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(54) Title: TRIAZOLE ϵ -AMINO ACIDS

(57) Abstract: The invention provides 1,2,3-triazole ϵ -amino acids as well as peptides and polypeptides having at least one 1,2,3--triazole ϵ -amino acid. The peptides of the invention can be linear or cyclic. In some embodiments, the cyclic peptides of the invention can assemble into supramolecular structures that can be used be used therapeutically or for small molecule transport and the modulation of small molecule transport within and between cells.



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TRIAZOLE ϵ -AMINO ACIDS

This application claims priority to provisional application U.S. Ser. No. 60/485,961, filed July 9, 2003 by M. Reza Ghadiri and W. Seth Horne, and entitled "TRIAZOLE ϵ -AMINO ACIDS", the contents of which is hereby incorporated by reference in its entirety. This application further relates to U.S. Ser. No. 60/288,990, filed May 4, 2001, PCT Application PCT/US02/14329, filed May 6, 2002, U.S. Ser. No. 60/378,395, filed May 6, 2002 and to U.S. Ser. No. 60/378,256, filed May 6, 2002, the contents of all which are hereby incorporated by reference in their entirety.

Statement Regarding Federally Sponsored Research or Development

The invention described herein was made with United States Government support under Grant Number GM52190 awarded by the National Institutes of General Medical Sciences. The United States Government may have certain rights in this invention.

Field

The invention relates to 1,2,3-triazole ϵ -amino acids and peptides or polypeptides having at least one 1,2,3-triazole ϵ -amino acid.

Background

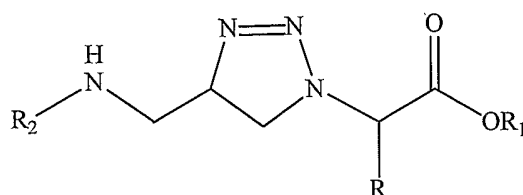
The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art, or relevant, to the presently described or claimed inventions, or that any publication or document that is specifically or implicitly referenced is prior art.

Peptides and polypeptides are increasingly used both *in vitro* and *in vivo* for diagnostic and therapeutic purposes. However, peptides and polypeptides made from natural amino acids are often prone to protease degradation and provide limited opportunities for attachment of functional groups and other useful moieties. Similarly, the peptidyl backbone formed from natural amino acids provides few useful attachment sites for addition of such functional groups and other useful moieties. While some modified amino acids can be used, synthesis of peptides from such modified amino acids can be expensive and is sometimes problematic. Hence, new types of amino acids are needed that can readily be synthesized, that can easily be incorporated into peptides and that provide convenient attachment sites for functional groups and other useful moieties.

Summary

The inventions described and claimed herein have many attributes and embodiments including, but not limited to, those set forth or described or referenced in this Summary. The inventions described and claimed herein are not limited to or by the features or embodiments identified in this Summary, which is included for purposes of illustration only and not restriction.

The invention provides a 1,2,3-triazole ϵ -amino acid of formula I:



wherein:

R is any amino acid side chain;

R₁ is H, a protecting group or an amino acid; and

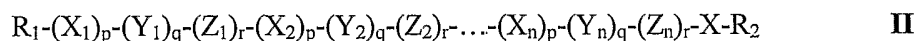
R₂ is H, a protecting group or an amino acid.

The invention also provides a peptide having at least one 1,2,3-triazole ϵ -amino acid of formula I. The peptide can be a linear or cyclic peptide.

The invention also provides a composition comprising a carrier and a peptide comprising at least one 1,2,3-triazole ϵ -amino acid of formula I. The peptide can be a linear or cyclic peptide.

In another embodiment, the invention provides cyclic peptides that can self-assemble into supramolecular structures such as nanotubes. The epsilon amino acid residues of these peptides provide linkage points for surface functionalization of the nanotubes.

Hence, the invention provides a cyclic peptide that has an amino acid sequence comprising formula II:

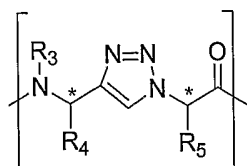


wherein:

each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



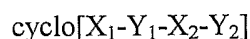
R₃, R₄, and R₅ are separately any amino acid, functional group, protecting group;

each Y is any α amino acid residue;

each Z is any β amino acid residue; and

R₁ and R₂ can separately be a hydrogen atom, hydroxy group, protecting group or R₁ and R₂ can be linked to form a cyclic peptide when there are at least three residues in the peptide.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **III**:



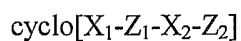
III

wherein:

each X is an ε amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ε amino acid and where each Y has the same chirality as the all the other Y groups.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **IV**:



IV

wherein:

each X is an ε amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Z is a β amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acids and where each Z has the same chirality as the all the other Z groups.

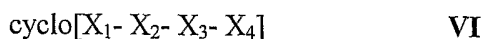
- 5 The invention also provides a cyclic peptide that has an amino acid sequence comprising formula V:



- 10 wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups.

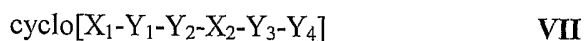
- The invention also provides a cyclic peptide that has an amino acid sequence
15 comprising formula VI:



wherein each X is separately an ϵ amino acid with alternating R,R or S,S substitution pattern throughout the peptide.

20

- The invention also provides a cyclic peptide that has an amino acid sequence comprising formula VII:



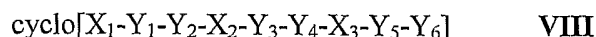
25

wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

- each Y is an α amino acid with either R or S chirality, but with chirality
30 opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **VIII**:

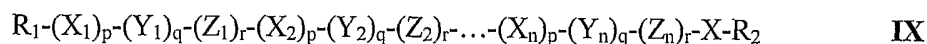


5 wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **IX**:



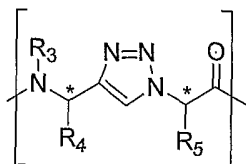
15

wherein:

each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



20

R_3 , R_4 , and R_5 are separately any amino acid, functional group, protecting group;

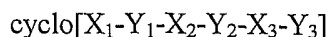
each Y is any α amino acid residue;

each Z is any β amino acid residue; and

25 R_1 and R_2 can separately be a hydrogen atom, hydroxy group, protecting group or R_1 and R_2 can be linked to form a cyclic peptide when there are at least two residues in the peptide.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **X**:

30



X

wherein:

- each X is an ϵ amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X
groups; and
each Y is an α amino acid with either R or S chirality, but with chirality
opposite to that of the X ϵ amino acid and where each Y has the same
chirality as the all the other Y groups.

In some embodiments, the cyclic peptides of the invention can self-assemble into a
supramolecular structure. Such cyclic peptides are useful for small molecule transport, and
for treating a variety of conditions including microbial infections, fungal infections, viral
infections, and cancer.

The invention also provides a composition having a carrier and any of the cyclic
peptides of the invention is also provided herein. The carrier can be a pharmaceutically
effective carrier.

Brief Description of the Figures

Figure 1. Selected region of ^1H NMR spectrum of **1** (a) in DMSO, (b) 1.0 mM in
CDCl₃, (c) 0.50 mM in CDCl₃, and (d) 0.25 mM in CDCl₃. The peaks in DMSO show sharp
splitting, while the signals in CDCl₃ are broad and show concentration-dependent chemical
shifts.

Figure 2. Crystal structure of **1**: (a) single molecule viewed from the top (solvent
omitted), (b) crystal packing viewed along the tube axis (solvent omitted), (c) one tube
viewed from the side (solvent omitted), and (d) expanded view of the interaction between two
rings with heteroatom-heteroatom distances for indicated hydrogen-bonding moieties labeled
in Å (protons omitted).

Figure 3. Structure of compound II, a compound of formula III (Fig. 3A); and
Compound III, a compound according to formula VI (Fig. 3B).

Figure 4. Structure of compound IV, a compound of formula V (Fig. 4A); and
compound V, a compound formula V (Fig. 4B).

Figure 5. Structure of compound VI, a compound according to formula V (Fig. 5A);
and compound VII, a compound according to formula III (Fig. 5B).

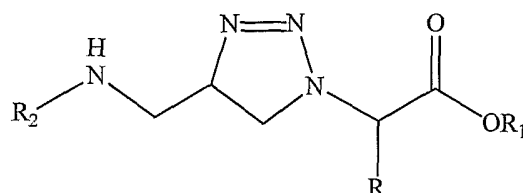
Figure 6. Structure of compound VIII (Fig.6A), a compound according to formula IX and compound IX (Fig. 6B), a compound according to formula II.

Figure 7. Structure of compound X (Fig. 7A), a compound according to formula II; and compound XI (Fig. 7B), a compound according to formula IX.

5 **Figure 8.** Structure of compound XII, a compound according to formula VII (Fig. 8A); and compound XIII, a compound according to formula VII (Fig. 8B).

Detailed Description

10 The present invention provides peptides and polypeptides having at least one 1,2,3-triazole ϵ -amino acid. The invention also provides compositions of peptides or polypeptides having at least one 1,2,3-triazole ϵ -amino acid. Formula I provides an example of a 1,2,3-triazole ϵ -amino acid.



15

wherein:

R is any amino acid side chain;

R₁ is H, a protecting group or an amino acid; and

20 R₂ is H, a protecting group or an amino acid.

Definitions

The term "amino acid," includes the residues of the natural α -amino acids (e.g. Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Hyl, Hyp, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in D or L form, as well as β -amino acids, 1,2,3-triazole ϵ -amino acids, synthetic and unnatural amino acids. Many types of amino acid residues are useful in the cyclic peptides and the invention is not limited to natural, genetically-encoded amino acids. Examples of amino acids that can be utilized in the cyclic peptide described herein can be found, for example, in Fasman, 1989, CRC Practical Handbook of Biochemistry and

30 Molecular Biology, CRC Press, Inc., and the references cited therein. Another source of a

wide variety of amino acid residues is provided by the website of RSP Amino Acids Analogues, Inc. (www.amino-acids.com).

The term "mammal," as used herein, refers to an animal, in general, a warm-blooded animal, which is susceptible to or has a microbial infection. Mammals include cattle, buffalo, sheep, goats, pigs, horses, dogs, cats, rats, rabbits, mice, and humans. Also included are other livestock, domesticated animals and captive animals. The term "farm animals" includes chickens, turkeys, fish, and other farmed animals.

The term "protecting group" as used herein includes any substituent, moiety, and the like which can protect a potentially reactive functional group from one or more undesired chemical transformation. A protecting group can, for example, exist as any temporary adduct (e.g. covalent attachment) to a target molecule that can be selectively removed either a chemical or enzymatic method. Suitable protecting groups encompass any described in the literature, or later developed, including those described in Protective Groups in Organic Synthesis, Greene, T. W.; Wuts, P. G. M., John Wiley & Sons, New York, N.Y., (3rd Edition, 1999), which is hereby incorporated by reference herein in its entirety.

As used herein, a "nanotube" or "nanotubule" is a small tubule that may spontaneously form from the 1,2,3-triazole ϵ -amino acids and peptides of the present invention. The present cyclic peptides are believed to stack to form supramolecular structures composed of nanotubes. Nanotubes and molecular structures described herein may be used as membrane channels, for example to facilitate ion and small molecule transport. Thus, the 1,2,3-triazole ϵ -amino acids and peptides, including cyclic peptides, can be employed as drug carriers and agents for the delivery or modulation of small molecule transport in and between cells. Hydrogen bonding between peptides is believed to help drive the self-assembly of the supramolecular structures from cyclic peptides. Each nanotube has a pore in the center of the tube that is surrounded by the series of peptide backbones of the stacked cyclic peptides that form the nanotubes. The size of the pore depends upon the number of amino acids in the cyclic peptides that form the nanotube. In general, depending on the ring size of the cyclic peptides employed, ions, sugars, and other small molecules can travel through the pores of the nanotubes. Larger molecules can also flow through pores formed from larger cyclic peptides and supramolecular structures formed of aggregates of nanotubes. For example, in some embodiments the supramolecular structure is thought to be a barrel-like structure composed of clusters of nanotubes. In other embodiments, the supramolecular structure is thought to be a "carpet" or "carpet-like" arrangement of nanotubes. A "carpet" or "carpet-like" arrangement of one or more nanotubes is where the

nanotube(s) adopt orientations that can be approximately or somewhat parallel to the plane of the membrane structure. However, when in such a carpet-like arrangement, the nanotubes can assume other orientations relative to the plane of the membrane. For example, the nanotubes may be situated on the surface of the membrane, or may be partially or fully
5 contained within the interior of the membrane. In one example, the carpet-like nanotubes can be oriented with a tilt angle of up to approximately $70 \pm 5^\circ$ from the membrane normal. These carpet or carpet-like arrangements may be formed because the contemplated cyclic peptides can possess clusters of hydrophilic and hydrophobic residues, that upon aggregation can form supramolecular structures with, for example, a hydrophilic side or face and a hydrophobic
10 side or face. A hydrophilic face of a nanotube can, for example, be positioned so that it is in contact with the hydrophilic portions of the membrane or with the aqueous environment. A hydrophobic face of a nanotube can, for example, be in contact with the hydrophobic portions of the membrane. See PCT Application PCT/US02/14329, filed May 6, 2002 (e.g. Figure 2A-D).

