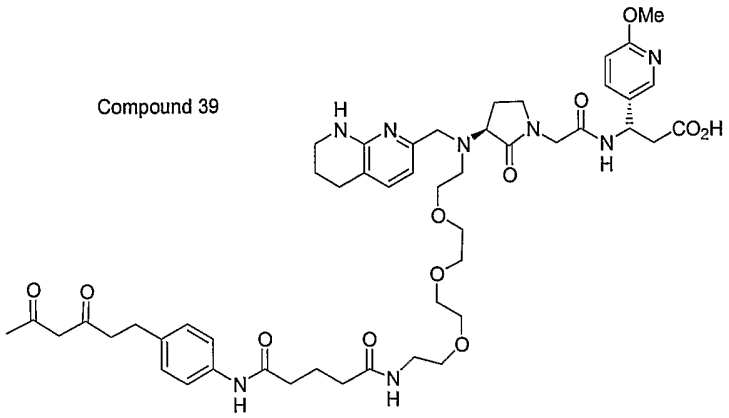
Compound 37



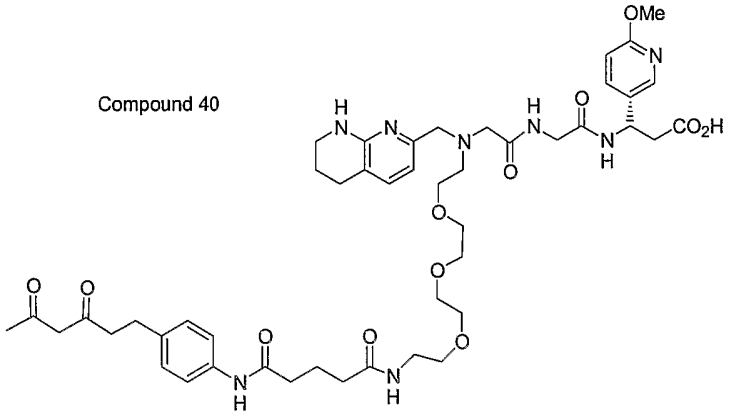
Compound 38



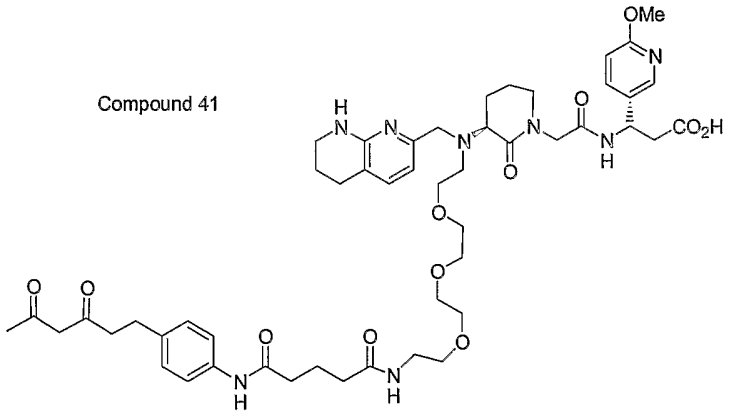
Compound 39



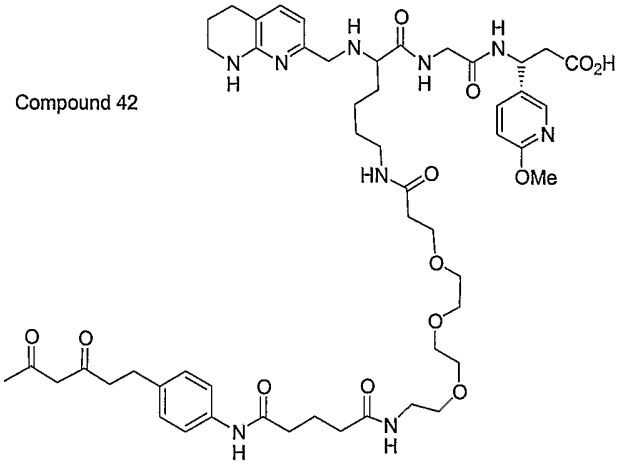
Compound 40



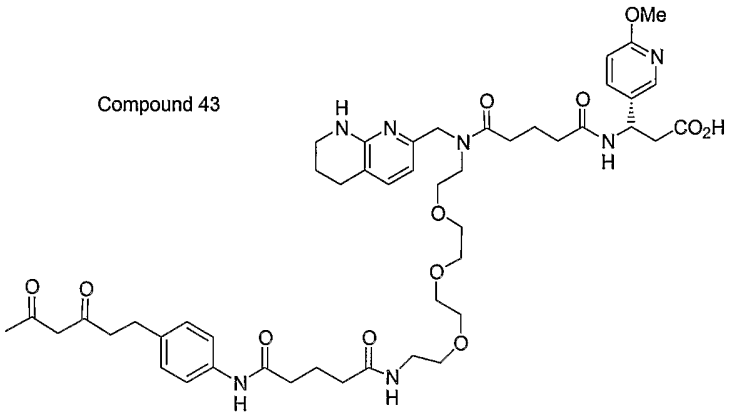
Compound 41



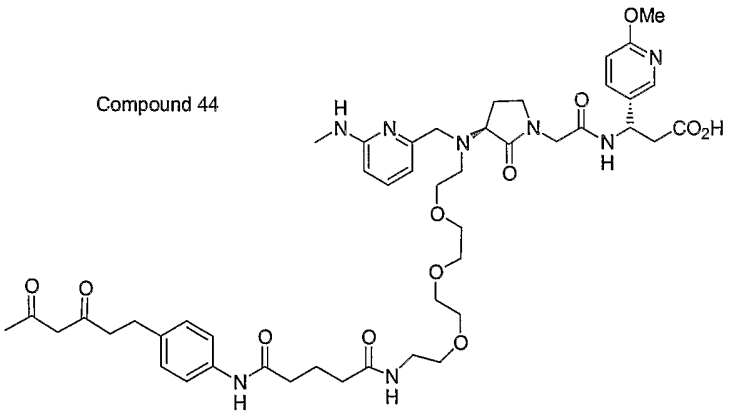
Compound 42



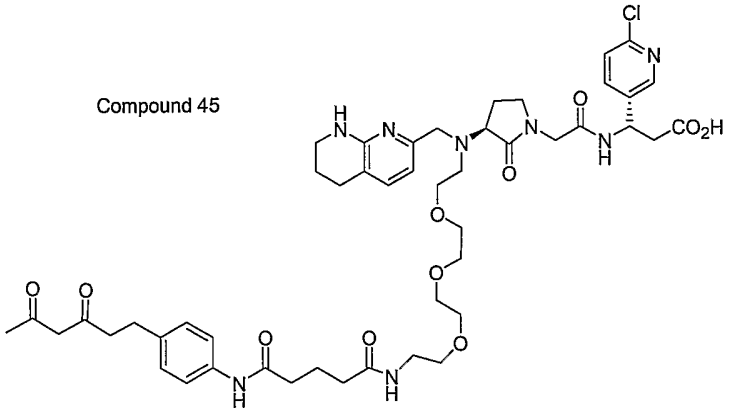
Compound 43



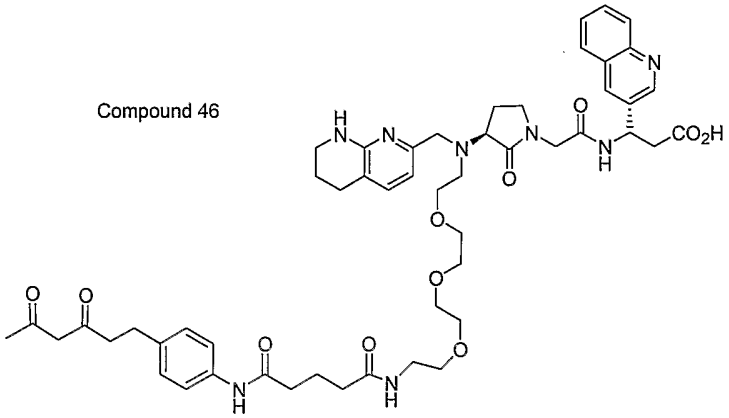
Compound 44



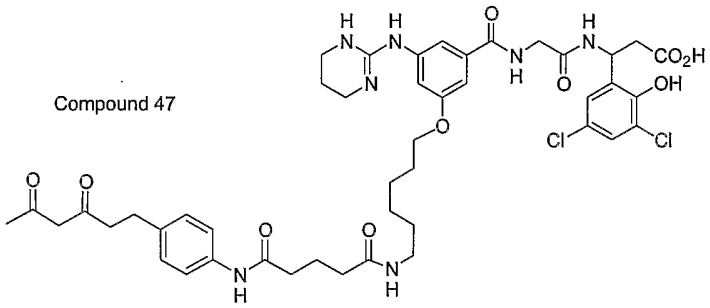
Compound 45



Compound 46



Compound 47



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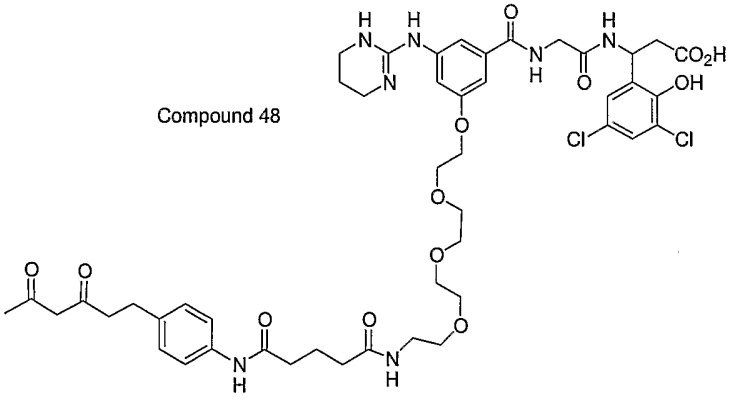

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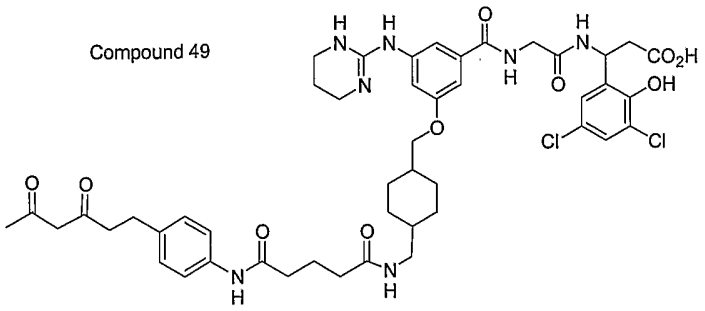
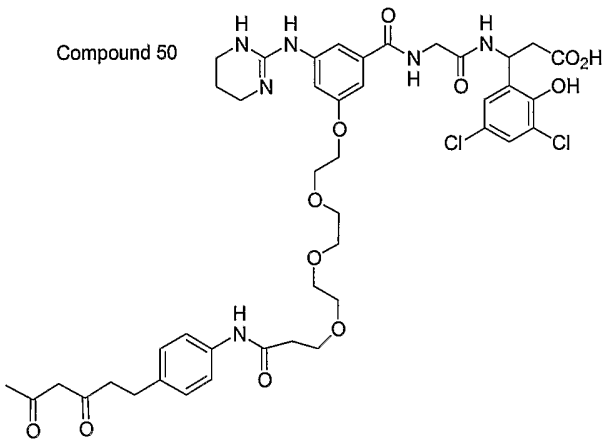
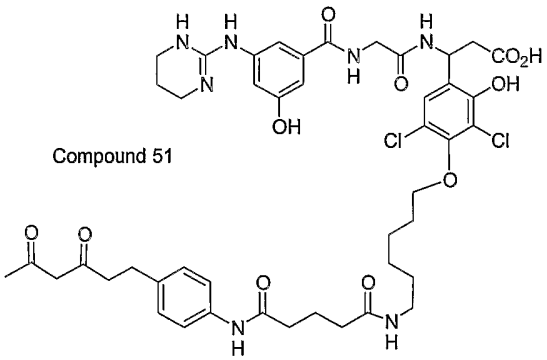


Compound 50



Compound 51



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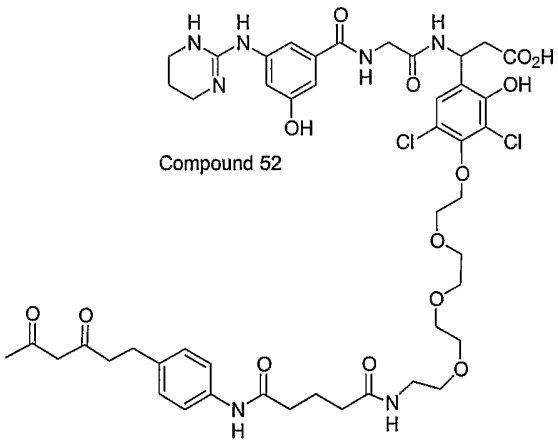
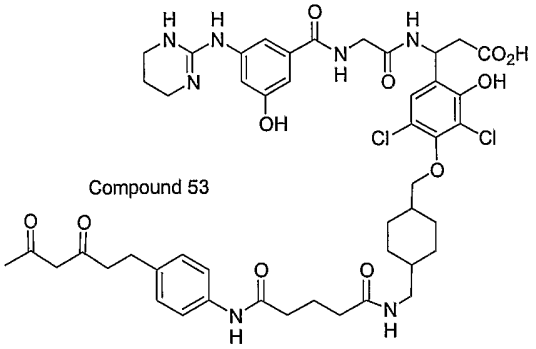
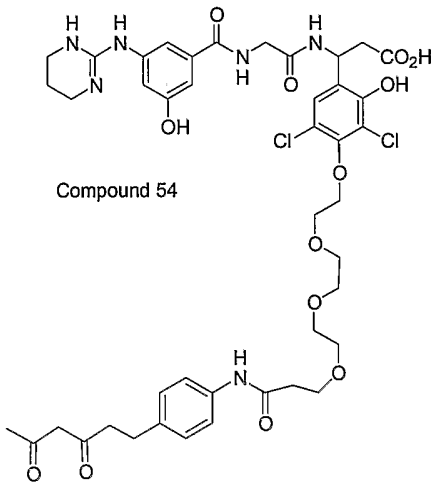
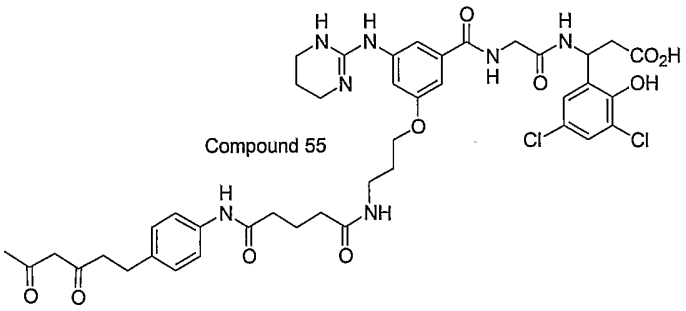




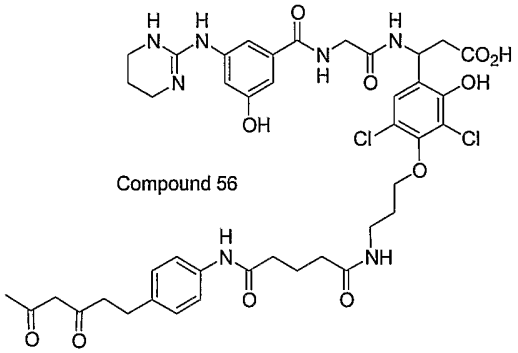
Compound 53



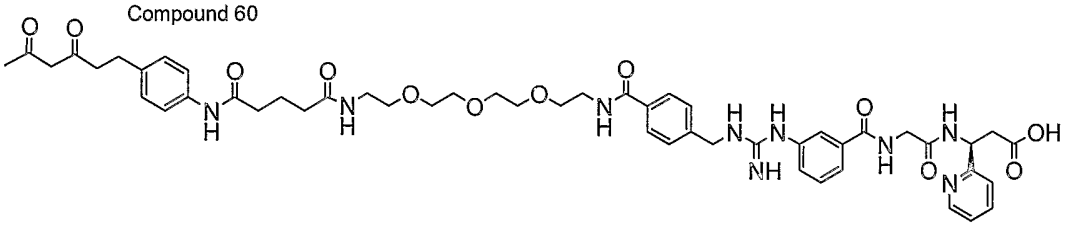
Compound 54



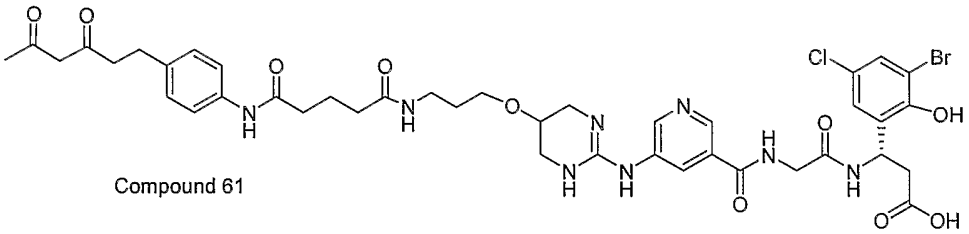
Compound 55



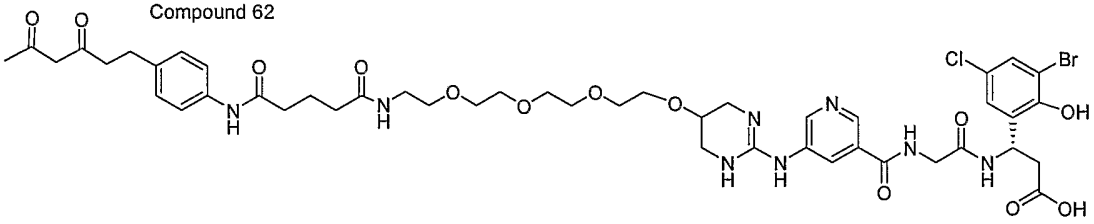
Compound 56



Compound 60



Compound 61



Compound 62

[00108] The following terms as defined below are used throughout this disclosure.

[00109] In general, "substituted" refers to a group as defined below in which one or more bonds to a hydrogen atom contained therein are replaced by a bond to non-hydrogen or non-carbon atoms such as, but not limited to, a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. Substituted alkyl groups and also substituted cycloalkyl groups and others also include groups in which one or more bonds to a carbon(s) or hydrogen(s) atom is replaced by a bond to a heteroatom such as oxygen in carbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles. As employed herein, a group which is "optionally substituted" may be substituted or unsubstituted. Thus, e.g., "optionally substituted alkyl" refers to both substituted alkyl groups and unsubstituted alkyl groups.

[00110] However, substituted functional groups such as substituted amidine, guanidine, urea, thioruea, and the like may also include substituents in place of hydrogen such as substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, and N-protecting groups, and the like.

[00111] The term "N-protecting group" or "N-protected" as used herein refers to those groups intended to protect the N-terminus of an amino acid or peptide or to protect an amino group (alone or as part of another functional group) against undesirable reactions during synthetic procedures. Commonly used N-protecting groups are disclosed in Greene, "Protective Groups In Organic Synthesis," (John Wiley & Sons, New York (1981)), which is hereby incorporated by reference. N-protecting groups comprise acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, α-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl

and the like; carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, α,α-di methyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2,-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl and the like; alkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl and the like; nitro groups; and silyl groups such as trimethylsilyl and the like. Preferred N-protecting groups are formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, benzyl, 9-fluorenylmethyloxycarbonyl (Fmoc), t-butyloxycarbonyl (Boc) and benzyloxycarbonyl (Cbz).

[00112] The phrase "unsubstituted alkyl" refers to alkyl groups that do not contain heteroatoms. Thus the phrase includes straight chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The phrase also includes branched chain isomers of straight chain alkyl groups, including but not limited to, the following which are provided by way of example: $-CH(CH_3)_2$, $-CH(CH_3)(CH_2CH_3)$, $-CH(CH_2CH_3)_2$, $-C(CH_3)_3$, $-C(CH_2CH_3)_3$, $-CH_2CH(CH_3)_2$, $-CH_2CH(CH_3)(CH_2CH_3)$, $-CH_2CH(CH_2CH_3)_2$, $-CH_2C(CH_3)_3$, $-CH_2C(CH_2CH_3)_3$, $-CH(CH_3)CH(CH_3)(CH_2CH_3)$, $-CH_2CH_2CH(CH_3)_2$, $-CH_2CH_2CH(CH_3)(CH_2CH_3)$, $-CH_2CH_2CH(CH_2CH_3)_2$, $-CH_2CH_2C(CH_3)_3$, $-CH_2CH_2C(CH_2CH_3)_3$, $-CH(CH_3)CH_2CH(CH_3)_2$, $-CH(CH_3)CH(CH_3)CH(CH_3)_2$, $-CH(CH_2CH_3)CH(CH_3)CH(CH_3)(CH_2CH_3)$, and others. The phrase does not include cycloalkyl groups. Thus, the phrase unsubstituted alkyl groups includes primary alkyl groups, secondary alkyl groups, and tertiary alkyl groups. Unsubstituted alkyl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound. Preferred unsubstituted alkyl groups

include straight and branched chain alkyl groups having 1 to 20 carbon atoms. More preferred such unsubstituted alkyl groups have from 1 to 10 carbon atoms while even more preferred such groups have from 1 to 5 carbon atoms. Most preferred unsubstituted alkyl groups include straight and branched chain alkyl groups having from 1 to 3 carbon atoms and include methyl, ethyl, propyl, and $-CH(CH_3)_2$.

[00113] The phrase "substituted alkyl" refers to an unsubstituted alkyl group as defined above in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen and non-carbon atoms such as, but not limited to, a halogen atom in halides such as F, Cl, Br, and I; and oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. Substituted alkyl groups also include groups in which one or more bonds to a carbon(s) or hydrogen(s) atom is replaced by a bond to a heteroatom such as oxygen in carbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles. Preferred substituted alkyl groups include, among others, alkyl groups in which one or more bonds to a carbon or hydrogen atom is/are replaced by one or more bonds to fluorine atoms. One example of a substituted alkyl group is the trifluoromethyl group and other alkyl groups that contain the trifluoromethyl group. Other alkyl groups include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a hydroxyl, alkoxy, aryloxy group, or heterocyclyloxy group. Still other alkyl groups include alkyl groups that have an amine, alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine, diarylamine, heterocyclylamine, (alkyl)(heterocyclyl)amine, (aryl)(heterocyclyl)amine, or diheterocyclylamine group.

[00114] The phrase "unsubstituted cycloalkyl" refers to cyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and such

rings substituted with straight and branched chain alkyl groups as defined above. The phrase also includes polycyclic alkyl groups such as, but not limited to, adamantyl norbornyl, and bicyclo[2.2.2]octyl and the like, as well as such rings substituted with straight and branched chain alkyl groups as defined above. Thus, the phrase would include methylcyclohexyl groups among others. The phrase does not include cyclic alkyl groups containing heteroatoms. Unsubstituted cycloalkyl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound. Preferred unsubstituted cycloalkyl groups have from 3 to 20 carbon atoms. More preferred such unsubstituted alkyl groups have from 3 to 8 carbon atoms while even more preferred such groups have from 3 to 7 carbon atoms.

[00115] The phrase "substituted cycloalkyl" " has the same meaning with respect to unsubstituted cycloalkyl groups that substituted alkyl groups had with respect to unsubstituted alkyl groups. Thus, the phrase includes, but is not limited to, oxocyclohexyl, chlorocyclohexyl, hydroxycyclopentyl, and chloromethylcyclohexyl groups.

[00116] The phrase "unsubstituted aryl" refers to aryl groups that do not contain heteroatoms. Thus the phrase includes, but is not limited to, groups such as phenyl, biphenyl, anthracenyl, naphthenyl by way of example. Although the phrase "unsubstituted aryl" includes groups containing condensed rings such as naphthalene, it does not include aryl groups that have other groups such as alkyl or halo groups bonded to one of the ring members, as aryl groups such as tolyl are considered herein to be substituted aryl groups as described below. A preferred unsubstituted aryl group is phenyl. Unsubstituted aryl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound, however.

[00117] The phrase "substituted aryl group" has the same meaning with respect to unsubstituted aryl groups that substituted alkyl groups had with respect to unsubstituted alkyl groups. However, a substituted aryl group also includes aryl groups in which one of the aromatic carbons is bonded to one of the non-carbon or non-hydrogen atoms described above and also includes aryl groups in which one or more aromatic carbons

of the aryl group is bonded to a substituted and/or unsubstituted alkyl, alkenyl, or alkynyl group as defined herein. This includes bonding arrangements in which two carbon atoms of an aryl group are bonded to two atoms of an alkyl, alkenyl, or alkynyl group to define a fused ring system (e.g. dihydronaphthyl or tetrahydronaphthyl). Thus, the phrase "substituted aryl" includes, but is not limited to tolyl, and hydroxyphenyl among others.

[00118] The phrase "unsubstituted alkenyl" refers to straight and branched chain and cyclic groups such as those described with respect to unsubstituted alkyl groups as defined above, except that at least one double bond exists between two carbon atoms. Examples include, but are not limited to vinyl, $-CH=C(H)(CH_3)$, $-CH=C(CH_3)_2$, $-C(CH_3)=C(H)_2$, $-C(CH_3)=C(H)(CH_3)$, $-C(CH_2CH_3)=CH_2$, cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl among others.

[00119] The phrase "substituted alkenyl" has the same meaning with respect to unsubstituted alkenyl groups that substituted alkyl groups had with respect to unsubstituted alkyl groups. A substituted alkenyl group includes alkenyl groups in which a non-carbon or non-hydrogen atom is bonded to a carbon double bonded to another carbon and those in which one of the non-carbon or non-hydrogen atoms is bonded to a carbon not involved in a double bond to another carbon. For example, $-CH=CH-OCH_3$ and $-CH=CH-CH_2-OH$ are both substituted alkenyls. Oxoalkenyls wherein a CH_2 group is replaced by a carbonyl, such as in $-CH=CH-C(O)-CH_3$, are also substituted alkenyls.

[00120] The phrase "unsubstituted alkynyl" refers to straight and branched chain groups such as those described with respect to unsubstituted alkyl groups as defined above, except that at least one triple bond exists between two carbon atoms. Examples include, but are not limited to $-C\equiv C(H)$, $-C\equiv C(CH_3)$, $-C\equiv C(CH_2CH_3)$, $-C(H_2)C\equiv C(H)$, $-C(H)_2C\equiv C(CH_3)$, and $-C(H)_2C\equiv C(CH_2CH_3)$ among others.

[00121] The phrase "substituted alkynyl" has the same meaning with respect to unsubstituted alkynyl groups that substituted alkyl groups had with respect to unsubstituted alkyl groups. A substituted alkynyl group includes alkynyl groups in

which a non-carbon or non-hydrogen atom is bonded to a carbon triple bonded to another carbon and those in which a non-carbon or non-hydrogen atom is bonded to a carbon not involved in a triple bond to another carbon. Examples include, but are not limited to, oxoalkynyls wherein a CH_2 group is replaced by a carbonyl, such as in -C(O)-CH≡CH-CH_3 and -C(O)-CH_2-CH≡CH among others.

[00122] The phrase "unsubstituted aralkyl" refers to unsubstituted alkyl groups as defined above in which a hydrogen or carbon bond of the unsubstituted alkyl group is replaced with a bond to an aryl group as defined above. For example, methyl (-CH_3) is an unsubstituted alkyl group. If a hydrogen atom of the methyl group is replaced by a bond to a phenyl group, such as if the carbon of the methyl were bonded to a carbon of benzene, then the compound is an unsubstituted aralkyl group (*i.e.*, a benzyl group). Thus the phrase includes, but is not limited to, groups such as benzyl, diphenylmethyl, and 1-phenylethyl (-CH(C_6H_5)(CH_3)) among others.

[00123] The phrase "substituted aralkyl" has the same meaning with respect to unsubstituted aralkyl groups that substituted aryl groups had with respect to unsubstituted aryl groups. However, a substituted aralkyl group also includes groups in which a carbon or hydrogen bond of the alkyl part of the group is replaced by a bond to a non-carbon or a non-hydrogen atom. Examples of substituted aralkyl groups include, but are not limited to, -CH_2C(=O)(C_6H_5), and -CH_2(2-methylphenyl) among others.

[00124] The phrase "unsubstituted heteroalkyl" refers to unsubstituted alkyl groups as defined above in which the carbon chain is interrupted by one or more heteroatoms chosen from N, O, and S. Unsubstituted heteroalkyls containing N may have NH or N(unsubstituted alkyl) in the carbon chain. Thus, unsubstituted heteroalkyls include alkoxy, alkoxyalkyl, alkoxyalkoxy, thioether, alkylaminoalkyl, aminoalkyloxy, and other such groups. Preferred unsubstituted heteroalkyl groups contain 1-5 heteroatoms, and particularly 1-3 heteroatoms. Preferred unsubstituted heteroalkyls include, for example, alkoxyalkoxyalkoxy groups such as ethyloxyethyloxyethyloxy.

[00125] The phrase "substituted heteroalkyl" has the same meaning with respect to unsubstituted heteroalkyl groups that substituted alkyl groups have with respect to unsubstituted alkyl groups.

[00126] The phrase "unsubstituted heteroalkenyl" refers to unsubstituted alkenyl groups as defined above in which the carbon chain is interrupted by one or more heteroatoms chosen from N, O, and S. Unsubstituted heteroalkenyls containing N may have NH or N(unsubstituted alkyl or alkenyl) in the carbon chain.

[00127] The phrase "substituted heteroalkenyl" has the same meaning with respect to unsubstituted heteroalkenyl groups that substituted heteroalkyl groups have with respect to unsubstituted heteroalkyl groups.

[00128] The phrase "unsubstituted heterocyclyl" refers to both aromatic and nonaromatic ring compounds including monocyclic, bicyclic, and polycyclic ring compounds such as, but not limited to, quinuclidyl, containing 3 or more ring members of which one or more is a heteroatom such as, but not limited to, N, O, and S. Although the phrase "unsubstituted heterocyclyl" includes condensed heterocyclic rings such as benzimidazolyl, it does not include heterocyclyl groups that have other groups such as alkyl or halo groups bonded to one of the ring members as compounds such as 2-methylbenzimidazolyl are substituted heterocyclyl groups. Examples of heterocyclyl groups include, but are not limited to: unsaturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but not limited to pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl etc.), tetrazolyl, (e.g. 1H-tetrazolyl, 2H tetrazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl; condensed unsaturated heterocyclic groups containing 1 to 4 nitrogen atoms such as, but not limited to, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl; unsaturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, oxazolyl, isoxazolyl, oxadiazolyl (e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2

oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, morpholinyl; unsaturated condensed heterocyclic groups containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, benzoxazolyl, benzoxadiazolyl, benzoxazinyl (e.g. 2H-1,4-benzoxazinyl etc.); unsaturated 3 to 8 membered rings containing 1 to 3 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolyl, isothiazolyl, thiadiazolyl (e.g. 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolodinyl; saturated and unsaturated 3 to 8 membered rings containing 1 to 2 sulfur atoms such as, but not limited to, thienyl, dihydrodithiinyl, dihydrodithionyl, tetrahydrothiophene, tetrahydrothiopyran; unsaturated condensed heterocyclic rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, benzothiazolyl, benzothiadiazolyl, benzothiazinyl (e.g. 2H-1,4-benzothiazinyl, etc.), dihydrobenzothiazinyl (e.g. 2H-3,4-dihydrobenzothiazinyl, etc.), unsaturated 3 to 8 membered rings containing oxygen atoms such as, but not limited to furyl; unsaturated condensed heterocyclic rings containing 1 to 2 oxygen atoms such as benzodioxolyl (e.g. 1,3-benzodioxoyl, etc.); unsaturated 3 to 8 membered rings containing an oxygen atom and 1 to 2 sulfur atoms such as, but not limited to, dihydrooxathiinyl; saturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 2 sulfur atoms such as 1,4-oxathiane; unsaturated condensed rings containing 1 to 2 sulfur atoms such as benzothienyl, benzodithiinyl; and unsaturated condensed heterocyclic rings containing an oxygen atom and 1 to 2 oxygen atoms such as benzoxathiinyl. Heterocyclyl group also include those described above in which one or more S atoms in the ring is double-bonded to one or two oxygen atoms (sulfoxides and sulfones). For example, heterocyclyl groups include tetrahydrothiophene, tetrahydrothiophene oxide, and tetrahydrothiophene 1,1-dioxide. Preferred heterocyclyl groups contain 5 or 6 ring members. More preferred heterocyclyl groups include morpholine, piperazine, piperidine, pyrrolidine, imidazole, pyrazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, thiomorpholine, thiomorpholine in which the S atom of the thiomorpholine is bonded to one or more O atoms, pyrrole, homopiperazine, oxazolidin-2-one, pyrrolidin-2-one, oxazole, quinuclidine, thiazole, isoxazole, furan, and tetrahydrofuran.

