CYCLIC CELL ADHESION MODULATION COMPOUNDS

BACKGROUND OF THE INVENTION

This application is a continuation-in-part application of Serial No. 07/550,330, filed July 9, 1990.

1. Field of Invention

The present invention relates to novel cyclic peptides and peptidomimetic compounds which are characterized by cell adhesion modulation activity. The compounds have application to the study and treatment of disease conditions mediated by cell adhesion. Specifically, the compounds have application to the study, diagnosis, treatment or prevention of diseases and conditions such as cardiovascular disease, harmful platelet aggregation, neoplastic disease including metastasis of neoplastic growth, wound healing, inflammation and auto-immune disease or other diseases or conditions involving cell adhesion.

2. Background of the Invention

The extracellular matrix is the major component of connective tissue of all mammals. The extracellular matrix provides for structural integrity, and promotes cell migration and cellular differentiation. As part of these functions, the extracellular matrix has been shown to support adhesion for various types of cells *in vitro*. Molecules such as collagens, fibronectin, vitronectin, laminin, von

Willebrand factor, thrombospondin, bone sialoprotein, fibrinogen, and tenacin have been found to possess this property of mediating cell adhesion.

The above cell-adhesive molecules have been found to exhibit a structural similarity in their respective binding sites, each of which contains the amino acid sequence arginine-glycine-aspartic acid, or RGD using single letter nomenclature (*infra*). The cell-binding site in fibronectin has been reproduced synthetically and establishes the RGD sequence as the essential structure recognized by cells in fibronectin. Changes in the peptide as small as the exchange of alanine for glycine or glutamic acid for aspartic acid which constitutes the addition of a single methyl or methylene group to the tripeptide, eliminates these activities (Pierschbacher & Ruoslahti, *PNAS*, **1981**, 81, 5985). In turn, the cellular receptor site for fibronectin has been identified for various cells. In addition, cellular receptors that recognize RGD-containing sequences in other extracellular matrix proteins (e.g., the vitronectin receptor and the platelet gpIIb/IIIa receptor) have been identified.

Such cellular receptors, responsive to RGD-containing proteinaceous compounds, have been characterized. The complete, primary structure of the fibronectin receptor has been deduced from cDNA, and physical properties have been determined (Argraves, *et al.*, *J.Biol. Chem.* **1986**, 261, 12922; Argraves *et al.*, *J.Cell Biol.* **1987**, 105, 1183). The protein exists at the cell surface as a heterodimeric complex (although the larger polypeptide is enzymatically processed) having both polypeptide chains inserted into the membrane. Each chain extends 30-40 residues into the cytoplasmic space, and at least one of the cytoplasmic peptides appears to interact with the cytoskeleton (Horwitz *et al.*, *Nature* **1986**, 320, 531). The larger of the two polypeptides, the α subunit, contains a number of regions that are structurally similar to calmodulin and that apparently mediate the binding

of calcium to the receptor. The presence of such divalent cations is required for the receptor to bind ligand. The β subunit is somewhat smaller and conformationally compact due to numerous intrachain disulfide bonds. The cytoplasmic domain of the β subunit contains a potentially phosphorylated tyrosine (Hirst *et al.*, *PNAS-USA* **1986**, 83, 6470; Tamkun *et al.*, *Cell* **1986**, 46, 271).

Other RGD-directed receptors, as well as other "orphan" receptors the ligand for which is unknown, have also been characterized. This putative RGD commonality of the ligand matrix proteins has revealed a superfamily of cell surface receptor proteins that share a high degree of structural similarity and probably also functional similarity. The members of this superfamily of cell surface proteins collectively are known as the integrins. The integrins are α, β heterodimeric cell surface proteins which can be grouped on the basis of the identity of their β subunit. Each integrin can interact with single or multiple ligands. The β subunit, as disclosed above for the fibronectin receptor, is compact due to a high degree of cross-linking. The first group of integrins, β_1 molecules, includes the very late activation antigen (VLA) proteins, which themselves include the fibronectin receptor (VLA-5), collagen receptor (VLA-2), laminin receptor, and a receptor (VLA-4) which binds to the vascular cell adhesion molecule-1 (VCAM-1) on cytokine activated endothelial cells. The second group (β_2 subfamily) includes the lymphocyte associated antigen-1 (LFA-1), macrophage antigen-1 (MAC-1), and p150,95. The third group (β_3 subfamily) includes the vitronectin receptor, the platelet glycoprotein gpIIb/IIIa. To date, seven β chains have been described. For reviews, see Hynes, *Cell* **1987**, 48, 549; Hemler, *Immunol. Today* **1988**, 9, 109; Springer *et al.*, *Annu. Rev. Immunol.* **1987**, 5, 223; Kishimoto *et al.*, *Leukocyte Adhesion Molecules*, T.A. Springer, D.C. Anderson, A.S. Rosenthal, and R. Rothlein, Eds., Springer-Verlag, New York, 1989, pp. 7-43.

The RGD-directed receptor present on platelets (gpIIb/IIIa) that binds fibronectin, vitronectin, fibrinogen, and von Willebrand factor has also been purified. The receptor is thus not specific to one extracellular matrix protein, as is the fibronectin receptor. It has been proposed that this lack of specificity is correlated to the lack of conformational specificity in the ligands. Other work has suggested that specificity can be achieved with relatively short, conformationally restricted synthetic peptides containing the RGD sequence. For a literature summary, see: Pierschbacher *et al., Nature* **1984**, 309, 30; Pierschbacher *et al., PNAS-USA* **1984**, 81, 5985; Ruoslahti *et al., Cell* **1986**, 44, 517; Pierschbacher *et al., J.Biol.Chem.* **1987**, 262, 17294; Hynes, *Cell* **1987**, 48, 549; Ruoslahti, *Ann. Rev. Biochem.* **1988**, 57, 375. It has also been proposed that the receptor affinity for its peptide ligand may be altered as the stereoconformation, or three-dimensional shape, of the peptide is restricted, typically by cyclization. Pierschbacher and Ruoslahti, PCT International Publication WO 89/05150 (1989). However, the publication states that the cyclic peptide of the invention (see Figure 2 and Example V) was ineffective in inhibiting attachment to fibronectin.

A limited number of compounds containing sequences of natural amino acids or derivatives other than RGD may also possess the capability for affecting cell adhesion. These non-RGD-containing peptides are not well characterized. See, Graf, J. *et al., Cell* **1987**, 48, 989; Kloezewiak, M. *et al., Biochemistry* **1984**, 12, 1767; Wayner, E.A., *et al., J. Cell. Biol.* **1989**, 109, 1321.

All publications, patents and other reference materials to which reference is made in the present specification are incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention relates to compounds having activity as cell adhesion modulators. Some of the compounds contain the amino acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp or RGD). Others contain non-RGD sequences, including but not limited to the RCD sequence and "reverse orientation" forms of amino acid residues. The compounds, in one aspect, sufficiently mimic extra-cellular matrix ligands or other cell adhesion ligands so as to bind to cell surface receptors. Such receptors include integrin receptors, in general, including the fibronectin, collagen, laminin, LFA-1, MAC-1, p150,95, vitronectin and gpIIb/IIIa receptors. The novel compounds have been found to modulate cell adhesion by competing, for example, with ligands containing the appropriate amino acid sequence and by binding to ligand-directed receptors on cell surfaces. The cell adhesive protein, such as (but not limited to) fibronectin, is sufficiently inhibited from binding to the cell's receptor so as to prevent or reduce cell adhesion. Other uses include enhancing cell adhesion by using the compounds to attach cells to a surface, or by other promotion of cell adhesion. The useful compounds herein described function as cell-adhesion modulators.

One object of the present invention is to provide novel compounds which act to modulate cell adhesion.

Another object of the present invention is to provide novel RGD-containing compounds which are capable of binding to a cellular receptor.

Another object of the present invention is to provide novel non-RGD-containing compounds which contain one or more "reverse orientation" amino acid residues and which are capable of binding to a cellular receptor.

Another object of the present invention is to provide novel non-glycine containing, e.g., RCD-containing, compounds which are capable of binding to a cellular receptor.

Another object of the present invention is to provide new uses for known peptides containing the sequence RGD as cell adhesion modulators.

Another object of the present invention is to provide a novel method for modulating cell adhesion using novel compounds.

Another object of the present invention is to provide compounds having extraordinarily high potencies in modulating cell adhesion to integrin receptors, including specifically inhibition of cell adhesion to the fibronectin receptor. Thus, in one regard, the present invention includes compounds having an IC_{50} of less than about 500 μM as established in a U937 fibronectin adhesion assay; and in another regard, the invention includes compounds having an IC_{50} of less than about 100 μM in such assay. The invention also includes methods for obtaining (either *in vitro* or *in vivo*) such fibronectin receptor adhesion inhibition, and integrin receptor adhesion inhibition. The compounds of the present invention accomplish strong inhibition, at low concentrations, with an IC_{50} of less than about 500 μM, or alternatively less than about 100 μM.

Another object of the present invention is to provide compounds having high potencies in modulating leukocyte adhesion to endothelial cells. Thus, in one regard, the present invention includes compounds having an IC_{50} of less than about 200 μM as established in a Jurkat-endothelial cell adhesion assay; and in another regard, the invention includes compounds having an IC_{50} of less than about 10 μM in such assay. The invention also includes methods for obtaining (either *in vitro* or *in vivo*) such leukocyte receptor adhesion inhibition. The compounds of the present invention accomplish strong inhibition using disclosed compounds, at low concentrations, with an IC_{50} of less than about 200μM, or alternatively less than about 10 μM.

Another object of the present invention is to provide novel compounds, formulations, and methods which may be used in the study, diagnosis, treatment or prevention of diseases and conditions which relate to cell adhesion, including but not limited to rheumatoid arthritis, asthma, allergies, adult respiratory distress syndrome (ARDS), cardiovascular disease, thrombosis or harmful platelet aggregation, reocclusion following thrombolysis, allograft rejection, neoplastic disease including metastasis of neoplastic growth, wound healing, Type I diabetes, inflammatory and immunoinflammatory conditions including ophthalmic inflammatory conditions and inflammatory bowel disease (e.g., ulcerative colitis and regional enteritis), and autoimmune diseases.

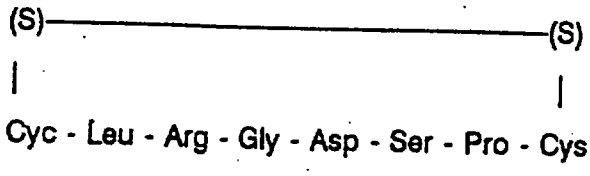
Another object is to provide derivative compounds, such as, but not limited to, antibodies and anti-idiotype antibodies to the compounds disclosed and claimed in order to study, diagnose, treat or prevent diseases and conditions which relate to cell adhesion, including but not limited to rheumatoid arthritis, asthma, allergies, ARDS, cardiovascular disease, thrombosis or harmful platelet aggregation,

neoplastic disease including metastasis of neoplastic growth, wound healing, Type I diabetes, inflammatory conditions and autoimmune diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1a and 1b are diagrams representing chemical reactions for the manufacture of amide-linked cyclic peptides.

FIGURE 2 shows the results of an assay demonstrating cell adhesion inhibition by the representative cyclic polypeptide,



DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention are those having the property of modulating cell adhesion.

While cell adhesion is required for certain normal physiological functions, there are situations in which cell adhesion is undesirable, or in which modulated cell adhesion is desirable.

Altered leukocyte-endothelial interactions are implicated in adult respiratory distress syndrome (ARDS). Here, the attachment of inappropriate cells to the lung lining induces an inflammatory response. This results in lung injury, ARDS and in some cases, asthma. Preliminary *in vitro* results show that such detrimental attachment, in which the leukocyte adheres to endothelial cells or the lung extracellular matrix, is mediated by RGD-containing protein and integrin receptors on the leukocyte. In this situation, peptides or other compounds with a binding affinity to integrin receptors are desirable as competitive antagonists and should be useful in treating ARDS and asthma.

Cell adhesion also contributes to metastasis of cancerous tumors. Metastasis has been called "the major underlying cause of death from cancer." Welch, *et al., Intern. J. Cancer* **1989**, 43, 449. An RGD-containing peptide which would prevent cell adhesion to basement membrane components may be useful to prevent or eliminate metastasis. See, Humphries, M.J. *et al., Science* **1986**, 223, 469; Liotta, L.A., *Cancer Res.* **1986**, 46, 1; Roose, E., *Biochem. Biophys. Acta*. **1986**, 738, 263. A peptide or other compound with suitable affinity for RGD receptors should likewise have anti-metastasis utility.

Harmful blood clotting is also caused by inappropriate cell adhesion, particularly cell adhesion to the extracellular matrix. The attachment, spreading and aggregation of platelets on extracellular matrices are central events in thrombus formation. These events can be regulated by the family of platelet adhesive glycoproteins, fibrinogen, fibronectin, and von Willebrand factor. Fibrinogen functions as a cofactor for platelet aggregation, while fibronectin supports platelet attachment and spreading reactions. Von Willebrand factor is important in platelet attachment to and spreading on subendothelial matrices. Plow *et al., PNAS-USA* 1985, 82, 8057. A peptide or other compound which would function as an antagonist and bind to cell receptors which recognize the matrix glycoprotein RGD site would be beneficial as a thrombotic.

Other physiological conditions may be treated by stimulatory modulation of cell adhesion. Wound healing, for example, is undesirably prolonged when insufficient cell adhesion occurs. A peptide or other compound with suitable affinity for integrin receptors attached, for example, to a suitably positioned matrix or surface, may be able to promote beneficial cell adhesion and resultant wound healing by binding cells with the appropriate RGD-recognizing receptor. Also, in prosthetic implantation, such peptides or other compounds coating the prosthesis would provide a means for covering the prosthesis with a surface of cells. This cell surface would provide a surface compatible with the prosthesis, and thus minimize rejection that might otherwise occur due to stimulation of the immune system by the prosthesis itself.

The cell adhesion modulation compounds of the present invention are represented in part by amino acid sequence formulas wherein the individual amino acids are represented by their standard three-letter or alternatively one-letter abbreviations as follows:

Amino Acid	Three-letter symbol	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Where abbreviations such as the foregoing are used without an indication of enantiomeric structure, either the l- or d-enantiomers may suitably be utilized, although the l-enantiomer is preferred for amino acids having the normal (non-reversed) peptide bond orientation, and the d-enantiomer is preferred for amino acids having a reversed orientation (see discussion below).

Additional abbreviations used herein for analogs of amino acids and amino acid mimetic compounds include:

β-Adamantylalanine	(Ada)-Ala
β-Alanine (3-aminopropionic acid)	β-Ala
α-Aminoisobutyric acid (2-methylalanine)	Aib
d-3-(2'-Naphthyl)alanine	d-Nal
l-3-(2'-Naphthyl)alanine	l-Nal
3,4-Dehydroproline	Dhp
Gamma-aminopentane-1,5-dioic acid	3-Glu
Homoarginine	homoR
Homocysteine	homoC
Homoproline	homoP
Homoserine	homoS
4-Hydroxyproline	Hyp
3-Mercaptopropionic acid (des-α-amino cysteine)	Mpr
1-β-Mercapto-β,β-cyclopentamethylenepropionic acid	PMP
Nicotinyl lysine	Nic-Lys
Norarginine ($H_2NC(=NH)NH(CH_2)_2CH(NH_2)CO_2H$)	norArg

Norleucine	Nle
Ornithine	Orn
Penicillamine (β,β-dimethylcysteine)	Pen
Pyroglutamic acid	pyroGlu
Sarcosine	Sar
3-Thioproline or l-thiazolidine-4-carboxylic acid	3-thioPro

Additional abbreviations used herein include:

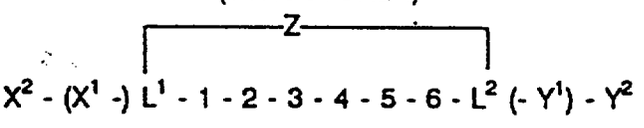
(1,1-ACC): 1-Amino-1-cyclohexanecarboxylic acid
(Ada): 1-Adamantaneacetic acid
(Ada-CA): 1-Adamantanecarboxylic acid
(AMBA): 4-(Aminomethyl)benzoic acid
(AnB): 4-Aminobutyric acid
(AnC): 6-Aminocaproic acid
ARDS: Adult respiratory distress syndrome
BOC: tert-butyloxycarbonyl
BS: Bovine serum albumin
Cbz: Benzyloxycarbonyl
(CHA): 3-(Cyclohexyl)-L-Alanine
CHAc: 3-Cyclohexylacetic acid
Chx: Cyclohexyl ester
(CPA): Cyclohexylphenylacetic acid
DCC: dicyclohexylcarbodimide
DCM: Dichloromethane
DMF: Dimethylformamide
DIEA: Diisopropylethylamine
(DTC): L-5,5-dimethylthiazoline-4-carboxylic acid
(1-FCA):1-fluorenecarboxylic acid
(9-FCA): 9-fluorenecarboxylic acid
(9-FA):9-fluoreneacetic acid
(Fm): Fluorenylmethyl ester
(FMOC): Fluorenylmethyloxycarbonyl
FN: Fibronectin

(HCA): Hydrocinnamic acid
ICAM-1: Intercellular adhesion molecule 1
IC_{50}: Inhibitory concentration, concentration at which adhesion is inhibited to 50% of control level
IPA: isopropyl alcohol
(3-Me-Ada): 3-Methyl-1-adamantaneacetic acid
(MTC): L-2-methylthiazolidine-4-carboxylic acid
(NACA): 3-Noradamantanecarboxylic acid
(Naph-Ac): 1-Naphthylacetic acid
(NB-Ac): 2-Norbornaneacetic acid
(norAda-CA): 3-Noradamantanecarboxylic acid
(PhAc): Phenylacetic acid
PyE: Pyroglutamic acid
(QC): Quinaldic acid
(TA): 3-β-Thienyl-L-Alanine
(TC): DL-thiazolidine-2-carboxylic acid
TEA: Triethylamine
TFA: Trifluoroacetic acid
(TCA): 1,4-thiazene-3-carboxylic acid
(TTC): L-tetrahydrothiazine-4-carboxylic acid
VLA: Very late activation antigens

A. Cyclic Normal Orientation Compounds

The first class of compounds of the invention is represented by the formula:

(STRUCTURE I)



and pharmaceutically acceptable salts thereof, wherein

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid analog or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety or bond between L^1 and L^2;

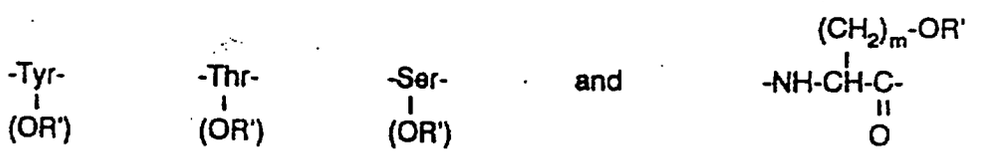
1 is optional and, where present, is selected from Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TC, TCA, DTC, MTC, TTC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, d-Nal, l-Nal, Sar and (Ada)-Ala;

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N,N'-dialkylArg, and N,N,N'-trialkylArg wherein the alkyl has one to four carbons (such as N-methylArg, N,N'-dimethylArg, and N,N-dimethylArg);

3 is selected from Gly, and Sar;

4 is selected from Asp, Glu, and the lower alkyl, aralkyl, aryl esters, OFm esters, O-cyclohexyl esters, O-benzyl esters, of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Leu,



wherein m is 2, 3 or 4;

6 is optional and, where present, is selected from Pro, 3-thioPro, 1,1-ACC, Dhp, DTC, TCC, TC, MTC, TCA, Hyp, homoPro and Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, TA, d-Nal, l-Nal and isonipecotic acid;

X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids and amino acid analogs (such as AMBA, AnC, AnB, and ω-amino-lower alkyl carboxylic acids);

X^2 is an optional N^{α}-substituent selected from R' (including hydrogen) and R'CO-;

Y^2 is an optional carboxyl-terminal substituent selected from -OR' (including hydroxyl), $-NR'_2$ (including $-NH_2$ and NHR'), $-NHNH_2$ and -SR';

and wherein each R' is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of $-NR'_2$, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

In the above structure I, a bridge is formed via the cyclizing moiety Z between L^1 and L^2 such that the compound is cyclized. It will be appreciated, both here and in structures depicted below in this disclosure, that the angular corners on connecting lines such as those shown in Structure I as linking Z with L^1 and L^2 are not intended, at the corners, to represent methylene residues. Therefore, each of L^1 and L^2 is chosen so as to provide a functional group suitable for the formation of a cyclizing bridge. As is discussed in more detail hereinafter, preferred functional groups include thiol, amino and carboxyl groups and their residues. Such a functional group may be provided by the side-chain, or by the terminal α-

amino (in L^1) or terminal carboxyl (in L^2) group, of a natural amino acid residue or an analog thereof (including a homolog or stereoisomer thereof; see e.g. Table 1 above); or it may be provided by an "amino acid mimetic" moiety, i.e., an organic residue which includes a suitable cyclizing functional group and which is covalently linked through a peptide (i.e., amide) bond to the amino terminus (in the case of L^1) and/or to the carboxyl terminus (in the case of L^2) of the residue sequence (NH_2-)1-2-3-4-5-6(-COOH).

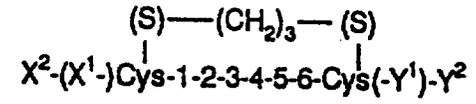
The bridging residues L^1 and L^2 are, in one embodiment, each preferably selected from Cys, Pen, homoC, and, for L^1, additionally Mpr and PMP. Each of these residues provides a side chain sulfhydryl group particularly suitable as a reactive precursor functional group for the formation of a cyclizing bridge between L^1 and L^2. Specifically, in the case of these residues, the cyclic bridge may be formed via oxidative coupling (loss of hydrogens) to form a disulfide bond between the side chain sulfur groups (in which the cyclizing moiety Z is a simple bond between the two sulfur atoms). This may also be depicted generally for compounds wherein, for example, both L^1 and L^2 are Cys residues as follows:

$$\begin{array}{c} \overset{(S)}{|}\!\!\!\text{Cys} - 1 - 2 - 3 - 4 - 5 - 6 - \text{Cys}\!\!\!\overset{|}{(S)} \\ X^2 - (X^1\text{-}) \qquad\qquad\qquad\qquad (\text{-}Y^1)\text{-}Y^2 \end{array}$$

wherein (as in other similar depictions used herein) the side chain functional group portion (here a sulfur atom in both instances) appears in parentheses above the residue having the side chain.