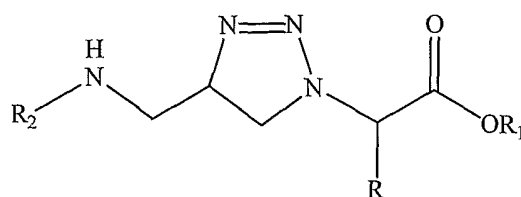
15 According to the present invention, "supramolecular structures" are multi-subunit structures, e.g. nanotubes, barrels and carpets of nanotubules, which are believed to be formed through "noncovalent" assembly of cyclic peptides. Supramolecular structures may be contrasted with molecular or polymeric systems in which the products are based on covalent bond formation between reactants or monomers. The proposed peptide
20 supramolecular structures are thermodynamically controlled assemblies that can undergo reversible structural assembly and disassembly. Such assembly-disassembly will depend, for example, on the environment, subunit structure, side group selection, side group interaction, and the nature and combination of noncovalent forces operating on the system. In contrast, covalent polymeric structures have been used to design kinetically stable structures rather
25 than structures that assemble and dissemble in response to the environment. Hence, one attractive feature of the present compositions containing peptides that can form supramolecular structures is their ability to select amongst various cell membrane types. Such selection is driven by favorable thermodynamic forces determined by the composition of the cyclic peptide relative to the cell membrane environment and the molecular and/or
30 supramolecular constituents of the cell membrane. See PCT Application PCT/US02/14329, filed May 6, 2002 (e.g. Figure 2A-D).

The term "substantially no" with reference to self-assembly, hemolysis, toxicity or cellular lysis, or the like, means that little or no self-assembly, hemolysis, toxicity, cellular

lysis or the like is present at the tested or desired peptide dosage or concentration. By way of example, "substantially no" hemolysis can mean that less than about 20%, alternatively less than 15% or less than 10%, no undesirable, or no detectable, hemolysis at the tested or desired peptide dosage or concentration has occurred. Similarly, "substantially no" toxicity or lysis can mean that less than about 20%, alternatively less than 15% or less than 10%, no undesirable, or no detectable, toxicity or lysis at the tested or desired peptide dosage or concentration has occurred. In other embodiments, "substantially no" hemolysis, toxicity or lysis means that less than about 5%, or no detectable, hemolysis, toxicity or lysis, etc., at the tested or desired peptide dosage or concentration has occurred.

1,2,3-Triazole ϵ -Amino Acids

The present invention provides 1,2,3-triazole ϵ -amino acids, for example, 1,2,3-triazole ϵ -amino acids having Formula I.



I

wherein:

R is any amino acid side chain;

R₁ is H, a protecting group or an amino acid; and

R₂ is H, a protecting group or an amino acid.

The R amino acid side chains can be any natural or synthetic side chain known to one of skill in the art. Thus, the 1,2,3-triazole ϵ -amino acids can have R groups such as hydroxy; linear or branched C₁-C₆-alkyl, alkenyl, alkynyl; hydroxy-C₁-C₆-alkyl; amino-C₁-C₆-alkyl; C₁-C₆-alkyloxy, C₁-C₆-alkoxy-alkyl; C₁-C₆-amino; mono- or di-C₁-C₆-alkylamino; carboxamido; carboxamido-C₁-C₆-alkyl; sulfonamido; sulfonamido-C₁-C₆-alkyl, urea, cyano, fluoro, thio; C₁-C₆-alkylthio; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C₁-C₆ and heteroaryl-C₁-C₆-alkyl and similar R groups.

Peptides, Peptide Variants, and Derivatives Thereof

The present invention provides peptides and polypeptides having at least one 1,2,3-triazole ϵ -amino acid. The invention also provides compositions of peptides or polypeptides
5 having at least one 1,2,3-triazole ϵ -amino acid. Such peptides and polypeptides can be linear or cyclic.

In one embodiment, the invention provides cyclic peptides that have at least one 1,2,3-triazole ϵ -amino acid and an amino acid sequence of alternating D- and L-amino acids that is between four to about sixteen, alternatively about six to about sixteen amino acids in
10 length. Alternatively, the cyclic peptides of the present invention can have at least one 1,2,3-triazole ϵ -amino acid and between three to about ten β -amino acids. In general, the cyclic D, L-peptides do not include the amino acids proline and glycine. According to the invention, β -amino acids can be substituted at the α - or β -carbons, or both. Mono-substituted β -amino acids of either S or R chirality can be employed for the construction of cyclic β -peptides,
15 provided that the cyclic beta peptide is homochiral. Disubstituted β -amino acids employed in the present invention must have the relative R,R or S,S diastereomeric configuration, provided that the β -amino acid residues in a cyclic peptide structure are homochiral. Cyclic peptides having β -amino acids generally have at least one β -amino acid with at least one polar side chain.

20 The cyclic peptides of the present invention are believed to undergo self-assembly to form supramolecular structures that, upon assembly in or on a microbial, fungal or cancer cell membrane, or in association with a virus or viral membrane, can cause depolarization and/or permeablization and/or destabilization of the microbial, fungal, or cancer cell membrane, or virus. In some cases, the cyclic peptides cause death, for example, by lysis, of the microbial
25 cell, fungal cell, or cancer cell or virus. Self-assembly into supramolecular structures is thought to occur by stacking of the cyclic peptides in an anti-parallel fashion or a parallel fashion with formation of β -sheet hydrogen bonds between adjacent cyclic peptides. However, it is believed that the preferred cyclic peptides do not readily self-assemble into supramolecular structures in normal mammalian cellular membranes as measured, for
30 example, in an assay for toxicity in mammalian cells or hemolysis of mammalian red blood cells at tested or therapeutically effective doses.

Peptides of the present invention can be made from at least one 1,2,3-triazole ϵ -amino acid and can include α -amino acids or β -amino acids. The amino acid sequence of cyclic

peptides includes at least one polar amino acid in the case of D,L-amino acid cyclic peptides, or at least one polar side chain in the case of cyclic β -peptides. The percentage of polar amino acids can range, for example, from about 25% or 33% to about 65% or 88%. However, in some embodiments a majority of the amino acids are polar. For example, the percentage of polar amino acids can be from about 50% to about 88% of the total number of amino acids. The exact number of polar and nonpolar amino acids depends on the size and the properties sought for a given cyclic peptide. In some embodiments, the size of the cyclic peptides is about six to about ten D,L-amino acids or three to about ten β -amino acids. In other embodiments, the size for the present cyclic peptides is about six to about eight D,L-amino acids or four to about six β -amino acids. Thus, for example, an eight residue cyclic peptide of the invention can have at least one, alternatively, two to seven polar D- and/or L-amino acids. Other eight residue cyclic peptides will have three to five polar D- and/or L-amino acids for example. Preferred eight residue cyclic peptides have three, four or five polar amino acids. In some embodiments, for example, six residue cyclic peptides of the invention can have two to five polar D- and/or L-amino acids. Other six residue cyclic peptides may have three to four polar D- and/or L-amino acids. At least one of these polar D- or L-amino acids may be adjacent to at least one other polar D- or L-amino acid. Alternatively, at least one polar D- or L-amino acid may be adjacent only to nonpolar D- or L-amino acids. Beta peptides having about four to about eight β -amino acids may have, for example, about two to twelve polar side chains, depending on the level of α and β backbone substitution.

The cyclic D- L-peptides of the invention generally have about 25% to about 88% ionizable amino acid residues. In some embodiments, the percentage of ionizable amino acids can be from about 33% or 50% to about 65% or 88% of the total number of D- and/or L-amino acids. Thus, for example, a six or eight residue cyclic peptide can have at least one, or alternatively two or three or more ionizable D- and/or L-amino acids. In other embodiments, the cyclic peptides of the invention can have four to six ionizable D- and/or L-amino acids. Such an ionizable D- or L-amino acid can be adjacent to at least one other polar or ionizable D- or L-amino acid. Alternatively, the cyclic peptides of the invention can have at least one ionizable D- or L-amino acid that is adjacent only to nonpolar D- or L-amino acids. The cyclic β -peptides of the invention generally have about 25% to about 88% ionizable amino acid side chains. In some embodiments, the percentage of ionizable amino acid side chains can be from about 33% or 50% to about 65% or 88% of the total number of amino acid side chains.

Thus, for example, a four to six residue cyclic β -peptide can have at least one, or alternatively two or three or more ionizable amino acid side chains. In other embodiments, the cyclic β -peptides of the invention can have four to six ionizable amino acid side chains.

The cyclic peptides of the invention can have nonpolar D- and/or L-amino acid residues. The number of non-polar amino acids chosen can vary as the size of the peptide varies and as the selected target membrane (e.g. microbial, fungal, cancer cell) environment, or virus or viral membrane, varies. The cyclic peptides of the invention generally have about 12% to about 75% D- and L-nonpolar amino acids. In some embodiments, the percentage of nonpolar amino acids can be from about 50% to about 67% or 75% of the total number of D- and L-amino acids. Thus, for example, an eight residue cyclic peptide of the invention can have at least one, alternatively, two to seven nonpolar D- and/or L-amino acids. Other eight residue cyclic peptides may have three to five nonpolar D- and/or L-amino acids. In some embodiments, for example, six residue cyclic peptides of the invention have two to five nonpolar D- and/or L-amino acids. Other six residue cyclic peptides may have three to four nonpolar D- and/or L-amino acids. At least one of these nonpolar D- or L-amino acids may be adjacent to at least one other nonpolar D- or L-amino acid. Alternatively, at least one nonpolar D- or L-amino acid may be adjacent only to polar D- or L-amino acids. In general, the cyclic peptides do not include the amino acid proline or glycine, but certain cyclic peptides may have good activity even though proline or glycine is included.

According to the invention, β -amino acids can have non-polar side chains at the α - or β -carbons, or both. The number of non-polar amino acid side chains chosen can vary as the size of the peptide varies and as the selected target or target membrane environment varies. The cyclic β -peptides of the invention generally have about 12% to about 75% nonpolar amino acid side chains. In some embodiments, the percentage of nonpolar amino acid side chains can be from about 50% to about 67% or 75% of the total number of amino acid side chains. Thus, for example, an eight residue cyclic β -peptide of the invention can have at least one, alternatively, two to seven nonpolar amino acid side chains. Other eight residue cyclic β -peptides may have three to five nonpolar amino acid side chains. In some embodiments, for example, six residue cyclic β -peptides of the invention have two to five nonpolar amino acid side chains. Other six residue cyclic β -peptides may have three to four nonpolar amino acid side chains.

While the peptides and polypeptides of the invention have at least one 1,2,3-triazole ϵ -amino acid, they can also contain a variety of other amino acids. Amino acids used in the

peptides and polypeptides of the invention can be genetically encoded amino acids, naturally occurring non-genetically encoded amino acids, or synthetic amino acids. Both L- and D-enantiomers of any of the above are utilized in cyclic peptides. The amino acid notations used herein for the twenty genetically encoded L-amino acids and some examples of non-

5 encoded amino acids are provided in Table 1.

Table 1

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val
B-Alanine		Bala
2,3-Diaminopropionic acid		Dpr
A-Aminoisobutyric acid		Aib
N-Methylglycine (sarcosine)		MeGly
Ornithine		Orn

Amino Acid	One-Letter Symbol	Common Abbreviation
Citrulline		Cit
t-Butylalanine		t-BuA
t-Butylglycine		t-BuG
N-methylisoleucine		MeIle
Phenylglycine		Phg
Cyclohexylalanine		Cha
Norleucine		Nle
Naphthylalanine		Nal
Pyridylalanine		
3-Benzothienyl alanine		
4-Chlorophenylalanine		Phe(4-Cl)
2-Fluorophenylalanine		Phe(2-F)
3-Fluorophenylalanine		Phe(3-F)
4-Fluorophenylalanine		Phe(4-F)
Penicillamine		Pen
1,2,3,4-Tetrahydro-isoquinoline-3-carboxylic acid		Tic
B-2-thienylalanine		Thi
Methionine sulfoxide		MSO
Homoarginine		Harg
N-acetyl lysine		AcLys
2,4-Diamino butyric acid		Dbu
P-Aminophenylalanine		Phe(pNH ₂)
N-methylvaline		MeVal
Homocysteine		Hcys
Homoserine		Hser
E-Amino hexanoic acid		Aha
Δ -Amino valeric acid		Ava
2,3-Diaminobutyric acid		Dab

Certain commonly encountered amino acids that are not genetically encoded and that can be present in the peptides of the invention include, but are not limited to, β -alanine (b-Ala) and other omega-amino acids such as 3-aminopropionic acid (Dap), 2,3-

diaminopropionic acid (Dpr), 4-aminobutyric acid and so forth; α -aminoisobutyric acid (Aib); ϵ -aminohexanoic acid (Aha); δ -aminovaleric acid (Ava); methylglycine (MeGly); ornithine (Orn); citrulline (Cit); t-butylalanine (t-BuA); t-butylglycine (t-BuG); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); 2-naphthylalanine (2-Nal); 4-chlorophenylalanine (Phe(4-Cl)); 2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β -2-thienylalanine (Thi); methionine sulfoxide (MSO); homoarginine (hArg); N-acetyl lysine (AcLys); 2,3-diaminobutyric acid (Dab); 2,3-diaminobutyric acid (Dbu); p-aminophenylalanine (Phe(pNH₂)); N-methyl valine (MeVal); homocysteine (hCys) and homoserine (hSer). Additional amino acid analogs contemplated include phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate, hippuric acid, octahydroindole-2-carboxylic acid, statine, α -methyl-alanine, para-benzoyl-phenylalanine, propargylglycine, and sarcosine. Peptides that are encompassed within the scope of the invention can have any of foregoing amino acids in the L- or D- configuration, or any other amino acid known to one of skill in the art.

