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(57) Abstract

Cyclized integrin receptor antagonists useful in modulating cell adhesion, including adhesion related to fibronectin, as well as leukocyte adhesion to endothelial cells, are disclosed. Methods for synthesizing, testing, formulating, and using the compounds as therapeutic agents are also disclosed.

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PEPTIDE INHIBITORS OF CELL ADHESION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is related to a PCT application published as WO 92/00995, published January 23, 1992 and hereby incorporated in its entirety by reference.

BACKGROUND OF THE INVENTION

Field of Invention

The present invention relates to novel cyclic peptides and peptidomimetic compounds which are characterized by cell adhesion modulation activity.

Description of Related Art

The extracellular matrix (ECM) is the major component of connective tissue which provides for structural integrity, promotes cell migration and differentiation. As part of these functions, extracellular matrix molecules such as fibronectin, collagen, laminin, von Willebrand factor, thrombospondin, fibrinogen, and tenascin have been shown to support adhesion of cells in vitro. This adhesive interaction is critical for a number of biological processes including hemostasis, thrombosis, wound healing, tumor metastasis, immunity and inflammation.

Fibronectin (FN) is the prototype ECM molecule. The major cell attachment site in the fibronectin molecule has been reproduced synthetically with the amino acid sequence arginine-glycine-aspartic acid, or RGD using single letter nomenclature. Peptides containing the RGD sequence which

either inhibit or promote cell adhesion have been described (US Patent Nos. 4,589,881;4,661,111; 4,517,686; 4,683,291; 4,578,079; 4,614,517; and 4,792,525). Changes in the peptide as small as the exchange of alanine for glycine or glutamic acids for aspartic acid, which constitute the addition of a single methyl or methylene group to the tripeptide, eliminates these activities (Pierschbacher et al, PNAS, 81:5985 (1984)). Recently, a second FN cell binding domain has been identified within the alternatively spliced region of the A chain of the molecule. A ten amino acid recognition sequence (GPEILDVPST) (SEQ. ID. NO.:1) in FN has been shown to be the site which interacts with cells (Wayner et al., J. Cell Biol., 109:1321 (1989); Guan et al., Cell, 60:53 (1990)).

The receptors which recognize these sites on FN belong to a gene superfamily called integrins which consist of heterodimeric complexes of non-covalently associated alpha and beta subunits. A common β subunit combines with unique α subunits to form an adhesion receptor of defined specificity. Eight beta subunits have been cloned and sequenced to date. The $\beta 1$ subfamily, also known as the VLA family (Very Late Activation Antigens), binds to ECM molecules such as FN, collagen and laminin. For reviews, see, Hynes, Cell, 48:549 (1987); Hemler, Annu. Rev. Immunol., 8:365 (1990). Leukocyte interaction with FN at the two spatially separate binding domains is mediated by two distinct integrins. The RGD site is recognized by the integrin $\alpha 5\beta 1$, while, EILDV (SEQ. ID. NO.:2) is recognized by $\alpha 4\beta 1$ (Pytela et al., Cell, 40:191 (1985); Wayner et al., J. Cell Biol. 109:1321 (1989); Guan et al, Cell 60:53 (1990)).

Vascular endothelial cells form the interface between blood and tissues and control the passage of leukocytes as well as plasma fluid into tissues. A variety of signals generated at the site of inflammation can activate both endothelial cells as well as circulating leukocytes so that they become more adhesive to one another. Following this

initial adhesion the leukocytes migrate into the tissues to perform host defense functions. Several adhesion molecules have been identified which are involved in leukocyte--endothelial interactions. On the leukocytes, members of the β2 integrin subfamily, which includes CD11a/CD18, CD11b/CD18, and CD11c/CD18, have been shown to play an important role in this process. In the β1 subfamily, in addition to binding to fibronectin, α4β1 interacts with a cytokine inducible molecule on endothelial cells termed vascular cell adhesion molecule (VCAM). Other molecules on endothelial cells which bind to the leukocytes include ICAM-1, ICAM-2, E-selectin and P-selectin (Carlos and Harlan, Immunol. Rev., 114:1 (1990); Osborn, L., Cell, 62:3 (1990); Springer T., Nature, 346:425 (1990); Geng et al., Nature, 347:757 (1990); Stoolman, L. Cell, 56:907 (1989)).

Recent data supports an important role for the integrin α4β1 in inflammation. *In vitro* data show that antibodies to α4 block adhesion of lymphocytes to synovial endothelial cells; this adhesion plays a potential role in rheumatoid arthritis (van Dinther-Janssen et al, J. Immunol., 147:4207 (1991)). Studies in which monoclonal antibodies to α4 block adhesion of basophils and eosinophils to cytokine activated endothelial cells (Walsh et al, J. Immunol., 146:3419 (1991); Bochner et al, J. Exp. Med., 173:1553 (1991)) imply a potential role of α4 in allergy and asthma. Additionally, *in vivo* studies have shown that experimental autoimmune encephalomyelitis can be blocked by anti-α4 monoclonal antibodies (Yednock et al., Nature, 356:63 (1992)). Migration of leukocytes to an inflammatory site can also be blocked by anti-α4 monoclonal antibodies (Issekutz et al., J. Immunol., 147:4178 (1991)). Lastly, in a model of contact hypersensitivity, peptides GRGDSP (SEQ. ID. NO.:3) or EILDV (SEQ. ID. NO.:2) block ear swelling when administered with sensitized cells into a challenged naive recipient mouse suggesting that both α4β1 and possibly α5β1 are involved in this inflammatory response (Ferguson et al., Proc. Natl. Acad. Sci. USA, 88:8072 (1991)). Thus, α4β1 and

$\alpha 5\beta 1$ are important receptor targets for control of inflammatory diseases.

SUMMARY OF THE INVENTION

The present invention relates to compounds having activity as cell adhesion modulators. The compounds do not contain the amino acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp or RGD), i.e., the RGD tripeptide epitope. In fact, some of the compounds do not have any of the three amino acids of the RGD epitope.

The compounds, in one aspect, sufficiently mimic extracellular 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, p95, 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 objective of the present invention is to provide novel compounds which act to modulate cell adhesion.

Another objective of the present invention is to provide novel non-RGD-containing compounds which are capable of binding to a cellular receptor which modulates cell adhesion.

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

Another objective of the present invention is to provide compounds which bind to a cellular adhesion molecule or integrin receptor.

Another objective of the present invention is to provide compounds having extraordinarily high potencies in modulating cell adhesion to integrin receptors, including 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 objective 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. Compounds with activity below 10 μM are most preferred, below 100 μM are not as preferred, below 500 μM lesser preferred and above 500 μM least preferred. 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 at low concentrations, with an IC_{50} of less than about 250μM, or alternatively less than about 50 μM.

Another object of the present invention is to provide novel compounds which modulate cell adhesion by binding to cell adhesion molecules or integrin receptors wherein said

compound is resistant to degradation *in vivo* due to inclusion of peptidomimetic residues, modified amino acids or D-isomers of amino acids.

Another objective 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 involve or 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, graft versus host disease, organ transplantation, septic shock, reperfusion injury, psoriasis, eczema, contact dermatitis and other skin inflammatory diseases, osteoporosis, osteoarthritis, atherosclerosis, neoplastic disease including metastasis of neoplastic or cancerous growth, wound healing enhancement, treatment of certain eye diseases such as detaching retina, Type I diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), inflammatory and immunoinflammatory conditions including ophthalmic inflammatory conditions and inflammatory bowel disease (e.g., ulcerative colitis and regional enteritis), and other autoimmune diseases.

The cell adhesion protein fibronectin (FN) has been implicated in the binding of capacitated sperm to oocytes (Fusi and Bronson, J. Androl., 13:28-35 (1992)). Thus, another object of the present invention is to provide compounds which may be used as contraceptives by inhibiting the binding of sperm to oocytes. The present invention also provides a possible means of diagnosing infertility resulting from defective adhesion of sperm to oocytes.

Another objective is to provide derivative compounds, such as, but not limited to, antibodies and anti-idiotype antibodies to the compounds disclosed in order to study, diagnose, treat or prevent the above-described diseases and conditions which relate to cell adhesion.

Another objective of the present invention is to provide a matrix which can be used to purify proteins, polysaccharides or other compounds which specifically bind to the cyclic peptides of the present invention with high affinity.

DETAILED DESCRIPTION OF THE INVENTION

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 a number of inflammatory diseases where inappropriate attachment of leukocytes leads to further injury of affected tissue. *In vitro* results show that such detrimental attachment, in which the leukocyte adheres to endothelial cells or to the extracellular matrix, is mediated by 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 inflammatory diseases including ARDS, asthma and rheumatoid arthritis.

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, 43:449 (1989). An RGD-containing peptide which would prevent cell adhesion to basement membrane components may be useful to prevent or eliminate metastasis. See, Humphries et al, Science, 223:469 (1986); Liotta, Cancer Res., 46:1 (1986); Roose, Biochem. Biophys. Acta., 738:263 (1986). A peptide or other compound with suitable affinity for RGD receptors should likewise have anti-metastasis utility.

Harmful blood clotting is also caused by increased cell adhesion. 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, 82:8057 (1985). 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 an anti-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 when 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 biocompatible surface to the prosthesis. Implantation of a prosthesis coated with a compound of the present invention would result in the prosthesis acquiring a covering of cells. This cell layer bound to the prosthesis would minimize rejection that might otherwise occur due to stimulation of the immune system by the prosthesis itself. As another example, coating of prosthetic devices which are used in connection with the circulatory system with a compound of the present invention which stimulates endothelial cell adhesion, especially on a surface exposed to blood flow, would enhance seeding of endothelial cells to form a layer on the blood-exposed surface of the device. When completely formed, the endothelial layer would prevent damage to blood cells often observed to be caused by non-endothelialized prostheses.

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.

Where such abbreviations for amino acids are used without an indication of enantiomeric structure, either the l- or d-enantiomers may suitably be utilized.

Additional abbreviations used herein include:

1,1-ACC: 1-Amino-1-cyclohexanecarboxylic acid
Ada: 1-Adamantaneacetic acid
(Ada)-Ala: β-Adamantylalanine
Ada-CA: 1-Adamantanecarboxylic acid
Aib: α-Aminoisobutyric acid (2-methylalanine)
β-Ala: β-Alanine (3-aminopropionic acid)
β-Asp: β-Aspartic acid
(β-CN)A: β-cyano-alanine
AMBA: 4-(Aminomethyl)benzoic acid
AnB: 4-Aminobutyric acid
AnC: 6-Aminocaproic acid
(AMP): 2-aminomethylpyridine
ARDS: Adult respiratory distress syndrome
BOC: tert-butyloxycarbonyl
[(3-Br)Tyr]: 3-bromo-tyrosine
BOP: benzotriazol-1-yl-tris(dimethylamino)-phosphonium hexafluorophosphate
BSA: Bovine serum albumin
CBO:cis-Bicyclo[3,3,0]octane-2-carboxylic acid
Cbz: Benzyloxycarbonyl
CHA: 3-(Cyclohexyl)-Alanine
CHAc: 3-Cyclohexylacetic acid
Chx: Cyclohexyl ester
Cl-Ala:chloroalanine
CPA: Cyclohexylphenylacetic acid
dA: D-alanine
DCC: dicyclohexylcarbodiimide
DCM: Dichloromethane
Dhp: 3,4-Dehydro-proline
DIEA: Diisopropylethylamine
DMEM: Dulbecco's Modified Eagle's Medium
DMF: Dimethylformamide
d-Nal: D-3-(2'-naphthyl)alanine
Dpr: diaminopropane
DTC: L-5,5-dimethylthiazoline-4-carboxylic acid
dV: D-valine
1-FCA:1-fluorenecarboxylic acid
9-FCA: 9-fluorenecarboxylic acid