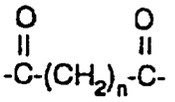
It is particularly preferred in such embodiments that L^1 and L^2 be Cys or Mpr. The combination of L^1 = Pen and L^2 = Cys is, in embodiments containing the sequence Arg-Gly-Asp, not preferred in the practice of the present invention.

The cyclizing bridge may also be formed via a hydrocarbon moiety, for example a (poly)methylene bridge moiety of the form $-(CH_2)_n-$ where n is an integer of from 1 to 8, preferably 1 to about 4. One type of such bridge is represented below, wherein a cyclic compound with three methylene residues (representing Z) between two cysteine side-chain sulfur atoms (representing L^1 and L^2) is depicted:

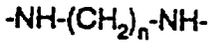


See L. Fieser and M. Fieser, *Reagents for Organic Synthesis*; J. Wiley and Sons: 1967; Vol. 1, pp. 356-357; Fieser, *J. Amer. Chem. Soc.*, 1959, 96, 1945.

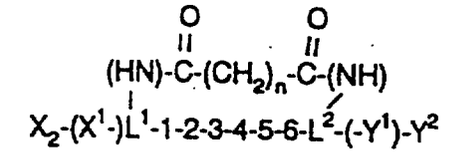
In other preferred embodiments of the invention, diketo and diamino linking groups Z such as those of the form



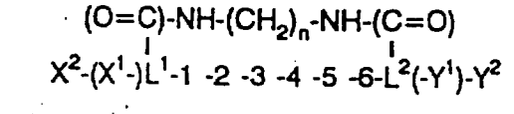
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wherein n is as defined above, may also conveniently be used to link, for example, side chain amino (as in Lys) or carbonyl (as in Glu or Asp) residues, respectively, on L^1 and L^2 to yield structures exemplified by

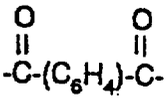


and

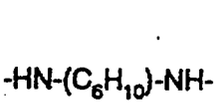


(Here, as elsewhere, the side chain functional groups (amino and carbonyl) on L^1 and L^2 are depicted in parentheses above the residue abbreviation.)

The foregoing are but examples of suitable hydrocarbon-containing bridges, and other forms will also be apparent to those skilled in the art. Where the cyclizing moiety Z includes a portion with such a hydrocarbon form, it may be branched and may, where of a size appropriate to form a stable structure (particularly, where Z comprises two or more methylene moieties) also include one or more heteroatom-containing substituents including hydroxyl, amino, nitro, alkoxyl and halo substituents. Such substituents may be used to affect the solubility and/or biodistribution characteristics of the subject compounds. Aromatic or cycloalkyl hydrocarbon-containing bridge groups may also be utilized in the Z position, as for example diketo or diamino structures such as

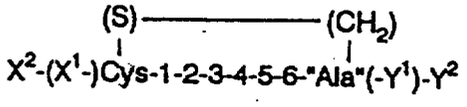


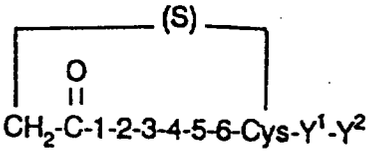
or



Simple hydrocarbon moieties of from 1 to about 4 carbons are preferred for hydrocarbon portions of such Z-moieties.

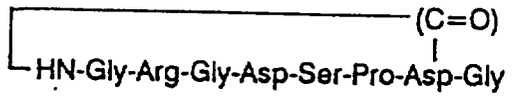
The cyclizing bridge between L^1 and L^2 may also be formed via a monosulfide (thioether) linkage, as exemplified below.



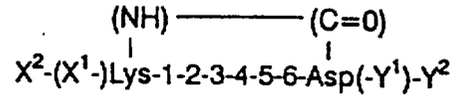


In such a case, L^1 can be formed from a residue of α,β-dehydroalanine, and L^2 from a residue of cysteine, which may be reacted to yield a lanthionine-like thioether linkage. In this regard, see Palmer, *et al.*, In *Peptides–Chemistry, Structure Biology*, Rivier & Marshall, Ed., Escom. Leider, 1990; pp. 616-618; and Jung, *op. cit.*, pp. 865-869.

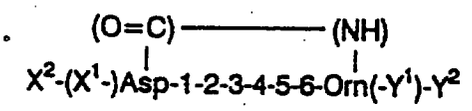
Alternatively, L^1 and L^2 may be chosen from other amino acids or analogs or amino acid mimetics which provide functional groups suitable for the formation of a side chain or as the amino- or carboxyl-terminus of an amino acid or analog residue. For example, L^2 may be selected from Asp, Glu, or other amino acids or analogs which provide a suitable side chain carboxyl group for cyclic linkage, through formation of an amide bond in a condensation reaction, with an amino group (e.g., an N^{α}-amino group, or a side chain amino group as on, for example, Lys or Orn) on L^1, provided, however, that the structure



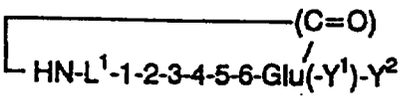
is not included. The cyclizing moiety Z will in such cases be a simple bond between L^1 and L^2. Likewise, an amino acid residue L^2 may provide a carboxyl group from its carboxyl terminus for amide linkage with either a side chain amino or α-amino group on an amino acid residue or analog L^1; or the direction of the amide linkage may be reversed where L^1 provides a side chain carboxyl group and L^2 provides a side chain amino group. Such structures may be exemplified as follows:

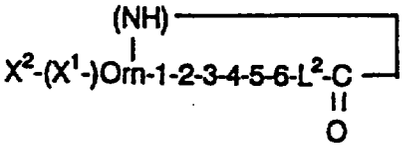


[wherein the side chain amino and carbonyl groups of L^1 (Lys) and L^2 (Asp) are directly bonded];

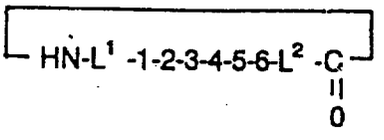


[wherein amide bond direction (from side chains of L^1 and L^2) is reversed];





[wherein the depicted amino terminus of L^1 is directly bonded to the side chain carboxyl group of Glu (L^2), or the depicted carboxyl terminus of L^2 is directly bonded to the side chain amino group of Orn (L^1); here, as elsewhere, the corners of the bent connecting line depicting these bonds do not represent methylene groups];

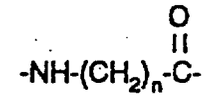


[wherein the depicted α-amino terminus at L^1 is directly bonded to the depicted carboxyl terminus at L^2, such that an amide bond is formed in the peptide "backbone" of the compound.]

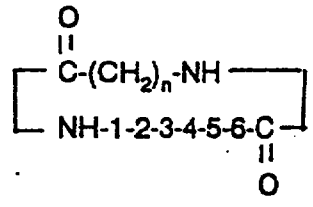
Analogs of amino acid residues may also be utilized for L^1 and/or L^2, as for example homologs (wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other reactive precursor functional group for cyclization), d-enantiomers of amino acids, analogs having variant side chains with appropriate functional groups (as for example β-cyanoalanine, canavanine, djenkolic acid, l-azaserine or gamma-methyleneglutamic acid), or other amino acid

analogs (see for example the table of amino acid analogs and mimetic compounds given above).

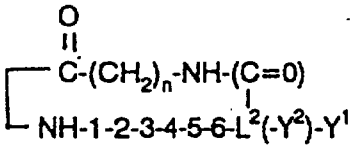
Amino acid mimetic structures that are capable of being covalently bonded through an amide bond to a carboxyl and/or amino terminus of the residue sequence 1-2-3-4-5-6, and which provide a suitable precursor functional group for cyclization (through Z), may also be employed in positions L^1 and/or L^2. Such amino acid mimetic structures include organic species containing one or more heteroatoms including at least one functional group (preferably a reactive heteroatom-containing functional group) precursor suitable for cyclization. Examples include residues of the form



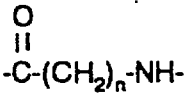
wherein n ranges from 1 to about 8, and preferably from 1 to 4, as for example residues of β-alanine and gamma-aminobutyric acid. (Where n is 1, the amino acid glycine, rather than an α-amino acid mimetic, results.) Such a structure may, similar to the amino acids and amino acid analogs discussed above, be utilized as L^1 (wherein the carbonyl group depicted above, formed for example from a carboxyl precursor, conveniently forms an amide (peptide-mimetic) linkage with the amino terminus of residue 2 or, if present, residue 1, or it may be utilized as L^2 (wherein the depicted amino group may engage in an amide linkage with the carboxyl terminus of the terminal residue 4, 5 or 6). If only one such linking residue L is used, it may serve as both L^1 and L^2 (and thereby include Z) in that cyclization can be achieved through formation of two amide bonds, one at each terminus of the sequence 1-2-3-4-5-6. Such structures may be exemplified by the form



where the N^{α}-terminus and the carboxyl terminus of the sequence 1-2-3-4-5-6 are bonded directly to, respectively, the carbonyl residue and the amino residue of the amino acid mimetic linking group depicted immediately above to form two peptide-mimetic amide bonds. Likewise, cyclization can be achieved with such an amino acid mimetic linking moiety wherein a side chain functional group on a second linking moiety appended (as L^1 or L^2) to one terminus of the numbered sequence 1-2-3-4-5-6 (as for example an amino or carboxyl side chain group) engages in bonding to the mimetic moiety, and the mimetic moiety (as L^2 or L^1) cyclizes the compound to the remaining terminal residue of the numbered sequence. This may be exemplified by structures of the form

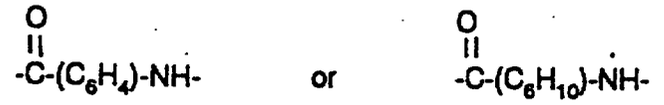


wherein L^2 (as for example Asp) provides the side chain carbonyl group depicted in parenthesis, residue 1 provides the depicted N^{α}-terminal amino group, and the amino acid mimetic linking moiety



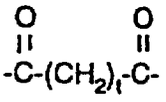
serves as L^1.

Amino acid mimetic structures containing aromatic, cycloalkyl or other linking portions can also be utilized as L^1 and/or L^2, such as structures of the form

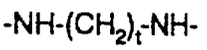


Similarly, the heterobifunctional (keto-amino) structures depicted above may also serve as a Z-group in linking complementary side chain functional groups on L^1 and L^2 (e.g., a side chain amino group on L^1 and a side chain carboxyl group on L^2) through two amide bond structures.

As will be discussed in more detail below, homobifunctional structures such as those of the form



and



where t is an integer of from 1 to about 8, and more preferably 1 to 3, may also serve individually as amino acid mimetic structures where a "reverse" amino acid sequence occurs within the structure 1-2-3-4-5-6 (see Structures II and III below). In such cases, the numbered portion of such a structure will display two carboxyl termini or two amino termini (rather than one of each type), and L^1 and L^2 may be selected together to be a homobifunctional linking moiety such as one of those depicted above.

Other forms of cyclization include those wherein Z comprises a secondary amino structure (obtained, for example, upon reduction of the carbonyl portion of an amide linkage to a methylene group). For example, a secondary amino linking structure may be formed by reaction of an aldehyde functional group on L^1 (e.g., an aldehyde formed by reduction of a side chain carboxyl group on Asp or Glu) or on L^2 (e.g., an aldehyde formed from the terminal carboxyl group of L^2) with a

suitable amino group (on, e.g., the side chain or N^{α}- terminus of L^2 or L^1). Methods for the preparation and incorporation of such aldehyde-bearing residues in peptide structures are described by Fehrentz, J-A and Castro, B., *Synthesis* **1983**, 676; and by Sasaki, Y. and Coy, D.H., *Peptides* **1987**, 8, 119. Thus, where the carboxyl terminus of L^2 is of the reduced aldehyde form and is bonded to the amino terminus of L^1 in a reductive alkylation reaction, a peptide bond isostere structure ($-NHCH_2-$) will result in the cyclic backbone of the product.

Other means of cyclization through appropriate choices of L^1, L^2 and Z will be recognized by those skilled in the art and are included in the scope of the present invention.

It is also specifically contemplated that the foregoing discussion of cyclizing moieties (Z), bridging residues (L^1 and L^2), substituents, amino acid analogs, amino acid mimetics, cyclization methods, and the like are applicable, *mutatis mutandis*, to the other structural formulas discussed hereinafter.

Residue 1 in structure I is most preferably Leu; residue 2 is most preferably Arg; residue 3 is most preferably Gly; residue 4 is most preferably Asp; residue 5 is most preferably Ser; and residue 6 is preferably Pro or 3-thioPro. The sequence Leu-Arg-Gly-Asp-Ser-Pro is most preferred for residues 1-2-3-4-5-6.

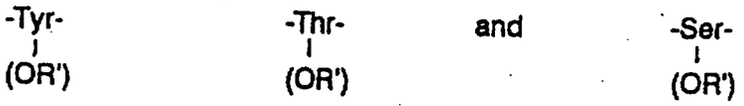
Also particularly preferred for residue 4 is Glu where residue 2 is norArg. Thus, the sequence Leu-norArg-Gly-Glu-Ser-Pro is also preferred for residues 1-2-3-4-5-6.

X^1 and Y^1 are each optional in structure I. Where present, they are preferably each independently selected so as to enhance the activity of the resultant compound

and/or to preserve the compound against metabolism in, for example, the *in vivo* environment and thereby increase the effective half-life of the compound. In this regard, the use of one or more d-amino acids, most preferably at one or more terminal residue position in the compound (i.e., at the amino-most and/or carboxyl-most residue position in X^1 or Y^1) are believed to stabilize the compound against metabolism by proteolytic or other enzymes in the body. Specific preferred residues for position X^1 include Gly-, Phe-, Leu-, Asn-, Val-, Try-, 1- or 2-naphthylalanine, cyclohexylAla-, AMBA, AnC, AnB and ω-amino-lower alkyl carboxylic acids, Aib-, Ser-Tyr-Asn-, Ala-Thr-Val-, and p-chloro-Phe-. Preferred residues for position Y^1 include -Ala, -Ala-Ser, -Ala-Ser-Ser, -Ala-Ser-Ser-Lys, -Ala-Ser-Ser-Lys-Pro, -Thr, -Thr-Phe, -Aib, -p-chloro-Phe, AMBA, AnC, AnB, ω-amino-lower alkyl carboxylic acids, 1- or 2-naphythlalanine, and -(cyclohexylAla). Such X^1 and Y^1 groups are preferred also in the corresponding positions given in the structural formulas described hereinafter.

Where a substituent X^2 or Y^2 incorporating R' other than hydrogen is used, e.g., acyl groups R'CO or amino groups of the form R'NH, preferred substituents include those derived from bulky compounds such as adamantaneacetic acid, adamantanecarboxylic acid, 1- or 2-naphthylacetic acid, 2-norbornaneacetic acid, 3-noradamantanecarboxylic acid, 3-methyladamataneacetic acid, and 1- or 2-adamantylamine. Other suitable R' groups are those derived from acids such as 9-fluoreneacetic acid, 1-fluorenecarboxylic acid, 9-fluorenecarboxylic acid, phenylacetic, hydroxycinnamic acid, quinaldic acid, cyclohexylacetic acid, and 3-mercaptopropionic acid.

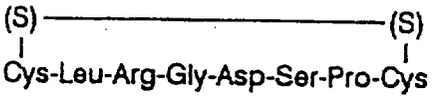
Structures exemplified by the forms



such as those set forth with respect to residue 5 in Structure I, represent derivatives of amino acid residues wherein the side chain hydroxyl group (shown in parentheses) is optionally substituted with a group of the form R' which can be other than hydrogen as defined above.

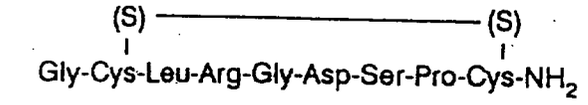
Where such substituted residues are employed in position 5 of structure I, R' is preferably selected from hydrogen and C_1 through C_8 lower alkyls, particularly methyl and ethyl alkyl moieties.

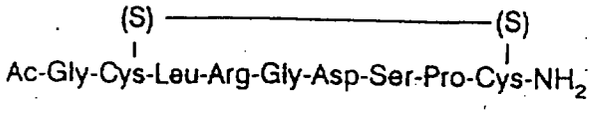
A particularly preferred compound within the scope of structure I includes:

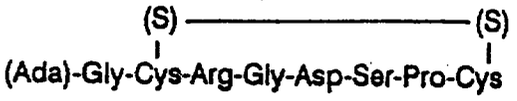


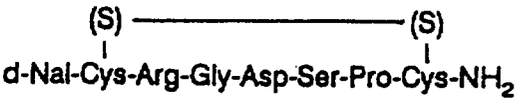
wherein the shorthand structure -Cys-, consistent with similar usage elsewhere in this description, represents a cysteine residue with its side chain sulfur atom separately depicted, and likewise the structure (S)–(S) represents a disulfide bond. The compound depicted has been shown to be active in inhibiting cell adhesion to fibronectin.

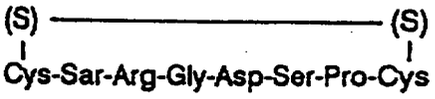
Other preferred compounds within the scope of structure 1 include:

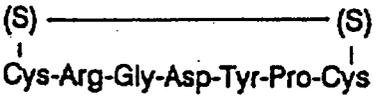


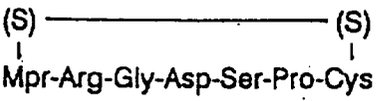


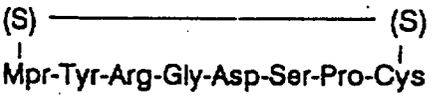

(S)
(S)
(Ada)-Gly-Cys-Arg-Gly-Asp-Ser-Pro-Cys

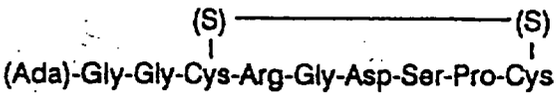

(S)
(S)
d-Nal-Cys-Arg-Gly-Asp-Ser-Pro-Cys-NH2

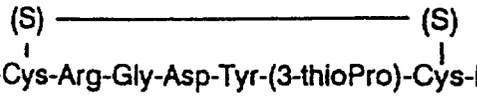

(S)
(S)
Cys-Sar-Arg-Gly-Asp-Ser-Pro-Cys

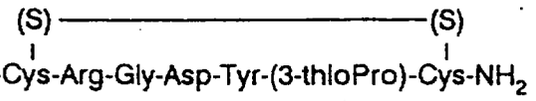

(S)
(S)
Cys-Arg-Gly-Asp-Tyr-Pro-Cys

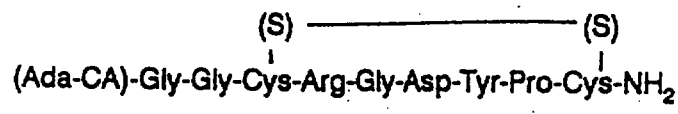

(S)
(S)
Mpr-Arg-Gly-Asp-Ser-Pro-Cys

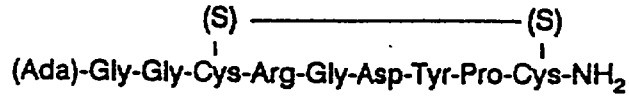

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(S)
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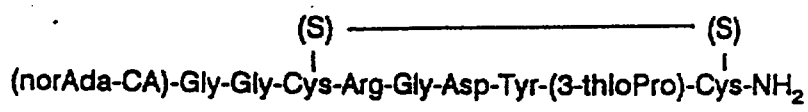

(S)
(S)
(Ada)-Gly-Gly-Cys-Arg-Gly-Asp-Ser-Pro-Cys

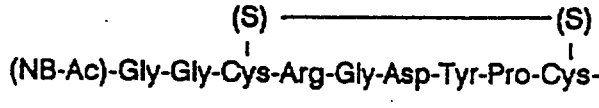

(S)
(S)
(Ada)-Gly-Gly-Cys-Arg-Gly-Asp-Tyr-(3-thioPro)-Cys-NH2

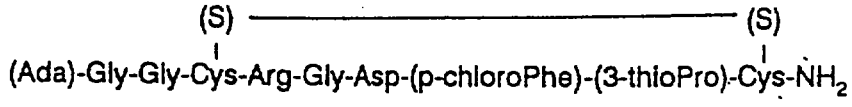

(S)
(S)
(Ada-CA)-Gly-Gly-Cys-Arg-Gly-Asp-Tyr-(3-thioPro)-Cys-NH2

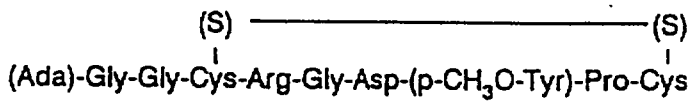


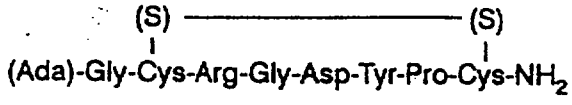








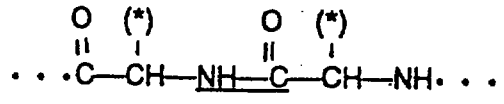




wherein similar shorthand notation, as elsewhere in this disclosure, has been utilized.

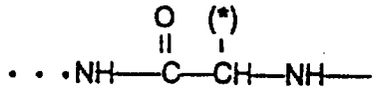
B. Cyclic Reversed Orientation Compounds

A "reversed" or "retro" peptide sequence as disclosed herein refers to that part of an overall sequence of covalently-bonded amino acid residues (or analogs or mimetics thereof) wherein the normal carboxyl-to amino direction of peptide bond formation in the amino acid backbone has been reversed such that, reading in the conventional left-to-right direction, the amino portion of the peptide bond (as underlined below) precedes (rather than follows) the carbonyl portion:

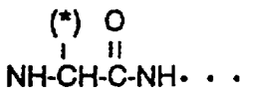


wherein "*" represents a side chain group. See, generally, Goodman, M. and M. Chorev, *Accounts of Chem. Res.* **1979**, 12, 423. Compounds containing such sequences are referred to herein as "reversed" peptides.