[00129] The phrase "substituted heterocyclyl" refers to an unsubstituted heterocyclyl group as defined above in which one of the ring members is bonded to a non-hydrogen atom such as described above with respect to substituted alkyl groups and substituted aryl groups. Examples, include, but are not limited to, 2-methylbenzimidazolyl, 5-methylbenzimidazolyl, 5-chlorobenzthiazolyl, 1-methyl piperazinyl, and 2-chloropyridyl among others.

[00130] The phrase "unsubstituted heterocyclylalkyl" refers to unsubstituted alkyl groups as defined above in which a hydrogen or carbon bond of the unsubstituted alkyl group is replaced with a bond to a heterocyclyl group as defined above. For example, methyl ($-CH_3$) is an unsubstituted alkyl group. If a hydrogen atom of the methyl group is replaced by a bond to a heterocyclyl group, such as if the carbon of the methyl were bonded to carbon 2 of pyridine (one of the carbons bonded to the N of the pyridine) or carbons 3 or 4 of the pyridine, then the compound is an unsubstituted heterocyclylalkyl group.

[00131] The phrase "substituted heterocyclylalkyl" has the same meaning with respect to unsubstituted heterocyclylalkyl groups that substituted aralkyl groups had with respect to unsubstituted aralkyl groups. However, a substituted heterocyclylalkyl group also includes groups in which a non-hydrogen atom is bonded to a heteroatom in the heterocyclyl group of the heterocyclylalkyl group such as, but not limited to, a nitrogen atom in the piperidine ring of a piperidinylalkyl group.

[00132] The phrase "unsubstituted alkoxy" refers to a hydroxyl group (-OH) in which the bond to the hydrogen atom is replaced by a bond to a carbon atom of an otherwise unsubstituted alkyl group as defined above.

[00133] The phrase "substituted alkoxy" refers to a hydroxyl group (-OH) in which the bond to the hydrogen atom is replaced by a bond to a carbon atom of an otherwise substituted alkyl group as defined above.

[00134] A "pharmaceutically acceptable salt" includes a salt with an inorganic base, organic base, inorganic acid, organic acid, or basic or acidic amino acid. As salts of

inorganic bases, the invention includes, for example, alkali metals such as sodium or potassium; alkaline earth metals such as calcium and magnesium or aluminum; and ammonia. As salts of organic bases, the invention includes, for example, trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, and triethanolamine. As salts of inorganic acids, the instant invention includes, for example, hydrochloric acid, hydroboric acid, nitric acid, sulfuric acid, and phosphoric acid. As salts of organic acids, the instant invention includes, for example, formic acid, acetic acid, trifluoroacetic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid. As salts of basic amino acids, the instant invention includes, for example, arginine, lysine and ornithine. Acidic amino acids include, for example, aspartic acid and glutamic acid.

[00135] Compounds of the present invention include enriched or resolved optical isomers at any or all asymmetric atoms as are apparent from the depictions. Both racemic and diastereomeric mixtures, as well as the individual optical isomers can be isolated or synthesized so as to be substantially free of their enantiomeric or diastereomeric partners. All such stereoisomers are within the scope of the invention.

[00136] The target molecule to which the targeting agent of the targeting compound binds is preferably a non-immunoglobulin molecule or is an immunoglobulin molecule where the target moiety is outside the immunoglobulin combining site. It is not intended to exclude from the inventive compounds those targeting agents that function as antigens and, therefore, bind to an immunoglobulin combining site. Such targeting agents are included herein provided the targeting agents also bind to a non-immunoglobulin molecule and/or a target moiety located outside the combining site of an immunoglobulin molecule. In general, the target molecule can be any type of molecule including organic, inorganic, protein, lipid, carbohydrate, nucleic acid and the like.

[00137] The target molecule is preferably a biomolecule such as a protein, carbohydrate, lipid or nucleic acid. The target molecule can be associated with a cell ("cell surface expressed"), or other particle ("particle surface expressed") such as a

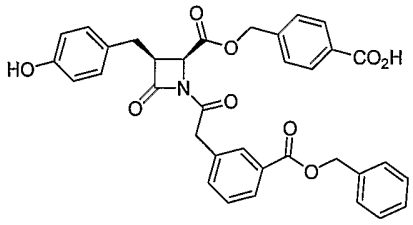
virus, or may be extracellular. If associated with a cell or particle, the target molecule is preferably expressed on the surface of the cell or particle in a manner that allows the targeting agent of the targeting compound to make contact with the surface receptor from the fluid phase of the body.

[00138] In some preferred embodiments, the target molecule is predominantly or exclusively associated with a pathological condition or diseased cell, tissue or fluid. Thus, the targeting agent of a present targeting compound can be used to deliver the targeting compound to a diseased tissue by targeting the cell, an extracellular matrix biomolecule or a fluid biomolecule. Exemplary target molecules disclosed hereinafter in the Examples include integrins (Example 1), cytokine receptors (Examples 2, 3 and 7), cytokines (Example 4), vitamin receptors (Example 5), cell surface enzymes (Example 6), and HIV-1 virus and HIV-1 virus infected cells (Examples 8 and 11), and the like.

[00139] In other preferred embodiments, the target molecule is associated with an infectious agent and is expressed on the surface of a microbial cell or on the surface of a viral particle. As such, targeting compositions, such as antibody targeting compositions, in which the targeting agent can bind to the cell surface expressed or particle expressed infectious agent can be used as an anti-microbial, by targeting microbial agents inside the body or on the surface (e.g., skin) of an individual. In the latter case, the invention compound can be applied topically.

[00140] Antibody targeting agents specific for a microbial target molecule also can be used as an anti-microbial agent in vitro. Accordingly, a method of reducing the infectivity of microbial cells or viral particles present on a surface is provided. Some methods include contacting the surface of a microbial cell or viral particle with an effective amount of the invention targeting compound. The targeting compound in such methods includes a targeting agent specific for a receptor on the microbial cell or virus particle. Applicable surfaces are any surfaces in vitro such as a counter top, condom, and the like.

[00141] Another preferred target molecule for targeting compounds of the invention is prostate specific antigen (PSA), a serine protease that has been implicated in a variety of disease states including prostate cancer, breast cancer and bone metastasis. Specific inhibitors of PSA which bind to the active site of PSA are known. See Adlington et al., J. Med. Chem., 2001, 44:1491-1508 and WO 98/25895 to Anderson. A specific inhibitor of PST is shown below as compound 61.



61

[00142] A targeting agent, in addition to its ability to bind a target molecule, may be characterized in having one or more biological activities, each activity characterized as a detectable biological affect on the functioning of a cell organ or organism. Thus, in addition to being a targeting agent, such compounds can be considered biological agents. For example, the integrin targeting agents shown as compounds 1, 2, 3 and 33 above not only target an integrin, but have integrin antagonist biological activity. In some embodiments, however, a targeting agent may be a pure binding agent without biological activity.

[00143] The targeting compounds of the invention include a targeting agent that is covalently linked to a polymer such as an antibody. Such targeting compounds may have one or more biological activities associated with the targeting compound. The biological activity may be an inherent feature of the targeting agent itself or may be provided by a biological agent distinct from the targeting agent in the targeting compound. The biological agent may be associated covalently or non-covalently with the other molecules or portions of the targeting compound, although covalent linkage is preferred. The biological agent may be linked to either the targeting agent, the polymer, or both by means well known in the art. For example, see Kiaris et al., Eur. J.

Cancer 37:620-628 (2001) and Schally et al. Eur. J. Endocrin. 141:1-14 (1989), which describe various conjugates between peptide hormone targeting agents and doxorubicin. See also, Canevari et al., Ann Oncol 1994 Oct;5(8):698-701; Rihova, Folia Microbiol (Praha) 1995;40(4):367-84; Vitetta, Princess Takamatsu Symp 1988;19:333-40; and Ghose et al., Crit Rev Ther Drug Carrier Syst 1987;3(4):263-359. Thus, in some embodiments, the polymer-targeting agent targeting compounds of the invention may include a functional component in the form of a targeting agent that has inherent biological activity. In such embodiments, the targeting agent may be linked to a combining site of the antibody or antibody fragment and the targeting agent is the functional component that exhibits the biological activity. In other embodiments, the targeting compound includes a targeting agent linked to a combining site of an antibody or antibody fragment, and also includes a separate functional component that is preferably attached or linked to the targeting compound through a covalent bond.

[00144] A targeting agent or biological agent can be linked to a polymer targeting compound of the invention using a linkage that is labile under certain conditions. The labile linkage may be between the polymer and the targeting agent or biological agent, while if a linker is present, the labile linkage may be between the polymer and the linker, the targeting agent or biological agent and the linker, within the linker, or combinations thereof.

[00145] Labile linkers include, reversible covalent bonds, pH sensitive linkages (acid or base sensitive), enzyme sensitive linkages, degradation sensitive linkers, photosensitive linkers, sand the like, and combinations thereof. These features are also characteristic of a prodrug which can be considered as a type of labile linker. A variety of labile linkers have been previously designed. For example, prodrugs can be formed using compounds having carboxylic acid moieties that slowly degrade by hydrolysis as described in U.S. Patent No. 5,498,729.

[00146] The particular design of a labile linker may be used to direct release of the biological agent after it has reached the intended target. For example, a linkage may be designed to direct release in a particular intracellular compartment or in an extracellular compartment in which polymer targeting compounds may accumulate. An acid-labile

linker such as a cis-aconitic acid linker can take advantage of the acidic environment of different intracellular compartments such as the endosomes encountered during receptor mediated endocytosis and the lysosomes. See Shen et al., Biochem. Biophys. Res. Commun. (1981) 102:1048-1054; Yang et al., J. Natl. Canc. Inst. (1988) 80: 1154-1159. A peptide spacer arm located within or at the ends of a linker can be used to effect release of a targeting agent or biological agent by the action of a peptidase such as a lysosomal peptidase. See e.g., Trouet et al., Proc. Natl. Acad. Sci. (1982) 79: 626-629.

[00147] Particular targeting agents may or may not possess biological activity depending on the context of their use. For example, the therapeutic drug doxorubicin, which is a DNA intercalator, can be a targeting agent for double stranded DNA when the drug is covalently linked to, e.g., an antibody and applied to DNA in a cell-free form. Doxorubicin, however, may not be considered a targeting agent with respect to a cell while the drug is covalently linked to an antibody unless the compound can be taken up by the cell. In the latter case, doxorubicin may have biological activity following uptake if the drug can access DNA in the cell nucleus.

[00148] Biological agent functional components include, but are not limited to, small molecule drugs (a pharmaceutical organic compound of about 5,000 daltons or less), organic molecules, proteins, peptides, peptidomimetics, glycoproteins, proteoglycans, lipids glycolipids, phospholipids, lipopolysaccharides, nucleic acids, proteoglycans, carbohydrates, and the like. Biological agents may be anti-neoplastic, anti-microbial, a hormone, an effector, and the like. Such compounds include well known therapeutic compounds such as the anti-neoplastic agents paclitaxel, daunorubicin, doxorubicin, carminomycin, 4'-epiadriamycin, 4-demethoxy-daunomycin, 11 -deoxydaunorubicin, 13-deoxydaunorubicin, adriamycin-14-benzoate, adriamycin-14-octanoate, adriamycin-14-naphthaleneacetate, vinblastine, vincristine, mitomycin C, N-methyl mitomycin C, bleomycin A_2, dideazatetrahydrofolic acid, aminopterin, methotrexate, cholchicine and cisplatin, and the like. Anti-microbial agents include aminoglycosides including gentamicin, antiviral compounds such as rifampicin, 3'-azido-3'-deoxythymidine (AZT) and acylovir, antifungal agents such as

azoles including fluconazole, polyene macrolides such as amphotericin B, and candicidin, anti-parasitic compounds such as antimonials, and the like. Hormones may include toxins such as diphtheria toxin, cytokines such as CSF, GSF, GMCSF, TNF, erythropoietin, immunomodulators or cytokines such as the interferons or interleukins, a neuropeptide, reproductive hormone such as HGH, FSH, or LH, thyroid hormone, neurotransmitters such as acetylcholine, hormone receptors such as the estrogen receptor. Also included are non-steroidal anti-inflammatories such as indomethacin, salicylic acid acetate, ibuprofen, sulindac, piroxicam, and naproxen, and anesthetics or analgesics. Also included are radioisotopes such as those useful for imaging as well as for therapy.

[00149] Biological agent functional components for use in the targeting compounds of the invention can be naturally occurring or synthetic. Biological agents can be biologically active in their native state, or be biologically inactive or in a latent precursor state and acquire biological or therapeutic activity when a portion of the biological agent is hydrolyzed, cleaved or is otherwise modified. The prodrug can be delivered at the surface of a cell or intracellulary using targeting compounds of the invention where it can then be activated. In this regard, the biological agent can be a "prodrug," meaning that prodrug molecules capable of being converted to drugs (active therapeutic compounds) by certain chemical or enzymatic modifications of their structure. In the prodrug approach, site-specific drug delivery can be obtained from tissue-specific activation of a prodrug, which is the result of metabolism by an enzyme that is either unique for the tissue or present at a higher concentration (compared with other tissues); thus, it activates the prodrug more efficiently.

[00150] Photodynamic treatment may be used to activate a prodrug by cleaving a photosensitive linker or by activating a photoresponsive enzyme (acyl enzyme hydrolysis) as described previously (see U.S. Patent No. 5,114,851 and 5,218,137). Photodynamic treatment also may be used to rapidly inactivate a drug in sites where the drug activity is not desired (e.g. in non-target tissues). Various means of covalently modifying a drug to form a prodrug are well known in the art.

[00151] Targeting agents may be covalently linked to the polymer, e.g., antibody combining site, directly or through the aid of a linker. An appropriate linker can be chosen to provide sufficient distance between the targeting agent and the antibody combining site in order for the targeting agent to be able to bind to its target molecule. This distance depends on several factors including, for example, the distance from the outermost surface of the antibody combining site to the reactive side chain in the combining site, and the nature of the targeting agent. Generally, the linker will be between about 5 to 250 angstroms (0.5 to 25 ηm) in length, with 10 or more angstroms (1.0 nm) being more preferred, although shorter linkers of about 3 angstroms (0.3 nm) in length may be sufficient if the amino acid side chain is very near to the outermost portion of the combining site and/or the targeting agent or biological agent includes a segment that can function as a part of a linker.

[00152] Linker length may also be viewed in terms of the number of linear atoms (cyclic moieties such as aromatic rings and the like to be counted by taking the shortest route). Linker length under this measure is generally about 10 to 200 atoms and more typically about 30 or more atoms, although shorter linkers of two or more atoms may be sufficient if the reactive amino acid side chain is very near to the outermost portion of the combining site. Generally, linkers with a linear stretch of at least about 9 atoms are sufficient. Other linker considerations include effect on physical or pharmacokinetic properties of the resulting targeting compound or targeting agent-linker, solubility, lipophilicity, hydrophilicity, hydrophobicity, stability (more or less stable as well as planned degradation), rigidity, flexibility, immunogenicity, modulation of antibody binding, chemical compatibility with targeting agent, ability to be incorporated into a micelle or liposome, and the like.

[00153] In targeting compounds where a linker is present between the polymer and the targeting agent, the targeting compound may be prepared by several approaches. In one approach, a targeting agent-linker compound and/or biological agent-linker compound is synthesized with a linker that includes one or more reactive groups designed for covalent reaction with a side chain of an amino acid in the combining site of an antibody. The agent-linker compound and antibody are combined under

conditions where the linker reactive group forms a covalent bond with the amino acid side chain.

[00154] In another approach, linking can be achieved by synthesizing a polymer-linker compound comprising a polymer and a linker wherein the linker includes one or more reactive groups designed for covalent reaction with an appropriate chemical moiety of the targeting agent or biological agent. The targeting agent or biological agent may need to be modified to provide the appropriate moiety for reaction with the linker reactive group. The polymer-linker and targeting agent and/or biological agent are combined under conditions where the linker reactive group covalently links to the targeting and/or biological agent.

[00155] A further approach for forming an antibody targeting compound of the invention uses a dual linker design. In one embodiment, the an agent-linker compound is synthesized which comprises a targeting agent and/or a biological agent and a linker with a reactive group. A polymer-linker compound such as an antibody-linker compound is synthesized which comprises an antibody and a linker with a chemical group susceptible to reactivity with the reactive group of the agent-linker of the first step. These two linker containing compounds are then combined under conditions whereby the linkers covalently link, forming the targeting compound, e.g., antibody targeting compound.

[00156] In another embodiment, a polymer-linker compound is synthesized which comprises a polymer and a linker with a reactive group. A targeting agent and/or biological agent-linker compound is prepared which comprises the agent and a linker with a chemical group susceptible to reactivity with the reactive group of the polymer-linker of the first step. These two linker containing compounds are then combined under conditions whereby the linkers covalently link, forming the polymer targeting compound. "Susceptible" as used herein with reference to a chemical moiety indicates that the chemical moiety will covalently bond with a compatible reactive group. Thus, an electrophilic group is susceptible to covalent bonding with a nucleophillic group and vice versa.

[00157] As discussed, the linker may be first conjugated to the targeting agent and then the targeting agent-linker conjugated to the polymer, e.g., to the antibody combining site. Alternatively, the linker may be conjugated first to the antibody combining site and the antibody-linker conjugated to the targeting agent. Numerous means well known in the art can be used to attach a linker to the targeting agent or antibody combining site. Exemplary functional groups that can be involved in the linkage include, for example, esters, amides, ethers, phosphates, amino, keto, amidine, guanidine, imines, eneamines, phosphates, phosphonates, epoxides, aziridines, thioepoxides, masked or protected diketones (ketals for example), lactams, haloketones, aldehydes, thiocarbamate, thioamide, thioester, sulfide, disulfide, phosphoramide, sulfonamide, urea, thioruea, carbamate, carbonate, hydroxamide, and the like.

[00158] The linker includes any atom from the group C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof. The linker also may include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. The linker also may include one or more ring structures. As used herein a "ring structure" includes saturated, unsaturated, and aromatic carbocyclic rings and saturated, unsaturated, and aromatic heterocyclic rings. The ring structures may be mono-, bi-, or polycyclic, and include fused or unfused rings. Further, the ring structures are optionally substituted with functional groups well known in the art including, but not limited to halogen, oxo, -OH, -CHO, -COOH, $-NO_2$, -CN, $-NH_2$, $-C(O)NH_2$, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. Combinations of the above groups and rings may also be present in the linkers of the targeting compounds of the invention.

[00159] The general design of a embodiment of a unbranched linker for use in preparing targeting compounds of the present invention is shown in FIG. 2A. The linker is of the formula

$$X-Y-Z$$

Wherein X is a connecting chain, Y is a recognition group and Z is a reactive group. Figure 2B-E shows various targeting agent-linker compounds with the linker X, Y and Z portions identified. The linker may be linear or branched. In some embodiments, the linker has a linear stretch of between 5-200 or 10-200 atoms although in other embodiments, longer linker lengths may be used. One or more targeting agents may be linked to X. In some embodiments, where more than one targeting agent is linked and a branched linker is used, some of the targeting agents may be linked to different branches of the linker. However, it should be understood that linkers used in the compounds of the invention may have one or more recognition groups, one or more reactive groups and one or more connecting chains and combinations thereof. Connecting chains may branch from another connecting chain or from a recognition group.

[00160] The connecting chain X of the linker includes any atom from the group C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof. X also may include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. In some embodiments, X may include one or more ring structures. In a preferred embodiment, X includes a repeating ether unit of between 2-100 units. Various embodiments of X are shown in FIG. 9.

[00161] The recognition group Y of the linker is optional and if present is located between the reactive group and the connecting chain. In preferred embodiments, Y is located from 1-20 atoms from Z. Although not wishing to be bound by any theory, it is believed that the recognition group acts to properly position the reactive group into, e.g., the antibody combining site or other binding site on the polymer so that it may react with a reactive amino acid side chain. FIG. 8 shows a variety of exemplary recognition groups with one or more homo or hetero ring structures of five or six atoms. Larger ring structures also may be used. One or more targeting agents may be linked to Y. In some embodiments, a linker may be used to link the targeting agent to Y. In embodiments where two or more targeting agents are used, one or more can be attached to both X and Y. More than one targeting agent also can be attached to Y.

[00162] The linker reactive group Z includes any nucleophilic or electrophilic group. In a preferred embodiment Z is capable of forming a covalent bond with a reactive side chain of a polymer such as an antibody or other protein. In some embodiments, Z includes one or more C=O, groups arranged to form a diketone, an acyl beta-lactam, an active ester, haloketone, a cyclohexyl diketone group, an aldehyde or maleimide. Other groups may include lactone, anhydride, an alpha-haloacetamide or an epoxide. Exemplary linker electrophilic reactive groups that can covalently bond to a reactive nucleophilic group (e.g. lysine or cysteine side chain) in the combining site of an antibody include acyl beta-lactam, simple diketone, succinimide active ester, maleimide, haloacetamide with linker, haloketone, cyclohexyl diketone, aldehyde, amidine, guanidine, imine, eneamine, phosphate, phosphonate, epoxide, aziridine, thioepoxide, a masked or protected diketone (a ketal for example), lactam, sulfonate, and the like masked C=O groups such as imine, ketal, acetal and any other known electrophilic group. A preferred linker reactive group includes one or more C=O, groups arranged to form a acyl beta-lactam, simple diketone, succinimide active ester, maleimide, haloacetamide with linker, haloketone, cyclohexyl diketone, or aldehyde.

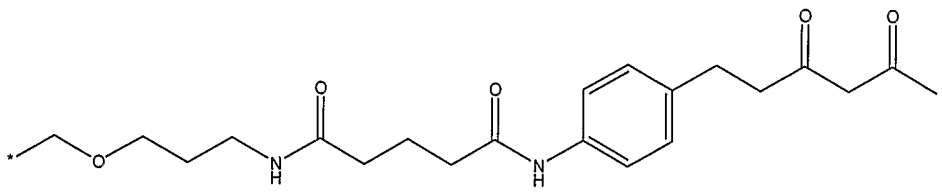
[00163] Z may be a group that forms a reversible or nonreversible covalent bond. In some embodiments, reversible covalent bonds may be formed using diketone Z groups such as those shown in FIG. 6. R_1 and R_2 and R_3 in structures A-C of FIG. 6 represent substituents which can be C, H, N, O, P, S, Si, halogen (F, Cl, Br, I) or a salt thereof. These substituents also may include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. R_2 and R_3 also could from a ring structure as exemplified in structures B and C. X in FIG. 6 could be a heteroatom. Other Z groups that form reversible covalent bonds include the diketone amidine, imine, and other reactive groups shown in structures B and G of FIG. 7. FIG. 7 also includes the structures of other preferred linker reactive groups.

[00164] Z reactive groups that form a nonreversible covalent bond with a polymer reactive site such as the combining site of an antibody include structures D-G in FIG. 6

and structures A, C and D of FIG. 7. Such structures are useful for nonreversibly attaching a targeting agent-linker to a reactive nucleophilic group (e.g. lysine or cysteine side chain) in the combining site of an antibody.

[00165] It should be understood that the above described reversible and nonreversible covalent linking chemistry can also be applied to link a targeting agent or biological agent to a polymer in the absence of a linker or to link a targeting agent or biological agent to a linker (e.g. to the connecting chain of the linker). For example, a targeting agent can be linked to a linker to form a targeting agent-linker by placing a suitable reactive group Z type element such as an appropriate nucleophilic or electrophilic group on either the linker or the targeting agent and a suitable reactive moiety such as an amino or sulfhydral group on the other of the two.

[00166] A preferred linker for use in targeting compounds of the invention and for preparing targeting agent-linker compounds includes a 1,3-diketone reactive group as Z. Another preferred linker is one where the connecting chain X includes a repeating ether unit of between 2-100 units. Linkers in which the recognition group Y is present are preferred with Y located preferably between 1-20 atoms from the reactive group Z. Such a linker attached to the core of an integrin targeting RGD peptidomimetic moiety such as those described above, can have the structure as shown below in compound **89**, where n is from 1-100 or more and preferably is 1, 2, or 4, and more preferably is 3. In some embodiments, the linker is a repeating polymer such as polyethylene glycol.



compound **89**

[00167] The linker reactive group or similar such reactive group that may be inherent in the targeting agent, is chosen for use with a particular polymer, especially an antibody. For example, a chemical moiety for modification by an aldolase antibody may be a ketone, diketone, beta lactam, active ester haloketone, lactone, anhydride,

maleimide, alpha-haloacetamide, cyclohexyl diketone, epoxide, aldehyde, amidine, guanidine, imine, eneamine, phosphate, phosphonate, epoxide, aziridine, thioepoxide, masked or protected diketone (ketal for example), lactam, haloketone, aldehyde, and the like. A 1,3-diketone configuration such as the diketone shown in compounds **80** (see FIG. 2) or **83** (see FIG. 13), is especially preferred as a substrate for modification by an aldolase antibody. Conversely, an antibody with a diketone engineered into a combining site, will form a covalent bond with a linker reactive group having an amine.

[00168] A linker reactive group chemical moiety (Z)suitable for covalent modification by a reactive sulfhydryl group in an antibody may be a disulfide, aryl halide, maleimide, alpha-haloacetamide, isocyanate, epoxide, thioester, active ester, amidine, guanidine, imine, eneamine, phosphate, phosphonate, epoxide, aziridine, thioepoxide, masked or protected diketone (ketal for example), lactam, haloketone, aldehyde, and the like. The chemical structures of various targeting agent-linker compounds which include a linker with a 1,3 diketone as the reactive group are shown in FIGs. 2-5.

[00169] One of skill in the art will readily appreciate that reactive amino acid side chains in polymers which are proteins (e.g., antibodies) may possess an electrophilic group that reacts with a nucleophilic group on the targeting agent or its linker, whereas in other embodiments a reactive nucleophilic group in an amino acid side chain of a protein or protein fragment reacts with an electrophilic group in a targeting agent or linker. Thus, polymer binding site side chains such as antibody or antibody fragment combining site side chains may be substituted with an electrophile (e.g., FIGs. 6 and 7) and this group may be used to react with a nucleophile on the targeting agent or its linker (e.g., NH_2). In this embodiment, the protein and targeting agent each have a partial linker with appropriate reactive moieties at each end so that the two ends of the partial linker can form the full linker, thus creating the complete targeting compound.

[00170] One of skill in the art also will readily appreciate that two or more targeting agents may be linked to a single polymer binding site. The two targeting agents may be the same or may be different with respect to their specificity for a particular target. In one embodiment, each targeting agent may be linked to a separate reactive side chain of

an amino acid in a polymer binding site such as an antibody combining site. In a preferred embodiment, the two targeting agents are attached to a branched or linear linker which then links both targeting agents to the same reactive amino acid side chain in the antibody combining site. Each branch of a branched linker may in some embodiments comprise a linear stretch of between 5-100 atoms. By way of example, the structures disclosed in FIGs. 3-5 show embodiments of branched linkers with two targeting agents linked to a different branch of the linker, which has a 1,3-diketone as the reactive group. As shown in these embodiments, the branch point may be in the connecting chain or in the recognition group (if present).

[00171] "Antibody" as used herein includes immunoglobulins which are the product of B cells and variants thereof as well as the T cell receptor (TcR) which is the product of T cells and variants thereof. An immunoglobulin is a protein comprising one or more polypeptides substantially encoded by the immunoglobulin kappa and lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Also subclasses of the heavy chain are known. For example, IgG heavy chains in humans can be any of IgG1, IgG2, IgG3 and IgG4 subclass.

[00172] A typical immunoglobulin structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively. The amino acids of an antibody may be natural or nonnatural.

[00173] Antibodies exist as full length intact antibodies or as a number of well-characterized fragments produced by digestion with various peptidases or chemicals. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab')_2$, a dimer of Fab which itself is a light chain joined to V_H-CH_1

by a disulfide bond. The $F(ab')_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $F(ab')_2$ dimer into an Fab' monomer. The Fab' monomer is essentially a Fab fragment with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that any of a variety of antibody fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo or antibodies and fragments obtained by using recombinant DNA methodologies. Antibody fragments produced by recombinant techniques may include fragments known by proteolytic processing or may be unique fragments not available or previously known by proteolyic processing. Whole antibody and antibody fragments also may contain natural as well as unnatural amino acids.

[00174] The T cell receptor (TcR) is a disulfide linked heterodimer composed of α or β chains or, on a minority of T cells, γ or δ chains. The two chains are generally disulfide-bonded just outside the T cell plasma membrane in a short extended stretch of amino acids resembling the antibody hinge region. Each TcR chain is composed of one antibody-like variable domain ($V\alpha$ or $V\beta$) and one constant domain ($C\alpha$ or $C\beta$). The full TcR has a molecular mass of about 95 kDa with the individual chains varying in size from 35 to 47 kDa. Also encompassed within the meaning of TCR are portions of the receptor such as the variable regions of this receptor that can be produced as a soluble protein using methods well known in the art. For example, U.S. Patent No. 6,080,840 describes a soluble T cell receptor (TcR) prepared by splicing the extracellular domains of a TcR to the glycosyl phosphatidylinositol (GPI) membrane anchor sequences of Thy-1. The molecule is expressed in the absence of CD3 on the cell surface, and can be cleaved from the membrane by treatment with phosphatidylinositol specific phospholipase C (PI-PLC). The soluble TcR also may be prepared by coupling the TcR variable domains to an antibody heavy chain CH_2 or CH_3 domain, essentially as described in U.S. Patent No. 5,216,132 or as soluble TcR single

chains as described by Schusta et al. Nature Biotech. 18,754-759 (2000) or Holler et al. Proc. Natl. Acad. Sci (USA) 97:5387-5392 (2000). The TcR "antibodies" as soluble products may be used in place of antibody for making the compounds of the invention. The combining site of the TcR can be identified by reference to CDR regions and other framework residues using the same methods discussed above for antibodies.