9-FA:9-fluoreneacetic acid
5-FINC:5-fluoroindole carboxylic acid
Fm: Fluorenylmethyl ester
FMOC: Fluorenylmethyloxycarbonyl
FN: Fibronectin
GAC: Guanidine-acetic acid
3-Glu: Gamma-aminopentane-1,5-dioic acid
HCA: Hydrocinnamic acid
HOBt: 1-hydroxybenzotriazole
HomoC: Homocysteine
HomoP: homoproline
HomoR: homoarginine
HomoS: Homoserine
Hyp: 4-Hydroxyproline
ICAM-1: Intercellular adhesion molecule 1
IC_{50}: Inhibitory concentration, concentration at which adhesion is inhibited to 50% of control level
1-Nal: 1-3-(2'-Naphthyl)alanine
IPA: isopropyl alcohol
Isonipecotic acid: 4-piperidinecarboxylic acid
3-Me-Ada: 3-Methyl-1-adamantaneacetic acid
mono MeR: N-methyl-arginine
Mpr: 3-Mercaptopropionic acid (des-α-amino cysteine)
MTC: L-2-methylthiazolidine-4-carboxylic acid
NACA: 3-Noradamantanecarboxylic acid
Naph-Ac: 1-Naphthylacetic acid
NB-Ac: 2-Norbornaneacetic acid
Nic-Lys: Nicotinyl lysine
Nle: Norleucine
[(N-Me)R]:N-methyl arginine
norAda-CA: 3-Noradamantanecarboxylic acid
norArg: Norarginine ($H_2NC(=NH)NH(CH_2)_2CH(NH_2)CO_2H$)
Orn: Ornithine
O-Cys: Cysteic acid
Pen: Penicillamine (β,β-dimethylcysteine)
PhAc: Phenylacetic acid
PMP: 1-(β-Mercapto-β,β-cyclopentamethylene)propinonic acid
PyE: Pyroglutamic acid
pyroGlu: Pyroglutamic acid
QC: Quinaldic acid
R.T.: Room Temperature (about 24°C)
Sar: Sarcosine
SLE: Systemic lupus erythematosus
TA: 3-β-Thienyl-Alanine
TC: DL-thiazolidine-2-carboxylic acid
TCA: 1,4-thiazene-3-carboxylic acid
TEA: Triethylamine
TFA: Trifluoroacetic acid
(thiop): 3-thioproline or

1-thiazolidine-4-carboxylic acid)
TIC: 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
tlc: thin layer chromatography
TTC: L-tetrahydrothiazine-4-carboxylic acid
VLA: Very late activation antigens

Definitions

As used herein, the following words and/or phrases have the following meanings:

"Analog" means a compound which is a derivative of a parent compound in which chemical substituents are appended to a backbone recognizable as the parent compound. Furthermore, the parent compound is derivatized in a manner such that it retains its basic chemical function. Thus, an "amino acid analog" is an amino acid which is derivatized at a side chain carbon or nitrogen, N-derivatized at the nitrogen bonded to the α-carbon and the like, but which retains the ability to form peptide bonds. It is noted that D-enantiomers of amino acids are thus encompassed by this definition. As further examples, "arginine analog" is a compound which consists of an arginine backbone and substituents appended thereto. Thus, the genus of arginine analogs includes, but is not limited to, the compounds N-methylArg, N-lower alkyl-Arg, N,N-dimethyl-Arg, N,N-di-lower alkyl-Arg, homoArg, norArg, side-chain guanidinyl substituted N-nitro-Arg, N^{ω}-nitro-Arg, N,N'-dimethyl-Arg, N,N'-di-lower alkyl Arg, Arginine derivatized at the β, γ or δ carbon with nitro-, alkyl-, aryl-, nitroalkyl- or nitroaryl- groups, and the like.

"Phenylalanine analogs" include those compounds which have halogen, methyl or lower alkyl, nitro or hydroxyl substituents attached to the phenyl ring; non-exclusive examples being p-nitro-Phe, p-halo-Phe, p-amino-Phe and

pentafluoro-Phe. Di-substituted analogs, e.g. dichlorophenylalanine, o,m-dimethylphenylalanine and the like are encompassed as phenylalanine analogs, as are heterodisubstituted analogs, e.g. o-methyl-m-chloro-phenylalanine. As noted above, N-alkyl substituted compounds such as N-methyl-Phe are encompassed.

"Tyrosine analogs" would be homologous to phenylalanine analogs, for instance, 3-bromo-Tyr, 3,5-dibromo-Tyr and 3,5-diiodo-Tyr, and also encompass derivatives of the ring hydroxyl such as O-methyl-tyrosine, O-lower alkyl-tyrosine, etc.

"Proline analogs" include sulfur-containing compounds such as 3-thioproline and also compounds such as homoproline, hydroxyproline, 3,4-dihydroxyproline, DL-thiazolidine-2-carboxylic acid, 1,4-tetrahydrothiazine-3-carboxylic acid, L-5,5-dimethylthiazoline-4-carboxylic acid and 1,3-tetrahydrothiazine-4-carboxylic acid, L-tetrahydrothiazine-4-carboxylic acid and 1,3-, 1,4- and 1,5-thiazepine-carboxylic acids.

"Aspartate analogs" and "glutamate analogs" include esters of the ω-carboxylic acid function of these amino acids.

"Lysine analogs" include amides of the α-amino group and of the ε-amino group and alkyl derivatives of the α-amino group and the ε-amino group. Also encompassed as lysine analogs are DpR, ornithine, homolysine and similar analogs of these and related amino acids.

A. Description of the Compounds

In one aspect, the present invention is directed to a compound of the formula



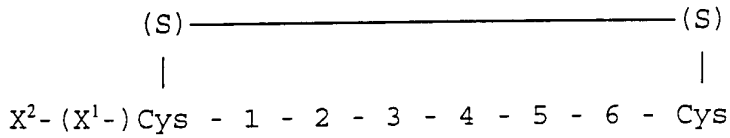
(I)

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 that in structures depicted below in this disclosure, "αHN" represents an α amino group of the amino terminal amino acid in a sequence. Similarly, terminal α-carboxyls are denoted in structural representations as "αC=O". Side chain functional groups are indicated the structural representations in parenthesis.

L^1 and L^2 are chosen so that each contains a functional group which contributes to the formation of the cyclizing bridge moiety Z. Thus, Z is formed from functional groups contributed by L^1 and L^2 and may also contain additional atoms and spacer groups. As is discussed in more detail below, preferred functional groups include thiol, amino and carboxyl groups. Such functional groups may be borne on the side chain of amino acids or amino acid analogs, or may constitute the α-amino group (in L^1) or α-carboxy group (in L^2) thereof. Alternatively, the functional group contributing to the cyclization may be provided by a non-peptide cyclizing linker moiety which is covalently linked to residues 1 and/or 6.

In preferred embodiments of the invention, the bridging residues L^1 and L^2 are each selected from the residues Cys, Pen, and homoC. For L^1, additional preferred residues are Mpr and PMP. All of these residues contain a sulfhydryl group. For L^2 an additional preferred residue is

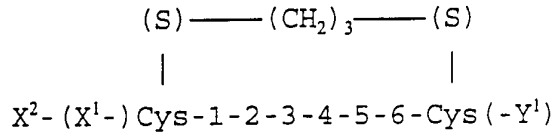
mercapto-ethylamine (MEA). If MEA is used, Y^1 and Y^2 is absent. Thus, the bridging cyclization can be accomplished by oxidative coupling of the sulfhydryls to form a disulfide bond between residues L^1 and L^2. In such a case, the cyclizing moiety Z is a covalent bond between the two sulfur atoms. This may also be depicted generally for compounds wherein, for example, both L^1 and L^2 are Cys residues as follows:



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.

Particularly preferred embodiments are those such that L^1 is Cys or Mpr and L^2 is Cys.

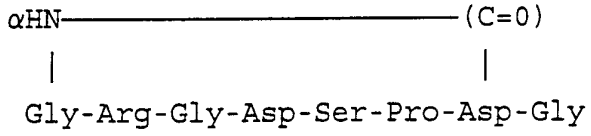
The cyclizing bridge may also be formed by a hydrocarbon moiety, for example a (poly)methylene bridge moiety of the form $-(CH_2)_n-$ where n is an integer of from 1 to 8, preferably 1 to about 4. One type of such bridge is represented below, wherein a cyclic compound with three methylene residues (representing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



(See, L. Fieser et al., "Reagents for Organic Synthesis", Vol. 1, pp. 356-357, J. Wiley and

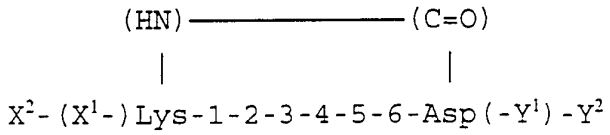
Sons: (1967); Fieser, J. Amer. Chem. Soc., 96:1945 (1959)).

In another preferred embodiment, L^1 and L^2 may be chosen from other amino acids or analogs or amino acid mimetics which provide, as functional groups suitable for the formation of a cyclizing moiety, a side chain or 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

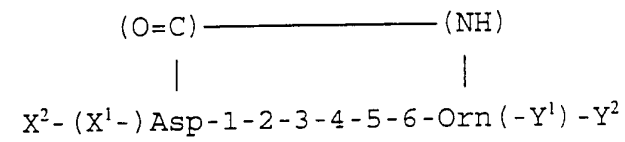


is not included. The cyclizing moiety Z will in such cases be a simple bond between L^1 and L^2.

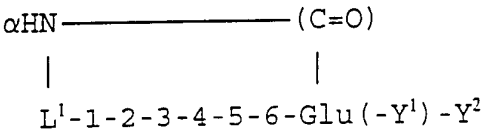
Likewise, an amino acid residue L^2 may provide a carboxyl group from its carboxyl terminus for amide linkage with either a side chain amino or α-amino group on an amino acid residue or analog L^1; or the direction of the amide linkage may be reversed where L^1 provides a side chain carboxyl group and L^2 provides a side chain amino group. Such structures may be exemplified as follows:



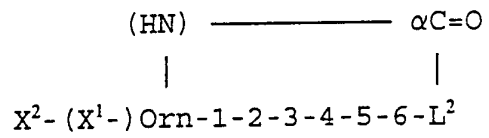
wherein the side chain amino and carbonyl groups of L^1 (Lys) and L^2 (Asp) are directly bonded;



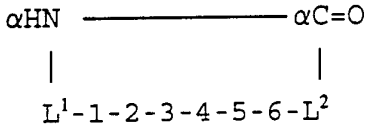
wherein amide bond direction (from side chains of L^1 and L^2) is reversed;



or

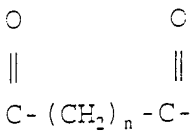


wherein the depicted amino terminus of L^1 is directly bonded to the side chain carboxyl group of Glu (L^2), or the depicted carboxyl terminus of L^2 is directly bonded to the side chain amino group of Orn (L^1);

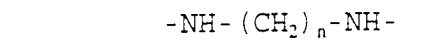


wherein the depicted α-amino terminus at L^1 is directly bonded to the depicted carboxyl terminus at L^2, such that an amide bond is formed in the peptide "backbone" of the compound.

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

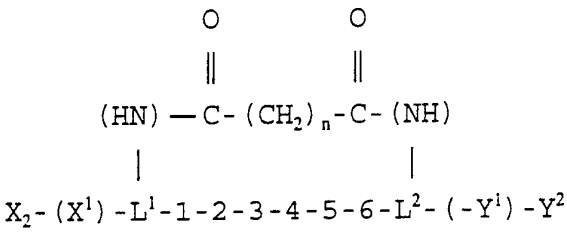


and

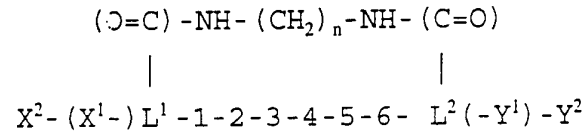


wherein n is as defined above, may also be used.

Diketo linkers can be used to join, for example, the ε-amino groups of lysine residues, while diamino linkers are conveniently employed to cyclize the δ-carboxy groups of glutamic acid or aspartic acid residues. Such examples yield compounds having the 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.