The reversed orientation peptides described herein include (a) those wherein one or more amino-terminal residues are converted to a reversed ("rev") orientation (thus yielding a second "carboxyl terminus" at the left-most portion of the molecule), and (b) those wherein one or more carboxyl-terminal residues are converted to a reversed ("rev") orientation (yielding a second "amino terminus" at the right-most portion of the molecule). It will be seen that a normal peptide (amide) bond cannot be formed at the interface between a normal orientation residue and a reverse orientation residue:

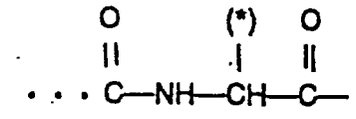


(reverse orientation portion [structure a(1)])

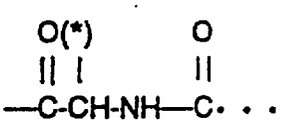


(normal orientation portion [structure a(2)]

Case (a): N-terminal residue(s) converted to reverse orientation.



(normal orientation portion [structure b(1)]



(reverse orientation portion [structure b(2)]

Case (b): C-terminal residue(s) converted to reversed orientation.

Therefore, certain reversed peptide compounds of the invention can be formed by utilizing an appropriate amino acid mimetic moiety to link the two adjacent portions of the sequences depicted above utilizing a reversed peptide (reversed amide) bond. In case (a) above, a central residue of a diketo compound, as shown by moiety 3 in Structure II below, may conveniently be utilized to link structures a(1) and a(2) with two amide bonds to achieve a peptidomimetic structure. In case (b) above, a central residue of a diamino compound, as shown by moiety 3 in Structure III below, will likewise be useful to link structures b(1) and b(2) with two amide bonds to form a peptidomimetic structure. With reference also to Structure I above, it will be seen that such central residues are most preferably utilized at residue position 3.

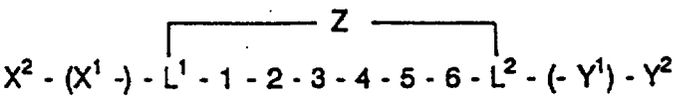
The reversed direction of bonding in such compounds will generally, in addition, require inversion of the enantiomeric configuration of the reversed amino acid residues in order to maintain a spatial orientation of side chains that is similar to

that of the non-reversed peptide. The configuration of amino acids in the reversed portion of the peptides is preferably d, and the configuration of the non-reversed portion is preferably l. Opposite or mixed configurations are acceptable when appropriate to optimize a binding activity.

1. <u>Diketo Cyclic Reversed Peptides</u>

Thus, a second class of compounds of the invention is represented by the formula:

(STRUCTURE II)



and pharmaceutically acceptable salts thereof, wherein:

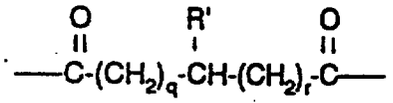
L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid derivative or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety or bond between L^1 and L^2;

1 is optional, and where present, is of reversed orientation and is selected from Sar, Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TC, TCA, DTC, MTC, TTC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, l-Nal, d-Nal and (Ada)-Ala;

2 is of reversed orientation and is selected from Arg, nitroArg, homoArg, p-aminomethyl-Phe, norArg N-alkylArg, N,N-dialkylArg, N,N'-dialkylArg, N,N,N'-trialkylArg wherein the alkyl has one to four carbons (such as N-methyl, N,N'-dimethyl, and N,N-dimethyl);

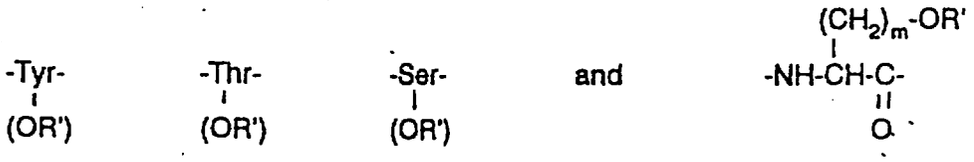
3 is a moiety suitable for linking reversed-orientation residue 2 with residue 4, and is preferably of the form



wherein q and r are independently integers of 0, 1 or 2;

4 is selected from Asp, Glu, the lower alkyl, aralkyl, aryl esters, OFm esters, O-cyclohexyl esters, O-benzyl esters, of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Leu



wherein m is 2, 3 or 4;

6 is optional and, where present, is selected from Pro, 3-thioPro, 1,1-ACC, Dhp, DTC, TCC, TC, MTC, TCA, Hyp, homoPro and Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, TA, L-Nal, d-Nal, isonipecotic acid;

X^1 and Y^1 are each optional and, where present, are independently selected from sequences of from 1 to 4 d- or l-amino acids and amino acid analogs such as AMBA, AnC, AnB, and ω-amino-lower alkyl carboxylic acids;

X_2 is an optional substituent selected from R'_2N- (including R'HN- and H_2N-), R'O- (including hydroxyl), H_2NNH- and R'S-;

Y^2 is an optional carboxyl-terminal substituent selected from -OR' (including hydroxyl), -NR'_2 (including -NH_2 and -NHR'), -$NHNH_2$ and -SR';

and wherein each R; is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of -NR'_2, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

In the above Structure II, cyclization may generally be achieved in the manners described above for Structure I. However, it is to be noted that the orientation of peptide bonding in the compounds of Structure II is, in residues to the left of residue number 3, reversed in direction such that a carboxyl "terminus" group is exposed in residue 2 or, if present, residue 1, rather than an amino group. Therefore, linking groups in position L^1 may utilize for bonding to this carboxyl terminus an amino moiety, as for example a diamino moiety such as those in preferred structures of the form

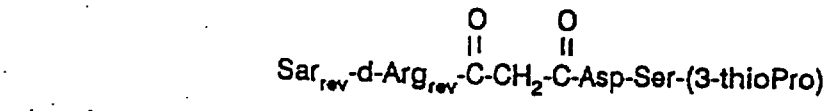
$$-NH-(CH_2)_t-NH-$$

wherein t is 1, 2 or 3, which can simultaneously provide as (L^2) a second amino moiety for cyclizing bonding to the normal-orientation carboxyl terminus of residue number 4 or, if present, 5 or 6.

Other forms of linking groups, including Z groups, and various combination of bonding between terminal functional groups and side chain function groups on L^1 and L^2, will be recognized by those skilled in the art in view of the present

disclosure. In particular, the discussion above with respect to use of amino acid analogs and amino acid mimetic structures is applicable also here.

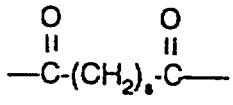
Residues L^1, 1, 2 and Y^1 are preferably d-amino acids. Residue 1 in Structure II is most preferably absent or Sar, and is secondarily preferred to be d-forms of Leu, Tyr or Gly; residue 2 is more preferably Arg (particularly d-Arg); in residue 3, q and r are most preferably zero and R' is most preferably hydrogen, with lower alkyl also being preferred; residue 4 is most preferably Asp; residue 5 is most preferably Ser; and residue 6 is most preferably 3-thioPro. The sequence



wherein the subscript "rev" indicates a reverse-form residue, is especially preferred for residues 1-2-3-4-5-6.

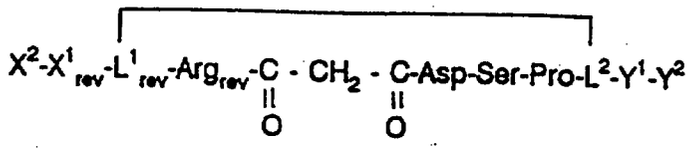
As with Structure I, X^1 and Y^1 are both optional, and are selected from the same constituents as those of X^1 and Y^1 of Structure I (with appropriate utilization of reversed orientation residues in X^1). Optional substituent X^2 is of a form suitable for bonding to, typically, a carboxyl terminus on X^1 or L^1.

For group 3 in Structure II, an especially preferred residue is



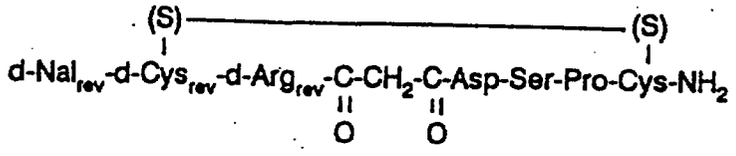
wherein s is an integer of from 1 to about 5, preferably 1 to 3, and most preferably 1.

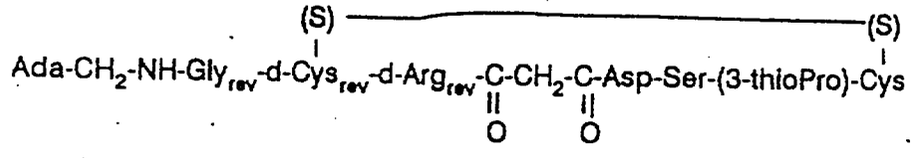
A particularly preferred compound class of Structure II is:



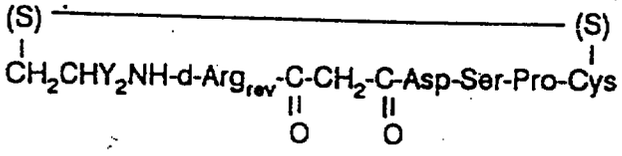
wherein R' in X^1 and Y^1 is preferably hydrogen or lower alkyl.

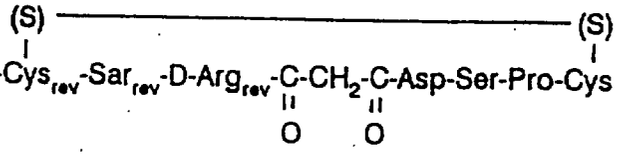
Specifically preferred compounds include:

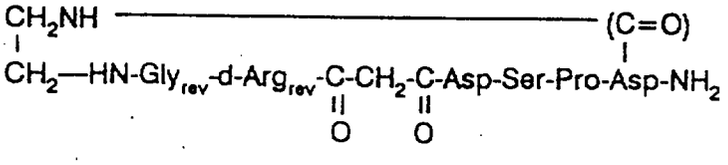




wherein "Ada" represents adamantyl notwithstanding the abbreviation used throughout the specification;





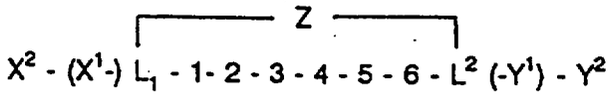


As with Structure I, derivatives of the peptides of Structure II may be useful in the generation of antigens which, in turn, may be useful to generate antibodies. These antibodies will in some cases themselves be effective in inhibiting cell adhesion or modulating immune activity by acting as receptors for matrix proteins or other ligands, or, if anti-idiotypic, by acting to block cellular receptors.

2. <u>Diamino Cyclic Reversed Compounds</u>

The compounds of class III of the invention are represented by the formula:

(STRUCTURE III)



and pharmaceutically acceptable salts thereof, wherein:

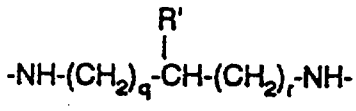
L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid derivative or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety between L^1 and L^2;

1 is optional and, where present, is selected from Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TC, TCA, DTC, MTC, TTC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, l-Nal, d-Nal, (Ada)-Ala and Sar;

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N,N'-dialkylArg, and N,N,N'-trialkylArg, wherein the alkyl has one to four carbon atoms (such as N-methyl, N,N'dimethyl, and N,N-dimethyl);

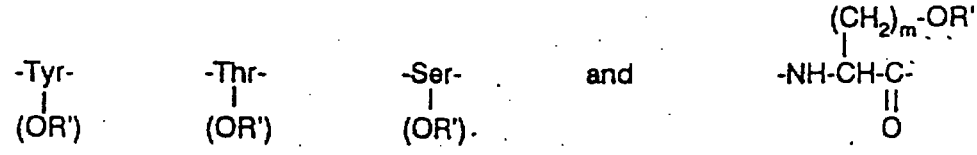
3 is a moiety suitable for linking residue 2 with reversed-orientation residue 4, and is preferably of the form



wherein q and r are independently integers of 0, 1 or 2;

4 is of reversed orientation and is selected from Asp, Glu, and the lower alkyl, aralkyl, aryl esters, OFm esters, O-cyclohexyl esters, O-benzyl esters, of the foregoing two amino acids;

5 is optional and, where present, is of reversed orientation and is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe,



wherein m is 2, 3 or 4;

6 is optional and, where present, is of reversed orientation and is selected from Pro, 3-thioPro, 1,1-ACC, Dhp, Hyp, DTC, TCC, TC, TCA, MTC, homoPro, Phe, o-, m- and p-halo-Phe, p-nitro-Phe, TA, l-Nal, d-Nal, and isonipecotic acid;

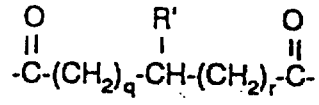
X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids and amino acid analogs (such as AMBA, AnC, AnB, and ω-amino-lower alkyl carboxylic acids);

X^2 is an optional N^{α}-substituent selected from R' (including hydrogen) and R'CO-;

Y^2 is an optional substituent selected from -R' (including hydrogen) and -COR';

and wherein each R' is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of -NR'_2, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

In the above Structure III, cyclization may generally be achieved in the manners described above with respect to Structures I and II. Further, it will be seen that the right-most "terminus" exposed by residue number 4 (or, if present, 5 or 6) is an amino moiety rather than the carboxyl moiety of a non-reverse-orientation sequence, and that a linking group L^1 bearing a carboxyl group and suitable for forming an amide bond with this right-most terminus will be appropriate. Such structures include those of the form



where q and r are as defined above; such a structure can, in view of its second carboxyl group, also serve as L^1 by bonding to the amino terminus of residue number 2 or, if present, residue 1. Other forms of linking groups L^1 and L^2, and Z groups, will be recognized in view, for example, of the discussion above with respect to Structures I and II. L^2 may in such a case be selected from Mpr and PMP.

For group 3 in Structure III, an especially preferred residue is -HN-$(CH_2)_s$-NH-, wherein s is an integer of from 1 to about 5, preferably 1 to 3, and most preferably 1.

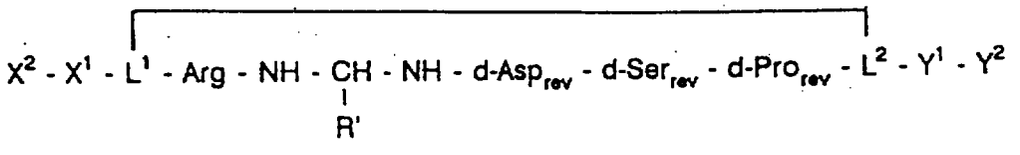
Residues 4, 5, 6, L^2 and Y_1 in Structure III are preferably d-amino acids. Residue 1 in Structure III is most preferably absent or Sar and is secondarily preferred to be Leu, Tyr or Gly; residue 2 is most preferably Arg; in residue 3, q and r are most preferably zero and R' is most preferably hydrogen, with lower alkyl also being preferred; residue 4 is most preferably d-Asp, residue 5 is most preferably d-Ser, residue 6 is most preferably d-Pro. The sequence

Sar-Arg-NH-$(CH_2)_t$-NH-d-Asp-d-Ser-d-Pro,

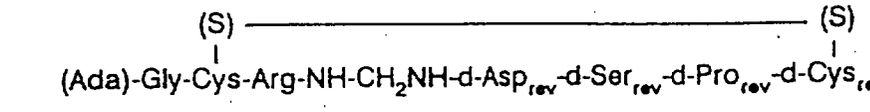
where t is 1, 2 or 3, is most preferred for residues 1-2-3-4-5-6.

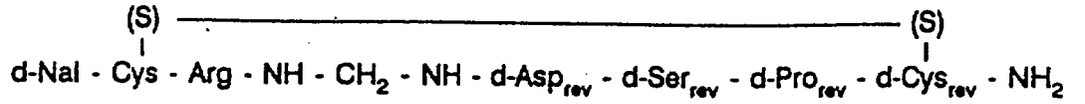
As with Structures I and II, X^1 and Y^1 are both optional, and are selected from the same constituents as those of X^1 and Y^1 of Structures I and II (with appropriate consideration of reversed orientation residues in Y^1). Optional substituent Y^2 is of a form suitable for bonding to, typically, an amino terminus of Y^1 or L^2.

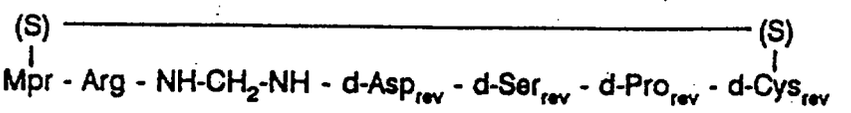
A particularly preferred compound of Structure III is:

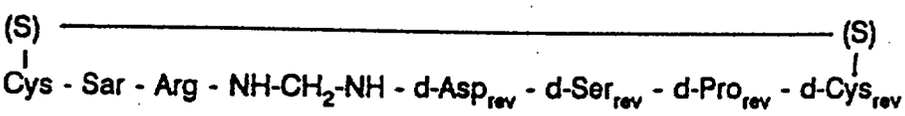


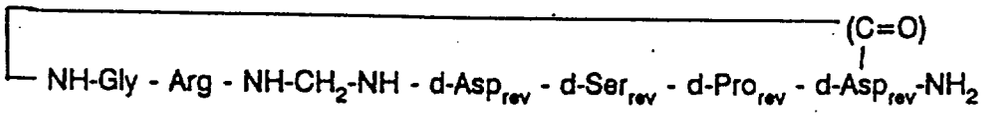
More specifically, preferred compounds are:











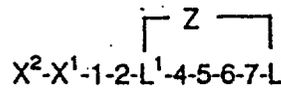
As with Structures I and II derivatives of the peptides of Structure III may be useful in the generation of antigens which, in turn, may be useful to generate antibodies. These antibodies will, in some cases, themselves be effective in inhibiting cell adhesion or modulating immune activity by acting as receptors for matrix proteins or other ligands or, if anti-idiotypic, by acting to block cellular receptors.

C. RCD-Containing Cyclic Compounds

Compounds of this class containing, for example, the sequence RCD were synthesized and found to be effective in modulating cell adhesion activity. This demonstrates that the residue glycine is not necessary to adhesion modulation activity and that residues such as cysteine may replace glycine in the peptide while maintaining activity. Moreover, it is an aspect of the invention that cysteine or other cyclizing residues are able in this regard to facilitate cyclization via disulfide or other side chain bridging.

The compounds of Class IV of the invention are represented by the formula:

(STRUCTURE IV)



and pharmaceutically acceptable salts thereof, wherein

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid analog or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

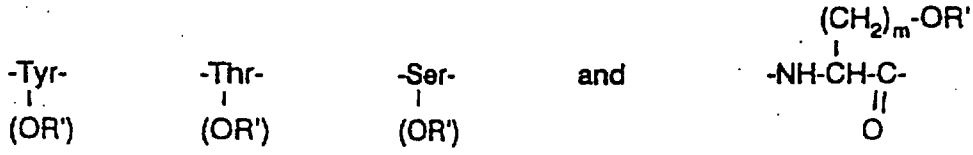
X is a cyclizing moiety or bond between L^1 and L^2;

1 is optional and, where present is selected from Leu, Sar, d-Nal, l-Nal, Tyr, Phe, Ile, Pro, 3-thioPro, TTC, TCA, DTC, MTC, TC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, and (Ada)-Ala;

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N,N'-dialkylArg, and N,N,N'-trialkylArg wherein the alkyl has one to four carbon atoms (such as N-methyl, N,N'-dimethyl, and N,N-dimethyl);

4 is selected from Asp, Glu, the lower alkyl, aralkyl, aryl esters, OFm esters, O-cyclohexyl esters, O-benzyl esters, of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Asn, Asp, Met



wherein m is 2, 3 or 4;

6 is optional and, where present, is selected from Pro, 3-thioPro, TA, DTC, TTC, TC, MTC, TCA, 1,1-ACC, Dhp, Hyp, homoPro, Phe, and Thr, Tyr, Val, d-Nal, l-Nal, CHA, Ser, Asn, Glu, o, m, and p-halo-Phe, and isonipecotic acid;

7 is optional and, where present, is Pro, 3-thioPro, TA, DTC, TTC, TC, MTC, TCA, Ala, Gly, Ser, Phe, and Leu.

X_1 and Y_1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids or amino acid analogs (such as AMBA, AnC, AnB, and ω-amino-lower alkyl carboxylic acids).

X^2 is an optional N^{α}-substituent selected from R'- (including hydrogen) and R'CO-; and

Y^2 is an optional carboxyl-terminal substituent selected from -OR' (including hydroxyl), -NR'_2 (including -NH_2 and -NHR'), -$NHNH_2$ and -SR';

and wherein each R' is individually a pharmaceutically suitable substituent group, preferably one selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of -NR'_2, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

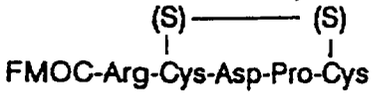
It is particularly preferred that residue number 1 be absent or Leu; that residue 2 be Arg; that residue L^1 be Cys; that residue 4 be Asp; that residue 5 be absent or Ser; that residue 6 be absent, Pro, 3-thioPro; that residue L^2 be Cys. Thus, the structures

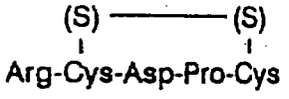
Leu-Arg-Cys-Asp-Ser-Pro-Cys,

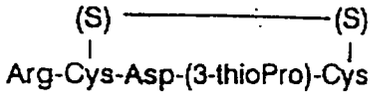
Arg-Cys-Asp-(p-chloro-Phe)-(3-thioPro)-Cys, and

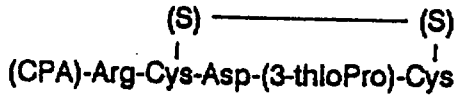
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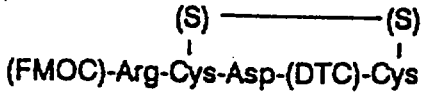
are particularly preferred for residues 1-2-L^1-4-5-6-7-L^2. The compounds

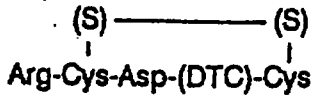


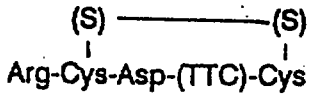


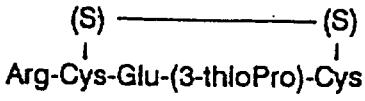


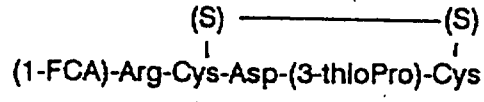


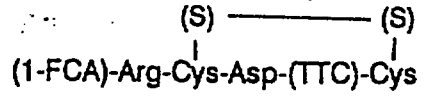












are particularly preferred.