[00175] Recombinant antibodies may be conventional full length antibodies, antibody fragments known from proteolytic digestion, unique antibody fragments such as Fv or single chain Fv (scFv), domain deleted antibodies, and the like. An Fv antibody is about 50 Kd in size and comprises the variable regions of the light and heavy chain. A single chain Fv ("scFv") polypeptide is a covalently linked V_H::V_L heterodimer which may be expressed from a nucleic acid including V_H- and V_L-encoding sequences either joined directly or joined by a peptide-encoding linker. See Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85:5879-5883. A number of structures for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778.

[00176] The combining site refers to the part of an antibody molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. The antibody variable regions comprise three highly divergent stretches referred to as "hypervariable regions" or "complementarity determining regions" (CDRs) which are interposed between more conserved flanking stretches known as "framework regions" (FRs). In an antibody molecule, the three hypervariable regions of a light chain (LCDR1, LCDR2, and LCDR3) and the three hypervariable regions of a heavy chain (HCDR1, HCDR2 and HCDR3) are disposed relative to each other in three dimensional space to form an antigen binding surface or pocket. The antibody combining site therefore represents the amino acids that make up the CDRs of an antibody and any framework residues that make up the binding site pocket.

[00177] Antibodies that contain two heavy chains and two light chains bivalent in that they have two combining sites. A typical natural bivalent antibody is an IgG. Antibodies may be multi-valent as in the case of dimeric forms of IgA and the pentameric IgM molecule. Antibodies may also be univalent such as, for example, in the case of Fab or Fab' fragments.

[00178] The identity of the amino acid residues in a particular antibody that make up the combining site can be determined using methods well known in the art. For example, antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services; Johnson, G and Wu, TT (2001) Kabat Database and its applications: future directions. Nucleic Acids Research, 29: 205-206; http://immuno.bme.nwa.edu). The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others, (see Chothia and Lesk, J. Mol. Biol. 196, 901 (1987), Chothia et al., Nature 342, 877 (1989), and Tramontano et al., J. Mol. Biol. 215, 175 (1990)). Other methods include the "AbM definition" which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys) or the "contact definition" of CDRs by Macallum et al., ("Antibody-antigen interactions: contact analysis and binding site topography," J Mol Biol. 1996 Oct 11;262(5):732-45). The following chart identifies CDRs based upon various known definitions.

Loop	Kabat	AbM	Chothia	Contact
L1	L24 -- L34	L24 -- L34	L24 -- L34	L30 -- L36
L2	L50 -- L56	L50 -- L56	L50 -- L56	L46 -- L55
L3	L89 -- L97	L89 -- L97	L89 -- L97	L89 -- L96
H1 (Kabat Numbering)	H31 -- H35B	H26 -- H35B	H26 -- H32..34	H30 -- H35B
H1 (Chothia Numbering)	H31 -- H35	H26 -- H35	H26 -- H32	H30 -- H35
H2	H50 -- H65	H50 -- H58	H52 -- H56	H47 -- H58

H3	H95 -- H102	H95 -- H102	H95 -- H102	H93 -- H101

General guidelines by which one may identify the CDRs in an antibody from sequence alone are as follows:

LCDR1:

Start - Approximately residue 24.

Residue before is always a Cys.

Residue after is always a Trp. Typically TRP is followed with TYR-GLN, but also may be followed by LEU-GLN, PHE-GLN, or TYR-LEU.

Length is 10 to 17 residues.

LCDR2:

Start - 16 residues after the end of L1.

Sequence before is generally ILE-TYR, but also may be VAL-TYR, ILE-LYS, or ILE-PHE.

Length is generally 7 residues.

LCDR3:

Start – generally 33 residues after end of L2.

Residue before is a Cys.

Sequence after is PHE-GLY-X-GLY.

Length is 7 to 11 residues.

HCDR1:

Start – at approximately residue 26 (four residues after a CYS) [Chothia / AbM definition] Kabat definition starts 5 residues later.

Sequence before is CYS-X-X-X.

Residues after is a TRP, typically followed by VAL, but also followed by ILE, or ALA.

Length is 10 to 12 residues under AbM definition while Chothia definition excludes the last 4 residues.

HCDR2:

Start - 15 residues after the end of Kabat /AbM definition of CDR-H1.
Sequence before typically LEU-GLU-TRP-ILE-GLY (SEQ ID NO. 1), but a number of variations are possible.
Sequence after is LYS/ARG-LEU/ILE/VAL/PHE/THR/ALA-THR/SER/ILE/ALA
Length is 16 to 19 residues under Kabat definition (AbM definition ends 7 residues earlier).

HCDR3:

Start –33 residues after end of CDR-H2 (two residues after a CYS).
Sequence before is CYS-X-X (typically CYS-ALA-ARG).
Sequence after is TRP-GLY-X-GLY.
Length is 3 to 25 residues.

[00179] The identity of the amino acid residues in a particular antibody that are outside the CDRs, but nonetheless make up part of the combining site by having a side chain that is part of the lining of the combining site (i.e., it is available to linkage through the combining site), can be determined using methods well known in the art such as molecular modeling and X-ray crystallography. See e.g., Riechmann et al., (1988) Nature, 332:323-327. The aldolase antibody mouse mAb 38C2, which has a reactive lysine near to but outside HCDR3, is an example of such an antibody.

[00180] The reactive residue of the antibody combining site may be naturally associated with the antibody such as when the residue is encoded by nucleic acid present in the lymphoid cell first identified to make the antibody. Alternatively, the amino acid residue may arise by purposely mutating the DNA so as to encode the particular residue (see, e.g., WO 01/22922 to Meares et al.). The reactive residue may be a non-natural residue that may arise by biosynthetic incorporation using a unique codon, tRNA and aminoacyl-tRNA as already discussed. In another approach, the amino acid residue or its reactive elements (e.g., a nucleophilic amino group or sulfhydryl group) may be attached to an amino acid residue in the antibody combining

site. Thus, covalent linkage with the antibody occurring "through an amino acid residue in the combining site of the antibody" as used herein means that linkage can be directly to an amino acid residue of an antibody combining site or through a chemical moiety that is linked to a side chain of an amino acid residue of an antibody combining site.

[00181] As discussed, antibodies that can be used in preparing the antibody targeting compounds of the invention require a reactive side chain in the antibody combining site. A reactive side chain may be present or be placed by mutation in any antibody. Catalytic antibodies are a preferred source of such antibodies. Such antibodies include aldolase antibodies, beta lactamase antibodies, esterase antibodies, amidase antibodies, and the like.

[00182] A reactive lysine in an antibody combining site may be covalently linked to a ketone, diketone, beta lactam, active ester haloketone, lactone, anhydride, maleimide, epoxide, aldehyde amidine, guanidine, imines, eneamines, phosphates, phosphonates, epoxides, aziridines, thioepoxides, masked or protected diketones (ketals for example), lactams, haloketones, aldehydes, and the like, associated with a targeting agent or linker-targeting agent. An exemplary and preferred such antibody is an aldolase antibody such as the mouse monoclonal antibody mAb 38C2 and other like catalytic antibodies as well as suitably humanized and chimeric versions of such antibodies. Mouse mAb 38C2 is the prototype of a new class of catalytic antibodies that were generated by reactive immunization and mechanistically mimic natural aldolase enzymes (Barbas et al., 1997, Science 278, 2085-2092). Through a reactive lysine, these antibodies catalyze aldol and retro-aldol reactions using the enamine mechanism of natural aldolases (Wagner et al., 1995, Science 270, 1797-1800; Barbas et al., 1997, Science 278, 2085-2092; Zhong et al., 1999, Angew. Chem. Int. Ed. 38, 3738-3741; Karlstrom et al., 2000, Proc. Natl. Acad. Sci. U.S.A., 973878-3883). In addition to their versatility and efficacy in synthetic organic chemistry (e.g., Hoffmann et al., 1998, J. Am. Chem. Soc. 120, 2768-2779 ; Sinha et al., 1998, Proc. Natl. Acad. Sci. U.S.A. 95, 14603-14608), aldolase antibodies have been used to activate camptothecin, doxorubicin, and etoposide prodrugs in vitro and in vivo as an anti-cancer strategy

(Shabat et al., 1999, Proc. Natl. Acad. Sci. U.S.A. 96, 6925-6930 and ,2001, Proc. Natl. Acad. Sci. U.S.A. 98, 7528-7533).

[00183] In another example, the reactive amino acid of an antibody combining site may be a reactive cysteine, serine or tyrosine residue. For cysteines, the resulting antibody may form a covalent linkage with maleimide-containing components or other thiol-reactive groups such as iodoacetamides, aryl halides, disulfhydryls and the like. Reactive cysteines may be found in thioesterase catalytic antibodies as described by Janda et al., Proc. Natl. Acad. Sci. (USA) 91:2532-2536, (1994). For other esterase antibodies see Wirsching et al., Science 270:1775-82 (1995). Reactive amino acid-containing antibodies may be prepared by means well known in the art including mutating an antibody combining site residue to encode for the reactive amino acid or chemically derivatizing an amino acid side chain in an antibody combining site with a linker that contains the reactive group.

[00184] Antibodies suitable for use herein may be obtained by conventional immunization, reactive immunization in vivo, or by reactive selection in vitro, such as with phage display. Antibodies may be produced in humans or in other animal species. Antibodies from one species of animal may be modified to reflect another species of animal. For example, human chimeric antibodies are those in which at least one region of the antibody is from a human immunoglobulin. A human chimeric antibody is typically understood to have variable regions from a non-human animal, e.g. a rodent, with the constant regions from a human. In contrast, a humanized antibody uses CDRs from the non-human antibody with most or all of the variable framework regions from and all the constant regions from a human immunoglobulin. Chimeric and humanized antibodies may be prepared by methods well known in the art including CDR grafting approaches (see, e.g., U.S. Patent Nos. 5,843,708; 6,180,370; 5,693,762; 5,585,089; 5,530,101), chain shuffling strategies (see e.g., U.S. Patent No. 5,565,332; Rader et al., Proc. Natl. Acad. Sci. USA (1998) 95:8910-8915), molecular modeling strategies (U.S. Patent No. 5,639,641), and the like.

[00185] Unlike typical chemical derivatization of antibodies, those derived from reactive immunization can be specifically labeled in their binding site at a defined

position, facilitating the rapid and controlled preparation of a homogeneous product. In addition, unlike chemical derivatization of antibodies, those derived from reactive immunization with 1,3-diketones are reversible. Due to this reversibility, a diketone derivative of a targeting compound bound to mAb 38C2 can be released from the antibody through competition with the covalent binding hapten JW (Wagner et al., 1995, Science 270, 1797-800), or related compounds. This allows one to immediately neutralize the conjugate in vivo in case of an adverse reaction. Alternatively, non-reversible covalent linkage is possible such as with aldolase antibodies and beta lactam derivatives of the targeting compound. Unlike typical anti-hapten antibodies, covalent diketone binding antibodies have the advantage that the covalent linkage that is formed between the diketone and antibody is stable to large changes in pH, either extremes of low pH 3 or high pH 11. Such pH shifts do not release the targeting compound from the antibody. This is an advantage for tumor targeting since tumors typically exhibit reduced pH as compared to normal tissues. The added stability of covalent binding antibodies covalently linked to their targeting agent should provide additional advantages in terms of formulation, delivery, and long term storage.

[00186] A targeting compound of the present invention can be made using techniques well known in the art. Typically, synthesis of a targeting agent which also is a functional component (biological agent) is the first step. The targeting agent (also functional component in this case) is then derivatized for linkage to a connecting component (the linker) which is then combined with the antibody. One of skill in the art will readily appreciate that the specific synthetic steps used depend upon the exact nature of the three components.

[00187] By way of example, as a first step, targeting agent-linker compounds shown as Compounds 15 and 4, were made as shown in Schemes 1 (FIG. 10) and 2 (FIG. 11), respectively, as derivatized versions of the integrin targeting agents shown as Compounds 1 and 2, above. Compounds 15 and 4 were derivatized (relative to Compounds 1 and 2) by addition of a portion of the linker (connecting component). Scheme 3 (FIG. 12) shows additional synthetic steps by which a complete linker with a

diketone reactive moiety was added to derivatized targeting agent Compound 15 to obtain targeting compounds 80 and 82.

[00188] Synthesis of compound 80 was achieved starting from compound 14 in three steps. Compound 14 was converted to 15 as shown in Scheme 1 and the crude product was reacted with an N-hydroxysuccinimide (NHS) -ester of the diketone compound 23 in CH_3CN-DMF in the presence of Et_3N. Purification over silica gel ($CH2Cl_2$-MeOH, 9:1) afforded pure compound 80.

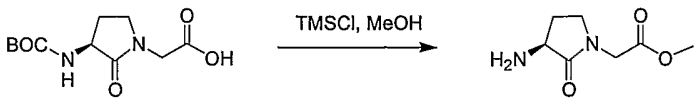
[00189] Compound 82 was synthesized from 14 in five steps (Schemes 2 and 3). Deprotection of the BOC group in compound 14 followed by reaction with the NHS ester of the bivalent linker 24 afforded compound 25, which was then deprotected and reacted with 23 as above to afford compound 82.

[00190] Synthesis of integrin targeting component-linker compounds 83 and 84 is shown in Scheme 4 (FIG. 13). Compounds 83 and 84 were each synthesized in one step from compound 4 (FIG. 13, scheme 4). Linking of Compound 4 was achieved with the appropriate activated NHS-ester.

[00191] Targeting agent-linker compounds, such as compounds 82, 83, and 84 where the linker includes a diketone reactive moiety, can be incubated with 0.5 equiv. of an aldolase antibody such as mAb 38C2 to produce antibody targeting compounds. Additional examples are set forth below.

[00192] Additional integrin RGD peptidomimetic targeting agent – linker conjugates of formula I can be synthesized in accordance with scheme 5 (FIG. 15), scheme 6 (FIG. 16), scheme 7 (FIG. 17), scheme 8 (FIG. 18), scheme 9 (FIG. 19), or scheme 10 (FIG. 20). Compound 58 may be prepared from 3-(2-Benzyloxycarbonylamino-acetylamino)-3-(4-hydroxy-phenyl)-propionic acid methyl ester (compound 57) as shown in scheme 5 (FIG. 15). RGD peptidomimetic targeting agent – linker conjugate shown as compound 46 may be synthesized from compound 59 in accordance with Scheme 6 (FIG. 16). Compound 59 may be produced from (S)-

3-(BOC-amino)-2-oxo-1-pyrrolidine – acetic acid (Chem-Impex International, Wood Dale, IL) as shown below.



Compound 59

RGD peptidomimetic targeting agent – linker conjugate shown as compound 86 may be synthesized in accordance with Scheme 7 (FIG. 17). Compounds 87 (scheme 8), 46 (scheme 9), and 88 (scheme 10) may be prepared by similar routes as shown in FIG.s 18, 19, and 20, respectively. Compounds of formula I without addition of the linker may be prepared as described in U.S. patent no. 6,211,184; or 5,952,341; and in PCT publication WO 00226717; WO 00226227; WO 00051686; or WO 00051968.

[00193] Also provided are targeting agent-linker compounds for covalently linking to a combining site of an antibody. The linker is of sufficient length to allow the targeting agent to bind to the target molecule when the targeting agent is linked through the linker to an antibody. In some embodiments, the targeting agent-linker compound includes one or more targeting agents specific for a target molecule with a linker of the formula X – Y – Z. The makeup of linker components X, Y and Z are as described above. If two or more targeting agents are included in the targeting agent-linker compound, the various targeting agents may be attached directly to the linker or the linker may be branched with targeting agents attached to different linker branches.

[00194] Also provided is a targeting agent-linker compound that can be noncovalently associated with the combining site of an antibody. This compound can be used in conjunction with a suitable antibody to form a targeting compound of the invention. Such targeting agent-linker compounds comprise two or more targeting agents covalently linked via a linker to an antigen recognized by the antibody. The linker may linear or branched and should be of sufficient length to allow the targeting

agent(s) to bind to the target molecule when the targeting agent(s) is linked through the linker to the antibody.

[00195] In some embodiments, the linker includes any of C, H, N, O, P, S, Si, F, Cl, Br, and I, or a salt thereof. The linker also may include a group such as an alkyl, alkenyl, alkynyl, oxoalkyl, oxoalkenyl, oxoalkynyl, aminoalkyl, aminoalkenyl, aminoalkynyl, sulfoalkyl, sulfoalkenyl, or sulfoalkynyl group, phosphoalkyl, phosphoalkenyl, phosphoalkynyl group. The linker also may include one or more ring structures. Combinations of the above groups and rings may also be present in the linkers of the targeting compounds of the invention. In some embodiments, the linker has a linear stretch of between 2-200 atoms although in other embodiments, longer linker lengths may be used. One or more targeting agents may be linked to the linker and if a branched linker is used, some of the targeting agents may be linked to different branches of the linker.

[00196] In some embodiments, the targeting agent of the targeting agent-linker compound is biologically active while in other embodiments, the targeting agent-linker compound further includes a separate biological agent, which is preferably covalently linked to the targeting agent. In some embodiments, the biological agent may be linked to the targeting agent or to the linker using essentially the same approaches used to link the targeting agent to the linker or using other approaches well known in the art.

[00197] The antigen of the linker can be any antigen which can be bound by an available antibody. Antigens are well known in the art and include, an organic compound, a drug, a biomolecule such as a protein, peptide, peptidomimetic, glycoprotein, proteoglycan, lipid, glycolipid, nucleic acid, carbohydrates, and the like as well as combinations of these molecules.

[00198] The present invention also includes methods of modifying the combining site of an antibody to generate binding specificity for a particular target molecule. Such methods include covalently linking a reactive amino acid side chain in the combining site of the antibody to a chemical moiety on a linker of a targeting agent-linker compound where the targeting agent is specific for the target molecule. The chemical

moiety of the linker is sufficiently distanced from the targeting agent so that the targeting agent can bind to the target molecule when the targeting agent-linker compound is covalently linked to the antibody combining site. Typically, the antibody will not be considered specific for the target molecule. In a preferred embodiment, the antibody prior to covalent linking would have an affinity for the target molecule of less than about 1×10^{-5} moles/liter. However, after the antibody is covalently linked to the targeting agent-linker compound, the modified antibody preferably has an affinity for the target molecule of at least about 1×10^{-6} moles/liter, more preferably at least about 1×10^{-7} moles/liter, even more preferably at least 1×10^{-8} moles/liter, yet even more preferably at least 1×10^{-9} moles/liter, most preferably at least about 1×10^{-10} moles/liter.

[00199] The present invention also includes methods of altering at least one physical or biological characteristic of a targeting agent, biological agent or linker. The methods include covalently linking the targeting agent or biological agent to the combining site of an antibody as described above. In some embodiments, the targeting agent or biological agent is linked to the antibody combining site though a linker, the characteristics of which are described above. The method is particularly useful for linking small targeting or biological agents of 5 Kd or less. However, the method also works for larger such molecules. Characteristics of the targeting agent or biological agent can include binding affinity, susceptibility to degradation, such as by proteases, pharmocokinetics, pharmacodynamics, immunogenicity, solubility, solubility, lipophilicity, hydrophilicity, hydrophobicity, stability (more or less stable as well as planned degradation), rigidity, flexibility, modulation of antibody binding, and the like.

[00200] As used herein, pharmacokinetics refers to the concentration an administered compound in the serum over time. Pharmacodynamics refers to the concentration of an administered compound in target and nontarget tissues over time and the effects on the target tissue (efficacy) and the non-target tissue (toxicity). Improvements in, for example, pharmacokinetics or pharmacodynamics can be designed for a particular targeting agent or biological agent such as by using labile

linkages or by modifying the chemical nature of any linker (changing solubility, charge, etc.).

[00201] The biological characteristic of a targeting compound of the invention may be modified to obtain improved pharmaceutical or other characteristics. This may be achieved by altering one or more chemical characteristics of the targeting agent or biological agent, the linker or the polymer. A preferred approach is to chemically modify one or more chemical characteristics of the linker. By altering chemical characteristics of the compound including the linker, one can obtain improved features such as improvement in pharmockinetics, pharmacodynamics, solubility, immunogenicity and the like.

[00202] The targeting compounds of the present invention have many uses. For example, the polymer (e.g., antibody) portion of a targeting compound may generally extend the half-life of a smaller sized targeting agent in vivo. Also, the biological potency of a particular targeting agent may be increased by the addition of effector function(s) provided by the polymer portion of the targeting compound (e.g., complement mediated effector functions). In addition, the targeting agent, through its increased size conferred by linkage to the polymer, may enable the targeting agent to function as a competitive inhibitor in situations where it would otherwise fail to do so. Thus, in one aspect, the invention provides a method for increasing the effective circulating half-life of a targeting agent. The method includes linking the targeting agent to a polymer such as an antibody using a linking group as set forth above. In another aspect, the invention provides a method of redirecting an antibody to a specific target. The method includes linking an antibody to a targeting agent through a linker as set forth above.

[00203] The invention also provides a method of treating or preventing a disease or condition in an individual wherein said disease or condition involves cells, tissue or fluid that expresses a target molecule. The method includes administering to a subject such as a patient, a therapeutically effective amount of a targeting compound of the invention. The subject may be an animal such as a mammal. In some embodiments, the subject is a human. The compound may include a biological agent that is the same

or is distinct from the targeting agent and which may take any of the forms or activities described herein. In some preferred embodiments, the target molecule is an integrin and the disease is a carcinoma. The association of integrin expression in carcinomas is well known in the art (See, e.g., United States Patent Nos. 5,753,230 and 5,766,591, the disclosures of which are incorporated herein by reference). For therapeutic use in humans, a human, humanized, or human chimeric antibody is a preferred as the antibody component of the targeting compound. An antibody with a human IgG4 constant region also is preferred if agonist activity is desired.

[00204] In addition to therapeutic applications, antibody targeting compounds of the invention may also be used for the imaging of cells such as tumor cells or tissues (e.g., an extracellular matrix biomolecule) as is well known in the art. Accordingly, provided is a method of imaging cells or tissue (e.g., an extracellular matrix biomolecule) in an individual. In such methods, the cells or tissue expresses a target molecule. The method includes administering to a subject an antibody targeting compound of the invention linked to a detectable label. A detectable label for use in such methods can be a radioisotope or may be a non-radioisotope such as may be used in nuclear magnetic resonance (NMR) imaging. In the latter case, one may link the antibody targeting agent to chelates e.g., diethylenetriaminepentaacetate (DTPA) of the paramagnetic metal gadolinium essentially as described in Simkins et al., Nat. Med., 4(5):623-6 (1998).

[00205] The binding of a mixture of compound 80 and 38C2 to human Karposi's sarcoma SLK cells was studied. Compound 80 effectively mediated cell surface binding of 38C2. No binding of 38C2 was detectable in the absence of compound 80. Control experiments confirmed that the 1,3- diketone moiety is required for binding of compound 80 to 38C2. After independent i.p. and i.v. injections, respectively, compound 80 and 38C2 form an integrin $\alpha_v\beta_3$ targeting conjugate *in vivo*. In these experiments, the circulatory half-life of compound 80 was extended by more than two orders of magnitude through binding to 38C2. Combination of compound 80 and 38C2 effectively inhibited tumor growth in a mouse model of human Karposi's sarcoma, whereas either compound 80 or 38C2 alone were less effective or not effective at all.

[00206] The present invention also provides methods of targeting a biological activity to cells, tissue (e.g., an extracellular matrix biomolecule) or a biolomolecule in the fluid of a subject. The method includes administering to the subject, a targeting compound that includes a targeting agent specific for the cells, tissue extracellular matrix biomolecule or fluid biomolecule. The targeting agent is covalently linked to an amino acid residue in the combining site of an antibody. In some embodiments, a linker is used to link the targeting agent to the antibody. The targeting agent is not an antibody. In some embodiments, the compound has a biological activity while in other embodiments, an biologically active molecule that is not the targeting agent is included as a component of the compound.

[00207] The invention methods of targeting a biological activity to cells, tissue or a biolomolecule in the fluid of a subject and use of such approaches for therapy and/or imaging may be achieved by administering the targeting agent – linker conjugate to the individual and allowing it to form a covalent compound with the combining site of an appropriate antibody in vivo. The antibody portion of the targeting compound that forms in vivo may be may be administered to the individual before, at the same time, or after administration of the targeting agent – linker conjugate. As aleardy discussed, the targeting agent may include a linker/reactive moiety or the antibody combining site may be suitably modified to covalently link to the targeting agent. Alternatively, or in addition, the antibody may be present in the circulation of the individual following immunization with an appropriate immunogen. For example, catalytic antibodies may be generated by immunizing with a reactive intermediate of the substrate conjugated to a carrier protein. See Lerner et al. Acta Chem Scand 50(8): 672-678 (1996). In particular, aldolase catalytic antibodies may be generated by administering with keyhole limpet hemocyanin linked to a diketone moiety as described by Wagner et al., Science 270(5243):1775-1782 (1995).

[00208] Targeting agents of the invention also may conjugate to circulating proteins and cells endogenous to the individual following administration of the targeting agent to the individual. The targeting agents of the invention may have reactive groups for linking to endogenous serum proteins and cells as described previously in U.S. Patent

nos. 5,612,034; 6,277,583; 6,329,336; 6,277,863; 6,107,489; 6,087,375; 5,840,733; 6,103,233; 5,843,440; 5,942,620; 6,403,324 and 6,437,092.

[00209] A targeting compound of the present invention can be administered as a pharmaceutical or medicament that includes a targeting compound of the invention formulated with a pharmaceutically acceptable carrier. Accordingly, the compounds may be used in the manufacture of a medicament or pharmaceutical composition. Pharmaceutical compositions of the invention may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. Liquid formulations may be buffered, isotonic, aqueous solutions. Powders also may be sprayed in dry form. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water, or buffered sodium or ammonium acetate solution. Such formulations are especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride, sodium citrate, and the like.

[00210] Alternatively, compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, or

an aqueous or non-aqueous suspension. For rectal administration, the invention compounds may be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

[00211] Compounds of the invention may be formulated to include other medically useful drugs or biological agents. The compounds also may be administered in conjunction with the administration of other drugs or biological agents useful for the disease or condition that the invention compounds are directed.

[00212] As employed herein, the phrase "an effective amount," refers to a dose sufficient to provide concentrations high enough to impart a beneficial effect on the recipient thereof. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated, the severity of the disorder, the activity of the specific compound, the route of administration, the rate of clearance of the compound, the duration of treatment, the drugs used in combination or coincident with the compound, the age, body weight, sex, diet, and general health of the subject, and like factors well known in the medical arts and sciences. Various general considerations taken into account in determining the "therapeutically effective amount" are known to those of skill in the art and are described, e.g., in Gilman et al., eds., Goodman And Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990. Dosage levels typically fall in the range of about 0.001 up to 100 mg/kg/day; with levels in the range of about 0.05 up to 10 mg/kg/day are generally applicable. A compound can be administered parenterally, such as intravascularly, intravenously, intraarterially, intramuscularly, subcutaneously, or the like. Administration can also be orally, nasally, rectally, transdermally or inhalationally via an aerosol. The composition may be administered as a bolus, or slowly infused.

[00213] The administration of an antibody-targeting agent conjugate to an immunocompetent individual may result in the production of antibodies against the conjugate. Such antibodies may be directed to the antibody itself, such as the variable region including the antibody idiotype as well as to the targeting agent or any linker

used to conjugate the targeting agent to the antibody. Reducing the immunogenicity of the antibody-targeting agent conjugate can be addressed by methods well known in the art such as by attaching long chain polyethylene glycol (PEG)-based spacers, and the like, to the antibody-targeting agent. Long chain PEG and other polymers are known for their ability to mask foreign epitopes, resulting in the reduced immunogenicity of therapeutic proteins that display foreign epitopes (Katre et al., 1990, *J. Immunol.* **144**, 209-213; Francis et al., 1998, *Int. J. Hematol.* **68**, 1-18). As noted, PEG can be a linker as well, thus providing both linker function and reduced immunogenicity in a targeting compound of the invention. Alternatively, or in addition, the individual administered the antibody-targeting agent conjugate may be administered an immunosuppressent such as cyclosporin A, anti-CD3 antibody, and the like.

[00214] A method of screening a chemical library for agonists or antagonists of a receptor is further provided. The method includes linking individual members of the chemical library to the polymer, e.g., the combining site of an antibody and then testing the antibody linked library for binding to the receptor or for inhibition of binding between the receptor and a ligand for the receptor. By this approach, the present antibody targeting compounds provide a new format for high throughput screening to identify candidate small molecule chemicals such as drugs peptides peptidomimetics, organic compounds, and the like, that function for example, as antagonists or agonists. The relative small size of a useful candidate chemical molecule typically requires indirect screening such as in displacement or competition formats. As provided herein, one can build the chemical library on a polymer, e.g., antibody format, by linking individual drugs in the library to a combining site of an antibody.

[00215] Polymer-tagged libraries may be prepared by synthesizing chemical candidates with a suitable linker comprising a particular linker moiety designed for covalent interaction with a particular polymer, e.g., an antibody. Such linkers may include a diketone moiety to be used in conjunction with an aldolase antibody that includes a reactive lysine in the combining site. One skilled in the art would readily understand that other linkers and linker moieties (e.g., biotin) which have been described herein are clearly useful for this purpose.

[00216] Polymer-tagged chemical libraries such as antibody combining site-tagged chemical libraries thus prepared can be used, for example, in receptor assays or cell bioassays where binding of each compound in the library may be monitored by detecting the linked antibody. Detection of the antibody portion of each compound may be accomplished by methods of antibody detection well known in the art. For example, the antibody may be linked to a detectable moiety such as an enzyme, fluorophore, radioisotope, and the like. Indirect systems can also be used such as biotin-streptavidin. Libraries can be screened on cells or impure antigens such as viral lysates as well as on purified antigens. For example, libraries can be tested for binding or inhibition of binding using as the target, lysates run on protein gels, with the analysis focussed on a particular gel band. In the case where the receptor is expressed on a cell, binding or inhibition of binding can be determined by detecting cellular signaling events occurring (or not occurring as in the case of inhibition) downstream of said binding or inhibition of binding. Downstream cellular signaling can be detected with the aid of a reporter gene as is well known in the art (see, e.g., U.S. Patent No. 5,618,720 and 5,670,113).

[00217] Screening of polymer tagged chemical libraries can be readily adapted for use with high throughput instruments. Screening may be done in vitro or in vivo. Furthermore, a biological display library such as a peptide phage library may be used to prepare a polymer-tagged library such as an antibody combining site-tagged library. In such cases, the site of attachment of the linker moiety (e.g., diketone) can be the fusion point of the library to the biological carrier.

[00218] The screening by inhibition of binding also can be applied to analyze and identify targeting compounds that differ in structure but bind to the same target moiety. For example, in the case of integrin peptidomimetics, one may define an integrin targeting compound comprising, an RGD peptidomimetic - polymer complex with the following requirements:

a) the RGD peptidomimetic binds to one or both of a particular integrin (e.g. $\alpha_v\beta_3$ and $\alpha_v\beta_5$);

b) the polymer (e.g., antibody) does not bind to the integrin (e.g., $\alpha_v\beta_3$ or $\alpha_v\beta_5$);

c) the complex results from an association between the RGD peptidomimetic and thepolymer (e.g., combining site of the antibody); and

d) the integrin targeting compound competes for binding between the integrin (e.g., $\alpha_v\beta_3$ and $\alpha_v\beta_5$) and its protein target (e.g., vitronectin, and fibrinogen). In this example, the association between the RGD peptidomimetic and the polymer (e.g., combining site of the antibody) may be covalent or noncovalent.