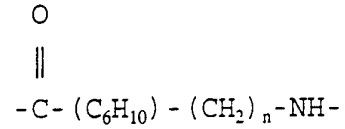
The foregoing are but examples of suitable hydrocarbon-containing bridges, and other forms will also be apparent to those skilled in the art. Where the cyclizing moiety Z includes a portion with such a hydrocarbon form, it may be branched and may, where of a size appropriate to form a stable structure (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



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

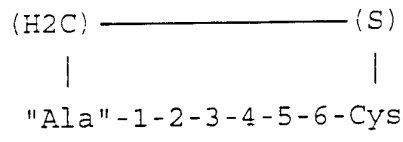
It is of course possible for the bridging moiety to be heterobifunctional, that is, to have a keto group at one end and an amino group at the other. Thus, one may employ as a bridging moiety a structure which includes many of the elements previously discussed, as drawn below:



The cyclizing bridge between L^1 and L^2 can also be formed via a monosulfide (thioether) linkage, as exemplified below. One method for making such a linkage is to use cysteine at L^1 or L^2 and to use a residue providing a bromo-acetic

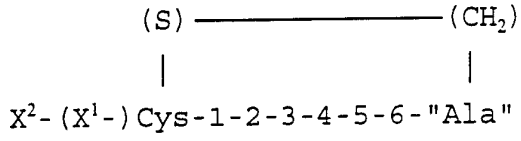
acid [or $Br-CH_2-(CH_2)_nC-$ where (n=0-4) in general] functional group at the other linking site (See,

Barker et al., J. Med. Chem., 35:2040-2048 (1992)).



Alternatively, L^1 can be an α,β dehydroalanine and L^2 can be a cysteine residue. Reaction of the two yields a lanthionine-like thioether linkage.

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

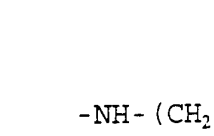


In this regard, see, Palmer et al., in "Peptides--Chemistry, Structure, Biology", pp. 616-618, Rivier & Marshall, Ed., Escom. Leider (1990); and Jung, op. cit., pp. 865-869.

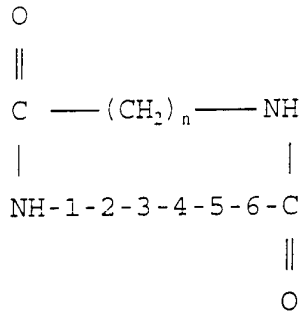
Analogs of amino acid residues may also be utilized for L^1 and/or L^2, as for example homologs (wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other reactive precursor functional group for cyclization), d-enantiomers of amino acids, analogs having variant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

Amino acid-mimetic structures that are capable of being covalently bonded through an

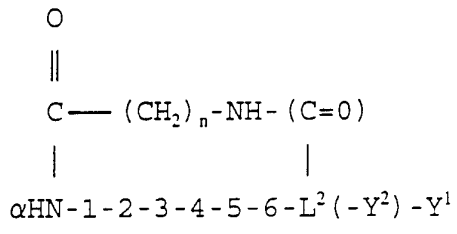
amide bond to a carboxyl and/or amino terminus of the residue sequence 1-2-3-4-5-6, and which provide a suitable precursor functional group for cyclization (through Z), may also be employed in positions L^1 and/or L^2. Such amino acid-mimetic structures include organic species containing one or more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



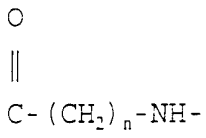
wherein n ranges from 1 to about 8, and preferably from 1 to 4, as for example residues of β-alanine and gamma-aminobutyric acid. (Where n is 1, the amino acid glycine, rather than an α-amino acid mimetic, results.) Such a structure may, similar to the amino acids and amino acid analogs discussed above, be utilized as L^2 (wherein the carbonyl group depicted above, formed for example from a carboxyl precursor, conveniently forms an amide linkage with the amino terminus of residue 2 or, if present, residue 1), or it may be utilized as L^1 (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 result in the exemplifying 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.

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 absent; residue 2 is most preferably Arg; residue 3 is most preferably Ala; residue 4 is most preferably Asp; residue 5 is most preferably 3-thioproline (thiop); and residue 6 is preferably absent. The sequence Arg-Ala-Asp-(thiop) (SEQ. ID. NO.:9),

residues 1-4) is most preferred for residues 1-2-3-4-5-6.

Also particularly preferred is a sequence wherein residue 1 is absent, residue 2 is absent, residue 3 is Asp, residue 4 is (thiop) and residues 5 and 6 are both absent. Thus, the sequence Asp-(thiop) is also preferred for residues 1-2-3-4-5-6.

A third particularly preferred sequence is one in which X^1 is Gly, residue 1 and residue 2 are both absent, residue 3 is Asp, residue 4 is (thiop) and residues 5 and 6 are both absent.

A fourth particularly preferred sequence is one in which X^2 is (1-FCA), X^1 and residues 1 and 2 are all absent, residue 3 is Asp, residue 4 is (thiop) and residues 5 and 6 are both absent.

A fifth particularly preferred compound is one wherein X^2 is Fmoc, X^1 is Arg, residues 1 and 2 are both absent, residue 3 is AnB, residue 4 is (thiop) and residues 5 and 6 are both absent.

A sixth particularly preferred compound is one wherein X^2 is absent, X^1 is Arg, residues 1 and 2 are both absent, residue 3 is Ala, residue 4 is (thiop) and residues 5 and 6 are both absent.

A seventh particularly preferred compound is one wherein X^1 and X^2 are absent, residue 1 is absent, residue 2 is Arg, residue 3 is d-Ala, residue 4 is Asp, residue 5 is absent and residue 6 is (thiop).

An eighth particularly preferred compound is one wherein residue 1 is absent, residue 2 is Arg, residue 3 is Ala, residue 4 is Leu, residue 5 is absent and residue 6 is (thiop).

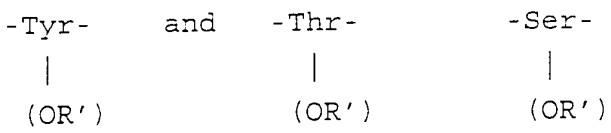
X^1 and Y^1 are each optional in structure I. Where present, they are preferably each independently selected so as to enhance the

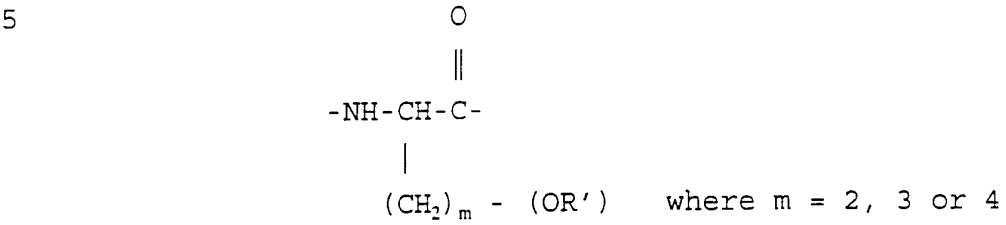
activity of the resultant compound and/or to preserve the compound against metabolism in, for example, the *in vivo* environment and to increase the effective half-life of the compound. In this regard, the use of one or more d-amino acids, most preferably at one or more terminal residue position in the compound (i.e., at the amino-most and/or carboxyl-most residue position, or 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, Tyr, Ala, Arg, His, 1- or 2-naphthylalanine, cyclohexyl-Ala-, 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 -(cyclohexyl)Ala. 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., for X^2 including acyl groups R'CO, especially formic acid, acetic acid and other lower alkyl carboxylic acids, including linear mixed-function carboxylic acids which contain nitrogen and sulfur (e.g. 3-mercaptopropionic acid) are preferred. For Y^2 including amino groups of the form R'NH, especially lower alkyl amines are especially preferred. Additional preferred substituents for X^2 include those derived from bulky compounds such as adamantaneacetic acid, adamantanecarboxylic acid, 1- or 2-naphthylacetic

acid, 2-norbornaneacetic acid, 3-noradamantane-carboxylic acid, 3-methyladamataneacetic acid. Additional preferred substituents for Y^2 include lower alkyl amines, aryl amines, 1- or 2-adamantylamine and amino acids having the α-carboxylic acid replaced by a tetrazole group. Each R' is individually a pharmaceutically suitable substituent group, preferably one selected from the group consisting of hydrogen, 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, formic acid, acetic acid, heterocyclic carboxylic acids, aryl carboxylic acids, heteroaromatic carboxylic acids, alkyl carboxylic acids, alkenyl carboxylic acids, alkynyl carboxylic acids, other mixed-function sulfur and nitrogen containing linear carboxylic acids, adamantyl, fluorenyl, 1-FCA, 9-FCA, 9-FA, FMOC, Ada, Ada-CA, NACA, 3-Me-Ada, (NB)-Ac, PhAc, Naph-Ac, HCA, QC, CPA, DTC, TCA, AMBA, other multi-ring aromatic and heteroaromatic carboxylic and acetic acids, QC, CPA, BOC, 5-FINC, and CBO.

Structures exemplified by the forms

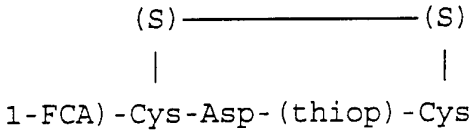




such as those set forth with respect to residue 5 in Structure I, represent derivatives of amino acid residues wherein the side chain hydroxyl group (shown in parentheses) is optionally substituted with a group of the form R' which can be other than hydrogen as defined above.

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:



(SEQ. ID. NO.:4) 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 are those wherein residue number 1 is absent or Leu; residue 2 is Arg; residue L^1 is Cys; residue 4 is Asp; residue 5 is absent or Ser; residue 6 is absent, Pro or (thiop); residue L^2 is Cys. The compounds in the following Table 1 are particularly preferred.

Selected compounds in Table 1 are listed in the Sequence Listing as follows: cmpd. 2, (SEQ. ID. NO.:13); cmpd. 3, (SEQ. ID. NO.:5); cmpds. 23, 33, 48, 49, 51, 54, 55, 62 and 92, (SEQ. ID. NO.:4); cmpd. 29, (SEQ. ID. NO.:6); cmpd. 42, (SEQ. ID. NO.:7); cmpd. 50, (SEQ. ID. NO.:8); cmpds. 65 and 66, (SEQ. ID. NO.:9); cmpd. 67, (SEQ. ID. NO.:10); cmpd. 68, (SEQ. ID. NO.:11); cmpd. 69, (SEQ. ID. NO.:12); cmpd. 71, (SEQ. ID. NO.:14); cmpd. 75, (SEQ. ID. NO.:15); cmpd. 77, (SEQ. ID. NO.:16); cmpds. 78 and 79, (SEQ. ID. NO.:17); cmpd. 80, (SEQ. ID. NO.:18); cmpd. 81, (SEQ. ID. NO.:19); cmpd. 82, (SEQ. ID. NO.:20); cmpd. 84, (SEQ. ID. NO.:21); cmpd. 85, (SEQ. ID. NO.:22); cmpd. 86, (SEQ. ID. NO.:23); cmpd. 87, (SEQ. ID. NO.:24); cmpd. 89, (SEQ. ID. NO.:25).