As discussed above in conjunction with Structure I, linking residues L^1 and L^2 other than Cys, and Z groups other than simple bonds, may also be usefully employed in the context of Structure IV. It will be seen in this regard that the presence of residue numbers 2 and 4 on either side of L^1 will typically require cyclization

(through Z) to L^2 through a side chain or other functional group on L^1 that is not engaged in bonding to residues 2 or 4. Residue L^2 may more generally be engaged in cyclization through either a terminal (typically, carboxyl) functional group or a side chain functional group.

Preferred residues for position X^1 include Gly-, Phe-, Leu-, Asn-, Val-, Try, 1- or 2-naphthylalanine, cyclohexylAla-, AMBA, AnC, AnB, ω-amino lower alkylcarboxylic acids, Aib-, Ser-Tyr-Asn-, Ala-Thr-Val-, and p-chloro-Phe-. Preferred residues for position Y^1 include -Ala, -Ala-Ser, -Ala-Ser-Ser, -Ala-Ser-Ser-Lys, -Ala-Ser-Ser-Lys-Pro, -Thr, -Thr-Phe, -Aib, -p-chloro-Phe, AMBA, AnC, AnB, ω-amino-lower alkyl carboxylic acids, 1- or 2-naphthylalanine, and -(cyclohexylAla).

Where a substituent X^2 or Y^2 incorporating R' other than hydrogen is used, e.g., acyl groups R'CO or amino groups of the form R'NH, preferred substituents include those derived from bulky compounds such as adamantaneacetic acid, adamantanecarboxylic acid, 1- or 2-naphthylacetic acid, 2-norbornaneacetic acid, 3-noradamantanecarboxylic acid, 3-methyladamataneacetic acid, and 1- or 2-adamantylamine. Other suitable R groups are those derived from acids such as from 9-fluoreneacetic acid, 2-fluorenecarboxylic acid, 9-fluorenecarboxylic acid, phenylacetic, hydroxycinnamic acid, quinaldic acid, cyclohexyl acetic acid, and 3-mercaptopropionic acid.

As with Structures I, II and III, derivatives of the peptides of Structure IV may be useful in the generation of antigens which, in turn, may be useful to generate antibodies. These antibodies will, in some cases, themselves be effective in inhibiting cell adhesion or modulating immune activity by acting as receptors for

matrix proteins or other ligands or, if anti-idiotypic, by acting to block cellular receptors.

D. Examples

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. As set forth above, all publications to which reference is made are incorporated herein by reference.

1. Synthesis and Formulation of Compounds

The "backbones," i.e., the peptide-bond linked portions of the cyclic compounds of the invention were generally synthesized using solid phase peptide synthesis, and then cyclized using a procedure which, where necessary, selectively removed protective groups from only the residues involved in cyclizing. In this way, the peptide sequence in the compound was not changed or lengthened, but the peptide was properly cyclized. Other methods for synthesis and cyclization are known in the art and may be employed in the preparation of the cyclic compounds and formulations disclosed herein.

Thus, peptide sequences in the compounds of this invention may be synthesized by the solid phase peptide synthesis (for example, BOC or FMOC) method, by solution phase synthesis, or by other techniques known in the art including combinations of the foregoing methods. The BOC and FMOC methods, which are established and widely used, are described in the following references:

Merrifield, *J. Am.Chem.Soc.*, 1963, 88, 2149;

Meienhofer, *Hormonal Proteins and Peptides*, C.H. Li, Ed., Academic Press, 1983, pp. 48-267;

Barany and Merrifield, In *The Peptides*, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980, pp. 3-285.

In the preferred solid phase synthesis method, a peptide of a desired length and sequence is produced through the stepwise addition of amino acids to a growing peptide chain which is covalently bound to a solid resin particle. Automated synthesis may be employed in this method.

In the preferred application of this method the C-terminal end of the growing peptide chain is covalently bound to a resin particle and amino acids having protected α-amino groups are added in the stepwise manner indicated above. A preferred α-amino protecting group is the tert-butyloxycarbonyl (BOC) group, which is stable to the condensation conditions and yet is readily removable without destruction of the peptide bonds or racemization of chiral centers in the peptide chain. At the end of the procedure the product peptide is cleaved from the resin, and any remaining protecting groups are removed by treatment under acidic conditions such as, for example, with a mixture of hydrobromic acid and trifluoroacetic acid, with trifluoromethane sulfonic acid or with liquified hydrofluoric acid. For the present cyclic peptides, hydrofluoric acid was typically used.

The preferred steps for solid phase peptide synthesis using the symmetric anhydride method are shown in Table 1, below. The preferred steps for solid phase peptide synthesis using the active ester method are shown in Table 2, below. The preferred steps using the DCC method are shown in Table 3, below.

Table 1: Solid Phase Peptide Synthesis

(symmetrical anhydride method)

Step	Reagent	Vol.* (ml)	Time (min)
1	DCM wash (3 times)	15	1 (per wash)
2	TFA-DCM	15	1.5
3	TFA-DCM	15	30
4	DCM wash (6 times)	15	1 (per wash)
5	TEA-DCM	15	1.5
6	DCM wash (6 times)	15	1 (per wash)
7	Symmetrical anhydride (0.8 meq) of BOC-amino acid in DCM	10	120 or until monitoring shows complete reaction
8	Recouple if necessary by repeating Steps 4-7		
9	DCM wash (3 times)	15	1 (per wash)

* The volume given is for synthesis using 0.4 meq. of growing peptide chain on 1 gram of resin.

Table 2: Solid Phase Peptide Synthesis

(active ester method)

Step	Reagent	Vol.* (ml)	Time (min)
1	DCM wash (3 times)	15	1 (per wash)
2	TFA-DCM	15	1.5
3	TFA-DCM	15	30
4	DCM-wash (6 times)	15	1 (per wash)
5	DIEA-DCM (2 times)	15	1.5 (per wash)
6	DCM wash (6 times)	15	1 (per wash)
7	DMF wash (3 times)	15	1 (per wash)
8	BOC-amino acid active ester (0.8 meq) in DMF	7	2 hr or until monitoring shows complete reaction
9	Recouple if necessary by repeating Steps 4-9		
10	DMF wash (6 times)	15	1 (per wash)
11	DCM wash (3 times)	15	1 (per wash)

* The volume given is for the synthesis using 0.4 meq. of growing peptide chain on one gram of resin.

Table 3: Solid Phase Peptide Synthesis (DCC method)

Step	Reagent	Vol.* (ml)	Time (min)
1	DCM wash (3 times)	15	1 (per wash)
2	50% TFA-DCM	15	1
3	50% TFA-DCM	15	20
4	DCM wash (3 times)	15	1
5	IPA wash (2 times)	15	1
6	DCM wash (3 times)	15	1
7	10% TEA-DCM	15	1
8	10% TEA-DCM	15	5
9	DCM wash (4 times)	15	1 (per wash)
10	BOC AA (1.2 meq.) in DCM (or DMF)		120
11	DCC (1.2 meq., 0.5 M) in DCM		
12	Recouple if necessary by repeating Steps 4-11		
13	DCM wash (2 times)	15	1 (per wash)
14	50% CH_3OH-DCM wash	15	1
15	DCM wash (3 times)	15	1 (per wash)
16	IPA wash (2 times)	15	1 (per wash)
17	DCM wash (3 times)	15	1 (per wash)

* The volume given is for the synthesis using 0.6 meq. of growing peptide chain on one gram of resin.

Alternatively, peptide sequences or portions thereof may be synthesized in solution. See, e.g., M. Mutter and E. Bayer, in "*The Liquid Phase Method for Peptide Synthesis*"; E. Gross and J. Meienhofer, Eds.; *The Peptides*; Academic Press: 1980; pp. 285-332, for one example of peptide synthesis in solution.

General strategies for preparing cyclic compounds of the invention typically involve selective protection of side chains or termini so that cyclization may be accomplished. Essentially, the peptide bond linked linear backbone of the compound is wholly or partially constructed using one type of protection means on potentially reactive groups (e.g., side groups) on residues not involved in cyclization, with the residues bearing functional groups to be cyclized being protected with another type ("orthogonol protection"). The moieties to be cyclized are then chemically revealed without disturbing the other protective groups. The revealed moieties are then appropriately activated and cyclized. Following this, the remainder of the peptide bond linked portion of the compound is completed (if the partial backbone was cyclized), deprotected, cleaved from the resin (when appropriate) and purified. In this way, functional groups not intended for cyclization are not prematurely exposed and cyclization occurs in the proper location.

For example, a peptide may be synthesized on a resin using FMOC protection on the side chains, or on the terminus or other portion, which will be used for cyclization. Preferred steps for solid phase peptide synthesis using such FMOC orthogonal protection are set forth in Table 4 below. The remainder of the potentially reactive groups of the peptide may be BOC protected. Upon cyclization, the FMOC-protected side chain/termini are deprotected, without deprotection of the BOC-protected moieties. Inappropriate cyclization or other

modification is thereby prevented. If cyclization is to be performed prior to completion of the backbone construction, the temporary terminus should be blocked with a protecting group stable to the deprotection and cyclization conditions for the involved cyclized functional groups. Following cyclization, the temporary terminus is then deprotected and the peptide construction is completed.

Table 4: Solid Phase Peptide Synthesis (FMOC method)

Step	Reagent	Vol.* (ml)	Time (min)
1	DMC wash (2 times)	15	1 (per wash)
2	DMF wash (2 times)	15	1 (per wash)
3	Deprotect: DMF: piperidine (4:1)	15	20
4	DMF wash (4 times)	15	1 (per wash)
5	DCM wash (3 times)	15	1 (per wash)
6	Symmetrical anhydride of FMOC amino acid in DCM (or DMF:DCM) (with 2-3 fold equivalent excess of anhydride)	15	15
7	Add 1.0 mL of 10% DIEA in DCM	1	15 or until monitoring shows coupling complete
8	Recouple if necessary by repeating Steps 5-7		
9	DMF wash (5 times)	15	1 (per wash)
10	IPA wash (5 times)	15	1 (per wash)
11	DCM wash (5 times)	15	1 (per wash)

* The volume given is for synthesis using 0.4 meq. growing peptide chain on one gram of resin.

The compounds after cleavage from the resin are isolated and purified by means well known in the art. For example, the cleaved compound/resin mixture is washed in turn with several portions of diethyl ether and then extracted with several portions of aqueous acetic acid. The resin may then be discarded. The aqueous acetic acid extracts are combined, concentrated, resuspended in water and lyophilized. Following this, the crude compound can be purified by reversed phase high pressure liquid chromatography (HPLC), or by size exclusion chromatography, partition chromatography on polysaccharide gel media such as Sephadex G10 or G25, or counter current distribution.

The composition of the final compound may be confirmed by amino acid analysis after degradation of the compound by standard means, by amino acid sequencing techniques, or by FAB-MS techniques. For the examples herein, compounds were usually purified by reverse-phase HPLC and subjected to amino acid analysis.

One important aspect in final purification is the removal of fluoride. Even small amounts of fluoride may alter the biological profile of the peptides. Generally, ion exchange chromatography, using AG_3-4X acetate for example, to exchange the fluoride salt, is used. The subject compound is dissolved in water and passed over an acetate-form resin. The eluate is collected, and lyophilized to dryness. One skilled in the art will recognize that in some cases, such as, for example, where fluoride is removed through reverse-phase chromatography, or other methods, or where FMOC protection has been utilized without HF cleavage, fluoride removal using ion exchange chromatography is unnecessary.

Salts of carboxyl groups of the product compounds may be prepared in the usual manner by contacting the compound with one or more equivalents of a desired

base such as, for example, a metallic hydroxide base such as, for example, sodium hydroxide; a metal carbonate or bicarbonate base such as, for example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like. In all such procedures and in other synthetic procedures provided herein, the pH should be kept below approximately 8 in order to avoid complications such as racemization, deamidation, peptide degradation or other undesirable side reactions.

Acid salts of the compounds may be prepared by contacting the polypeptide with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid, acetic acid or citric acid.

Esters of carboxyl groups of the compounds may be prepared by any of the usual means known in the art for converting a carboxylic acid or precursor to an ester. One preferred method for preparing esters of the present compounds, when using the Merrifield synthesis technique described above, is to cleave the completed peptide sequence from the resin in the presence of the desired alcohol either under basic or acidic conditions, depending upon the resin. Thus the C-terminal end of the peptide when freed from the resin is directly esterified without isolation of the free acid. Alternatively, especially where the desired compound contains one or more glutamic acid or aspartic acid residues, C-terminal amino acid esters may be made using solution phase synthesis wherein the C-terminal residue bearing the desired ester functionality is incorporated.

Amides of the compounds of the present invention may also be prepared by techniques well known in the art for converting a carboxylic acid group or precursor to an amide. A preferred method for amide formation at the C-terminal

carboxyl group is to synthesize the peptides by solid phase methods on an appropriate resin, for example a 4-methylbenzhydrylamine or a benzhydrylamine resin, and thereafter to cleave the polypeptide from a solid support with an appropriate acid. If the desired amide is to include a secondary or tertiary amino group, then the amide may be synthesized using solution phase techniques wherein an aminated C-terminal residue bearing the desired amide functionality is incorporated.

N-Acyl derivatives of an amino group of the present peptides may be prepared by utilizing an N-acyl protected amino acid for the final condensation, or by acylating a protected or unprotected functional group in the compound. Acylation may be carried out using standard acylating reagents such as acyl halides, anhydrides, acyl imidazoles, and the like prior to deprotection of the residue side chains.

The coupling reaction is generally carried out at 0° - 25° C, deprotection at room temperature, and cleavage at a reduced temperature of -5° to 0° C (especially with HF cleavage). The exact temperature for any particular reaction will, of course, be dependent upon the substrates, reagents, solvents and so forth, all being well within the skill of the practitioner. Illustrative reaction conditions for these processes may be gleaned from the examples.

The compounds of this invention may also be synthesized using any techniques that are known to those in the synthetic arts, for example, those described in HoubenWeyl, *Methoden Der Organischen Chemie*; Georg-Thieme-Verlag: Stuttgart, 1974; pp. 1-806, or by recombinant DNA technology when only natural amino acids are used.

* * * *

Presented below are examples wherein cyclization of the present compounds is achieved with a disulfide linkage between side chain sulfur atom. Example 1 relates general procedures as applied to two representative RGD-containing peptides, while Examples 2 and 3 give procedures for preparing disulfide linked compounds according to Structures II and III above.

EXAMPLE 1

General Procedure for Synthesis of Disulfide Linked Cyclic Compounds

Peptides of this class were synthesized and cyclized as the disulfide. Such peptides include:



experimental details for which follow.

All t-butyloxycarbonyl protected amino acids with chiral centers were of the L-configuration. Such protected amino acids included the t-butyloxycarbonyl derivatives of Ser(O-benzyl), Asp(β-cyclohexyl ester), Arg(tosyl), Cys (S-*p*-methylbenzyl) and Pen(S-*p*-methoxybenzyl).

Peptide Synthesis. Stepwise build up of the peptides depicted above on the BOC-Cys (S-*p*-methylbenzyl)-Merrifield resin was done automatically on a Beckman 990C peptide synthesizer (Beckman instrument Company, Palo Alto, California 94304) and using 2 equivalents of protected amino acids for each equivalent of peptide on-resin. Automated synthesis was performed according to the general procedures published with the synthesizer.

Trifluoroacetic acid (50% in DCM) was used for deblocking. Triethyl amine (10% in DCM) was used for neutralization. Resin washing was accomplished by application of MeOH-DCM (50:50), isopropyl alcohol and methylene chloride at the other steps. Couplings (usually 120 minutes per coupling) were mediated by N,N'-dicyclohexyl-carbodiimide (DCC) in either DCM, DMF, or mixtures thereof depending on the solubility of the respective amino acid residue being coupled.

The coupled peptides were cleaved from the peptide resin by treatment with distilled anhydrous HF (10 ml/g peptide resin) in the presence of anisole (1 ml/g peptide resin) and dimethyl sulfide (0.5 ml/g) as scavengers. The reaction was carried out at -5°C for one hour. After removal of the HF under reduced pressure, the resin was washed three times with diethyl ether. The peptide was extracted from the resin with 1N acetic acid and then lyophilized.

Cyclization to the Disulfide Using Iodine. Approximately 2 grams of the respective cleaved peptides were dissolved in 500 ml 80% acetic acid. While the solution was stirring, a solution of I_2, at a 10-fold molar excess over peptide, in glacial acetic acid was added dropwise over 30 minutes. The mixture was stirred for another hour. After this, ascorbic acid was added until the reddish solution turned colorless. The solution was then concentrated by evaporation (Rotavap, Buchi Corporation), and the residue redissolved in water. The aqueous solution was then lyophilized.

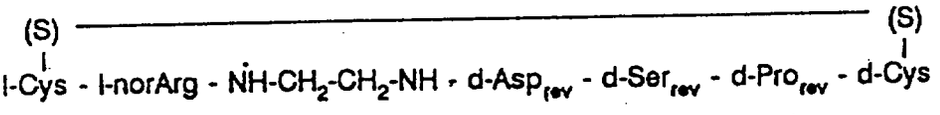
Purification. The crude cyclic peptide was purified using preparative RP-HPLC (Waters Delta Prep 3000, Millipore Corporation, Millford, Mass.) according to the manufacturer's general instructions. Such peptides can be generally purified using a linear gradient of increasing acetonitrile concentration in TEAP (1%

triethylamoronium phosphate, pH~2.3) as mobile phase. The collected fractions of pure peptide are reapplied to the HPLC column and then eluted again with 0.5% acetic acid to change the phosphate salt form of the peptide to the desired acetate form. The peptides were then dissolved in water, and fluoride was removed via ion exchange chromatography as described above if necessary. Highly purified fractions were pooled and lyophilized.

EXAMPLE 2

Synthesis of Disulfide Linked, Diamino Cyclic Reversed Peptides

In this example, the following peptide is synthesized:



Solution phase BOC and FMOC methods are used in this synthesis. The product from each step prior to HF cleavage is purified by silica gel chromatography. The general approach is to synthesize the reversed form of the peptide and attach it to the partially protected diaminopropane moiety, followed with coupling amino acids in the normal orientation to the deprotected diaminopropane coupled to the reversed peptide segment. The peptide is then deprotected with HF, the resulting crude dicysteine containing product cyclized, and the disulfide compound purified.

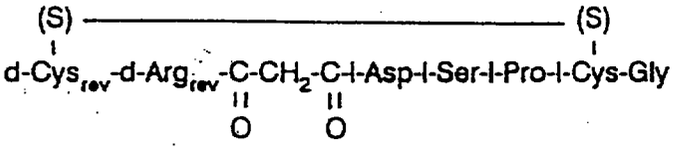
N-BOC-d-aspartic acid is protected with α-9-fluorenylmethyl ester, and the β-cyclohexyl ester is N-deprotected with 1:1 TFA/DCM. The resulting ammonium trifluoroacetate is coupled to N-BOC-O-benzyl-d-serine (1.05 eq.) using

benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent, 1.2 meq.), 1-hydroxybenzotriazole hydrate (HOBt, 0.12 meq.), and diisopropylethylamine (DIEA, 4 meq.) in DMF. This dipeptide product is N-deprotected and coupled as above sequentially to N-BOC-d-proline and N-BOC-S-4-methylbenzyl-d-cysteine. The OFm ester of the aspartic acid residue is deprotected with 20% piperidine/DMF and the free carboxylate product coupled to 1-(9-fluorenylmethyl-carboxamide)-2-aminoethane with BOP activation. The product of this reaction is coupled sequentially to N-α-FMOC-N^g-tosyl-l-(nor)arginine and N-BOC-S-4-methylbenzyl-l-cysteine, using 20% piperidine/DMF deprotection and activation with BOP. After a final deprotection with HF, the resulting cysteine sulfhydryl are cyclized in an iodine oxidation procedure to produce the final product depicted above.

EXAMPLE 3

Synthesis of Disulfide Linked, Diketo Cyclic Reversed Peptides

In this example, the following peptide is synthesized:



Solution phase BOC and FMOC methods are used in this synthesis. The product from each step prior to HF cleavage is purified by silica gel chromatography. As can be seen, this peptide contains reversed (rev) forms of d-Cys and d-Arg. The general approach is to synthesize the l-configuration segment of the peptide and then attach, through an amide bond, a residue of malonic acid mono-t-butyl ester. The synthesis is then continued by coupling amino acids in the reversed

orientation to the deprotected malonate. The compound is then deprotected with HF, the resulting crude dicysteine-containing product cyclized and the disulfide compound purified.

BOC-Gly(O-benzyl) ester is N-deprotected with 1:1 trifluoroacetic acid/methylene chloride. The resulting ammonium trifluoroacetate is coupled to N-BOC-S-4-methyl-benzyl-l-cysteine (1.05 eq.) using benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorosphosphate (BOP reagent, 1.2 eq.), 1-hydroxybenzotriazole hydrate (HOBt, 0.12 eq.), and diisopropylethylamine (DIEA, 4 eq.) in dimethylformamide (DMF).

This dipeptide product is N-deprotected and coupled as above sequentially to the following compounds: N-BOC-l-proline, N-BOC-O-benzyl-l-serine, N-BOC-(β-cyclohexyl ester)-l-aspartic acid, and malonic acid mono-t-butyl ester.