[00219] Also provided is an immunoassay method for determining the amount of analyte in a sample (wherein the polymer is an antibody). Such methods include:

(a) forming, in a medium containing a sample, a complex between the analyte and at least one antibody specific for the analyte;

(b) analyzing the medium to detect the amount of the complex; and

(c) relating the amount of the complex to the amount of analyte in the sample.

Such methods may also include forming the complex with at least one antibody that is specific for the analyte. The specificity of the antibody is provided by a non-antibody targeting agent specific for the analyte which is covalently linked to a reactive amino acid in the combining site of the antibody. Thus, the antibody targeting compounds of the invention can be used in immunoassays for detecting and measuring the amount of an analyte in a sample as has been done previously with conventionally prepared polyclonal or monoclonal antibodies. Such assays are well known in the art and include RIA, EIA, Western, ELISA, and the like. The assay formats may be competitive or non-competitive and may be direct or indirect. The antibody targeting compound can be used in the liquid phase and/or can be bound to a solid phase carrier. Carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, natural and modified cellulose, polyacrylamide, agarose, magnetite, and the like. The nature of the carrier can be either soluble or insoluble. The antibody targeting compound may be detectably labeled in any of various ways well known in the art. U.S. Patent Nos. 4,659,678; 4,780,423; and 4,298,685 are exemplary of such assays.

[00220] Viewed in general terms, the amount of an analyte in a sample can be determined by forming, in a medium containing the sample, a complex between the analyte and at least one polymer, such as an antibody, specific for the analyte. The medium is then analyzed to determine the amount of the complex that is formed. Finally, the amount of complex formed is then related to the amount of analyte in the sample. As already described, this general approach can take many forms such as direct and indirect, homogenous or heterogeneous, and competitive and noncompetitive. In all cases, the antibody targeting compounds of the invention may be used to replace functions provided by conventionally prepared antibodies.

[00221] Also provided is a direct or indirect binding assay where the presence of an analyte is determined using a polymer (e.g., antibody) specific for the analyte. In such methods, the presence of the analyte is determined using an antibody specific for the analyte. The antibody specificity results from a non-antibody targeting agent that is specific for the analyte, and the targeting agent is covalently linked to a reactive amino acid in the combining site of the antibody. Thus, antibody-targeting compounds of the invention can be used in qualitative assays in place of conventionally prepared antibodies.

[00222] It would be readily evident that the compounds of the invention find use not only in human medical therapy and diagnosis but also in veterinary, agricultural, environmental and other disciplines.

[00223] Also provided are methods of inhibiting or reducing the ability of a targeting agent or biological agent to cross a cell membrane. In these methods an antibody targeting compound is formed by covalently linking the polymer, e.g., the combining site of an antibody, that does not itself cross the cell membrane to the targeting agent or biological agent, wherein linkage of the polymer to the targeting agent or biological agent reduces or inhibits the ability of the agent to cross the cell membrane. Antibodies that are not directed to cell surface internalizing receptors are a preferred source of polymers that do not cross cell membranes.

[00224] Further provided are methods of mediating intracellular delivery of a intracellularly active drug. In these methods, a targeting compound is prepared wherein said compound includes one or more targeting agents or one or more biological agents or both covalently linked via a linker to the combining site of the antibody. The targeting agents or biological agents are characterized in that they bind to a cell receptor and mediate internalization of the agent. The targeting compound also includes a drug that is active intracellularly. Intracellular drug delivery occurs when a cell expressing the receptor contacts the targeting compound, e.g., antibody targeting compound. The contacting results in internalization of the targeting agent and delivery of said drug intracellularly.

[00225] This approach uses takes advantage of receptor mediated endocytosis (i.e., receptor mediated internalization) to deliver the antibody targeting compound intracellularly. Cell surface receptors that mediate internalization of binding ligands are well known in the art and include, for example, integrins, HER2, EGF receptor, folic acid receptor, and the like. Internalization assays are readily available and can be evaluated using fluorescent detection methods.

[00226] In some embodiments, the intracellularly active drug is a prodrug that becomes active when said drug contacts an intracellular compartment. The targeting compound may include an intracellular trafficking signal to direct the internalized targeting compound to a particular intracellular compartment. Many proteins contain one or more targeting sequences that serve as a trafficking signal or address to target the protein to the correct intracellular site. Receptors at the destination also may be involved in the trafficking process.

[00227] The sequences that direct proteins and other compounds to different intracellular sites such as endoplasmic reticulum, endosome, golgi, or nucleus, and the like, are well known in the art. For example, endoplasmic reticulum trafficking signals include a KDEL or KKXX sequence, golgi trafficking signals include a GRIP domain (see Munro et al., *Curr Biol* 9: 377-379, 1999), lysosomal trafficking signals (from golgi) include mannose-6-phosphate modified oligosaccharides, and nuclear localization trafficking signals which include one or two short positively charged

sequences, e.g., lysine or arginine rich (see, Penco et al. Biotech Appl Biochem 34:151-159 2001).

[00228] The versatility of the invention is illustrated by the following Examples which illustrate preferred embodiments of the invention and are not limiting of the claims or specification in any way.

EXAMPLE 1: Antibody targeting compound comprising an RGD peptidomimetic targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00229] An integrin targeting compound was formed based on the formation of a reversible covalent bond between a diketone linker derivative of an RGD peptidomimetic and the reactive lysine of mouse mAb 38C2. Mouse mAb 38C2 is the prototype for a new class of catalytic antibodies generated by reactive immunization and mechanistically mimic natural aldolase enzymes (Barbas et al., *Science* **278**, 2085-2092, 1997). Through a reactive lysine, these antibodies catalyze aldol and retro-aldol reactions using the enamine mechanism of natural aldolases (Wagner et al., *Science* **270**, 1797-1800, 1995; Barbas et al., *Science* **278**, 2085-2092, 1997; Zhong et al., *Angew. Chem. Int. Ed.* **38**, 3738-3741, 1999). In addition to their versatility and efficacy in synthetic organic chemistry, aldolase antibodies have been used in the activation of camptothecin, doxorubicin, and etoposide prodrugs *in vitro* and *in vivo* as an anti-cancer strategy (Shabat et al., *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6925-6930, 1999); Shabat, D. *et al. Proc. Natl. Acad. Sci. U.S.A.* **98**, 7528-7533, 2001). Yet another feature of these antibodies, namely their ability to bind diketones covalently, has remained largely unexplored.

[00230] The RGD peptidomimetic used (see Compound 1) is specific for human integrin with a high binding affinity for $\alpha_v\beta_3$ at 0.9 nM and $\alpha_v\beta_5$ at 0.6 nM (specificity exhibited by minimal $a_{IIb}b_3$ binding) (Miller et al., supra). A diketone linker modified version of Compound 1, designated as compound 80, was prepared as described above.

[00231] A peptidomimetic RGD antagonist with known activity for both $\alpha_v\beta_3$ or $\alpha_v\beta_5$ binding is desirable because some of these compounds bind both murine and human integrins. Such species cross reactivity affords preclinical *in vivo* studies in animal angiogenesis models prior to human trials. In addition, the targeting compound may be used for the therapy of Kaposi's sarcoma which is associated with $\alpha_v\beta_3$ integrin.

[00232] Compound 80 was linked to antibody 38C2 by the following procedure: One milliliter antibody 38C2 in phosphate buffered saline (10mg/mL) was added to 12 microliters of a 10 mg/mL stock solution of compound 80 and the resulting mixture was maintained at room temperature for 2 hours prior to use.

[00233] The binding of a mixture of compound 80 and 38C2 to SLK cells was evaluated. Compound 80 effectively mediated cell surface binding of 38C2. No binding of 38C2 was detectable in the absence of compound 80. Control experiments confirmed that the diketone moiety of the linker is required for binding of compound 80 to 38C2. It was determined that compound 80 retains the integrin specificity of the integrin targeting component, i.e., no binding to $a_{IIb}b_3$ in ELISA was detected while binding to $\alpha_v\beta_3$ and $\alpha_v\beta_3$ was found to be strong. Independent i.p. and i.v. injections of the targeting compound prepared with compound 80 and 38C2 versus each component alone into mice demonstrated integrin targeting *in vivo*. In these experiments, the serum half-life of compound 80 was extended by more than two orders of magnitude through binding to 38C2. Free compound 80 not bound to antibody had a serum half-life of only minutes while the combination of antibody and small molecule could be detected in the serum sampled from eye bleeds after several days.

EXAMPLE 2: Antibody targeting compound comprising IL-4 as targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00234] Kaposi's sarcoma tumor cells, among other human epithelial tumor cells, express interleukin-4 (IL-4) receptors that can be targeted with a recombinant chimeric protein consisting of IL-4 and a truncated form of bacterial toxin called *Pseudomonas*

exotoxin (Husain et al., 1999, *Nat. Med.* **5**, 817-822). Based on these studies, an IL-4 targeting compound for targeting mAb 38C2 to Kaposi's sarcoma tumor cells is prepared. A linker with a diketone reactive group is conjugated to a lysine side chain of IL-2 using a lysine reactive moiety such as N-hydroxysuccinimide (NHS). Alternatively, a recombinant IL-2 with an added free cysteine is used for conjugation to cysteine reactive moieties such as maleimide. To reduce immunogenicity associated with the linker portion of the targeting compound, the spacer (i.e. linker connecting chain) between the diketone reactive group on one end and the NHS or maleimide group on the other, is a polyethylene glycol (PEG) chain. Long chain PEG and other polymers are known for their ability to mask foreign epitopes, resulting in the reduced immunogenicity of therapeutic proteins that display foreign epitopes (Katre et al., 1990, *J. Immunol.* **144**, 209-213; Francis et al., 1998, *Int. J. Hematol.* **68**, 1-18). Not more than one to two diketones should be conjugated to the IL-4 in order to avoid clearance of cross-linked antibodies (Rehlaender and Cho, 1998, *Pharm. Res.* **15**, 1652-1656). Other interleukins such as IL-2 can be used in place of IL-4 as the targeting agent. While IL-4 can be used primarily as a targeting module, an enhancement of its pharmacological effect (Lussow et al., 1996, *Transplantation* **62**, 1703-1708) may result from IL-2 receptor triggering due to the prolonged serum half-life of the interleukin obtained through its linkage to an antibody.

EXAMPLE 3: Antibody targeting compound comprising VEGF-R2 binding peptide as targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00235] Vascular endothelial growth factor (VEGF) is a key modulator of tumor angiogenesis. Induced by hypoxia, VEGF expression is upregulated through the induction of VEGF mRNA transcription in the tumor. Following production and release by the tumor, VEGF diffuses to endothelial cells of nearby preexisting blood vessels, which display VEGF receptors (VEGFR). VEGF binds to two tyrosine kinase receptors, VEGFR-1 and VEGFR-2, which are expressed predominantly on endothelial cells. Activation of endothelial cells is associated with the binding of VEGF to VEGFR-2, whereas VEGFR-1 probably functions as a decoy receptor that regulates the local concentration of VEGF (Neufeld et al., 1999, *FASEB J.* **13**, 9-22). Following

activation, the endothelial cells proliferate, migrate directionally toward the tumor, and eventually roll up and interconnect to form new blood vessels. Anti-angiogenic drugs that interfere with the interaction of VEGF and VEGR-2 are promising candidates for cancer therapy (Klohs and Hamby, 1999, *Curr. Opin. Biotechnol.* **10**, 544-549). Binétruy-Tournaire et al. (2000, *EMBO J.* **19**, 1525-1533) identified the VEGFR-2 binding linear peptide ATWLPPR (SEQ ID NO: 2) through phage display of peptide libraries. ATWLPPR (SEQ ID NO: 2) effectively interfered with VEGF binding to VEGFR-2 and inhibited VEGF-mediated angiogenesis.

[00236] An antibody targeting compound comprising VEGF-R2 binding peptide is prepared by synthesizing the peptide with an additional Cys residue at the amino or carboxy terminus, resulting in a peptide with the sequence ATWLPPRC (SEQ ID NO: 3) and CATWLPPR (SEQ ID NO: 4), respectively. These thiol-modified peptides are reacted with a maleimide/diketone linker (FIG. 14) to produce peptide-linker-diketo and diketo-linker-peptide. Incubation of these diketone derivatives with mAb38C2 results in a covalent linkage between the VEGFR-2 peptide and the antibody combining site. The resulting antibody - VEGFR-2 targeting compound is used to target endothelial cells that express VEGFR-2 such as in tumor angiogenesis. The compound prolongs the half-life of the peptide and equips it with antibody effector function.

EXAMPLE 4: Antibody targeting compound comprising neutralizing RNA aptamer as targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00237] Using the process of SELEX (Systematic Evolution of Ligands by Exponential Enrichment), RNA and DNA aptamers to a variety of molecular targets have been generated (Jayasena, 1999, *Clin. Chem.* **45**, 1628-1650). For example, 2' fluoropyrimidine RNA aptamers that include about 25 nucleotides and that bind VEGF with an affinity in the 100-pM range were described (Ruckman et al., 1999, *J. Biol. Chem.* **32**, 20556-20567). Like the peptide described in the previous example, the aptamers were found to interfere with the interaction of VEGF and VEGFR-2.

[00238] An antibody targeting compound comprising VEGF RNA aptamer is prepared using commercially available thiol-derivatized nucleotides such as 5'-

phosphorothioate. A phosphorothioate group is a modified phosphate group with one of the oxygen atoms replaced by a sulfur atom. The thiol-modified nucleotide within the RNA aptamer is reacted with a maleimide diketone (e.g., FIG. 14) to produce an RNA aptamer targeting-diketone linker compound. Alternatively, a primary amino group is introduced into the RNA aptamer using commercially available amino modifiers. A nucleotide labeled with a primary amino group within the RNA aptamer is reacted with a linker that has N-hydroxysuccinimide diketone as the reactive group. Incubation of the diketone derivatives with mAb38C2 results in a covalent linkage between the RNA aptamer and the antibody combining site. The resulting antibody – RNA aptamer VEGFR-2 targeting compound is used to target endothelial cells that express VEGFR-2 such as in tumor angiogenesis. The compound prolongs the half-life of the RNA aptamer and equips it with antibody effector function.

EXAMPLE 5: Antibody targeting compound comprising folate as targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00239] The folate receptor mediates the uptake of folic acid into cells by endocytosis. It is overexpressed on a variety of epithelial tumor cells (Leamon and Low, 2001, *Drug Discov. Today* **6**, 44-51). For example, greater than 90 % of ovarian carcinomas express the folate receptor (Sudimack and Lee, 2000, *Adv. Drug Deliv. Rev.* **41**, 147-162). Mabs directed to the folate receptor, for example Mov18 and Mov19, have been evaluated as drugs for ovarian cancer therapy (Coney et al., 1994, *Cancer Res.* **54**, 2448-2455; Molthoff et al., 1997, *Cancer* **80**, 2712-2720). Folate-mediated targeting of cancer cells over expressing the folate receptor is an alternative strategy (Leamon and Low, 2001, *Drug Discov. Today* **6**, 44-51). For example, chemotherapeutic drugs such as maytansinoids (Ladino et al., 1997, *Int. J. Cancer* **73**, 859-864), are conjugated to folate for selective chemotherapy.

[00240] A targeting agent-linker compound comprising folate derivatized with a diketone shown in FIG. 2E is linked to mAb 38C2 and is used to target ovarian cancer cells. Because a majority of ovarian tumor cells also express integrins $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$, in addition to the folate receptor, a dual targeting compound may be used for

treatment. A targeting agent-linker compound comprising folate and an RGD peptidomimetic antagonist are together derivatized with a single diketone linker to form the dual targeting compound shown in FIG. 4B. The targeting agent-linker is linked to mAb 38C2 and is used to target ovarian cancer cells.

EXAMPLE 6: Antibody targeting compound comprising an inhibitor of prostatic acid phosphatase or prostate-specific antigen as targeting agent covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00241] Prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA), a serine protease, are expressed on the cell surface of prostate tumor cells and are used as markers for prostate cancer. Mabs directed to PAP and PSA have long been considered promising drugs for prostate cancer therapy (Chang et al., 1999, *Curr. Opin. Urol.* **9**, 391-395). More recently, small synthetic molecules that are specific inhibitors of PAP (Beers et al., 1996, *Bioorg. Med. Chem.* **4**, 1693-1701) and PSA (Adlington et al., 2001, *J. Med. Chem.* **44**, 1491-1508) have been reported. Other cell surface enzymes specific for prostate tumor cells, such as the recently identified serine protease hepsin (Magee et al., 2001, *Cancer Res.* **61**, 5692-5696), also can be used as a target after specific small synthetic molecules or peptides targeting agents are identified.

[00242] A targeting agent-linker compound comprising a PAP and/or PSA inhibitor is derivatized with a diketone linker to form the compound shown in FIG. 2C). The targeting agent-linker is linked to mAb 38C2 and is used to target prostate cancer.

EXAMPLE 7: Antibody targeting compound comprising thrombopoietin mimetic peptides or small-molecule agonists of the thrombopoietin receptor covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00243] The cell surface thrombopoietin receptor (cMpl, TPOR) is a member of the hematopoietic growth factor receptor superfamily. Thrombopoietin (TPO), the cytokine that binds to the thrombopoietin receptor, plays a central role in

megakaryopoiesis and platelet production. Therapeutically, recombinant TPO is being tested in the clinic for the treatment of thrombocytopenia resulting from chemotherapy and bone marrow transplantation. As a therapeutic compound, TPO suffers from a relatively short half-life *in vivo* and from manufacturing and formulation shortcomings.

[00244] A TPO targeting agent antibody compound is prepared to treat treatment of thrombocytopenia resulting from chemotherapy and bone marrow transplantation. The TPO mimetic peptide AF12505 with the sequence IEGPTLRQWLAARA (SEQ ID NO: 5), which has been reported to mimic the activity of recombinant TPO (Cwirla et al., 1997, *Science*, 276:1696-9), is synthesized with an additional Cys residue added to the amino terminus to produce CIEGPTLRQWLAARA (SEQ ID NO: 6). This thiol-labeled peptide is then reacted with a maleimide/diketone linker (FIG. 14) to produce TPO peptide-linker (diketone) compound. Incubation of this diketone derivative with mAb38C2 generates an antibody-TPO receptor targeting compound.

[00245] *In vitro* assays are used to demonstrate that the targeted antibody binds live cells expressing the TPOR and stimulated megakaryocyte colony formation to a greater extent than the peptide AF12505. Other TPO mimetic peptides are known in the art and can also be used as the TPO receptor targeting agent. In addition, small-molecule mimetics with TPO receptor binding have recently been described by Kimura et. al (*FEBS Lett*, 1998, :428(3):250-4.) also may be used in preparing TPOR targeting compounds.

[00246] The above approach can be similarly applied to target the erythropoietin (EPO) receptor using EPO targeting mimetics that have increased therapeutic efficacy (Middleton et al., *J Biol Chem.*, 1999, 274(20):14163-9; Johnson et al., Nephrol Dial Transplant., 2000, 15(9):1274-7).

EXAMPLE 8: Antibody targeting compound comprising T-20 peptide or small-molecules that bind the envelope proteins of HIV-1 covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00247] T-20, N-Acetyl-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (SEQ ID NO: 7), a synthetic peptide corresponding to a region of the transmembrane subunit of the HIV-1 envelope protein, blocks cell fusion and viral entry at concentrations of less than 2 ng/mL in vitro. When administered intravenously, T-20 (monotherapy), the peptide decreases plasma HIV RNA levels demonstrating that viral entry can be successfully blocked in vivo. Administration of T-20 provides potent inhibition of HIV replication comparable to anti-retroviral regimens approved at present (Kilby et al., *Nat Med.,* 1998, 4(11):1302-7). This peptide drug suffers from a short half-life *in vivo* of approximately 2 hrs.

[00248] An antibody targeting compound using the T-20 peptide as targeting agent was produced to increase the valency, potency, and half-life of T-20. The T-20 peptide was synthesized with an additional Cys residue at the carboxy terminus, the resulting modified T-20 peptide having the sequence N-Acetyl-YTSLIHSLIEESQNQQEKNE QELLELDKWASLWNWFC (SEQ ID NO: 8). This thiol-labeled peptide was then reacted with a maleimide/diketone linker (FIG. 14) to produce a T-20-Cys-linker compound. Incubation of this targeting agent-diketone linker with Ab38C2 resulted in a covalent linkage between the peptide and the antibody. *In vitro* assays demonstrated that the targeted antibody demonstrated increased potency in inhibiting HIV-1 entry and infection.

[00249] In addition to peptides that target the envelope proteins of HIV-1, a number of small-molecules that bind the envelope proteins have been described. For example, the betulinic acid derivative IC9564 is a potent anti-human immunodeficiency virus (anti-HIV) compound that can inhibit both HIV primary isolates and laboratory-adapted strains. Evidence suggests that HIV-1 gp120 plays a key role in the anti-HIV-1 activity of IC9564 (Holz-Smith et al., *Antimicrob Agents Chemother.,* 2001, 45(1):60-6.) Preparing an antibody targeting compound in which IC9564 is the targeting agent is expected to have increased activity over IC9564 itself by increasing valency, half-life, and by directing immune killing of HIV-1 infected cells based on the constant region of the antibody chosen. Similarly, recent X-ray crystallographic determination of the HIV-1 envelope glycoprotein gp41 core structure opened up a new avenue to discover

antiviral agents for chemotherapy of HIV-1 infection and AIDS. Compounds with the best fit for docking into the hydrophobic cavity within the gp41 core and with maximum possible interactions with the target site can also be improved by addition of a diketone arm and covalent linkage to an antibody. Several compounds of this class have been identified (Debnath et al., *J Med Chem.*, 1999, 42(17):3203-9).

EXAMPLE 9: Antibody Targeting compound formation in vivo via Transgenic expression of the antibody and administration of the targeting agent-linker derivative.

[00250] Within the scope of the methods of the present invention is in vivo formation of the targeting compounds of the invention. In one approach, mAb 38C2 is produced in vivo from an inducible transgene and a targeting agent-linker derivative (e.g. diketone linker) is administered. Using gene delivery vectors, such as adenoviruses, cDNAs encoding light and heavy chain or a single-chain fragment of mAb 38C2 can be introduced into a host organism to establish the antibody transgene. This approach allows increased flexibility in treatment. For example, a patient with a general risk of cancer chooses to receive the transgene prior to the actual detection of the disease. Once cancer is diagnosed, expression of the reactive antibody (e.g. mAb 38C2) is induced in the patient and a targeting agent –linker derivative (e.g. diketone linker), where the targeting agent is specifically designed for targeting and affecting the diagnosed cancer, is administered. Ideally, both transgene induction and drug administration are accomplished orally, thus avoiding hospitalization.

EXAMPLE 10: Antibody targeting compound libraries with improved detectability.

[00251] Screening of small molecule or peptide antagonist, agonists, or simple binding molecules is often hampered by the assay available for the detection of the binding event. Often, displacement or competition assays are required where the small molecule displaces or competes with the binding of another molecule to the target site. The assay must frequently be specifically designed for the specific target molecule.

The direct detection of a small molecule binding to either a cell surface or a protein is often not possible.

[00252] This problem is addressed by preparing the library in the form of antibody targeting compounds. To this end, small molecule libraries are synthesized with an appended reactive group such as a diketone or a high affinity tag such as biotin. Incubation of the tagged molecule with the target allows simple and sensitive detection of the binding event, accomplished using an enzyme-linked or fluorophore labeled antibody (e.g. 38C2 for the diketone) or streptavidin (for biotin). These types of assays are readily adapted for high throughput screening of compound and peptide libraries. The advantage of this direct screening of tagged molecules is that the detection method is sensitive and standardized over the diversity of possible cell surface molecules and protein or other soluble protein targets. Once identified, the attachment site of the linker arm does not need to be designed since it pre-exists in the tagged molecule. Therefore direct addition of the covalent binding antibody provides the novel therapeutic agent. In cases where a biotin tag is used for detection, the biotin arm is readily exchanged for a diketone arm for direct addition of the covalent binding antibody providing the novel therapeutic agent. If the library is a biological display library such as a peptide phage library, the site of attachment of the diketone arm is at the point where the peptide library resides are joined to the phage coat protein.

EXAMPLE 11: Antibody targeting compound comprising TAK-779 small-molecules that bind the envelope proteins of HIV-1covalently linked to the combining site of aldolase monoclonal antibody 38C2.

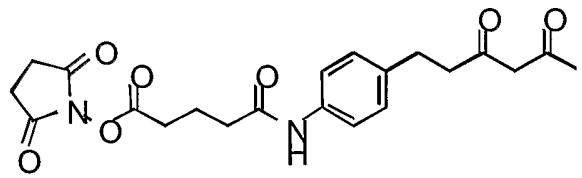
[00253] The β-chemokine receptor CCR5 is an attractive target for inhibition of macrophage-tropic (CCR5-using or R5) HIV-1 replication because individuals having a nonfunctional receptor (a homozygous 32-bp deletion in the CCR5 coding region) are apparently normal, but are resistant to infection with R5 HIV-1. Compound 85 is a low molecular weight (Mr 531.13) nonpeptide CCR5-antagonist (Baba et al., (1999, Proc. Natl. Acad. Sci. USA, 96, 5698–5703). A targeting agent-linker compound was prepared by derivatizing compound 85 with a diketo linker to yield the compound

shown in FIG. 2D. Compound 81 was incubated with Mab 38C2 to generate an antibody CCR5 targeting compound (based on compound 85). This compound displayed highly potent and selective inhibition of R5 HIV-1 replication and bound specifically to CCR5 expressing cells. The antibody CCR5 targeting compound also displayed increased valency, increased biological potency, and increased serum half-life over that of compound 85 itself.

[00254] Other CCR5 antagonists (Shiraishi, et al., 2000, *J. Med. Chem.*, *43,* 2049-2063) can also be modified for reaction with covalent binding antibodies to produce targeting compounds of the invention. A wide variety of chemokine receptor antagonists may also be modified using this approach.

EXAMPLE 12: Antibody targeting compound comprising LHRH peptide covalently linked to the combining site of aldolase monoclonal antibody 38C2.

[00255] [D-Lys6] LH-RH antagonist Glp-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2 (SEQ ID NO: 9) (100 micromoles) was dissolved in 1 mL anhydrous DMF. One equivalent of NHS-diketone linker (compound 35) was added with stirring overnight. Solvent was evaporated in vacuo, and the product was purified by HPLC. The resulting [D-Lys6] LH-RH- diketone linker compound was used directly for coupling to antibody 38C2. The resulting covalently-modified antibody specifically bound the OV-1063 human epithelial ovarian cancer line known to express the LH-RH receptor.



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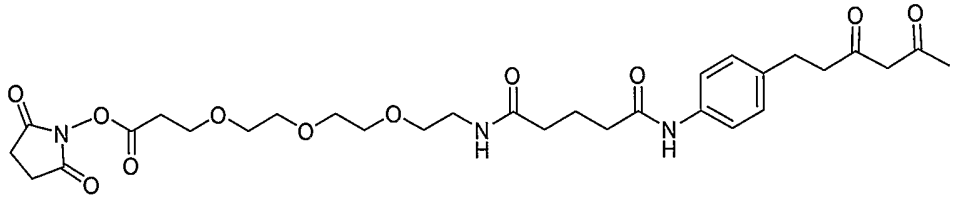
EXAMPLE 13: Preparation of 3-{2-[2-(2-{4-[4-(3,5-Dioxo-hexyl)-phenylcarbamoyl]-butyrylamino}-ethoxy)-ethoxy]-ethoxy}-propionic acid 2,5-dioxo-pyrrolidin-1-yl ester:



[00256] 3-{2-[2-(2-Hydroxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: Na metal (catalytic) was added to a stirring solution of acrylic acid tert-butyl ester (6.7 mL, 46 mmol), and 2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethanol (20.7 g, 138 mmol) in THF (100 mL) at 0 °C and the mixture was stirred overnight. Solvent was removed and the remaining oil dissolved in EtOAc (100 mL). The organic layer was washed with water (3 × 50 mL), and dried over Na_2SO_4 and the solvent removed in vacuo to give an oil which corresponds to the title compound that would be used as is for the next step. (M + 1) = 279.

[00257] 3-{2-[2-(2-Tosylsulfonyloxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: Tosyl chloride (22.3 g, 117 mmol) was added in portions to a stirring solution of 3-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (16.3 g, 58.6 mmol) and pyridine 60 mL in (240 mL) and the mixture was stirred overnight. The reaction was quenched with water (300 mL) and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layer was washed with HCl (1M, 100 mL), water (100 mL), and dried over Na_2SO_4 and the solvent was removed in vacuo to give an oil which corresponds to the title compound that would be used as is for the next step. (M + 1) = 433.

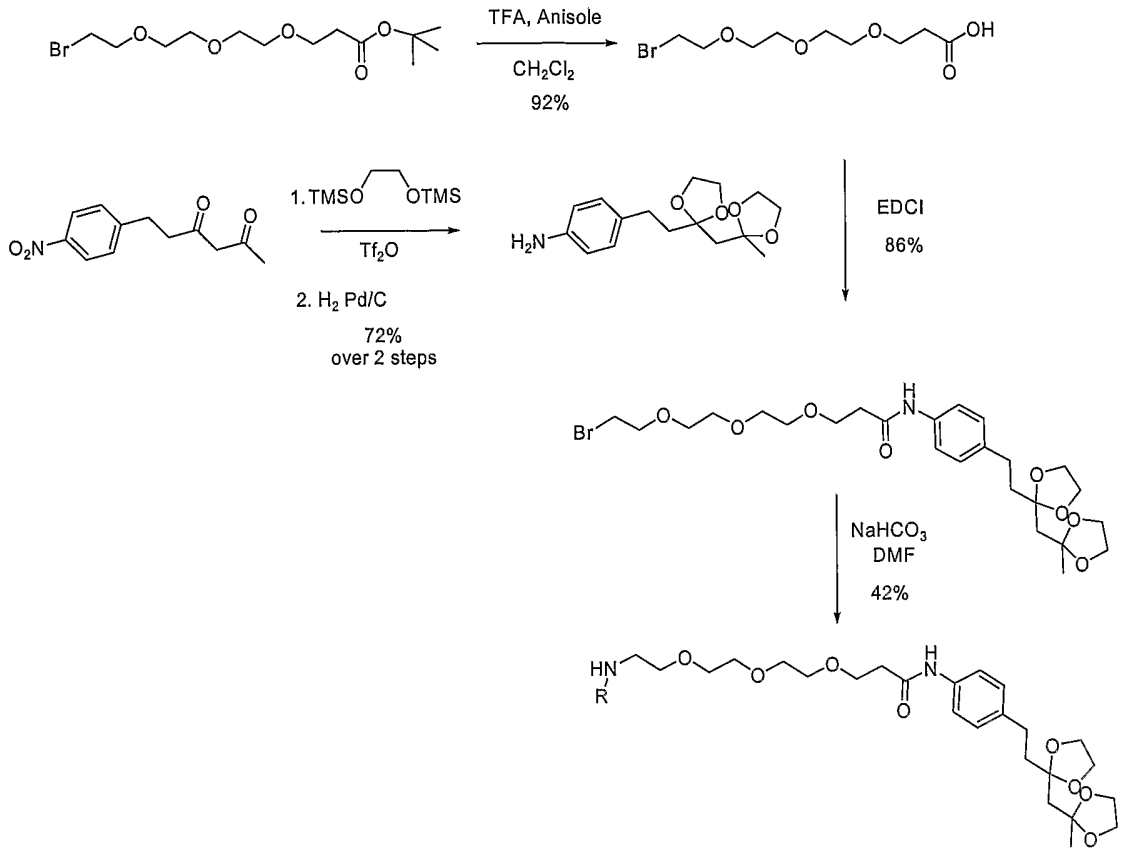
[00258] 3-{2-[2-(2-Amino-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: NaN_3 (35g, 538 mmol) was added to a stirring solution of 3-{2-[2-(2-tosylsulfonyloxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (20g, 46 mmol) in DMF (150 mL) and the reaction was stirred overnight. Reaction was diluted with water (200 mL) and extracted with EtOAc (4 × 100 mL). The organic layer was

washed with water (100 mL) and brine (100 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to give an oil. Column chromatography EtOAc/Hex (1:4) gave an oil which corresponds to the 3-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester, (M + 1) = 304. This oil was hydrogenated using Pd (5% on carbon) in EtOAc under hydrogen (1 atm.) over 3 days. The catalyst was removed by filtration and solvent removed in vacuo to give an oil corresponding to the tithe compound, (M + 1) = 278.