Table 1

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
1	C*(NiC-Lys)GDSPC*		8 -20'-> 28	9.9	99.9
2	C*R(Sar)DSPC*		0 -20'-> 20	10.9	96.9
3	C*RGNSPC*		0 -20'-> 20	9.2	98.1
4	RC*NPC*		0 -20'-> 20	7.5	97.8
5	FmocKC*DPC*		40 -20'-> 60	8.9	97.3
6	KC*DPC*		2 -30'-> 32	3.9	82.9
7	FmocAC*D(thiop)C*		50 -20'-> 70	12.5	99.7
8	FmocFC*D(thiop)C*		70 -20'-> 90	8.1	99.4
9	FmocHC*D(thiop)C*		55 -20'-> 75	9.3	*69.2
10	FmocLC*D(thiop)C*		55 -20'-> 75	11.0	99.0
11	FmocRC*A(thiop)C*		50 -20'-> 70	7.6	97.4
12	FmocRC*L(thiop)C*		55 -20'-> 75	10.2	95.3
13	FmocRC*F(thiop)C*		55 -20'-> 75	10.3	98.6
14	AC*D(thiop)C*		0 -30'-> 30	5.2	88.5
15	RC*A(thiop)C*		0 -20'-> 20	10.1	*64.3

SUBSTITUTE SHEET (RULE 26)

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
16	LC*D(thiop)C*		2 -30'-> 32	13.9	87.4
17	FC*D(thiop)C*		2 -20'-> 22	17.1	88.1
18	RC*F(thiop)C*		2 -20'-> 32	27.1	77.7
19	HC*D(thiop)C*		2 -30'-> 32	21.0	*61.2
20	RC*L(thiop)C*		2 -30'-> 32	22.9	*54.6
21	FmocGC*D(thiop)C*		50 -30'-> 70	6.5	99.2
22	FmocPC*D(thiop)C*		45 -20'-> 65	10.8	99.7
23	AdaC*D(thiop)C*		45 -20'-> 65	6.3	99.7
24	FmocAC*A(thiop)C*		60 -20'-> 80	6.2	96.4
25	(1-FCA)AC*D(thiop)C*	715.90	45 -20'-> 65	7.1	98.1
26	(1-FCA)C*D(thiop)C*	645	45 -20'-> 65	8.7	99.0
27	(1-FCA)RC*[(p-Cl)Phe](thiop)C*		60 -20'-> 80	7.1	98.5
28	GC*D(thiop)C*		40 -20'-> 60	8.0	95.7
29	C*GKGESPC*		2 -20'-> 22	6.4	96.8
30	(1FCA)(Sar)C*D(thiop)C*		45 -20'-> 65	6.3	99.1
31	(thiop)C*D(thiop)C*		2 -20'-> 22	15.5	99.5
32	PC*D(thiop)C*		2 -20'-> 22	7.0	88.9
33	(Sar)C*D(thiop)C*		2 -20'-> 22	6.6	93.5

SUBSTITUTE SHEET (RULE 26)

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
34	FmocRC*(thiop)C*		55 -20'-> 75	6.9	99.1
35	(1-FCA)(thiop)C*D(thiop)C*		50 -20'-> 70	5.9	86.7
36	FmocK-AdaC*D(thiop)C*	980	75 -20'-> 95	6.8	97.7
37	FmocRC*(BD)(thiop)C*	831	45 -20'-> 65	12.2	96.7
38	FmocK(AnB)C*D(thiop)C*		45 -20'-> 65	6.8	98.5
39	[(N-Me)R]C*D(thiop)C*		2 -20'-> 22	8.2	92.9
40	C*RGA[(p-Cl)Phe](thiop)C*		35 -20'-> 55	6.5	98.1
41	AdaGGC*RGA[(p-Cl)Phe](thiop)C*		60 -20'-> 80	8.0	96.6
42	C*AGD[(p-Cl)Phe](thiop)C*		30 -20'-> 50	6.2	97.2
43	AdaGGC*AGD[(p-Cl)Phe](thiop)C*		55 -20'-> 75	5.4	97.2
44	FmocRC*(AnB)(thiop)C*	800.89	50 -20'-> 70	7.0	99.3
45	FmocRC*(AiB)(thiop)C*		45 -20'-> 65	7.9	96.6
46	FmocRC*V(thiop)C*		60 -20'-> 80	6.7	98.1
47	FmocRC*(β-CN)A(thiop)C*		75 -20'-> 95	10.3	89.6
48	(GAC)C*D(thiop)C*		2 -20'-> 22	13.6	96.1
49	(DTC)C*D(thiop)C*		15 -20'-> 35	10.0	89.8

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
50	C*GRGA[(p-Cl)Phe](thiop)C*		30 -20'-> 50	7.5	99.2
51	FmocC*D(thiop)C*		50 -20'-> 70	8.9	89.5
52	(TCA)C*D(thiop)C*		2 -20'-> 22	9.1	98.1
53	(1-FCA)GC*D(thiop)C*	701.94	45 -20'-> 65	6.4	97.8
54	(5-FINC)C*D(thiop)C*		35 -20'-> 55	9.7	98.6
55	(CBO)C*D(thiop)C*		35 -20'-> 55	8.8	95.7
56	FmocKPC*D(thiop)C*		45 -20'-> 65	8.7	98.3
57	RC*(AnB)(thiop)C*		2 -20'-> 22	12.3	91.1
58	DC*R(thiop)C*		2 -20'-> 22	9.9	89.6
59	KPC*D(thiop)C*		2 -20'-> 22	10.7	88.7
60	(1-FCA)KC*E(thiop)C*		45 -20'-> 65	6.3	99.7
61	RC*(O-Cys)(thiop)C*		2 -20'-> 22	19.1	99.6
62	(1-FCA)C*(thiop)DC*		55 -20'-> 75	5.8	86.9
63	FmocRC*N(thiop)C*		40 -20'-> 60	11.0	99.0
64	RC*(β-Ala)(thiop)C*		20 -20'-> 40	10.1	84.7
65	R*AD(thiop)D*		2 -20'-> 22	12.8	89.4
66	(Anb)*RAD(thiop)D*	658.25	2 -20'-> 22	9.9	92.9

SUBSTITUTE SHEET (RULE 26)

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
67	(Anb)*RVD(thiop)D*		5 -20'-> 25	10.8	99.8
68	(1-FCA)K*AD(thiop)D*	737.27	45 -20'-> 65	6.4	94.7
69	G*RAD(thiop)D*	630.19	2 -20'-> 22	7.0	96.1
70	(1-FCA)K*(dA)D(thiop)D*		40 -20'-> 60	10.1	98.0
71	(1-FCA)Orn*AD(thiop)D*		40 -20'-> 60	8.5	98.8
72	(1-FCA)(dK)*AD(thiop)(dD)*		40 -20'-> 60	9.3	95.2
73	(1-FCA)(dK*)(dA)(dD)(dP)(dD*)		40 -20'-> 60	8.1	96.4
74	(1-FCA)(dK*)AD(thiop)D*		40 -20'-> 60	8.5	97.2
75	G*RVD(thiop)D*		2 -20'-> 22	12.1	99.6
76	(Anb)*R(D-Nal)D(thiop)D*		30 -20'-> 50	9.3	96.0
77	(Anb)*RFD(thiop)D*		15 -20'-> 35	6.9	98.4
78	(1-FCA)K*ADPD*		45 -20'-> 65	6.5	99.9
79	(1-FCA)K*D(thiop)D*	666.19	45 -20'-> 65	6.3	98.1
80	G*RAL(thiop)D*		15 -20'-> 35	12.1	83.1
81	(Aib)*RAD(thiop)D*	658.7	2 -20'-> 22	10.3	97.4
82	G*RFD(thiop)D*		10 -20'-> 30	10.9	98.6
83	RGC*D(thiop)C*		2 -20'-> 22	8.4	97.8
84	G*R[(3-Br)Tyr]D(thiop)D*		10 -20'-> 30	12.2	99.6
85	G*R[(3-Br)Tyr](thiop)C*		2 -20'-> 22	7.6	97.6
86	G*R[(3-pyridyl)A]D(thiop)D*		2 -20'-> 22	12.0	98.9
87	(AMBA)*RAD(thiop)D*		10 -20'-> 30	10.8	92.2

SUBSTITUTE SHEET (RULE 26)

Cpd. No.	Sequence	Mass Spec.	Gradient (%A-min->%B)	Retention Time (min)	%Purity
88	(1-FCA)C*D(TIC)C*	689.06	50 -20'-> 70	8.4	98.8
89	G*MD(thiop)D*		2 -20'-> 22	8.1	91.1
90	[(N-Me)R]C*L(thiop)C*	621.05	15 -20'-> 35	11.7	99.2
91	RC*Y(thiop)C*		10 -20'-> 30	12.6	98.0
92	(1-FCA)C*D(thiop)C*-NH2	644	55 -20'-> 75	7.8	99.9

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. It will be seen in this regard that the presence of residues 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 the adjacent residues. 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-, Tyr, 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-noradamantane-carboxylic acid, 3-methyladamataneacetic acid for X^2, and 1- or 2-adamantylamine for Y^2. Other suitable R' groups are those derived from lower alkyl amines, lower arylamines or acids such as from 9-fluoreneacetic acid, 1-fluorenecarboxylic acid, 4-fluorenecarboxylic acid, 2-

fluorenecarboxylic acid, 9-fluorene- carboxylic acid, phenylacetic, hydroxycinnamic acid, quinaldic acid, formic acid, acetic acid, trifluoroacetic acid, cyclohexyl acetic acid, and 3-mercaptopropionic acid. In general, one would choose an acid moiety for X^2 and a basic moiety for Y^2.

Derivatives of the compounds of Structure I 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. 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), by inhalation or by suppository, parenterally (e.g., intramuscularly, intravenously, subcutaneously or intradermally), or by inhalation, and in the form of either solid or liquid 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. A unit dose is defined as 1 to 3000 mg for a human patient.

Useful pharmaceutical carriers for the preparation of the pharmaceutical compositions hereof can be solids, liquids or mixtures thereof; 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 or lipoprotein vesicles or adding additional terminal amino acids), sustained release formulations, erodable formulations, implantable devices or components thereof, microsphere 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, 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 and pp. 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 might be 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 might 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; contraception through inhibition of fertilization and embryo implantation; as well as conditions wherein increased cell binding is desired, as in wound healing or prosthetic implantation situations as discussed in more detail above.

The compounds of the present invention might find use in the diagnosis of diseases which result from abnormal cell adhesion. For example, excessive adhesion of leukocytes to endothelial cells or to exposed extracellular matrix in blood vessels has been implicated in early stages of atherosclerosis. Thus, a person demonstrating excessive binding of leukocytes to endothelial cells might be at risk for developing occluded arteries. One might detect this risk factor by determining which species of the compound of the present invention is able to inhibit the binding of leukocytes to endothelial cells, then

measuring the binding of that compound to endothelial cells of the patient thought to be at risk.

Furthermore, the compounds of the present invention might find use in the diagnosis of autoimmune diseases caused by antibodies which bind to cell adhesion molecules or which bind to receptors for cell adhesion molecules. For example, if a disease is caused by antibodies binding to a cell adhesion molecule mimicked by a compound of structure I, then a diagnostic test for the presence of such antibodies is easily performed by immunoassay of blood or serum from a patient using the compound of structure I bound to a substrate so as to capture the antibodies. The bound antibody can be detected by the means typical of the art such as a labelled second antibody directed to the Fc portion of human antibodies or using labelled Fc-binding proteins from bacteria (protein A or protein G). In the alternative situation, where the compound of structure I binds to the same receptor as the disease-causing antibody, a competitive immunoassay format can be used. In this format, the compound I is labelled and competition for binding to receptor protein attached to the substrate can be measured.

In addition, derivatives of the present compounds might be useful in the generation of antigens which are prepared by coupling the peptides to a carrier protein. Animals are then immunized with this complex thereby generating antibodies to the peptides. 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.

Furthermore, the compounds of the present invention might be used to produce matrices for purifying substances which bind to the compounds of the present invention with high affinity. Such a matrix could be produced, for example, by covalently attaching a compound of the present invention to a derivatized chromatographic support. In one embodiment of this aspect of the invention, a cyclic peptide listed in Table 1 which contains a free amino group can be coupled to a cyanogen bromide activated chromatography resin, such as that available from Pharmacia, (Uppsala, Sweden, cat. no. 52-1153-00-AK). If neccessary, an amino group can be introduced into the desired peptide, either by addition of a lysine residue, or by addition of another amine-containing residue. Alternatively, of course, carbodiimide-activated resin can be used in conjunction with cyclic peptides bearing free carboxyl functions.

The peptide is coupled using the protocol essentially as provided by the manufacturer. The cyclic peptide-derivatized resin can then be used to purify proteins, polysaccharides or the like which may bind the cyclic peptide with high affinity. Such a purification would be accomplished by contacting the cyclic peptide-derivatized resin with a sample containing the compound to be affinity purified under conditions which allow formation of the specific complex, washing of the complex bound to the resin with a solution which removes unwanted substances, but leaves the complex intact, and then eluting the substance to be purified by washing the resin with a solution which disrupts the complex.

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.

EXAMPLE 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 cyclization. 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. Unless otherwise noted, the methods described in PCT International Publication No. WO 92/00995, published 23 January 1992, are generally applicable to synthesis of the peptides of the present invention.

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., 88:2149 (1963);

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

Barany et al, in "The Peptides", pp. 3-285, E. Gross and J. Meienhofer, Eds., Academic Press, New York (1980).

Experimental:

SPECIFIC SYNTHESIS EXAMPLES

N,N-dialkyl-arginines

N,N-dimethyl-arginine, N,N'-dimethyl-arginine and N,N'-diethyl-arginine were synthesized using the general procedure set forth in PCT International Publication No. WO 92/00995, published on January 23, 1992 and herein incorporated by reference.