Amino acids that are substitutable for each other generally reside within similar classes or subclasses. As known to one of skill in the art, amino acids can be placed into different classes depending primarily upon the chemical and physical properties of the amino acid side chain. For example, some amino acids are generally considered to be hydrophilic or polar amino acids and others are considered to be hydrophobic or nonpolar amino acids. Polar amino acids include amino acids having acidic, basic or hydrophilic side chains and nonpolar amino acids include amino acids having aromatic or hydrophobic side chains. Nonpolar amino acids may be further subdivided to include, among others, aliphatic amino acids. The definitions of the classes of amino acids as used herein are as follows:

“Nonpolar Amino Acid” refers to an amino acid having a side chain that is uncharged at physiological pH, that is not polar and that is generally repelled by aqueous solution. Examples of genetically encoded hydrophobic amino acids include Ala, Ile, Leu, Met, Trp, Tyr and Val. Examples of non-genetically encoded nonpolar amino acids include t-BuA, Cha and Nle.

“Aromatic Amino Acid” refers to a nonpolar amino acid having a side chain containing at least one ring having a conjugated π -electron system (aromatic group). The aromatic group may be further substituted with substituent groups such as alkyl, alkenyl, alkynyl, hydroxyl, sulfonyl, nitro and amino groups, as well as others. Examples of genetically encoded aromatic amino acids include phenylalanine, tyrosine and tryptophan.

Commonly encountered non-genetically encoded aromatic amino acids include phenylglycine, 2-naphthylalanine, β -2-thienylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 4-chlorophenylalanine, 2-fluorophenylalanine, 3-fluorophenylalanine and 4-fluorophenylalanine.

- 5 “Aliphatic Amino Acid” refers to a nonpolar amino acid having a saturated or unsaturated straight chain, branched or cyclic hydrocarbon side chain. Examples of genetically encoded aliphatic amino acids include Ala, Leu, Val and Ile. Examples of non-encoded aliphatic amino acids include Nle.

- 10 “Polar Amino Acid” refers to a hydrophilic amino acid having a side chain that is charged or uncharged at physiological pH and that has a bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids are generally hydrophilic, meaning that they have an amino acid having a side chain that is attracted by aqueous solution. Examples of genetically encoded polar amino acids include asparagine, cysteine, glutamine, lysine and serine. Examples of non-genetically encoded
- 15 polar amino acids include citrulline, homocysteine, N-acetyl lysine and methionine sulfoxide.

 “Acidic Amino Acid” refers to a hydrophilic amino acid having a side chain pK value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Examples of genetically encoded acidic amino acids include aspartic acid (aspartate) and glutamic acid (glutamate).

- 20 “Basic Amino Acid” refers to a hydrophilic amino acid having a side chain pK value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Examples of genetically encoded basic amino acids include arginine, lysine and histidine. Examples of non-genetically encoded basic amino acids include the non-cyclic amino acids ornithine, 2,3-
- 25 diaminopropionic acid, 2,4-diaminobutyric acid and homoarginine.

- “Ionizable Amino Acid” refers to an amino acid that can be charged at a physiological pH. Such ionizable amino acids include acidic and basic amino acids, for example, D-aspartic acid, D-glutamic acid, D-histidine, D-arginine, D-lysine, D-hydroxylysine, D-ornithine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine, L-lysine, L-
- 30 hydroxylysine or L-ornithine.

As will be appreciated by those having skill in the art, the above classifications are not absolute. Several amino acids exhibit more than one characteristic property, and can therefore be included in more than one category. For example, tyrosine has both a nonpolar aromatic ring and a polar hydroxyl group. Thus, tyrosine has several characteristics that

could be described as nonpolar, aromatic and polar. However, the nonpolar ring is dominant and so tyrosine is generally considered to be nonpolar. Similarly, in addition to being able to form disulfide linkages, cysteine also has nonpolar character. Thus, while not strictly classified as a hydrophobic or nonpolar amino acid, in many instances cysteine can be used to confer hydrophobicity or nonpolarity to a peptide.

The classifications of the above-described genetically encoded and non-encoded amino acids are summarized in Table 2, below. It is to be understood that Table 2 is for illustrative purposes only and does not purport to be an exhaustive list of amino acid residues that may comprise the peptides and peptide analogues described herein. Other amino acid residues that are useful for making the peptides described herein can be found, e.g., in Fasman, 1989, CRC Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Inc., and the references cited therein. Another source of amino acid residues is provided by the website of RSP Amino Acids Analogues, Inc. (www.amino-acids.com). Amino acids not specifically mentioned herein can be conveniently classified into the above-described categories on the basis of known behavior and/or their characteristic chemical and/or physical properties as compared with amino acids specifically identified.

TABLE 2

Classification	Genetically Encoded	Genetically Non-Encoded
Nonpolar		
Aromatic	F, Y, W	Phg, Nal, Thi, Tic, Phe(4-Cl), Phe(2-F), Phe(3-F), Phe(4-F), Pyridyl Ala, Benzothienyl Ala
Aliphatic	A, V, L, I	t-BuA, t-BuG, MeIle, Nle, MeVal, Cha, bAla, MeGly, Aib
Other Nonpolar	M, G, P	
Polar		
Acidic	D, E	
Basic	H, K, R	Dpr, Orn, hArg, Phe(p-NH ₂), DBU, A ₂ BU
Neutral Polar	S, T, Y, Q, N, D, E, H, R, K, C	Cit, AcLys, MSO, hSer, Orn, Hcys
Cysteine-Like	C	Pen, hCys, β -methyl Cys

In some embodiments, polar amino acids contemplated by the present invention include, for example, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, homocysteine, lysine, hydroxylysine, ornithine, serine, threonine, the corresponding
5 β -amino acids, and structurally related amino acids. In one embodiment the polar amino is an ionizable amino acid such as arginine, aspartic acid, glutamic acid, histidine, hydroxylysine, lysine, or ornithine.

Examples of nonpolar or nonpolar amino acid residues that can be utilized include, for example, alanine, valine, leucine, methionine, isoleucine, phenylalanine, tryptophan, tyrosine
10 and the like.

In addition, the amino acid sequence of a peptide can be modified so as to result in a peptide variant that includes the substitution of at least one amino acid residue in the peptide for another amino acid residue, including substitutions that utilize the D rather than L form.

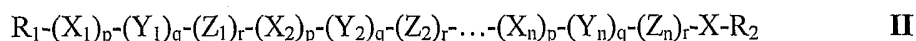
One or more of the residues of the peptide can be exchanged for another, to alter,
15 enhance or preserve the biological activity of the peptide. Such a variant can have, for example, at least about 10% of the biological activity of the corresponding non-variant peptide. Conservative amino acid substitutions are often utilized, i.e., substitutions of amino acids with similar chemical and physical properties, as described above.

Hence, for example, conservative amino acids substitutions involve exchanging
20 aspartic acid for glutamic acid; exchanging lysine for arginine or histidine; exchanging one nonpolar amino acid (alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, valine) for another; and exchanging one polar amino acid (aspartic acid, asparagine, glutamic acid, glutamine, glycine, serine, threonine, etc.) for another. After the substitutions are introduced, the variants are screened for biological activity.

25 The present isolated, purified peptides or variants thereof, can be synthesized *in vitro*, e.g., by the solid phase peptide synthetic method or by enzyme catalyzed peptide synthesis or with the aid of recombinant DNA technology. Solid phase peptide synthetic method is an established and widely used method, which is described in references such as the following: Stewart et al., Solid Phase Peptide Synthesis, W. H. Freeman Co., San Francisco (1969);
30 Merrifield, J. Am. Chem. Soc. 85 2149 (1963); Meienhofer in "Hormonal Proteins and Peptides," ed.; C.H. Li, Vol.2 (Academic Press, 1973), pp.48-267; and Bavaay and Merrifield, "The Peptides," eds. E. Gross and F. Meienhofer, Vol.2 (Academic Press, 1980) pp.3-285. These peptides can be further purified by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or

on an anion-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; ligand affinity chromatography; or crystallization or precipitation from non-polar solvent or nonpolar/polar solvent mixtures. Purification by crystallization or precipitation is preferred.

- 5 In one embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula **II**:

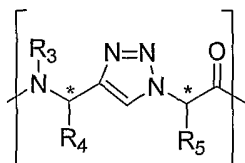


wherein:

- 10 each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



R₃, R₄, and R₅ are separately any amino acid, functional group, protecting group,;

- 15 each Y is any α amino acid residue;

each Z is any β amino acid residue; and

R₁ and R₂ can separately be a hydrogen atom, hydroxy group, protecting group or R₁ and R₂ can be linked to form a cyclic peptide when there are at least three residues in the peptide.

20

In some embodiments, R₅ is an amino acid. In some embodiments, R₃ and R₄ are chiral propargyl amines. Example of synthesis of chiral propargylamines are provided in Rae, Alastair; Ker, James; Tabor, Alethea B.; Castro, Jose L.; Parsons, Simon. *Tetrahedron Letters* **1998**, 39(36), 6561-6564, which is incorporated by reference herein.

25

Cyclic peptides capable of self assembly

In some embodiments, the invention provides cyclic peptides that can self-assemble into supramolecular structures. For example, such self-assembling peptides of the invention can have an amino acid sequence having formula **III**:

30



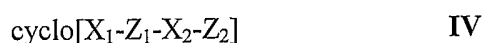
wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

In another example, such self-assembling peptides of the invention can have an amino acid sequence having formula IV:

10



wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Z is a β amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acids and where each Z has the same chirality as the all the other Z groups.

In another example, such self-assembling peptides of the invention can have an amino acid sequence having formula V:

20

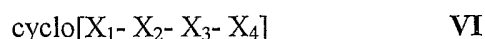


wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups.

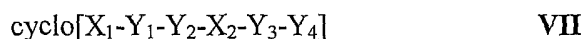
In another example, such self-assembling peptides of the invention can have an amino acid sequence having formula VI:

30



wherein each X is separately an ϵ amino acid with alternating R,R or S,S substitution pattern throughout the peptide.

In another example, such self-assembling peptides of the invention can have an amino acid sequence having formula **VII**:



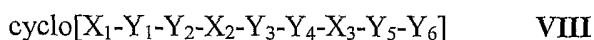
wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

15

In another example, such self-assembling peptides of the invention can have an amino acid sequence having formula **VIII**:



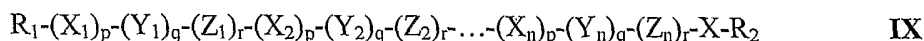
20 wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

25

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **IX**:



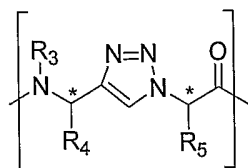
30

wherein:

each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



R₃, R₄, and R₅ are separately any amino acid, functional group, protecting group;

5 each Y is any α amino acid residue;

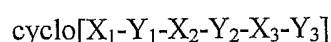
each Z is any β amino acid residue; and

R₁ and R₂ can separately be a hydrogen atom, hydroxy group, protecting group or R₁ and R₂ can be linked to form a cyclic peptide when there are at least two residues in the peptide.

10 In some embodiments, R₅ is an amino acid. In some embodiments, R₃ and R₄ are chiral propargyl amines.

The invention also provides a cyclic peptide that has an amino acid sequence comprising formula **X**:

15



X

wherein:

20 each X is an ε amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ε amino acid and where each Y has the same chirality as the all the other Y groups.

25 As described above, in certain embodiments cyclic peptides are utilized. In other embodiments, linear peptides or polypeptides are utilized. To identify highly active cyclic or linear peptides that have little or no undesired toxicity for mammalian cells, individual peptides, or libraries of peptides can be made and the individual peptides from those libraries can be screened for activities such as anti-microbial activity, anti-fungal, anti-viral, anti-cancer activity and/or toxicity against normal mammalian cells. For example, libraries of
30 peptides can be made using a one-bead-one-compound strategy provided by Lam et al. (97

Chem. Rev. 411-448 (1997) or synthesized on macrobeads by a split and pool method of Furka, et al. (37 Int. J. Pept. Prot. Res. 487-493(1991)). Mass spectrometric sequence analysis techniques enable rapid identification of every peptide within a given library. *See*, Biemann, K. 193 Methods Enzymol. 455 (1990). In general, synthetic operations, including peptide cyclization, are performed on solid support to avoid laborious and difficult to automate solution-phase operations. Moreover, the final product of the synthesis regimen is generally sufficiently pure for biological assays without laborious purification procedures. Peptide yields from each synthesis can be sufficient for performing 50 to 100 assays. Rapid, automatic mass-spectrometry-based peptide sequence analysis can be performed to identify peptide sequences that have high activity and to discard peptide sequences with low activity.