[00259] 3-{2-[2-(2-{4-[4-(3,5-Dioxo-hexyl)-phenylcarbamoyl]-butyrylamino}-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: A solution of 4-[4-(3,5-dioxo-hexyl)-phenylcarbamoyl]-butyric acid 2,5-dioxo-pyrrolidin-1-yl ester (1.5 g, 3.6 mmol), 3-{2-[2-(2-amino-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (1.0 g, 3.6 mmol) and DIEA (1.3 μL, 7.2 mmol) in CH_2Cl_2 (10 mL) was stirred at rt overnight. The solvent was removed in vacuo and the residual oil purified using column chromatography EtOAc/MeOH (95:5) to give the title compound as a transparent oil, (M + 1) = 579.

[00260] 3-{2-[2-(2-{4-[4-(3,5-Dioxo-hexyl)-phenylcarbamoyl]-butyrylamino}-ethoxy)-ethoxy]-ethoxy}-propionic acid 2,5-dioxo-pyrrolidin-1-yl ester: 3-{2-[2-(2-{4-[4-(3,5-Dioxo-hexyl)-phenylcarbamoyl]-butyrylamino}-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (400 mg, 0.692 mmol) was dissolved in TFA/CH_2Cl_2 (1:1, 3 mL) and the mixture stirred overnight. The solvent was removed to give an oil as the acid intermediate. This oil was dissolved in CH_2Cl_2 (4 mL) containing DIEA (569 μL, 3.09 mmol), N-hydroxysuccinimide (119 mg, 1.03 mmol) and EDC (197 mg, 1.0 mmol) and the mixture stirred over the night. The solvent was removed and the residual oil was purified using column chromatography EtOAc/MeOH (95:5) to give an oil as the title compound, (M + 1) = 620.

EXAMPLE 14: Synthesis of a Generic Tether



[00261] 4-{2-[2-(2-Methyl-[1,3]dioxolan-2-ylmethyl)-[1,3]dioxolan-2-yl]-ethyl}-phenylamine: A clean oven dried flask was charget with the 6-(4-nitro-phenyl)-hexane-2,4-dione (3.7g, 15.72 mmol), dry CH_2Cl_2 (20 mL) followed by bisTMS ethylene glycol (38.5 mL, 157.3 mL) were added to the flask and the resulting solution was cooled to -50 °C with stirring under argon. TMSOTf (300 μl) was added to the reaction mixture and the solution was stirred at -5 °C for 6h. Reaction was quenched with pyridine (10 mL) and poured into sat. $NaHCO_3$. The mixture was extracted with EtOAc; the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated to give a liquid that was concentrated under high vacuum to give a brown solid. The crude product was purified by flash chromatography with a gradient of 30-40% EtOAc in Hexanes to give the nitro diketal (3.5g, 72%) a pale yellow solid. 1.6g of the nitrodiketal was dissolved in EtOAc (25 mL) and hydrogenated on a Parr shaker starting with 50 p.s.i of hydrogen. After two hours the reaction was filetered through a pad of celite, the celite was washed thoroughly with CH_2Cl_2/MeOH and combined

organics were concentrated to give title compound (1.46 g, 100%) as an oil that solidifies upon standing.

[00262] 3-{2-[2-(2-Bromo-ethoxy)-ethoxy]-ethoxy}-N-(4-{2-[2-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,3]dioxolan-2-yl]-ethyl}-phenyl)-propionamide: EDCI·HCl (1.04g, 5.46 mmol) was added to a stirring solution of 3-{2-[2-(2-bromo-ethoxy)-ethoxy]-ethoxy}-propionic acid (1.41g, 4.97 mmol) and 4-{2-[2-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,3]dioxolan-2-yl]-ethyl}-phenylamine (1.46g, 4.97 mmol) in dry CH_2Cl_2. The resulting solution was stirred under argon for 16h. The reaction was poured into water the organic layer was washed with water, brine, dried (Na2SO4) and concentrated to give pale brown oil that was purified by flash chromatography using a gradient of 80-90% EtOAc in Hexanes to give 3-{2-[2-(2-bromo-ethoxy)-ethoxy]-ethoxy}-N-(4-{2-[2-(2-methyl-[1,3]dioxolan-2-ylmethyl)-[1,3]dioxolan-2-yl]-ethyl}-phenyl)-propionamide (2.4 g, 86%) as a colorless oil.

[00263] 3-{2-[2-(2-Hydroxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: Na (0.1 g, cat.) was added in portions to a stirring solution of triethyleneglycol (64 mL, 0.479 mol) in dry THF (250 mL). Upon disappearance of Na, *t*-butyl acrylate (24 mL, 0.165 mol) was added to the reaction and the resulting solution was stirred under argon for 20h. Reaction mixture was neutralized with 1N HCl and concentrated in vacuo. The residue was suspended in brine and extracted with EtOAc (3X). Combined organics were washed with brine, dried (Na_2SO_4) and concentrated to give 36.7 g (80%) of title compound as colorless oil.

[00264] 3-{2-[2-(2-Bromo-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester: A solution of 3-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (4g, 14.37 mmol) and CBr_4 (5.71g, 17.24 mmol) in CH_2Cl_2 (60 mL) was cooled to 0 °C. A solution of PPh_3 (5.65g, 21.55 mmol) was added dropwise to the above solution. Upon completion of addition the solution was stirred at 0 °C for 5 min at which the ice bath was removed and the solution was stirred at room temperature for 30 min. The reaction mixture was concentrated and the residue was triturated with Et_2O to precipitate triphenylphosphine oxide. The precipitate was filtered off and the residue treated in the above manner several times until most of the triphenylphosphine

oxide is removed. The crude product was purified by flash chromatography with a gradient of 25-35% EtOAc in Hexanes to give title compound (4.1g, 84%) as a colorless oil.

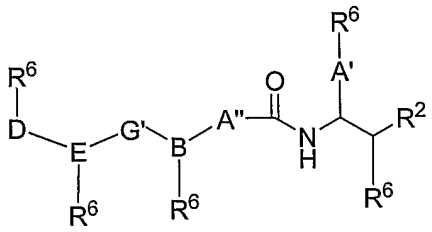
[00265] 3-[2-(2-{2-[(1H-Benzoimidazol-2-ylmethyl)-amino]-ethoxy}-ethoxy)-ethoxy]-propionic acid tert-butyl ester: DIEA (5 mL) was added to a suspension of aminomethyl benzimidazole dihydrochloride hydrate (645mg , 2.93 mmol) in dry DMF (5 mL). $NaHCO_3$ (984.5 mg, 11.72 mmol) was added to the resulting suspension followed by a slow addition of a solution of 3-{2-[2-(2-bromo-ethoxy)-ethoxy]-ethoxy}-propionic acid tert-butyl ester (1.0 g, 2.93 mmol) in dry DMF (5 mL). The resulting solution was stirred under argon at room temperature for 72h. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried Na_2SO_4, concentrated and the residue purified by flash chromatography using a gradient of 2.5-5% MeOH/CH_2Cl_2 with 0.1% Et_3N to give title compound (270 mg, 23%) as a brown oil.

[00266] The invention thus has been disclosed broadly and illustrated in reference to representative embodiments described above. Those skilled in the art will recognize that various modifications can be made to the present invention without departing from the spirit and scope thereof. All publications, patent applications, and issued patents, are herein incorporated by reference to the same extent as if each individual publication, patent application or issued patent were specifically and individually indicated to be incorporated by reference in its entirety. Definitions that are contained in text incorporated by reference are excluded to the extent that they contradict definitions in this disclosure. All structures shown herein are contemplated to provide all enantiomers.

CLAIMS

WHAT IS CLAIMED IS:

1. A compound having formula I, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof:



Formula I

wherein

G' is a covalent bond or $-(CH_2)_a-T-(CH_2)_b-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, and a and b are each independently 0 to 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is $T-(CH_2)_j-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is $-(CH_2)_d$-L'-U wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is substituted by R^6 at one of U, L', or $-(CH_2)_d$-;

E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11}, and wherein when U is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group;

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, $(C_{1-8}$

alkyl)$_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, (C_{1-8} alkyl)$_o$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl)$_o$ amino, (aryl)$_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$S(O)_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -$N(R^5)$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$N(R^5)$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$N(R^5)$ - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, (C_{1-6} alkyl)$_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, $(aryl)_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, (aryl)$_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

R^6 is, at a first occurrence, a linker moiety having the formula -J-G-K, and is, at a second occurrence R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K is a reactive group; and

wherein if J is an unsubstituted C_{1-21} alkyl and K is $-NH_2$, -SH, $-OS(OH)_3$, or -COOH, G is present;

R^7 is $-C(O)R^8$, $-C(O)-CR'_2-R^9$, $-C(S)R^8$, $-S(O)_o$ OR', $-S(O)_o$ NR'R", -PO(OR'), $-PO(OR')_2$, $-B(OR')_2$, $-NO_2$, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", $-NR'SO_2$ R', -NR'OR', $-OCR'_2$ C(O)OR', $-OCR'_2OC(O)-R'$, $-OCR'_2C$ (O)NR'_2, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', CN, $-S(O)_r$ R', $S(O)_o$ NR'_2, $-C(O)R'C(O)NR'_2$ or $-CO_2$ R';

R^{11} is H, halogen, $-OR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R'$, $-CONR'_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', -C(O)-NR'$_2$, -C(O)-OR^{13}, -S(O)$_o$-R' or -S(O)$_o$-NR'$_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or -C(O)OR^{13};

R''' is -H, or substituted or unsubstituted C_{1-6} alkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

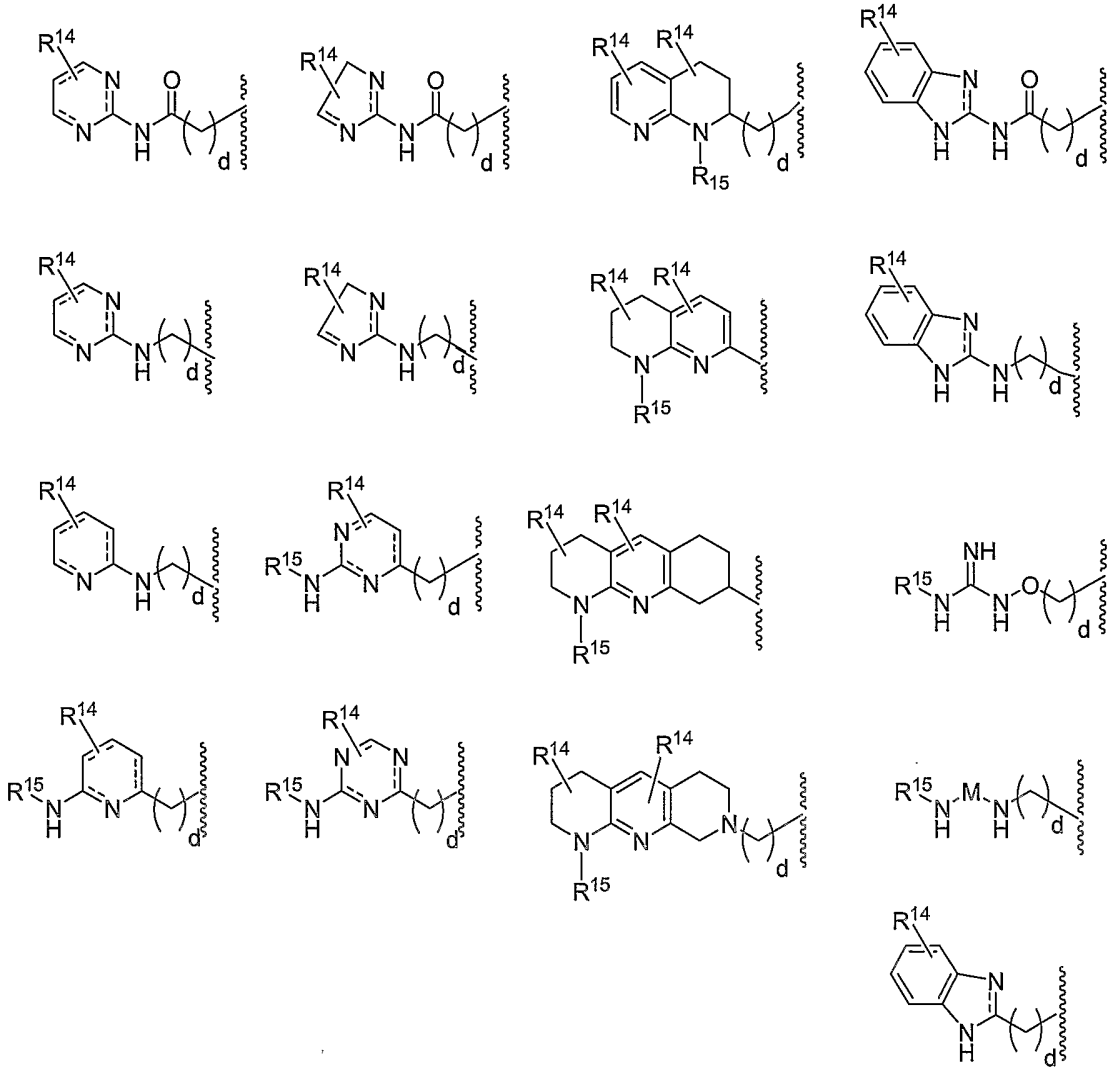
q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

2. The compound according to claim 1, wherein D is substituted or unsubstituted heterocyclyl.

3. The compound according to claim 3, wherein the heterocyclyl is a nitrogen-containing heterocyclyl.

4. The compound according to claim 1, wherein D is a structure selected from:

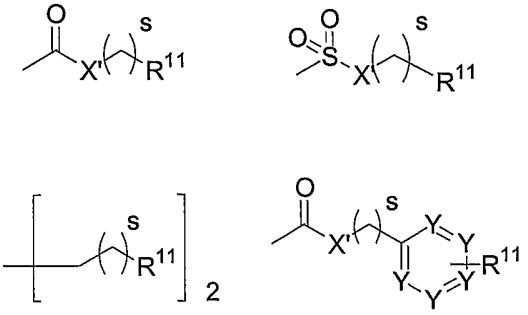


wherein,

M is -C(=O)-, -C(=S)-, -C(=NR$^{'''}$)-, -C(=N-C(=O)-O-(C_{1-6} alkyl)), or -C(=N-C(=O)-O-(C_{1-6} alkyl)(C_{6-14} aryl));

R^{14} is -H, halogen, -OR^{16}, -SR^{16}, -CN, -$NR^{16}R^{17}$, -NO_2, -CF_3, $CF_3S(O)_r$-, -CO_2R^{16}, -C(O)R^{16}, -C(O)$NR^{16}{}_2$, C_{1-10} alkyl optionally substituted by halogen, -$O(CH_2)_{0-1}CF_3$, or $R^{18}S(O)_2O$-;

R^{15} is –H or optionally substituted C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} cycloalkyl, C_{3-8} heterocyclyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} heterocyclyl-C_{1-6} alkyl, aryl C_{1-6} alkyl, or a structure selected from the group consisting of,



wherein

X' is NH, O, or CH_2;

each Y is independently CH or, at up to 3 occurrences, N; and

s is 0 to 5;

R^{16} is, at each occurrence, independently H, C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

R^{17} is, at each occurrence, independently R^{16}, -C(O)R^{16} or -C(O)OR^{16} ;

R^{18} is, at each occurrence, independently C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

each dashed line is absent or represents a single bond; and

D is substituted by R^6 in place of hydrogen at a CH or NH.

5. The compound according to claim 1 wherein R^2 is –COOH.

6. The compound according to claim 1, wherein J is substituted or unsubstituted and is selected from the group consisting of -R^{22} - $[CH_2$-CH_2-O$]_t$ - R^{23}-, -R^{22}-cycloalkyl- R^{23}-, -R^{22}-aryl- R^{23}-, and -R^{22}-heterocyclyl-R^{23}- wherein

R^{22} and R^{23} are independently a covalent bond, -O-, -S-, -NR^a-, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50}

heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

R^a, at each occurrence, is independently hydrogen, substituted or unsubstituted C_{1-10} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-6} alkyl, or substituted or unsubstituted aryl–C_{0-6} alkyl;

t = 0-50;

and the size of R^{22} and R^{23} are such that the backbone length of J remains about 200 atoms or less.

7. The compound accoding to claim 6, wherein

R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-O-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_u-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_u-NR^a-(CH_2)_v-$, $-(CH_2)_u-O-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-NR^a-(CH_2)_v-$, or $-(CH_2)_u-P(O)(OR^a)-O-(CH_2)_v-$;

R^{23} is -O-, -S-, $-NR^a-$, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

u and v are independently 0-20;

and the values of t, u, and v are such that the backbone length of J remains about 200 atoms or less.

8. The compound according to claim 7, wherein R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-O-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$,

$-(CH_2)_u-NR^a-(CH_2)_v-$, $-(CH_2)_u-O-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-(CH_2)_v-$, or $-(CH_2)_u-S(O)_{0-2}-NR^a-(CH_2)_v-$.

9. The compound according to claim 7, wherein R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, or $-(CH_2)_u-NR^a-(CH_2)_v$.

10. The compound according to claim 6 wherein R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkylene or substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene.

11. The compound according to claim 1, wherein J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl in the backbone.

12. The compound according to claim 11, wherein J has the formula

$$-R^{22}-[CH_2-CH_2-O]_t - R^{23}-$$

wherein

R^{22} is $-(CH_2)_v-$, $-C(O)-(CH_2)_v-$, $-C(O)-O-(CH_2)_v-$, $-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(O)-NR^a-$, $-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(S)-NR^a-$, $-NR^a-(CH_2)_v-$, $-O-(CH_2)_v-$, $-S(O)_{0-2}-(CH_2)_v-$, $-S(O)_{0-2}-NR^a-(CH_2)_v-$, $-(CH_2)_v-S(O)_{0-2}-NR^a-$, or $-P(O)(OR^a)-O-(CH_2)_v-$;

R^a, at each occurrence, is independently -H, substituted or unsubstituted C_{1-8} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl–C_{0-4} alkyl;

R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkyl, substituted or unsubstituted straight or branched chain C_{1-49} heteroalkyl, or substituted or unsubstituted straight or branched chain C_{2-50} alkenyl;

t = 2-50; and

v = 0-20;

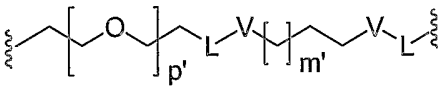
and wherein the values of t and v are such that the backbone length of J remains about 200 atoms or less.

13. The compound according to claim 1, wherein J is optionally substituted and has the formula: $-[CH_2-L]_{n'}-$, wherein n' is 1-100 and L is, at each occurrence, independently $-CH_2-$, -O-, -NR'-, $-SiR'_2-$, $-S(O)_{0-2}-$, or a covalent bond.

14. The compound according to claim 13, wherein J is substituted.

15. The compound according to claim 13, wherein L is, at each occurrence, independently $-CH_2-$, -O-, -NR'-, or a covalent bond.

16. The compound according to claim 15, wherein J has the formula:



wherein

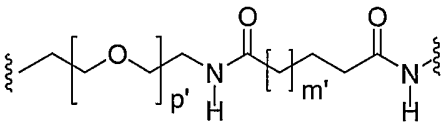
V is –C(O)-, $-CH_2-$, or -C(S)-;

m' and p' are each independently 1 up to about 50, such that the backbone length of J remains about 200 atoms or less.

17. The compound according to claim 16, wherein the backbone length of J is about 100 atoms or less.

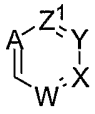
18. The compound according to claim 16, wherein the backbone length of J is about 30 atoms or less.

19. The compound according to claim 16, wherein J has the structure:



20. The compound according to claim 1, wherein G is present.

21. The compound according to claim 1, wherein the ring structure of G has the optionally substituted formula:



or


wherein,

A, W, X, Y, and Z^1 are independently carbon or nitrogen; and

Z^2 is carbon, nitrogen, oxygen, or sulfur; and

G is attached to J and K independently through a covalent bond or substituted or unsubstituted C_{1-6} alkyl or heteroalkyl; and wherein

no more than four of A, W, X, Y, Z^1, or Z^2 are simultaneously nitrogen.

22. The compound according to claim 21, wherein A, W, X, Y, and Z^1 are each carbon.

23. The compound according to claim 21, wherein the ring structure of G is substituted or unsubstituted phenylalkyl and is attached to K at the alkyl.

24. The compound according to claim 1, wherein K is substituted alkyl, substituted cycloalkyl, substituted aryl, substituted arylalkyl, substituted heterocyclyl, or substituted heterocyclylalkyl, wherein at least one substituent is a 1,3-diketone moiety, an acyl beta-lactam, an active ester, an alpha-haloketone, an aldehyde, a maleimide, a lactone, an anhydride, an alpha-haloacetamide, or an epoxide.

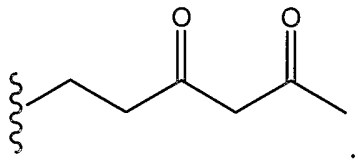
25. The compound according to claim 24, wherein K is substituted alkyl.

26. The compound according to claim 24, wherein the at least one substituent is a 1,3-diketone moiety.

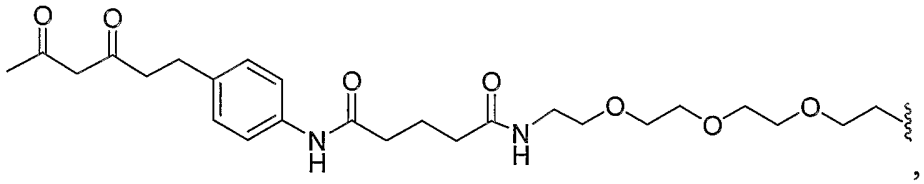
27. The compound according to claim 26, wherein the 1,3-diketone moiety is separated from G by C_{1-5} alkyl.

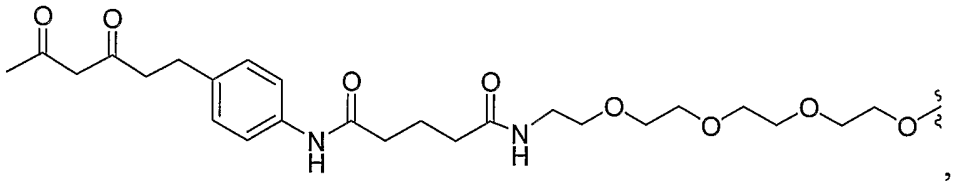
28. The compound according to claim 26, wherein the 1,3-diketone moiety is separated from G by C_{2-4} alkyl.

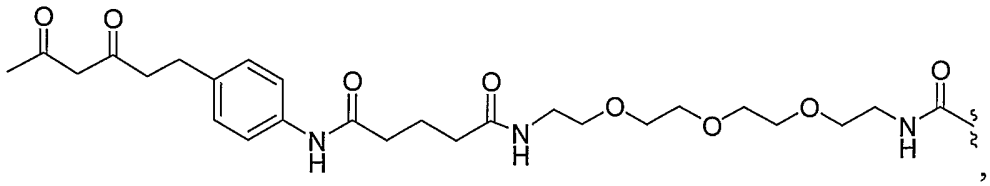
29. The compound according to claim 24, wherein K has the structure:

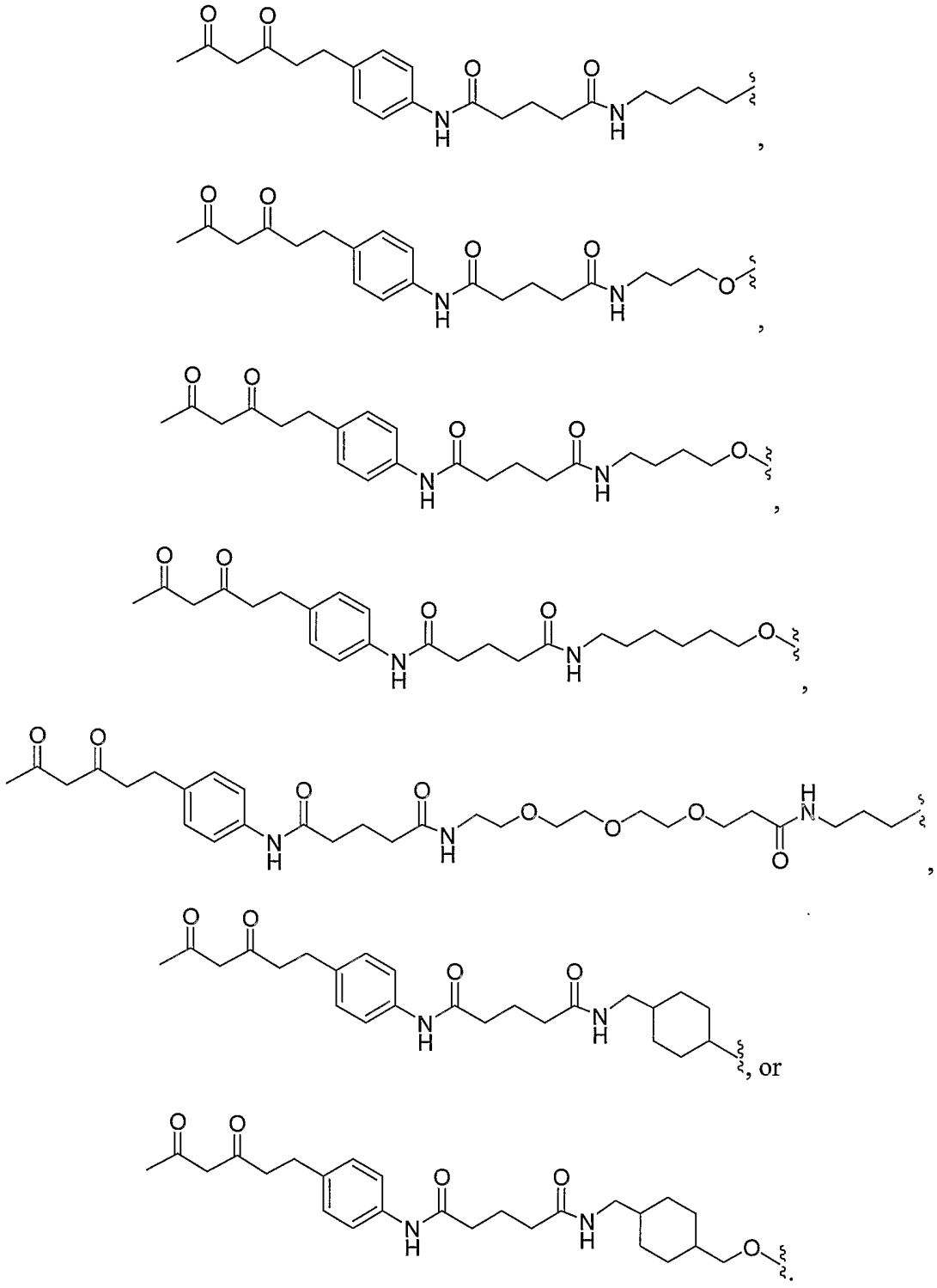


30. The compound according to claim 1 wherein R^6 has the structure:

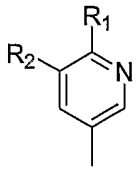








31. The compound according to claim 1, wherein one or more of R^{19}, R^{20} and R^{21} is

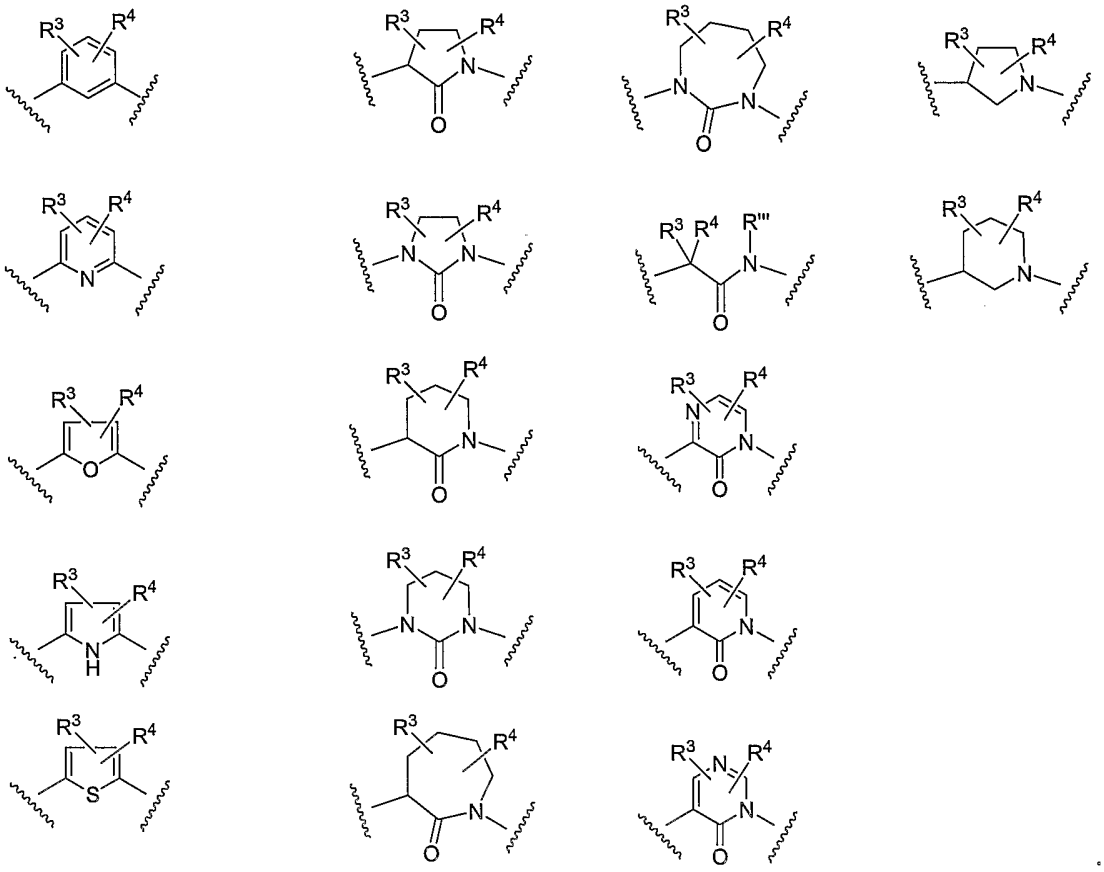


wherein

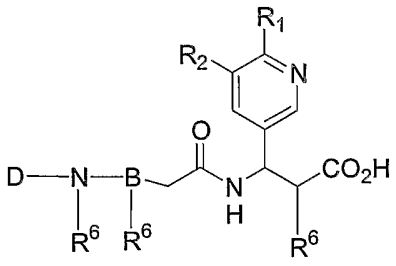
R_1 and R_2 are independently -H, halogen, $-OR^{12}$, -CN, $-NR^8R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R^8$, $-CONR^8{}_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$-, or R_1 and R_2 together form a fused unsubstituted or substituted aryl.