Protection of the modified arginine: The material from above is suitable for BOC-protection without crystallization or other purification. One equivalent of the amino acid is dissolved in 1 eq. of 1 N NaOH and an equal volume of dioxane. BOC-Anhydride (1.1 eq.) was dissolved in dioxane and stirred at R.T. for 4 hrs while maintaining pH 9 by addition of 1 N NaOH when necessary. The reaction is followed by tlc for the disappearance of the starting material (visualized with ninhydrin spray). When the reaction was complete acetic acid was added to pH

5. After stirring for 15 minutes the product was isolated following lyophilization.

Tlc System -- methanol/ammonium hydroxide, 1:1 Dimethyl-Arginine at Rf 0.6, Diethyl-Arginine at Rf 0.8.

Proline Analogs

The syntheses of (D,L)-thiazolidine-2-carboxylic acid, N-BOC-(D,L)-thiazolidine-2-carboxylic acid, N-BOC-(L)-5,5-dimethyl-thiazolidine-4-carboxylic acid, (L)-2-methylthiazolidine-4-carboxylic acid and N-BOC-(L)-2-methylthiazolidine-4-carboxylic acid are set forth in PCT International Publication No. WO 92/00995, published on January 23, 1992 and previously incorporated by reference.

L-1,3-Tetrahydrothiazine-4-carboxylic acid

Procedure:

To 5 g homocysteine thiolactone HCl (Sigma) in 75 ml of water 3 ml of 1N HCl and 16 ml of 37% aqueous formaldehyde solution (Aldrich) were added. After stirring for 2 days at room temperature the solution was concentrated to dryness (bath temperature 40 - 50 °C). The product was dissolved in 100 ml of 3Å ethanol, filtered and concentrated to 25 ml *in vacuo*. An equal volume of ethyl acetate was added dropwise and the mixture was allowed to sit in the freezer overnight. The off-white or tan crystals were washed with ethyl acetate and dried under vacuum to afford about 3.0 g (50.4 % yield). The crude product was dissolved in a minimum amount of hot 3Å ethanol and cooled in ice-bath. After crystallization began an additional volume of ethyl acetate was stirred in and the mixture was

cooled in an ice-bath for 4 hrs. The product crystals were collected, washed with ethyl acetate and dried *in vacuo* to afford 2 gm L-1,3-tetrahydrothiazine-4-carboxylic acid.

Analytical:
Silica gel tlc in System A (Butanol/Acetic acid/Water/Pyridine 4:1:2:1): product Rf 0.4.
melting point: 208-210 °C dec.
Rotation [water] -14.03° d=1.

N-BOC-(L)-1,3-Tetrahydrothiazine-4-carboxylic acid

Procedure:

To 3 gm of L-1,3-tetrahydrothiazine-4-carboxylic acid 16.3 mmol) in 50 ml water, 17 ml of 1N NaOH and 50 ml of dioxane (peroxide free) were added 3.8 gm $(BOC)_2O$ (17.4 mmol) in 10 ml of dioxane. The pH was of the reaction was maintained at 8-9 with 1N NaOH and the reaction was stirred overnight. Overnight reaction was found to be incomplete. Additional $(BOC)_2O$ (0.38 gm) was added and the mixture was stirred for an additional 4 hrs to complete the reaction. The reaction mixture was concentrated *in vacuo* to half volume and extracted with hexane (2x50 ml). The hexane layers were discarded. The aqueous layer was cooled with an ice-bath, acidified to pH 3 with 1N $NaHSO_4$ and extracted with three 50 ml portions of ethyl acetate. The pooled ethyl acetate layers were back washed with water (3x50 ml), dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* to give a spontaneously crystallizing light amber oil. A small amount of hexane was used to break up and facilitate collection of the solids. The product

was dried under vacuum to give 3.6 gm of an off-white powder.

Analytical:
Silica gel tlc,system A; product Rf 0.8.
M.P. 112°-113°C. Rotation in acetone, -162.4°, d=1.

Anal. Calcd. for $C_{10}H_{17}N_1O_4S_1$; F.W. 247: C, 48.58; H, 6.88; N, 5.67. Found: C, 48.52; H, 6.94; N, 5.67.

(L)-1,4-Tetrahydrothiazine-3-carboxylic acid

Step 1: To a solution of sodium (16.8 gm) in 1.5 l liquid ammonia L-cystine (Sigma, 38 gm) was added in small portions over 1/2 hr until the blue color was permanently discharged. Bromoethanol (Aldrich, 56 gm) was added slowly over 45 min and stirred overnight as the ammonia was allowed to evaporate. Step 2: The residue from step 1 was dissolved in 1500 ml of conc. HCl and heated to 90-95°C for 7 hr. The solution was concentrated *in vacuo* and the solid collected. The solid was slurried in 1200 ml of isopropyl alcohol and filtered. The mother liquor was concentrated to a slush, filtered and the filter cake air dried. Step 3: The step 2 solid (45 gm) was dissolved in 1 l DMF and 750 ml of triethylamine was added. The mixture was heated for 2.5 hr at 90-95°C and then concentrated to dryness on a rotary evaporator. The solids were dissolved in 1.5 l water and applied to an 800 ml column of Amberlite IR-120 H+ resin. After washing until neutral the product was eluted with 1.5 N aqueous ammonium hydroxide. The product was concentrated to dryness, taken up in water, treated with carbon, filtered through a celite

pad and diluted slowly with about 2 volumes of acetone. The crystals were collected, washed with acetone and air dried to afford 16.2 gm of the desired (L)-1,4-tetrahydrothiazine-3-carboxylic acid.

N-BOC-(L)-1,4-Tetrahydrothiazine-3-carboxylic acid

To a mixture of (L)-1,4-tetrahydrothiazine-3-carboxylic acid (16 gm) dissolved in 1:1 dioxane/1N NaOH was added $(BOC)_2O$ (24 gm) dissolved in 10 ml dioxane. The pH was maintained at 9 and allowed to stand at room temperature overnight. The solution was concentrated to 1/2 volume and extracted in succession with 3 portions of 50 ml each of hexane. The aqueous layer was diluted to 300 ml with water, cooled and acidified to pH 3 with $NaHSO_4$. The solids were collected, washed with water and dried over P_2O_5 to afford 22.4 gm of a white powder of the desired N-BOC-(L)-1,4-Tetrahydrothiazine-3-carboxylic acid.

Amino acid precursors were purchased from BACHEM (Torrance, California). DCC was from Sigma Co. (St. Louis, Mo); TFA was from Halocarbon Co. (New York, NY). Triethylamine was from Fisher Scientific (Fairlane, NJ). 4-Methylbenzhydrylamine resin was from CBA Inc. (Boulder. CO). Other reagents were of analytical grade or better.

All peptides were synthesized by the solid phase method (Barany et al, The Peptides, E. Gross and J. Meienhofer eds., Volume 2, Part A, Chapter 1, Academic Press, Inc., 1-284 (1979)) with a Beckman Automated peptide synthesizer (System 990, Beckman Instruments, Inc., Palo

Alto, California) using BOC/DCC chemistry (Barany et al, The Peptides, E. Gross and J. Meienhofer eds., Volume 2, Part A, Chapter 1, Academic Press, Inc., 1-284 (1979)). Other activating agents include DCC/HOBt (D.L. Nyugen and B. Castro, "Peptide Chemistry", pp. 231-238 Protein Research Foundation Press, Osaka (1987)) or BOP or BOP/HOBt (D. Hudson, J. Org. Chem., 53:617-624 (1988)).

Attachment of N-BOC-S-(4-methylbenzyl)-Cysteine (BOC-Cys-(4-MeBzl) to the chloromethyl polystyrene resin (Merrifield resin) was done in the presence of potassium fluoride (Horiki, Chem. Lett. (#2):166-168 (1978)).

General Procedures for Synthesis of Cyclic Peptides

Peptide Synthesis: BOC-Cys(4-MeBzl)-polystyrene resin (for C-terminal carboxylic acids) or 4-methylbenzhydrylamine resin (for C-terminal carboxamides) was used for the stepwise assembly of the product peptides using the BOC amino acid procedure in Table 2. Related procedures can be found in the published International Patent Application WO 92/00995, which was previously incorporated by reference. Following coupling of the last amino acid the N-terminal BOC protecting group was removed by mixing the resin with TFA:DCM (1:1) for 20 minutes. Following rinsing in order with DCM (3X), MeOH (2X), DCM (3X) the resin was air dried.

Cleavage: The BOC-deprotected peptides on-resin were cleaved by stirring at -5 to 0°C with a cocktail of distilled anhydrous HF (10 ml/g resin), anisole (1 ml/g resin) and dimethyl sulfide (0.5 ml/g resin). After one hour the HF was evaporated under reduced pressure. The cleaved peptide/resin mixture was washed three

times with diethyl ether and then extracted with 80% aq. acetic acid. The combined extracts (200 ml/g resin) were pooled and carried on to the cyclization step.

Cyclization: The formation of the intramolecular disulfide bridge was accomplished by using the iodine oxidation method (Wunch et al, Int. J. Peptide Protein Res., 32:368-383 (1988), Bodanszky, Int. J. Peptide Protein Res., 25:449-474 (1985)). Saturated I_2 in glacial acetic acid was added dropwise to the stirred crude peptide in 80% aqueous acetic acid until the solution turned light brown. After stirring for 1 hr at room temperature, the excess iodine was quenched by adding saturated aqueous ascorbic acid. The cyclized peptide was concentrated *in vacuo*, resuspended in water and lyophilized.

Purification: The cyclic peptide was purified using a Waters Delta Prep 3000 system (Waters, Milford, Massachusetts) equipped with Vydac C_{18} column (15-20 mm, 5X30 cm ID), using a linear gradient of increasing acetonitrile concentration in 1% triethylammonium phosphate (TEAP, pH 2.3) as mobile phase. The appropriate fractions were pooled to give the pure peptide as a phosphate salt. The peptide salt was applied again to the column and eluted with a 0.5% aqueous HOAc in acetonitrile to afford the desired acetate salt form.

Analysis: The purified peptides were analyzed using a Beckman System 126 (Detector module 166), equipped with a Beckman C_{18} column (RPC18 Ultrasphere, 5 μm, 4.6 x 150 mm ID). Elution was performed with buffer A = 0.1 M sodium phosphate in water, pH 4.4 - 4.5, buffer B = 60% acetonitrile in buffer A, using a linear gradient of buffer B and a flow rate of 1 ml/minute. The

time of the gradient was adjusted for each peptide, being 20 or 30 minutes, to provide elution of the peptide near the middle of the gradient. Retention times of each of the preferred peptides are shown in the table of preferred compounds (Table 1).

Fmoc Removal: Piperidine (6 ml) is added to 24 ml of DMF solution containing 1 g FMOC peptide to give a 20% piperidine in DMF. The solution is stirred for twenty minutes at room temperature and then evaporated *in vacuo* until dry. The residue is dissolved in ethyl acetate/water (1:1) and the water layer is collected. The water layer is back extracted twice with ethyl acetate, filtered and lyophilized.

TABLE 2

Schedule for Solid Phase Peptide Synthesis
(TFA deprotection/DCC coupling)

Step	Reagent	Vol*(ml)	Time(min)
1	DCM wash (3X)	20	0.5
2	TFA-DCM (1:1)	20	1
3	TFA-DCM (1:1)	20	20
4	DCM wash (3X)	20	0.5
5	MeOH wash (2X)	20	0.5
6	DCM wash (3X)	20	0.5
7	TEA-DCM (1:9)	20	1
8	TEA-DCM (1:9)	20	5
9	DCM wash (3X)	20	0.5
10	MeOH wash (2X)	20	0.5
11	DCM wash (3X)	20	0.5
12A	BOC-AA (3.2 mM, 2 equiv.) in DCM (DMF)**	10	
12B	DCC in DCM (0.5M)	6.4	120***
13	DCM wash (2X)	20	0.5
14	DCM-MeOH (1:1) (2X)	20	0.5
15	TEA-DCM (1:9)	20	0.5
16	MeOH wash (2X)	20	0.5
17	DCM wash (3X)	20	0.5
18	Ac2O in DCM (1:3)	20	20
19	DCM wash (3X)	20	0.5
20	MeOH wash (2X)	20	0.5
21	DCM wash (3X)	20	0.5

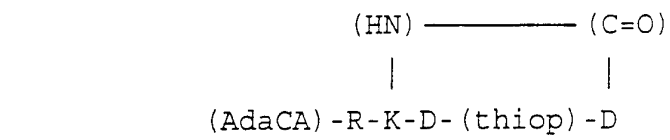
* The volume given is for the synthesis using 2 g of resin with the substitution of 0.8 mM/g of resin.