The synthetic approach employed can provide individually separable and identifiable peptide sequences to avoid the use of combinatorial library mixtures and laborious deconvolution techniques. However, libraries of impure mixtures of peptides can also be generated for testing. Impure preparations of peptides can be used for quick screening of combinations of sequences. When a mixture of peptides shows activity, the peptides in the mixture can either be individually isolated and tested or pure peptides having sequences known to be present in the impure mixture can be individually prepared and tested.

Salts of carboxyl groups of a peptide or peptide variant of the invention may be prepared in the usual manner by contacting the peptide with one or more equivalents of a desired base such as, for example, a metallic hydroxide base, e.g., sodium hydroxide; a metal carbonate or bicarbonate base such as, for example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like.

N-acyl derivatives of an amino group of the peptide or peptide variants may be prepared by utilizing an N-acyl protected amino acid for the final condensation, or by acylating a protected or unprotected peptide. O-acyl derivatives may be prepared, for example, by acylation of a free hydroxy peptide or peptide resin. Either acylation may be carried out using standard acylating reagents such as acyl halides, anhydrides, acyl imidazoles, and the like. Both N-acylation and O-acylation may be carried out together, if desired.

Acid addition salts of the peptide or variant peptide, or of amino residues of the peptide or variant peptide, may be prepared by contacting the peptide or amine with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid. Esters of carboxyl groups of the peptides may also be prepared by any of the usual methods known in the art.

The invention also contemplates cyclic and linear peptides with at least one 1,2,3-triazole ϵ -amino acid that are also composed of one or more β amino acids. Such β -amino acids can be substituted along their peptide backbones by one to two substituents. Such substituents can include cycloalkyl, cycloalkenyl, and heterocyclic rings that encompass the α and β carbons of the β -peptide backbone. These rings can be, for example, C₃-C₈ cycloalkyl, cycloalkenyl or heterocyclic rings having one or more nitrogen atoms as the sole heteroatom, and can be substituted or unsubstituted. The substituents on the ring or on the α and β carbons of the β -peptide can be, for example, hydroxy, linear or branched C₁-C₆-alkyl, alkenyl, alkynyl; hydroxy-C₁-C₆-alkyl; amino-C₁-C₆-alkyl; C₁-C₆-alkyloxy, C₁-C₆-alkoxy-alkyl; C₁-C₆-amino; mono- or di-C₁-C₆-alkylamino; carboxamido; carboxamido-C₁-C₆-alkyl; sulfonamido; sulfonamido-C₁-C₆-alkyl, urea, cyano, fluoro, thio; C₁-C₆-alkylthio; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C₁-C₆ and heteroaryl-C₁-C₆-alkyl and the like.

15 **Therapeutic Agents**

The present invention provides small peptides and compositions that selectively kill or inhibit the growth of target cells or organisms, preferably without substantial or undesired toxicity toward normal mammalian cells. The present invention includes cyclic peptides, and pharmaceutical compositions comprising cyclic peptides with at least one 1,2,3-triazole ϵ -amino acid, and with either a sequence of alternating D-, and L- α -amino acids, a sequence of alternating α -amino acids and ϵ -amino acids, or a sequence of β -amino acids, that have flat, ring-shaped conformations. Such ring-shaped conformations project the amino acid side chains of the cyclic peptides away from the center of the ring and orient the amide backbone approximately perpendicular to the plane of the ring structure. It is believed that under conditions that favor hydrogen bonding, such as side chain charge neutralization through interactions with cell membrane constituents and/or contact with low dielectric constant environments of cell membranes, the cyclic peptides can self-assemble via intermolecular hydrogen bonding to form supramolecular structures. Cyclic peptides that simply contain one or more D-amino acids do not adopt a flat ring-shaped conformation and do not have the backbone conformation needed for self-assembly of the cyclic peptide into supramolecular structures. Target microbial organisms against which the present cyclic peptides are effective include microbes, including any single cell organism or parasite that has a cellular membrane and that can infect a mammal. For example, target microbial organisms include bacteria,

helminths, protozoa, yeast strains and other single cell organisms. Targets include both gram-negative and gram-positive bacteria, as well as fungal and cancer cell types, and viruses.

Differences in environment, for example, the difference in composition of different cellular membranes, can be relied on to influence the course and nature of the proposed assembly process. This feature is used in the present invention both to target and to optimize the anti-microbial, anti-fungal, anti-viral or anti-cancer activity of selected cyclic peptides against particular microbial species or cancer cells or other target cells, while providing substantially no toxicity, or no undesired toxicity, in normal mammalian cells at therapeutically effective doses and dose regimens. Supramolecular structures are believed to respond to their immediate environment through dynamic self-assembling/disassembling processes to quickly find the most thermodynamically favored assembly. It is believed that, during assembly, peptide supramolecular structures sense and respond to the environment of a cellular membrane by sampling various topologically related assemblies. In non-target mammalian membranes at therapeutically desirable concentrations, it is believed that the preferred cyclic peptides of the invention do not or cannot adopt a thermodynamically favorable supramolecular structure. Thus, mammalian membranes are not substantially or undesirably affected by the presence of such cyclic peptides. However, in selected microbial, fungal, or cancer cell membranes the present cyclic peptides are believed to form unique energetically favorable supramolecular structures that destabilize (e.g., lyse), permeabilize and/or depolarize the microbial or cancer cell membrane, thereby disrupting microbial transmembrane ion and electrical gradients and other vital functions, and quickly leading to cell death.

Changes in amino acid sequence of a cyclic peptide can be utilized to create differences at the supramolecular level. Thus, changes in the structure of a cyclic peptide may constrain peptide interaction and limit formation of supramolecular structures to particular cellular membranes that have particular membrane constituents, membrane partitioning properties, uptake properties, and the like.

Another feature of the present self-assembling peptide supramolecular structures is believed to be the potential for a given cyclic peptide to form a number of diastereomeric nanotube assemblies. This property stems from the fact that backbone-backbone hydrogen bonding are believed primarily to direct the self-assembly of the nanotube structure. Differently stacked subunits can give rise to topoisomeric supramolecular structures that share the same or nearly the same tubular β -sheet-like hydrogen bonded backbone structure.

The variety of supramolecular structures assembled from a single cyclic peptide minimizes the probability that microbes or cancer cells can develop resistance to these agents.

By varying the peptide sequence while retaining at least one 1,2,3-triazole ϵ -amino acid and either a cyclic D- and L- α -peptide backbone, or a cyclic β -peptide backbone, a multitude of cyclic peptides can quickly be screened or evaluated for the ability to selectively target and assemble in microbial, fungal, or cancer cell membranes. They can also be screened and evaluated for anti-microbial, anti-fungal, or anti-cancer activity by testing for increased membrane permeability, depolarization, or destabilization.

In addition, therapeutic agents can be linked to the linear or cyclic peptides and polypeptides of the invention, for example, through at least one 1,2,3-triazole ϵ -amino acid present in the peptide or polypeptide. Such therapeutic agents can be any therapeutic agent available to one of skill in the art. For example, the therapeutic agent can be a small molecule, a peptide, a polypeptide, a glycoprotein, a lipoprotein or a nucleic acid. The therapeutic agent can be an anti-cancer agent, an anti-microbial agent, an anti-inflammatory agent, a pain reliever, an antihistamine, a bronchodilator or other agent.

In some embodiments, an antibiotic can be linked to the peptides of the invention, such as aminoglycosides (e.g., streptomycin, gentamicin, sisomicin, tobramycin and amikacin), ansamycins (e.g. rifamycin), antimycotics (e.g. polyenes and benzofuran derivatives), β -lactams (e.g. penicillins and cephalosporins), chloramphenicol (including thiamphenol and azidamphenicol), lincosamides (lincomycin, clindamycin), macrolides (erythromycin, oleandomycin, spiramycin), polymyxins, bacitracins, tyrothycin, capreomycin, vancomycin, tetracyclines (including oxytetracycline, minocycline, doxycycline), phosphomycin and fusidic acid.

25 Dosages, Formulations and Routes of Administration for the Peptides

The peptides or polypeptides of the invention, including their salts, are administered so as to achieve a reduction in at least one symptom associated with an infection, indication or disease, or a decrease in the amount of antibody associated with the indication or disease.

To achieve the desired effect(s), the peptide, a variant thereof or a combination thereof, may be administered as single or divided dosages, for example, of at least about 0.01 mg/kg to about 500 to 750 mg/kg, of at least about 0.01 mg/kg to about 300 to 500 mg/kg, at least about 0.1 mg/kg to about 100 to 300 mg/kg or at least about 1 mg/kg to about 50 to 100 mg/kg of body weight, or at least about 1 mg/kg to about 20 mg/kg of body weight, although

other dosages may provide beneficial results. The amount administered will vary depending on various factors including, but not limited to, the peptide chosen, the disease, the weight, the physical condition, the health, the age of the mammal, whether prevention or treatment is to be achieved, and if the peptide is chemically modified. Such factors can be readily
5 determined by the clinician employing animal models or other test systems that are available in the art.

Administration of the therapeutic agents in accordance with the present invention may be in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the
10 administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the peptides of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

To prepare the composition, peptides are synthesized or otherwise obtained, purified
15 as necessary or desired and then lyophilized and stabilized. The peptide can then be adjusted to the appropriate concentration, and optionally combined with other agents. The absolute weight of a given peptide included in a unit dose can vary widely. For example, about 0.01 to about 2 g, or about 0.1 to about 500 mg, of at least one peptide of the invention, or a plurality of peptides specific for a particular cell type can be administered. Alternatively, the
20 unit dosage can vary from about 0.01 g to about 50 g, from about 0.01 g to about 35 g, from about 0.1 g to about 25 g, from about 0.5 g to about 12 g, from about 0.5 g to about 8 g, from about 0.5 g to about 4 g, or from about 0.5 g to about 2 g.

Daily doses of the cyclic peptides of the invention can vary as well. Such daily doses can range, for example, from about 0.1 g/day to about 50 g/day, from about 0.1 g/day to
25 about 25 g/day, from about 0.1 g/day to about 12 g/day, from about 0.5 g/day to about 8 g/day, from about 0.5 g/day to about 4 g/day, from about 0.5 g/day to about 1 g/day, and from about 0.5 g/day to about 2 g/day.

Thus, one or more suitable unit dosage forms comprising the therapeutic peptides of the invention can be administered by a variety of routes including oral, parenteral (including
30 subcutaneous, intravenous, intramuscular and intraperitoneal), rectal, dermal, transdermal, intrathoracic, intrapulmonary and intranasal (respiratory) routes. The therapeutic peptides may also be formulated for sustained release (for example, using microencapsulation, see WO 94/ 07529, and U.S. Patent No.4,962,091). The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the

methods well known to the pharmaceutical arts. Such methods may include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

5 When the therapeutic peptides of the invention are prepared for oral administration, they are generally combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. For oral administration, the peptides may be present as a powder, a granular formulation, a solution, a suspension, an emulsion or in a natural or synthetic polymer or resin for ingestion of the active ingredients
10 from a chewing gum. The active peptides may also be presented as a bolus, electuary or paste. Orally administered therapeutic peptides of the invention can also be formulated for sustained release, e.g., the peptides can be coated, micro-encapsulated, or otherwise placed within a sustained delivery device. The total active ingredients in such formulations comprise from 0.1 to 99.9% by weight of the formulation.

15 By "pharmaceutically acceptable" it is meant a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Pharmaceutical formulations containing the therapeutic peptides of the invention can be prepared by procedures known in the art using well-known and readily available
20 ingredients. For example, the peptide can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, solutions, suspensions, powders, aerosols and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include buffers, as well as fillers and extenders such as starch, cellulose, sugars, mannitol, and silicic derivatives. Binding agents can also be included such as carboxymethyl cellulose,
25 hydroxymethylcellulose, hydroxypropyl methylcellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone. Moisturizing agents can be included such as glycerol, disintegrating agents such as calcium carbonate and sodium bicarbonate. Agents for retarding dissolution can also be included such as paraffin. Resorption accelerators such as quaternary ammonium compounds can also be included. Surface active agents such as cetyl
30 alcohol and glycerol monostearate can be included. Adsorptive carriers such as kaolin and bentonite can be added. Lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols can also be included. Preservatives may also be added. The compositions of the invention can also contain thickening agents such as cellulose and/or cellulose

derivatives. They may also contain gums such as xanthan, guar or carbo gum or gum arabic, or alternatively polyethylene glycols, bentones and montmorillonites, and the like.

For example, tablets or caplets containing the peptides of the invention can include buffering agents such as calcium carbonate, magnesium oxide and magnesium carbonate.