32. The compound according to claim 1 wherein R^{19}, R^{20} and R^{21} are independently selected from C_{3-6} cycloalkyl, aryl, and heterocyclyl optionally substituted by one or more of R^{11}.

33. The compound according to claim 1 wherein B is a structure selected from:

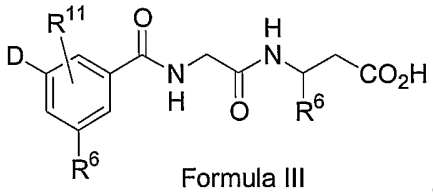


34. The compound according to claim 31 having formula II,



Formula II

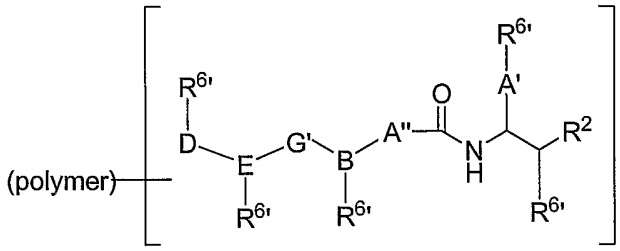
35. The compound according to claim 1 having formula III,



Formula III

36. An integrin targeting compound having formula IV,

stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof:



Formula IV

wherein,

G' is a covalent bond or $-(CH_2)_a-T-(CH_2)_b-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, and a and b are each independently 0 – 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is $T-(CH_2)_j-$ wherein

T is -O-, -N(R''')-, $-S(O)_q-$,-CHR'''-, $-CH_2-$, -C(O)-, $-S(O)_2N(R''')-$, or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is $-(CH_2)_d$-L'-U wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is substituted by $R^{6'}$ at one of U, L', or $-(CH_2)_d$-;

E is nitrogen, carbon, phosphorous, amide, amidine, guanidine, or aryl or heteroaryl, optionally substituted with R^{11}, and wherein when U is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group;

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl,

nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-$S(O)_q$, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, (C_{1-8} alkyl)$_o$ aminocarbonyloxy, oxo, (aryl C_{1-8} alkyl)$_o$ amino, (aryl)$_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$S(O)_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -$N(R^5)$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$N(R^5)$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -$N(R^5)$ - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, (C_{1-6} alkyl)$_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, $(aryl)_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, (aryl)$_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

$R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein,

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K' is an attachment moiety including about 1 to about 20 carbon atoms and is covalently linked to the polymer;

R^7 is $-C(O)R^8$, $-C(O)-CR'_2-R^9$, $-C(S)R^8$, $-S(O)_o$ OR', $-S(O)_o$ NR'R", -PO(OR'), $-PO(OR')_2$, $-B(OR')_2$, $-NO_2$, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", $-NR'SO_2$ R', -NR'OR', $-OCR'_2$ C(O)OR', $-OCR'_2OC(O)$-R', $-OCR'_2C$ (O)NR'_2, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', -CN, $-S(O)_r$ R', $S(O)_o$ NR'_2, -C(O)R'C(O)NR'_2 or $-CO_2$ R';

R^{11} is H, halogen, $-OR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R'$, $-CONR'_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', -C(O)-NR'$_2$, -C(O)-OR^{13}, -S(O)$_o$-R' or -S(O)$_o$-NR'$_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or -C(O)OR^{13};

R''' is -H, or substituted or unsubstituted C_{1-6} alkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

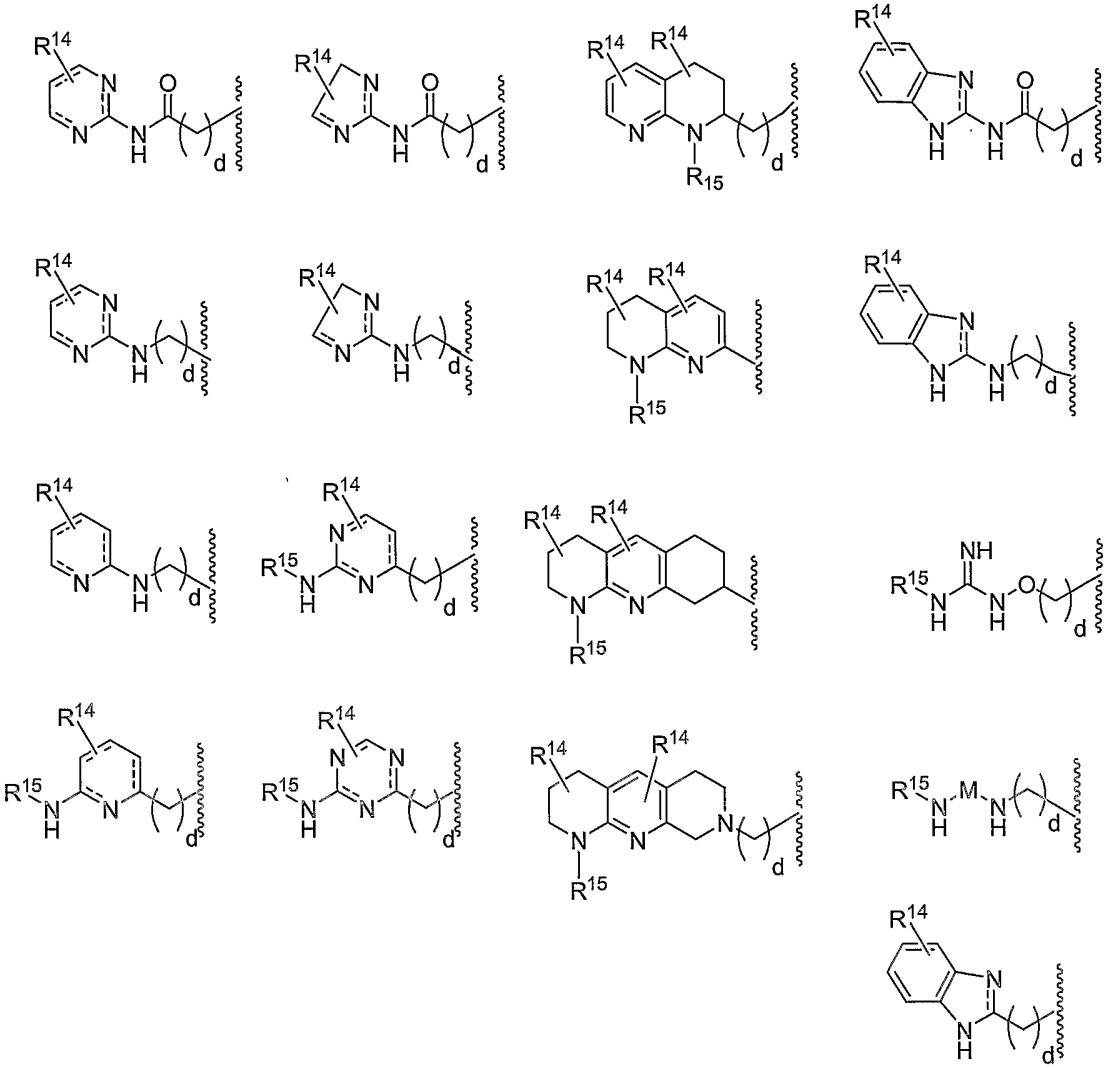
q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

37. The compound according to claim 36, wherein D is substituted or unsubstituted heterocyclyl.

38. The compound according to claim 37, wherein the heterocyclyl is a nitrogen-containing heterocyclyl

39. The compound according to claim 36, wherein D is a structure selected from:

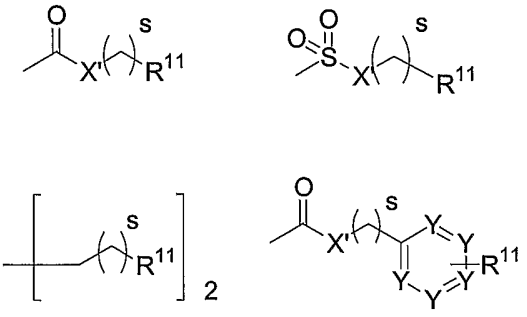


wherein,

M is -C(=O)-, -C(=S)-, -C(=NR'')-, -C(=N-C(=O)-O-(C_{1-6} alkyl)), or -C(=N-C(=O)-O-(C_{1-6} alkyl)(C_{6-14} aryl));

R^{14} is -H, halogen, -OR^{16}, -SR^{16}, -CN, -$NR^{16}R^{17}$, -NO_2, -CF_3, $CF_3S(O)_r$-, -CO_2R^{16}, -$C(O)R^{16}$, -$C(O)NR^{16}_2$, C_{1-10} alkyl optionally substituted by halogen, -$O(CH_2)_{0-1}CF_3$, or $R^{18}S(O)_2O$-;

R^{15} is –H or optionally substituted C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} cycloalkyl, C_{3-8} heterocyclyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} heterocyclyl-C_{1-6} alkyl, aryl C_{1-6} alkyl, or a structure selected from the group consisting of,



wherein

X' is NH, O, or CH_2;

each Y is independently CH or, at up to 3 occurrences, N;

and

s is 0 to 5;

R^{16} is, at each occurrence, independently H, C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

R^{17} is, at each occurrence, independently R^{16}, $-C(O)R^{16}$ or $-C(O)OR^{16}$;

R^{18} is, at each occurrence, independently C_{1-6} alkyl, heterocyclyl-C_{0-6} alkyl, C_{3-7} cycloalkyl-C_{0-6} alkyl, or aryl-C_{0-6} alkyl;

each dashed line is absent or represents a single bond; and

D is substituted by $R^{6'}$ in place of hydrogen at a CH or NH.

40. The compound according to claim 36, wherein J is substituted or unsubstituted and is selected from the group consisting of $-R^{22}-[CH_2-CH_2-O]_t-R^{23}-$, $-R^{22}$-cycloalkyl-$R^{23}-$, $-R^{22}$-aryl-$R^{23}-$, and $-R^{22}$-heterocyclyl-$R^{23}-$ wherein

R^{22} and R^{23} are independently a covalent bond, -O-, -S-, -NR^a-, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

R^a, at each occurrence, is independently hydrogen, substituted or unsubstituted C_{1-10} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-6} alkyl, or substituted or unsubstituted aryl–C_{0-6} alkyl;

t = 0-50;

and the size of R^{22} and R^{23} are such that the backbone length of J remains about 200 atoms or less.

41. The compound accoding to claim 40, wherein

R^{22} is -$(CH_2)_v$-, -$(CH_2)_u$-C(O)-$(CH_2)_v$-, -$(CH_2)_u$-C(O)-O-$(CH_2)_v$-, -$(CH_2)_u$-C(O)-NR^a-$(CH_2)_v$-, -$(CH_2)_u$-C(S)-NR^a-$(CH_2)_v$-, -$(CH_2)_u$-NR^a-$(CH_2)_v$-, -$(CH_2)_u$-O-$(CH_2)_v$-, -$(CH_2)_u$-$S(O)_{0-2}$-$(CH_2)_v$-, -$(CH_2)_u$-$S(O)_{0-2}$-NR^a-$(CH_2)_v$-, or -$(CH_2)_u$-P(O)(OR^a)-O-$(CH_2)_v$-;

R^{23} is -O-, -S-, -NR^a-, substituted or unsubstituted straight or branched chain C_{1-50} alkylene, substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene, substituted or unsubstituted straight or branched chain C_{2-50} alkenylene, or substituted or unsubstituted C_{2-50} heteroalkenylene;

u and v are independently 0-20;

and the values of t, u, and v are such that the backbone length of J remains about 200 atoms or less.

42. The compound according to claim 41, wherein R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-O-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_u-NR^a-(CH_2)_v-$, $-(CH_2)_u-O-(CH_2)_v-$, $-(CH_2)_u-S(O)_{0-2}-(CH_2)_v-$, or $-(CH_2)_u-S(O)_{0-2}-NR^a-(CH_2)_v-$.

43. The compound according to claim 41, wherein R^{22} is $-(CH_2)_v-$, $-(CH_2)_u-C(O)-(CH_2)_v-$, $-(CH_2)_u-C(O)-NR^a-(CH_2)_v-$, or $-(CH_2)_u-NR^a-(CH_2)_v$.

44. The compound according to claim 40 wherein R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkylene or substituted or unsubstituted straight or branched chain C_{1-50} heteroalkylene.

45. The compound according to claim 36, wherein

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl in the backbone;

-J-G-K'- is attached to D or E; and

polymer is an antibody comprising one or more combining sites wherein K' is covalently linked to one of the one or more combining sites.

46. The compound according to claim 45, wherein J has the formula

$$-R^{22}-[CH_2-CH_2-O]_t - R^{23}-$$

wherein

R^{22} is $-(CH_2)_v-$, $-C(O)-(CH_2)_v-$, $-C(O)-O-(CH_2)_v-$, $-C(O)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(O)-NR^a-$, $-C(S)-NR^a-(CH_2)_v-$, $-(CH_2)_v-C(S)-NR^a-$, $-NR^a-(CH_2)_v-$, $-O-(CH_2)_v-$,

$-S(O)_{0-2}-(CH_2)_v-$, $-S(O)_{0-2}-NR^a-(CH_2)_v-$, $-(CH_2)_v-S(O)_{0-2}-NR^a-$, or $-P(O)(OR^a)-O-(CH_2)_v-$;

R^a, at each occurrence, is independently -H, substituted or unsubstituted C_{1-8} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl–C_{0-4} alkyl;

R^{23} is substituted or unsubstituted straight or branched chain C_{1-50} alkyl, substituted or unsubstituted straight or branched chain C_{1-49} heteroalkyl, or substituted or unsubstituted straight or branched chain C_{2-50} alkenyl;

t = 2-50; and

v = 0-20;

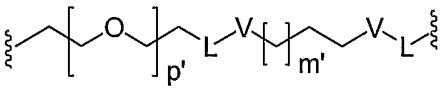
and wherein the values of t and v are such that the backbone length of J remains about 200 atoms or less.

47. The compound according to claim 36, wherein J has the formula: $-[CH_2-L]_{n'}-$, wherein n' is 1-100 and L is, at each occurrence, independently $-CH_2-$, -O-, $-NR^8-$, -Si-, $-S(O)_{0-2}-$, or a covalent bond.

48. The compound according to claim 47, wherein J is substituted.

49. The compound according to claim 47, wherein L is, at each occurrence, independently $-CH_2-$, -O-, -NR'-, or a covalent bond.

50. The compound according to claim 49, wherein J has the formula:



wherein

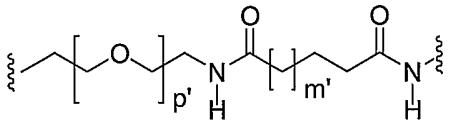
V is –C(O)-, $-CH_2-$, or -C(S)-;

m' and p' are each independently 1 up to about 50, such that the backbone length of J remains about 200 atoms or less.

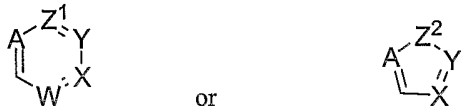
51. The compound according to claim 50, wherein the backbone length of J is about 100 atoms or less.

52. The compound according to claim 50, wherein the backbone length of J is about 30 atoms or less.

53. The compound according to claim 50, wherein J has the structure:



54. The compound according to claim 36, wherein the ring structure of the recognition group has the optionally substituted formula:



or

wherein,

A, W, X, Y, and Z^1 are independently carbon or nitrogen; and

Z^2 is carbon, nitrogen, oxygen, or sulfur; and

the recognition group is independently attached to J and the attachment moiety through a covalent bond or substituted or unsubstituted C_{1-6} alkyl; wherein

no more than four of A, W, X, Y, Z^1, or Z^2 are simultaneously nitrogen.

55. The compound according to claim 54, wherein A, W, X, Y, and Z^1 are each carbon.

56. The compound according to claim 36, wherein the attachment moiety K' is substituted or unsubstituted straight or branched chain C_{1-20} alkyl or C_{2-20} alkenyl, or substituted or unsubstituted straight or branched chain C_{1-20} heteroalkyl or C_{2-20} heteroalkenyl.

57. The compound according to claim 36, wherein the attachment moiety K' is substituted or unsubstituted straight or branched chain C_{1-20} alkyl or C_{2-20} alkenyl.

58. The compound according to claim 36, wherein the attachment moiety K' is substituted or unsubstituted straight chain C_{3-10} alkyl or C_{3-10} alkenyl.

59. The compound according to claim 36, wherein the polymer is a protein.

60. The compound according to claim 59, wherein the protein is an antibody.

61. The compound according to claim 60, wherein said antibody is full length.

62. The compound according to claim 60, wherein said antibody is a fragment of a full length antibody.

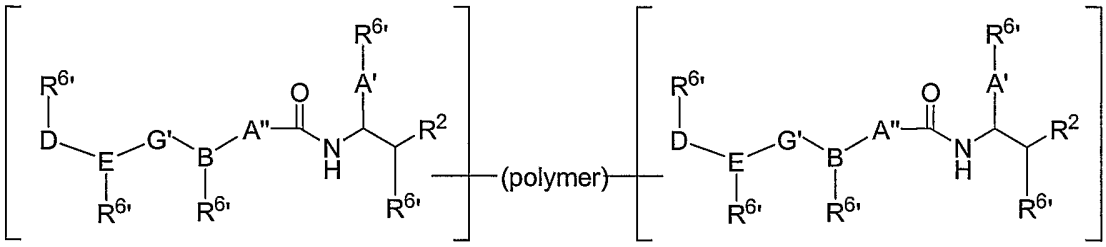
63. The compound according to claim 62, wherein said fragment of a full length antibody is Fab, Fab' $F(ab')_2$, Fv or sFv.

64. The compound according to claim 60, wherein said antibody is a human antibody, humanized antibody or chimeric human antibody.

65. The compound according to claim 60, wherein said antibody is a catalytic antibody.

66. The compound according to claim 59, wherein the protein is serum albumin.

67. An integrin targeting compound having formula V, stereoisomers thereof, tautomers thereof, and pharmaceutically acceptable salts thereof:



Formula V

wherein,

G' is a covalent bond or $-(CH_2)_a$-T-$(CH_2)_b$- wherein

T is -O-, -N(R''')-, $-S(O)_q$-,-CHR'''-, $-CH_2$-, -C(O)-, $-S(O)_2N(R''')$- , or –C(N)R'''-, and a and b are each independently 0 – 4, provided that the sum of a and b is not more than 6;

B is absent or is a C_{1-2} heteroalkyl or a 5-11 membered aromatic or nonaromatic mono- or polycyclic ring system containing 0 to 6 double bonds, and containing 0 to 6 heteroatoms chosen from N, O and S, and wherein the ring system and heteroalkyl are either unsubstituted or substituted on a carbon or nitrogen atom with one or more groups selected from R''', R^3, or R^4;

A'' is T-$(CH_2)_j$- wherein

T is -O-, -N(R''')-, $-S(O)_q$-,-CHR'''-, $-CH_2$-, -C(O)-, $-S(O)_2N(R''')$- , or –C(N)R'''-, or a covalent bond; and

j is 0 to 3;

A' is substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, or a covalent bond;

D is -$(CH_2)_d$-L'-U, wherein

U is substituted or unsubstituted heterocyclyl, substituted aryl, substituted or unsubstituted amidine, substituted or unsubstituted urea or thiourea, or substituted or unsubstituted guanidine, wherein the substituted aryl has one or more nitrogen-containing substituents;

L' is absent or is -O-, -NH-, or -C(O)-;

d is 0 to 4, provided that the sum of a, b, and d is not more than 8; and

D is substituted by $R^{6'}$ at one of U, L', or -$(CH_2)_d$-;

E is nitrogen, carbon, phosphorous, amide, amidine, or guanidine, wherein when D is amidine or guanidine and E is amidine or guanidine, U and E are separated by at least one methylene group;

R^2 is R^7, or Q-C_{1-4} alkyl, Q-C_{2-4} alkenyl, or Q-C_{2-4} alkynyl substituted by R^7;

R^3 is -H, halo, C_{1-10} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloheteroalkyl, C_{3-8} cycloalkyl C_{1-6} alkyl, C_{3-8} cycloheteroalkyl C_{1-6} alkyl, aryl, aryl C_{1-8} alkyl, amino, amino C_{1-8} alkyl, C_{1-3} acylamino, C_{1-3} acylamino C_{1-8} alkyl, $(C_{1-6}$ alkyl$)_o$ amino, $(C_{1-6}$ alkyl$)_o$ amino C_{1-8} alkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkoxycarbonyl C_{1-6} alkyl, hydroxycarbonyl-C_{1-6} alkyloxy, hydroxy, hydroxy C_{1-6} alkyl, C_{1-6} alkyloxy-C_{1-6} alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C_{1-8} alkyl-S(O)$_q$, $(C_{1-8}$ alkyl$)_o$ aminocarbonyl, C_{1-8} alkyloxycarbonylamino, $(C_{1-8}$ alkyl$)_o$ aminocarbonyloxy,

oxo, (aryl C_{1-8} alkyl)$_o$ amino, (aryl)$_o$ amino, aryl C_{1-8} alkylsulfonylamino or C_{1-8} alkylsulfonylamino;

R^4 is H, aryl, aryl-$(CH_2)_p$-, aryl-$(CH_2)_n$ -O- $(CH_2)_m$ -, aryl-$(CH_2)_n$ --S(O)$_q$ - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -C(O) -N(R^5) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) -C(O) - $(CH_2)_m$ -, aryl-$(CH_2)_n$ -N(R^5) - $(CH_2)_m$ -, halogen, hydroxyl, C_{1-8} alkylcarbonylamino, aryl C_{1-5} alkoxy, C_{1-5} alkoxycarbonyl, (C_{1-8} alkyl)$_o$ aminocarbonyl, C_{1-6} alkylcarbonyloxy, C_{3-8} cycloalkyl, oxo, (C_{1-6} alkyl)$_o$ amino, amino C_{1-6} alkyl, arylaminocarbonyl, aryl C_{1-5} alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C_{1-6} alkyl, hydroxycarbonyl, hydroxycarbonyl C_{1-6} alkyl, and C_{1-8} alkyl, either unsubstituted or substituted with R^3.

R^5 is H, aryl, aryl-$(CH_2)_p$ -, hydroxyl, C_{1-5} alkoxy, aminocarbonyl, C_{3-8} cycloalkyl, amino C_{1-6} alkyl, (aryl)$_q$ aminocarbonyl, (aryl C_{1-5} alkyl)$_q$ aminocarbonyl, hydroxycarbonyl C_{1-6} alkyl, C_{1-8} alkyl, aryl C_{1-6} alkyl, (C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, (aryl C_{1-6} alkyl)$_q$ amino C_{1-6} alkyl, C_{1-8} alkylsulfonyl, C_{1-8} alkoxycarbonyl, aryloxycarbonyl, aryl C_{1-8} alkoxycarbonyl, C_{1-8} alkylcarbonyl, arylcarbonyl, aryl C_{1-6} alkylcarbonyl, (C_{1-8} alkyl)$_q$ aminocarbonyl, aminosulfonyl, C_{1-8} alkylaminosulfonyl, (aryl)$_q$ aminosulfonylamino, (aryl C_{1-8} alkyl)$_q$ aminosulfonyl, C_{1-6} alkylsulfonyl, arylsulfonyl, aryl C_{1-6} alkylsulfonyl, aryl C_{1-6} alkylcarbonyl, C_{1-6} alkylthiocarbonyl, arylthiocarbonyl, or aryl C_{1-6} alkylthiocarbonyl, wherein any of the alkyl groups may be unsubstituted or substituted with R^3;

on each targeting agent, $R^{6'}$ is, at a first occurrence, -J-G-K'-, and is, at a second occurrence, R^{19}, and is, at a third occurrence, R^{20}, and is, at a fourth occurrence, R^{21}; and is, at a fifth occurrence, R^{24}; wherein,

J is an optionally substituted linear or branched connecting chain having from about 1 to about 200 atoms in its backbone and optionally including a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted aryl, or a substituted or unsubstituted heterocyclyl in the backbone;

G is an optionally present recognition group comprising at least a ring structure; and

K' is an attachment moiety including about 1 to about 20 carbon atoms and is covalently linked to the polymer;

R^7 is $-C(O)R^8$, $-C(O)-CR'_2-R^9$, $-C(S)R^8$, $-S(O)_o$ OR', $-S(O)_o$ NR'R", -PO(OR'), $-PO(OR')_2$, $-B(OR')_2$, $-NO_2$, or substituted or unsubstituted tetrazole, substituted or unsubstituted imidazole, substituted or unsubstituted oxazole or substituted or unsubstituted triazole;

R^8 is -OR', -NR'R", $-NR'SO_2$ R', -NR'OR', $-OCR'_2$ C(O)OR', $-OCR'_2OC(O)-R'$, $-OCR'_2C$ (O)NR'_2, unsubstituted C_{1-5} alkyl, or an amino acid attached via its amino group and having its carboxyl group optionally protected;

R^9 is -OR', CN, $-S(O)_r$ R', $S(O)_o$ NR'_2, $-C(O)R'C(O)NR'_2$ or $-CO_2$ R';

R^{11} is H, halogen, $-OR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $-S(O)_r-CF_3$, $-CO_2R'$, $-CONR'_2$, Q-C_{0-6} alkyl-, Q-C_{1-6} oxoalkyl-, Q-C_{2-6} alkenyl-, Q-C_{2-6} alkynyl-, Q-C_{0-6} alkyloxy-, Q-C_{0-6} alkylamino- or Q-C_{0-6} alkyl-$S(O)_r$ -;

Q is -H, substituted or unsubstituted C_{3-6} cycloalkyl, substituted or unsubstituted heterocyclyl, or substituted or unsubstituted aryl;

R^{12} is R', -C(O)-R', $-C(O)-NR'_2$, $-C(O)-OR^{13}$, $-S(O)_o-R'$ or $S(O)_o-NR'_2$; and

R^{13} is -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted arylalkyl;

R^{19}, R^{20}, R^{21}, and R^{24} are each independently –H, optionally substituted C_{1-6} alkyl, C_{1-6} oxoalkyl, C_{2-6} alkenyl, C_{3-4} oxoalkenyl, C_{3-4} oxoalkynyl, C_{2-4} alkynyl, C_{3-6} cycloalkyl, aryl, or heterocyclyl;

R' is, at each occurrence, independently -H, substituted or unsubstituted C_{1-6} alkyl, substituted or unsubstituted C_{3-7} cycloalkyl-C_{0-4} alkyl, or substituted or unsubstituted aryl-C_{0-4} alkyl;

R" is R', -C(O)R' or -C(O)OR^{13};

R''' is -H, or substituted or unsubstituted C_{1-6} alkyl;

m at each occurrence is independently 0 to 3;

n at each occurrence is independently 1 to 3;

o at each occurrence is independently 1 or 2;

p at each occurrence is independently 1 to 4;

q at each occurrence is independently 0 to 2; and

r at each occurrence is indepencdently 0 to 2.

68. A pharmaceutical formulation comprising the targeting compound of any of claims 1, 36 or 67 and a pharmaceutically acceptable carrier.

69. A method of producing an integrin RGD peptidomimetic targeting compound, comprising covalently linking the compound of claim 1 to a polymer.

70. The method according to claim 69 wherein the polymer is a protein.

71. The method according to claim 69 wherein the polymer is at least one combining site of a multi-valent antibody.

72. A method according to claim 69 wherein K is a 1,3-diketone.

73. A method according to claim 71 wherein the antibody is an aldolase antibody.

74. A method according to claim 71 wherein said antibody is bivalent and said compound of formula I is linked to each combining site.

75. A compound produced by the method of claim 69.

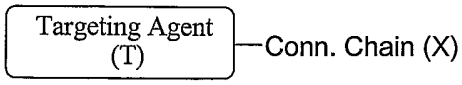
76. A method of treating or preventing a disease or condition that involves integrin $\alpha_v\beta_3$ or $\alpha_v\beta_5$ in an individual, said method comprising administering to the individual a therapeutically effective amount of the compound of claim 36, 67, or 75 wherein said therapeutic component reduces the symptoms associated with said disease or condition.

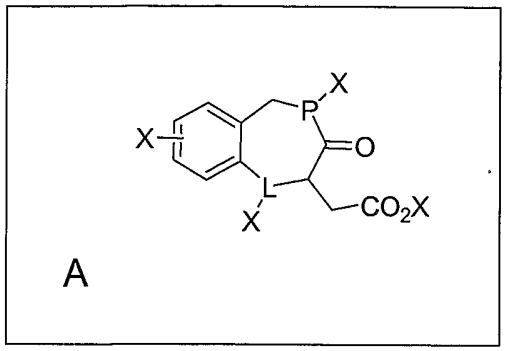
77. The method of claim 76 wherein said disease or condition involves a defect in angiogenesis, bone metabolism, inflammation or cell growth.

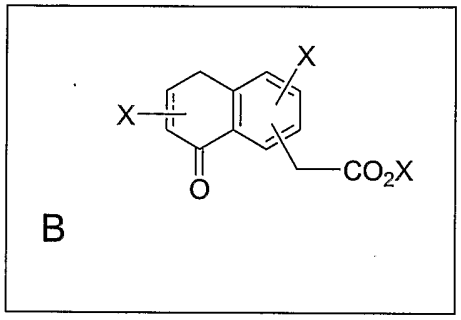
78. The method of claim 76 wherein said disease or condition is cancer.

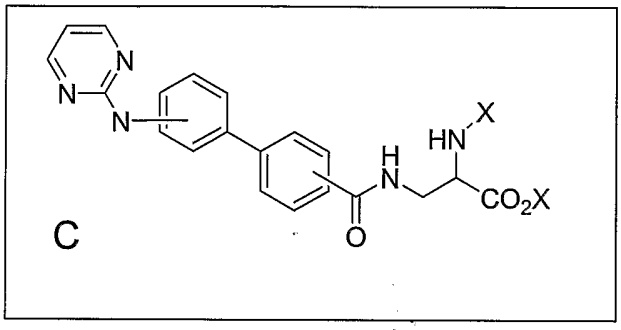
79. A method of antagonizing $\alpha_v\beta_3$ or $\alpha_v\beta_5$ function in vivo comprising comprising administering to the individual an antagonizing amount of the compound of claim 36, 67, or 75.