** DMF added where BOC-AA is insoluble in DCM alone.

*** When the coupling is determined incomplete by the ninhydrin test, recoupling is necessary with a mixture consisting of amino acid, BOP reagent in DMF and DIEA in a 1:1:3 ratio for one hour. After recoupling, the resin is filtered and the ninhydrin test is performed to determine whether coupling is complete. If not the procedure is repeated until the test shows the coupling is complete.

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 DDC 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 Asp 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 Bolin, (Bolin et al., Organic Preparations and Procedures Intern., 21:67-74 (1989)) with certain modifications.

N-BOC-O$^{\beta}$-9-fluorenylmethyl asparate.

8.31 g (25.7 mmol) of N-BOC-O$^{\alpha}$-benzylaspartate 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), 25% $NaHCO_3$ (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$^{\beta}$-9-fluorenylmethyl aspartate, with a melting point of 135-137°C.

N-BOC-O-fluorenylmethyl-glutamate (gamma ester).

N-BOC-O$^{\alpha}$-benzylglutamate (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 described above for the aspartate. This yielded N-BOC-O$^{\alpha}$-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 produce 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 RK*D(thiop)D* 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-Asp(OFm)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-(3-thiop), BOC-Asp(O-benzyl), BOC-Lys(N^{ϵ}-FMOC), and BOC-Arg(N^{g}-tos). 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 2, 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 Arg with 50% TFA in DCM, and neutralization with 5% DIEA in DCM, the protected peptide on resin was reacted with activated AdaCA. 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 capped with (AdaCA), DCC and HOBt in DMF. The peptide then was cyclized by forming an amide linkage between the β-carboxyl group of Asp 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).

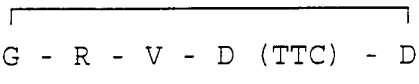
The final cyclic compound was removed from the resin by treatment with HF in the presence of 10% anisole for 1 hr at 0°C. After evaporation of the HF, the residue was washed with diethyl ether and extracted from the resin with 5% HOAc in H_2O. The aqueous extract was lyophilized to yield the crude peptide.

(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.

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-Asp(Fm), BOC-1,4-TTC, BOC-Asp(O-benzyl), BOC-Val, BOC-Arg(N^g-tosyl) and N-FMOC-Gly were used in the synthesis.

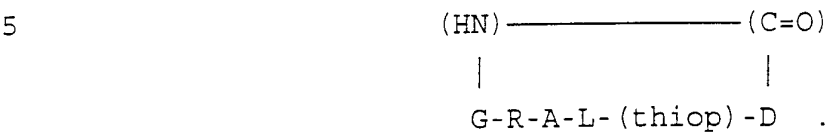
The cyclization between the terminal amino group of Gly and the β-carboxyl group of Asp^6 was performed according to the general amide cyclization procedure described above.

The cyclic compound then was cleaved from the resin by HF and 10% anisole for 1 hr at 0°C. Following evaporation to 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 produce compound, HPLC purity of 99.7%, white powder.

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

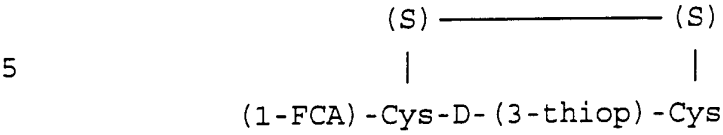
In this example, the following compound is synthesized (SEQ. ID. NO.:18):



The compound is made using the procedure described in PCT Application WO 92/00995, published January 23, 1992.

Synthesis of Cyclic Disulfide Compounds

In this example, the following compound was prepared (SEQ. ID. NO.:4):



1-FCA was purchased from Alrich Chemical Company (Milwaukee, WI). All amino acids, amino acid derivatives and analogs and unnatural amino acids 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). Triethylamine 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-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 2 above. At the end of the synthesis the N-terminal BOC protecting group was removed using TFA:DCM (1:1) for 30 min.

The compound was then cleaved from the resin with HF, the disulfide was formed as described earlier and purified as usual.

Synthesis of Cyclic Peptides Containing Carboxy-terminal NHNR' (especially AMP) or Tetrazole

In general, peptides having the carboxy-terminus modified by amidation to NHNR', in particular, those compounds having AMP derivatives of the carboxyl-terminus, or those peptides in which the carboxyl-terminus has been replaced by tetrazole, are synthesized by a slight variation of the technique by which the other peptides of the present invention are made. To make these compounds, that portion of the cyclic peptide except for the carboxy-terminal amino acid (i.e. except Y^1) is synthesized by the solid-phase method described above and cleaved from the resin. The peptide is then coupled to the appropriately derivatized carboxyl-terminal amino acid, using solution-phase techniques. This procedure is described in the published PCT

patent application WO 92/000995, previously incorporated by reference. Details of solution phase peptide synthesis techniques can be found in Bodanszky, "Peptide Synthesis", Springer-Verlag, New York (1984).

Synthesis of amino acids having the α-carboxylic acid replaced by tetrazole can be performed according to Langry (Langry, K.C., J. Org. Chem. 56:2400-2404 (1991)).

EXAMPLE 2

CELL ADHESION INHIBITION ASSAYS:

U937 Cell Fibronectin Adhesion Assay

The following assay established the activity of the present compounds in inhibiting cell adhesion in a representative *in vitro* system. This assay is a competition assay in which both fibronectin and a test compound are 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 incubated, washed and stained for quantitation of attached cells. The present assay directly demonstrates the anti-cell adhesion activity and adhesion modulatory activity of the present compounds.

The cell line U937 was purchased from American Type Culture Collection (Rockville, MD). The cells are cultured in RPMI media (J.R. Scientific Company, Woodland Hills, CA) containing 10% fetal calf serum. Fibronectin was purified from human plasma according to the procedures of Engvall et al., Int. J. Cancer, 20:1-4 (1977).

Microtiter plates (96-well, Falcon) were coated overnight at 4°C with 0.1 ml of a 5 μg/ml

fibronectin in phosphate buffered saline (PBS). As a control, 5 μg/ml bovine serum albumin (BSA) was added to wells. Unbound proteins were removed from plates by washing with PBS. To block non-reacted sites, the plates are coated with 100 μl of a 2.5 mg/ml BSA solution in PBS for one hour at 37°C. U937 cells were collected and washed twice in Hanks' Balanced Salt Solution (HBSS). The cells were counted and adjusted to 2.5×10^6 cells/ml in Dulbecco's Modified Eagle's Medium (DMEM) plus BSA (2.5 mg/ml) for the cell attachment assay. The test compounds were dissolved in DMEM-BSA and the pH adjusted to 7.4 with 7.5% sodium bicarbonate. The compounds (100 μl) were generally added to the FN-coated wells, at 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.95 and 0.98 μg/ml final concentration. The concentrations used for testing were adjusted depending on the potency of the peptide. U937 cells (100 μl) were added to the wells and the plates were incubated for one hour at 37°C. Following this incubation the plates were washed once with PBS and the attached cells were fixed with 3% paraformaldehyde 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, CA). This procedure is a modification of previously published method, Cardarelli et al., PNAS-USA 83:2647-2651 (1986).

Jurkat-CS-1 Adhesion Assay (Jurkat-α_4 assay)

The CS-1 derived peptides CLHPGEILDVPST (SEQ. ID. NO.:26) and CLHGPIELVSDPT (SEQ. ID. NO.:27) were immobilized onto microtiter plates using the heterobifunctional crosslinker 3-(2-pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP) (Sigma, St. Louis, MO) according to published methods (Pierschbacher, PNAS-USA 80:1224 (1983)). Briefly, microtiter plates were coated at room temperature with 20 μg/ml human serum albumin (HSA) for 2 hours then derivatized with 10 μg/ml SPDP for 1 hour. After removal of excess unbound reagents the cysteine containing peptides were added and allowed to crosslink overnight at 4° C. The following day the cell adhesion assay was carried out as specified in the "U937 Cell Fibronectin Adhesion Assay", except that the human T-lymphocyte cell line, Jurkat, was used.

Jurkat-Endothelial Cell Adhesion Assay

The following assay established the activity of the present compounds in inhibiting cell-cell adhesion in a representative *in vitro* system. This assay measures the adhesive interactions of a T-cell line, Jurkat, to endothelial cell monolayers in the presence of test compounds. The test compounds are 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 present assay directly demonstrates the cell adhesion inhibitory activity and adhesion 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 growth 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 are refed every 2-3 days, reaching confluence by day 4 to 6. The cells are monitored for factor VIII antigen and our results show that at passage 12, the cells are positive for this antigen. The endothelial cells are not used following passage 12.

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

Confluent endothelial monolayers grown in 96-well tissue culture plates are 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 are washed twice with HBSS and 0.1 ml of DMEM-HSA solution are added. Jurkat cells (5×10^5 cells) are combined with the appropriate concentration of peptide and 0.1 ml of the Jurkat cell-peptide mixture are added to the endothelial cell monolayers. Generally, 250, 50, 10 and 2 μM peptide concentrations are tested. With several potent peptides the IC_{50} is determined by testing the peptides at 50, 10, 2 and 0.4 μM. The plates are placed on ice for 5 minutes to allow for Jurkat cell settling and the plates are incubated

at 37°C for 20 minutes. Following this incubation, the monolayers are washed twice with PBS containing 1 mM calcium chloride and 1 mM magnesium chloride and the plates are read using a Pandex Fluorescence Concentration Analyzer (Baxter, Mundelein, IL). Fluorescence in each well is measured as Arbitrary Fluorescence Units and percent adhesion in the absence of peptide is adjusted to 100% and the % adhesion in the presence of peptides is calculated. Monolayers are also fixed in 3% paraformaldehyde and evaluated microscopically to verify the adhesion.

The cell adhesion assay was performed as outlined above in "U937-FN assay" except that the human T lymphocyte cell line, Jurkat, was used in place of the U937 cells.

Results of U937 Cell Adhesion to Fibronectin

Potency is expressed in μM units. Activity is defined in this assay as an IC_{50} below 500 μM. This should be taken to mean that compounds that require a higher molarity to inhibit adhesion by 50 % are still active and of interest but are of overall lesser interest because of the high dose expected to be required when given *in vivo* to humans. Compounds with activity below 10 μM are most preferred, below 100 μM are not as preferred, below 500 μM lesser preferred and above 500 μM least preferred.

Thus an aspect of the present invention is to provide compounds having extraordinarily high potencies in modulating cell adhesion, including but not limited to inhibition of cell adhesion to fibronectin. Data are also provided to show that compounds not containing an RGD sequence are effective inhibitors of adhesion to FN.

Results of Jurkat-Endothelial Cell Adhesion

Results of the inhibition of Jurkat cell adhesion to IL-1 stimulated endothelial cells. Activity (A) in this assay is defined arbitrarily as an IC_{50} below 250 μM; and inactivity (I) as IC_{50} > 250 μM. As above, this does not mean that the compounds with an IC_{50} > 250 μM are actually inactive but rather they are not potent enough to be as practical for human use as those with lower IC_{50}.

Thus an aspect of the present invention is to provide compounds having extraordinarily high potencies in modulating cell adhesion, including but not limited to inhibition of T-cell adhesion to endothelial cells. The exact receptors involved in this interaction and the specific receptors targeted by the test compounds include, but are not limited to, $\alpha_4\beta_1$, $\alpha_4\beta_7$, on the leukocyte and VCAM-1 on the endothelial cells. Lastly, data are provided to show that compounds not containing an RGD sequence are effective inhibitors of cell-cell adhesion.

The following Table 3 shows results from three of the above assays using data from various compounds of the present invention.