The t-butyl ester of the malonate is deprotected with 1:1 TFA/DCM and the freed carboxylate product is activated (using the BOP coupling procedure described above) and coupled to N^g-tosyl-d-arginine, t-butyl ester (free N^α-amine). The t-butyl ester of the resulting arginine-containing product is deprotected with 1:1 TFA/DCM and the free carboxylate product is again activated and coupled to S-4-methylbenzyl-d-cysteine with BOP reagent.

The product of this reaction is deprotected first with 1:1 TFA/DMC and then treated with HF, and the resulting cysteine sulfhydryl are cyclized using the iodine oxidation procedure to produce the compound depicted above.

* * * *

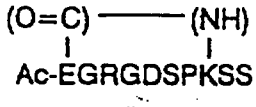
Presented below are examples of peptides which were cyclized using an amide bridge. Example 4 is of a side chain-to-side chain amide bridge wherein the δ-carboxyl group of Glu was condensed with the ϵ-amino group of Lys. Example 5 is of a side chain-to-backbone amide bridge wherein the terminal α-amino group of Gly was condensed with the β-carboxyl group of Asp. In both of these examples, the amino acid backbone was fully assembled, and then cyclized, on a resin.

Example 6 shows cyclization using an amide bridge as an intermediate step. The amino acid backbone is partially assembled and then cyclized using an amide bridge. After assembly of the amide bridge, the amino acid chain is completed.

EXAMPLE 4

Synthesis of Amide Linked Cyclic Compounds (Side Chain-Side Chain Linkage)

In this example, the following compound was synthesized:



All amino acids and amino acid derivatives were purchased from BACHEM (Torrance, California). 9-Fluorenylmethanol and DCC were obtained from Sigma Chemical Co. (St. Louis, Missouri). Diisopropylethylamine and 4-(dimethylamino)-pyridine were obtained from Aldrich (Milwaukee, Wisconsin). Unless otherwise noted, other reagents were of analytical grade and used without further purification.

All residues were linked by the solid phase method using BOC protection. The side chain carboxyl groups of Asp and Glu were protected as fluorenylmethyl

esters and the ϵ-amino group of Lys and α-amino group of Gly were protected as N-FMOC. The amide bridge between the two side chains (on Glu and Lys) was synthesized while the peptide was bound on the resin. This procedure is represented by Figure 1a.

(a) Preparation of N-BOC-O-9-fluorenylmethyl omega-esters of aspartic and glutamic acids.

The N-BOC-O-9-fluorenylmethyl omega-esters of aspartic and glutamic acids were prepared following the procedure as generally described by R. Bolin, C.T. Wang, and A.M. Felix, *Organic Preparations and Procedures Intern.* **1989**, 21, pp. 67-74, with certain modifications.

N-BOC-O$^{\beta}$-9-fluorenylmethyl asparate. 8.31 g (25.7 mmol) of N-BOC-O$^{\alpha}$-benzyl-aspartate and 4.80 g (24.5 mmol) of 9-fluorenylmethanol were dissolved in 150 ml DCM. The solution was chilled in an ice bath. 30 mg (0.24 mmol) of 4-(dimethylamino)pyridine was added to the solution followed by addition of 5.31 g (25.7 mmol) DCC in portions, over 10 minutes. The resulting mixture was stirred for one hour with continued cooling. The precipitated n,n'-dicyclohexylurea was removed by filtration and the filtrate was diluted with 250 ml DCM. This solution was extracted with (in order) 10% citric acid (2 x 50 ml), H_2O (1 x 50 ml), 2.5% $NaHCO_3$ (2x50 ml), H_2O (1 x 50 ml), brine (1 x 50 ml). The solution was then dried over $MgSO_4$, and concentrated to an oily residue. Recrystallization from methanol/ether/petroleum ether (1:3:10) yielded 10.85 g (84%) N-BOC-O$^{\alpha}$-benzyl-O$^{\beta}$-fluorenylmethyl-aspartate, with a melting point of 74-77°C.

5.5 g (10.9 mmol) of the above product was then dissolved in 150 ml warmed methanol, and hydrogenated over 300 mg of 20% $Pd(OH)_2/C$ for 1.5 hr at room temperature and a pressure of 35-40 psi. The catalyst was filtered off and the solvent was evaporated *in vacuo*. The residual oil was redissolved in 200 ml diethyl ether and extracted with (in order) 1% $NaHCO_3$ (3 x 50 ml), H_2O (1 x 50 ml), 5% citric acid (2 x 50 ml), and brine (1 x 50 ml). The ether layer was dried over $MgSO_4$ and concentrated. Recrystallization from diethyl ether/petroleum ether yielded 3.53 g of N-BOC-O^{β}-9-fluorenylmethyl aspartate, with a melting point of 135-137°C.

N-BOC-O-fluorenylmethyl-glutamate (gamma ester). N-BOC-O^{α}-BENZYL-GLUTAMATE (4.5 G, 13.3 mol) and 9-fluorenyl-methanol (2.5 g, 12.5 mmol) were dissolved in 100 ml DCM. The solution was stirred and chilled in an ice bath. To the solution, 15.5 mg (0.13 mmol) of 4-(dimethylamino)-pyridine and 2.75 g (13.3 mmol) of DCC were added, and the resulting mixture was stirred for 4 hr with continued cooling. Precipitated N,N'-dicyclohexylurea was filtered off and filtrate was diluted with 200 ml DCM. The solution was extracted and treated in the same manner as was for the aspartate described above. This yielded N-BOC-O^{α}-benzyl-O-fluorenylmethylglutamate (gamma ester) (4.2 g), with a melting point of 97-99.5°C.

4.0 g (7.75 mmol) of the foregoing product was hydrogenated over 125 mg of 10% Pd/C in 200 ml mixture of MeOH/EtOH/IPA (2:1:1) for 2 hr at room temperature at 40 psi. The reaction mixture was filtered to remove the catalyst, and concentrated to an oily residue. The residue was then mixed with 150 ml diethyl ether and combined aqueous layers were back-extracted with diethyl ether (2 x 40 ml). The combined ether layers were dried over $MgSO_4$, filtered, and concentrated to a

white form. N-BOC-O-fluorenylmethylglutamate (gamma ester) (2.3 g) was obtained by recrystallizing the crude residue from diethyl ether/petroleum ether (1:10), melting point 123.5-126°C.

(b) Synthesis of protected EGRGRDSPKSS peptide sequence.

Synthesis of the above peptide was performed using, in conjunction, an automated peptide synthesizer (System 990, Beckman Instruments, Inc., Palo Alto, California) and a manual peptide synthesis apparatus (S.C. Glass Tech, Bonica, California).

BOC-Ser(Bzl)OCH_2-PAM resin (1.0 g, 0.75 mmol) from Applied Biosystems (Foster City, California) was used as the starting resin. The following amino acids were used in the synthesis: BOC-l-Ser(benzyl), BOC-l-Lys(N^{ϵ}-FMOC), BOC-l-Pro, BOC-l-Asp(O-benzyl), BOC-Gly, BOC-Arg(N^{g}-tos), and BOC-Glu(Fm). Excess amino acid (2-3 fold) was used for each coupling. The peptide chain was constructed on the Beckman peptide synthesizer using BOC chemistry with the stepwise addition of each amino acid following the standardized cycle similar to that presented in Table 3, with adjustments for scale. 50% TFA in DCM, 5% DIEA in DCM, and 0.5 M of DCC in DCM were used as deprotecting agent, neutralizer, and activating agent, respectively, for each coupling.

(c) Capping of peptide sequence.

Following the removal of the BOC group from the N-terminal Glu with 50% TFA in DCM, and neutralization with 5% DIEA in DCM, the protected peptide on resin was transferred to a manual apparatus for the terminal acetylation, or "capping". The N-terminal deprotected side chain protected peptide on resin was washed with

MeOH (2 x 1 min), DCM (3 x 1 min); neutralized with 5% DIEA again in DCM (1 x 1 min, 1 x 20 min); washed with DCM (3 x 1 min); and acetylated (capped) with 50% Ac_2O in DCM (1 min wash, 20 min acetylation). The peptide then was cyclized by forming an amide linkage between the δ-carboxyl group of Glu and the ϵ-amino group of Lys by the general procedure below.

(d) General cyclization procedure for formation of the amide bridge.

After the construction of the peptide chain, the amidating cyclization was carried out according to the following protocol. Filtering was performed between each step: (1) MeOH (2 x 1 min); (2) DCM (3 x 1 min); (3) 20% piperidine in DMF, wash for 1 min, and deprotection for 20 min; (4) DMF (2 x 1 min); (5) MeOH (2 x 1 min); (6) DCM (3 x 1 min); (7) BOP reagent (4 equiv.) in DMF (20 ml/gram of resin), stir for 2 min, and add DIEA (2% of DMF volume), stir for 4 hrs (the completion of the cyclization reaction was monitored by the ninhydrin test; if the reaction was judged incomplete at 4 hrs, the reaction was continued until the ninhydrin test was negative); (8) DMF (2 x 1 min); (9) DCM (2 x 1 min); (10) MeOH (2 x 1 min).

The final cyclic compound was removed from the resin by treatment with HF in the presence of 10% anisole for 1 hr at 0°C. After evaporation of the HF, the residue was washed with diethyl ether and extracted from the resin with 5% HOAc in H_2O. The aqueous extract was lyophilized to yield the crude peptide (730 mg).

(e) Purification.

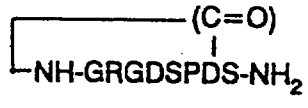
The compound was purified using a Waters Delta Prep 3000 system (Waters, Milford, MA) equipped with a C_{18} column, using a linear gradient of increasing

acetonitrile concentration in TEAP (pH 2.2 to 2.4) as the mobile phase. The collected fractions of the pure compound were pooled and applied again to the C_{18} column. This time the sample was eluted with 0.5% HOAc to convert the phosphate salt form of the peptide to the desired acetate form. The pure peptide fractions were pooled, concentrated *in vacuo*, redissolved in water and lyophilized to give 92.9 mg of peptide, 98.7% HPLC purity, white powder.

EXAMPLE 5

Synthesis of Amide-Linked Cyclic Compounds (Backbone-Side Chain Linkage)

In this example, the following compound was synthesized:



The manual synthesis of the above compound began with 4-methylbenzhydrylamine resin (2.0 g, 1.4 mmol) from CBA, Inc. (Boulder, Colorado). The peptide chain was assembled by using the BOC procedure described in the synthesis of the compound of Example 4 above. BOC-l-Asp(Fm), BOC-l-Pro, BOC-l-Ser(Benzyl), BOC-Gly, BOC-l-Arg(N^g-tosyl) and N-FMOC-Gly were used in the synthesis.

The cyclization between the terminal amino group of Gly and the β-carboxyl group of Asp^7 was performed according to the general amide cyclization procedure described in the preceding Example 4.

The cyclic compound then was cleaved from the resin by HF and 10% anisole for 1 hr at 0°C. Following evaporation of the HF, the mixture was washed with diethyl

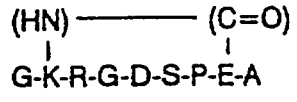
ether (ether layer discarded) and extracted with 1N HOAc. The aqueous extract was lyophilized to yield 1.23 g of the crude compound.

Purification of the compound was achieved using a Waters preparative HPLC system with a C_{18} column, following the method described in the preceding example. Yield was 678 mg pure product compound, HPLC purity of 99.7%, white powder.

EXAMPLE 6

Synthesis of Amide Linked Cyclic Compounds
(Cyclization Prior to Complete Chain Assembly)

In this example, the following compound is synthesized:



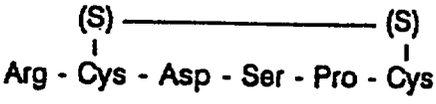
Here, cyclization can be performed when the chain is partially assembled, using orthogonal protection methods as described above. Figure 1b is a diagram representing suitable procedures. The chain is partially assembled using the DCC method (Table 3) with primarily BOC protection until the BOC-Lys(FMOC), representing L^1 in the eventual product compound, is added. The BOC-Glu(Fm) and BOC-Lys(FMOC) are substituted for BOC-Glu(Chx) and BOC-Lys(Cl-Cbz). At this time the chain assembly is interrupted and the compound is cyclized while bound to the resin. Cyclization proceeds by treating the compound with 20% piperidine in DCM to deprotect the Glu and Lys residues, followed by filtering and washing, and by then by reaction with BOP in DMF and DIEA until the compound on the resin is ninhydrin negative. The amino-terminal glycine residue is then

added to the cyclized compound, and the resulting product cleaved from the resin and worked up as described generally in the foregoing examples.

EXAMPLE 7

Synthesis of Cyclic Disulfide Compounds Containing RCD

In this example, the following compound was prepared:



All amino acids, amino acid derivatives and amino acid mimetic were purchased from BACHEM (Torrance, California). DCC was from Sigma Chemical Co. (St. Louis, Missouri). Trifluoroacetic acid was from Halocarbon Co. (New York, New York). Triethyl-amine was from Fisher Scientific (Fair Lawn, New Jersey). Other reagents were obtained from conventional sources and of analytical grade.

All peptides were synthesized by the solid phase method with a Beckman automated peptide synthesizer (System 990, Beckman Instruments, Inc., Palo Alto, California) using BOC chemistry.

Attachment of N-BOC-S-*p*-methylbenzyl-l-cysteine (BOC-Cys(4-MeBzl)) to the chloromethylpolystyrene resin (Merrifield resin) was done in the presence of potassium fluoride. BOC-Cys(4-MeBzl) (0.9 molar eq.) was reacted with swelled Merrifield resin (Bio-Rad lab., Richmond, California) (1.0 molar eq.) in DMF in presence of KF (1.8 molar eq.) at 80°C for 16 hr. The resin then was filtered, washed, and dried. The molar substitution of the resin was determined by weight.

The sequential elaboration of the peptide chain on the BOC-Cys(4-MeBzl) resin was carried out stepwise using the BOC procedure according to the procedure in Table 5, below. At the end of the synthesis the N-terminal BOC protecting group was removed using TFA:DCM (1:1) for 30 min.

TABLE 5:

Solid Phase Peptide Synthesis
For RCD-Containing Compounds
(TFA deprotection/DCC coupling)

Step	Reagent	Vol*(ml)	Time**(min)
1	DMC wash (3x)	20	1
2	TFA-DCM (1:1)	20	1
3	TFA-DCM (1:1)	20	20
4	DCM wash (3x)	20	1
5	MeOH wash (2x)	20	1
6	DCM wash (3x)	20	1
7	DIEA-DCM (1:9)	20	1
8	DIEA-DCM (1:9)	20	5
9	DCM wash (3x)	20	1
10	MeOH wash (3x)	20	1
11	DCM wash (3x)	20	1
12	BOC-AA (3.2 mM, 2-fold excess in DCM plus DCC in DCM (0.5 M)		120
13	DCM wash (2x)	20	1
14	DCM-MeOH (1:1) (2x)	20	1
15	DIEA-DCM (1:9)	20	1
16	MeOH wash (2x)	20	1
17	DCM wash (2x)	20	1
18	Ac_2O in DCM (1:3)	20	20
19	DCM wash (3x)	20	1
20	MeOH wash (2x)	20	1

* The volume given is for the synthesis using 2 g of resin with the substitution of 0.8 mM/g of resin.

** All times given in repeated wash steps are "per wash".

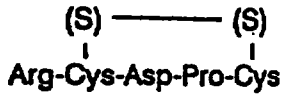
Synthesis and Cleavage. For the synthesis of the title compound, the following amino acids were used: N-BOC-l-proline, N-BOC-l-serine(benzyl), N-BOC-l-aspartic acid-β-cyclohexyl ester, N-BOC-l-cysteine-(4-methylbenzyl), and N-BOC-N^g-tosyl-l-arginine. Starting with 2.0 g (1.6 mmol) N-BOC-Cys(4-MeBzl) resin and through the stepwise elaboration of the protected peptide chain, 3.4 g of the peptide resin was obtained. The peptide was removed from the resin by treatment with HF in the presence of 10% anisole and 5% of dimethylsulfide for 1 hr. at 0°C. Following evaporation of the HF, the peptide resin residue was washed in turn with diethyl ether (discarded ether wash) and extracted with 5% HOAc in H_2O (50 ml x 4). The aqueous acetic acid solution was lyophilized to yield 780 mg of the crude, noncyclic peptide (Ellman test positive, Stewart and Young, *Solid Phase Peptide Synthesis*, 2nd ed. p. 116).

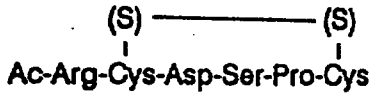
Cyclization. The formation of the intramolecular disulfide bridge was accomplished by using the iodine oxidation method. The crude peptide was dissolved in 300 ml of 80% acetic acid in H_2O. The peptide solution was titrated with I_2-glacial acetic acid (saturated) until the solution turned light brown in color, and stirred for 1-2 hr. at room temperature. The excess iodine was removed by adding ascorbic acid-water solution. The peptide solution was then concentrated *in vacuo*. The residue was redissolved in water and lyophilized to obtain the crude cyclic compound.

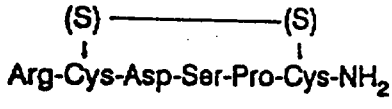
Purification. The compound was purified on a 25 x 2.5 cm SP Sephadex c-25 (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) column. The column was first equilibrated with 10 mM NH_4OAc buffer (pH 3.5) The compound was eluted with NH_4OAc buffer in a linear gradient of increasing NH_4OAc concentration with 10-300

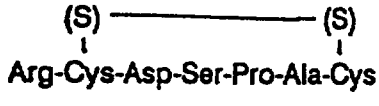
mM (pH3.5-6.5). The fractions which contained the pure product were pooled and lyophilized. The dried residue was redissolved in H_2O and lyophilized thrice to ensure the removal of the NH_4OAc salt to yield 106 mg product (HPLC, 95% pure).

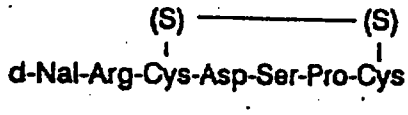
Other RCD Peptides. In a similar fashion, the following compounds of the invention are also synthesized:

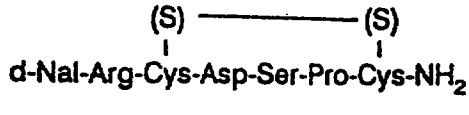


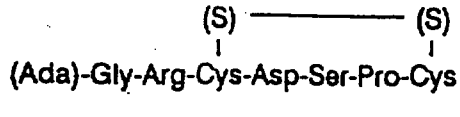


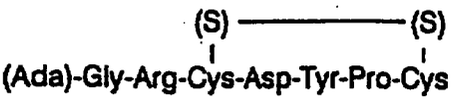


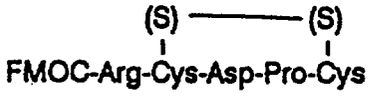


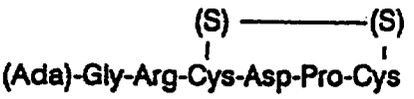


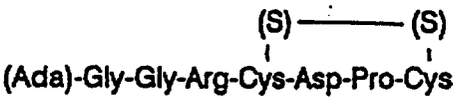


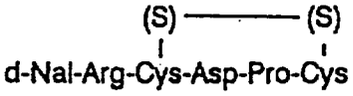


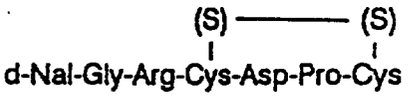

(S)
(S)
(Ada)-Gly-Arg-Cys-Asp-Tyr-Pro-Cys

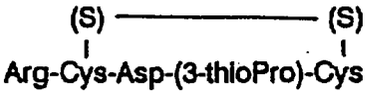

(S)
(S)
FMOC-Arg-Cys-Asp-Pro-Cys

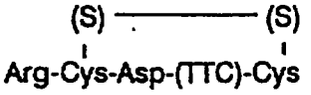

(S)
(S)
(Ada)-Gly-Arg-Cys-Asp-Pro-Cys

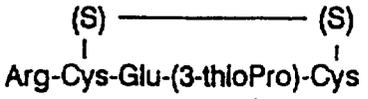

(S)
(S)
(Ada)-Gly-Gly-Arg-Cys-Asp-Pro-Cys


(S)
(S)
d-Nal-Arg-Cys-Asp-Pro-Cys


(S)
(S)
d-Nal-Gly-Arg-Cys-Asp-Pro-Cys


(S)
(S)
Arg-Cys-Asp-(3-thioPro)-Cys


(S)
(S)
Arg-Cys-Asp-(TTC)-Cys

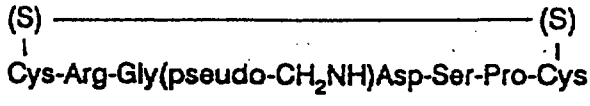

(S)
(S)
Arg-Cys-Glu-(3-thioPro)-Cys

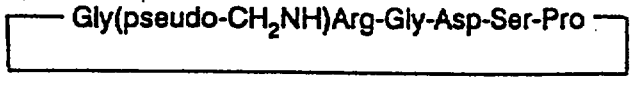
The peptides containing D-Nal are purified using a Waters Delta Prep 3000 system (Waters, Milford, Massachusetts) equipped with a C_{18} column, using a linear gradient of increasing acetonitrile concentration in TEAP (pH 2.2 to 2.4) as the mobile phase. The collected fractions of the pure peptide are then eluted again with 0.5% HOAc in the same HPLC system to exchange the phosphate salt form of the peptide to the desired acetate form.

Example 8

Synthesis of Peptide Bond Isostere Compounds

Cell adhesion of modulation compounds containing peptide bond isostere structures of the following formulas were synthesized:





BOC amino acid aldehydes used in this synthesis were prepared by the method of J-A Fehrentz and B. Castro, *Synthesis* **1983**, 676. The introduction of the pseudo CH_2NH peptide bond was done by utilizing the reductive alkylation reaction in solid phase (Y. Sasaki and D.H. Coy, *Peptides*, **1987**, 8, 119.

Preparation of BOC-Glycinal. Synthesis of BOC-glycinal from BOC-glycine involved the steps of forming the N-methoxy-N-methylamide of BOC-protected glycine, followed by reduction to the aldehyde compound. Since the aldehyde was highly reactive it was prepared immediately before use.