5 Caplets and tablets can also include inactive ingredients such as cellulose, pre-gelatinized starch, silicon dioxide, hydroxy propyl methyl cellulose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide, benzoic acid, citric acid, corn starch, mineral oil, polypropylene glycol, sodium phosphate, zinc stearate, and the like. Hard or soft
10 gelatin capsules containing at least one peptide of the invention can contain inactive ingredients such as gelatin, microcrystalline cellulose, sodium lauryl sulfate, starch, talc, and titanium dioxide, and the like, as well as liquid vehicles such as polyethylene glycols (PEGs) and vegetable oil. Moreover, enteric-coated caplets or tablets containing one or more peptides of the invention are designed to resist disintegration in the stomach and dissolve in the more neutral to alkaline environment of the duodenum.

15 The therapeutic peptides of the invention can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous, intraperitoneal or intravenous routes. The pharmaceutical formulations of the therapeutic peptides of the invention can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an
20 emulsion or suspension or salve.

Thus, the therapeutic peptides may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion containers or in multi-dose containers. As noted above, preservatives can be added to help maintain the shelf life
25 of the dosage form. The active peptides and other ingredients may form suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active peptides and other ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.
30

These formulations can contain pharmaceutically acceptable carriers, vehicles and adjuvants that are well known in the art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents such as acetone, ethanol, isopropyl alcohol, glycol

ethers such as the products sold under the name "Dowanol," polyglycols and polyethylene glycols, C₁-C₄ alkyl esters of short-chain acids, ethyl or isopropyl lactate, fatty acid triglycerides such as the products marketed under the name "Miglyol," isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

- 5 It is possible to add, if necessary, an adjuvant chosen from antioxidants, surfactants, other preservatives, film-forming, keratolytic or comedolytic agents, perfumes, flavorings and colorings. Antioxidants such as t-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α -tocopherol and its derivatives can be added.

- Also contemplated are combination products that include one or more peptides of the
10 present invention and one or more other anti-microbial, anti-fungal, anti-viral, or anti-cancer agents. For example, a variety of antibiotics can be included in the pharmaceutical compositions of the invention, such as aminoglycosides (e.g., streptomycin, gentamicin, sisomicin, tobramycin and amikacin), ansamycins (e.g. rifamycin), antimycotics (e.g. polyenes and benzofuran derivatives), β -lactams (e.g. penicillins and cephalosporins),
15 chloramphenicol (including thiamphenol and azidamphenicol), lincosamides (lincomycin, clindamycin), macrolides (erythromycin, oleandomycin, spiramycin), polymyxins, bacitracins, tyrothycin, capreomycin, vancomycin, tetracyclines (including oxytetracycline, minocycline, doxycycline), phosphomycin and fusidic acid.

- Additionally, the peptides are well suited to formulation as sustained release dosage
20 forms and the like. The formulations can be so constituted that they release the active peptide, for example, in a particular part of the intestinal or respiratory tract, possibly over a period of time. Coatings, envelopes, and protective matrices may be made, for example, from polymeric substances, such as polylactide-glycolates, liposomes, microemulsions, microparticles, nanoparticles, or waxes. These coatings, envelopes, and protective matrices
25 are useful to coat indwelling devices, e.g., stents, catheters, peritoneal dialysis tubing, draining devices and the like.

- For topical administration, the therapeutic agents may be formulated as is known in the art for direct application to a target area. Forms chiefly conditioned for topical application take the form, for example, of creams, milks, gels, dispersion or microemulsions,
30 lotions thickened to a greater or lesser extent, impregnated pads, ointments or sticks, aerosol formulations (e.g., sprays or foams), soaps, detergents, lotions or cakes of soap. Other conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols. Thus, the

therapeutic peptides of the invention can be delivered via patches or bandages for dermal administration. Alternatively, the peptide can be formulated to be part of an adhesive polymer, such as polyacrylate or acrylate/vinyl acetate copolymer. For long-term applications it might be desirable to use microporous and/or breathable backing laminates, so
5 hydration or maceration of the skin can be minimized. The backing layer can be any appropriate thickness that will provide the desired protective and support functions. A suitable thickness will generally be from about 10 to about 200 microns.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated
10 with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active peptides can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or 4,051,842. The percent by weight of a therapeutic agent of the invention present in a topical formulation will depend on various factors, but generally
15 will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-85% by weight.

Drops, such as eye drops or nose drops, may be formulated with one or more of the therapeutic peptides in an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently
20 delivered from pressurized packs. Drops can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The therapeutic peptide may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may be formulated as a lozenge further
25 comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the composition of the present invention in a suitable liquid carrier.

The pharmaceutical formulations of the present invention may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents,
30 and salts of the type that are available in the art. Examples of such substances include normal saline solutions such as physiologically buffered saline solutions and water. Specific non-limiting examples of the carriers and/or diluents that are useful in the pharmaceutical formulations of the present invention include water and physiologically acceptable buffered saline solutions such as phosphate buffered saline solutions pH 7.0-8.0.

The peptides of the invention can also be administered to the respiratory tract. Thus, the present invention also provides aerosol pharmaceutical formulations and dosage forms for use in the methods of the invention. In general, such dosage forms comprise an amount of at least one of the agents of the invention effective to treat or prevent the clinical symptoms of a specific infection, indication or disease. Any statistically significant attenuation of one or more symptoms of an infection, indication or disease that has been treated pursuant to the method of the present invention is considered to be a treatment of such infection, indication or disease within the scope of the invention.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the therapeutic agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator, or a metered-dose inhaler (see, for example, the pressurized metered dose inhaler (MDI) and the dry powder inhaler disclosed in Newman, S. P. in Aerosols and the Lung, Clarke, S. W. and Davia, D. eds., pp. 197-224, Butterworths, London, England, 1984).

Therapeutic peptides of the present invention can also be administered in an aqueous solution when administered in an aerosol or inhaled form. Thus, other aerosol pharmaceutical formulations may comprise, for example, a physiologically acceptable buffered saline solution containing between about 0.1 mg/ml and about 100 mg/ml of one or more of the peptides of the present invention specific for the indication or disease to be treated. Dry aerosol in the form of finely divided solid peptide or nucleic acid particles that are not dissolved or suspended in a liquid are also useful in the practice of the present invention. Peptides of the present invention may be formulated as dusting powders and comprise finely divided particles having an average particle size of between about 1 and 5 μm , alternatively between 2 and 3 μm . Finely divided particles may be prepared by pulverization and screen filtration using techniques well known in the art. The particles may be administered by inhaling a predetermined quantity of the finely divided material, which can be in the form of a powder. It will be appreciated that the unit content of active ingredient or ingredients contained in an individual aerosol dose of each dosage form need not in itself constitute an effective amount for treating the particular infection, indication or disease since the necessary effective amount can be reached by administration of a plurality of dosage units. Moreover, the effective amount may be achieved using less than the dose in the dosage form, either individually, or in a series of administrations.

For administration to the upper (nasal) or lower respiratory tract by inhalation, the therapeutic peptides of the invention are conveniently delivered from a nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, 5 dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Nebulizers include, but are not limited to, those described in U.S. Patent Nos. 4,624,251; 3,703,173; 3,561,444; and 4,635,627. Aerosol delivery systems of the type disclosed herein are available from numerous commercial sources including Fisons Corporation (Bedford, 10 Mass.), Schering Corp. (Kenilworth, NJ) and American Pharmoseal Co., (Valencia, CA). For intra-nasal administration, the therapeutic agent may also be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

Furthermore, the active ingredients may also be used in combination with other 15 therapeutic agents, for example, pain relievers, anti-inflammatory agents, antihistamines, bronchodilators and the like, whether for the conditions described or some other condition.

The present invention further pertains to a packaged pharmaceutical composition for controlling microbial, fungal, or viral infections such as a kit or other container. The kit or container holds a therapeutically effective amount of a pharmaceutical composition for 20 controlling microbial infections and instructions for using the pharmaceutical composition for control of the microbial infection. The pharmaceutical composition includes at least one peptide of the present invention, in a therapeutically effective amount such that microbial infection is controlled.

25 The invention is further illustrated by the following non-limiting Examples.

EXAMPLE 1: Materials and Methods

General. 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), Fmoc-phenylalanine, leucine, and trityl chloride resin were purchased from 30 Novabiochem. Trifluoromethanesulfonic anhydride was purchased from Lancaster. All other reagents were purchased from Aldrich or Fisher. All reagents and solvents were used as received unless otherwise noted. All NMR spectra were obtained on a Varian Inova-400 MHz

or Bruker AMX-500 MHz spectrometer. Electrospray ionization mass spectrometry infusion experiments were carried out on Hitachi M-8000 ion trap and Agilent 1100 quadrupole mass spectrometers.

Prop-2-ynyl-carbamic acid, 9H-fluoren-9-ylmethyl ester (3). This compound was synthesized using procedures similar to those described in Tong, G.; Lawlor, J. M.; Tregear, G. W.; Haralambidis, J. *J. Org. Chem.* **1993**, 58, 2223-2231. Fmoc-*N*-hydroxysuccinimide (2.0 g, 5.9 mmol) was suspended in THF (12 mL). The solution was cooled on an ice bath, and propargylamine (0.427 mL, 6.23 mmol) was added dropwise. The reaction was stirred and allowed to warm to room temperature over 2 h. The THF was removed under vacuum. The residue was dissolved in EtOAc (150 mL) and washed with water (3 x 50 mL). The organic layer was dried and concentrated. The crude solid was recrystallized from EtOAc to yield 1.164 g (71%) of the desired product as white needles. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.88 (d, *J* = 8 Hz, 2H), 7.78 (t, *J* = 6 Hz, 1H), 7.68 (d, *J* = 7 Hz, 2H), 7.41 (t, *J* = 7 Hz, 2H), 7.32 (dt, *J* = 1, 7 Hz, 2H), 4.31 (d, *J* = 7 Hz, 2H), 4.21 (t, *J* = 7 Hz, 1H), 3.77 (dd, *J* = 2, 6 Hz, 2H), 3.10 (t, *J* = 2 Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 155.9, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 81.4, 73.1, 65.7, 46.6, 29.8; ESI-MS (*m/z*) 278 [M+H]⁺ (MW_{calcd} = 277).

2-(R)-{4-[(9H-Fluoren-9-ylmethoxycarbonylamino)-methyl]-[1,2,3]-triazol-1-yl}-4-methyl-pentanoic acid (6). Azido-D-leucine (5) (157 mg, 1.0 mmol) was used as a starting material for this synthesis. Lundquist IV, J. T.; Pelletier, J. C. *Org Lett.* **2001**, 3, 781-783. The azido-D-leucine (5) and Fmoc-propargylamine (3) (277 mg, 1.0 mmol) were dissolved in degassed acetonitrile (4 mL). To this solution, 2,6-lutidine (0.233 mL, 2.0 mmol) and diisopropylethylamine (0.348 mL, 2.0 mmol) were added under argon. Copper(I) iodide (19 mg, 0.1 mmol) was then added to the solution. The reaction was stirred under argon for 3 h. Most of the MeCN was removed under vacuum, and the resulting residue was dissolved in EtOAc (150 mL). The solution was washed with 9:1 saturated NH₄Cl:NH₄OH and then twice with 0.5 N HCl. The organic phase was dried and concentrated to yield 420 mg (97%) of the desired product as a white solid, which was used without further purification. An analytically pure sample was obtained by recrystallization from hot EtOAc/hexanes. [α]_D²² = -8.9 (*c* = 10 mg/mL in CHCl₃); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.00 (s, 1H), 7.88 (d, *J* = 7 Hz and s, total 3H), 7.69 (d, *J* = 7 Hz, 2H), 7.40 (t, *J* = 7 Hz, 2H), 7.31 (t, *J* = 7 Hz, 2H), 5.39 (dd, *J* = 4, 11 Hz, 1H), 4.4-4.2 (m, 3H), 3.7-3.1 (broad s, 1H), 2.2-2.1 (m, 1H), 2.0-1.8 (m, 1H), 1.2-1.1 (m, 1H), 0.83 (dd, *J* = 7, 23 Hz, 6H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ

170.7, 156.2, 145.0, 143.9, 140.7, 127.6, 127.1, 125.2, 122.6, 120.1, 65.6, 60.5, 46.7, 36.0, 24.3, 22.6, 20.9 (signal for methylene carbon on isobutyl side chain is buried under solvent peaks at about 39 ppm and was identified by a cross-peak observed in an HMQC experiment); ESI-MS (m/z) 435.2 $[M+H]^+$ ($MW_{\text{calcd}} = 434.2$).

Linear Peptide 8. (a) Loading of resin: Fmoc-phenylalanine (482 mg, 1.245 mmol) and diisopropylethylamine (0.217 mL, 1.245 mmol) were dissolved in CH_2Cl_2 (5 mL) and added to trityl chloride resin (500 mg, 1.66 mmol/g max loading). The mixture was agitated on a shaker for 4 hours. The vessel was then drained, and the resin was washed with 8:2:1 CH_2Cl_2 :MeOH:DIEA (2 x 10 min), CH_2Cl_2 (3 x 1 min), and Et_2O . After drying under vacuum, loading was quantified by UV quantification of Fmoc release. Final loading was found to be 1.0 mmol/g.