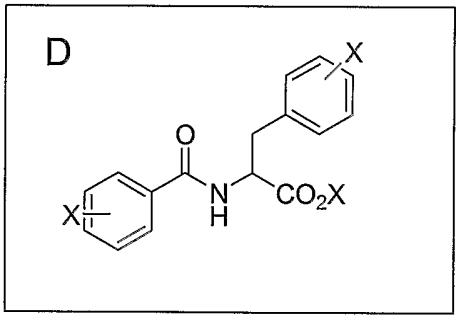
Figure 1

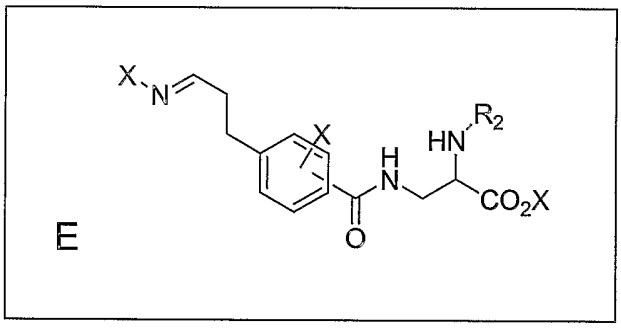












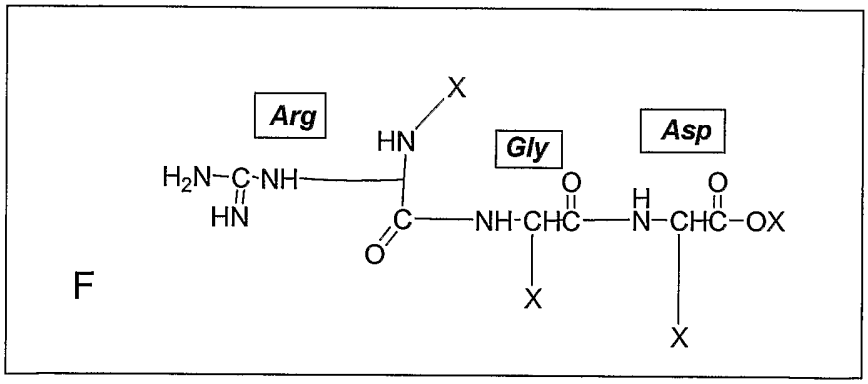
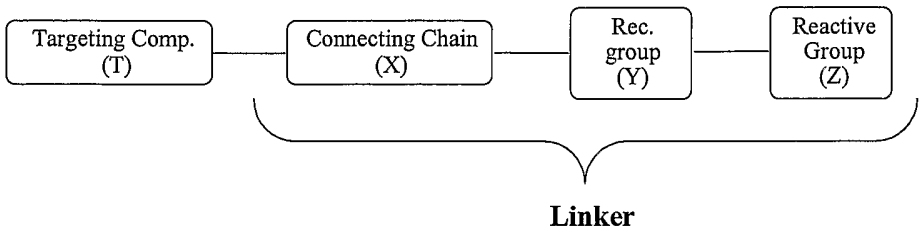
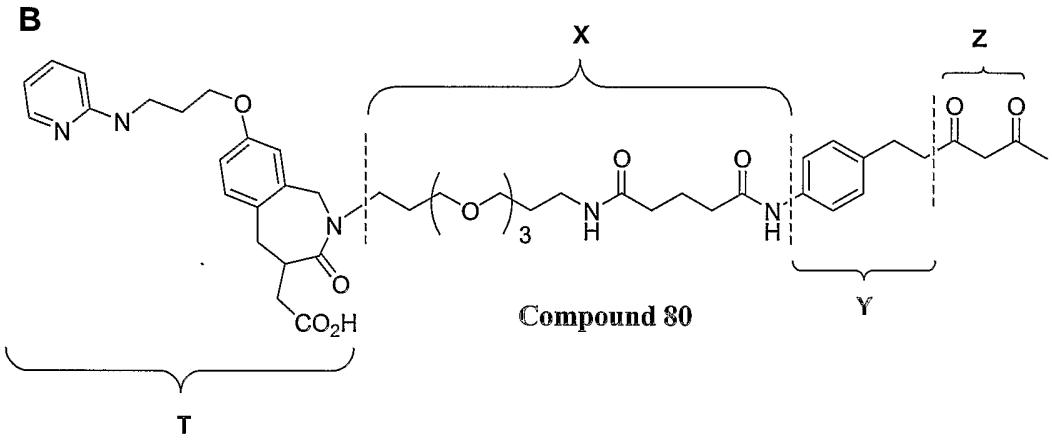


Figure 2

A


Targeting Comp. (T)
Connecting Chain (X)
Rec. group (Y)
Reactive Group (Z)
Linker

B


X
Z
Y
T
Compound 80

C

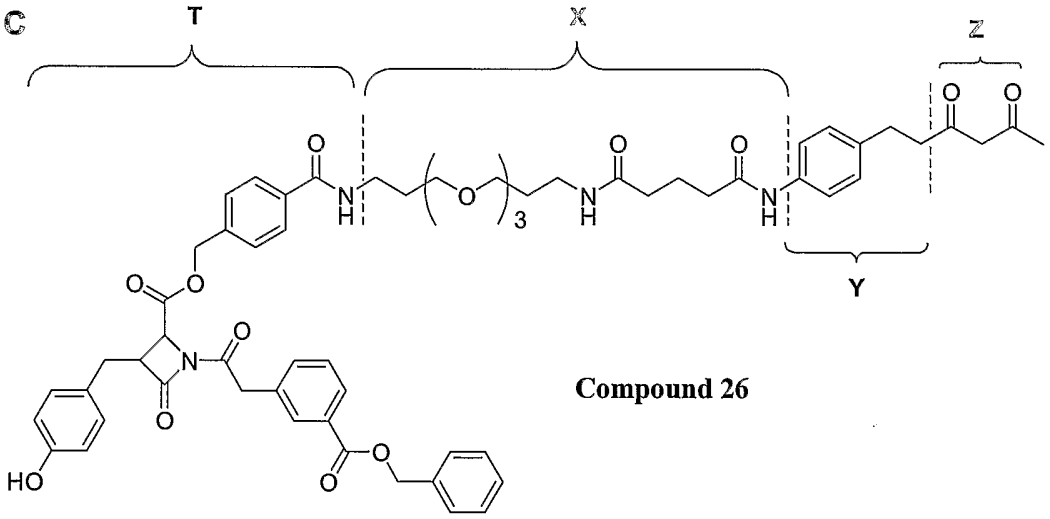
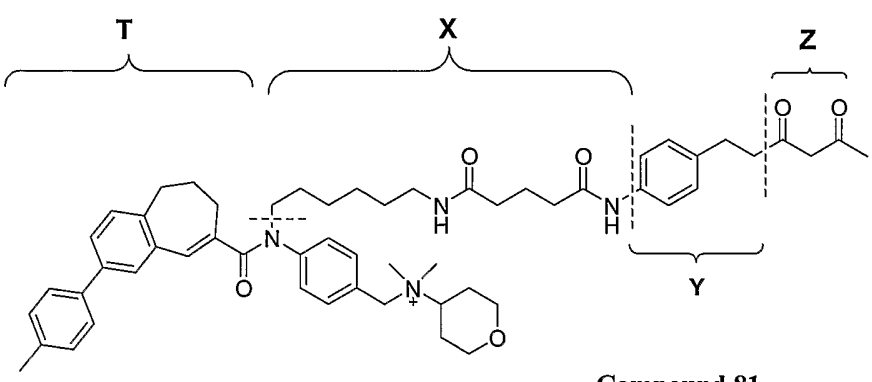

T
X
Z
Y
Compound 26

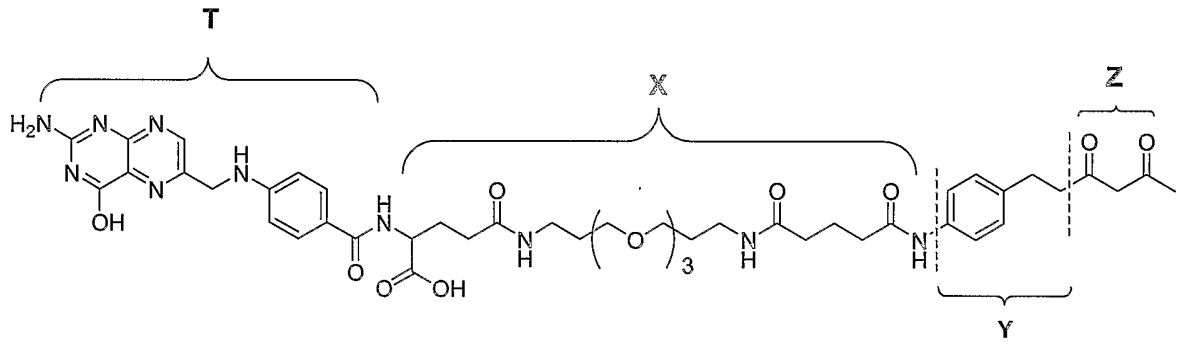
Figure 2 (Cont'd.)

D



Compound 81

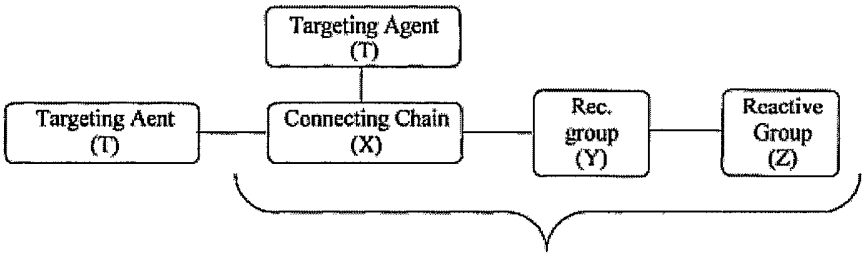
E



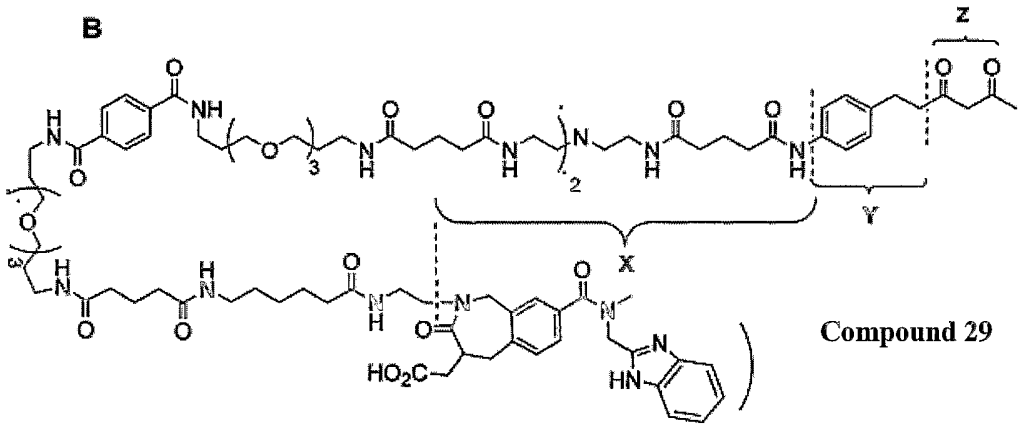
Compound 28

Figure 3

A

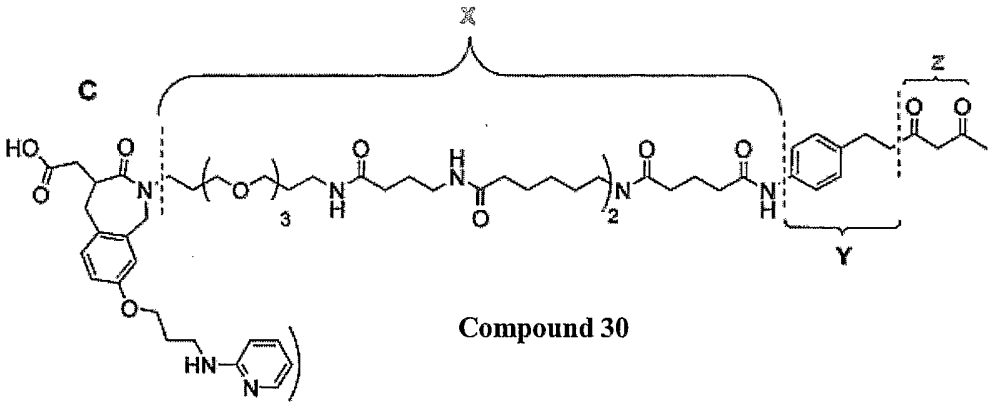


B



Compound 29

C



Compound 30

Figure 4

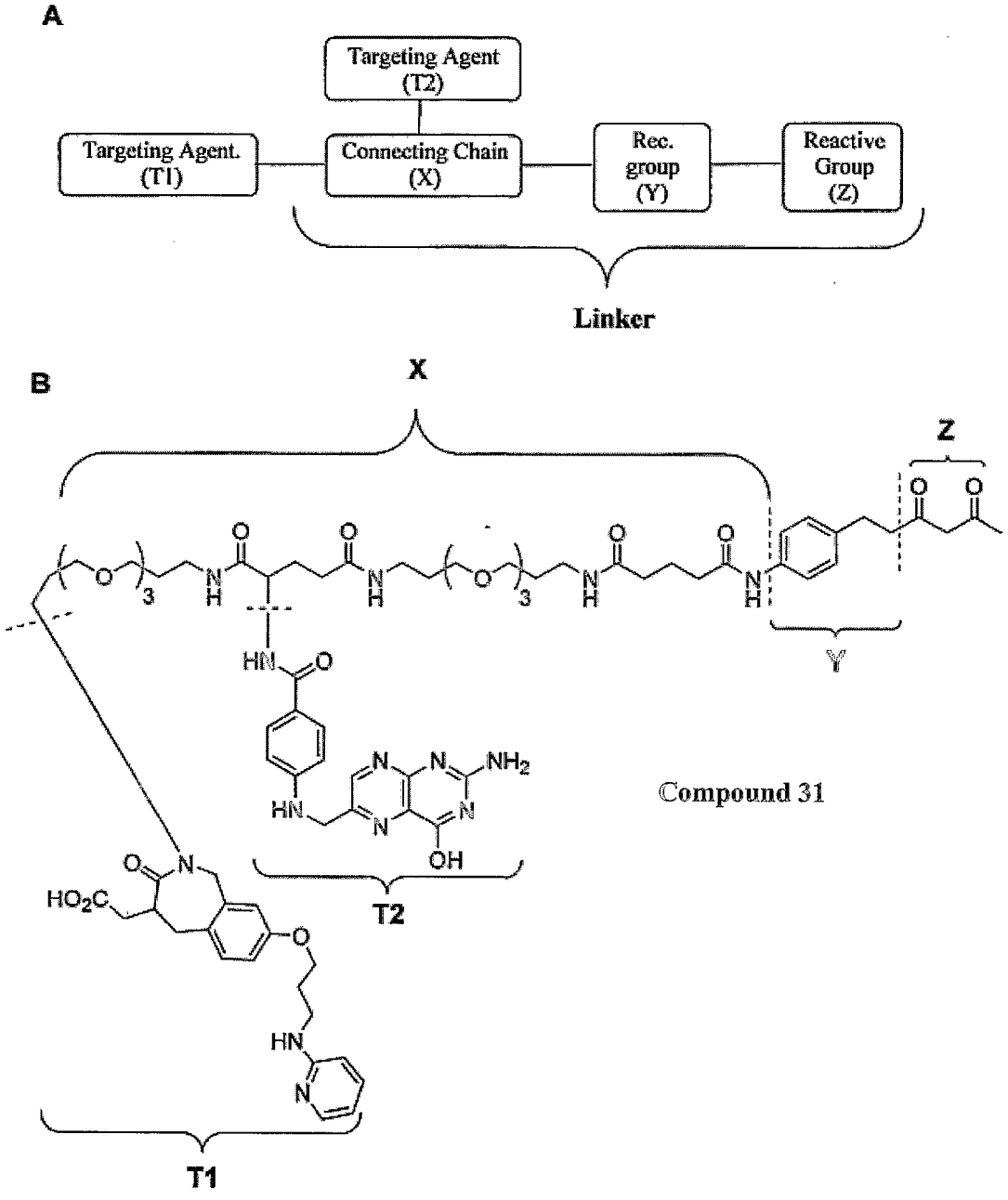
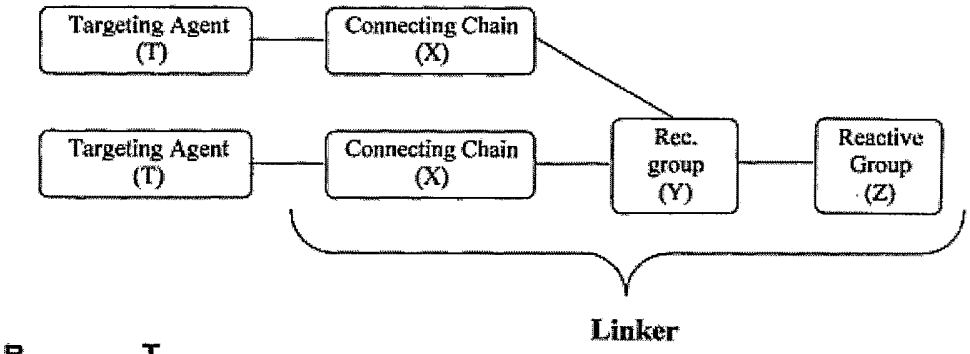

A
Targeting Agent (T2)
Targeting Agent. (T1)
Connecting Chain (X)
Rec. group (Y)
Reactive Group (Z)
Linker
B
X
Z
Y
Compound 31
T2
T1

Figure 5

A


Targeting Agent (T)
Connecting Chain (X)
Targeting Agent (T)
Connecting Chain (X)
Rec. group (Y)
Reactive Group (Z)
Linker

B

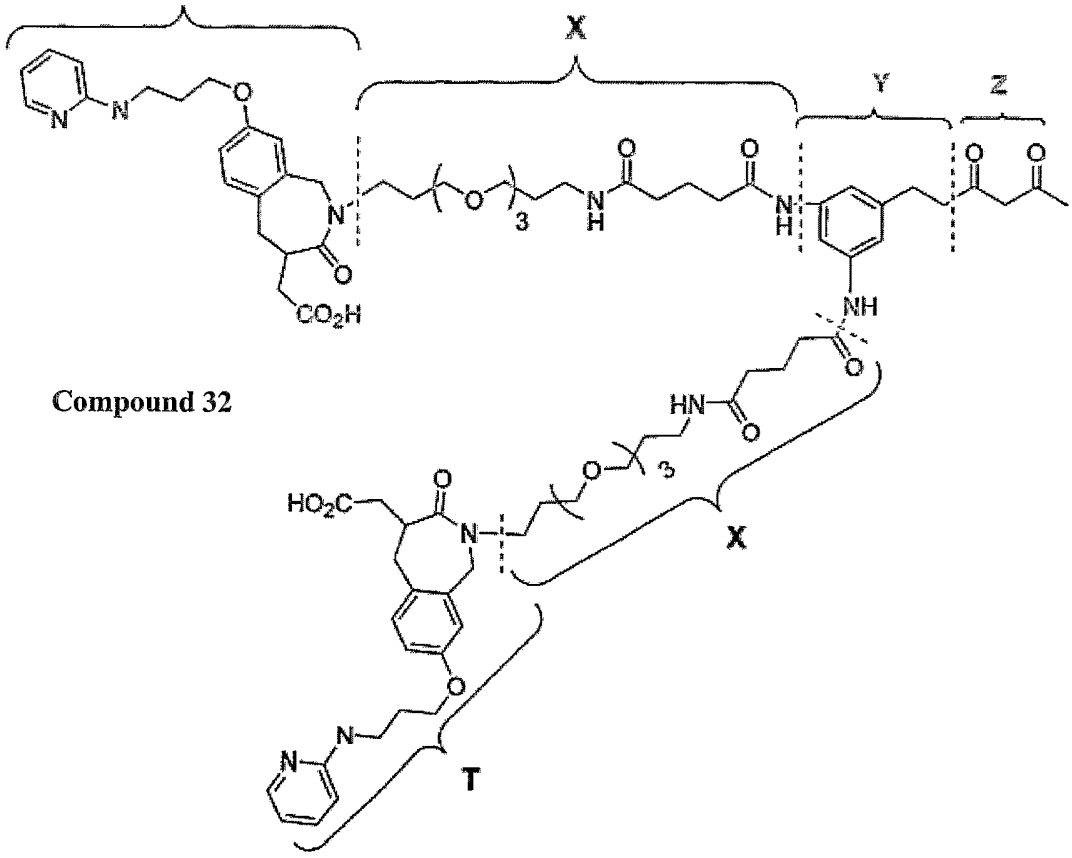

T
X
Y
Z
Compound 32

Figure 6

Linker Reactive Groups (Z)

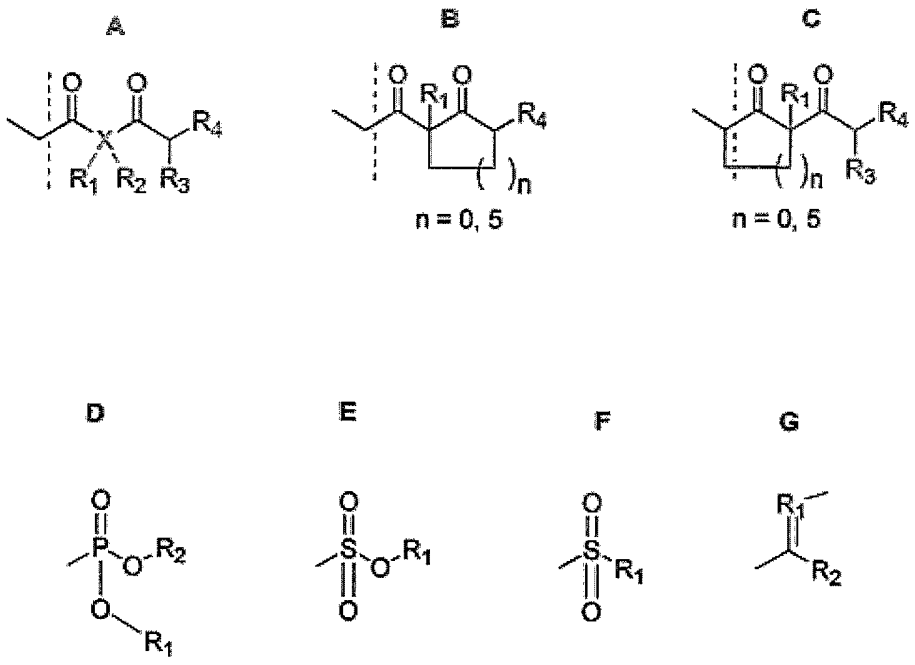
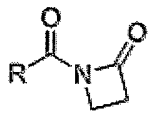
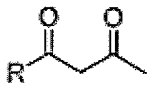


Figure 7

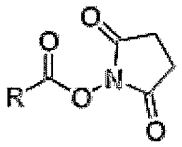
A



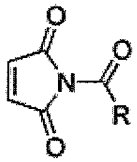
B



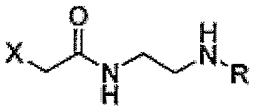
C



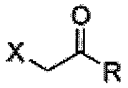
D



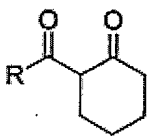
E



F



G

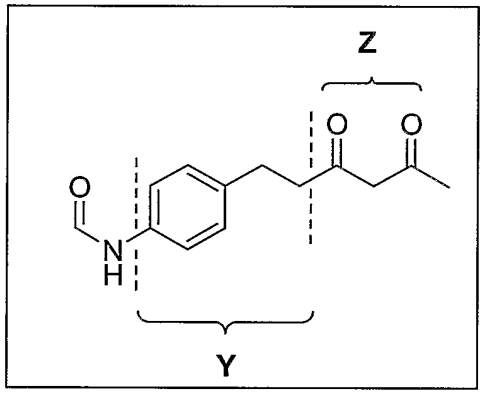


H

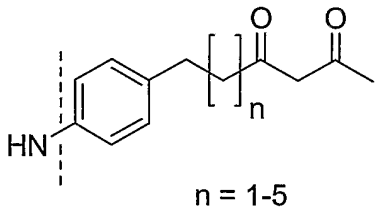
Figure 8

Linker Recognition Groups (Y)

A

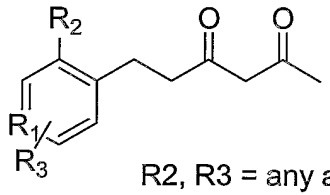


B



n = 1-5

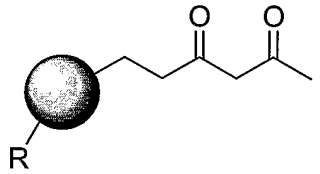
C

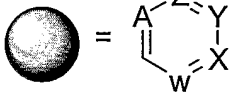


R2, R3 = any atom

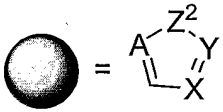
R1 = O-R,
S-R, N-R, CH2-R

D





A, W, X, Y and Z^1 are Independently C or N

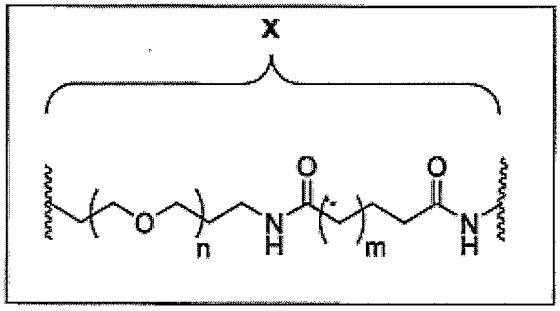


A, X and Y are Independently C or N; Z^2 is C, N, O or S

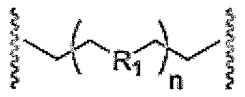
Figure 9

Linker Connecting Chain (X)

A

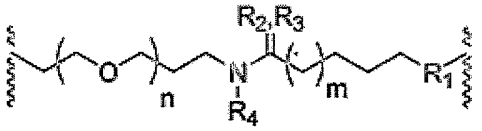


B



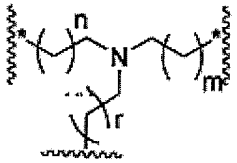
R1 = O, CH2, NR_1R_2, Si, S, S(O), $S(O)_2$

D



C

Branched Chain:



E

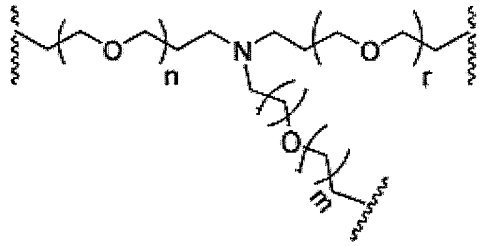


FIG. 10 (Scheme 1)

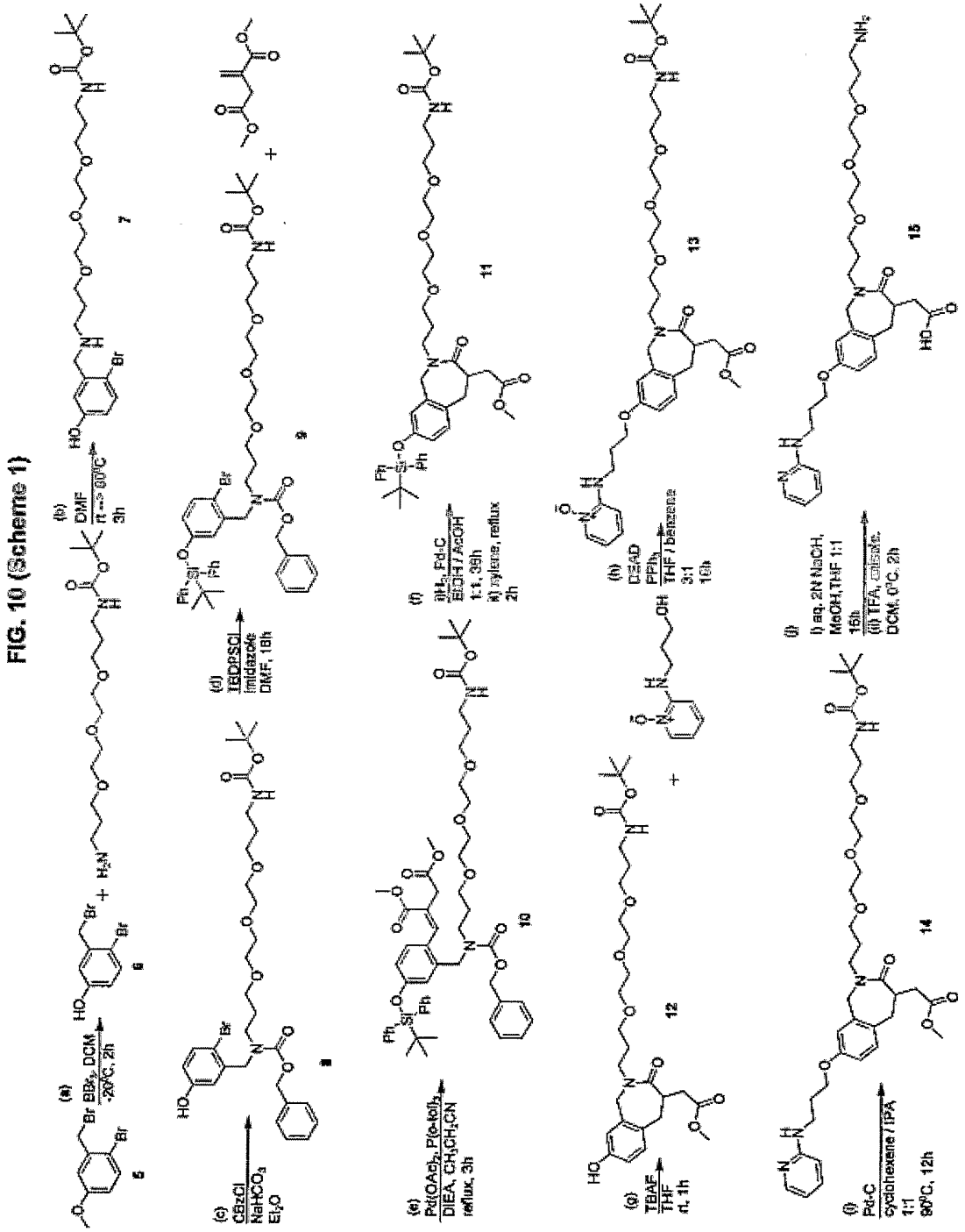


FIG. 11 (Scheme 2)