8.75 g (50 mmol) of BOC-glycine dissolved in 150 ml DCM and 6.95 ml (50 mmol) of TEA was added to the solution, and stirred. 17.42 g (50 mmol) BOP reagent (Richelieu Biotechnologies, QC, Canada) was added, followed a few minutes later with O,N-dimethyl-hydroxylamine hydrochloride (5.6 g, 55 mmol) and TEA (7.67 g, 55 mmol). The reaction was complete in 60 min, and the result was verified by TLC monitoring. A small amount of TEA was used to neutralize the mixture (adjust the pH to above 7.0) in order to allow the reaction to go to completion. The mixture was diluted to 500 ml with DCM. The solution was washed in turn with 3N HCl (3 x 100 ml), saturated $NaHCO_3$ (3 x 100 ml), and brine (3 x 100 ml). The DCM layer was dried over $MgSO_4$ and concentrated to obtain the crude product. Recrystallization from water afforded 5.85 g N^{α}-(BOC)-glycine N-methoxy-N-methylamide (m.p. 92-95°C). This product was dried thoroughly *in vacuo* over P_2O_5 before use in the following reaction.

To obtain the BOC-glycinal (BOC-NH-CH_2-CHO), $LiALH_4$ (569.3 mg, 15 mmol) was added to a stirred solution of the above compound (2.18g, 10 mmol) in 100 ml anhydrous THF (Aldrich). Reduction was completed in 15 min. The mixture was hydrolyzed with a solution of $KHSO_4$ (2.72 g, 20 mmol) in H_2O (50 mL). Then, the THF was evaporated, and the aqueous solution was extracted with diethyl ether (1 x 300 ml, 2 x 100 ml). The combined diethyl ether layers were washed in turn with 3N HCl (3 x 70 ml), 5% $NaHCO_3$ (3 x 50 ml), brine (1 x 70 ml), and dried over $MgSO_4$. The solution was concentrated to leave a light yellow oily residue, which was tested by IR for the presence of the desired aldehyde. The final product was redissolved in anhydrous diethyl ether and stored under N_2 gas at -20°C until used.

Peptide Synthesis. All residues were incorporated by the solid phase method using the BOC procedure described earlier, except for the reduced bond isosters linkage which was prepared as described below. Following the introduction of the reduced bond isosters to the peptide on-resin, the remainder of the synthesis was continued as before. The peptide fragment preceding the CH_2NH bond were assembled in a Beckman Peptide Synthesizer (System 990).

Introduction of the CH_2NH bond. The following procedure was next carried out:

(1) The peptide-resin was transferred to a manual apparatus after the removal of the BOC group from the partially assembled peptide chain.

(2) The resin was washed in turn with DCM (3 x 1 min), MeOH (2 x 1 min), DCM (3 x 1 min), and DMF (2 x 1 min).

(3) BOC-glycinal (> 3 equiv.) was added in 15 ml DMF containing 1% AcOH.

(4) 10 ml (10 mmol, 4 equiv.) of 1M $NaBH_3CN$ in THF (Aldrich) was added portion-wise in 30 min and further stirred for 1 hr for the reductive alkylation.

(5) The resin was washed in turn with DMF (2 x 1 min), DCM (3 x 1 min), MeOH (2 x 1 min), and tested for NH_2 content with the ninhydrin assay.

The coupling of the additional amino acids after the CH_2NH bond was continued using the standard BOC procedure in a manual apparatus. The peptide was removed from the resin by treatment with HF in presence of 10% anisole and 5% dimethylsulfide for 1 hr at 0°C. The residue was washed with diethyl ether, filtered,

and extracted with 1 M HOAc in H_2O (50 ml x 4). The solution was lyophilized to yield the crude noncyclic peptide (780 mg).

Cyclization. With respect to the first title compound, the formation of the intramolecular disulfide bridge was accomplished by using iodine oxidation method. The noncyclic peptide (Ellman positive) was dissolved in 300 ml of 80% HOAc. The peptide solution was titrated with I_2-glacial acetic acid until the solution turned light brown in color, and stirred for 1-2 hr at room temperature. The excess iodine was removed by adding ascorbic acid-water solution. The peptide solution was then concentrated *in vacuo*. The residue was redissolved in water and lyophilized to obtain the crude cyclic peptide.

Both peptides were purified using techniques as described in the foregoing examples.

By using the synthetic techniques described above, cyclic compounds of the invention in which linkage (through L^1, L^2 and Z) includes a secondary amine structure may analogously be prepared. It is most advantageous, from a synthetic standpoint, to incorporate the isostere bond linkage prior to final cyclization of the compound. In such a case, cyclization is completed as a final step (after assembly of the desired residues including the isostere bond) through formation of, typically, a peptide bond at a point within the sequence 1-2-3-4-5-6.

* * * * *

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2. Therapeutic Utility

In the practice of the therapeutic methods of the present invention, an effective amount of the active compound, including derivatives or salts thereof, or a pharmaceutical composition containing the same, as described below, is administered via any of the usual and acceptable methods known in the art, either singly or in combination with another compound or compounds of the present invention or other pharmaceutical agents such as immunosuppressants, antihistamines, corticosteroids, and the like. These compounds or compositions can thus be administered orally, sublingually, topically (e.g., on the skin or in the eyes), parenterally (e.g., intramuscularly, intravenously, subcutaneously or intradermally), or by inhalation, and in the form of either solid, liquid or gaseous dosage including tablets, suspensions, and aerosols, as is discussed in more detail below. The administration can be conducted in single unit dosage form with continuous therapy or in single dose therapy ad libitum.

Useful pharmaceutical carriers for the preparation of he pharmaceutical compositions hereof can be solids, liquids or gases; thus, the compositions can take the form of tablets, pills, capsules, powders, enterically coated or other protected formulations (such as binding on ion exchange resins or other carriers, or packaging in lipid protein vesicles or adding additional terminal amino acids), sustained release formulations, solutions (e.g., ophthalmic drops), suspensions, elixirs, aerosols, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic) for injectable solutions. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include starch,

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cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical expedients such as sterilization and may contain conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers, and the like. Suitable pharmaceutical carriers and their formulations are described in Martin, *Remington's Pharmaceutical Sciences*; 15th Ed.; Mack Publishing Co., Easton, 1975, see, e.g., pp. 1405-1412, 1461-1487. Such compositions will, in general, contain an effective amount of the active compound together with a suitable amount of carrier so as to prepare the proper dosage form for proper administration to the host.

In one preferred embodiment, the therapeutic methods of the present invention are practiced when the relief of symptoms is specifically required or perhaps imminent; in another preferred embodiment, the method hereof is effectively practiced as continuous or prophylactic treatment.

In the practice of the therapeutic methods of the invention, the particular dosage of pharmaceutical composition to be administered to the subject will depend on a variety of considerations including the nature of the disease, the severity thereof, the schedule of administration, the age and physical characteristics of the subject, and so forth. Proper dosages may be established using clinical approaches familiar to the medicinal arts. It is presently believed that dosages in the range of 0.1 to 100 mg of compound per kilogram of subject body weight will be useful, and a range of 1 to 100 mg per kg generally preferred, where administration is by injection or ingestion. Topical dosages may utilize formulations containing

generally as low as 0.1 mg of compound per ml of liquid carrier or excipient, with multiple daily applications being appropriate.

The compounds and therapeutic or pharmaceutical compositions of the invention are useful in the study or treatment of diseases or other conditions which are mediated by the binding of integrin receptors to ligands, including conditions involving inappropriate (e.g., excessive or insufficient) binding of cells to natural or other ligands. Such diseases and conditions include inflammatory diseases such as rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases (e.g., ulcerative colitis and regional enteritis) and ophthalmic inflammatory diseases; autoimmune diseases; thrombosis or inappropriate platelet aggregation conditions, and cardiovascular disease; prevention of occlusion following thrombolysis; neoplastic disease including metastasis conditions; as well as conditions wherein increased cell binding is desired, as in wound healing or prosthetic implantation situations as discussed in more detail above.

In addition, derivatives of the present compounds may be useful in the generation of antigens which, in turn, may be useful to generate antibodies. These antibodies will, in some cases, themselves be effective in inhibiting cell adhesion or modulating immune activity by acting as receptors for matrix proteins or other cell adhesion ligands, or, if anti-idiotypic, by acting to block cellular receptors.

EXAMPLE 9

Cell Adhesion Inhibition Assay (I)

The following assay established the activity of the present compounds in inhibiting cell adhesion in a representative *in vitro* system. The assay was a competition assay in which both fibronectin and a test compound were present. Microtiter plates were first precoated with fibronectin. The test peptide was then added in increasing concentrations with cells known to contain the fibronectin receptor. The plates were then washed and stained for quantitation of attached cells. The present assay directly demonstrates the anti-cell adhesion activity and modulatory activity of the present compounds. Additionally, by immobilizing the peptide on a surface, one could adhere appropriate cells to that surface. Other cell adhesion modulation activity, and utilities pertinent thereto, will be apparent to those skilled in the art.

The cell line U937 was purchased from American Type Tissue Culture Collection. The cells were cultured in RPMI media (J.R. Scientific Company, Woodland Hills, California 95695) containing 10% fetal calf serum. Fibronectin was purified from human plasma according to the procedure of Engvall, E. and Ruoslahti, E., *Int. J. Cancer* 1977, 20, 1.

Microtiter plates (96-well, Falcon) were coated overnight at 4°C with 5 μg/ml fibronectin (FN) (for a total volume of 0.1 ml) or, as a control, 5 μg/ml bovine serum albumin (BSA) diluted in phosphate buffered saline (PBS, 0.01 M $NaPO_4$ in 0.9% NaCl at pH 7.2 to 7.4). Unbound proteins were removed from plates by washing with PBS. The plates were then coated with 100 μl of PBS containing 2.5 mg/ml BSA for one hour at 37°C. This procedure is a modification of a previously

published method, Cardarelli, P.M. and M.D. Pierschbacher, *PNAS-USA* 1986, 83, 2647. The containment in the wells of functional amounts of immobilized protein has been confirmed by independent assay of fibroblast attachment and ELISA (Engvall, E., *Methods Enzymol.* 1980, 70, 419), although the actual amount of protein bound to the plate in these assays was not determined.

A U937 culture was collected and washed two times with Hanks Balanced Salt Solution. The cells were counted and adjusted to 1.5 x 10^6 cells per ml in Dulbecco's Modified Eagles Medium (DMEM) plus BSA (2.5 mg/ml) for cell attachment assay. Subject compounds were then dissolved in DMEM and BSA, and the pH was adjusted to 7.4 with 7.5% sodium bicarbonate. The compounds (100 μl) were added to FN-coated wells, at 1.5, 0.75, 0.375, 0.188, 0.094, 0.047, 0.023, 0.012, 0.006 and 0.003 mg/ml final concentration and U937 cells (100 μl) were added per well. The plates were then incubated at 37°C for 60 minutes. Following this incubation, the plates were washed once with PBS. Attached cells were fixed with 3% parformaldehyde in PBS and stained with 0.5% toluidine blue in 3.7% formaldehyde. The cells were stained overnight at room temperature and the optical density at 590 nm of toluidine blue-stained cells was determined using a vertical pathway spectrophotometer to quantitate attachment (VMAX Kinetic Microplate Reader, Molecular Devices, Menlo Park, California 94025).

Results. Table 6, below, shows the results of the cell adhesion inhibition assay. Potency is expressed in μM units. Figure 2 is a diagram representing the curve of cell adhesion inhibition for the compound

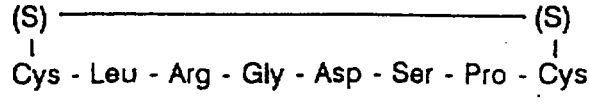


TABLE 6

ACTIVITY OF COMPOUNDS IN THE U937 - FIBRONECTIN ADHESION ASSAY[1]

Peptide #	Sequence	IC_{50}, μM
1	C*LRGDSPC*	909
2	GC*LRGDSPC*-NH_2	468
3	GC*L(homoA)GDSPC*-NH_2	1000-1500
4	C*GRGDSPC*	597
5	C*LRGDSP(Pen)*-NH_2	100-1500
6	C*(Nle)RGDSPC*-NH_2	383
7	C*(dL)RGDSPC*-NH_2	924
8	C*RGDC*	1461
9	C*RGDSC*	1104
10	C*RGDSPC*	140
11	(Mpr*)GRGDSPC*	330
12	(Mpr*)LRGDSPC*	611
13	(Mpr*)LRGDTPC*	870
14	C*YRGDSPC*	198
15	C*GRGDVPC*	494
16	C*GRGDAPC*	829
17	E*GRGDSPK*SS	1059
18	Ac-E*GRGDSPK*SS	171
19	K*RGDSPD*SS	1084
20	(dC*)(dR)G(dD)(dS)(dP)(dC*)	597
21	C*(Sar)RGDSPC*	136
22	G*RGDSPD*-NH_2	112
23	(Ada)-C*GRGDSPC*	272
24	VTC*GRGDSPC*A-NH_2	50
25	VSC*GRGDSPC*A-NH_2	636
26	(dC*)(dP)(dS)(dD)G(dR)(dC*)	1161
27	C*RGDSPC*-NH_2	242

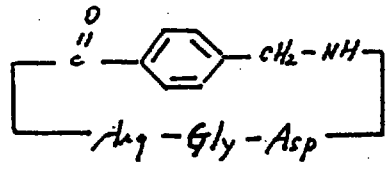
Asterisk ("*") following residue abbreviation designates linking residue that provides functional group for cyclization. Functional group precursors are as follows: C*, side chain sulfhydryl; Pen*, side chain sulfhydryl; Mpr, 3-mercapto (sulfhydryl) group; K*, side chain amino group D*, side chain carboxyl group; G*, α-amino group; R*, α-amino group. "d" indicates the D-enantiomer.

Peptide #	Sequence	IC_{50},μM
28	A(norVal)SC*GRGDSPC*A-NH_2	632
29	C*GRGD[Thr(OCH_3)]PC*	255
30	C*YRGDSPC*-NH_2	1024
31	C*GRGDSPC*-NH_2	2719
32	(Mpr*)YRGDSPC*	240
33	(Mpr*)RGDSPC*	231
34	(d-Nal)-C*RGDSPC*	236
35	RC*DSPC*	1040
36	C*RGDYPC*	30
37	(Ada)-GGGC*RGDSPC*	85
38	(Ada)-GGC*RGDSPC*	45
39	(Ada)-GC*RGDSPC*	58
40	(d-Nal)-C*RGDSPC*	136
41	(PMP)*-RGDSPC*	227
42	(FMOC)-RC*DPC*	107
43	Sar*RGDSPD*	103
44	C*RGDTPC*	91
45	C*YRGDVPC*-Alb-NH_2	106
46	R*GDSPE*-NH_2	91
47	G*RGDSPD*-NH_2	134
48	R*GDSPD*	60
49	RC*DPC*	32
50	C*RGDFPC*	35
51	(Ada)-GGC*RGDY-(3-thioPro)-C*NH_2	7
52	(Ada)-GGC*RGDYPC*-NH_2	15
53	(Ada-CA)-GGC*RGDY-(3-thioPro)-C*NH_2	25
54	(Ada)-GGC*RGDYPC*-NH_2	39
55	(Ada)-GGC*RGDVPC*	30
56	(Ada)-GC*RGDVPC*	54
57	(Ada)-C*RGDVPC*	115
58	(Ada)-GGC*RGDYPC*	42
59	(Ada)-GC*RGDYPC*	81
60	(FMOC)-GGC*RGDSPC*	53
61	(Ada)-GC*RGDSPC*-NH_2	86
62	C*RGDS(1,1-ACC)C*	148
63	C*RGDWC*	57
64	(Ada)-C*RGDC*	267
65	(Ada)-GGC*RGDWC*	178
66	(Ada)-GGC*RGDPC*	69

Peptide #	Sequence	IC_{50},μM
67	(Ada)-GGC*RGDVC*	148
68	(Ada)-C*GRGDS-(3-thioPro)C*-NH_2	421
69	(Mpr*)-RGDY-(3-thioPro)C*-NH_2	102
70	C*RGD(TA)C*	190
71	C*RGD(CHA)C*	160
72	(AdaCA)C*GRGDS-(3-thioPro)C*	161
73	C*RGD(p-nitro-Phe)PC*	20
74	C*RGDFHypC*	655
75	C*RGD(p-chloro-Phe)PC*	12
76	C*RGDF(d-Nal)C*	428
77	RC*DFPC*	622
78	C*RGD(p-fluoro-Phe)-(3-thioPro)C*	22
79	(1-FCA)RC*D(p-fluoro-Phe)-(3-thio)-ProC*	249
80	(HCA)RC*D-(3-thioPro)C*	69
81	(Ada)-(AMBA)-C*RGDY-(3-thioPro)-C*-NH_2	179
82	(Ada)-(AnB)-C*RGDY-(3-thioPro)-C*NH_2	68
83	(Ada)-(AnC)-C*RGDY-(3-thioPro)-C*NH_2	84
84	(Ada)-GGC*RGDF-(3-thioPro)-C*-NH_2	15
85	(QC)GGC*RGDF-(3-thioPro)-C*-NH_2	138
86	(HCA)-GGC*RGDF-(3-thioPro)-C*-NH_2	107
87	(PhAc)-GGC*RGDF-(3-thioPro)C*-NH_2	117
88	(NaphAc)-GGC*RGDF-(3-thioPro)C*-NH_2	83
89	C*RGD(p-chloro-Phe)-(3-thioPro)C*-NH_2	15
90	(Ada)-GGC*RGD(p-chloro-Phe)-(3-thioPro)C*-NH_2	5
91	(1-FCA)-GGC*RGD(p-chloro-Phe)-(3-thioPro)-C*	37
92	(CPA)-CC*RGDFPC*	55
93	(NACA)GGC*RGDYPC*	116
94	(NBAC)GGC*RGDYPC*	155
95	(Ada)-GGC*RGDY(1,1-ACC)C*	301
96	(Ada)-GGC*RGDY(p-OCH_3-Tyr)PC*-NH_2	9
97	(Ada)-GC*RGDYPC*-NH_2	5
98	(FMOC)RC*D-(3-thioPro)C*	16
99	RC*D(Hyp)C*	41
100	RC*DFC*	438
101	RC*D(TA)C*	258
102	RC*D(CHA)C*	182
103	RC*DYC*	318

Peptide #	Sequence	IC_{50}, μM
104	RC*DVC*	35
105	RC*D(L-Nal)C*	463
106	RC*EPC*	23
107	RC*D-(3-thioPro)C*	4
108	(Ada-CA)-RC*D-(3-thioPro)C*	4
109	(NACA)-RC*D-(3-thioPro)C*	16
110	(CPA)-RC*D-(3-thioPro)C*	8
111	(9-FCA)-RC*D-(3-thioPro)C*	14
112	(3-Me-Ada)-RC*D-(3-thioPro)C*	41
113	RC*D-(3-thioPro)C*	66
114	(FMOC)-R(HomoC)*D-(3-thioPro)C*	78
115	HomoRC*D-(3-thioPro)C*	100
116	C*RGDLPC*	210
117	C*RGDY-(3-thioPro)-Pen*-NH_2	249
118	Pen*RGDY-(3-thioPro)-Pen*-NH_2	121
119	C*VRGDVC*	561
120	(FMOC)-RC*D(DTC)C*	10
121	RC*D(DTC)C*	10
122	RC*D(TTC)C*	6
123	(Ada)-RC*E-(3-thioPro)C*	164
124	(AdaCA)-RC*E-(3-thioPro)C*	47
125	RC*E-(3-thioPro)C*	10
126	(Ada)-GGC*(HomoR)GD(p-chloro-Phe)-(3-thioPro)C*	272
127	(Ada)-GG(HomoC*)RGD(p-chloro-Phe)-(3-thioPro)C*	10
128	HomoC*RGD(p-chloro-Phe)-(3-thioPro)C*-NH_2	96
129	K(AnB)-(AnB)-RC*D-(3-thioPro)C*	66
130	K(AnC)RC*D-(3-thioPro)C*	33
131	(FMOC)-R(HomoC)*D-(3-thioPro)-HomoC*	245
132	(1-FCA)RC*D(TTC)C*	92
133	(FMOC)RC*D(MTC)C*	387
134	(1-FCA)C*RGDF-(3-thioPro)C*	106
135	(FMOC)RC*DNAC*	66
136	C*RGD(CH_3O-Tyr)PC*	108
137	(Ada)-GGC*RGD-(3-thioPro)C*	4
138	C*RGD-(p-chloro-Phe)(DTC)-C*-NH_2	10
139	C*RGD-(p-chloro-Phe)(TTC)-C*-NH_2	52

In addition, the following compound was tested and found to have an IC_{50} of 251 μM.

140  251

The following compounds of Table 7 have also been synthesized and tested in the cell adhesion inhibition assay described above. Specific activity levels were not established inasmuch as the IC_{50} of the compounds was determined to be in excess of 1.5 mg/ml. Thus, although such compounds are believed to be active as cell adhesion modulators at higher dosage levels, they are presently not as highly preferred as the compounds exemplified above.

TABLE 7

ADDITIONAL COMPOUNDS[2]

Sequence

(Pen*)LRGDSPC*
Ac-GC*LRGDSPC*-NH_2
(Pen*)LRGDSP(Pen*)-NH_2
C*GRGDC*
C*GRGDSC*
C*(norVal)RGDSPC*
C*IRGDSPC*
YC*LRGDSPC*-NH_2
LC*LRGDSPC*-NH_2
FC*LRGDSPC*-NH_2
LC*LRGDSPC*
FC*LRGDSPC*

[2] See notes to Table 6.