(b) Peptide Synthesis: The resin (150 mg, 0.100 mmol) prepared above was placed in a sintered glass peptide synthesis vessel and swollen in CH_2Cl_2 for 45 min. The resin was washed twice with DMF, treated with 20% piperidine/DMF (2 x 8 min) to remove the Fmoc group, and washed again with DMF (3x). A solution of **6** (241 mg, 0.555 mmol), diisopropylcarbodiimide (70 mg, 0.555 mmol), and HOBt, H_2O (85 mg, 0.555 mmol) in DMF (2 mL) was added to the vessel. The resin suspension was agitated for 30 min, drained, and washed with DMF (3x). After Fmoc deprotection and washing as described above, a solution of Fmoc-phenylalanine (233 mg, 0.602 mmol), HBTU (210 mg, 0.553 mmol), and diisopropylethylamine (0.263 mL, 1.501 mmol) in DMF (2 mL) was added. The coupling was carried out for 30 min followed by washing and Fmoc deprotection. The final coupling of **6** was carried out under the same conditions as the first coupling.

After removal of the terminal Fmoc, the resin was washed with DMF (3x) and then CH_2Cl_2 (3x). Product was cleaved from the resin by treatment with 5% TFA in CH_2Cl_2 (5 x 3 mL) followed by washing with CH_2Cl_2 and MeOH. The acid solution and subsequent washes were combined and concentrated under vacuum. Water was added to the oily residue, and the resulting suspension was frozen and lyophilized. The crude product was purified by preparative reverse-phase HPLC on a C18 column to yield 46 mg of **8** (66% based on resin loading). MALDI-FTMS (m/z) 701.3886 $[M+H]^+$ ($MW_{\text{calcd}} = 701.3882$).

Macrocycle 1. Linear precursor **8** (29 mg, 0.041 mmol) was dissolved in DMF (29 mL). PyBOP (32 mg, 0.061 mmol), HOAT (0.123 mL of 0.5 M solution in DMF, 0.061 mmol), and DIEA (0.043 mL, 0.245 mmol) were added to the solution. After stirring for 90 min, the DMF was removed under vacuum. The resulting residue was triturated with 4:1

water:acetonitrile (3 x 10 mL). The remaining solid was dried under vacuum to yield 18 mg (65%) of **1**. ¹H NMR (500 MHz, DMSO- *d*₆) δ 9.06 (d, *J* = 7 Hz, 2H), 8.66 (dd, *J* = 4, 8 Hz, 2H), 7.91 (s, 2H), 7.32-7.26 (m, 8H), 7.24-7.18 (m, 2H), 5.33 (t, *J* = 7 Hz, 2H), 4.63 (dd, *J* = 8, 16 Hz, 2H), 4.45-4.35 (m, 2H), 4.00 (dd, *J* = 4, 16 Hz, 2H), 3.00 (dd, *J* = 4, 14 Hz, 2H), 2.77 (dd, *J* = 11, 14 Hz, 2H), 1.6-1.5 (m, 2H), 1.5-1.4 (m, 2H), 0.9-0.8, (m, 2H), 0.70 (t, *J* = 7 Hz, 12H); ¹³C NMR (125 MHz, DMSO- *d*₆) δ 170.9, 167.7, 146.1, 137.7, 129.1, 128.1, 126.4, 120.4, 60.5, 55.4, 43.1, 37.0, 34.4, 24.0, 22.1, 21.8; MALDI-FTMS (*m/z*) 683.3774 [*M*+*H*]⁺ (*MW*_{calcd}) 683.3776).

Crystal Data Collection and Structure Determination. Crystals of **1** were prepared by slow evaporation of a saturated in solution in ethanol. Each crystal was mounted on a cryo-loop with paratone-N oil. Data were collected on an Raxis IV image plate detector equipped with Osmic confocal mirrors and Xstream cryo-device (100K) using Cu K α radiation (λ = 1.5418 Å) from a Ru200 X-ray generator operated at 50 kV, 100 mA. Data were processed using MSC Crystal Clear. The space group was determined to be *P*1 with cell dimensions *a* = 5.51 Å, *b* = 12.59 Å, *c* = 14.71 Å, α = 83.30°, β = 72.61°, γ = 81.88°. The data set was 96% complete and included 2356 reflections of which 320 were unique. The scaling and averaging gave an *R*_{merge} of 7.4%. The mean *I*/ σ was 6.7, and the average multiplicity for the data set was 7.3. Each cell contains **1**, EtOH (C₃₆H₄₆N₁₀O₄·C₂H₆O).

The structure was solved by molecular replacement and restrained refinement using the Collaborative Computational Project Number 4: *Acta Crystallogr.* **1994**, *D50*, 760-763; Xtalview: McRee, D. E. *J. Mol. Graphics* **1992**, *10*, 44-46. The search model was generated from an energy-minimized structure of **1** calculated in the Discover module of InsightII. Geometric restraints for the triazole portion were assembled from a survey of 1,4-disubstituted 1,2,3-triazoles in the Cambridge Structural Database. Hydrogen atoms were used in the refinement but were fixed to moving C, N, and O atoms. After several cycles of restrained refinement, electron density for the ethanol was located in a $|F_o| - |F_c|$ map and modeled. The final model gave an *R*_{factor} of 16.7% for all data and an *R*_{free} value of 18.0% for 10% of the data set aside throughout structure solution and refinement. The mean *B* value for the model was 17.8 Å² with rms deviations from ideal bond lengths and angles of 0.02 Å and 2.6°, respectively.

Variable-Concentration ¹H NMR. A 1.0 mM stock solution of **1** was prepared in CDCl₃. Serial 2-fold dilutions were carried out to produce 0.5, 0.25, 0.125, and 0.0625 mM stocks. A 0.6 mL amount of each solution was transferred to separate NMR tubes. These tubes were

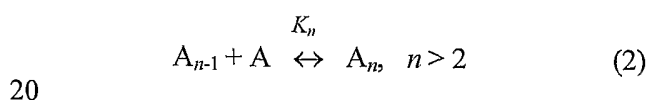
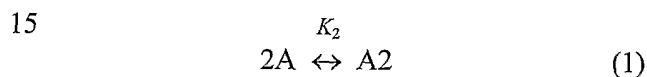
placed on an autosampler, and ^1H NMR spectra were acquired on a Varian 400 MHz instrument. As all peaks are broad, each chemical shift was recorded as the midpoint of the observed signal. The recorded data is summarized in Table 3.

5 **Table 3: Concentration Dependent ^1H NMR Chemical Shifts (in Hz)**

signal	δ 1.0 mM	δ 0.50 mM	δ 0.25 mM	δ 0.125 mM	δ 0.0625 mM
Leu ϵ CH ₃	292	296	300	303	308
Leu γ CH	378	387	399	408	<i>a</i>
Leu β CH ₂ (1)	772	777	781	788	<i>a</i>
Phe β CH ₂ (1)	1233	1226	1222	1219	<i>a</i>
backbone CH ₂ (1)	1650	1659	1673	1683	1706
backbone CH ₂ (2)	1857	1854	1849	1841	1831
Phe α CH	1911	1907	1904	1901	1899
Leu α CH	2141	2124	2107	2087	2059
amide NH (1)	3212	3147	3086	<i>a</i>	<i>a</i>

a Signal was either buried under another peak or not visible above noise at the lowest concentration acquisition.

10 **Derivation of Apparent Equilibrium Constants.** The model developed by LaPlanche et al. was used to determine association constants for the initial dimerization and higher order aggregation. LaPlanche, L. A.; Thompson, H. B.; Rogers, M. T. *J. Phys. Chem.* **1965**, 69, 1482-1488.



In the above expressions, A represents peptide monomer and A_n represents n peptide monomers in a hydrogen-bonded assembly. In brief, the simplifying assumptions used in the modeling are (1) the monomer-dimer equilibrium has a unique binding constant K_2 , (2) the association constants K_n for higher order aggregation events are all equal, (3) the chemical shift of a proton in assembly A_n does not depend on n when $n \geq 2$, (4) $[A_n] < [A_{n-1}]$ for $n > 2$, and (5) the amount of hydrogen bonding by the solvent is negligible. Also, although 1 exhibited parallel stacking in the solid state, antiparallel stacking is also possible. To reduce calculations to a manageable level, antiparallel species were not included.

From the equations derived by LaPlanche, the chemical shift δ_{calcd} for a given proton in the above system satisfies

$$\delta_{\text{calcd}} = (\delta_1 - \delta_n) x + \delta_n \quad (3)$$

$$x = \left[\frac{1 + \frac{K_2 A_1}{1 - K_n A_1}}{1 + \frac{K_2 A_1 (2 - K_2 A_1)}{1 - K_n A_1}} \right] \quad (4)$$

where A_1 represents the mole fraction of free monomer in solution, δ_1 is the chemical shift of the proton in the monomer, and δ_n is the chemical shift in aggregate species. A_1 for given values of C , K_2 , and K_n is found by solving the root of

$$[K_n^2(C+1) - K_n K_2 C - K_n K_2] A_1^3 + [-2K_n(C+1) - K_n^2 C + K_2 C + 2K_2] A_1^2 + [(C+1) + 2K_n C] A_1 - C = 0 \quad (5)$$

where C is defined as the fraction of moles of total peptide added to moles of solvent. As equation 5 has only one real root subject to the physical constraints of the experiment, an A_1 value for a given pair of K_2 and K_n can be determined explicitly for any experimental concentration. Thus, experimental $\{\delta_{\text{obsd}}, C\}$ pairs for a given signal in the NMR spectrum can be used in equation 3 to find corresponding δ_1 and δ_n by linear

regression analysis. K_2 and K_n were used as parameters in the above model to fit δ_{obsd} measured for nine protons in **1** at five concentrations in CDCl_3 . The objective function was defined as the sum of the squares of the residuals between δ_{calcd} and δ_{obsd} for all signals. Nonlinear numerical minimization algorithms were used to find the K_2 and K_n values
5 showing the best overall agreement with experimental data.

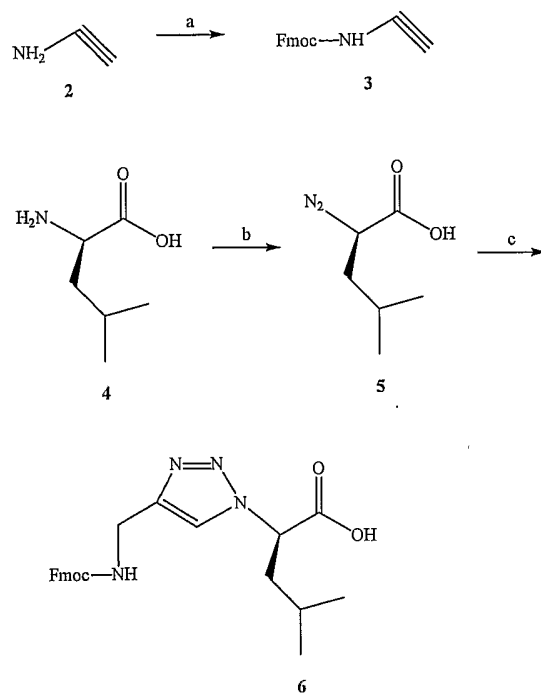
Supporting Information Available: Atomic coordinates for **1** (CIF format). This material is available free of charge via the Internet at <http://pubs.acs.org>. See any current masthead page for ordering information and Web access instructions.

10

EXAMPLE 2: Peptides with 1,2,3-Triazole ϵ -Amino Acids

The macroheterocyclic peptide **1** employed in the present study was designed based on similar structural considerations that have been previously noted for the cyclic D,L-R-
15 peptide nanotube analogues. An even number of amino acids with alternating CR stereochemistry was employed to instill a preference for adoption of a flat ring conformation in solution. In this conformation the side chains are presented on the exterior of the macrocycle with the amide backbone oriented perpendicular to the plane of the ring. This provides complementary hydrogen bond donor and acceptor pairs on each face of the ring
20 structure enabling cyclic peptide self-assembly. Molecular modeling suggested that within this peptide framework the triazole backbone moiety could adopt a number of conformations that would be productive with respect to the intermolecular hydrogen bond-directed stacking.

The required 1,2,3-triazole ϵ -amino acid precursor **6**, suitable for use in peptide synthesis, was readily synthesized in three steps from the corresponding optically pure free
25 amino acid in 81% overall yield (Scheme 1).

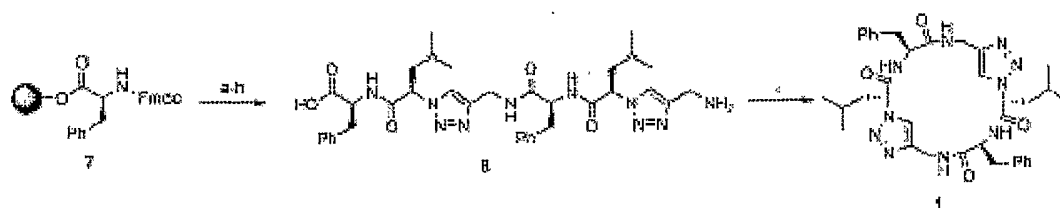


Scheme 1

- 5 Reagents used for Scheme 1 were as follows: (a) Fmoc-*N*-hydroxysuccinimide (71%); (b) $(F_3CSO_2)_2O$, NaN_3 , then $CuSO_4$, K_2CO_3 (84%); (c) 3, CuI , diisopropylethylamine, 2,6-lutidine (97%).