Selected compounds in Table 3 are listed in the Sequence Listing as follows: cmpd. 2, (SEQ. ID. NO.:13); cmpd. 3, (SEQ. ID. NO.:5); cmpds. 23, 33, 48, 49, 51, 54, 55, 62 and 92, (SEQ. ID. NO.:4); cmpd. 29, (SEQ. ID. NO.:6); cmpd. 42, (SEQ. ID. NO.:7); cmpd. 50, (SEQ. ID. NO.:8); cmpds. 65 and 66, (SEQ. ID. NO.:9); cmpd. 67, (SEQ. ID. NO.:10); cmpd. 68, (SEQ. ID. NO.:11); cmpd. 69, (SEQ. ID. NO.:12); cmpd. 71, (SEQ. ID. NO.:14); cmpd. 75, (SEQ. ID. NO.:15); cmpd. 77, (SEQ. ID. NO.:16); cmpds. 78 and 79, (SEQ. ID. NO.:17); cmpd. 80, (SEQ. ID. NO.:18); cmpd. 81,

(SEQ. ID. NO.:19); cmpd. 82, (SEQ. ID. NO.:20); cmpd. 84, (SEQ. ID. NO.:21); cmpd. 85, (SEQ. ID. NO.:22); cmpd. 86, (SEQ. ID. NO.:23); cmpd. 87, (SEQ. ID. NO.:24); cmpd. 89, (SEQ. ID. NO.:25).

Table 3

CMPD	SEQUENCE	IC_{50} (μM) FN-U937	Jrkt-EC	Jrkt-α4
1	C*(NIC Lys)GDSPC*	>500	nd[a]	nd
2	C*R(Sar)DSPC*	>500	nd	nd
3	C*RGNSPC*	>500	nd	nd
4	RC*NPC*	17	116	21
5	FmocKC*DPC*	>500	>200	nd
6	KC*DPC*	712	>10	nd
7	FmocAC*D(thiop)C*	585	5	nd
8	FmocFC*D(thiop)C*	>500	6	nd
9	FmocHC*D(thiop)C*	>500	190	nd
10	FmocLC*D(thiop)C*	>500	8	nd
11	FmocRC*A(thiop)C*	25	15	nd
12	FmocRC*L(thiop)C*	>500	34	nd
13	FmocRC*F(thiop)C*	15	18	nd
14	AC*D(thiop)C*	124	17	3
15	RC*A(thiop)C*	25	37	4
16	LC*D(thiop)C*	>500	15	1.3
17	FC*D(thiop)C*	388	46	0.85
18	RC*F(thiop)C*	37	65	9
19	HC*D(thiop)C*	637	25	4.7
20	RC*L(thiop)C*	69	55	4
21	FmocGC*D(thiop)C*	320	1	nd

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CMPD	SEQUENCE	IC_{50} (μM) FN-U937	Jrkt-EC	Jrkt-α4
22	FmocPC*D(thiop)C*	129	<2	24
23	AdaC*D(thiop)C*	>500	2	0.104
24	FmocAC*A(thiop)C*	>500	5	0.572
25	(1-FCA)AC*D(thiop)C*	460	<.4	0.9
26	(1-FCA)C*D(thiop)C*	>500	2	0.9
27	(1-FCA)RC*[(p-Cl)Phe](thiop)C*	>500	5	nd
28	GC*D(thiop)C*	57	49	2.8
29	C*GKGESPC	>500	nd	nd
30	(1-FCA)(Sar)C*D(thiop)C*	278	<0.4	4
31	(thiop)C*D(thiop)C*	51	25	0.255
32	PC*D(thiop)C*	433	9	0.729
33	(Sar)C*D(thiop)C*	119	>50	3.4
34	FmocRC*(thiop)C*	>500	>50	nd
35	(1-FCA)(thiop)C*D(thiop)C*	27	1	nd
36	FmocK(AdaC)*D(thiop)C*	>500	13	nd
37	FmocRC*(βD)(thiop)C*	>500	4	0.306
38	FmocK(AnB)C*D(thiop)C*	>500	1	nd
39	(N-MeR)C*D(thiop)C*	2	24	2
40	C*RGA[(p-Cl)Phe](thiop)C*	>500	50	nd
41	AdaGGC*RGA[(p-Cl)Phe](thiop)C*	1>50	140	nd
42	C*AGD[(p-Cl)Phe](thiop)C*	>500	>50	nd
43	AdaGGC*AGD[(p-Cl)Phe](thiop)C*	>500	>50	nd

CMPD	SEQUENCE	IC_{50} (μM) FN-U937	Jrkt-EC	Jrkt-α4
44	FmocRC*(AnB)(thiop)C*	21	4	nd
45	FmocRC*(AiB)(thiop)C*	>500	>50	nd
46	FmocRC*V(thiop)C*	31	26	nd
47	FmocRC*[(β-CN)A](thiop)C*	>500	>250	nd
48	(GAC)C*D(thiop)C*	460	>50	nd
49	(DTC)C*D(thiop)C*	>500	2	nd
50	C*GRGA[(p-Cl)Phe](thiop)C*	>500	>50	nd
51	FmocC*D(thiop)C*	>500	8	0.315
52	(TCA)C*D(thiop)C*	656	18	0.097
53	(1-FCA)GC*D(thiop)C*	603	0.6	nd
54	(5-FINC)C*D(thiop)C*	>500	23	nd
55	(CBO)C*D(thiop)C*	>500	1	nd
56	FmocKPC*D(thiop)C*	>500	2	nd
57	RC*(AnB)(thiop)C*	67	8	nd
58	DC*R(thiop)C*	594	>50	nd
59	KPC*D(thiop)C*	603	>50	nd
60	(1-FCA)KC*E(thiop)C*	>25	2	nd
61	RC*(O-Cys)(thiop)C*	>50	32	nd
62	(1-FCA)C*(thiop)DC*	>50	>50	nd
63	FmocRC*N(thiop)C*	20	15	nd
64	RC*(β-Ala)(thiop)C*	119	>50	nd
65	R*AD(thiop)D*	>50	>250	>250

SUBSTITUTE SHEET (RULE 26)

CMPD	SEQUENCE	IC_{50} (μM) FN-U937	Jrkt-EC	Jrkt-α4
66	(Anb)*RAD(thiop)D*	21	>250	51
67	(Anb)*RVD(thiop)D*	12	>250	56
68	(1-FCA)K*AD(thiop)D*	>50	>500	106
69	G*RAD(thiop)D*	0.088	>250	>250
70	(1-FCA)K*(dA)D(thiop)D*	>50	>250	nd
71	(1-FCA)Orn*AD(thiop)D*	>50	>250	nd
72	(1-FCA)(dK)*AD(thiop)(dD)*	>50	>250	nd
73	(1-FCA)(dK*)(dA)(dD)(dP)(dD*)	>50	>250	>250
74	(1-FCA)(dK*)AD(thiop)D*	>50	>250	nd
75	G*RVD(thiop)D*	0.198	>250	>100
76	(Anb)*R(D-Nal)D(thiop)D*	56	>250	nd
77	(Anb)*RFD(thiop)D*	15	>250	nd
78	(1-FCA)K*ADPD*	>50	>250	nd
79	(1-FCA)K*D(thiop)D*	500	>250	nd
80	G*RAL(thiop)D*	14	>250	>100
81	(Aib)*RAD(thiop)D*	73	>250	nd
82	G*RFD(thiop)D*	0.445	>250	>100
83	RGC*D(thiop)C*	170	111	8
84	G*R(3-Br-Tyr)D(thiop)D*	4	>250(d)[b]	nd
85	G*R(3-Br-Tyr)(thiop)C*	>50	>250	nd
86	G*R[(3-pyridyl)A]D(thiop)D*	12	223	nd
87	(AMBA)*RAD(thiop)D*	268	>250	nd

SUBSTITUTE SHEET (RULE 26)

CMPD	SEQUENCE	IC_{50} (μM) FN-U937	Jrkt-EC	Jrkt-α4
88	(1-FCA)C*D(TIC)C*	>50	11	nd
89	G*MD(thiop)D*	>50	>250	nd
90	[(N-Me)R]C*L(thiop)C*	6	11	nd
91	RC*Y(thiop)C*	20	250	nd
92	(1-FCA)C*D(thiop)C*-NH2	>100	>100*	64

[a]not done.

[b]>250(d) detached endothelial cell monolayers at a concentration of 250 μM or greater.

The invention being thus described, various changes to the materials and methods used to make and use the invention set forth in the description will be obvious to one of skill in the art. Such modifications are to be considered encompassed within the scope of the invention as claimed below.

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Chang, Shiu-Lan N.
Carderelli, Pina M.
Lobl, Thomas J.

(ii) TITLE OF INVENTION: Peptide Inhibitors of Cell Adhesion

(iii) NUMBER OF SEQUENCES: 28

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Birch, Stewart, Kolasch & Birch
(B) STREET: P.O. Box 747
(C) CITY: Falls Church
(D) STATE: Virginia
(E) COUNTRY: USA
(F) ZIP: 22040-3487

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/001,773
(B) FILING DATE: 08-JAN-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Murphy Jr., Gerald M.
(B) REGISTRATION NUMBER: 28,977
(C) REFERENCE/DOCKET NUMBER: 485-103P

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 703-241-1300
(B) TELEFAX: 703-241-2848
(C) TELEX: 248345

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /label= peptide
/note= "Cell recognition site in Fibronectin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /label= peptide
/note= "alpha-4-beta-1 integrin recognition
peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Ile Leu Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /label= peptide
/note= "blocking peptide in contact hypersensitivity challenge experiment."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Arg Gly Asp Ser Pro
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label=modified_aa
/note= "derivatized with 1-FCA, Ada, GAC, DTC, Fmoc, 5-FINC, CBO or sarcosine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION:
/label=modified_aa
/note= "thioproline"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "can be c-terminal amidated, e.g. cmpd. 92 in Tables 1 and 3, or c-terminal derivatized with AMP as in claim 8."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asp Xaa Cys
1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /label= peptide
/note= "cmpd. 3 in Tables 1 and 3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Arg Gly Asn Ser Pro Cys
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..8
(D) OTHER INFORMATION: /label= peptide
/note= "cmpd. 29 in Tables 1 and 3."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Gly Lys Gly Glu Ser Pro Cys
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "p-Cl phenylalanine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Ala Gly Asp Phe Xaa Cys
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION:
/label= modified_aa
/note= "p-Cl phenylalanine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION:
/label= modified_aa

/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Gly Arg Ala Gly Ala Phe Xaa Cys
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "can be derivatized with Anb"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa

/note= "derivatized with Anb"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Val Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:

/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Arg Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION:
/label= modified_aa
/note= "sarcosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Arg Xaa Asp Ser Pro Cys
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "ornithine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4

(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Xaa Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Arg Val Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "derivatized with Anb"

(ix) FEATURE:
(A) NAME/KEY: Modified-site

(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Phe Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "derivatized with 1-FCA"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "can be thioproline, see cmpd. 79"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys Ala Asp Pro Asp
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gly Arg Ala Leu Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "derivatized with Aib"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Arg Phe Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION:
/label= modified_aa
/note= "3-Br tyrosine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Arg Tyr Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION:
/label= modified_aa
/note= "3-Br tyrosine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Arg Tyr Xaa Cys
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION:
/label= modified_aa
/note= "3-pyridyl alanine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Arg Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "derivatized with AMBA"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Ala Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "thioproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Met Asp Xaa Asp
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: peptide
(B) LOCATION: 1...13
(D) OTHER INFORMATION: /label= peptide
/note= "CS-1 derived peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Leu His Pro Gly Glu Ile Leu Asp Val Pro
1 5 10

Ser Thr

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: peptide
(B) LOCATION: 1...13

(D) OTHER INFORMATION: /label= peptide
/note= "CS-1 derived peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Leu His Gly Pro Ile Glu Leu Val Ser Asp
1 5 10

Pro Thr

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION:
/label= modified_aa
/note= "derivatized with 1-FCA
or acetylated."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION:
/label= modified_aa
/note= "c-terminal amidated."