YC*LRGDSPC*
(d-Nal)C*LRGDSPC*
C*GRGD(Sar)PC*
C*GRGDS(Hyp)C*
C*GRGDS(homoP)C*
C*GRGDS(Dhp)C*
C*(dR)G(dD)(dS)(dP)C*
C*TRYRGDQDATMSC*
C*(Nic-Lys)GDSPC*
C*FRGDSPC*
R*GDSPD*-NH_2
Ac-D*RGDSPK*-NH_2
C*RGD(d-Phe)-PC*
C*RGD(d-p-chloro-Phe)-PC*
C*RGEYPC*
RC*DPC*-NH_2
(FMOC)-RC*D(1,1-ACC)C*
C*PRGDPC*
YIGSRC*DDC*
(FMOC)-RC*DPC*
(Ada)-C*RGDPC*
C*RGD-(p-chloro-Phe)(MTC)-C*-NH_2
RC*D(dNal)C*
WRC*DNC*
(FMOC)-RC*DWC*
C*RGD(d-Nal)C*
C*RGD(l-Nal)C*
(Ada)-C*RGESPC*
(Ada)-C*GRESPC*-NH_2
C*RGESPC*
C*GRG-SPC*-NH_2
RC*D(MTC)C*
RC*D(TC)C*
C*(HomoR)GDF-(3-thioPro)C*-NH_2
R(dC)*DP(dC)*
N-Me-ArgC*D-(3-thioPro)C*-NH_2
R*GDC-NH-$(CH_2)_3$-CO*

Thus, an aspect of the present invention is to provide compounds having extraordinarily high potencies in modulating cell adhesion to integrin receptors, including specifically inhibition of cell adhesion to the fibronectin receptor. The invention also includes methods for obtaining (either *in vitro* or *in vivo*) such fibronectin receptor adhesion inhibition, and integrin receptor adhesion inhibition. The disclosed compounds accomplish strong inhibition at desirably low concentrations, with an IC_{50} of less than about 500 μM, or alternatively less than about 100 μM. Likewise, another aspect of the invention is to provide such compounds, and such methods for obtaining integrin receptors adhesion inhibition, with IC_{50} potency levels at least as low as about 1 μM, and alternatively at least as low as about 4 μM.

EXAMPLE 10

Cell Adhesion Inhibition Assay II

The following assay established the activity of the present compound in inhibiting cell-cell adhesion in a representative *in vitro* system. This assay measures the adhesive interactions of T-cells to endothelial cell monolayers in the presence of a test compound. The test compound is added in increasing concentrations with T-cells and this is added to endothelial cell monolayers. The plates are incubated, washed and the percentage of attached cells is quantitated. The assay directly demonstrates the anti-cell adhesion activity and modulatory activity of the present compounds.

Human umbilical vein endothelial cells were purchased from Clonetics (San Diego, CA) at passage number 2. The cells were grown on 0.5% porcine skin gelatin pre-coated flasks (Sigma, St. Louis, MO) in EGM-UV media (Clonetics, San Diego, CA) supplemented with 10% fetal bovine serum. Cells were refed every 2-3 days

reaching confluence by day 4 to 6. The cells were monitored for factor VIII antigen and our results showed that at passage 12, the cells were positive for this antigen. The endothelial cells were not used following passage 14-15.

The T-cell line Jurkat was obtained from American Type Tissue Culture Collection (Rockville, MD). The cells were cultured in RPMI media containing 10% fetal calf serum. The cells were washed twice with Hank's Balanced Salt Solution (HBSS) and resuspended in Dulbecco's Minimal Eagle's Media (DMEM) containing 2.5 mg/ml Human Serum Albumin (HSA). The Jurkat cells ($1x10^6$ cells/ml) were stained with 10 μg/ml fluorescein diacetate (Sigma, St. Louis, MO) in HSSS containing 5% fetal calf serum. The cells were stained for 15 minutes in the dark at room temperature, washed 2 times, and resuspended in a DMEM-HSA solution.

Confluent endothelial monolayers grown in 96-well tissue culture plates were stimulated for 4 hours at 37°C with 0.1 ng/ml (~50 U/ml) recombinant IL-1 (Amgen, Thousand Oaks, CA). Following this incubation, the monolayers were washed twice with HBSS and 0.1 ml of DMEM-HSA solution was added. Jurkat cells (5×10^5 cells) were combined with appropriate concentrations of a peptide to be tested and 0.1 ml of the Jurkat-peptide mixture were added to the endothelial cell monolayers. Generally, 200 and 10 μM peptide concentrations were tested. With some representative peptides IC50 was determined by testing the peptides at 200, 40, 8, 1.6, 0.32, and 0.064 μM. The plates were placed on ice for 5 minutes to allow for Jurkat cell settling and then incubated at 37°C for 20 minutes. Following this incubation, the monolayers were washed twice with PBS containing 1 mM calcium chloride and 1 mM magnesium chloride and the plates were read using a Pandex Fluorescence Concentration Analyzer (Baxter, Mundelein, IL). Fluorescence in each well was measured as arbitrary fluorescence units and

percent adhesion in the absence of a peptide was adjusted to 100% and the percentage adhesion in the presence of the peptide was calculated. Monolayers were also fixed in 3% paraformaldehyde and evaluated microscopically to verify adhesion.

Results

Table 8 shows the results of the cell adhesion inhibition assay using the Jurkat-Endothelial cells. Potency is expressed in terms of percentage inhibition at 200 μM and 10 μM concentrations of the tested peptides. Where peptides are determined to be very active, IC_{50} (expressed as μM units) are also given.

TABLE 8

ACTIVITY OF COMPOUNDS IN THE JURKAT-ENDOTHELIAL CELL ADHESION ASSAY

PEPTIDE #	SEQUENCE	200 μM%	10 μM%	IC_{50} μm
14	C*YRGDSPC*	21	31	4
23	(Ada)-C*GRGDSPC*	9		4
42	(FMOC)-RC*DPC*	12	41	17
141	C*LRGESPC*	17	60	
142	C*LRGDTPC*	25	98	
143	C*VRGDSPC*	16	64	
144	(Ada)-C*RGDSPC*	49	95	
145	(FMOC)-RC*DSPC*	34	81	
146	(FMOC)-RC*D-(3-thioPro)C*	22	24	3
147	RC*D-(3-thioPro)C*	15	57	41
148	(Ada)-RC*DPC*	29	58	22
149	(FMOC)-RC*D(Hyp)C*	16	34	16
150	(Ada-CA)-RC*DPC*	37	44	39
151	(Ada)-RC*DFC*	22	83	
152	(Ada)-RC*DNC*	68	72	
153	(Ada)-C*GRGDS-(3-thioPro)C*	14	22	1

PEPTIDE #	SEQUENCE	200 μM%	10 μM%	IC_{50} μm
154	(FMOC)-RC*EPC*	10	42	4
155	(FMOC)-RC*DNC*	20	96	
156	(FMOC)-RC*(DTC)C*	32	90	
157	(Ada)-C*GRGES-(3-thioPro)C*	21	30	
158	(Ada-CA)-C*GRGES-(3-thioPro)C*	14	57	
159	(Ada)-C*GRGESPC*	20	47	
160	(Ada)-(HomoR)C*D-(3-thioPro)C*	13	40	
161	(FMOC)-RC*D-(3-thioPro)(HomoC*)	35	53	
162	(NaphAc)-RC*D-(3-thioPro)C*	37	52	
163	(9-FA)-RC*D-(3-thioPro)C*	12	16	0.7
164	(1-FCA)-RC*D-(3-thioPro)C*	9	16	0.09
165	(FMOC)-RC*E-(3-thioPro)C*	12	30	2
166	(FMOC)-RC*D(TTC)C*	11	18	0.9

Thus, another aspect of the present invention is to provide compounds having high potencies in modulating leukocyte adhesion to endothelial cells.

In one regard, the present invention includes compounds having an IC_{50} of less than about 200 μM as established in a Jurkat-Endothelial cell adhesion assay; and in another regard, the invention includes compounds having an IC_{50} of less than about 10 μM in such assay. The invention also includes methods for obtaining (either *in vitro* or *in vivo*) such leukocyte receptor adhesion inhibition. The disclosed compounds accomplish strong inhibition at low concentrations, with an IC_{50} of less than about 200 μM, or alternatively less than about 10 μM.. Likewise, a further aspect of the invention is to provide such compounds, and such methods for obtaining leukocyte receptors adhesion inhibition, with IC_{50} potency levels at least as low as about 0.1 μM, and alternately at least as low as about 50 μM.

PREPARATION 1

(d,l)-Thiozolidine-2-carboxylic Acid

(TC)

A solution consisting of cysteamine hydrochloride (56.8 g, 0.5 mole), triethylamine (50.6 g, 0.5 mole), glyoxylic acid (37 g, 0.4 mole), and 150 ml ethanol was refluxed for 2.5 hrs. Upon completion of reaction, the reaction mixture was cooled to collect solids. The solids were washed with ethanol and recrystallized from boiling water (~100 ml) to afford the title product which was washed with water and dried *in vacuo* at 40°C. The yield of pure product amounted to 25 g (47%): mp 195°C (dec); Anal. Calcd. for $C_4H_7N_1S_1O_2$: C, 36.07; H, 5.29; N, 10.52. Found: C, 36.16; H, 5.34; N, 10.53.

PREPARATION 2

l-2-Methylthiazolidine-4-carboxylic Acid

(MTC)

To l-cysteine (hydrochloride monohydrate; 10 g, 63.45 mmole) dissolved in 35 ml water was added acetaldehyde (4 ml, 71.5 m mole) at ice-bath temperature. After stirring in the cold for 2 days, pyridine (10 ml) and ethanol 950 ml) were added to the reaction mixture. The resultant mixture was further allowed to stand at room temperature for two days. The mixture was diluted with ethanol (100 ml) and upon standing overnight, the title compound (3.6 g) was obtained as a gelatinous product. The material was dried *in vacuo* at 40°; m.p. 145-152°C.

PREPARATION 3

N-BOC-(d,l)-thiazolidine-2-carboxylic acid

To a solution consisting of thiazolidine-2-carboxylic acid of preparation 1 (5 g, 37.5 m mole), 70 ml water, 37 ml 1NNaOH, and 70 ml dioxane, was added di-t-butyl-dicarbonate $(BOC)_2O$(8.2 g, 37.5 mmole) diluted with 30 ml dioxane. The mixture was stirred at ambient temperature for 4 hrs, with the pH being maintained between 8 and 9. Additional $(BOC)_2O$ (3.75 mmole) was added to the mixture. Stirring continued for 16 hr. at pH 9. The reaction mixture was concentrated to ½ volume and extracted with hexane (x2). The organic extract was discarded and the aqueous phase (chilled with ice) was acidified to pH 3 with I N $NaHSO_4$. The acidified solution was then extracted with ethyl acetate (x3). The ethyl acetate extract was washed with water (x3), dried over anhydrous $MgSO_4$ and filtered. After concentration of the solvent, there was obtained the title compound as a white powder. The yield of product amounted to 6.9 g (79%). m.p. 84-86°C. Anal. Calcd for $C_9H_{15}N_1O_4Si$; C, 46.39; H, 6.43; N, 6.00. Found: C, 46.46; H, 6.53; N, 6.03.

PREPARATION 4

N-BOC-(l)-5,5-dimethylthiazolidine-4-carboxylic Acid (N-BOC-DTC)

The method of Preparation 3 was repeated, except that l-5,5-dimethylthiazolidine-4-carboxylic acid (available from Sigma Chemicals) was used as the starting material in lieu of (d,l)-thiazolidine-2-carboxylic acid. The title compound was thus prepared, m.p. 104-108°C. Anal. Calcd for $C_{11}H_{19}N_1O_4S_1$: C, 50.54; H, 7.27; N, 5.35. Found: C, 50.69; H, 7.35; N, 5.38.

PREPARATION 5

N-BOC-(l)-2-methylthiazolidine-4-carboxylic Acid

The method of preparation 3 was repeated, except that l-2-methylthiazolidine -4-carboxylic acid (Preparation 2) was used as the starting material in lieu of (d,l)-thiazolidine-2-carboxylic acid. The title compound was thus prepared, m.p. 93-96°C. Anal. Calcd for $C_{10}H_{17}N_1O_4S_1$: C, 48.55; H, 6.93; N, 5.66. Found: C, 48.68; H, 6.96; N, 5.74.

PREPARATIONS 6-9

N,N (or N')-Dialkyl-l-arginines

l-Ornithine HCl (1 eq.), 1 eq. S-methylisothiouronium salts (Schenck, M., *Archiv. der Pharmazie*, 1911, 249, 478) or 1 eq. of (alkyl- or dialkyl amino)-(alkylimino)methanesulfonic acid (Arzeno, H.B. *et al., Syn. Com.*, 1990, 20, 3433; Maryanoff, C.A. *et al., J. Org. Chem.*, 1986, 51, 1882), 2 eq. 1N NaOH were combined and, after dissolution, the mixture was allowed to stand at room temperature for 5-10 days. The reaction progress was monitored by tlc (methanol/ammonium hydroxide 1:1) and leveled off at approximately 90% reaction. The reaction mixture was diluted with 1 vol. water, adjusted to pH 5 with aq HCl and applied to a column of Amberlite IR-120 H+ resin (5 eq.). The column was washed with water until neutral or negative to chloride and sulfate test. The amino acids were eluted following the sequential treatment with 2 column volumes each of 0.15 N and 0.25 N ammonium hydroxide, then with 3 column volumes of 0.3 N ammonium hydroxide. Fractions were taken starting when the ammonia front exits the column. Early fractions were a mixture of both Ornithine and the product. Later fractions contained pure products. The purification was followed by tlc. The fractions containing pure product were

combined, concentrated *in vacuo* at 40°C and freeze dried. This hygroscopic material was crystallized as follows: The amino acid was dissolved in the minimum amount of HCl or acetic acid and diluted with 1 volume of ethanol. Acetone was then added slowly to turbidity (2-3 vol.). The solution was cooled and the product was collected, washed with acetone and dried under vacuum. The title compounds were thus prepared.

N-BOC Protection:

The method of preparation 3 was repeated, except that the various alkylated arginines were used as the starting materials. BOC-protected amino acids corresponding to the title compounds were obtained. These were N,N-dimethyl-l-arginine, N,N'-dimethyl-l-arginine, and N,N'-diethyl-l-arginine.

The foregoing examples are given to enable those skilled in the art to more fully understand and practice the present invention. They should not be construed as a limitation upon the scope of the invention, which is set forth in the appended claims, but merely as being illustrative and representative thereof.

CYCLIC CELL ADHESION MODULATION COMPOUNDS

CLAIMS

1. A compound of the formula

$$X^2 - (X^1 -) \overset{\overset{\Large Z}{\frown}}{L1 - 1 - 2 - 3 - 4 - 5 - 6 - L2} (- Y^1) - Y^2$$

and pharmaceutically acceptable salts thereof, wherein

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid analog or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety or bond between L^1 and L^2;

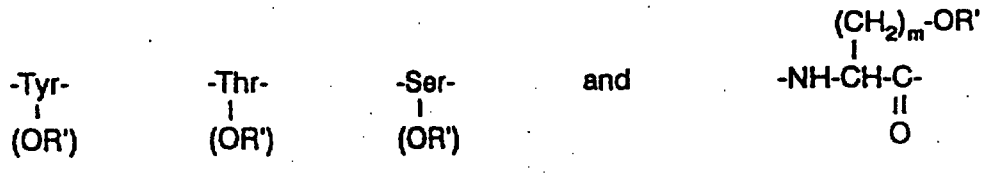
1 is optional and, where present, is selected from Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TTC, TCA, DTC, MTC, TC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, d-Nal, l-Nal, Sar and (Ada)-Ala;

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N,N'-dialkylArg, N,N,N'-trialkylArg, wherein the alkyl has from one to four carbon atoms;

3 is selected from Gly, and Sar;

4 is selected from Asp, Glu, and the alkyl, aralkyl, aryl esters of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m- and p-halo-Phe, p-nitro-Phe, Leu



wherein m is 2, 3 or 4;

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* C L M S *

6 is optional and, where present, is selected from Pro, 3-thioPro, 1,1-ACC, Dhp, DTC, TTC, TC, MTC, TCA, Hyp, homoPro and Phe, *p*-halo-Phe, *p*-nitro-Phe, TA, d-Nal, l-Nal, isonipecotic acid;

X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids and amino acid analogs;

X^2 is an optional N^{α}-substituent selected from R' (including hydrogen) and R'CO-;

Y^2 is an optional carboxyl-terminal substituent selected from -OR' (including hydroxyl), $-NR'_2$ (including $-NH_2$ and -NHR'), $'NHNH_2$ and -SR';

and wherein each R' is individually a member selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of $-NR'_2$, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

2. A compound of claim 1 wherein L^1 and L^2 are each Cys or homoCys, and cyclization is achieved through a disulfide bond.

3. A compound of claim 1 wherein L^1 and L^2 each provide sulfur-containing functional groups, and Z is a hydrocarbon bridge between said sulfur-groups.

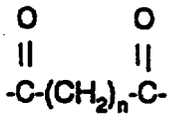
4. A compound of claim 1 wherein Z is a diketo, a diamino or a keto-amino moiety.

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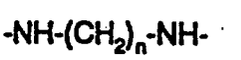


5. A compound of claim 4 wherein Z is of the form



wherein n is from 1 to 8.

6. A compound of claim 4 wherein Z is of the form



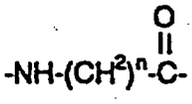
wherein n is an integer of from 1 to 8.

7. A compound of claim 1 wherein the linkage between L^1 and L^2 is a monosulfide linkage.

8. A compound of claim 1 wherein L^1 is directly bonded to L^2 via an amide bond.

9. A compound of claim 1 wherein one or both of L^1 and L^2 is an amino acid mimetic structure.

10. A compound of claim 9 wherein said amino acid mimetic structure is



and n is an integer of from 1 to abouf 8.

11. A compound of claim 1 wherein the sequence 1-2-3-4-5-6 is of the structure:

Leu-Arg-Gly-Asp-Ser-Pro or Arg-Gly-Asp-Phe-3-thioPro

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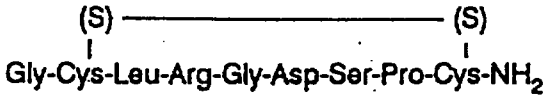


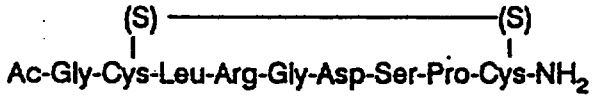


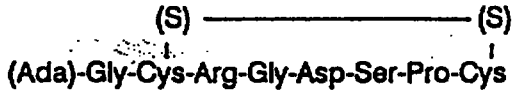
12. A compound of claim 1 wherein X_2 is selected from the group consisting of 1-adamantaneacetyl, 1-admantanecarbonyl, 9-fluoreneacetyl, 9-fluorenecarbonyl, 1-fluorenocarbonyl, fluorenylmethyloxycarbonyl, 3-noradamantanecarbonyl, 3-methyladmantane acetyl, 2-norbornaneacetyl, phenylacetyl, 1-naphthylacetyl, hydrocinnamyl, quinaldyl, cyclohexylacetyl, and 3-mercaptopropionyl.

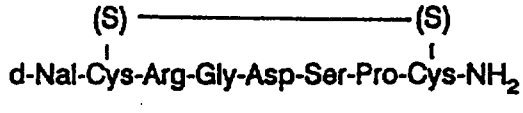
13. A compound of claim 1 wherein X_1 and Y_1 are each selected from the group consisting of l-3-(2'-naphthyl)-alanine, l-3-(2'-naphthyl-)alanine., AMBA, AnC, AnB and ω-amino-lower alkyl carboxylic acids.