Commercially available propargylamine (2) was protected as the *N*-fluorenylmethyl carbamate (3) by treatment with Fmoc-NHS. Tong, G.; Lawlor, J. M.;
 10 Tregear, G. W.; Haralambidis, J. J. *Org. Chem.* 1993, 58, 2223-2231. The free amino acid D-leucine (4) was converted to α -azido acid 5 by copper(II)-catalyzed diazo transfer from in situ generated triflyl azide. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* 1996, 37, 6029-6032; Lundquist IV, J. T.; Pelletier, J. C. *Org Lett.* 2001, 3, 781-783. Alkyne 3 and azide 5 in the presence of catalytic amounts of copper(I) and base underwent a rapid 1,3
 15 dipolar cycloaddition reaction to afford triazole 6 in 97% isolated yield. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed. Engl.* 2002, 41, 2596-2599; Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* 2002, 67, 3057-3064.

The synthesis of the triazole backbone modified linear peptide was performed on polystyrene solid support using the acid labile trityl linker and Fmoc-phenylalanine as the
 20 first residue (Scheme 2).

**Scheme 2****Compound 1**

Reagents used for Scheme 2 were as follows: (a) 20% piperidine/DMF; (b) **6**, DIC, HOBT;
 5 (c) 20% piperidine/DMF; (d) Fmoc-Phe-OH, HBTU, DIEA; (e) 20% piperidine/DMF; (f) **6**,
 DIC, HOBT; (g) 20% piperidine/DMF; (h) 5% TFA/DCM; (i) PyBOP, HOAT, DIEA.

Solid phase Fmoc peptide synthesis was carried out using standard protocols except
 for coupling of the triazole residues which were performed under base free conditions to
 minimize racemization. (Coupling reactions of **6** in the presence of diisopropylethylamine
 10 led to substantial racemization at the stereocenter adjacent to the triazole.) The linear
 peptide **8** was cleaved from the resin by treatment with 5% TFA/CH₂Cl₂ and purified by RP-
 HPLC (66% isolated yield). Exposure of the linear peptide in DMF to activating agents
 (PyBOP, HOAT, DIEA) resulted in a rapid macrolactamization yielding **1**. Pure peptide **1**
 was isolated in 65% yield after repeated trituration/crystallization from water/MeCN.

15 Techniques for synthesis of heterocyclic amino acids with backbone modifications are well
 known in the art, examples and protocols of which are illustrated in Seneci, P. Solid-Phase
 Synthesis and Combinatorial Technologies. John Wiley & Sons, Inc., 2001, New York.;
 Bannwarth, W., & Felder, E. Combinatorial Chemistry: A Practical Approach. Wiley-VCH,
 Weinheim., 2000; and Dorwald, F.Z. Organic Synthesis on Solid Phase: Supports, Linkers,
 20 Reactions. Wiley-VCH, Weinheim, New York., 2000, the contents of which are all herein
 incorporated by reference in their entireties.

¹H-NMR spectroscopy was employed to probe the aggregation propensity of **1** in
 solution. The spectrum in the polar solvent DMSO was sharp with well-resolved signals
 while in the nonpolar solvent chloroform peaks broaden significantly (Figure 1). Moreover,
 25 ¹H-NMR spectra in CDCl₃ displayed significant concentration dependent chemical shifts.
 These observations are consistent with hydrogen bond-mediated intermolecular aggregation
 giving rise to multiple supramolecular species in fast exchange on the NMR time scale. To
 approximate the equilibrium binding associations between interacting species in solution, ¹H-
 NMR spectra in CDCl₃ at a range of concentrations from 1.0 mM to 63 μM were analyzed
 30 according to the model developed by LaPlanche et al., J. Phys. Chem. 1965, 69, 1482-1488.

These analyses were used to derive apparent association constants for formation of dimeric ($K_2 = 8.6 \times 10^4 \text{ M}^{-1}$) and higher order aggregate species ($K_n = 3.8 \times 10^4 \text{ M}^{-1}$). The apparent equilibrium constants correspond to $6.7 \text{ kcal} \cdot \text{mol}^{-1}$ driving force for peptide dimerization and $6.2 \text{ kcal} \cdot \text{mol}^{-1}$ for each subunit added to from a higher order aggregate. The aggregation propensity of peptide 1 was also evident by electrospray ionization mass spectrometry. Infusion of a 0.5 mM solution of 1 in 9:1 acetonitrile/water gave rise to strong signals corresponding to monomeric $[1+H]^+$ (calcd = 683.4, obsd = 683.7), $[1+Na]^+$ (calcd = 705.4, obsd = 705.7) as well as noncovalent dimers $[1 \cdot 1+H]^+$ (calcd = 1365.8, obsd = 1365.5), and $[1 \cdot 1+Na]^+$ (calcd = 1387.7, obsd = 1387.2).

X-ray crystallography was employed to elucidate the structural characteristics of 1 in the solid-state. Small needle-like crystals with high aspect ratios were grown by slow evaporation from a saturated solution of 1 in ethanol and used to obtain X-ray diffraction data at -160°C to 1.8 Å resolution. As direct methods are not applicable at this resolution, the crystal structure was solved using molecular replacement techniques and restrained refinement to give a structure with $R = 16.7\%$ ($R_{\text{free}} = 18\%$). The crystal structure (Figure 2) indicates that the peptide subunits adopt the expected flat ring shape conformation and stack in a parallel fashion into tubular assemblies with ordered ethanol molecules filling the channel pores. It is noteworthy to point out that the nanotube solid-state structure does not seem to be an artifact of the crystal packing forces as a single cyclic peptide comprises the contents of the unit cell (P1). The triazole rings orient perpendicular to the overall macrocycle, lining the nanotube interior with π electron rich heteroaromatic moieties. Three of the four amide bonds in the ring backbone form a network of intermolecular hydrogen bonds with N to O distances of 3.3 Å. The remaining amide bond is involved in an apparent ethanol-mediated bridging hydrogen bond with N to O and O to O distances of 2.7 Å. The portion of the amide backbone involved in this bridging hydrogen bond is slightly tilted, relative to the rest of the ring, with the amide N-H pointing more toward the ethanol oxygen than along the tube axis. The peptide nanotube channel is roughly oval in shape with internal Van der Waals diameter ranging from 5.2 Å between the triazole rings to 6.8 Å between the phenylalanine α carbons. Volume calculations were carried out using GRASP: Nicholls, A.; Sharp, K. A.; Honig, B. *Proteins* 1991, 11, 281-296. These volume calculations suggest an approximately 80 Å^3 cavity size per macrocycle repeat along the tube axis with the ethanol filling about 60% of this space.

Additional cyclic peptides with 1,2,3-triazole ϵ -amino acids, including compounds II-XIII, from several genera of cyclic peptides were prepared as described above. Hence, the

invention describes the design, synthesis, and characterization of a new class of peptide based macrocycle incorporating 1,2,3-triazole ϵ -amino acids in the backbone. The synthesis is modular and straightforward with the protected triazole ϵ -amino acid readily prepared from the corresponding free amino acid. In the solid state, these molecules form a solvent filled
5 nanotube held together by an extended network of intermolecular amide backbone hydrogen bonds. NMR and mass spectrometry studies support similar behavior in solution and the gas phase.

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- (8) Coupling reactions of 6 in the presence of diisopropylethylamine led to substantial racemization at the stereocenter adjacent to the triazole.
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- (12) Volume calculations were carried out using GRASP: Nicholls, A.; Sharp, K. A.; Honig, B. Proteins 1991, 11, 281-296.
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All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

30

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the

invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein

5 without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in

10 the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an antibody" includes a plurality (for example, a solution of antibodies or a series of antibody preparations) of such antibodies, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or

15 embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

20 The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically

25 disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the

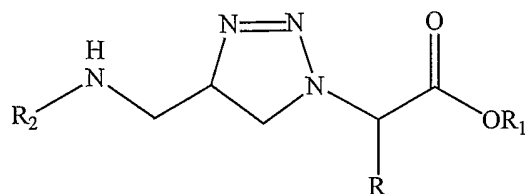
30 narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

The following statements of the invention are intended to characterize possible elements of the invention according to the foregoing description given in the specification. Because this application is a provisional application, these statements may become changed upon preparation and filing of a nonprovisional application. Such changes are not intended to
5 affect the scope of equivalents according to the claims issuing from the nonprovisional application, if such changes occur. According to 35 U.S.C. § 111(b), claims are not required for a provisional application. Consequently, the statements of the invention cannot be interpreted to be claims pursuant to 35 U.S.C. § 112.

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WHAT IS CLAIMED IS:

1. A cyclic peptide comprising at least one 1,2,3-triazole ϵ -amino acid of formula **I**:



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wherein:

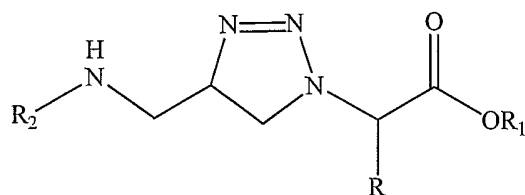
R is any amino acid side chain;

R₁ is H, a protecting group or an amino acid; and

10

R₂ is H, a protecting group or an amino acid.

2. A peptide comprising at least one 1,2,3-triazole ϵ -amino acid of formula **I**:



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wherein:

R is any amino acid side chain;

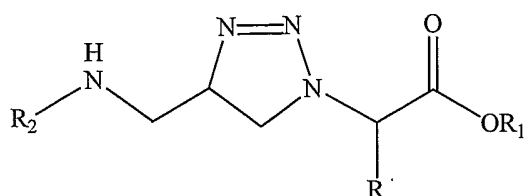
R₁ is H, a protecting group or an amino acid; and

R₂ is H, a protecting group or an amino acid.

20

3. The peptide of claim 2, wherein the peptide is a linear peptide.
4. The peptide of claim 2, wherein the peptide is a cyclic peptide.
5. A composition comprising a carrier and a peptide comprising at least one 1,2,3-triazole ϵ -amino acid of formula **I**:

25



wherein:

R is any amino acid side chain;

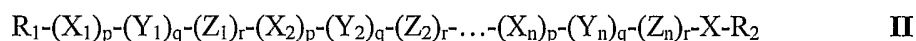
5 R₁ is H, a protecting group or an amino acid; and

R₂ is H, a protecting group or an amino acid.

6. The composition of claim 5, wherein the peptide is a linear peptide.

10 7. The composition of claim 5, wherein the peptide is a cyclic peptide.

8. A cyclic peptide that has an amino acid sequence comprising formula II:

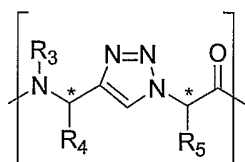


15 wherein:

each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



20 R₃, R₄, and R₅ are separately any amino acid, functional group, protecting group;

each Y is any α amino acid residue;

each Z is any β amino acid residue; and

25 R₁ and R₂ can separately be a hydrogen atom, hydroxy group, protecting group or R₁ and R₂ can be linked to form a cyclic peptide when there are at least three residues in the peptide.

9. The cyclic peptide of claim 8, wherein R₅ is an amino acid.

10. The cyclic peptide of claim 8, wherein R₃ and R₄ are chiral propargyl amines.
11. A cyclic peptide that has an amino acid sequence comprising formula III:
cyclo[X₁-Y₁-X₂-Y₂] III
wherein:
each X is an ε amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X
groups; and
each Y is an α amino acid with either R or S chirality, but with chirality
opposite to that of the X ε amino acid and where each Y has the same
chirality as the all the other Y groups.
12. The cyclic peptide of claim 11, wherein the peptide can self-assemble into a
supramolecular structure.
13. A cyclic peptide that has an amino acid sequence comprising formula IV:
cyclo[X₁-Z₁-X₂-Z₂] IV
wherein:
each X is an ε amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X groups;
and
each Z is a β amino acid with either R or S chirality, but with chirality
opposite to that of the X ε amino acids and where each Z has the same chirality as
the all the other Z groups.
14. The cyclic peptide of claim 13, wherein the peptide can self-assemble into a
supramolecular structure.
15. A cyclic peptide that has an amino acid sequence comprising formula V:
cyclo[X₁- X₂- X₃] V

wherein:

each X is an ε amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X groups.

- 5 16. The cyclic peptide of claim 15, wherein the peptide can self-assemble into a
supramolecular structure.

17. A cyclic peptide that has an amino acid sequence comprising formula VI:

10 cyclo[X₁-X₂-X₃-X₄] VI

wherein each X is separately an ε amino acid with alternating R,R or S,S substitution pattern throughout the peptide.