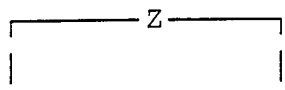
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Asp Val Cys
1

CLAIMS:

What is claimed is:

1. A compound of the formula:



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

or a pharmaceutically acceptable salt thereof, wherein

L^1 and L^2 are each, or are together, selected from the group consisting of Cys, Pen, Mpr, AnB, AnC, β-Ala, Lys, Orn, Dpr, Asp, Glu. a residue of an amino acid, an amino acid analog and 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;

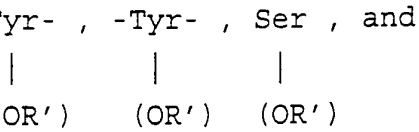
1 is optional and, when present, is selected from the group consisting of Leu, Tyr, Phe, Ile, Pro, a Pro analog, Gly, Ala, Val, norLeu, norVal, Trp, D-Nal, Sar, (Ada)-Ala, AnC, AnB, Lys, ω-amino-lower alkyl carboxylic acid, Gly, Ala, Gly-Gly, Ala-Ala, AnC-AnC, AnB-AnB, β-Ala, β-Ala-β-Ala, 1-Nal, TTC, TCA, DTC and MTC;

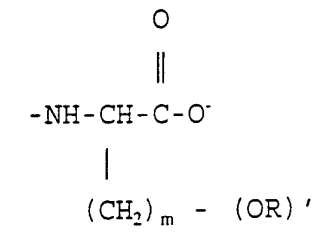
2 is optional and, when present, is selected from the group consisting of Arg, an Arg analog, Lys, a lysine analog, His, Ala, Gly, Sar, Leu, AnB, Phe, an analog of Phe, Pro, a Pro analog, TCA, TTC, DTC and Dpr;

3 is selected from the group consisting of Gly, Sar, Ala, an alanine analog, d-Ala, Ile, Val, d-Val, d-Nal, Phe, Lys, Arg, Asp, β-Asp, Asn, AiB, AnB, β-Ala, Glu, Met, Leu, Tyr, 3Br-Tyr, cysteic acid, 3,5-dibromo-Tyr, and 3,5-diiodo-Tyr;

4 is selected from the group consisting of Asp, d-Asp, Glu, Asn, Gln, Fm-esters of Asp and Glu, other substituted alkyl, aryl and alkaryl esters of Asp and Glu, alkyl, aryl and alkaryl amides of Asn and Gln, Gly, Ala, β-Ala, Leu, Val, AnB, Phe, o, m, p-halo-Phe, p-nitro-Phe, Arg, Cys, TIC, Pro, and thiop;

5 is optional and, when present, is selected from the group consisting of Ser, Thr, Trp, Ala, Val, Phe, o, m, p-halo-Phe, p-nitro-Phe, p-fluoro-Phe, p-Chloro-Phe, 3,5-dibromo-Tyr, p-Methoxy-Tyr,





where m is 2, 3 or 4;

6 is optional and, when present, is selected from Pro, d-Pro, thiop, 1,3-dithiazepine, 1,4-dithiazepine and 1,5-dithiazepine, a Pro analog, 1,1-ACC, Dhp, Hyp, homoPro, Phe, DTC, TTC, TC,

MTC, TCA, o-halo-Phe, m-halo-Phe, p-halo-Phe, p-nitro-Phe, isonipecotic acid, N-methylalanine and TIC;

X^1 is optional and, when present, is selected from the group consisting of sequences of from 1 to 4 D- or L-amino acids and amino acid analogs, Ada, AnC, AnB, CBO, ω-amino-lower alkyl-carboxylic acid and AMBA, lower alkyl, aromatic carboxylic acid, alkylaromatic carboxylic acid and SAR;

Y^1 is optional and, when present, is selected from the group consisting of sequences of from 1 to 4 D- or L-amino acids and amino acid analogs, Ada, AnC, AnB, CBO, ω-amino-lower alkyl-carboxylic acid, AMBA, aminomethylpyridine, lower alkyl amine, aromatic amine, alkylaromatic amine and SAR;

X^2 is an optional N^{α}-substituent R' or R'CO-, formic acid, acetic acid, heterocyclic carboxylic acids, aryl carboxylic acids, heteroaromatic carboxylic acids, alkyl carboxylic acids, alkenyl carboxylic acids, alkynyl carboxylic acids, other mixed-function sulfur and nitrogen containing linear carboxylic acids, Adamantyl, fluorenyl, 1-FCA, 9-FCA, 9-FA, FMOC, Ada, Ada-CA, NAcA, 3-Me-Ada, (NB)-Ac, PhAc, Naph-Ac, HCA, QC, CPA, DTC, TCA, AMBA, other multi-ring aromatic and heteroaromatic carboxylic and acetic acids, QC, CPA, BOC, 5-FINC, and CBO;

Y^2 is an optional carboxyl-terminal substituent selected from the group consisting of -OR', NHR', $NR'NH_2$, NHNR', $-NR'_2$, $-NHNH_2$, -SR', aminomethylpyridine and an amino acid having the α-carboxylic acid moiety replaced by a tetrazole;

and wherein each R' is individually a pharmaceutically suitable substituent group, preferably one selected from the group consisting

of 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,

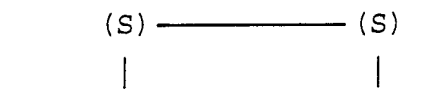
with the provisos that

(1) when 3 is Gly and when 2 is Arg or an analog of Arg, then 4 is not Asp, an analog of Asp, Glu or an analog of Glu and

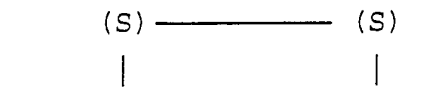
(2) when 3 is Gly and 4 is Asp, an analog of Asp, Glu or an analog of Glu, then 2 is not Arg or an analog of Arg.

2. The compound of claim 1, wherein L^1 and L^2 are each Cys, and cyclization is achieved through a disulfide bond, lactam, lanthionine-like monosulfide or other linkage completing the cyclizing bridge.

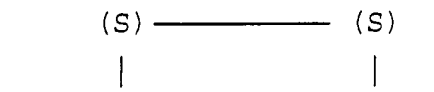
3. A compound of claim 1, wherein said compound has a structure selected from the group consisting of:



Arg-Cys-Ala-thiop-Cys ,



Arg-Cys-Phe-thiop-Cys ,



Arg-Cys-Leu-thiop-Cys ,

(S) ——— (S)
| |
methyl-Arg-Cys-Leu-thiop-Cys ,

(S) ——— (S)
| |
(5-FINC)-Cys-Asp-thiop-Cys (SEQ. ID. NO.:4) ,

(S) ——— (S)
| |
(1-FCA)-Cys-Asp-(TIC)-Cys (SEQ. ID. NO.:4) ,

(S) ——— (S)
| |
Ala-Cys-Asp-thiop-Cys ,

(S) ——— (S)
| |
Gly-Cys-Asp-thiop-Cys ,

(S) ——— (S)
| |
(1-FCA)thiop-Cys-Asp-thiop-Cys , and

(S) ——— (S)
| |
(TCA)-Cys-Asp-thiop-Cys (SEQ. ID. NO.:4) .

4. A compound of claim 1, wherein said compound has a structure selected from the group consisting of:



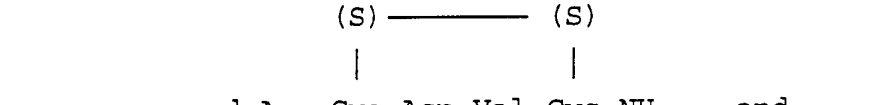
(1-FCA)-Cys-Asp-Val-Cys-NH_2 (SEQ. ID. NO.:28) ,



Ac-Cys-Asp-Val-Cys-NH_2 (SEQ. ID. NO.:28) ,



1-FCA-Cys-Asp-(thiop)-Cys-(AMP) (SEQ. ID. NO.:4) ,



d-Arg-Cys-Asp-Val-Cys-NH_2 , and



N-Me-Arg-Cys-Ala-Val-Cys-NH_2 .

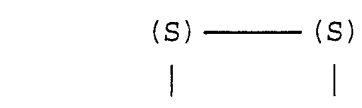
5. The compound of claim 1, wherein Z is a bifunctional linking group selected from the set consisting of diketo-, diamino- and heterobifunctional linking groups.

6. A compound of the formula



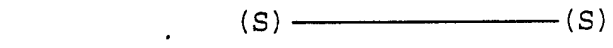
wherein X^1, X^2, 1, 2, L^1, 4, 5, 6, L^2, Y^1, and Y^2 are defined as in claim 1.

7. A compound of the formula



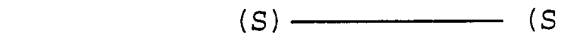
wherein X^2 is a protecting group and 6 is Pro or a derivative of Pro.

8. The compound according to claim 7 which has the formula



(SEQ. ID. NO.:4)

or



(SEQ. ID. NO.:4) .

9. A pharmaceutical composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition comprising the compound of claim 6 and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition comprising the compound of claim 7 and a pharmaceutically acceptable carrier.

12. A method of preventing or treating disease resulting from inappropriate cell adhesion to extracellular matrix which comprises administering to a patient an amount of the composition of claim 9 ranging from 0.1 to 100 mg/kg per dose.

13. A method of preventing or treating disease resulting from abnormal cell adhesion to extracellular matrix which comprises administering to a patient an effective amount of the composition of claim 10 so as to restore normal cell adhesion to extracellular matrix.

14. A method of preventing or treating disease resulting from abnormal intercellular adhesion which comprises administering to a patient an effective amount of the composition of claim 11 so as to restore normal intercellular adhesion.

15. A method for inhibiting adhesion of leukocytes to endothelial cells or to extracellular matrix which comprises administering an effective amount of the composition of claim 9 so as to inhibit adhesion of said leukocytes to endothelial cells or to extracellular matrix.

16. An antibody which specifically binds to the compound of claim 1.

17. A method of diagnosing disease resulting from the presence of antibodies which bind to endothelial cells which comprises:

i) immobilizing at least one compound as recited in claim 1 on a substrate to form a derivatized substrate;

ii) obtaining a blood or serum sample from a patient;

iii) contacting said derivatized substrate from (i) with said blood or serum sample from (ii) under conditions such that antibodies which are present that specifically bind to said endothelial cells would bind to said derivatized substrate; and

iv) detecting antibodies which bind to said derivatized substrate.

18. A method of diagnosing disease resulting from the presence of antibodies which bind to extracellular matrix which comprises:

i) immobilizing at least one compound as recited in claim 1 on a substrate to form a derivatized substrate;

ii) obtaining a blood or serum sample from a patient;

iii) contacting said derivatized substrate from (i) with said blood or serum sample from (ii) under conditions such that antibodies which are present that specifically bind to said endothelial cells would bind to said derivatized substrate; and

iv) detecting antibodies which bind to said derivatized substrate.

19. A method for diagnosing disease resulting from excessive adhesion of cells to endothelial cells which comprises:

i) obtaining from a patient a sample of endothelial tissue;

ii) contacting cells from said endothelial tissue with a compound as recited in claim 1; and

iii) quantitating the amount of said compound which is specifically bound by said endothelial cells.

20. A method for producing a biocompatible surface for a prosthetic device which comprises coating a surface of said prosthetic device with a compound as recited in claim 1.

21. A method according to claim 13, wherein said disease is selected from the group consisting of rheumatoid arthritis, asthma, allergies, adult respiratory distress syndrome, cardiovascular disease, thrombosis or harmful platelet aggregation, reocclusion following thrombolysis, allograft rejection, graft versus host disease, organ transplantation, septic shock, reperfusion injury, psoriasis, eczema, contact dermatitis and other skin inflammatory diseases, osteoporosis, osteoarthritis, atherosclerosis, neoplastic disease including metastasis of neoplastic or cancerous growth, eye diseases such as detaching retina, Type I diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), inflammatory and immunoinflammatory conditions including ophthalmic inflammatory conditions and inflammatory bowel disease, ulcerative colitis, regional enteritis, and other autoimmune diseases.

22. A method for contraception, inhibition of fertilization, inhibition of sperm maturation or inhibition of sperm capacitation which comprises administering to a subject an amount of a compound of claim 1 which is effective for effecting contraception, inhibiting fertilization, inhibiting sperm maturation or inhibiting sperm capacitation.

23. A matrix for the purification of macromolecules having affinity for a compound of claim 1, which comprises a compound of claim 1 covalently bound to an insoluble support.

24. A method for purifying macromolecules which specifically bind with high affinity to a compound of claim 1, which comprises:

i) providing an insoluble matrix derivatized with a compound of claim 1;

ii) contacting a sample containing a macromolecule having a high affinity for said compound of claim 1, under conditions favoring formation of a complex between said macromolecule and said compound of claim 1, said complex being thereby bound to said matrix;

iii) washing said matrix having said complex bound to it with a solution which will not disrupt said complex, so as to contaminants;

iv) eluting the desired substance by washing said support with a solution which disrupts the complex between said macromolecule and the compound of claim 1; and

v) recovering the macromolecule having high affinity for the compound of claim 1.