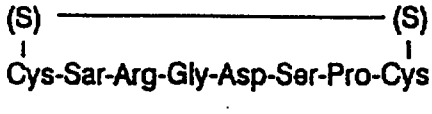
14. A compound of claim 1 selected from the group consisting of







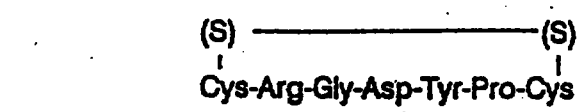


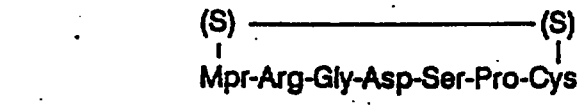


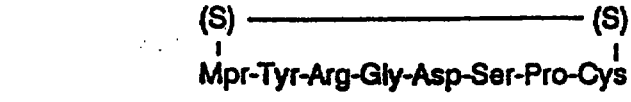
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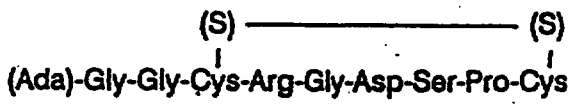


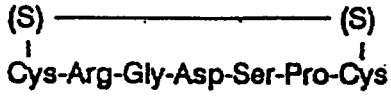


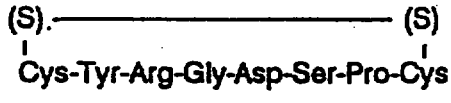


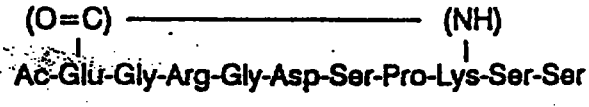


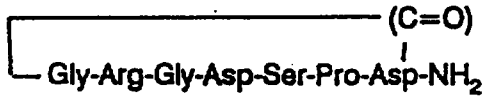


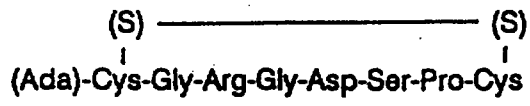








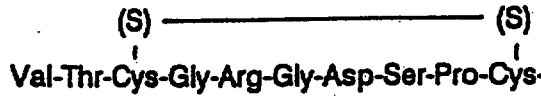




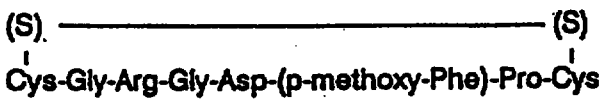
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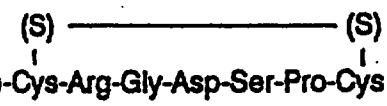


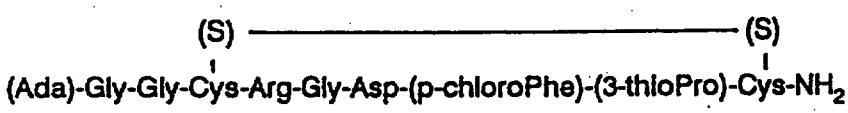


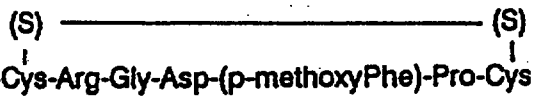


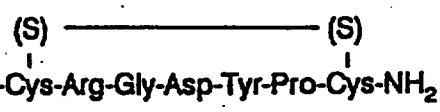




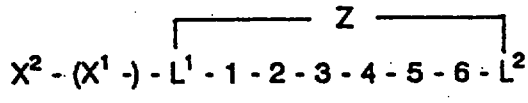








15. A compound of the formula



and pharmaceutically acceptable salts thereof, wherein:

* C L M S *

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid derivative or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety or bond between L^1 and L^2;

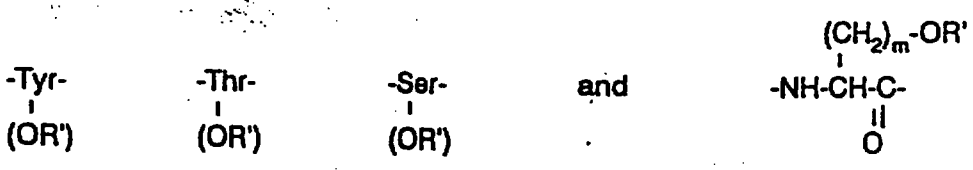
1 is optional, and where present, is of reversed orientation and is selected from Sar, Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TC, TCA, DTC, MTC, TTC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, L-Nal, D-Nal and (Ada)-Ala;

2 is of reversed orientation and is selected from Arg, homoArg, p-amino-methyl-Phe, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N,N,N'-dialkylArg, N,N'-trialkylArg wherein the alkyl has one to four carbon atoms;

3 is a moiety suitable for linking reversed-orientation residue 2 with residue 4;

4 is selected from Asp, Glu, and the lower alkyl, aralkyl, aryl esters, O-Fm esters, O-cyclohexyl esters, O-benzyl esters of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Leu



wherein m is 2, 3 or 4;

6 is optional and, where present, is selected from Pro, 3-thioPro, DTC, TCC, TC, MTC, TCA, 1,1-ACC, Dhp, Hyp, homoP, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, TA, d-Nal, isonipecotic acid;

* C L M S *

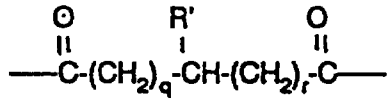
X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids and amino acid analogs;

X^2 is an optional substituent selected from R'_2N- (including R'HN- and H_2N-), R'O- (including hydrnoxyl), H_2NNH- and R'S-;

Y^2 is an optional carboxyl-terminal substituent selected from -OR' (including hydroxyl), $-NR'_2$ (including $-NH_2$ and 'NHR'), $'NHNH_2$ and 'SR';

and wherein each R' is individually a member selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of $-NR'_2$, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

16. A compound of claim 15 wherein residue 3 is a diketo molety of the form



wherein q and r are independently integers of 0, 1 or 2;

17. A compound of claim 16 wherein R' is hydrogen.

18. A compound of claim 15 wherein residue 2 and, if present, residue 1 are of the d-form.

19. A compound of claim 15 wherein one or both of L^1 and L^2 comprises a diamino molety.

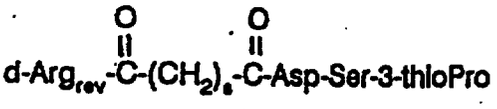
* C L M S *

20. A compound of claim 19 wherein L^1 and L^2 together comprise a diamino moiety of the form

$$-NH-(CH)_t-NH-$$

wherein t is 1, 2 or 3.

21. A compound of claim 15 wherein the sequence 2-3-4-5-6 is of the structure



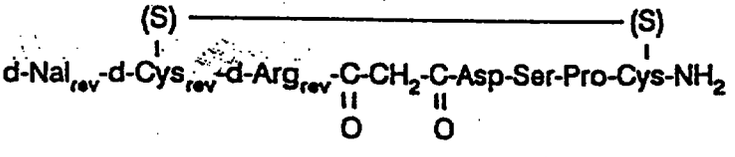
wherein s is an integer of from 1 to about 5.

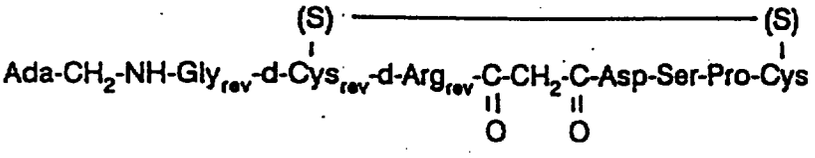
22. A compound of claim 15 wherein the cyclizing linkage between L^1 and L^2 comprises a structure selected from the group consisting of disulfide bonds, monosulfide bonds, amide bonds, and structures of the form

$$-S-(CH_2)_n-S-$$

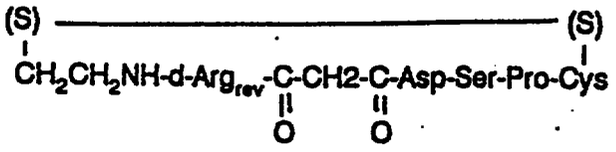
wherein n is an integer of from 1 to about 8.

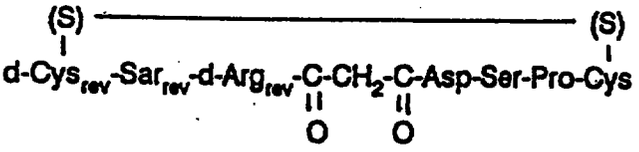
23. A compound of claim 15 selected from the group consisting of



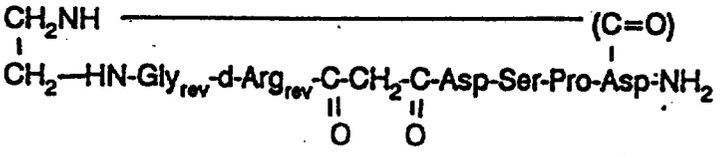




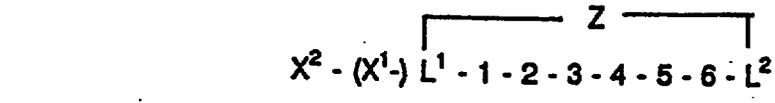




and



24. A compound of the formula



$$X^2 - (X^1-) L^1 - 1 - 2 - 3 - 4 - 5 - 6 - L^2 (-Y^1) - Y^2$$

and pharmaceutically acceptable salts thereof, wherein:

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid derivative or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety between L^1 and L^2;

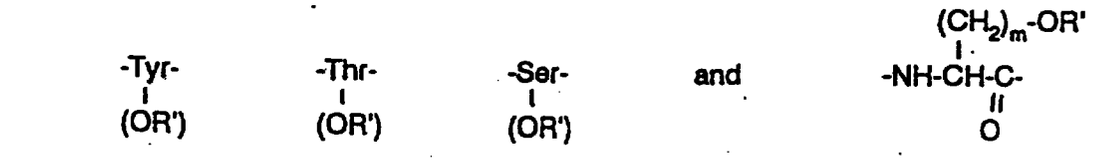
1 is optional and, where present, is selected from Leu, Tyr, Phe, Ile, Pro, 3-thioPro, TC, TCA, DTC, MTC, TTC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, l-Nal, d-Nal, (Ada)-Ala and Sar;

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N'-dialkylArg, N,N,N'-trialkylArg wherein the alkyl has one to four carbons;

3 is a moiety suitable for linking residue 2 with reversed-orientation residue 4;

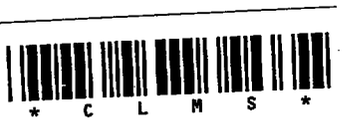
4 is of reversed orientation and is selected from Asp, Glu, the lower alkyl, aralkyl, aryl esters O-Fm esters, O-cyclohexyl esters, O-benzyl esters of the foregoing two amino acids;

5 is optional and, where present, is of reversed orientation and is selected from Ser, Thr, Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Leu



-Tyr-(OR') -Thr-(OR') -Ser-(OR') and -NH-CH(-$(CH_2)_m$-OR')-C(=O)-

wherein m is 2, 3 or 4;



6 is optional and, where present, is of reversed orientation and is selected from Pro, 3-thioPro, DTC, TCC, TC, MTC, TCA, 1,1-ACC, Dhp, Hyp, homoPro, Phe, p-halo-Phe, p-nitro-Phe, TA, d-Nal; l-Nal, and isonipecotic acid;

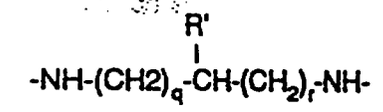
X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids and amino acid analogs;

X^2 is an optional N^{α}-substituent selected from R' (including hydrogen) and R'CO-;

Y^2 is an optional substituent selected from -R' (including hydrogen) and -COR';

and wherein each R' is individually a member selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8 lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls and C_3-C_{14} cycloalkyls, and, in the case of $-NR'_2$, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

25. A compound of claim 24 wherein residue 3 is a diamino moiety of form



wherein q and r are independently integers of 0, 1 or 2.

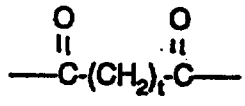
26. A compound of claim 25 wherein R' is hydrogen.

27. A compound of claim 24 wherein residues 4 and, if present, residues 5 and 6 are of the d-form.



28. A compound of claim 24 wherein one or both of L^1 and L^2 comprises a diketo moiety.

29. A compound of claim 28 wherein L^1 and L^2 together comprise a diketo moiety of the form



wherein t is 1, 2 or 3.

30. A compound of claim 24 wherein the sequence 2-3-4-5-6 is of the structure

$$Arg\text{-}NH\text{-}(CH_2)_s\text{-}NH\text{-}d\text{-}Asp_{rev}\text{-}d\text{-}Ser_{rev}\text{-}d\text{-}Pro_{rev}$$

wherein s is an integer of from 1 to about 5.

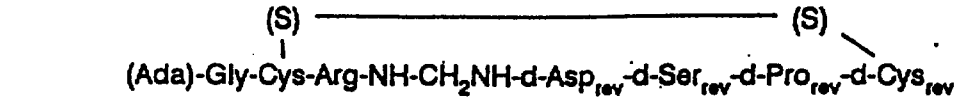
31. A compound of claim 24 wherein the cyclizing linkage between L^1 and L^2 comprises a structure selected from the group consisting of disulfide bonds, monosulfide bonds, amide bonds, and structures of the form

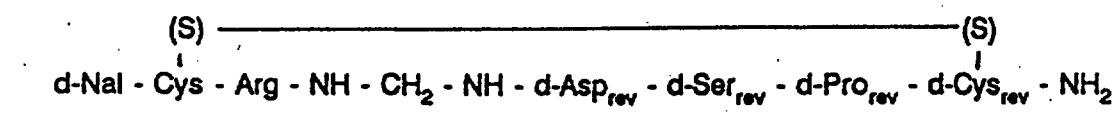
$$-S\text{-}(CH_2)_n\text{-}S-$$

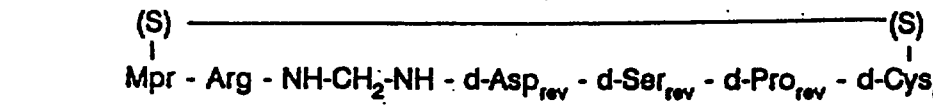
wherein n is an integer of from 1 to about 8.

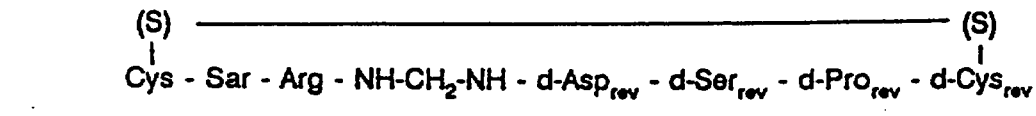
* C L M S *

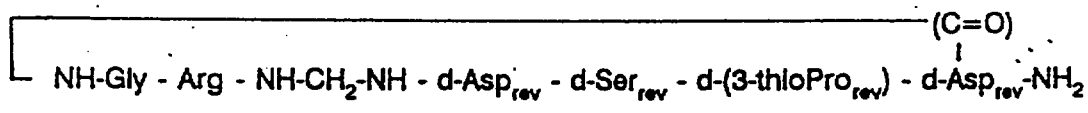
32. A compound of claim 24 selected from the group consisting of



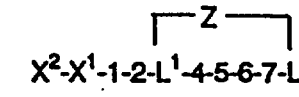








33. A compound of the formula



and pharmaceutically acceptable salts thereof, wherein

L^1 and L^2 are each, or are together, a residue of an amino acid, an amino acid analog or an amino acid mimetic having a functional group suitable for the formation of a cyclizing bridge between L^1 and L^2;

Z is a cyclizing moiety or bond between L^1 and L^2;

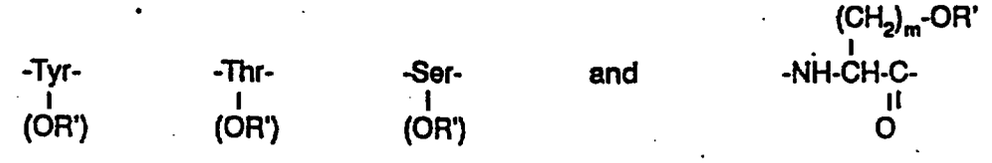
1 is optional and, where present, is selected from Leu, Sar, d-Nal, l-Nal, Tyr, Phe, Ile, Pro, 3-thioPro, TTC, TCA, DTC, MTC, TC, Gly, Ala, Val, norLeu, norVal, β-Ala, Trp, and (Ada)-Ala;

* C L M S *

2 is selected from Arg, homoArg, nitroArg, norArg, p-aminomethyl-Phe, N-alkylArg, N,N-dialkylArg, N-N'-dialkylArg, and N,N,N'-trialkylArg wherein the alkyl has one to four carbon atoms;

4 is selected from Asp, Glu, and the lower alkyl, aralkyl, aryl esters, O-Fm esters, O-cyclohexyl esters, O-benzyl esters, of the foregoing two amino acids;

5 is optional and, where present, is selected from Ser, Thr, Tyr, p-methoxy-Tyr, monohalo-Tyr, dihalo-Tyr, Trp, Ala, Val, Phe, o-, m-, and p-halo-Phe, p-nitro-Phe, Asn, Asp, Met



wherein m is 2, 3 or 4;

6 is optional and, where present, is selected from Pro, 3-thioPro, TA, DTC, TTC, TC, MTC, TCA, 1,1-ACC, Dhp, Hyp, homoPro, Phe, p-halo-Phe, p-nitro-Phe, Tyr, Val, d-Nal, l-Nal, CHA, Ser, Asn, Glu and Thr, o-, m-, p-halo-Phe, isonipecotic acid;

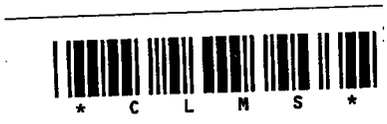
7 is optional and, where present, is Pro, 3-thioPro, TA, DTC, TTC, TC, MTC, TCA, Ala, Gly, Ser, Phe, and Leu.

X^1 and Y^1 are each optional and, where present, are independently selected from 1 to 4 d- or l-amino acids or amino acid analogs;

X^2 is an optional N^{α}-substituent selected from R'- (including hydrogen) and R'CO-; and

Y^2 is an optional carboxyl terminal substituent selected from -OR' (including hydroxyl), -NR'$_2$ (including -NH$_2$ and -NHR'), -NHNH$_2$ and -SR';

and wherein each R' is individually a member selected from hydrogen, from linear and branched, unsubstituted and substituted C_1-C_8



lower alkyls, C_2-C_8 alkenyls, C_2-C_8 alkynyls, C_6-C_{14} aryls, C_7-C_{14} alkaryls, C_7-C_{14} cycloalkaryls, and C_3-C_{14} cycloalkyls, and, in the case of -NR'_2, from cyclized groups forming (in an attachment with the nitrogen atom) a 5-8 membered heterocyclic ring optionally containing oxygen, nitrogen or sulfur as a further ring heteroatom.

34. A compound of claim 33 wherein L^1 and L^2 each provide a sulfur-containing functional group, and cyclization is achieved through a disulfide bond.

35. A compound of claim 33 wherein L^1 is Cys or homoC.

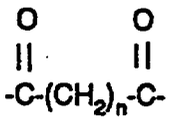
36. A compound of claim 33 wherein L^1 and L^2 each provide sulfur-containing functional groups, and Z is a hydrocarbon bridge between said sulfur groups.

37. A compound of claim 33 wherein the linkage between L^1 and L^2 is a monosulfide linkage.

38. A compound of claim 33 wherein Z is a diketo, a diamino or a keto-amino moiety.

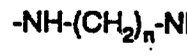
* C L M S *

39. A compound of claim 38 wherein Z is a diketo moiety of the form



wherein n is from 1 to 8.

40. A compound of claim 38 wherein Z is a diamino moiety of the form



wherein n is an integer of from 1 to 8.

41. A compound of claim 33 wherein L^1 is directly bonded to L^2 via an amide bond.

42. A compound of claim 33 wherein the sequence 1-2-L^1-4-5-6-L^2 is selected from structures of the form

Leu-Arg-Cys-Asp-Ser-Pro-Cys,

Arg-Cys-Asp-Ser-Pro-Cys,

Arg-Cys-Asp-Pro-Cys, and

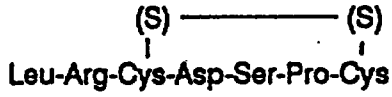
Arg-Cys-Asp-(3-thioPro)-Cys.

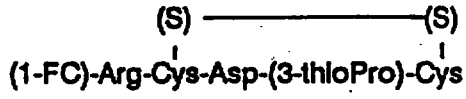
43. A compound of claim 33 wherein X_2 is selected from the group consisting of 1-adamantoneacetyl, 1-admantanecarbonyl, 9-fluoreneacetyl, 9-fluorenecarbonyl, 1-fluorenecarbonyl, fluorenylmethyloxycarbonyl, 3-noradamantanecarbonyl, 3-methyladmantaneacetyl, 2-norbornaneacetyl, phenylacetyl, 1-naphthylacetyl, hydrocinnamyl, quinaldyl, cyclohexylacetyl, and 3-mercaptopropionyl.

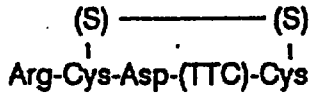
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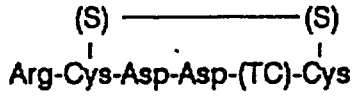
44. A compound of claim 33 wherein X1 is selected from the group consisting of l-3-(2'-naphthyl)-alanine, and l-3-(2'-naphthyl)alanine, AMBA, AnC, AnB, and ω-amino-lower alkyl carboxylic acids.

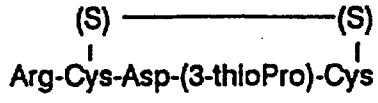
45. A compound of claim 33 selected from the group consisting of

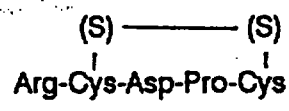




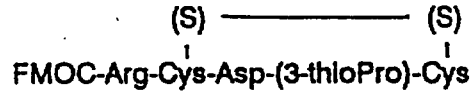








and



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46. A compound of claim 1 useful in modulating adhesion of a cell expressing a fibronectin receptor.

47. A compound of claim 15 useful in modulating adhesion of a cell expressing a fibronectin receptor.

48. A compound of claim 24 useful in modulating adhesion of a cell expressing a fibronectin receptor.

49. A compound of claim 33 useful in modulating adhesion of a cell expressing a fibronectin receptor.

50. A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 1.

51. The method of claim 50 wherein said cells express an integrin receptor.

52. The method of claim 50 wherein said cells express a receptor for fibronectin.

53. The method of claim 50 wherein said system is a mammalian system characterized by an excessive or inadequate level of cell adhesion.

54. A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 1, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions,

* C L M S *

adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions, reocclusion following thrombolysis, and allograft rejection.

55. A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 15.

56. The method of claim 55 wherein said cells express an integrin receptor.

57. The method of claim 55 wherein said cells express a receptor for fibronectin.

58. The method of claim 55 wherein said system is a mammalian system characterized by an excessive or inadequate level of cell adhesion.

59. A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 15, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions, reocclusion following thrombolysis, and allograft rejection.



60. A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 24.

61. The method of claim 60 wherein said cells express an integrin receptor.

62. The method of claim 60 wherein said cells express a receptor for fibronectin.

63. The method of claim 60 wherein said system is a mammalian system characterized by an excessive or inadequate level of cell adhesion.

64. A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 24, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions, reocclusion following thrombolysis, and allograft rejection.

65. A method of modulating cell adhesion comprising administering, in a system including cells expressing a receptor protein, an amount effective to modulate the adhesion of said cells of a compound of claim 33.

66. The method of claim 65 wherein said cells express an integrin receptor.

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67. The method of claim 65 wherein said cells express a receptor for fibronectin.

68. The method of claim 65 wherein said system is an animal system characterized by an excessive or inadequate level of cell adhesion.

69. A method of treating a mammalian condition comprising administering a therapeutically effective amount of a compound of claim 33, wherein said condition is selected from rheumatoid arthritis, asthma, allergy conditions, adult respiratory distress syndrome, inflammatory bowel diseases, ophthalmic inflammatory diseases, autoimmune diseases, thrombosis, platelet aggregation conditions, cardiovascular diseases, neoplastic diseases, wound healing conditions, and prosthetic implantation conditions, reocclusion following thrombolysis, and allograft rejection.

70. A compound of claim 1 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 500 μM.

71. A compound of claim 70 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 100 μM.

72. A compound of claim 15 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 500 μM.

73. A compound of claim 72 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 100 μM.

* C L M S *

74. A compound of claim 24 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 500 μM.

75. A compound of claim 74 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 100 μM.

76. A compound of claim 33 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 500 μM.

77. A compound of claim 76 having an IC_{50} in a U937 fibronectin adhesion assay of less than about 100 μM.

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