18. The cyclic peptide of claim 17, wherein the peptide can self-assemble into a
15 supramolecular structure.

19. A cyclic peptide that has an amino acid sequence comprising formula VII:

cyclo[X₁-Y₁-Y₂-X₂-Y₃-Y₄] VII

wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X groups; and

each Y is an α amino acid with either R or S chirality, but with chirality
 25 opposite to that of the X ε amino acid and where each Y has the same chirality as the
 all the other Y groups.

20. The cyclic peptide of claim 19, wherein the peptide can self-assemble into a supramolecular structure.

21. A cyclic peptide that has an amino acid sequence comprising formula **VIII**:

cyclo[X₁-Y₁-Y₂-X₂-Y₃-Y₄-X₃-Y₅-Y₆] **VIII**

wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S
chirality and where each X has the same chirality as the all the other X groups; and

5 each Y is an α amino acid with either R or S chirality, but with chirality
opposite to that of the X ϵ amino acid and where each Y has the same chirality as
the all the other Y groups.

22. The cyclic peptide of claim 21, wherein the peptide can self-assemble into a
supramolecular structure.

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23. A composition comprising a carrier and the cyclic peptide of any one of claims 8-
22.

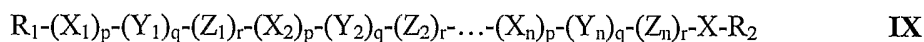
24. The composition of claim 23, wherein the carrier is a pharmaceutically effective
15 carrier.

25. A cyclic peptide of claim 1 comprising two 1,2,3-triazole ϵ -amino acids according
to formula I.

20 26. A cyclic peptide of claim 1 comprising at least four alternating α - and ϵ -amino
acids.

27. A cyclic peptide of claim 1 selected from any one of compounds 1-13.

25 28. A cyclic peptide that has an amino acid sequence comprising formula IX:

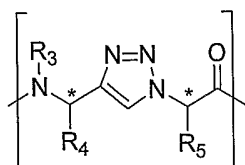


wherein:

30 each p, q, or r is separately an integer of 1 or 0;

at least one p is 1;

X is an epsilon amino acid residue of the following formula:



R_3 , R_4 , and R_5 are separately any amino acid, functional group, protecting group;

each Y is any α amino acid residue;

5 each Z is any β amino acid residue; and

R_1 and R_2 can separately be a hydrogen atom, hydroxy group, protecting group or R_1 and R_2 can be linked to form a cyclic peptide when there are at least two residues in the peptide.

10 29. The cyclic peptide of claim 28, wherein R_5 is an amino acid.

30. The cyclic peptide of claim 28, wherein R_3 and R_4 are chiral propargyl amines.

31. A cyclic peptide that has an amino acid sequence comprising formula X:

15

cyclo[$X_1-Y_1-X_2-Y_2-X_3-Y_3$]

X

wherein:

each X is an ϵ amino acid as described above with either an R,R or S,S chirality and where each X has the same chirality as the all the other X groups; and

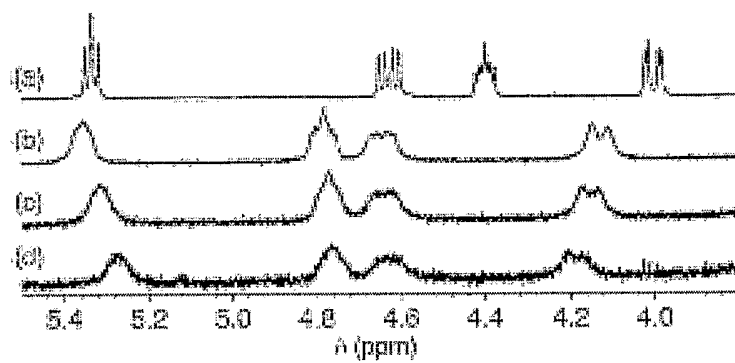
20

each Y is an α amino acid with either R or S chirality, but with chirality opposite to that of the X ϵ amino acid and where each Y has the same chirality as the all the other Y groups.

25 32. The cyclic peptide of claim 31, wherein the peptide can self-assemble into a supramolecular structure.

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5



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Fig. 1

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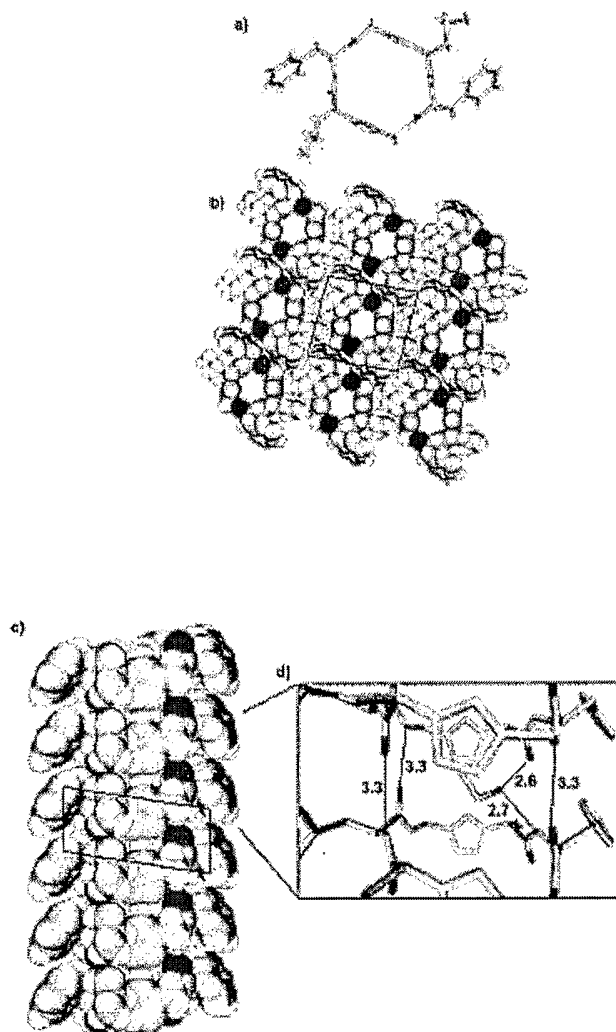
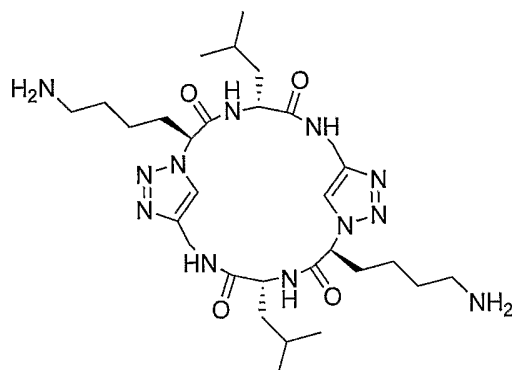
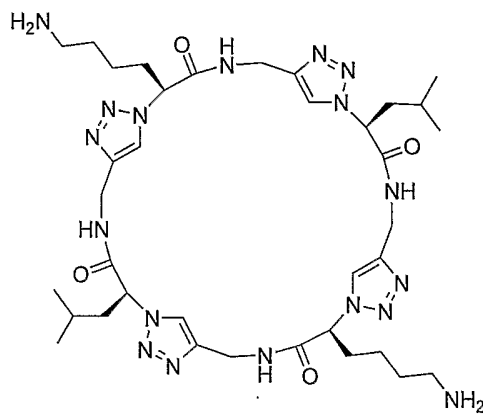


Fig. 2

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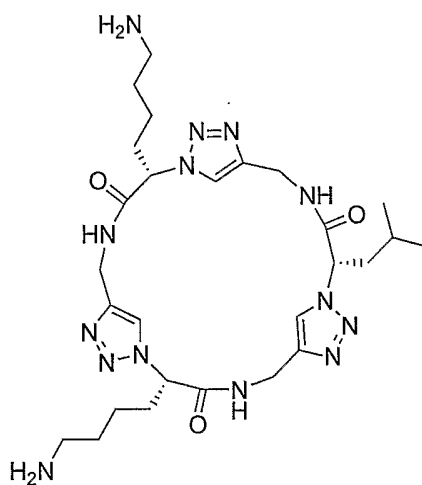
Compound II

Fig. 3A

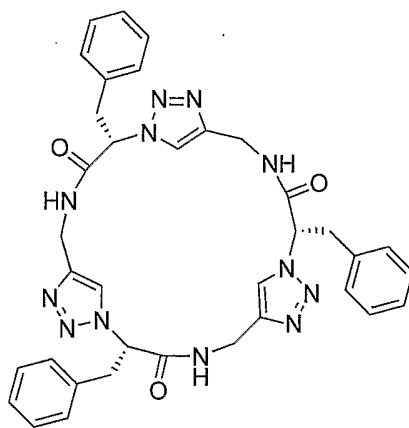
Compound III

Fig. 3B

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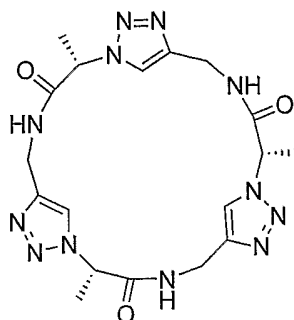
Compound IV

Fig. 4A

Compound V

Fig. 4B

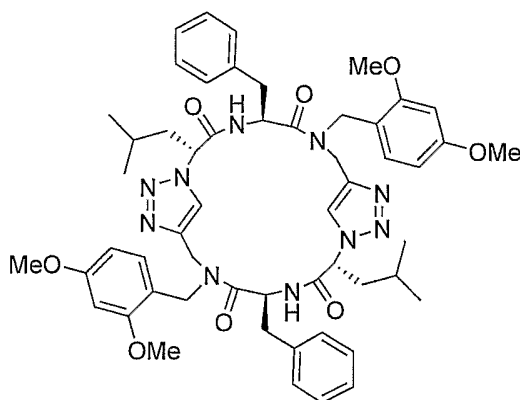
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Compound VI

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Fig. 5A

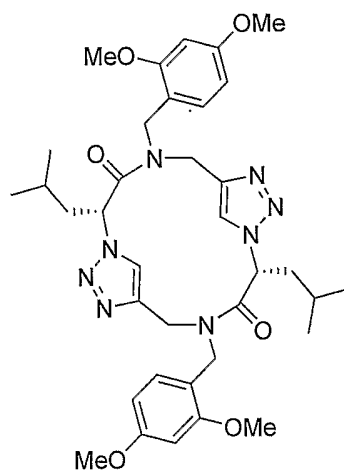
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Compound VII

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Fig. 5B

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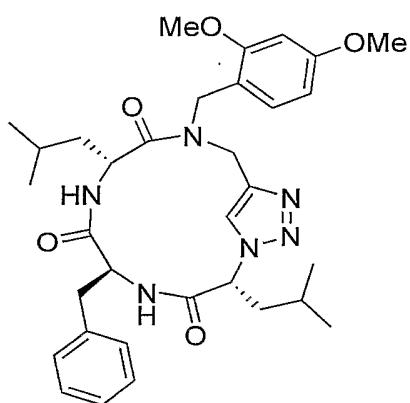


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Compound VIII

Fig. 6A

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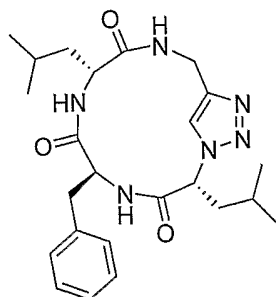


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Compound IX

Fig. 6B

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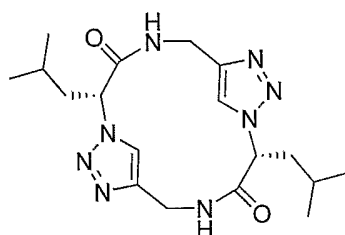
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Compound X

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Fig. 7A

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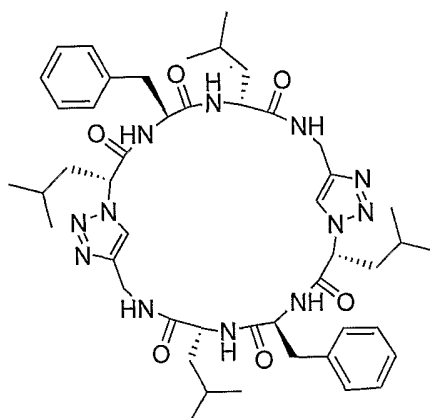


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Compound XI

Fig. 7B

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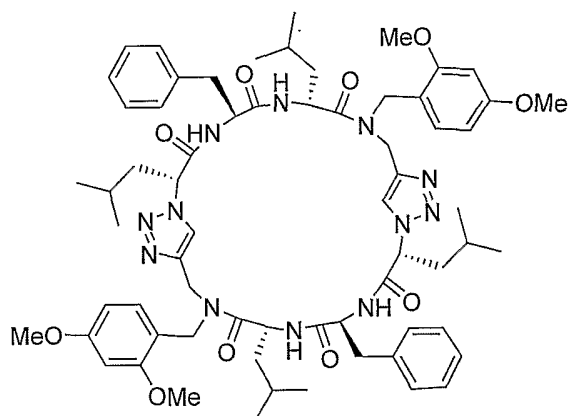


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Compound XII

Fig. 8A

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Compound XIII

Fig. 8B