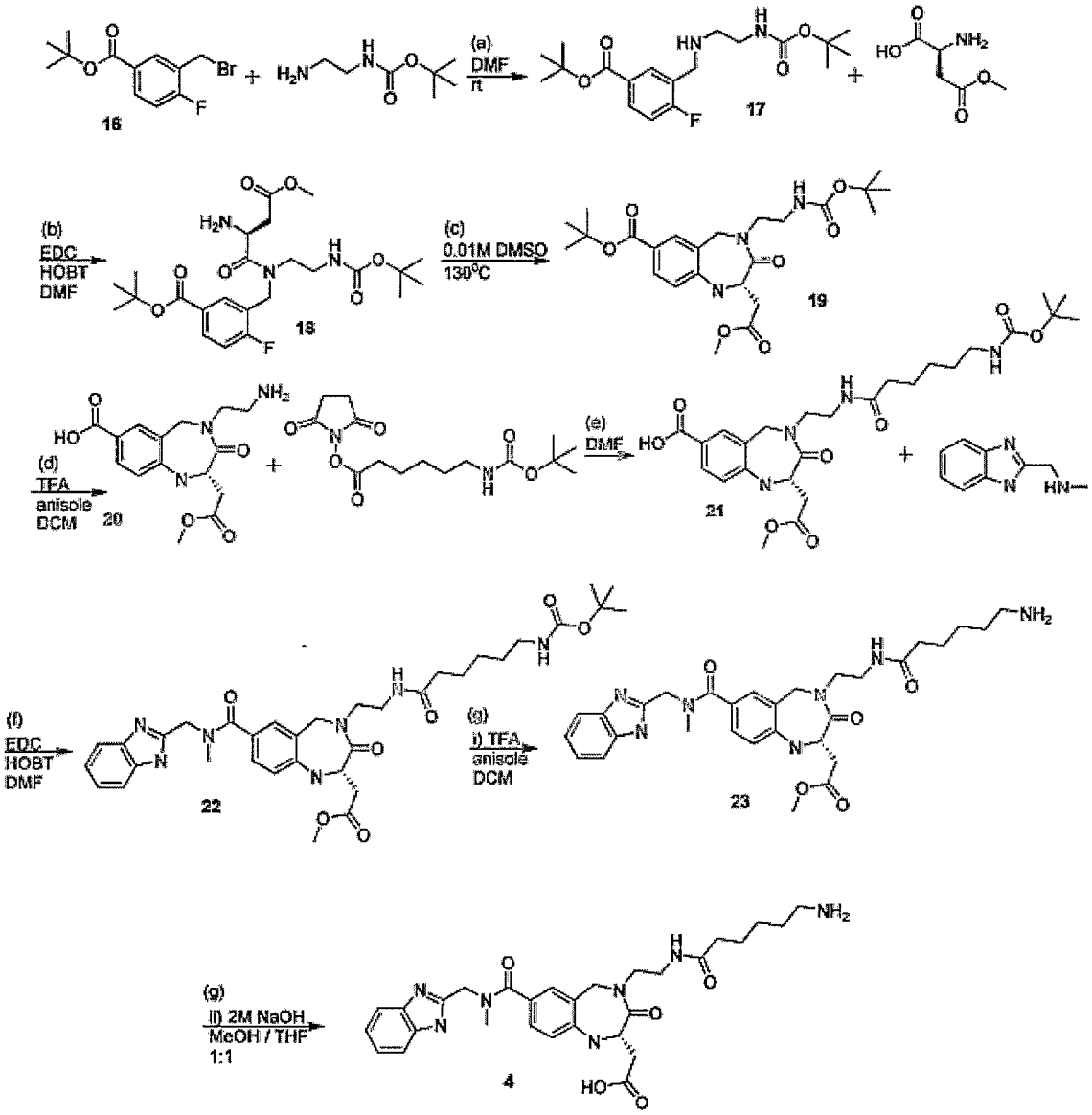


Figure 12 (Scheme 3)

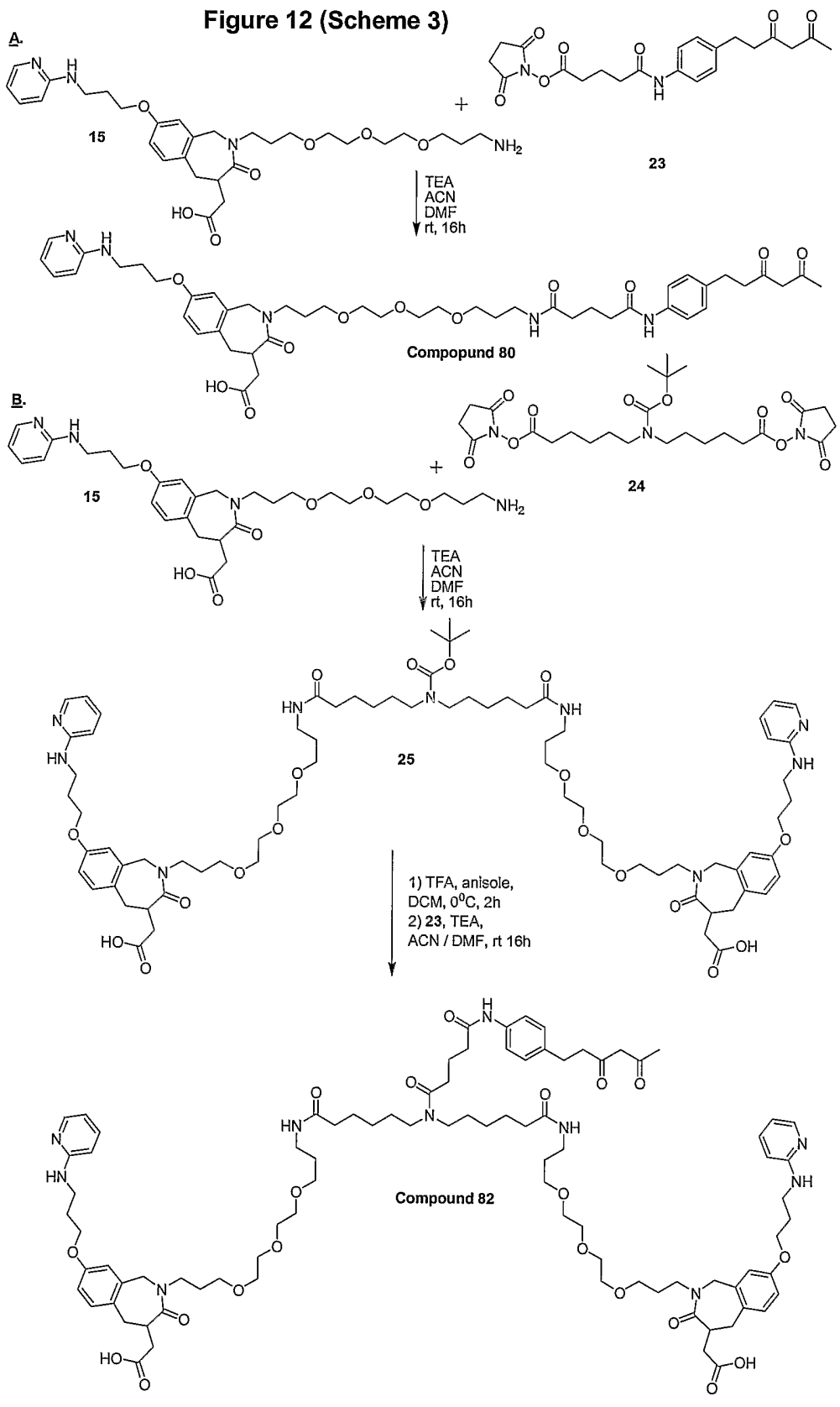


Figure 13 (Scheme 4)

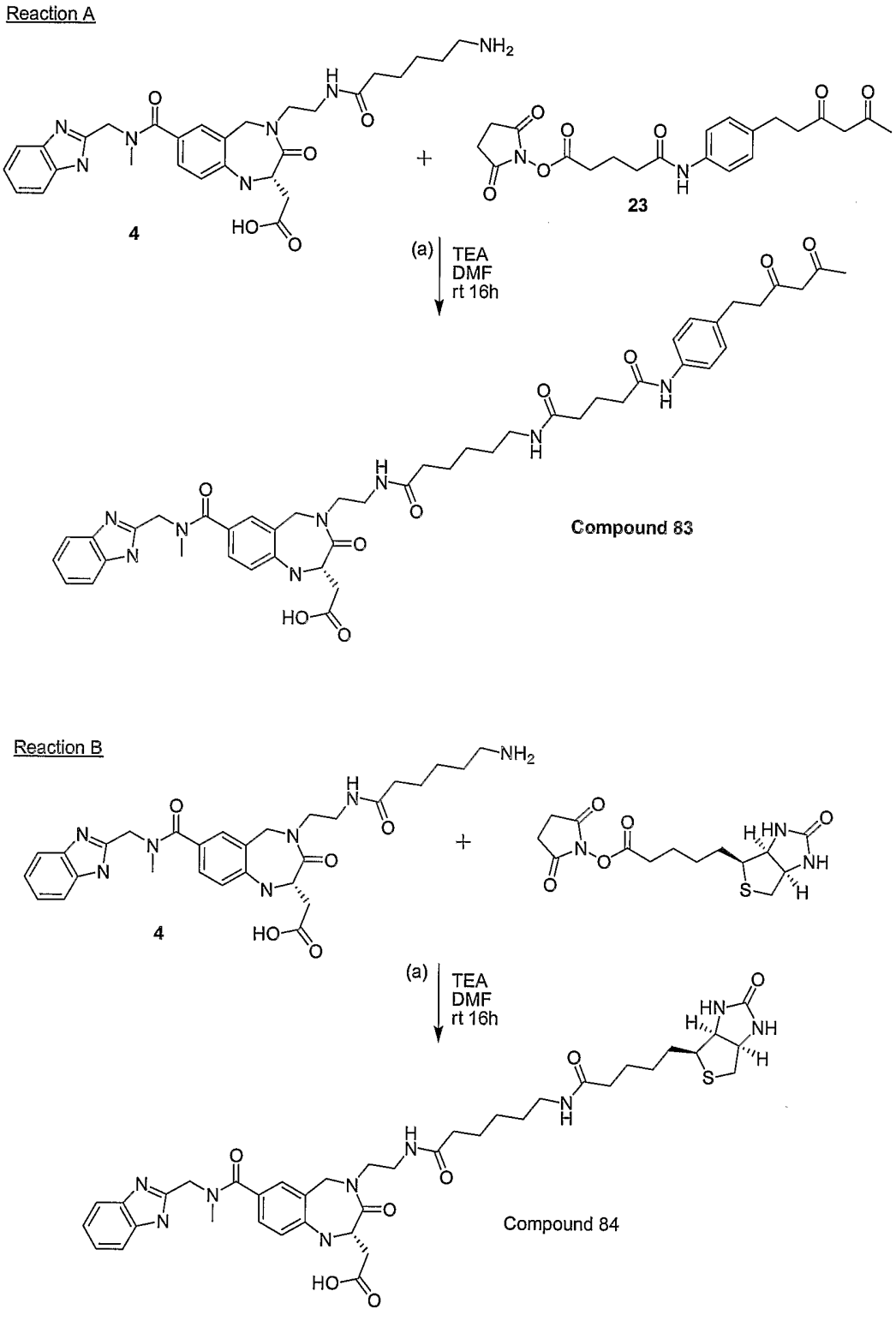


FIG. 14

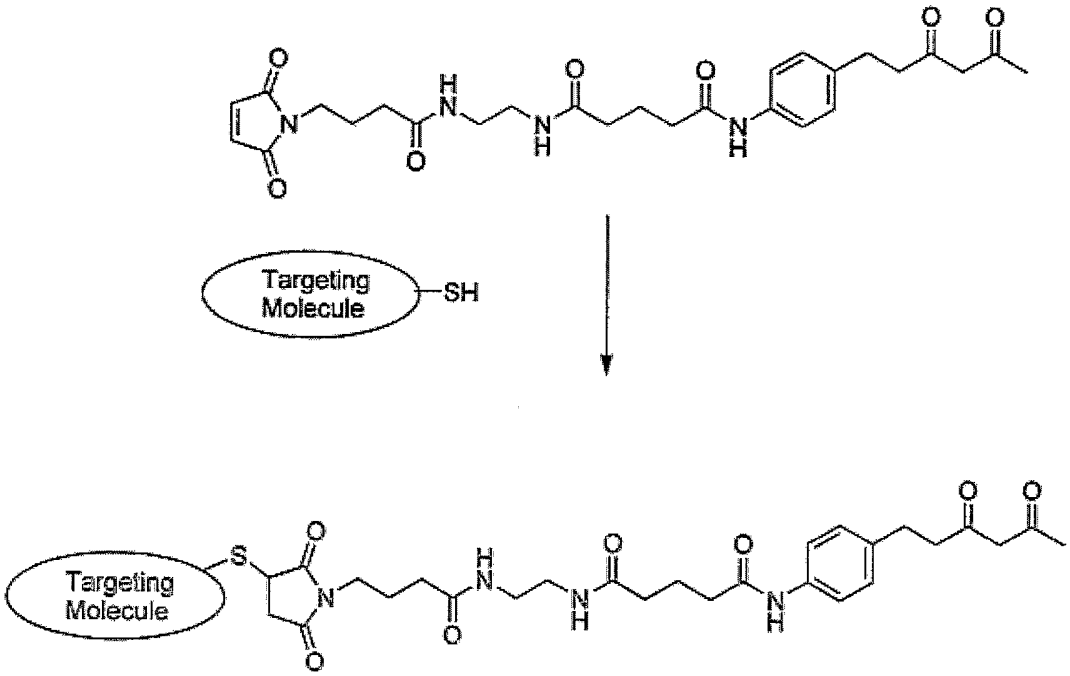


Figure 15 (Scheme 5)

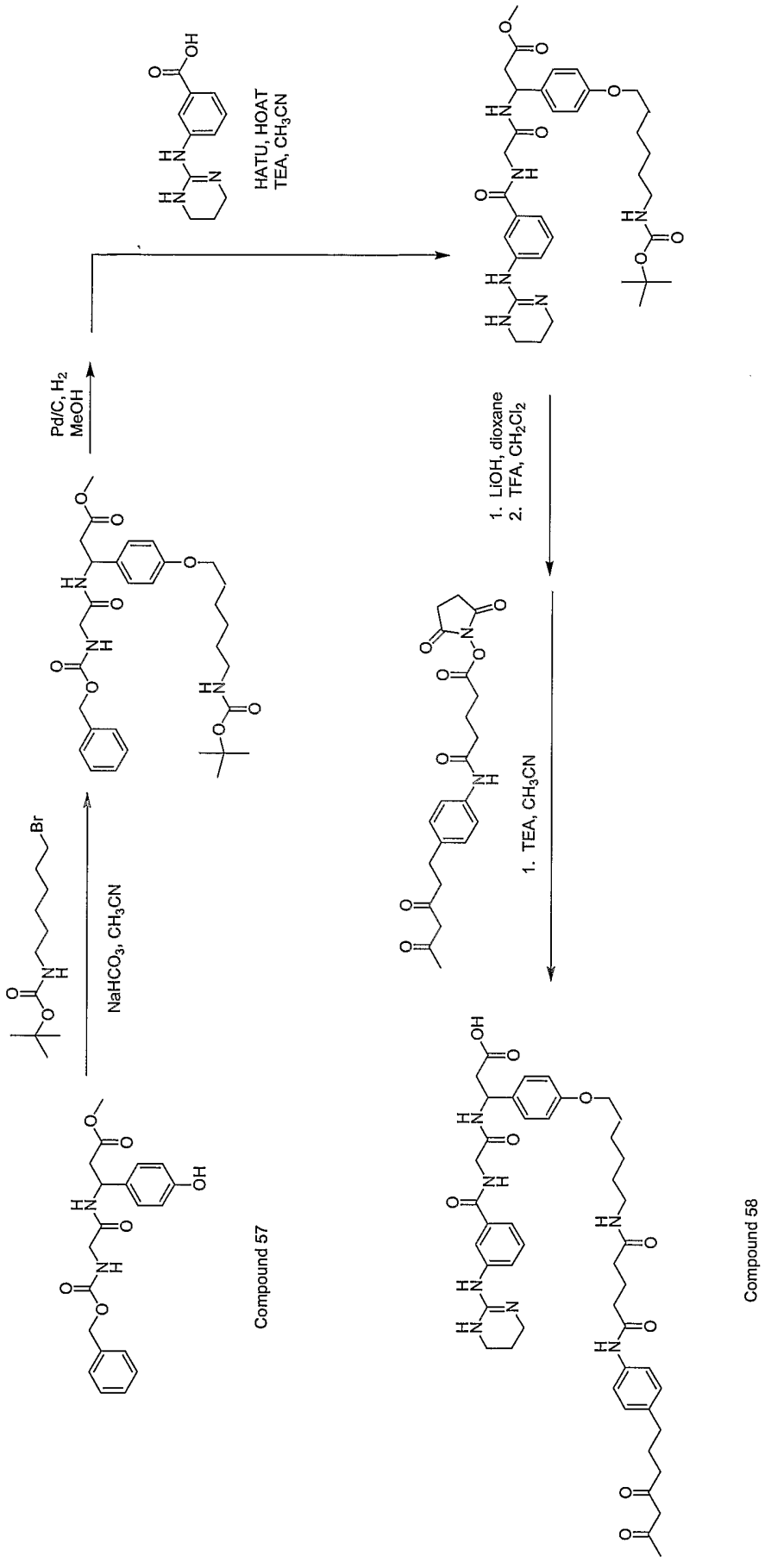


Figure 16 (Scheme 6)

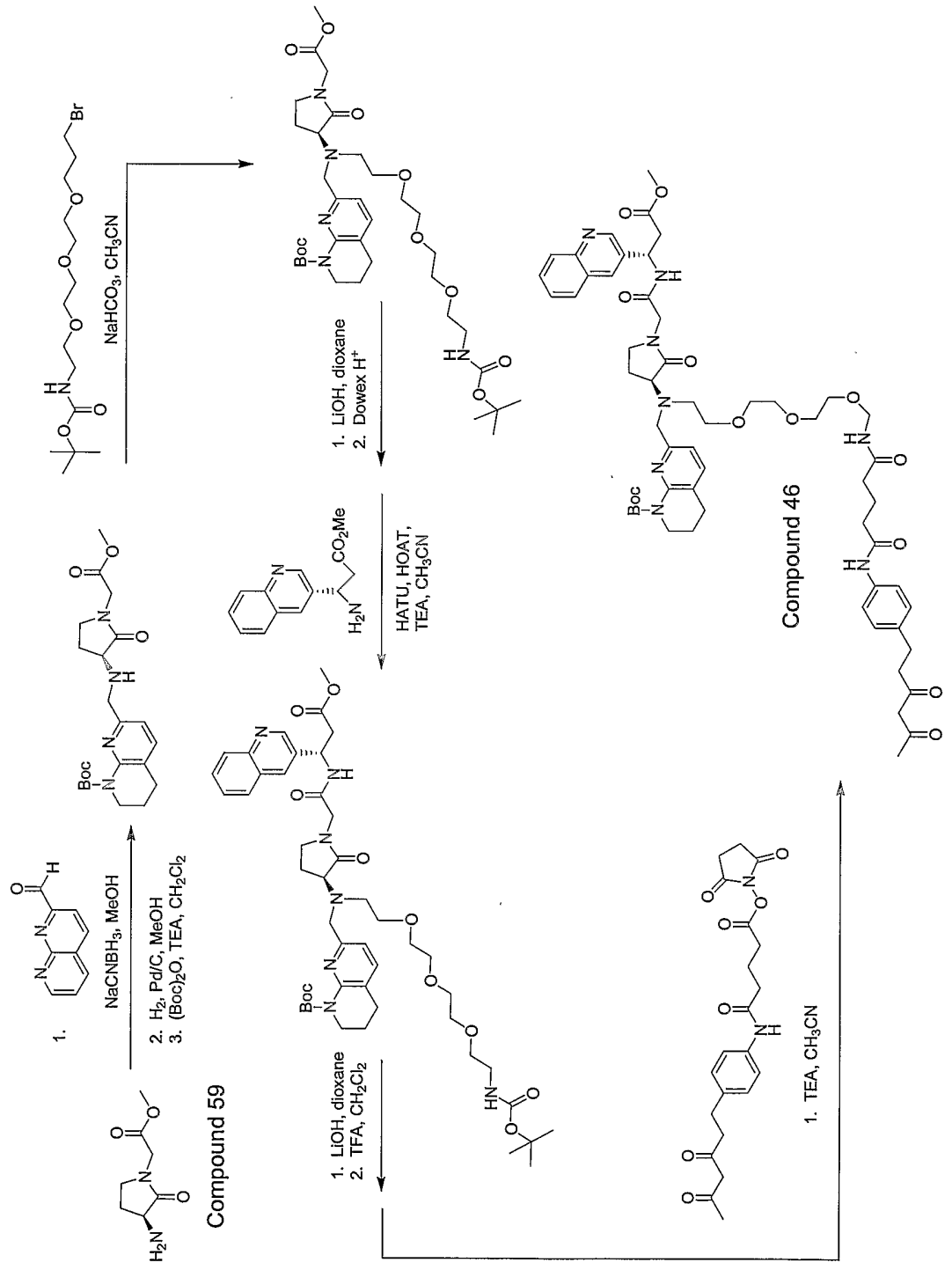


Figure 17 (Scheme 7)

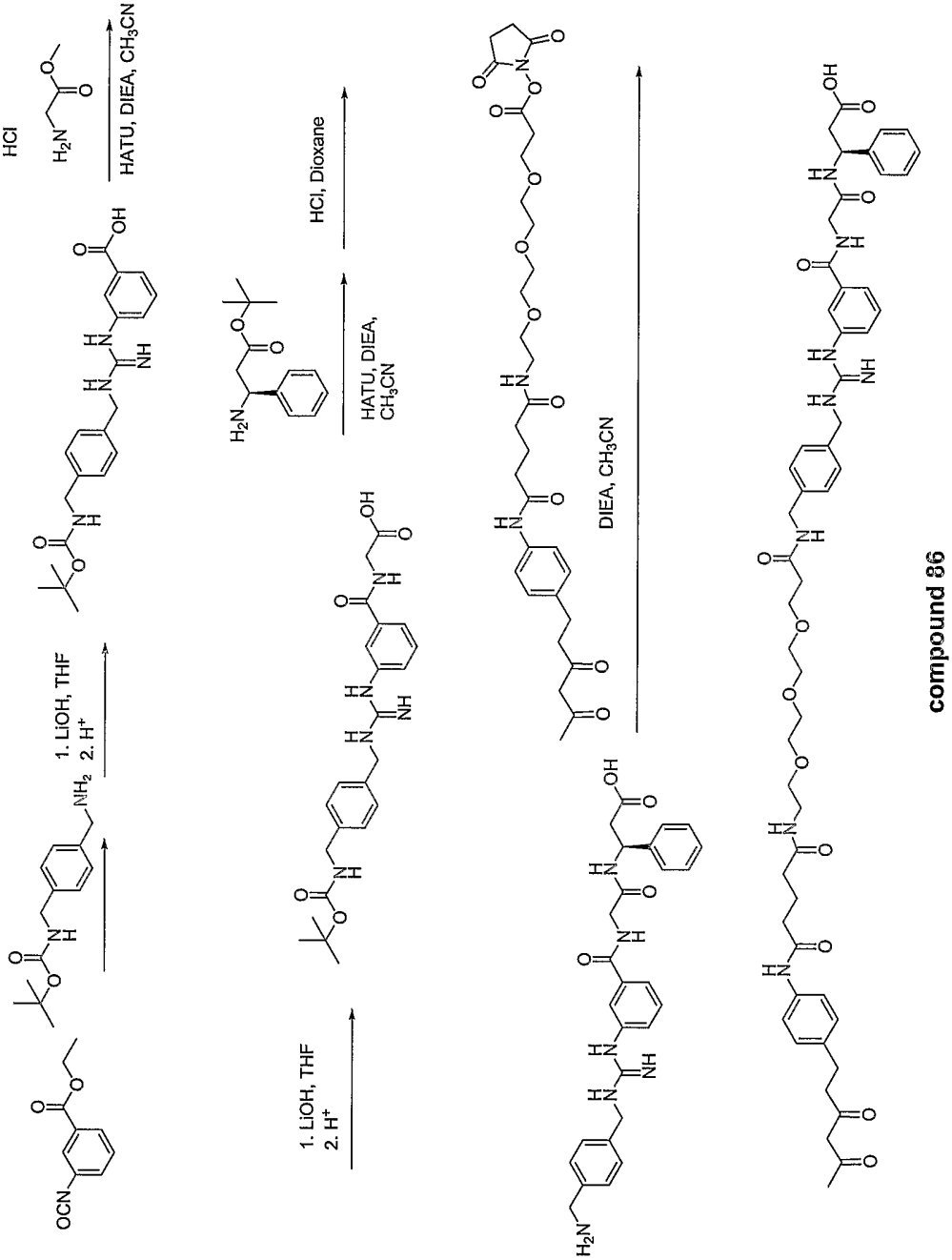


Figure 18 (Scheme 8)

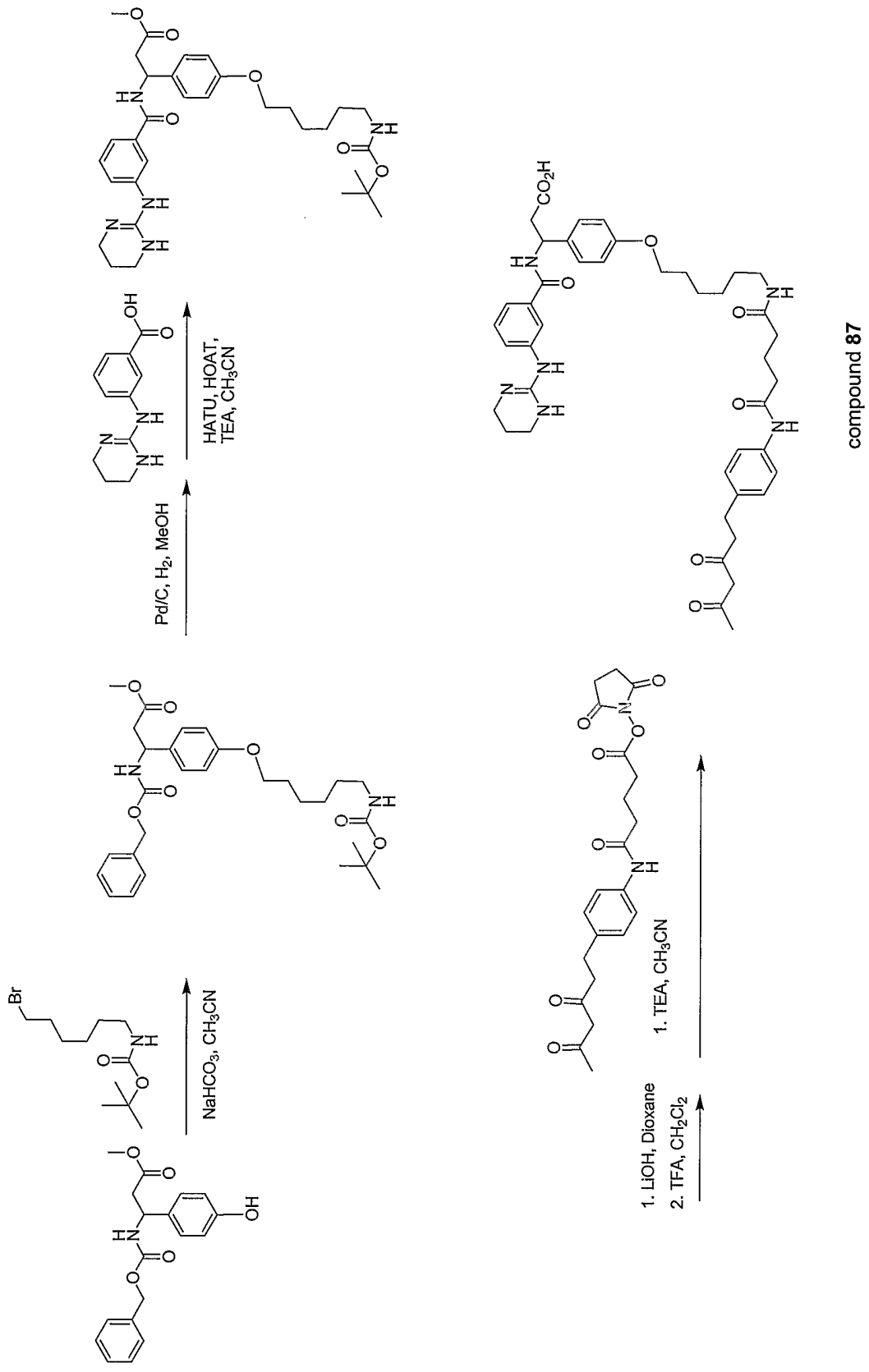


Figure 19 (Scheme 9)

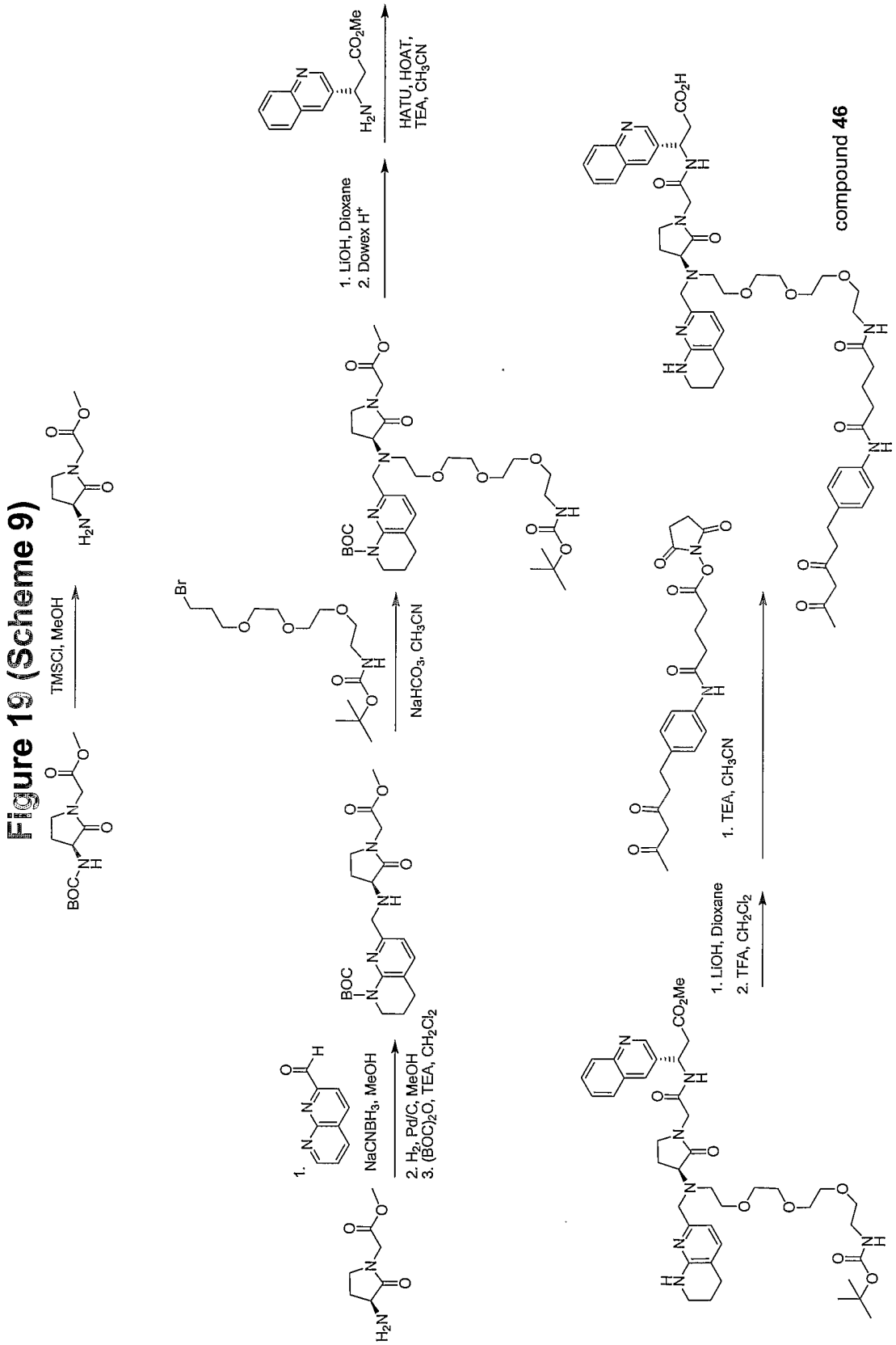


Figure 20 (Scheme 10)